Myocardial Function and Transmural Blood Flow During Coronary Venous Retroperfusion in Pigs

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Background. The degree of recovery of regional myocardial contraction during coronary venous retroperfusion has not been well established, particularly in the absence of coronary collateral channels. Therefore, the maximal functional benefit attainable with coronary venous retroperfusion was assessed in pigs by means of using selective pump retroperfusion of the left anterior descending artery to zero pressure.

Methods and Results. In eight anesthetized open-chest pigs during selective left anterior descending venous retroperfusion over a range of retroperfusion flows, regional myocardial function (percent systolic wall thickening by sonomicrometry) increased progressively to an average of 62% of control values at a retroperfusion flow rate 200% of control arterial flow. Progressive thickening of the end-diastolic dimension of the anterior wall was observed with increasing retroperfusion flow (from 8.7±0.9 to 10.7±2.3 mm, p<0.001). Perfusion pressures within the left anterior descending vein increased linearly with increased retroperfusion flow rates (up to 132±57 mm Hg with retroperfusion flow 200% of control). A gradual increase of retrograde left anterior descending arterial outflow was observed with increasing retroperfusion flows; however, the absolute amount (maximum, 8.3±4.1 ml/min) was much too low to explain the extent of functional recovery. Transmural myocardial capillary blood flows in the anterior wall with retroperfusion flows of 100% and 200% of control arterial flow were 0.22 and 0.42 ml/min/g with corresponding subendocardial blood flows of 0.14 and 0.29 ml/min/g; ratios of endocardium to epicardium were 0.51 and 0.61, respectively. Thus, capillary blood flows during selective retroperfusion were relatively low despite considerable restoration of regional systolic wall thickening, and a significant difference was noted in the slopes of the relations between regional systolic wall thickening and myocardial blood flow during retroperfusion and anterograde arterial perfusion (p<0.05). With retrograde injection of silicone elastomer at different retroperfusion pressures (50, 75, and 100 mm Hg) in three pigs, capillaries were well visualized, and profuse intramyocardial venous anastomotic connections were seen at the highest retroperfusion pressure (100 mm Hg), whereas there was filling of small venules but little capillary filling at the lowest retroperfusion pressure (50 mm Hg).

Conclusions. Considerable recovery of regional myocardial function with low regional capillary blood flows were observed during acute venous retroperfusion with high retroperfusion flows with arterial blood. These findings together with low levels of retrograde arterial outflow and visualization of retrograde capillary filling with a rich venous network provide evidence for possible oxygen delivery via the intramyocardial venous plexus. (Circulation 1992;86:1265–1279)

Key Words: myocardial blood flow • regional myocardial function • pressure-flow relation • retroperfusion

The coronary venous system has been considered an alternative route for delivering oxygen or drugs to the ischemic myocardium,1–8 and retroperfusion has been reported to improve global and regional myocardial function4–8 and myocardial oxygenation and energy metabolism9,10 and to reduce myocardial infarct size.11,12 In addition to its clinical application in unstable angina pectoris,13 retroperfusion has been grant HL-17682 from the National Heart, Lung, and Blood Institute. B.-H.O. is supported by a grant from the Seoul National University Hospital.

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All editorial decisions for this article, including selection of reviewers and the final decision, were made by Dr. Samuel Meerbaum, as guest editor. This procedure applies to all manuscripts with authors from the University of California San Diego or UCSD Medical Center.

Received May 20, 1991; revision accepted June 23, 1992.
used during percutaneous transluminal coronary angioplasty in high-risk patients\textsuperscript{14} and during coronary bypass surgery.\textsuperscript{15,16} Retroperfusion by surgical creation of an arterial anastomosis to a coronary vein also has been considered an alternative revascularization method to the coronary artery bypass graft in selected patients who have diffusely diseased or very small arteries with poor distal runoff, in whom there is little likelihood of long-term graft patency.\textsuperscript{17,18}

The potential extent of functional benefit, as well as the hemodynamic responses and retrograde drainage pattern during selective coronary venous retroperfusion, has not been well established, particularly in animal models with little coronary collateral circulation. Accordingly, we assessed the maximal functional benefit attainable with coronary venous retroperfusion in eight open-chest anesthetized pigs using selective pump retroperfusion of the left anterior descending coronary vein, with venting of the left anterior descending artery to zero pressure. The degree of recovery of regional contractile function, pressure–flow relations, retrograde arterial outflow, and myocardial blood flow distribution were evaluated over a range of retroperfusion flow rates. In three pigs, injection of silicone elastomer into the left anterior descending vein was performed at different retroperfusion pressures to evaluate the vascular microanatomy during retroperfusion.

**Methods**

The animals in this study were handled according to the animal welfare regulations of the American Heart Association and the University of California San Diego, and the experimental protocol was approved by the animal subjects committee of this institution.

**Animal Model and Surgical Preparation**

Eight swine (20–30 kg) were sedated with ketamine hydrochloride (30 mg/kg i.m.) and anesthetized with thiamylal sodium (20 mg/kg) administered through an ear vein. A tracheostomy was performed, and an endotracheal tube was positioned and connected to a respi- rator equipped with an isoflurane vaporizer. Anesthesia was maintained with isoflurane (1.5–2.5%) and 100% oxygen, and ventilation was adjusted to maintain Po$_2$, Pco$_2$, and pH within the following ranges: Po$_2$, 35±5 mm Hg; Pco$_2$, >150 mm Hg, and pH 7.40±0.10. Both carotid arteries were cannulated with large polyethylene catheters—one to supply the extracorporeal circuit, and the other for aortic pressure measurement and blood sampling. Both internal jugular veins were cannulated—one to infuse saline, and the other to return blood from the pump-perfused coronary circulation. Rectal temperature was measured periodically, and animals were kept on a circulating hot water pad to prevent hypothermia (body temperature, >36.8°C).

A left lateral thoracotomy was performed in the fourth intercostal space, and the pericardium was opened and sutured to cradle the heart. Bipolar electrodes were sutured to the left atrial appendage for electrical pacing (Medtronic 5800, Minneapolis, Minn.), and a fluid-filled catheter was inserted into the left atrium through the left atrial appendage for injection of microspheres. A micromanometer (Konigsberg P7, Pasadena, Calif.) and a fluid-filled catheter were placed in the left ventricle through the apex for measurement of left ventricular pressure.

A pair of ultrasonic crystals was implanted in the anterior wall within the perfusion bed of the left anterior descending artery (and the drainage bed of the left anterior descending vein) to measure wall thickness by standard techniques.\textsuperscript{19} To verify the stability of the preparation, a pair of ultrasonic crystals was also implanted in the lateral wall (control zone) within the perfusion bed of the normally perfused left circumflex artery to measure wall thickness. Dimensions were measured by the ultrasonic transit time technique\textsuperscript{19} (Triton Technologies, San Diego, Calif.).

The proximal left anterior descending artery and the left anterior descending vein at the junction of the great cardiac vein were dissected free from the surrounding tissue. After an infusion of heparin (15,000 IU initial dose, followed by 10,000 IU/hr i.v. bolus), the left anterior descending artery was ligated, rapidly cannulated, and perfused anterogradely with arterial blood by a pump circuit (Figure 1). The left anterior descending vein was ligated, cannulated retrogradely, and attached to the circuit so that it could be perfused with the pump or vented to zero pressure (Figure 1). Mean coronary arterial perfusion pressure during control left anterior descending arterial pump perfusion was measured through a distal side arm of the cannula and adjusted to match the aortic pressure. Mean coronary venous perfusion pressure during retroperfusion was also measured through a distal side arm of the cannula. Because the pressure decrease from the side arm to the cannula tip has been shown previously to be minimal (1.2 mm Hg at a flow rate of 100 ml/min in vitro),\textsuperscript{20} no correction was made.

The extracorporeal circuit was designed to allow rapid switching from anterograde to retrograde left anterior descending bed perfusion by a pump (Masterflex model 7523-00, Cole-Parmer Instruments, Chicago) (Figure 1). Whenever the vein was perfused retrogradely, the anterior descending artery was vented to zero pressure, and vice versa. The extracorporeal circuit included an occlusive roller pump, windkessel, and an ultrasonic in-line flow probe (Transonic Systems, Ithaca, N.Y.). The pump was calibrated by timed collection with graduated cylinder so that pump flow could be determined precisely with the calibrated dial and flowmeter. The blood drained from the coronary circulation was reinfused into the internal jugular vein with the other roller pump.

**Measurements**

Left ventricular pressure, aortic pressure, coronary perfusion pressure, thicknesses of anterior and lateral walls, and coronary blood flow were recorded on an eight-channel recorder (model 220, Brush, Cleveland, Ohio) and stored in an IBM PC/AT using the CODAS program (DATAQ Instruments, Akron, Ohio). Stored data were replayed later for digitization and analysis. At least 10 sequential beats were averaged for each measurement. End diastole was defined as the zero crossing point of the first derivative of the left ventricular pressure (dP/dt) before its maximum value. End systole was defined as the time of maximum wall thickness occurring within 20 msec before peak negative left ventricular dP/dt.\textsuperscript{21} Hemodynamic measurements in-
cluded left ventricular peak and end-diastolic pressures, peak positive and negative left ventricular dP/dt, mean coronary perfusion pressure and flow rate, and mean aortic pressure. Retrograde arterial outflow during retroperfusion was measured using timed collection with a graduated cylinder. For assessment of regional myocardial function, wall thicknesses were determined at end diastole and at end ejection as previously described, and systolic wall thickening was calculated as a percentage of the end-diastolic dimension.

Myocardial Blood Flow

Regional myocardial blood flow distribution during retroperfusion was measured with 15-μm microspheres (du Pont, Boston) labeled with one of the following radionuclides: $^{125}$I, $^{141}$Ce, $^{114}$In, $^{51}$Cr, $^{113}$Sn, $^{103}$Ru, $^{95}$Nb, and $^{48}$Sc. These studies were performed in six pigs with retroperfusion flows at 100% of control left anterior descending arterial flow, four of which also had measurements at retroperfusion flows at 200% of control left anterior descending arterial flow. For each radionuclide, 2–6 million spheres (1 ml volume) were injected into the left atrium for each experiment. The microspheres were suspended by the manufacturer in 10% dextran with Tween 80. Measurement of left ventricular pressure, wall thicknesses, and retroperfusion flow rate and pressure were performed throughout the injection and at a 2-minute arterial blood withdrawal period to document a hemodynamic steady state. Details of the measurement of blood flow in this laboratory have been published previously and followed the method described by Heymann et al. After the microspheres were injected, the perfusion bed of the left anterior descending vein during retroperfusion was determined by injecting 1% Trypan blue dye (Sigma Chemical Co.) through the coronary venous retroperfusion cannula without reverting to antegrade arterial perfusion to avoid washout of spheres.

The heart was then removed with the instrumentation intact, cleaned, and placed in 10% buffered formalin solution. Later, the atria, right ventricle, and epicardial fat were removed, and the left ventricle was sliced perpendicularly to the long axis to produce a 2-cm-thick ring of myocardium containing the retroperfusion bed zone sonomicrometer. The position of the ultrasonic crystals was carefully mapped to confirm proper orientation across the left ventricular wall and to ensure that the subendocardial crystal was near the endocardial surface (all crystals lay within the inner third of the ventricular wall). Each transmural plug from the retroperfusion and control zones was cut to include both ultrasonic crystals and then divided into transmural thirds and weighed. Gamma radiation was determined by a Packard Autogamma Spectrometer. The remainder of the ring was divided into additional transmural plugs and processed in a similar manner. We report only the blood flows at the two sonomicrometer locations (retroperfusion and control walls), but the remainder of the left ventricular ring was analyzed to ensure that the sonomicrometers were not in a border zone.

Results during retroperfusion were compared with those previously reported during antegrade left anterior descending arterial perfusion in pigs to evaluate differences in the relations between subendocardial blood flow and regional systolic wall thickening during antegrade and retrograde perfusion.

Retrograde Injection of Silicone Elastomer

In three additional pigs, silicone elastomer was injected retrogradely into the left anterior descending vein at a constant retroperfusion pressure of 50, 75, or 100 mm Hg to evaluate the vascular microanatomy during retroperfusion. The injection of silicone elastomer was performed using a method similar to that previously described. After cannulation of both left anterior descending artery and vein, the heart was arrested with saturated KCl solution given through a jugular vein, and a cardioplegic solution (2 mg/L nifedipine, 1 mg/ml papaverine, and 1.5 g/l 2,3-butanedionemonoxine) was perfused retrogradely into the left anterior descending vein at constant pressure to washout the blood and maintain diastolic arrest. After the
TABLE 1. Hemodynamic Data During Selective Retroperfusion

<table>
<thead>
<tr>
<th>Control arterial perfusion</th>
<th>Coronary venous retroperfusion (% of control flow rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>0</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>98±12</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>83±10</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>16±2</td>
</tr>
<tr>
<td>(+)-LV dP/dt (mm Hg/sec)</td>
<td>1,201±253</td>
</tr>
<tr>
<td>(-)-LV dP/dt (mm Hg/sec)</td>
<td>-1,021±215</td>
</tr>
<tr>
<td>Mean AoP (mm Hg)</td>
<td>67±16</td>
</tr>
</tbody>
</table>

LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; AoP, aortic pressure; (+)-LV dP/dt and (-)-LV dP/dt, peak positive (negative) first derivative of left ventricular pressure; ANOVA, analysis of variance during retroperfusion period. All values are mean±SD.

*p<0.05 vs. control antegrade perfusion.

Blood was cleared, silicone elastomer solution freshly catalyzed with 6.0% stannous 2-ethylhexoate and 3.0% ethyl silicate was allowed to perfuse the left anterior descending vein at the same retroperfusion pressure until the elastomer had completely hardened (approximately 70–80 minutes).

Transmural plugs approximately 4x4 mm in cross section were obtained from the left ventricular anterior wall (perfusion territory of the left anterior descending vein). Serial sections 70 μm thick were cut through the entire thickness of the plug using a sliding freezing microtome. After overnight dehydration with 100-proof ethyl alcohol, each section was placed in a labeled vial containing several milliliters of methyl salicylate to render the myocardium transparent and the silicone elastomer-filled microvasculature visible on light microscopy.

**Experimental Protocol**

Regional myocardial contractile function and coronary hemodynamics were measured in each animal during transient retroperfusion for 2-minute periods at progressively increased flow rates with the left anterior descending artery vented to 0 mm Hg, accompanied by measurements of retrograde arterial outflow. Left atrial pacing was performed at a constant rate throughout the study at rates of 90–110 beats per minute. Each step of retroperfusion was accomplished by rapidly switching the pump circuit to perfuse the left anterior descending vein while clamping the antegrade flow line. Between retroperfusion periods, antegrade perfusion of the left anterior descending artery was resumed until regional contractile function and hemodynamic findings had returned to preretroperfusion values (between 10 and 30 minutes for recovery of regional myocardial function, depending on the severity of ischemic dysfunction during retroperfusion). The flow rate during retroperfusion is reported as a percentage of that during control antegrade left anterior descending arterial perfusion and varied from 0% to 200% of control arterial flow. Measurements were performed during a steady state close to the end of each perfusion period. In a subset of animals, measurements at flows of 250% and 300% also were assessed.

**Myocardial Blood Flow–Regional Myocardial Function Relations**

In two different groups of pigs—one from a previous study and the other from the present experiment—the relations between subendocardial myocardial blood flow (microspheres) and systolic wall thickening were compared during normal antegrade perfusion (four pigs, prior study) and retroperfusion (six pigs, present study).

**Statistical Analysis**

All values are expressed as mean±SD. ANOVA with Tukey's test was used to compare values during retroperfusion. A paired t test with Bonferroni correction was used to compare values during antegrade perfusion and retroperfusion. Analysis of relations between regional myocardial function and regional myocardial blood flow during retroperfusion and normal antegrade perfusion was carried out by a comparison of the slope of each linear regression. A value of p<0.05 after Bonferroni correction or Tukey's test was considered statistically significant.

**Results**

Measurements were performed during retroperfusion with flow rates up to 200% of control antegrade left anterior descending arterial flow in eight animals, and measurements were made at 250% and 300% of left anterior descending arterial flow in five and four pigs, respectively.

**Global Hemodynamic Responses**

Hemodynamic data are summarized in Table 1. As the coronary venous retroperfusion flow rate was increased from 0%, increases in left ventricular systolic pressure, peak positive left ventricular dP/dt, and aortic pressure were observed. The left ventricular end-diastolic pressure remained elevated and peak negative left ventricular dP/dt depressed compared with values during antegrade left anterior descending arterial perfusion.

**Coronary Hemodynamic Responses During Retroperfusion**

Left anterior descending venous flow and perfusion pressure during retroperfusion are shown in Table 2. The average pump flow rate to the left anterior descending artery to maintain normal regional contractile function during control antegrade perfusion was 28 ml/min. Retroperfusion pressure increased linearly with increasing retroperfusion flow rates within a physiolog-
Table 2. Coronary Hemodynamic Data, Regional Myocardial Function, and Myocardial Blood Flow

<table>
<thead>
<tr>
<th></th>
<th>Control arterial perfusion</th>
<th>Coronary venous retroperfusion (% of control flow rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Pump flow (ml/min)</td>
<td>28±8</td>
<td>14±4</td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>79±19</td>
<td>17±10*</td>
</tr>
<tr>
<td>LAD artery outflow (ml/min)</td>
<td>4.4±2.1*</td>
<td>6.9±3.7*†</td>
</tr>
<tr>
<td>Fraction of inflow (%)</td>
<td>30±15*</td>
<td>23±11*</td>
</tr>
<tr>
<td>Oxygen saturation (%)</td>
<td>35.4±3.8</td>
<td>42.2±7.8</td>
</tr>
<tr>
<td>Posterior (control) wall</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT&lt;sub&gt;ed&lt;/sub&gt; (mm)</td>
<td>11.4±3.1</td>
<td>11.5±3.1</td>
</tr>
<tr>
<td>% WT</td>
<td>20.4±6.7</td>
<td>22.4±11.2</td>
</tr>
<tr>
<td>Anterior wall</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT&lt;sub&gt;ed&lt;/sub&gt; (mm)</td>
<td>9.7±1.8</td>
<td>8.7±0.9*</td>
</tr>
<tr>
<td>% WT</td>
<td>31.4±9.6</td>
<td>0.4±3.5*</td>
</tr>
<tr>
<td>% of control</td>
<td>100</td>
<td>-0.9±11.1*</td>
</tr>
<tr>
<td>MBF (ml/min/g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmural</td>
<td>0.22±0.10</td>
<td>0.42±0.10</td>
</tr>
<tr>
<td>Subendocardial</td>
<td>0.14±0.05</td>
<td>0.29±0.13</td>
</tr>
<tr>
<td>Subepicardial</td>
<td>0.31±0.17</td>
<td>0.51±0.20</td>
</tr>
<tr>
<td>Endo/epi ratio</td>
<td>0.51±0.25</td>
<td>0.60±0.31</td>
</tr>
</tbody>
</table>

LAD, left anterior descending; WT<sub>ed</sub>, end-diastolic wall thickness; % WT, systolic wall thickening expressed as a percent of end-diastolic thickness; MBF, myocardial blood flow measured with microspheres; ANOVA, analysis of variance during retroperfusion.

*p<0.05 vs. control antegrade perfusion.
†p<0.05 vs. retroperfusion with 0% flow.

dical pressure range but tended to increase more markedly at flows over 200% of control (Figure 2). Although individual variation among animals was evident, no clear evidence of autoregulation of the coronary venous bed was observed (Figure 2).

The amount of the left anterior descending arterial outflow collection during the left anterior descending venous retroperfusion was considerably smaller than the left anterior descending venous outflow during antegrade perfusion (6.9 versus 18.1 ml/min) and reached a plateau as retroperfusion flow rate was increased (Table 2). Oxygen saturation of blood in the left anterior descending arterial outflow showed a small increase with increasing retroperfusion flows; however, the values remained low, suggesting that the blood was of venous origin (Table 2).

Regional Myocardial Function During Selective Retroperfusion

Regional myocardial contractile function, expressed as percent systolic wall thickening of the anterior wall, gradually increased and then reached a plateau as

Figure 2. Relations between retrograde pressure and inflow to the left anterior descending vein during retroperfusion in all animals. Most experiments show relatively linear relations but with considerable variation among animals.
retroperfusion flow rates were increased (Figure 3A and Table 2). Mean systolic wall thickening with a retroperfusion flow of 200% of control arterial flow averaged 62% of the control value during anterograde perfusion. With retroperfusion flow at 250% and 300% (five and four animals, respectively), systolic wall thickening reached 70±26% and 72±32%, but neither value was significantly higher than that at retroperfusion flows of 200% of control flow.

End-diastolic wall thickness of the anterior wall increased progressively with increasing retroperfusion flows (Figure 3B). Mean end-diastolic wall thickness with retroperfusion flows of 100% and 200% of control arterial flow averaged 9.5 and 10.7 mm (both p<0.05), compared with 8.7 mm with 0% retroperfusion flow, although values were not significantly thicker than that with anterograde perfusion (9.7±1.8 mm).

The normally perfused posterior wall showed no significant changes in end-diastolic thickness (11.1–11.5 mm) or systolic wall thickening (20.6–22.4%) with retroperfusion and was not different compared with values with anterograde perfusion (11.4 mm and 20.4%) (Table 2).

**Myocardial Blood Flow and Regional Myocardial Function**

Myocardial capillary blood flow data during retroperfusion are summarized in Table 2. Despite considerable restoration of regional systolic wall thickening during retroperfusion, transmural and especially subendocardial blood flows were relatively low. There was a significant difference in the slopes of the relations between regional myocardial function and regional myocardial blood flow during retrograde versus anterograde perfusion.
sion (Figure 4; p<0.05). The slope during retroperfusion was steeper, indicating that regional function improved despite markedly reduced flows and smaller flow increases in the subendocardium with increased retroperfusion pump flow compared with anterograde pump perfusion (Figure 4).

Microanatomy of Coronary Venous System During Retroperfusion

The filling pattern of the coronary venous system, studied by retrograde injection of silicone elastomer, was largely dependent on the retroperfusion pressure. There was little capillary filling at the lowest perfusion pressure of 50 mm Hg, modest capillary filling at the intermediate perfusion pressure of 75 mm Hg, and almost complete capillary filling at the highest perfusion pressure of 100 mm Hg (Figure 5). A transmural gradient of capillary filling was also noted in the preparation with a perfusion pressure of 75 mm Hg, with complete capillary filling of the epicardial layer, modest filling of the midmyocardial, and little filling of the subendocardial layer (Figure 6). Capillary filling by retrograde injection at a perfusion pressure of 100 mm Hg was similar to that with anterograde arterial injection at the same perfusion pressure, but with retrograde injection the capillaries were more tortuous and the intercapillary distance appeared narrower (Figure 7). However, some amorphous silicone material, apparently extravasated from the intravascular space, was found in the preparation with the higher perfusion pressure (Figure 5). In addition, intramyocardial venous anastomotic connections to the thebesian system were suggested from the observation of clumps of silicone elastomer in both ventricular cavities.

Discussion

This study shows the extent of functional benefit, reflected as regional contraction, that is attainable by selective retrograde perfusion of the left anterior descending artery. Low retrograde left anterior descending arterial outflow was observed during such selective retroperfusion, despite low venous pressure in the artery. This discrepancy between the restoration of regional myocardial function (62% of the control value), suggesting substantial reduction of ischemia, and the low level of myocardial blood flow measured by both microspheres and outflow at high retroperfusion flow rates provides indirect evidence for a venous anastomotic network, supported by the anatomic observations during retrograde injection of silicone elastomer. Taken together, these findings suggest the possibility that oxygen delivery and nutrient exchange may occur via the extensive thin-walled intramyocardial venous plexus, in addition to retrograde capillary filling, during coronary venous retroperfusion.

Regional Myocardial Function During Selective Retroperfusion

The maximal functional benefit attainable with selective retroperfusion of the left anterior descending artery has not been well established. Verdouw et al.25 reported some restoration of regional myocardial function using M-mode echocardiography only with higher retroperfusion flow rates (60–75 ml/min) and pressures (80–120 mm Hg) during balloon occlusion of the coronary vein in pigs, but quantitative data on the degree of functional recovery were not reported. We found gradual restoration of regional myocardial function during retroperfusion flow rates up to 200% of control arterial inflow. Beneficial effects of various retroperfusion techniques have been established in other studies3–12; however, effects on regional myocardial function have been inconsistent, depending not only on the method of retroperfusion but also on the animal model used.12,25,26 The pig was used in the present experiments to eliminate the effect of coronary collateral circulation on regional myocardial function when anterograde left anterior descending artery flow was eliminated. Experimental
results using the pig have generally been less favorable than those using the dog.27-29 A difference that probably is the result of the absence of any significant coronary arterial collateral circulation in the pig.30-32 Also, in the pig, which has extensive venous interconnections including venovenous collaterals and thebesian pathways,33,34 higher retroperfusion flows and pressures appear to be necessary to partially restore regional myocardial function, perhaps related to venovenous shunting and the absence of coronary collateral circulation.

The significant restoration of regional myocardial contraction shown in this study strongly suggests that selective retroperfusion of the left anterior descending vein by the pump perfusion technique used in this study can improve regional ischemic dysfunction more than would be expected by the capillary blood flow levels measured with microspheres in the present and previous studies,29,35-37 which have indicated that 5-25% of retroperfusion flow reaches the regional capillary circulation. In isolated canine hearts, 51-85% of retroper-
fused blood containing radioactive microspheres was shunted directly into the right heart; in vivo, however, it was estimated that only 5% of the reperfusate reached the capillaries. In the present study, assuming a normal transmural flow of 1.0 ml/min/g, at 100% of control flow with pump retroperfusion, the capillary flow by microspheres was 22% of normal flow (Table 2), in agreement with a previous report. Based on other studies showing that regional myocardial contractile function is closely coupled to the amount of the nutrient blood flow delivered to the myocardium by regional anterograde coronary perfusion, particularly perfusion to the inner wall, the observed relation between regional contraction and the low subendocardial myocardial (capillary) flows were found to be shifted upward from the usual relation (Figure 4). This finding suggests that another source of nutrient flow may have contributed to the improvement in contraction. The observed relation between total retroperfusion flow and function (Figure 3A) indicates that increased shunting of the retroperfused blood must have occurred as retroperfusion flow rates increased, probably through the venovenous communications and the thebesian pathways by recruitment of more channels and/or distension of venules.

Although regional myocardial function increased progressively with increasing retroperfusion flows, there was variation among individual animals even after retroperfusion flow was normalized by the control arterial flow. Such variation may reflect differences in the extent of epicardial or intramyocardial coronary venous interconnections as well as in the retroperfusion bed size.

**Coronary Venous Hemodynamics During Retroperfusion**

The level of pressure in the coronary vein during retroperfusion is important for providing effective restoration of regional myocardial function, but it could also cause vascular damage, as suggested by the silicone injection studies at high pressure. The perfusion pressure is dependent on the capacitance and compliance of the individual coronary venous system. Some retroperfusion studies in dogs have reported increased vascular damage and myocardial hemorrhage with a retroperfusion pressure >60 mm Hg; however, Verduw et al observed improved regional myocardial function only with high flows and high pressures in the coronary vein of pigs.

Pressure–flow relations in the left anterior descending coronary vein during retroperfusion have not been well established, although linear relations have been reported during saline retroinjection in dogs and during pump retroperfusion of buffered solution in isolated rat hearts. The nearly linear pressure–flow relation in the left anterior descending vein that we observed during retroperfusion in most studies suggests that there is little capacity for autoregulation. These relations were variable in slope among animals, indicating the anatomic heterogeneity of the coronary venous system and the extent of venous interconnections, even within the same species.

The linear increase of end-diastolic thickness of the anterior wall with increase of the retroperfusion flow rates further suggests that blood volume increased within the vascular space, especially in the thin-walled...
venous plexus, and it is possible that some edema also occurred at high flow rates. A high venous pressure during retroperfusion could be transmitted directly to the capillary circulation causing vascular damage and subsequent myocardial edema and hemorrhage, although the extensive coronary venous interconnections and thebesian system observed in the pigs would be expected to play a significant role in decompressing high retroperfusion pressures. In addition, the marked increases of coronary venous pressure accompanying very high retroperfusion flow rates suggest that the bed was operating on a steep portion of the venous pressure-volume relation, with small changes in volume resulting in large changes in pressure. Myocardial edema with high retroperfusion flow rates might also contribute to the increases of the coronary venous pressure. In our study, as in a model used by Kay et al, the distal end of the left anterior descending artery was exposed to zero pressure to permit maximum retrograde drainage from the left anterior descending bed. This approach might tend to reduce high intramyocardial pressures. The longer-term effects of retroperfusion at the relatively
During retroperfusion, well with subendocardial network venous was quite (usually flow been used has been used because the blood oxygen saturation was quite low. However, the absolute flow was very low relative to the extent of functional recovery and to the high total retroperfusion to flow rates. The plateau at high flow rates (Figure 3A) suggests that there may be a maximum capacity of capillaries to handle retrograde flow, leading to increasing shunt flow through the venous network as total retrograde flow increases.

Regional Blood Flow and Its Distribution During Retroperfusion

Myocardial blood flow, measured with microspheres, has been used as an index of nutrient blood flow delivered to the regional myocardium, and it correlates well with regional myocardial function using antegrade pump perfusion in a porcine model. Many studies using different methods have attempted to evaluate myocardial blood flow distribution during retroperfusion, but the results have been inconsistent. Early studies using radioisotope scans showed increased uptake in the ischemic myocardium with several types of retroperfusion after left anterior descending coronary artery ligation, whereas studies using direct injection of microspheres into the coronary vein reported significant shunting of the retrogradely introduced microspheres. During antegrade perfusion with left atrial injection, a mixed sample of the microspheres enters the coronary arterioles with trapping in the capillaries, allowing measurement of capillary blood flow (usually called myocardial blood flow). However, during retroperfusion, an approach for measuring capillary blood flow has not been firmly established because a significant portion of microspheres injected into the coronary vein is shunted through the venovenous interconnections or directly into the ventricle through the thebesian system; 75% or ≥95% of retrogradely injected microspheres can be so shunted, depending on the experimental animal as well as the protocol used. During retroperfusion, we injected microspheres into the left atrium, so mixing occurred before the microspheres traversed the carotid artery and the perfusion circuit, and we measured capillary blood flow at the end of the study without reverting to antegrade perfusion to avoid dislodgment of already trapped microspheres. This approach should allow assessment of regional capillary flow since, with 15-µm spheres, the sizable fraction of flow that was shunted to venovenous connections or entered the thebesian veins would reach the right heart and then be trapped in the lungs. A small fraction of the microspheres used for timed flow collection are collected in the coronary venous effluent, and only that insignificant portion of microspheres shunted directly into the left ventricle might recirculate and affect measured myocardial blood flow. In our porcine model, the presence of silicone substance in the right ventricle substantiated that some shunting occurred through the thebesian channels and coronary venovenous interconnections.

The transmural distribution of capillary blood flow showed reduced subendocardial flows with some preferential distribution to the subepicardial myocardium [ratios of endocardium (endo) to epicardial (epi), 0.51 and 0.61 with retroperfusion flows of 100% and 200% of control left anterior descending arterial flow, respectively], which contrasts with studies showing no preferential distribution or preferential subendocardial dis-

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**FIGURE 6.** Capillary filling (C) of subepicardial (facing page, upper panel), midmyocardial (facing page, bottom panel), and subendocardial (above) layers by silicone elastomer at the pressure of 75 mm Hg shows transmural gradient of the extent of capillary filling. Venules (V) are visible in all the panels. Original magnification, ×100.
distribution (endo/epi ratio, 1.4) in dogs with patent coronary bypass graft to the venous bed. However, persistently decreased endo/epi ratios were observed in the ischemic zones of dogs subjected to 2 hours of diastolic synchronized retroperfusion, although the ratios were higher than in untreated animals. This pattern of distribution also is typical of ischemia with anterograde perfusion in most species. A retroperfusion study in the rat showed improved energy metabolism only in the subepicardium, suggesting that preferential nutrient flow reached the outer wall. Differences in findings among studies could originate from varying patterns of the coronary venous microcirculation between species as well as from different experimental protocols. For example, venting the left anterior descending artery to 0 mm Hg during venous retroperfusion in our study might have contributed to increasing flow to the subepicardium.
injections in our study cannot be directly extrapolated to the in vitro setting, they supported the possibility of preferential subepicardial perfusion by showing more capillary filling in the subepicardial layer than in the subendocardial layer at an injection pressure of 75 mm Hg (Figure 6). It should be recognized that the findings with in vitro injection in the noncontracting heart may not mimic those of the in vivo setting, although a physiological mean injection pressure and zero venous pressure were used in an attempt to produce a physiological pressure gradient in the nonbeating heart.

Relations Among Myocardial Blood Flow, Myocardial Function, and Oxygen Delivery

As mentioned, the relation observed between regional systolic wall thickening and subendocardial blood flow during retroperfusion was significantly steeper than that during anterograde perfusion, assuming linear relations, although maximum subendocardial capillary flows achieved during retroperfusion were relatively low (Figure 4). Thus, regional systolic wall thickening at low levels of subendocardial blood flow tended to be greater during retroperfusion than during anterograde perfusion, suggesting that myocardial blood flow measured by microspheres during retroperfusion does not fully reflect effective nutrient flow and oxygen delivery to the ischemic myocardium.

The Gregg (or "garden hose") effect might have selectively enhanced contraction during retroperfusion. However, this effect is likely to be minor. Studies concerned with the Gregg phenomenon in the pump-perfused left anterior descending bed in pigs are similar to the present preparation except that effects of anterograde flow were studied. In one such study concerned with the effects of overperfusion on regional and global myocardial function,51 flow was increased by a pump overperfusion, with coronary perfusion pressure increasing from 88 to 186 mm Hg and resulting in a doubling of subendocardial blood flow by the microsphere technique. Also, anterograde coronary blood flow was increased more than fourfold at a constant coronary perfusion pressure by adenosine infusion. No changes in regional or global left ventricular function were detected under either condition.51 In studies with anterograde pump perfusion, the end-diastolic wall thickness of the anterior wall increased from 8.4 to 9.5 mm with pump overperfusion and from 8.2 to 9.3 mm with adenosine infusion. In the present study, the increase in end-diastolic wall thickness was somewhat less, from 9.7 to 10.2 mm at 100% retrograde flow and to 10.7 mm at 200% of retrograde flow. Although we cannot exclude the possibility that increasing wall thickness by retrograde perfusion had a different mechanical effect than anterograde perfusion with similar changes in wall thickness, the likelihood of this possibility seems low.

Therefore, we hypothesize that retroperfusion might provide improved regional nutrient blood flow and oxygen delivery not only via direct flow at the capillary level (measured by microspheres) but also via other routes within the intramyocardial venous network, including the thin-walled venous plexus, sinusoids, and thebesian system, which could deliver oxygen and exchange nutrients with the adjacent myocardium by diffusion.34 The in vitro injection studies support the access of retroperfusion to an extensive intramyocardial venous system. This hypothesis is also supported by a report demonstrating oxygen diffusion through arteriolar and precapillary walls in the brain52 as well as by a mathematical model of oxygen exchange,53 but direct proof of this form of transport in the heart awaits further research.

Study Limitations and Clinical Implications

Several limitations and certain clinical implications of the present study deserve mention. The quantitation of nutritional myocardial blood flow during retroperfusion remains controversial. Because a significant portion of retrogradely delivered microspheres are shunted away from intramyocardial microcirculation and because anterogradely delivered microspheres through coronary arterial collateral circulation can be dislodged during retroperfusion, we used an animal model with little coronary arterial collateral circulation and injected microspheres into the left atrium during retroperfusion. However, it is likely that this method is not as accurate for quantitating nutritional blood flow as that which has been used during anterograde perfusion, and application of other techniques such as positron emission tomography54 that can evaluate myocardial metabolism in vivo during retroperfusion will be desirable in the future.

We used nonsynchronous retroperfusion for short periods of time to evaluate the maximal functional benefit that might be obtained by selective retroperfusion. Most techniques for clinical use have used synchronized retroperfusion, so that elevation of coronary venous pressure and retrograde flow occur in diastole when systolic compression by the ventricle (which would further elevate the venous pressure) is absent, thereby tending to reduce potential vascular damage and edema. We recognize that the type of retroperfusion used in our study produced nonphysiological increases in venous pressure; this could cause myocardial hemorrhage and edema, which, when sustained, might cause functional deterioration compared with the effects of synchronized retroperfusion with intermittent decompression of coronary venous pressure. Venting of the left anterior descending artery to 0 mm Hg during retroperfusion might be expected to lessen myocardial edema, but progressively increased diastolic wall thickness and extravasation of silicone substance at higher retroperfusion pressures indicated that insufficient decompression of the coronary venous system occurred with venting of the artery alone. In addition, the coronary vascular anatomy is different in the pig from that of humans with respect to coronary collateral circulation, the microcirculation, venous interconnections, and the thebesian system.35,38 Therefore, our approach is not considered to represent a clinically useful technique, and with high flows, it probably would be dangerous. Rather, this approach describes the maximum improvement in ischemic dysfunction that can be obtained experimentally, and it raises the question of whether an additional source for oxygen delivery may be available. Our findings also suggest that delivery of arterial blood as a retroperfusate may be necessary to restore depressed regional myocardial function, especially in an animal model such as the pig with minimal collateral
circulation, because pressure-controlled intermittent coronary sinus occlusion using venous blood was effective in reducing infarct size but not in restoring left ventricular function in the pig. 

Our results at flows <100% might have some bearing on the clinical situation in which a coronary graft to the venous bed occasionally is used in patients in whom there is little likelihood of success with arterial graft patency because substantial pressures and flow would be available to the venous bed in this setting.

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Circulation. 1992;86:1265-1279
doi: 10.1161/01.CIR.86.4.1265

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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