Recovery of Left Ventricular Segmental Function after Long-Term Reperfusion Following Temporary Coronary Occlusion in Conscious Dogs

Comparison of 2- and 4-Hour Occlusions

Larry R. Bush, L. Maximilian Buja, Wade Samowitz, Robert E. Rude, Mark Wathen, Gregory D. Tilton, and James T. Willerson

From the Departments of Internal Medicine (Cardiology Division) and Pathology at the University of Texas Health Science Center at Dallas, Texas

SUMMARY. We compared the recovery of left ventricular segmental function with long-term reperfusion after two periods of coronary artery occlusion in conscious dogs to determine the relationship between the severity and duration of a temporary ischemic insult and the potential for recovery of contractile function. Twenty-seven awake dogs, previously instrumented with ultrasonic crystals for measuring regional left ventricular net systolic wall thickening, underwent 2 (group I) or 4 (group II) hours of left anterior descending coronary artery occlusion, followed by 1 month of reperfusion. Dogs were studied 24 hours after reperfusion and at weekly intervals for 1 month, after which the contractile reserve of left ventricular segments was assessed by their response to dopamine and postextrasystolic potentiation. The myocardial infarctions produced in the experimental model were relatively small. Left ventricular segments were classified by their severity of contractile dysfunction 1 hour after left anterior descending occlusion: class 1, >67% pre-occlusion net systolic wall thickening; class 2, 0-66.9%; class 3, <0% (paradoxical thinning). Class 1 segments in both groups showed trivial changes in net systolic wall thickening and regional myocardial blood flow (measured with 9-15 μm microspheres) with left anterior descending occlusion and reperfusion. Class 2 segmental net systolic wall thickening was 32 ± 5 (SEM) and 30 ± 4% of control (P < 0.005 vs. control values) 1 hour after left anterior descending occlusion in groups I and II, respectively; endocardial blood flow to these segments decreased 56 ± 13% and 49 ± 12% (P < 0.05 vs. control values). Class 3 segments displayed severe dyskinesis (net systolic wall thickening = −43 ± 6% and −33 ± 5%, groups I and II) and reductions in endocardial blood flow during left anterior descending occlusion (−81 ± 10% and −78 ± 8% (P < 0.05)). Segmental function of class 2 and 3 segments in group I dogs improved significantly with long-term reperfusion, attaining values of net systolic wall thickening of 66 ± 9 and 26 ± 9% control, respectively, at 1 month. Mean values of net systolic wall thickening attained by class 2 and 3 segments in this group during postextrasystolic potentiation were 93 ± 7 and 50 ± 13, respectively. In contrast, the net systolic wall thickening of class 2 segments in group II dogs did not change significantly with long-term reperfusion (net systolic wall thickening = 37 ± 12 at 1 month) and the average net systolic wall thickening attained by these segments with postextrasystolic potentiation was 51 ± 10%. Class 3 segments in group II dogs underwent a reversal of paradoxical thinning, but were essentially akinetic (net systolic wall thickening = 12 ± 12%) after 1 month of reperfusion and did not demonstrate significant inotropic reserve. The extent of segmental necrosis paralleled the degree of contractile dysfunction in the three segment classes, but was not significantly different between groups I and II. Similarly, macrohistochemically determined area-at-risk and gross infarct size were not different between the two groups of dogs. Thus, reperfusion after 2 hours of left anterior descending occlusion eventually improves the contractile function at sites of moderate and severe dysfunction, whereas long-term reperfusion after 4 hours of left anterior descending occlusion does not restore contractile function to class 2 segments significantly, nor does it restore important degrees of contractile function to severely dyskinetic segments. These differences in recovery of contractile function apparently are not attributable to greater segmental necrosis, larger areas of infarction, or ultimate alterations in segmental coronary blood flow. (Circ Res 53: 248–263, 1983)
anesthetized preparations) as well as of variations in the duration of the observation period after reperfusion. In general, those who have studied the effects of reperfusion in short-term experiments report little beneficial effect (Banka et al., 1974; Lang et al., 1974), whereas evaluations in long-term studies have revealed that restoration of blood flow salvages significant amounts of myocardium from irreversible injury (Ginks et al., 1972; Theroux et al., 1976; Constantini et al., 1975).

Recently, emphasis has focused upon the recovery of contractile function of reversibly injured myocardium (Theroux et al., 1976; Roan et al., 1979; Kl珂ner et al., 1981). It has been shown that complete recovery of contractile and biochemical function of myocardium subjected to brief periods (less than 60 minutes) of ischemia requires several days (Kl珂ner et al., 1981; Swain et al., 1982). However, few studies have examined the long-term influence of reperfusion after longer periods (several hours) of coronary artery occlusion. Theroux et al. (1976) have described the alterations of “marginal” and “central” ischemic segments after 1 month of reperfusion following 2 hours of coronary occlusion in a small number of awake, but sedated, chronically instrumented dogs.

The role of coronary arterial spasm (Hillis and Braunwald, 1978) and thrombosis (Buja and Willerson, 1981; Oliva, 1981) in the pathogenesis of acute myocardial infarction is becoming increasingly recognized. In view of this and the growing attention that has focused upon surgical and pharmacological revascularization of ischemic myocardium (Rentrop et al., 1981), it is important to determine the potential of reperfusion for reversing ischemia-induced contractile dysfunction, and to identify the duration of occlusion after which coronary reperfusion does not restore physiologically important levels of contractile function. Accordingly, we evaluated the ability of long-term reperfusion to reverse regional left ventricular (LV) segmental dysfunction after two durations of experimental coronary artery occlusion in conscious dogs, previously instrumented with sonar crystals for measuring LV segmental wall thickening (Sasayama et al., 1976; Osakada et al., 1980). Epicardial crystals, attached to small (1 cm²) dacron patches, were sutured to the epicardial surface, while the smaller endocardial crystals were inserted into the subendocardium obliquely so as not to disturb the intervening myocardium. Placement of the endocardial crystals was guided by obtaining an optimal signal which was displayed on an oscilloscope (Theroux et al., 1974), and the crystal wire was secured with a purse string suture at the point of exit from the epicardium. In addition, epicardial pacing wires were attached to the surface of the right ventricle near the outflow tract. All wires and catheters were tunneled between the scapulae and exteriorized through two small incisions at the base of the neck. The chest was closed in layers and any pneumothorax was eliminated by suction drainage of the chest cavity. Dogs were allowed to recover for at least 1 week before experimental study. One or 2 days before the study, under brief thiamylal-induced anesthesia, polyethylene catheters were placed in the common carotid artery and external jugular vein to measure arterial blood pressure and administer drugs and/or fluids.

Study Protocol

After a sufficient recovery period and acclimatization to a standing sling, dogs were studied in the awake, unsedated state. Control measurements of aortic, LV, and arterial pressures and a lead II ECG were recorded on an Electronics for Medicine model VR-12 physiological recorder. The maximum rate of rise of LV intracavitary pressure (peak LV dp/dt) was obtained by electronic differentiation of the LV pressure pulse. Measurements of LV wall thickness were recorded on a Hewlett-Packard 8-channel recorder interfaced with a Tektronix (model 465) oscilloscope. Regional myocardial blood flow (RMBF) was measured with carbonized microspheres (9–15 μm in diameter) labeled isotopically with 125I, 57Co, 32P, 58Sr, 109Cd, and 55Co. Microspheres, suspended in 0.05% Tween-80,
were agitated vigorously by vortexing just before injection. One to 3 million microspheres were injected into the left atrium over an 8- to 10-second period. Starting 10 seconds before, and continuing for 90 seconds after microsphere injection, reference arterial blood was withdrawn from the carotid artery at a constant rate of 7.8 ml/min with a Harvard infusion/withdrawal pump. The order of the isotopes used was changed randomly.

Dogs underwent either 2 (group I) or 4 (group II) hours of LAD occlusion, produced by inflating the pneumatic balloon occluder around the LAD. Dogs were excluded from study if inflation of the occluder caused no change at any site at which wall thickness was measured, or if large, spontaneous improvement or reversal of dyskinesis occurred during LAD occlusion, suggesting unsuccessful or incomplete occlusion.

Hemodynamic and wall thickness measurements were recorded 5, 30, and 60 minutes after LAD occlusion and at hourly intervals thereafter (Fig. 1). Regional myocardial blood flow was measured 5 minutes and 2 (group I) or 4 (group II) hours after occlusion. Ten minutes before the occlusion was released, after hemodynamic and wall thickness measurements had been obtained, dogs were given an intravenous bolus injection of lidocaine (25 mg), followed by an infusion of 0.5 mg/min for 20 minutes (10 minutes each, before and after reperfusion) to minimize the risk of reperfusion-induced arrhythmias. Dogs were actually subjected to 130 (group I) or 250 minutes (group II) of LAD occlusion, although for simplicity, these occlusive periods hereafter will be referred to as '2 hour' or '4 hour,' respectively. Reflow was allowed by deflating the balloon occluder. If resistance to further inflation of the occluder at the end of the occlusive period was absent at this time, dogs were excluded from study for reasons similar to those cited above. Deflation of the balloon occluder was ensured by applying additional suction to the catheter, and further confirmation of release of the occlusion was provided by observing dramatic, upward shifts in end-diastolic wall thickness of previously hypokinetic or akinetic segments.

Dogs were observed for 2 hours following reperfusion. The lidocaine infusion was stopped 10 minutes after releasing the occlusion. Hemodynamic and wall thickness recordings were obtained just before, and 5 minutes after discontinuing the lidocaine infusion, as well as 1 and 2 hours after reperfusion, and RMBF was measured at the latter time point, after which dogs were removed to their quarters. Dogs were returned to the laboratory and studied 24 hours later and at weekly intervals for 4 weeks. Regional myocardial blood flow also was measured after 4 weeks of reperfusion. In addition to their weekly study periods, dogs were observed daily and maintained in good health.

At 4 weeks, hemodynamic and wall thickness measurements were recorded under resting conditions and during inotropic stimulation produced by graded intravenous infusions of dopamine and by elicitation of postextrasystolic potentiation (PESP) to assess the contractile reserve possessed by regional LV segments (Roan et al., 1979). Postextrasystolic potentiation was produced by delivering electrical stimuli (4.5 msec duration of 1.5-2 x threshold voltage) to the right ventricle with a Grass S88 stimulator, coupled with a Grass SIU5 stimulus isolation unit. Pulses were delivered at the shortest R-stimulus interval at which ventricular capture and a compensatory pause occurred (Fig. 1). Each dog received 5-8 premature stimuli, with a respite of 20 beats or more between stimuli. After a short rest period, graded infusions of dopamine were given at doses of 5, 10, and 20 µg/kg per min. Hemodynamic and wall thickness measurements were recorded after 5-minute infusions at each dose. Ten-minute rest intervals were allowed between successive infusions of dopamine.

**FIGURE 1.** Tracing demonstrating LV pressure, LV dP/dt, and LV segmental wall thickness alterations from six different segments at control, with LAD occlusion, at 1 hour LAD occlusion, with reperfusion for 4 weeks, and PESP after 4 weeks of reperfusion.
Post-mortem Tissue Analysis

At the conclusion of the study, dogs were given sodium heparin (5,000 U) intravenously, followed 5 minutes later by a lethal overdose of sodium pentobarbital. The hearts were excised quickly and rinsed under cold tap water. The balloon occluder around the LAD was examined and the patency of the coronary artery was determined by expelling heparinized blood or saline from a 7F polyethylene catheter positioned on the LAD proximal to the occlusion site, and observing the anterograde run-off in the epicardial vessels of the distal LAD bed. Next, to determine the size of the occluded bed (or "anatomical risk region"), we mounted the heart on a perfusion apparatus similar to that described previously (Reimer and Jennings, 1979; Bush et al., 1982). The aorta was cannulated and perfused with a warm solution of 0.5% (vol/vol) monastral blue dye (DuPont) in normal saline, and the LAD was cannulated and perfused simultaneously with a warm 1% solution of phosphate-buffered triphenyltetrazolium chloride (Lie et al., 1975), both at a perfusion pressure of 120 mm Hg. Thus, this method identified the area-at-risk as well as the extent of infarction therein.

After the subendocardial crystals had been removed, the heart was rinsed and sliced into 5–6 transverse sections. The epicardial crystals were left sutured to the myocardium so that crystal pairs could be identified later by wire color coding. The heart slices were fixed in 10% buffered formalin, weighed, and photographed on both sides to measure the extent of infarction and the size of the occluded bed and to record the orientation of the crystal pairs relative to the area-at-risk and infarct. Transmural sections (approximately 1 cm²) encompassing the segment between pairs of endocardial and epicardial crystals were dissected, trimmed of epicardial fat and large blood vessels, and divided into epicardial, midwall, and endocardial thirds. These sections were weighed and placed in scintillation vials containing 10% formalin. Triplicate samples of myocardium from the posterior free wall (Marcus et al., 1975), representing nonischemic control tissue, were taken for RMBF measurements and, when possible, blocks also were taken from myocardium exhibiting the maximal extent of transmural necrosis and termed "infarct center." Samples were counted for radioactivity in a Packard Autogamma scintillation spectrometer for 5 minutes with window settings set appropriately for each isotope (Heymann et al., 1977). Regional myocardial blood flow was calculated by a computer-assisted program and expressed in ml/min per g.

Quantitation of Segmental Necrosis, Area-at-Risk, and Infarct Size

After counting radioactivity for RMBF determinations, epicardial, midwall, and endocardial tissue samples were embedded in paraffin en bloc, sectioned, mounted, and stained with hematoxylin and eosin. Each section was photomicrographed and the areas comprising granulomatous tissue and fibrosis were interpreted as evidence of prior necrosis and thus termed "necrotic." The boundaries of "necrotic areas" were traced on photomicrographs, while the same thick sections were examined by light microscopy. The area of necrosis (relative to total cross-sectional area) was outlined and later measured by planimetry using a digitizing tablet (Talos) coupled with a PDP 11/05 (Digital Equipment) computer, as described previously (Roan et al., 1981). The term "total necrosis" represents the weighted average of the transmural segment, i.e., it takes into account differences in the weights of the three layers of tissue. These determinations were made without knowledge of the contractile, hemodynamic, or RMBF data.

The size of myocardial infarcts and areas-at-risk were measured planimetrically, similar to the technique described above. The bed-at-risk was defined as myocardium not stained by the blue dye which was injected via the aorta into all branches of the right and left coronary system proximal to the occlusion site in the LAD. Since the bed-at-risk was perfused simultaneously with tetrazolium solution, this region possessed stained and unstained tissue. These zones were designated uninfarcted (but jeopardized) and infarcted myocardium, respectively. Color slides of both faces of transverse heart slices were illuminated on a transluent digitizing table, and the outlines of infarcted, jeopardized ("area-at-risk"), and total LV myocardium were digitized. The fractions of total LV area representing the bed-at-risk and the infarcted tissue were multiplied by the weight of each left ventricular slice to obtain the percentage, by weight, of total LV that was infarcted and at risk.

Measurements of LV Segmental Function

Segmental LV wall thickness was measured in millimeters, assuming the speed of sound through myocardium to be 1.5 mm/µsec (Sasayama et al., 1976; Theroux et al., 1977). To correct for the variation in initial separation of crystal pairs, as well as the regional performance of the nonischemic ventricle (LeWinter et al., 1975), wall thickness measurements are expressed as a percent of occlusion, or control values (Roan et al., 1979, 1981).

Three measurements of wall thickness were made from which four variables describing regional segmental function were calculated. Left ventricular end-diastolic wall thickness (EDWTH) was determined immediately preceding the rapid upstroke of the LV pressure tracing and at the nadir of wall thinning caused by atrial contraction. This time point also defined as the point immediately before the upstroke of the LV dP/dt signal (Sasayama et al., 1976; Roan et al., 1979). The maximal extent of LV segmental wall thickening was defined between peak positive and negative LV dP/dt, which correspond closely to aortic valve opening and closure, respectively. When appropriate, the maximal extent of wall thinning (paradoxic wall motion) also was measured during this interval. The variable net systolic wall thickening (NET), as defined previously (Roan et al., 1979), equals the difference between maximal systolic wall thickness and EDWTH, minus the extent of paradoxic wall thinning, if present. Analog wall thickness tracings and hemodynamic recordings were digitized with a Graf/Pen (Science Accessories Corp.) coupled to a DEC 10 computer (Roan et al., 1979).

Calculations

In the analysis of regional LV contractile function, segments were classified according to their severity of dysfunction 1 hour after LAD occlusion: class 1 segments displayed minimal ischemic dysfunction, possessing 67% or greater of their pre-occlusion values for NET; class 2 consisted of segments that displayed moderate dysfunction, defined as those with NET values of 0–66.9% of control NET; class 3 segments showed severe contractile dysfunction, manifested by paradoxic systolic wall thinning. Segments in this class had NET values <0% of their control values.
Statistical Methods

All values are expressed as mean ± 1 SEM. Comparisons between group I and II dogs for certain variables were made with a two-tailed Student's group t-test. Repeated measured analyses within segment classes over time, or between classes within a time period, were performed by two-way analysis of variance (Barr et al., 1976) plus Duncan's multiple range test (Duncan, 1955). The probability (P) was considered to be statistically significant when less than 0.05. Comparisons of values of hemodynamic parameters between two specific time points (e.g., pre- vs. immediately post-occlusion) were made with a paired t-test (Zar, 1974). Comparisons with pre-occlusion control for NET and EDWTH data were made using a one-sample t-test, since the control value was constant (100%). However, to correct for multiple comparisons in these analyses (Bonferroni's method), P was considered to be statistically significant if <0.005 (Miller, 1966). Similarly, comparisons of NET values obtained during isotropic stimulation to resting values at 4 weeks were done using P < 0.01 as a level of statistical significance (Miller, 1966).

Results

Hemodynamic Changes

Heart rate increased acutely with LAD occlusion, from 105 ± 4 to 120 ± 6 beats/min in group I dogs (P = 0.012 paired t-test; P > 0.05 Duncan's multiple range test), and from 115 ± 4 to 118 ± 4 beats/min [P = not significant (NS)] in group II dogs (Table 1). Heart rate returned to normal as LAD occlusion ensued, but increased again during the first 24 hours of reperfusion, due largely to reperfusion-induced arrhythmias, which were characterized usually as unifocal or multifocal premature ventricular contractions. In view of the possible effects of premature ventricular contractions on global and regional contractile function (Vatner, 1978), care was taken to digitize LV wall motion during intervening periods of sinus rhythm or, if necessary, during stable idioventricular rhythms. If no intervening periods of normal sinus or stable idioventricular rhythms occurred at 24 hours (or any other time point), wall motion measurements were not obtained. In general, the incidence and severity of ventricular arrhythmias during the first 24 hours of reperfusion were greater in group II dogs, e.g., 10 of 14 (group II) vs. 5 of 12 (group I) [P = 0.103 (χ² analysis)]. Heart rate returned to normal after 2 weeks of reperfusion in both groups of dogs and was nearly identical to control values by 4 weeks. Mean left atrial pressure (ATM) increased from 9.5 ± 1.1 to 12.8 ± 2.0 mm Hg and from 8.4 ± 1.1 to 10.4 ± 1.3 mm Hg immediately (5 min) after LAD occlusion in group I and II dogs, respectively. These changes were significant by a paired t-test (P = 0.016 and 0.004, respectively), but not by Duncan's multiple range test. ATM remained elevated during LAD occlusion and early reperfusion, but gradually returned to normal levels after 1 month of reperfusion. No major changes in arterial blood pressure occurred during LAD occlusion or reperfusion in either group of dogs. The maximum rate of left ventricular pressure development (LV dP/dt) decreased acutely with LAD occlusion in both group I (3146 ± 89 to 2913 ± 117 mm Hg/sec) and group II dogs (3002 ± 153 to 2724 ± 125 mm Hg/sec). Again, these changes were statistically significant based upon a paired t-test comparison between control and 5-minute post-occlusion time points, but not by Duncan's multiple range analysis. Values for this parameter reached their nadir during early reperfusion in both groups and returned to normal in group I dogs, but remained depressed throughout the remainder of the study in group II dogs. The product of heart rate and systolic arterial pressure (X 1,000), an indirect index of myocardial energy demand, increased from 15.1 ± 0.9 to 16.9 ± 1.2 mm Hg * min immediately after LAD occlusion, reached a nadir of 13.9 ± 0.6 1 hour after reperfusion, and a maximum value of 17.9 ± 0.8 at 24 hours post-reperfusion in group I dogs. The double product declined with LAD occlusion in group II dogs, due largely to the higher control heart rates in this group. Values for this parameter declined progressively with increasing duration of occlusion, reaching a minimum value at 4 hours post-occlusion (14.1 ± 0.9) and a maximum value at 24 hours (18.7 ± 0.9).

Table 2 shows the hemodynamic responses to graded infusions of dopamine 4 weeks after reperfusion. Minimal changes in heart rate, left atrial pressure, or aortic pressure occurred during dopamine infusion at doses of 5 and 10 μg/kg per min, but peak LV dP/dt increased uniformly in both groups at doses of 10 and 20 μg/kg per min. The highest dose of dopamine also caused an increase in aortic pressure. These hemodynamic responses to dopamine agree with those reported previously (Goldberg, 1972).

Lidocaine, which was given to all dogs to minimize the incidence of reperfusion-induced arrhythmias, had little or no effect on hemodynamic parameters which were recorded after 20 minutes of infusion and 5 minutes after termination. Heart rate was 114 ± 6 and 120 ± 7 beats/min, mean aortic pressure was 108 ± 3 and 109 ± 4 mm Hg, and peak LV dP/dt was 2,723 ± 131 and 2,654 ± 126 mm Hg/sec before and after discontinuation of lidocaine, respectively. The dose of lidocaine and duration (20 minutes) of its administration in this study are substantially less than those reported to reduce experimental myocardial infarct size (Nasser et al., 1980).

LV Segmental Wall Thickness

Thirteen dogs in group I yielded a total of 51 LV regional segments, comprising 10 class I segments (obtained from nine dogs), 14 class II segments from nine dogs), and 27 class 3 segments (from 12 dogs). Eighteen class I segments (from 10 dogs), 24 class 2 segments (from 12 dogs), and 19 class 3 segments (from 12 dogs) were obtained from 14 group II dogs.
A total of 54 segments were eliminated from analysis due to technically inadequate signals either before or after beginning the study.

Changes in End-Diastolic Wall Thickness (EDWTH)

Group I. The control value of EDWTH for all segments in group I dogs was 9.75 ± 0.23 mm, in close agreement both with previously reported values for this variable (Sasayama et al., 1976; Osakada et al., 1980), and with actual left ventricular free wall thickness. In addition, there were no significant differences between segment classes in this parameter. The time-dependent changes in EDWTH during 2 hours of LAD occlusion and 4 weeks of reperfusion are shown in Figure 2. Segments with minimal contractile dysfunction (class 1) displayed insignificant changes in EDWTH during LAD occlusion or reperfusion, whereas hypokinetic (class 2) and dyskinetic (class 3) segments both demonstrated significant declines in EDWTH immediately after proximal LAD occlusion (Fig. 2). After 1 hour of occlusion, class 2 and 3 segments displayed 5.0 ± 1.1 and 6.4 ± 1.0% reductions of EDWTH, respectively. The EDWTH of class 2 and 3 segments was depressed maximally by 1 hour post-occlusion. Upon reperfusion, class 2 and 3 segmental EDWTH increased to values exceeding control values. Class 3 segments displayed the greatest increase in this parameter, reaching values of 124.0 ± 3.3% and 124.7 ± 3.3% at 1 and 2 hours post-reperfusion, respectively. The EDWTH of these (class 3) segments decreased after 24 hours of reperfusion, although they were still significantly elevated at this time. Class 2 segmental EDWTH also increased during reperfusion, but less than that in class 3 segments. The EDWTH of class 2 and 3 segments gradually returned toward control values over 4 weeks of reperfusion.

Group II. The mean control value of EDWTH in dogs that underwent 4 hours of LAD occlusion was 9.70 ± 0.24 mm. The changes in EDWTH which occurred during 4 hours of LAD occlusion and 1 month of reperfusion are shown in Figure 2. The EDWTH of minimally dysfunctional (class 1) segments declined slightly, but significantly, to 96.0 ± 0.96% of control after 1 hour of LAD occlusion. Similar to the pattern observed in group I dogs, class 3 segments displayed 5.0 ± 1.1% and 6.4 ± 1.0% reductions of EDWTH, respectively.
2 and 3 segments displayed even greater declines in EDWTH after coronary occlusion, reaching a nadir by 1 hour post-occlusion: 93.38 ± 1.06 and 91.31 ± 1.63%, respectively.

With reperfusion, the EDWTH of class 2 and 3 segments exceeded their control values, and this significant elevation of EDWTH in class 3 segments was maximal within the first hour after reperfusion, but persisted for 24 hours and gradually declined to near-normal during the ensuing month of reperfusion. This recovery of EDWTH to normal appeared to occur more slowly in dogs that underwent 4 hours of LAD occlusion, although the EDWTH of class 3 segments exceeded their control values, and this persistence for 24 hours and gradually declined to near-normal during the ensuing month of reperfusion. In a manner similar to that of class 2 segments, there was a significant increase in NET during the first 24 hours after reperfusion. During the first week of reperfusion, NET of class 2 segments increased from 24.3 ± 5.9% (at 24 hours) to 54.1 ± 8.0% (P < 0.05, 24 hours vs. 1 week) and improved further over the next 3 weeks. After 4 weeks of reperfusion, these segments recovered 65.5 ± 9.4% of their original (pre-occlusion) contractile function (P < 0.05 vs. values during occlusion and early reperfusion). After LAD occlusion, class 3 segments displayed paradoxical systolic wall thinning (NET = 43.1 ± 5.6 of control after 60 minutes post-occlusion). These segments showed small changes in NET during the remainder of occlusion and demonstrated a further (but not significant) decline in contractile function during the first 24 hours of reperfusion. In a manner similar to that of class 2 segments, there was a significant increase in NET during the first week following reperfusion, after which the mean value of NET for these segments was 0.4 ± 8.4% of control. Thus, the first week, class 3 segments, which originally were severely dysfunctional, became essentially akinetic.

Over the next 3 weeks, NET reached a value of 26.2 ± 9.1 at 4 weeks (P < 0.05 vs. 1 week value).

Changes in Net Systolic Wall Thickening (NET)

**Group I.** The mean control value for NET was 2.09 ± 0.12 mm for all segments in group I dogs. Thus, wall thickness increased 21% over EDWTH during systole (Fig. 1), which compares favorably with values reported previously (Sasayama et al., 1976; Osakada et al., 1980). Figure 3 shows the changes in NET during LAD occlusion and after 4 weeks of reperfusion. The contractile function of class 1 segments was depressed minimally during LAD occlusion (NET = 89.2 ± 6.4% of control at 60 minutes post-occlusion). The NET of these segments declined to 69.0 ± 6.7 at 24 hours post-reperfusion, but returned to near-control values by 4 weeks (NET = 89.5 ± 10.5%). Class 2 segmental function in this group of dogs was 31.6 ± 4.8% of control after 60 minutes of LAD occlusion and remained depressed during the first 24 hours after reperfusion. During the first week of reperfusion, NET of class 2 segments increased from 24.3 ± 5.9% (at 24 hours) to 54.1 ± 8.0% (P < 0.05, 24 hours vs. 1 week) and improved further over the next 3 weeks. After 4 weeks of reperfusion, these segments recovered 65.5 ± 9.4% of their original (pre-occlusion) contractile function (P < 0.05 vs. values during occlusion and early reperfusion). After LAD occlusion, class 3 segments displayed paradoxical systolic wall thinning (NET = 43.1 ± 5.6 of control at 60 minutes post-occlusion). These segments showed small changes in NET during the remainder of occlusion and demonstrated a further (but not significant) decline in contractile function during the first 24 hours of reperfusion. In a manner similar to that of class 2 segments, there was a significant increase in NET during the first week following reperfusion, after which the mean value of NET for these segments was 0.4 ± 8.4% of control. Thus, during the first week, class 3 segments, which originally were severely dysfunctional, became essentially akinetic. Over the next 3 weeks, NET reached a value of 26.2 ± 9.1 at 4 weeks (P < 0.05 vs. 1 week value).

**Group II.** The average control systolic wall thickness of all segments in dogs that underwent 4 hours of LAD occlusion was 1.83 ± 0.09 mm, representing an increase in wall thickness during systole of 19% above EDWTH. These values are not significantly different from those obtained for LV segments in group I.

Class 1 segments in group II dogs showed minimal...
decreases in NET during LAD occlusion \( [N = 90.8 \pm 3.9\% \text{ of control 1 hour after occlusion, (Fig. 3)}] \). After reperfusion, these segments showed further declines in NET. However, these values were not significantly different from control. After 4 weeks of reperfusion, class 1 segmental NET was 88.2 ± 14.8% of control.

Class 2 and 3 segments showed changes in NET during coronary occlusion and early reperfusion similar to those in group I dogs. However, unlike their group I counterparts, moderately dysfunctional (class 2) segments showed no significant improvement of contractile function throughout the month of reperfusion (NET = 36.6 ± 9.6 at 4 weeks). Class 3 segmental NET increased significantly between 24 hours and 1 week (-28.7 ± 11.1 to 10.4 ± 12.0; \( P < 0.05 \)), but there was no further improvement of contractile function in these segments during the ensuing 3 weeks. In fact, between 1 and 4 weeks post-reperfusion, class 3 segments vacillated between minimal systolic thickening and paradoxical thinning.

Comparisons between class 2 NET values at 4 weeks in groups I and II yielded a \( P \) value of 0.067 (NS). However, the overall recovery, or change in NET between 1 hour post-occlusion and 4 weeks post-reperfusion was significantly greater in group I dogs (\( P = 0.03 \)). Comparisons between group I and II dogs revealed no other significant differences in actual values of NET or the changes thereof over time after reperfusion.

Responses of Class 2 and 3 Segments to Inotropic Stimulation

**Group I.** The changes in segmental NET during inotropic stimulation with dopamine infusion and PESP after 4 weeks of reperfusion are shown in Figure 4. For technical reasons, one dog in this group was not given dopamine, and the segmental responses to PESP were not studied in two dogs. Therefore, the resting NET values of class 2 and 3 segments used for comparison with values obtained during inotropic stimulation were 75.5 ± 7.9 and 5.8 ± 9.7% of control, respectively. Consistent with the observed hemodynamic changes, the lowest dose of dopamine (5 \( \mu \text{g/kg per min} \)) affected NET minimally in both class 2 and 3 segments. Class 2 segmental NET increased to 88.5 ± 8.4 in response to the highest dose of dopamine, and to 92.7 ± 7.3 during PESP (\( P = 0.004 \)). The NET of class 3 segments increased significantly in response to dopamine at doses of 10 and 20 \( \mu \text{g/kg per min} \). The NET
of these segments also increased significantly with PESP, which again, produced the greatest inotropic response (25.8 ± 9.7 to 49.7 ± 12.7; P = 0.002).

Group II. Dopamine did not significantly increase the NET of class 2 or 3 segments in dogs that underwent 4 hours of ischemia, but class 2 NET did increase significantly from 36.5 ± 9.6 to 47.9 ± 10.2% of control (P = 0.005) in response to PESP (Fig. 4B). Class 3 segmental function did not increase significantly with either dopamine or PESP.

Regional Myocardial Blood Flow

Group I. The mean weights of epicardial, midwall, and endocardial samples for all segments in group I dogs were 0.89 ± 0.3 g, 0.80 ± 0.05 g, and 0.64 ± 0.04 g, respectively. Class 1 segments did not undergo significant changes in blood flow during LAD occlusion or reperfusion (Table 3), although the transmural distribution of flow changed due to a 19% decline (NS) in endocardial flow and a 42% increase in epicardial flow (NS). Endocardial blood flow to class 2 segments declined significantly after LAD occlusion, from 0.99 ± 0.08 ml/min per g to 0.56 ± 0.14 ml/min per g, 5 min after occlusion.

Class 3 segments sustained even greater declines in RMBF after LAD occlusion; endocardial flow in these segments decreased from 1.07 ± 0.08 to 0.38 ± 0.14 ml/min per g (P < 0.05) and subepicardial flow fell from 0.97 ± 0.08 ml/min per g to 0.45 ± 0.09 ml/min per g (P < 0.05). The transmural distribution of blood flow also decreased significantly after LAD occlusion. Blood flow in myocardium sampled from the infarct center decreased slightly more (although not significantly) than in class 3 segments; endocardial blood flow fell from 1.17 ± 0.13 ml/min per g to 0.23 ± 0.08 ml/min per g (P < 0.05). After 2 hours of LAD occlusion, blood flow to class 3 segments and the infarct center tended to be greater than at 5 minutes post-occlusion, although this increase was not significant (as determined by Duncan's multiple range test). The only significant change in RMBF that occurred between 5 minutes and 2 hours post-occlusion in group I dogs was a decline in epicardial flow from 1.30 ± 0.19 to 0.74 ± 0.09 ml/min per g, in class 1 segments. Blood flow to class 2 and 3 segments and the
Posterior wall

Class 3

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>5 min Occl</th>
<th>2 hr Occl</th>
<th>2 hr Rep</th>
<th>4 wk Rep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End</td>
<td>1.07 ± 0.16</td>
<td>1.30 ± 0.19*</td>
<td>0.74 ± 0.09bc</td>
<td>0.92 ± 0.13</td>
<td>1.02 ± 0.14bc</td>
</tr>
<tr>
<td>Mid</td>
<td>1.07 ± 0.09</td>
<td>1.30 ± 0.14*</td>
<td>1.06 ± 0.23</td>
<td>0.94 ± 0.20</td>
<td>1.17 ± 0.17</td>
</tr>
<tr>
<td>Epi</td>
<td>1.06 ± 0.09</td>
<td>1.39 ± 0.18bc</td>
<td>0.79 ± 0.15</td>
<td>1.03 ± 0.15</td>
<td>0.88 ± 0.12</td>
</tr>
<tr>
<td>End/Epi</td>
<td>1.07 ± 0.13</td>
<td>0.95 ± 0.10</td>
<td>1.10 ± 0.14</td>
<td>0.97 ± 0.14</td>
<td>1.27 ± 0.15</td>
</tr>
</tbody>
</table>

Class 2

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>End</td>
<td>12</td>
<td>0.99 ± 0.08</td>
<td>0.56 ± 0.14bc</td>
<td>0.37 ± 0.08bc</td>
<td>1.21 ± 0.11</td>
</tr>
<tr>
<td>Mid</td>
<td>1.04 ± 0.07</td>
<td>0.61 ± 0.11</td>
<td>0.52 ± 0.11</td>
<td>1.34 ± 0.18</td>
<td>0.77 ± 0.10bc</td>
</tr>
<tr>
<td>Epi</td>
<td>0.98 ± 0.07</td>
<td>0.74 ± 0.13</td>
<td>0.63 ± 0.11</td>
<td>0.96 ± 0.15</td>
<td>0.58 ± 0.09bc</td>
</tr>
<tr>
<td>End/Epi</td>
<td>1.04 ± 0.14</td>
<td>0.80 ± 0.16</td>
<td>0.73 ± 0.21</td>
<td>1.14 ± 0.11</td>
<td>1.51 ± 0.24</td>
</tr>
</tbody>
</table>

Class 3

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>End</td>
<td>1.07 ± 0.08</td>
<td>0.38 ± 0.14bc</td>
<td>0.52 ± 0.12bc</td>
<td>1.53 ± 0.16</td>
<td>0.59 ± 0.07bc</td>
</tr>
<tr>
<td>Mid</td>
<td>0.93 ± 0.05</td>
<td>0.32 ± 0.17bc</td>
<td>0.52 ± 0.10bc</td>
<td>1.43 ± 0.12</td>
<td>0.57 ± 0.13bc</td>
</tr>
<tr>
<td>Epi</td>
<td>0.97 ± 0.08</td>
<td>0.45 ± 0.09bc</td>
<td>0.56 ± 0.14bc</td>
<td>1.48 ± 0.11</td>
<td>0.48 ± 0.04bc</td>
</tr>
<tr>
<td>End/Epi</td>
<td>1.13 ± 0.08</td>
<td>0.67 ± 0.17*</td>
<td>1.18 ± 0.19</td>
<td>1.06 ± 0.14</td>
<td>1.34 ± 0.16</td>
</tr>
</tbody>
</table>

Infarct center

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>End</td>
<td>1.17 ± 0.13</td>
<td>0.23 ± 0.08bc</td>
<td>0.20 ± 0.05c</td>
<td>1.34 ± 0.36</td>
<td>0.40 ± 0.06bc</td>
</tr>
<tr>
<td>Mid</td>
<td>1.31 ± 0.12</td>
<td>0.26 ± 0.09bc</td>
<td>0.19 ± 0.08bc</td>
<td>1.43 ± 0.31</td>
<td>0.40 ± 0.09bc</td>
</tr>
<tr>
<td>Epi</td>
<td>0.97 ± 0.11</td>
<td>0.30 ± 0.11bc</td>
<td>0.28 ± 0.10bc</td>
<td>1.57 ± 0.24</td>
<td>0.43 ± 0.07bc</td>
</tr>
<tr>
<td>End/Epi</td>
<td>1.00 ± 0.09</td>
<td>0.65 ± 0.14</td>
<td>0.84 ± 0.14</td>
<td>0.77 ± 0.16</td>
<td>0.99 ± 0.12</td>
</tr>
</tbody>
</table>

Posterior wall

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>End</td>
<td>1.16 ± 0.06</td>
<td>1.32 ± 0.15*</td>
<td>1.00 ± 0.15</td>
<td>1.32 ± 0.17</td>
<td>1.20 ± 0.12</td>
</tr>
<tr>
<td>Mid</td>
<td>0.97 ± 0.10</td>
<td>1.35 ± 0.15*</td>
<td>1.14 ± 0.19</td>
<td>1.33 ± 0.14</td>
<td>1.10 ± 0.10</td>
</tr>
<tr>
<td>Epi</td>
<td>0.87 ± 0.07</td>
<td>1.24 ± 0.13*</td>
<td>0.98 ± 0.19</td>
<td>1.20 ± 0.14</td>
<td>0.95 ± 0.10</td>
</tr>
<tr>
<td>End/Epi</td>
<td>1.39 ± 0.13</td>
<td>1.10 ± 0.05</td>
<td>1.12 ± 0.08</td>
<td>1.16 ± 0.08</td>
<td>1.33 ± 0.09</td>
</tr>
</tbody>
</table>

End = subendocardium; Mid = midmyocardium; Epi = subepicardium; End/Epi = transmural blood flow ratio; Occl = occlusion; Rep = reperfusion.
Within time point and LV wall layer, values with the same letter superscript are not significantly different (P < 0.05) from each other (ANOVA and Duncan's multiple range test).
* Significantly different (P < 0.05) from control (ANOVA and Duncan's multiple range test).

Group II. The mean weights of epicardial, midwall, and endocardial LV segments were 0.89 ± 0.06 g, 0.79 ± 0.05 g, and 0.68 ± 0.06 g, respectively. These values did not differ significantly from the respective values in group I dogs. Blood flow to class 1 segments did not change significantly after LAD occlusion (Table 4). Endocardial blood flow in class 2 segments declined significantly during LAD occlusion from 1.31 ± 0.13 ml/min per g to 0.71 ± 0.12 ml/min per g, 5 minutes after LAD occlusion. Class 3 segments underwent greater reductions in RMBF during LAD occlusion. Endocardial blood flow fell from 1.27 ± 0.14 to 0.28 ± 0.08 ml/min per g after 5 minutes of LAD occlusion. Blood flow to severely dysfunctional segments and the infarct center increased between 5 minutes and 4 hours post-occlusion, although RMBF values in these regions were still significantly below control values. As with RMBF changes in group I dogs, although flows in ischemic segments were higher at the end of the occlusive period, a similar relationship between the decrements in flow and the degree of contractile dysfunction existed. Blood flow in class 3 segments returned to normal levels after 2 hours of reperfusion, but at 4 weeks, transmural blood flow was reduced significantly. Blood flow in the infarct center was slightly more reduced than in class 3 segments with LAD occlusion and the pattern of RMBF changes during immediate and late reperfusion periods was similar to those displayed by class 3 segments.

Myocardial Necrosis

Postmortem inspection of hearts revealed a small layer of fibrotic adhesions over epicardial crystals, but little myocardial necrosis caused by these crystals was observed. Similarly, endocardial crystals were surrounded by a thin (~0.5 mm thick) rim of necrosis.

Segmental Necrosis

Group I. The extent of histological necrosis in class 1, 2, and 3 LV segments, as well as in tissue samples taken from the infarct center, is shown in Table 5. Necrosis in the endocardial and midwall layers exceeded that in the epicardial layers, although this was not uniformly significant. The extent of endocardial necrosis was greatest in the infarct center (57.6 ± 10.8%) followed by that in class 3 (34.0 ± 7.0%), class 2 (22.5 ± 7.1), and class 1 segments (5.3 ± 2.1%). Thus, in general, the extent of segmental
necrosis paralleled the degree of contractile dysfunction in the three classes of segments.

**Group II.** As with group I dogs, necrosis was greatest in the endocardial layers, but this difference was significant in class 1 segments only (Table 5). Endocardial necrosis was greatest in class 3 segments (34.6 ± 6.8%) and infarct center (56.9 ± 14.9%); *P* < 0.05 INF vs. class 3), followed by class 2 (23.7 ± 6.2%) and class 1 (12.6 ± 6.5%). There was no significant difference in the extent of segmental necrosis between groups I and II within any segment class, at any level.

In most dogs, postmortem perfusion staining of hearts for delineation of the area supplied by the occluded LAD ("area-at-risk") and infarct size revealed a rim of subendocardial necrosis which penetrated the middle layer of the ischemic area to a variable extent. The mass of the bed-at-risk (expressed as a percentage of the total LV mass) was 19.7 ± 1.5% in group I, and 19.8 ± 2.4% in group II dogs (Table 6). Infarct size was not different in the two groups of dogs.

**Discussion**

Previously, we have demonstrated that moderately dysfunctional (class 2) or severely dyskinetic (class 3) LV segments undergo little or no improvement of contractile function during 1 week after permanent LAD occlusion in conscious dogs (Roan et al., 1979). In those studies, it was shown that class 2 segments (as defined in this study) display significant contractile reserve for as long as 24 hours after coronary occlusion, whereas class 3 segments exhibited contractile reserve for only 2 hours following LAD occlusion, suggesting that the time limit after occlusion for significant reversal of segmental dysfunction depends upon the duration of occlusion as well as the severity of ischemia-induced contractile dysfunction.

The results of the present study indicate that if reperfusion is instituted after 2 hours of LAD occlusion, class 2 segmental function is eventually restored to near pre-occlusion levels, whereas severely dysfunctional segments undergo a reversal of their paradoxical systolic wall thinning plus a recovery of 25% of their control NET. In contrast, 4 hours of LAD occlusion followed by 1 month of reperfusion results in persistent contractile dysfunction in class 2 segments which showed practically no improvement of contractile function. Although group II, class 3 segments underwent a reversal of their paradoxical wall thinning, they were essentially akinetic after 4 weeks of reperfusion. Unlike class 3 segments in group I dogs, however, these segments did not demonstrate further improvement of contractile function between 1 and 4 weeks post-reperfusion.

Our results generally are similar to those reported
Table 5

Extent of Histological Necrosis in LV Segments and Infarct Center

<table>
<thead>
<tr>
<th>Class</th>
<th>n</th>
<th>Epi</th>
<th>Mid</th>
<th>End</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>8</td>
<td>3.8 ± 2.0*</td>
<td>18 ± 1.0*</td>
<td>5.3 ± 1.8*</td>
<td>3.8 ± 1.8*</td>
</tr>
<tr>
<td>Group II</td>
<td>13</td>
<td>6.3 ± 2.2*</td>
<td>8.7 ± 3.2*</td>
<td>12.6 ± 6.5*</td>
<td>8.6 ± 2.9*</td>
</tr>
</tbody>
</table>

Class 2

| Group I | 9 | 8.5 ± 2.5* | 15.2 ± 5.9* | 22.5 ± 7.1* | 15.6 ± 4.8* |
| Group II | 19 | 17.1 ± 4.1* | 16.7 ± 5.8* | 23.7 ± 6.2* | 18.8 ± 4.6* |

Class 3

| Group | 21 | 17.6 ± 2.9* | 33.1 ± 4.6* | 34.0 ± 7.0* | 28.8 ± 4.3* |
| Group II | 17 | 19.9 ± 4.9* | 28.2 ± 6.5* | 34.6 ± 6.8* | 27.1 ± 5.6* |

Infarct center

| Group I | 9 | 20.8 ± 10.1* | 46.7 ± 10.2* | 57.6 ± 10.8* | 29.6 ± 7.4* |
| Group II | 7 | 23.4 ± 12.3* | 40.1 ± 13.5* | 56.9 ± 14.6* | 28.3 ± 11.8* |

n = number of segments.
Values shown represent the mean ± 1 SEM of percentage of cross-sectional area (see text). Value for "total" necrosis represents the weighted average of epi-, mid-, and endocardial values (see text for details).
Within the same group and LV wall layer, values with different letter superscripts are significantly different from each other (P < 0.05; ANOVA, Duncan’s multiple range test).
* = Indicates significant difference (P < 0.05) within a given group and class between LV wall layers.

Previously by Theroux et al. (1976), who compared the recovery of LV segmental systolic shortening in awake dogs subjected to 2 hours of left circumflex occlusion plus 1 month of reperfusion with a group that underwent 4 weeks of permanent coronary occlusion. In their study, "marginally ischemic" segments (which roughly correspond to our class 2 segments) exhibited late, but significant, eventual recovery of contractile function and end-diastolic length, while "ischemic" segments recovered approximately one-half of their original segmental shortening. In contrast, "marginally ischemic" and "ischemic" segments in dogs with permanent circumflex occlusion recovered significantly less contractile function than their corresponding segments in the reperfused group. Direct comparisons between the present study and that of Theroux et al. (1976) may be complicated by differences in classifying regional segments. Also, Theroux et al. (1976) measured subendocardial segmental shortening, rather than wall thickening, but Sasayama et al. (1976), and Heyndrickx et al. (1978) have shown that the two methods of examining segmental LV function result in parallel changes with experimental coronary occlusion.

A potential criticism of measuring wall thickening as an index of LV segmental function is that the method does not differentiate between true systolic wall thickening and spurious increases in this variable due to translational or rotational ("shear") movements of the LV wall. However, using a triangulation technique for studying segmental wall thickness, Osakada et al. (1980) have shown that, with careful placement of the endocardial crystal, the contribution of these factors to segmental wall thickness measurements is trivial. In this study, the endocardial crystals were removed before the heart was sliced, which precluded confirmation of the exact alignment of crystal pairs. It is likely that some segments were not aligned exactly parallel to the small axis of the heart. However, since we related changes in segmental wall thickening to each seg-

Table 6

Macrohistochemically Determined Infarct Size (g/100 g)

<table>
<thead>
<tr>
<th>Group</th>
<th>Area-at-risk LV</th>
<th>Infarcted LV myocardium</th>
<th>Infarcted LV Area-at-Risk</th>
<th>Total LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>19.7 ± 1.5* (11)</td>
<td>22.3 ± 4.9 (11)</td>
<td>5.0 ± 0.9 (13)</td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>19.8 ± 2.4 (8)</td>
<td>29.2 ± 7.0 (8)</td>
<td>7.1 ± 1.7 (11)</td>
<td></td>
</tr>
<tr>
<td>Group I vs. group II P</td>
<td>0.428</td>
<td>0.257</td>
<td>0.981</td>
<td></td>
</tr>
</tbody>
</table>

LV = left ventricle
The latent contractile reserve of regional LV segments was determined by their response to inotropic stimulation with dopamine infusion and premature electrical stimulation (Crozier et al., 1977; Roan et al., 1979). As shown previously, PESP produced the greatest inotropic response, regardless of group or segment class. Dopamine at doses of 10 or 20 μg/kg per min was variably effective in increasing NET. Dopamine, at 5 μg/kg per min did not produce a significant inotropic effect nor a consistent change in NET. The magnitude of the response to inotropic stimulation appeared to depend upon the degree of recovery of contractile function. Group I, class 2 segments exhibited significant contractile reserve, reaching a maximal NET of 93 ± 7% during PESP, suggesting that these segments might eventually regain nearly complete recovery of contractile function with either longer reperfusion periods or with selected pharmacological interventions. Also, the NET of group I, class 3 segments doubled in response to PESP. In comparison, the maximum values of NET attained by class 2 and 3 segments with PESP in group II dogs were 53 ± 11 and 35 ± 16% of their respective control NET, substantially less than their group I counterparts. In fact, the class 2 segments in group II resembled the group I, class 3 segments, with respect to eventual recovery of segmental function and maximal inotropic response. The small recovery of NET (12%) in class 3 segments after 4 hours of LAD occlusion and 4 weeks of reperfusion, coupled with their lack of response to inotropic stimulation, indicates that these segments possess little potential for important improvement of contractile function.

An important observation from these studies was that, regardless of segment class or duration of LAD occlusion, reperfusion exacerbated segmental dysfunction, an effect which persisted for 24 hours after reperfusion. This observation has important clinical and experimental implications. Clearly, detection of wall motion abnormalities early after a temporary ischemic event may result in inaccurate prognostication. Considerable experimental evidence exists showing that erroneous conclusions regarding the effects of reperfusion after 1 or more hours of experimental coronary occlusion can result from limiting the observation period following reflow to several hours (Lang et al., 1974; Weiner et al., 1976). The most consistent and dramatic improvements in segmental function occurred between 24 hours and 1 week after reperfusion. From the present data, it is not discernable whether segmental function improved gradually over this time. The following 3 weeks represent a period during which further, but more gradual improvements of segmental function occur. This pattern of recovery is consistent with the results of previous investigators (Theroux et al., 1976; Costantini et al., 1975).

Acute LAD occlusion caused a prompt, significant decline in EDWTH of both class 2 and 3 LV segments, which is interesting, since only the latter exhibited paradoxical systolic wall thinning. The decline in EDWTH in class 2 segments during ischemia may be related to the characteristically high resting tension of cardiac muscle (Abbott and Gordon, 1975). Previous studies have demonstrated that marginally ischemic segments undergo an increase in end-diastolic length, combined with a decline in systolic segmental shortening (Theroux et al., 1977). Decreases in EDWTH, which reflect regional LV wall dilatation probably resulted in increased wall stress in both class 2 and 3 segments; this may have allowed class 2 segments to exploit the Frank-Starling relationship (Theroux et al., 1974).

With reperfusion, the EDWTH of class 2 and 3 segments increased to values significantly higher than before or during LAD occlusion to a degree that seemed to be inversely related to the extent of ischemia-induced wall thinning. Interestingly, the elevated EDWTH during early reperfusion appeared to be related more to changes in blood flow than to systolic wall thickening since all segments displayed either no change or declines in NET during this interval.

The nature of the elevated EDWTH associated with reperfusion in class 2 and 3 is not clearly understood. Cell swelling, vascular endothelial damage, and interstitial edema have been observed in reperfused myocardium by several investigators (Bresnahan et al., 1974; Willerson et al., 1977). Willerson et al. (1977) have demonstrated, in isolated blood-perfused canine hearts, that 40 minutes of LAD occlusion after 20 minutes of reperfusion causes an increased mass, interstitial fluid accumulation, and identifies impaired cell volume regulation. Perhaps a component of the elevated EDWTH during reperfusion is also attributable to contracture. Gaasch et al. (1979) have reported that 2 hours of global ischemia followed by reperfusion in isolated blood-perfused canine hearts results in increased diastolic wall thickness and stiffness, which they attributed partly to ischemic contracture. Theroux et al. (1976) have also attributed elevated end-diastolic wall thickness of ischemic-reperfused segments to increased stiffness.

The results obtained from measurements of segmental histological necrosis, infarct size, and area-at-risk are important in several respects. It is apparent that increasing segmental contractile dysfunction was accompanied by significantly increased histological necrosis. However, increasing the duration of LAD occlusion from 2 to 4 hours, did not result in significantly greater histological necrosis in any...
segment class. When comparing class 2 segmental necrosis between group I and II dogs, it appears that the greatest difference (although not significantly different), occurred in the epicardial layer, whereas insignificant differences in necrosis were found in the midwall or endocardial layers (see Table 5). Consistent with histological findings, infarct size also was comparable between group I and II dogs, regardless of whether this parameter was expressed as a percent of the LV or anatomical area-at-risk. It was also important to confirm that the average areas-at-risk were not different (19.7 ± 1.5 vs. 19.8 ± 2.4% of LV mass) between group I and II dogs, since Lowe et al. (1978) and Jugdutt et al. (1979), have shown that the extent of ischemic injury is related to the size of the occluded coronary bed. Thus, the lack of recovery of class 2 segmental function in group II dogs cannot be attributed solely to increases in histological necrosis. These observations imply that, despite 4 weeks of reperfusion, apparently reversibly injured tissue in class 2 segments possesses some yet undefined defect in contractile function. Perhaps, even after 1 month of reperfusion, myocytes within these segments are still metabolically dysfunctional (e.g., unable to produce and/or utilize high energy phosphates), or possess a defect in calcium transport, or both. There is increasing experimental evidence to support suggestions that these segments possess a metabolic defect (Kloner et al., 1981; Swain et al., 1982); the lack of significant inotropic response of group II, class 2 segments to PESP could be caused by either or both mechanisms.

It should be noted that the segmental dysfunction observed within 2 and 4 hours of LAD occlusion and reperfusion was not accompanied by large infarcts. It is possible that more extensive necrosis and more severe contractile dysfunction would have occurred if we had produced larger areas of ischemia by occluding more proximally on the LAD, or by maintaining the occlusion for a longer period. However, in our study, we were able to obtain many severely and moderately dysfunctional segments by rendering, on the average, only one-fifth of the LV ischemic. It is difficult to predict what pattern of segmental dysfunction and recovery would have occurred if all segments underwent as severe ischemia as tissue sampled from the infarct center. However, RMBF in these two areas differed significantly only in endocardial flow during LAD occlusion. The apparent discrepancy between the small infarcts produced in groups I and II and the functional recovery of class 2 segments only serve to underscore the powerful influence of the early institution of reperfusion.

Previous studies from other laboratories (Kerber et al., 1975; Gallagher et al., 1980; Vatner, 1980), as well as our own (Roan et al., 1981), have demonstrated a correlation between the extent of regional LV segmental dysfunction and the degree of ischemia. In general, a similar relationship between these parameters existed in the present study. The regression equation for the combined groups I and II was:

\[ Y(\text{flow}) = 0.61 + 0.052 \times (\text{NET}) \]

\[ R = 0.476, P < 0.001 \]

Blood flow to class 3 segments and the infarct center increased between 5 minutes and either 2 or 4 hours in both groups of dogs. This increase was not statistically different from the 5-minute post-occlusion value and probably not important physiologically. Although class 3 segments in group II dogs displayed a spontaneous, progressive increase in NET during this interval, it is difficult to know how much of this is attributable to collateral flow (Schaper, 1971) or progressive stiffening. It is unlikely that these selective, small increases in NET were due to leakage or slippage of the balloon occluder, since one would expect simultaneous and comparable increases in NET to all segments if this were true. Furthermore, the criteria that we used to check the balloon occlusion eliminated obvious cases of balloon failure.

The increases in collateral blood flow to severely ischemic segments were comparable in groups I and II, although direct comparisons between end-occlusion flows may be inappropriate since these time points differed by 2 hours. However, it does not appear that the greater recovery of class 2 segmental function with long-term reperfusion in group I can be ascribed to differences in the severity of ischemia during LAD occlusion.

Although blood flow returned to near normal with acute reperfusion in class 2 segments, flow to the inner wall of these segments was depressed significantly at 4 weeks. Interestingly, blood flow in class 2 segments after 4 weeks of reperfusion was similar or slightly greater in group II dogs, indicating an apparent lack of correlation between blood flow and segmental function after 4 weeks of reperfusion. This depression of blood flow at 4 weeks was even more prominent in class 3 segments, suggesting that blood flow was diverted away from necrotic myocardium, an observation consistent with that reported previously by Hirzel et al. (1976), and Rivas et al. (1976).

An important assumption of the microsphere technique for measuring RMBF is that microspheres become permanently entrapped in the heart (Heymann et al., 1977). It has been suggested that microsphere loss from injured, necrotic myocardium occurs, based on disparate preocclusion flow values between nonischemic and involved regions of the LV. Changes in the density of injured or infarcted myocardium can occur due to edema, resulting in spuriously low blood flow values, or "apparent microsphere loss" (Reimer and Jennings, 1979). In addition, results of studies by Capurro et al. (1979) and Jugdutt et al. (1979) suggest that leaking of microspheres from necrotic myocardium occurs to a
variable degree, the extent of which depends upon the degree of ischemic injury. In addition, spuriously high RMBF values can result from replacement of infarcted myocardium by a smaller volume of scar tissue (Reimer and Jennings 1979). For calculated flows to have been increased artfactually, due to scar formation, one would expect pre-occlusion blood flow to be progressively higher from class 1 to class 3 (and infarct center) segments, since myocellular necrosis was greater in the latter. Although pre-occlusion blood flow in group II dogs tended to be higher in class 2 and 3 segments, which contained greater amounts of necrosis, these differences were not statistically significant. Moreover, the major focus of this study was to evaluate the recovery of segmental function with long-term reperfusion after 2 and 4 hours of coronary occlusion. Regional myocardial blood flow was measured primarily to verify that myocardial ischemia (and reperfusion) was produced in these LV segments and that progressively greater segmental dysfunction was associated with more intense ischemia, rather than to document absolute blood flow alterations occurring with varying periods of coronary arterial occlusion and reperfusion. Possible artifactual alterations in RMBF did not prevent us from achieving these objectives in this study.

In summary, the results of this study show that significant recovery of contractile function occurs at moderately dysfunctional LV segments with long-term reperfusion after 2 hours of LAD occlusion; severely dysfunctional segments exhibit a reversal of their paradoxical wall thinning plus a recovery of 26% of their pre-occlusion NET. Both class 2 and 3 segments at 4 weeks display significant contractile reserve, as assessed by their response to inotropic stimulation with dopamine and PESP. In contrast, class 2 segmental function is not significantly improved with 2 weeks of reperfusion after 4 hours of LAD occlusion. Despite a reversal of paradoxical wall thinning, class 3 segments in group II dogs were essentially akinetic after 4 weeks of reperfusion. The NET of class 2, but not class 3 segments, increased significantly during inotropic stimulation. Thus, significant return of segmental LV function occurs with 1 month of reperfusion after 2 hours, but not after 4 hours of proximal LAD occlusion.

We gratefully acknowledge the expert technical assistance of Michael Deguchi, Rebecca Lundwick, Coral Marsau, Janice McNatt, Judy Ober, David Perry, Gifford Ramsey, and Dorothy Thomas, the helpful advice of Dr. Peter G. Roan, and the statistical assistance of Kent Dana and Nancy Wilson. We also wish to thank Laurre Christian and Belinda Lambert for their assistance in preparing this manuscript. This work was supported by National Institutes of Health Ischemic SCOR Grant HL17669, American Heart Association (Texas Affiliate) Grants, and the Harry S. Mass Heart Fund.

Results of this study were presented in preliminary form at the 31st Annual Scientific Meeting of the American College of Cardiology, April 1982, in Atlanta, Georgia, and at the meetings of the American Federation for Clinical Research, May 7-10, 1982, Washington, D.C.
Bush et al. / Recovery of LV Segmental Function after Temporary Occlusion

INDEX TERMS: Experimental myocardial ischemia - Segmental LV function


INDEX TERMS: Experimental myocardial ischemia - Segmental LV function

Bush et al. / Recovery of LV Segmental Function after Temporary Occlusion
Recovery of left ventricular segmental function after long-term reperfusion following
temporary coronary occlusion in conscious dogs. Comparison of 2- and 4-hour occlusions.

doi: 10.1161/01.RES.53.2.248

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/53/2/248.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
Circulation Research 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 Research is online at:
http://circres.ahajournals.org/subscriptions/