Time Course of Coronary Endothelial Healing After Injury Due To Ischemia and Reperfusion

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Background Although it has been demonstrated in short-term preparations that ischemia with early reperfusion results in coronary vascular injury manifested by abnormal endothelium-dependent relaxation and increased permeability to plasma proteins, it has not been clear whether these abnormalities are permanent or reversible.

Methods and Results In a canine model, regional coronary ischemia was accomplished by 1 hour of left anterior descending coronary artery ligation, and follow-up studies were performed after reperfusion for 1 hour, 48 hours, 2 weeks, or 9 weeks. Vasorelaxation was measured in vitro with preconstricted epicardial coronary artery rings subjected to increasing concentrations of the endothelium-dependent vasodilator ADP and the endothelium-independent vasodilator nitroprusside. At 1 and 48 hours of reperfusion, relaxation of rings from the ischemic reperfused artery to ADP was blunted, but relaxation to nitroprusside was normal. At 2 weeks there was a nonsignificant trend toward a blunted response to ADP in the ischemic/reperfused rings, and at 9 weeks a completely normal response to ADP was observed. Coronary microvascular permeability was assessed by measurement of protein leak index (PLI), by using a double-isotope technique with autologous radiolabeled transferrin and erythrocytes. At 1 and 48 hours of reperfusion there were substantial increases in PLI in the previously ischemic regions, indicative of increased extravascular transferrin. There was a small increase in PLI at 2 weeks but a completely normal measurement at 9 weeks. Electron microscopy of ischemic/reperfused vessels demonstrated endothelial cell swelling and other abnormalities in epicardial arteries and the microcirculation at 48 hours of reperfusion but normal endothelium at 2 weeks of reperfusion.

Conclusions After 1 hour of regional coronary ischemia, coronary endothelial injury occurs early in reperfusion with abnormalities in epicardial coronary artery endothelium-dependent relaxation, coronary microvascular permeability, and both epicardial coronary artery and microvascular histology. This pattern of injury persists for at least 48 hours, but there is partial functional and complete histological recovery within 2 weeks and complete functional recovery within 9 weeks. (Circulation. 1994:90:2439-2447.)

Keywords • reperfusion • endothelium • infarction • microcirculation • ischemia

There is abnormal epicardial coronary artery endothelium-dependent relaxation after ischemia due to 1 hour of coronary artery ligation and 1 hour of reperfusion in experimental animal models.1-4 Endothelium-dependent relaxation is normal when coronary arteries are exposed to ischemia without reperfusion.1 Evidence of microvascular coronary endothelial injury manifested by increased permeability to serum proteins in regions exposed to ischemia and reperfusion has also been reported.5-7 Ultrastructural abnormalities in the endothelium in epicardial coronary arteries and in the coronary microcirculation after ischemia and reperfusion have been observed on electron micrographs at 1 hour of reperfusion in canine models.1,6

However, there is very little information available concerning the time course of reperfusion-induced coronary endothelial injury. Most models have used only 1 or 2 hours of reperfusion before the study of endothelial function or histology.1-7 If these models are relevant to clinical settings in which reperfusion injury could occur, it would be useful to know the outcome of the endothelial abnormalities over subsequent weeks or months. Whether healing occurs and when it occurs have not been clear. One laboratory has reported that abnormal epicardial coronary artery endothelium-dependent relaxation to platelets or platelet products is demonstrable in vitro 12 weeks after a transient episode of regional ischemia.8 Paradoxically, it has been reported that when the endothelium in coronary epicardial arteries has been injured by mechanical means in vivo, it regenerates within a week,9,10 with recovery of normal endothelium-dependent relaxation.10 There has been no information about the time course of reperfusion-induced coronary microvascular endothelial injury and healing.

We studied a canine model in which the left anterior descending coronary artery (LAD) was transiently ligated for 1 hour, and studies were performed at 1 hour, 48 hours, 2 weeks, or 9 weeks of reperfusion. The responsiveness of excised coronary artery epicardial segments to endothelium-dependent relaxation in vitro and measurement of coronary microvascular protein leak, an index of microvascular endothelial integrity in vivo, were performed at each time point. Electron microscopy was done at 48 hours and 2 weeks. We observed evidence of regional endothelial injury in the coronary epicardial arteries and microcirculation during the first 48 hours of reperfusion, with healing over subsequent weeks.

Methods

Surgical Procedures

Thirty-seven male mongrel dogs were anesthetized with pentobarbital sodium (30 mg/kg IV), intubated with an endotracheal tube, and mechanically ventilated. Using sterile technique, we performed a thoracotomy in the left fifth intercostal

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space and opened the pericardium. Catheters were placed in the descending aorta and left atrial appendage. Arterial blood gases were measured and ventilation was adjusted to maintain normal physiological levels. A 1-0 silk ligature was placed around the LAD just beyond the first diagonal branch. The ECG was continuously monitored by using needle electrodes placed in the chest wall. Aortic pressure was recorded through the descending aortic catheter. Arrhythmias during the experiments were not treated. After baseline measurements were obtained, the ligature on the LAD was tightened through a snare occluder for 60 minutes. During occlusion, we confirmed ischemia by noting paradoxical wall motion, a dusky discoloration of the ischemic region, and typical changes in the ECG.

Fifteen minutes before the end of the occlusion, radioactive microspheres were injected into the left atrium with sampling from the aorta for measurement of regional myocardial blood flow. For dogs that were maintained for 48 hours or more of reperfusion, the chest was then closed, air and blood were evacuated, and the animal was allowed to recover. Appro- priate IM doses of morphine sulfate were given by the investigators or the supervising veterinarian to relieve discomfort during the first 24 hours postoperatively.

Four dogs showed ventricular fibrillation during the first 30 minutes of reperfusion and were excluded from analysis. Dogs in which LAD occlusion did not reduce regional blood flow in the infarct region to \( \leq 30 \text{ mL} \cdot \text{min}^{-1} \) were excluded from analysis because of insufficient ischemia. Of 9 dogs reperfused for 1 hour, 6 were included in the study and 3 were rejected because of insufficient ischemia. Of 9 dogs reperfused for 48 hours, 8 were included and 1 rejected for inadequate ischemia. Of 7 dogs reperfused for 2 weeks, 5 were included in the study and 2 rejected for inadequate ischemia. All 5 dogs reperfused for 9 weeks were included in the study.

An arterial blood sample was drawn before induction of ischemia, and autologous serum protein (transferrin) and erythrocytes were labeled in vitro with \(^{113}\text{In}\) and \(^{99m}\text{Tc}\), respectively. The labeled transferrin and erythrocytes were injected into the aortic catheter during reperfusion. Sixty minutes later a blood sample was drawn from the aortic catheter, and the dog was killed immediately with an overdose of pentobarbital through the atrial catheter. The chest was opened and the heart was quickly removed.

An approximately 2.5-cm length of the epicardial portion of the LAD with the proximal end about 3 mm distal to the site where the ligature had been placed, was excised and carefully cleaned and trimmed. A similar-sized length of the epicardial portion of the left circumflex coronary artery (LCX) was also excised. The left ventricle was then sectioned into four rings cut perpendicular to its long axis. Each ventricular ring was sectioned into quadrants, and each quadrant was divided into endocardial and epicardial halves. Final tissue samples weighed approximately 1.0 g. The tissue samples were counted immediately for radiolabeled transferrin and erythrocyte activity in a Packard 5000 Series gamma counter.

**Determination of Myocardial Blood Flow**

To measure regional myocardial blood flow, radiolabeled microspheres (15.0 ± 3.0 μm in diameter) (New England Nuclear) suspended in 0.1% Tween 80 and 10% dextran (MW 40 000) were diluted in normal saline. Before injection, the total amount of injected microsphere activity was determined. After vigorous mechanical agitation to prevent clumping, 1.5 to 2 million microspheres were injected into the left atrial catheter over 4 seconds and the catheter was then flushed with saline. The injection syringe was later counted for residual activity, which was subtracted from the preinjection counts. Beginning 10 seconds before and continuing for 3 minutes 20 seconds after the injection of microspheres, we withdrew blood from the aorta at a rate of 2 mL/min into multiple tubes. At least 3 days after each dog was killed, when counts from the protein leak determinations were negligible, gamma emissions in the myocardial tissue and blood samples were counted. Using the reference sample method, as we have previously reported,\(^6,11\) we determined cardiac output and blood flow in each tissue sample.

**In Vitro Epicardial Coronary Artery Ring Studies**

Two or more 3-mm-wide rings were cut from each of the excised coronary artery segments as described previously. The rings were suspended between a stationary wire and a wire attached to a Grass FT03 force-displacement transducer. The bathing medium was Earle’s physiological salt solution (Sigma Chemical Co) at pH 7.4 maintained at 37°C and gassed with 95% O\(_2\) and 5% CO\(_2\). Rings were stretched to 5g of passive force and equilibrated for 1 hour. Maximal constriction to 80 mmol/L potassium chloride was then recorded for each ring, after which the rings were washed twice and tension allowed to return to baseline. Rings that did not develop at least a 2g contraction were considered damaged and were discarded. With rings that met this criterion, all studies were done in duplicate with two rings from the LAD and two rings from the LCX.

The rings were then submaximally contracted with 30 mmol/L potassium chloride and exposed to increasing concentrations of ADP (10\(^{-8}\) to 10\(^{-3}\) mol/L). After being washed twice, most rings were then exposed to increasing concentrations of sodium nitroprusside (10\(^{-9}\) to 10\(^{-6}\) mol/L). The relaxation concentration-response curves were plotted using the ratio of drug-induced relaxation to the precontracted tension. Evaluation of relaxation responses included determination of maximum relaxation, \(\Delta E\), and comparison of total concentration-response curves as we have described previously.\(^12\)

**Determination of Coronary Microvascular Protein Leak**

Microvascular permeability to serum proteins was assessed in vivo as protein leak index (PLI) by using methods we have previously described.\(^6,7\) In brief, the rate at which radiolabeled protein moves from the intravascular to the extravascular space is a function of vascular protein permeability, of protein concentration in the blood, and of perfused vascular surface area. Vascular permeability can be assessed by normalizing the rate of extravascular radioiodinated protein accumulation for intravascular radiolabeled protein concentration and for surface area. A reference blood sample is used to normalize for the concentration of radiolabeled protein (transferrin) in the blood. The radiolabeled erythrocyte concentration of each myocardial sample is used as a measure of the perfused vascular surface area.

Radiolabeled transferrin and erythrocytes were injected into the aorta at the designated reperfusion time point. Sixty minutes later a reference sample was drawn from the aorta, the dog was killed, and myocardial samples were quickly obtained. The radiolabeled transferrin and erythrocyte activities in each myocardial and blood sample were immediately determined in the gamma counter. The following calculations were made: (1) Extravascular protein activity equals total tissue protein activity minus intravascular protein activity; (2) Intravascular protein activity equals tissue blood weight times blood protein activity, where tissue blood weight equals tissue erythrocyte activity divided by erythrocyte activity per gram blood; (3) Blood protein activity equals protein counts per gram blood; and (4) PLI equals extravascular protein activity divided by intravascular protein activity.

**Electron Microscopy**

In 3 dogs, 2 reperfused for 48 hours and 1 for 2 weeks, no radiolabeled materials were injected during experiments. The heart was excised, and the presence of an infarction was confirmed visually. Each heart was perfused retrogradely at 30 to 40 cm H\(_2\)O pressure via the aorta with 1.5% glutaraldehyde in 0.1 mol/L cacodylate buffer at pH 7.3. The heart was
perfused for 40 minutes, after which sections of the LAD and LCx and both infarcted and normal myocardium were obtained. The tissue was subdivided into 1-mm³ pieces and postfixed in 1% osmium tetroxide in 0.1 mol/L cacodylate buffer (pH 7.3) at 4°C for 1 hour. En bloc 1% aqueous uranyl acetate staining was performed. The tissues were then dehydrated in acetone, cleared in propylene oxide, and infiltrated overnight with Embed/Araldite. The embedded tissues were cured 2 days at 70°C. Sections were cut on an ultramicrotome (LKB Instruments) and stained with 3% aqueous uranyl acetate and Reynold’s lead stain. After preliminary examination of multiple thick sections, thin sections were obtained and examined on an electron microscope (model 400T, Philips Electronic Instruments) at an accelerating voltage of 60 kV.

Statistical Analyses

Comparisons of percent relaxation and EC50 between LAD and LCx rings were made by paired t test. Total concentration-response curves of epicardial coronary ring relaxation were compared by ANOVA with replication. PLI as a function of myocardial flow during ischemia was compared with ANOVA using the Fisher least significant difference test. A value of P<.05 was considered statistically significant. All groups are expressed as mean±SEM.

Results

In Vitro Studies of Epicardial Coronary Artery Relaxation

Relaxation at 1 Hour of Reperfusion

The response to the endothelium-dependent vasodilator ADP at 1 hour of reperfusion in 6 dogs is shown in the top frame of Fig 1. The EC50 was 9.9±1.5x10⁻⁷ mol/L for the LAD rings and 4.4±0.9x10⁻⁷ mol/L for the LCx rings (P<.05). Maximum relaxation was 47±8% for the LAD versus 64±5% for the LCx, but the means were not significantly different. There was significant blunting of the response of the LAD versus the LCx at 10⁻⁷ mol/L ADP (P<.05) but not at other concentrations. However, when the entire concentration-response curves were compared by ANOVA with replications, there was a significant difference between the LAD and LCx responses to ADP (P<.05).

With the endothelium-independent vasodilator nitroprusside, at 1 hour of reperfusion in 6 dogs the concentration curves for the LAD and LCx were nearly superimposable. There were no significant differences in EC50, maximum relaxation, relaxation at any dose, or when the entire concentration-response curves were compared (data not shown).

Relaxation at 48 Hours of Reperfusion

Results with ADP at 48 hours of reperfusion in 8 dogs are shown in the second frame in Fig 1. The EC50 was significantly greater in the LAD (8.5±2.0x10⁻⁷ LAD versus 3.2±0.7x10⁻⁷ LCx, P<.05). There was a significant decrease in maximum relaxation in the LAD (53±4 LAD versus 72±5% LCx, P<.01). There were significant differences between the LAD and LCx at each concentration studied, except at 10⁻⁶ mol/L. When the entire concentration-response curves for the LAD and the LCx epicardial artery rings were compared, there was significantly less relaxation of the ischemic/reperfused LAD rings to ADP (P<.01). Rings from 3 dogs in this group were studied with nitroprusside, and there

![Fig 1. Concentration-response curves for the endothelium-dependent vasodilator ADP. Mean values±SEM are shown for percent relaxation of the ischemic/reperfused left anterior descending coronary artery (LAD) rings and the control left circumflex coronary artery (LCx) rings. Beginning with the top frame, results are in groups of dogs in which 1 hour of ischemia was followed by 1 hour, 48 hours, 2 weeks, and 9 weeks of reperfusion, respectively. Although there was substantial blunting of the response of the LAD to increasing doses of ADP at 1 hour and 48 hours of reperfusion, there were no significant differences between the LAD and LCx responses at 2 weeks or 9 weeks.](http://circ.ahajournals.org/doi/10.1161/01.CIR.88.8.2441)
**MBF (ml/100g/min)**

Fig 2. Mean values for protein leak index (PLI), measured during reperfusion, as a function of myocardial blood flow (MBF), measured during coronary ligation. The four groups of dogs shown were each exposed to 1 hour of left anterior descending coronary artery ligation followed by reperfusion. The reperfusion periods were 1 hour (n=6), 48 hours (n=8), 2 weeks (n=5), and 9 weeks (n=5). The vertical and horizontal bars designate standard errors for PLI and MBF, respectively, for each group. There were significant differences between the PLI in the most ischemic regions (≤20 ml·100 g⁻¹·min⁻¹) and the regions with normal flow during occlusion in the 1-hour, 48-hour, and 2-week groups, but not in the 9-week group (see text).

**Relaxation at 2 Weeks of Reperfusion**

Epicardial ring responses to ADP at 2 weeks of reperfusion in 5 dogs are shown in the third frame of Fig 1. Although there appeared to be a trend toward blunting of the LAD response, there were no significant differences in any measurement—including EC_{50} (5.2±1.1x10⁻⁷ LAD versus 2.7±0.2x10⁻⁷ LCx, P=NS)—between LAD and LCx at any concentration of ADP and when the entire concentration-response curves for the two arteries were compared. Rings from all 5 dogs were studied with nitroprusside, and there were no differences between LAD and LCx responses.

**Relaxation at 9 Weeks of Reperfusion**

Epicardial ring responses to ADP after 9 weeks of reperfusion were measured in 5 dogs (bottom frame of Fig 1). There were no trends toward differences or significant differences between the LAD and LCx at any concentrations or in EC_{50}. Comparisons of the entire concentration-response curves also were not significantly different. Rings from 2 dogs were studied with nitroprusside, and there were no apparent differences between LAD and LCx responses.

**In Vivo Studies of Coronary Microvascular Permeability**

In Fig 2 the mean PLI measured at the end of the reperfusion period is plotted as a function of the regional myocardial blood flow during ischemia for each group of dogs. The mean PLI values were grouped into regions according to myocardial blood flow during coronary occlusion (in mL·100 g⁻¹·min⁻¹) of ≤20, 21 to 50, 51 to 80, and >80, respectively. Curves of mean values are shown at 1 hour, 48 hours, 2 weeks, and 9 weeks of reperfusion. Typical measurements in individual dogs at each time point are shown in Fig 3.

At 1 and 48 hours of reperfusion, there were inverse relations between regional myocardial blood flow and PLI, with the highest PLIs in the regions with the most severe ischemia (Figs 2 and 3). At 1 hour, the mean PLI in the normal, nonischemic region (flows >80 mL·100 g⁻¹·min⁻¹) was significantly less than in the regions with flows of ≤20 mL·100 g⁻¹·min⁻¹ and 21 to 50 mL·100 g⁻¹·min⁻¹. At 48 hours, the flow in the normal region (>80 mL·100 g⁻¹·min⁻¹) was significantly less than in all the other regions. There were no significant differences at any flow level between the PLI at 1 hour and at 48 hours of reperfusion.

At 2 weeks, the PLI was significantly less within the central infarct regions than at 1 or 48 hours of reperfusion. However, although the difference was small, the 2-week PLI in the most severely ischemic region (flow ≤20 mL·100 g⁻¹·min⁻¹) was significantly greater than...
in the normal (flow >80 mL·100 g⁻¹·min⁻¹) region (P<.05). Within the normal regions, the PLI was significantly less at 2 weeks than at 48 hours of reperfusion, but there was no significant difference from the result in the normal region at 1 hour of reperfusion.

At 9 weeks of reperfusion, there were no statistically significant differences in PLI between the ischemic and nonischemic zones (Fig 2). The PLI in the central infarct region was significantly lower than in the corresponding regions at 1 hour and 48 hours but was not significantly different from the result at 2 weeks of reperfusion. In the normal regions, the PLI at 9 weeks of reperfusion was significantly less than at 48 hours of reperfusion but did not differ from results at 1 hour or 2 weeks of reperfusion.

Electron Microscopy

Electron microscopy was done in 2 dogs at 48 hours and 1 dog at 2 weeks of reperfusion. Abnormalities were observed in most photomicrographs of LAD or myocardial sections exposed to ischemia and reperfusion for 48 hours. However, the vascular abnormalities were generally not severe—in most cases the basic architecture of the endothelial cells was preserved and the interendothelial junctions were intact. The most common abnormal finding in the LAD, as illustrated in Fig 4, was swelling of endothelial cells with subendothelial edema, resulting in uneven protrusions of endothelial cells into the lumen. A few sections of the LAD showed denudation of some endothelial cells (Fig 5). Vacuoles were not a prominent feature in the endothelial cells at 48 hours, although we have found them to be present in previous studies we have done at 1 hour of reperfusion. Remarkably, the endothelial cells in venules and arterioles in the infarcted regions at 48 hours often lacked severe abnormalities. In Fig 6, which shows a venule in the midst of a severely damaged myocardial region, the endothelial cells appear to be mostly intact. Subtle abnormalities were noted in the microcirculation in some photomicrographs, with an example being the widened interendothelial junction in a venule from the infarcted zone shown in Fig 7. The sections examined at 48 hours, which were obtained from the LCx or from sections of myocardium that were not exposed to transient ischemia, were normal. The photomicrographs of the LAD and the microcirculation in the infarcted zones at 2 weeks were normal, except for evidence of myocardial damage in the infarcts.

Discussion

We and others have reported that in studies performed at 1 to 2 hours of reperfusion after 1 hour of coronary occlusion there is evidence of coronary endothelial injury.1-7,12-14 However, it has not been clear whether the abnormalities are transient or permanent. Therefore, we examined the time course of reperfusion-induced endothelial injury and healing by studying a series of reperfusion periods ranging from 1 hour to 9 weeks. We used three techniques: assessment of epicardial coronary artery endothelium-dependent relaxation...
in vitro; assessment of vascular permeability to serum proteins in vivo, an index of microvascular endothelial function; and transmission electron microscopy to evaluate structural integrity of the endothelium in both epicardial coronary arteries and the microcirculation.

There was blunted relaxation in the ischemic/reperfused epicardial coronary artery rings to the endothelium-dependent, guanylate cyclase–dependent, vasodilator ADP at 1 hour and 48 hours of reperfusion. However, although there was a trend toward slight blunting of the relaxation of the ischemic/reperfused rings to ADP at 2 weeks, there was no statistical evidence of differences from the responses of the normal rings. At 9 weeks the responses to ADP were equivalent in the rings exposed to ischemia and reperfusion and the normal rings. As expected, responses to the endothelium-independent vasodilator nitroprusside, which directly activates guanylate cyclase in vascular smooth muscle, were normal at all time periods. Therefore, exposure to ischemia followed by reperfusion resulted in transient injury to the epicardial coronary artery endothelium without alteration in the capacity of the vascular smooth muscle to respond to guanylate cyclase activation. The epicardial coronary endothelial dysfunction was present for at least 48 hours but was completely or nearly completely healed within 2 weeks.

The measurement of PLI has proved to be a highly sensitive method to detect regional microvascular endothelial injury in the coronary circulation. The use of radiolabeled erythrocytes minimizes the effect of regional variation in perfusion and allows intravascular protein (transferrin) to be distinguished from extravascular protein. Vascular permeability is assessed by normalizing the rate of extravascular radiolabeled protein accumulation for intravascular radiolabeled protein concentration and surface area. Evidence has been presented that protein leak estimation is more sensitive and specific as a measure of microvascular permeability than methods that measure plasma proteins without normalizing for intravascular volume with labeled erythrocytes.

At both 1 and 48 hours of reperfusion an inverse relation was present between regional blood flow during coronary ligation and the PLI. The mean PLI in the two most ischemic regions (≤50 mL·100 g⁻¹·min⁻¹) was approximately 2.5 times levels in the normally perfused regions (Fig 2). Thus, the microvascular injury present at 1 hour of reperfusion persisted for at least 48 hours.

When the PLI was measured after 2 weeks of reperfusion, the levels were markedly reduced from those observed at 48 hours in the regions that had been ischemic during coronary occlusion. However, a small statistically significant elevation in PLI was still present in the most ischemic region compared with the normal region. Therefore, substantial but not quite complete recovery of the injured microvascular endothelium had occurred by the end of 2 weeks. It is of interest that the decrease in PLI was not only noted in the previously ischemic regions, where it was most marked, but also occurred in the regions where flow was normal (>80
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mL·100 g⁻¹·min⁻¹) or only slightly decreased (51 to 80 mL·100 g⁻¹·min⁻¹). Apparently there was minimal functional injury at 48 hours in regions that were not transiently rendered ischemic. At 9 weeks of reperfusion there was no evidence of an inverse relation between myocardial blood flow during coronary occlusion and PLI during reperfusion—the PLI was low in all regions. Therefore, because by 9 weeks coronary microvascular permeability had returned completely to normal, the endothelium was no longer dysfunctional.

Information on the time course over days or weeks of coronary vascular injury due to ischemia and reperfusion has been sparse. Our results differ from a previous study in which endothelium-dependent relaxation of canine epicardial coronary rings transiently exposed to 1 hour of ischemia was studied 12 weeks afterward.⁸ In that study, responses to platelets and the platelet products ADP, serotonin, and thrombin were reported to be abnormal.⁸ Our results are in closer agreement with a report in which there was regrowth of coronary endothelium and return of function in vivo within 10 days after mechanical denudation in dogs¹⁰ and with a report of rapid regeneration of mechanically injured vascular endothelium in rats.⁹

There have been no previous studies on the long-term outcome of microvascular injury due to ischemia and reperfusion. Our findings with a highly sensitive estimate of coronary microvascular leak in vivo and assessment of epicardial coronary artery endothelium-dependent relaxation in vitro are evidence that there is functional recovery of endothelium damaged by exposure to ischemia and reperfusion, which is nearly complete within 2 weeks and complete within 9 weeks. Although recovery in these two different regions of the coronary circulation was parallel, there was some apparent regional variation. At 2 weeks there were no significant differences in epicardial artery relaxation, but a difference was still present in protein leak. This could reflect differences in rates of recovery or in sensitivity of the different measurements used. In addition, the microcirculation may be more susceptible to reperfusion injury than the epicardial coronary arteries.¹³

Electron microscopy demonstrated the presence of mild endothelial structural abnormalities at 48 hours of reperfusion. The functional abnormalities may have been more severe than the structural abnormalities at 48 hours of reperfusion. The electron micrographs at 2 weeks did not show endothelial abnormalities. Therefore, structural integrity was regained within 2 weeks. We previously reported abnormalities on electron microscopy at 1 hour of reperfusion.¹⁶ Normal findings at 12 weeks of reperfusion were reported by Pearson et al.⁸ Adhesion of leukocytes and release of leukocyte toxins, including reactive oxygen metabolites, is an attractive explanation for the endothelial dysfunction we observed in this model of coronary ischemia and reperfusion. There is massive accumulation of leukocytes the first few hours after reperfusion in the microcirculation¹⁶ and some infiltration of leukocytes in the large coronary arteries.¹⁷ Extensive and complex leuko-

FIG 6. Transmission electron photomicrograph in a section of infarcted myocardium obtained from a heart reperfused for 48 hours. Magnification is ×32,500. The venule in the center of the photomicrograph has largely intact endothelium, although there is extensive damage in the surrounding myocardium with loss of the usual striated appearance of the cardiac muscle. There is an endothelial cell at the upper right that has a pseudopod-like projection that could reflect injury, but this could also be fixation artifact.
cyte-endothelial interactions occur during reperfusion. We have reported that coronary microcirculatory injury can be reduced by scavengers of reactive oxygen metabolites and totally prevented by leukocyte filtering. However, protection from such measures may not be as readily demonstrable in the epicardial coronary arteries. The abnormal relaxation in the epicardial coronary arteries after ischemia and reperfusion is probably a result of reduced availability of endothelium-derived relaxing factor, which is likely to be nitric oxide or a closely related agent. We found no evidence of abnormal responsiveness of the guanylate cyclase system in ischemic/reperfused smooth muscle.

Our findings could have implications for clinical settings in which there is rapid reperfusion of occluded coronary arteries in patients with acute myocardial infarction. Although the initial occlusion itself may be related to localized acute endothelial abnormalities at the site of atherosclerotic plaques, early reperfusion, despite well-established benefits, could result in further coronary vascular endothelial injury even at sites with little or no preceding abnormality. Endothelial dysfunction due to ischemia and reperfusion is likely to increase susceptibility to local platelet aggregation. Platelet aggregation may compromise coronary perfusion and cause further myocardial necrosis or dysfunction. In addition, increased microvascular permeability could cause impairment of myocardial function due to tissue edema. Susceptibility to these abnormalities is likely to decrease rapidly within 9 weeks after the initial event as the endothelium recovers. Such a hypothesis is compatible with reports of a high incidence of new adverse coronary events after successful coronary reperfusion with a thrombolytic agent, and with increased responsiveness to ergonovine, a provocative stimulator of coronary artery spasm in the postinfarction period. However, extrapolation to clinical settings from animal models should be done only with caution and skepticism.

In summary, we present evidence that during reperfusion following an hour of ischemia due to coronary artery ligation in a canine model there is regional endothelial injury evident at 1 and 48 hours of reperfusion involving the epicardial coronary arteries and the coronary microcirculation. By 2 weeks of reperfusion, there is substantial functional recovery of the abnormalities in epicardial artery relaxation and microcirculatory permeability, and there are no longer histological abnormalities by electron microscopy. By 9 weeks of reperfusion complete functional recovery has occurred.

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**Fig 7.** Transmission electron photomicrograph in a section of infarcted myocardium obtained from a heart reperfused for 48 hours. The magnification is x115,000. The space at the lower left is the lumen of a venule. There is abnormal widening of the interendothelial cell junction in the center of the photomicrograph.


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