

Interrelationships between Regional Left Ventricular Function, Coronary Blood Flow, and Myocellular Necrosis during the Initial 24 Hours and 1 Week after Experimental Coronary Occlusion in Awake, Unsedated Dogs

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SUMMARY This study examined the relationships between left ventricular (LV) regional function, regional myocardial blood flow (RMBF), and myocellular necrosis after sudden proximal occlusion of the left anterior descending coronary artery (LAD) in 36 awake, unsedated dogs. Net wall thickening during systole (NET) was used to assess regional LV function, was expressed as percent control, and was measured with chronically implanted ultrasonic crystals. RMBF was measured with 8- to 10- μ m radioactive microspheres. In regions with a moderate degree of functional loss, NET fell to $35.3 \pm 2.2\%$ of control at 5 minutes when RMBF fell from 1.19 ± 0.08 to 0.86 ± 0.09 ml/g per min ($P < 0.05$). No significant change occurred in midwall or epicardial RMBF. The relationship between endocardial flow and NET was non-linear ($r = 0.69$, $P < 0.0001$). In these segments, subsequent changes in RMBF were unrelated to corresponding functional alterations through 24 hours. In segments with paradoxical systolic wall thinning RMBF fell in endocardial, midwall, and epicardial layers; endocardial ischemia was most severe (0.30 ± 0.05 ml/g per min). Segmental myocellular necrosis was most severe in the endocardial layer and correlated significantly with both RMBF and segmental function. Myocellular necrosis increased in severity as flow was reduced below 70–75% of normal. Thus, in this model of LV ischemia, (1) regional LV functional loss is most sensitive to reductions in endocardial RMBF; (2) subsequent increases in RMBF are largely unassociated with functional recovery; (3) transmural ischemia results in paradoxical systolic wall thinning. *Circ Res* 49: 31–40, 1981

ISCHEMIC impairment of left ventricular function plays a central role in the pathophysiology of ischemic heart disease (Page et al., 1971). Investigators have known for many years that loss of left ventricular contractile function occurs rapidly after coronary artery occlusion (Tennant and Wiggers, 1935). However, the quantitative interrelationships between regional left ventricular function and coronary flow are not well understood. The present study was performed to delineate the relationships between regional left ventricular ischemia, regional alterations in left ventricular function, and the corresponding extent of myocellular necrosis.

Previous investigators have documented the temporal and geometric changes in regional myocardial blood flow (RMBF) after coronary artery occlusion

in the dog (Becker et al., 1973; Hirzel et al., 1976). Others have defined the regional functional effects of coronary occlusion both early and late after myocardial infarction (Theroux et al., 1974) and have quantified left ventricular segmental inotropic reserve (Roan et al., 1979). However, the sequential assessment of changes in left ventricular regional myocardial blood flow and segmental function hours to days after coronary occlusion has not been reported previously. These acute relationships have been examined in the open-chest anesthetized pig (Stowe et al., 1978) and anesthetized dog models (Gallagher et al., 1978). The present study was performed using awake, unsedated dogs to avoid the possible confounding effects of thoracotomy and anesthesia in these previous studies.

We hypothesized that areas with severe early reductions in RMBF would develop correspondingly severe degrees of necrosis (Irvin and Cobb, 1977) and thus would be unlikely to demonstrate significant functional improvement with the re-establishment of RMBF through collateral channels. In contrast, in areas of myocardium with only moderate or mild early ischemia, permanent myocellular

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lar injury would be less. Thus, these areas would be more likely to demonstrate some functional recovery with subsequent increases in collateral RMBF. Accordingly, we sought to (1) quantify regional function and coronary flow relationships, (2) determine the functional effects of re-collateralization, and (3) determine the relationship between the severity of myocellular necrosis, the severity of regional flow reductions, and the resultant functional effect of myocellular necrosis.

Information obtained in the study of these relationships may be useful in understanding the pathophysiology of regional left ventricular dysfunction after acute myocardial infarction and in assessing the potential utility of interventions designed to increase regional coronary perfusion and thereby improve left ventricular function.

Methods

Surgical Preparation

Forty-two mongrel dogs were anesthetized with pentobarbital, 30 mg/kg, iv, intubated, and ventilated with a Harvard respirator. A thoracotomy was performed in the left 5th intercostal space under sterile conditions and the heart suspended in the pericardium. A Konigsberg (P22) catheter-tipped manometer (Konigsberg Instruments) was placed into the left ventricular (LV) cavity through an apical stab wound to obtain high fidelity intracavitary pressure recordings. A polyethylene catheter was inserted into the left atrium through the left atrial appendage. The left anterior descending artery (LAD) was carefully dissected free of the epicardium and an inflatable balloon occluder made of polyvinyl chloride placed around its proximal portion. The occluding balloon was fashioned from 8 Fr. Bardic infant feeding tube (C.R. Bard, Inc.). The distal end of the tube was heat-sealed, then immersed into hot water and inflated. The distal end was thus more compliant and, when tied around the coronary artery, acted as an effective occluder when inflated. During the surgery, the balloon was temporarily inflated for 15–20 seconds and the resultant area of cyanotic myocardium identified over the anterior portion of the left ventricle. To measure segmental left ventricular wall thickness, 5-MHz titanate-zirconate piezoelectric crystals (Transducer Products), 5 mm in diameter, were sutured to the epicardium, and smaller piezoelectric crystals, 3 mm in diameter, were inserted through the left ventricular wall into the endocardium. The position of the endocardial crystals was adjusted to obtain an optimal signal by the method of Franklin and co-workers (Theroux et al., 1974). Ordinarily, 4–5 pairs of such crystals were inserted within or near the area of cyanosis. One pair was inserted on the high lateral wall of the left ventricle. The various catheters and wires were exteriorized between the scapulae and the incision closed. Subsequently, the animals were studied 1–2 weeks after instru-

mentation surgery at a time when they appeared to have fully recovered. Under light anesthesia, a catheter was placed in the right common carotid artery 24 hours prior to the study.

Study Protocol

All dogs were studied in an awake, unsedated state. LAD occlusion was produced approximately 5–10 minutes after the acquisition control data. Two groups of dogs were defined: one group was allowed to survive 1 week ($n = 16$) following LAD occlusion and the second group for 24 hours ($n = 18$) following occlusion. Two dogs died suddenly between 8 and 24 hours. Dogs were assigned to either group in a nonsystematic fashion prior to the beginning of each study. (In six dogs, RMBF results are not included because technical problems precluded their measurement.) During the study period, the dogs were lightly restrained in a cradle-like device. In the control period, hemodynamic and segmental LV wall thickness measurements were obtained and radioactive microspheres injected for determination of regional myocardial blood flow (RMBF) (Heyman et al., 1977). Microspheres, 8–10 μ m in diameter, were suspended in a 0.05% solution of Tween-80, and were agitated vigorously in a Genie vortex mixer (Scientific Industries) prior to injection. Two to six million microspheres were given over a 10- to 15-second period into the left atrium. Fifteen seconds before and for 90 seconds after the microsphere injection, blood was withdrawn at a constant rate of 7.75 ml/min from the carotid artery with a Harvard pump (Harvard Apparatus). Microspheres were labeled with: ^{125}I , ^{46}Sc , ^{85}Sr , ^{90}Nb , ^{141}Ce (3M Co.) or ^{113}Sn or ^{57}Co (New England Nuclear). Combinations of isotopes were determined by availability of the particular microspheres. The isotopically labeled microspheres were given in random order. Following injection of the microspheres, hemodynamic and wall thickness data were obtained for a second time; no changes in these variables were observed from the determination prior to microsphere injection. Next, a pneumatic occluding balloon was inflated around the proximal LAD producing coronary occlusion as confirmed by the resultant alterations in segmental wall motion.

Five minutes after LAD occlusion, the analog wall thickness measurements were re-calibrated and another recording of hemodynamic and regional wall motion data was obtained. Then, a repeat microsphere injection was made into the left atrium. Subsequently, hemodynamic and regional LV wall thickness data were obtained at hourly intervals for 8 hours, after which time the animals were given disopyramide phosphate, 100–150 mg, po, to prevent ventricular fibrillation. Twenty-four hours after LAD occlusion, regional wall motion, hemodynamic variables, and myocardial blood flow were measured. The dogs, allowed to survive 1 week, had repeat measurements of the same variables.

Postmortem Tissue Preparation

At the conclusion of each study, the dogs were killed with a large overdose of pentobarbital. The hearts then were quickly removed and the occluding balloon examined. In each case, it was noted to be tightly inflated around the LAD. In 11 dogs, the hearts were suspended from a ring stand and the LADs perfused with saline under 100 mm Hg pressure—in each case the LAD was completely occluded and no distal flow occurred. Each heart was sliced into 4–5 1-cm sections and incubated in a warm solution of 2,3,4-triphenyltetrazolium chloride (TTC) for 15 minutes, then fixed in 10% buffered formalin (Lie et al., 1975). The heart slices were weighed and photographed on both their apical and basal aspects. Infarct size was determined by planimetry of areas of myocardium which did not stain with TTC (Lie et al., 1975). The epicardial crystals remained sutured to the myocardium and were identified postmortem by color-coding of the wires. Transmural blocks were taken to encompass myocardium approximately 1 cm on either side of each crystal site. After removal of epicardial fat, the blocks were divided into endocardial, midwall, and epicardial thirds. These sections were weighed to the nearest milligram and placed in a scintillation vial with 10% formalin for γ scintillation counting. Blocks were taken from each crystal site as well as from the center of the infarct, defined as an area with the maximal transmural extent of gross necrosis. Samples also were taken from normal-appearing posterior wall of the left ventricle. In some cases, the infarct was not large or confluent enough to allow a separate sample from the infarct center.

After counting, the epicardial, midwall, and endocardial samples from each site were embedded in paraffin as a single block, sectioned, mounted, and stained with hematoxylin and eosin. Photomicrographs were prepared for each section. The sections were examined by light microscopy and the boundaries of the necrotic areas traced on the photomicrographs. The relative area of necrosis was measured by planimetry with a Talos digitizing tablet (Talos Systems, Inc.) interfaced with a PDP 11/05 computer (Digital Equipment Corporation).

Measurements

Hemodynamic measurements were made in mm of mercury and included aortic systolic, diastolic, and mean pressure, as well as left atrial mean pressure. Heart rate and the maximal rate of rise of the left ventricular intracavitary pressure (dp/dt) in mm Hg/sec from the Konigsberg catheter also were obtained.

Segmental LV wall thickness was measured in mm assuming the speed of sound through myocardium to be 1.5 mm/ μ sec (Theroux et al., 1974). To correct for the variability in the initial separation of crystal pairs, as well as in regional performance of the nonischemic ventricle (LeWinter et al., 1975), measurements of wall thickness are expressed as a

percent of control values for each crystal pair or segment site. The analog tracings of wall thickness were digitized by hand with a Graf/Pen (Science Accessories Corp.), then stored and processed by computer (Digital Dec-10, Digital Equipment Corporation).

Three measurements of wall thickness were made from which four variables of regional performance were calculated. Left ventricular segmental end-diastolic wall thickness (EDWTH) was measured just prior to the rapid upstroke of the LV pressure tracing at the nadir of the transient wall thinning produced by atrial systole. The maximal extent of LV segmental wall thickening was measured between peak positive and peak negative LV dp/dt, times which approximate aortic valve opening and closure, respectively. If present, the maximal extent of systolic wall thinning (paradoxical wall motion) was also measured during this time. The variable NET was defined as the extent of net wall thickening, i.e., the extent of maximal systolic wall thickening minus the amount of paradoxical wall thinning, if any (Roan et al., 1979). Early diastolic wall thickening occurring after the time of peak negative LV dp/dt was frequently noted in ischemic regions but was not included in the quantitation of NET.

Scintillation vials were counted in a Packard multichannel γ scintillation counter for 5 minutes per vial with appropriate window settings for each isotope (Heymann et al., 1977).

Calculations

For the analysis of regional LV function, segments of myocardium were arbitrarily placed into three classes according to the loss of function sustained after proximal LAD occlusion. The boundaries of these classes were defined prior to the initiation of the study and have been presented elsewhere (Roan et al., 1979).

Class 1 consisted of segments with little or no functional impairment. Segments within this group retained 67% or greater net systolic wall thickening (NET) 5 minutes after proximal LAD occlusion.

Class 2 consisted of segments with a moderate degree of functional impairment. Segments within this class retained greater than 0% but less than 67% of control NET 5 minutes after LAD occlusion.

Class 3 consisted of segments with a severe degree of functional impairment manifested by paradoxical systolic thinning of the left ventricular wall (NET less than 0% of control) 5 minutes after LAD occlusion.

Regional myocardial blood flow (RMBF) was calculated by standard methods (Heyman et al., 1977) and expressed as ml/g per min. RMBF can be expected to vary as a result of both coronary occlusion and changes in myocardial oxygen demand estimated by heart rate-blood pressure product (Table 1). To remove the variability in RMBF contributed by changes in heart rate-blood pressure

TABLE 1 Hemodynamic Alterations after Proximal LAD Occlusion

Param	N1	Control	5 min*	1 Hr*	2 Hr*	3 Hr*	4 Hr*	5 Hr*	6 Hr*	7 Hr*	8 Hr*	24 Hr*	1 Wk*	N2
AOS	42	144±4	144±3	144±3	143±3	143±3	141±3	137±3†	134±3†	134±3†	132±3†	127±4†	132±4†	17
AOD	42	102±2	105±2	106±3	105±2	106±2	103±2	101±3	101±2	103±2	100±2†	88±3†	95±4†	17
AOM	42	122±2	122±3	123±2	122±2	124±2	118±4†	117±2†	115±2†	119±3	116±2†	106±3†	112±4†	17
ATM	42	12±1	14±1†	15±1†	15±1†	15±1†	15±1†	15±1\$	15±1†	15±1†	15±1†	13±1	15±2†	17
LV-dp/dt	42	2330±100	2720±100†	2650±100†	2660±100†	2640±80†	2530±90†	2420±90†	2360±100†	2260±90†	2270±90†	2200±90†	2480±150†	17
HR	42	112±3	127±4†	121±4	119±4	116±4	117±4†	125±4†	139±5†	149±4†	160±5†	137±6†	115±8	17
HR × BP × 10 ³	42	17±1	18±1†	18±1	17±1	17±1	17±1	18±1	19±1†	20±1†	21±1†	17±1	15±1	17

Abbreviations: AOS, AOD, AOM = aortic systolic, diastolic, and mean pressures (mm Hg); ATM = atrial mean pressure (mm Hg); dp/dt = maximal rate of rise of left ventricular intracavitary pressure (mm Hg/sec); HR = heart rate (beats/min); HR × BP = heart rate times systolic blood pressure product. N1 = number of observations at initiation of study; N2 = number of observations at 1 week.

* Indicates time after experimental coronary occlusion.

† Significantly different from control (Duncan's multiple range test, $P < 0.05$).

product, RMBF after occlusion was normalized to control conditions. This was done by adjusting each RMBF determination by the increment in normal posterior wall flow for each level for each dog. Segmental histological necrosis is expressed as a percent of the planimetric segment area. "Total" segmental necrosis refers to the weighted average of epicardial, midwall, and endocardial values.

Statistical Analysis

Comparisons over time and between classes were performed with a 2-way analysis of variance (Barr et al., 1976). Multiple comparisons within groups over time, or between groups during a time period, were performed with Duncan's multiple range test (Duncan, 1955). Correlations between regional LV function and regional myocardial blood flow were performed with a linear regression analysis (Barr et al., 1976). All values are expressed in terms of the mean ± SEM. The probability P was considered to be statistically significant if less than 0.05. Comparisons between pre-occlusion control for segmental function data were performed with a one-sample t -test, since the control value was a constant, i.e., 100%. To correct for multiple comparisons in such cases, P was considered to be statistically significant if less than 0.005 (Knoke, 1976).

Results

In each case, the occluding balloon was examined at the conclusion of the study and noted to be fully inflated. In 11 dogs, perfusion of the LAD with saline under 100 mm Hg pressure resulted in no distal flow. After removing the occluding balloon, flows ranged between 70 and 130 ml/min.

Hemodynamic Alterations after Proximal LAD Occlusion (Table 1)

The alterations that occurred in the seven measured hemodynamic variables as a consequence of proximal LAD occlusion are summarized in Table 1. Five minutes after LAD occlusion, left atrial mean pressure, heart rate, and the heart rate-systolic blood pressure product increased significantly. Peak LV dp/dt declined and remained depressed through 1 week, whereas mean left atrial pressure remained elevated except at 24 hours after LAD occlusion. Between 8 hours and 1 week after occlusion, aortic systolic, diastolic, and mean pressures declined below pre-occlusion levels.

LV Segmental Wall Function (Table 2)

Regional Systolic LV Wall Thickening

Segmental LV contractile function was assessed by the extent of systolic wall thickening. Three classes of segments were defined with either mild (class 1), moderate (class 2), or severe (class 3) loss of segmental function measured 5 minutes after LAD occlusion. Class 3 segments were those with

TABLE 2 Net Systolic Wall Thickening (NET) and End-Diastolic Wall Thickness (EDWTH) in Dogs through 1 Week after LAD Occlusion

Param	N1	5 min*	1 Hr*	2 Hr*	3 Hr*	4 Hr*	5 Hr*	6 Hr*	7 Hr*	8 Hr*	24 Hr*	1 Wk*	N2
NET 1	39	90.5±3.3	83.5±4.5†	79.4±4.5†	78.9±4.5†	79.4±4.6†	75.5±4.8†	76.8±4.7†	75.2±4.7†	74.6±5.9†	72.8±5.8†	85.1±10.1†	14
NET 2	66	35.3±2.2†	31.6±3.6†	31.0±3.9†	31.2±4.2†	27.4±4.4†	25.2±4.3†	23.9±5.2†	14.9±4.9†	19.7±4.9†	32.6±6.1†	41.1±9.3†	26
NET 3	82	-44.1±3.8†	-40.8±4.1†	-38.0±4.1†	-39.6±4.2†	-39.6±4.1†	-42.7±4.7†	-45.4±4.6†	-56.5±5.2†	-47.7±4.7†	-31.6±5.8†	-31.0±6.5†	26
EDWTH 1	39	97.4±0.7†	97.9±0.7	97.3±0.8	97.8±0.8†	97.3±0.9	98.6±0.8	99.7±0.9	100.7±1.0	100.2±0.9	100.1±1.9	101.6±6.3	14
EDWTH 2	66	97.0±0.9†	96.5±1.0†	96.4±1.0†	96.9±1.0†	97.5±1.1†	98.6±1.1†	100.4±1.2†	101.6±1.4†	101.0±1.2†	104.2±2.5†	109.3±5.7†	26
EDWTH 3	82	93.5±0.7†	92.3±0.7†	94.2±0.7†	95.2±1.2†	97.0±1.2†	97.8±1.1†	99.0±1.1†	100.6±1.2†	100.2±1.2†	100.6±2.2†	115.3±5.0†	26

Abbreviations: NET 1, NET 2, NET 3 = net systolic wall thickness (as percent control) in classes 1, 2, and 3. Classes are defined according to the loss of NET at 5 minutes after LAD occlusion. Class 1, NET $\geq 67\%$; class 2, $0 \leq$ NET $< 67\%$; class 3, $\leq 0\%$ (paradoxical). EDWTH 1, EDWTH 2, EDWTH 3 = end-diastolic wall thickness (as percent control) in classes 1, 2, and 3. N1 = number of crystal sites comprising each class at the initiation of the study, N2 = number of crystal sites at 1 week.

* Time after experimental coronary occlusion.

† Significantly different from pre-occlusion control (one-sample *t*-test, $P < 0.005$).

‡ Significantly different from 5-minute value (Duncan's multiple range test, $P < 0.05$).

predominantly paradoxical systolic wall thinning (NET wall thickening $< 0\%$ of control). Class 1 segmental function ($n = 39$) was $90.5 \pm 3.3\%$ (SEM) of control at 5 minutes after LAD occlusion; subsequently, a further significant, functional loss occurred between 2 and 24 hours, followed by a recovery between 24 hours and 1 week after LAD occlusion (Table 2). Segments within class 2 ($n = 66$) were depressed to $35.3 \pm 2.2\%$ of control at 5 minutes after occlusion, then demonstrated a further significant functional deterioration between 6 and 8 hours after LAD occlusion followed by a recovery to $41.1 \pm 9.3\%$ of control by 1 week. The degree of functional improvement between 8 hours ($19.7 \pm 4.9\%$) and 1 week and between 8 hours and 24 hours was statistically significant ($P < 0.05$) while that between 24 hours and 1 week was not. In a manner similar to LV segments in classes 1 and 2, class 3 segments ($n = 82$) deteriorated significantly at 7 and 8 hours after occlusion compared to values present at 5 minutes after occlusion (Table 2). A significant reduction in the extent of paradoxical systolic thinning occurred between 7 hours ($-56.5 \pm 5.2\%$) and 24 hours ($-31.6 \pm 5.8\%$) and also between 7 hours and 1 week ($-31.0 \pm 6.5\%$) after LAD occlusion. No significant changes occurred between 24 hours and 1 week.

LV Segmental Wall Thickness (EDWTH)

The alterations that occurred in EDWTH are summarized in Table 2. For class 1 segments, EDWTH fell significantly at 5 minutes, but no significant further change occurred. EDWTH of segments in class 2 fell below control levels for 3 hours, then exceeded the 5-minute value between 4 hours and 1 week after LAD occlusion. The increase in EDWTH between 24 hours ($104.2 \pm 2.5\%$) and 1 week ($109.3 \pm 5.7\%$) was statistically significant. Qualitatively similar changes in EDWTH occurred in segments with paradoxical systolic wall thinning (class 3)—an early loss of EDWTH persisted for 3 hours followed by a progressive increase through 1 week. At 1 week post-occlusion, EDWTH exceeded control levels ($P < 0.005$). Those segments with the greatest degree of functional impairment at 5 minutes after LAD occlusion subsequently demonstrated the greatest increase in EDWTH (Table 2).

Alterations in LV Regional Myocardial Blood Flow (RMBF) after Proximal LAD Occlusion

The mean weight of epicardial segments for all dogs was 0.88 ± 0.02 g, whereas that of midwall samples was 0.74 ± 0.02 g and that of endocardial samples was 0.73 ± 0.02 g.

RMBF Alterations after LAD Occlusion (Table 3)

Changes in absolute RMBF (in ml/g per min) are summarized in Table 3. Segments are classified by the degree of functional loss recorded 5 minutes after LAD occlusion (vide supra). RMBF in the

TABLE 3 Regional Myocardial Blood Flow (ml/g per min) and Distribution (ENDO/EPI) in Dogs Surviving 24 Hours or 1 Week after LAD Occlusion

		N1	Control	5 Min*	8 Hr*	24 Hr*	1 Wk	N2
Class 1	EPI	28	1.26 ± 0.11	1.26 ± 0.13	1.23 ± 0.11	1.20 ± 0.13	1.05 ± 0.09	9
	MID	28	1.45 ± 0.13	1.43 ± 0.13	1.33 ± 0.11	1.32 ± 0.11	1.12 ± 0.15	9
	ENDO	28	1.32 ± 0.13	1.23 ± 0.14	1.23 ± 0.11	1.16 ± 0.10	0.97 ± 0.14	9
ENDO/EPI		28	1.06 ± 0.05	1.02 ± 0.07	1.03 ± 0.07	1.10 ± 0.11	0.86 ± 0.07	9
Class 2	EPI	58	1.12 ± 0.08	1.01 ± 0.09	1.05 ± 0.08	1.09 ± 0.09	0.84 ± 0.07	21
	MID	58	1.25 ± 0.08	0.98 ± 0.09	1.02 ± 0.08	1.13 ± 0.11	0.78 ± 0.08†	21
	ENDO	58	1.19 ± 0.08	0.86 ± 0.09†	0.83 ± 0.08†	0.91 ± 0.11†	0.75 ± 0.09†	21
ENDO/EPI		58	1.09 ± 0.04	0.86 ± 0.04†	0.81 ± 0.05†	0.81 ± 0.05†	0.87 ± 0.08†	21
Class 3	EPI	61	1.02 ± 0.07	0.49 ± 0.05†	0.71 ± 0.07†	0.87 ± 0.08	0.65 ± 0.08†	22
	MID	61	1.11 ± 0.07	0.43 ± 0.05†	0.50 ± 0.06†	0.73 ± 0.08†	0.46 ± 0.08†	22
	ENDO	61	1.04 ± 0.07	0.30 ± 0.05†	0.37 ± 0.06†	0.54 ± 0.08†	0.42 ± 0.07†	22
ENDO/EPI		61	1.04 ± 0.05	0.54 ± 0.04†	0.49 ± 0.05†	0.55 ± 0.06†	0.60 ± 0.07†	22
Infarct Center	EPI	25	0.95 ± 0.09	0.30 ± 0.08†	0.32 ± 0.08†	0.45 ± 0.09†	0.41 ± 0.12†	8
	MID	25	0.95 ± 0.08	0.19 ± 0.06†	0.16 ± 0.05†	0.25 ± 0.06†	0.21 ± 0.08†	8
	ENDO	25	0.85 ± 0.09	0.13 ± 0.06†	0.16 ± 0.06†	0.26 ± 0.10†	0.18 ± 0.07†	8
ENDO/EPI		25	0.89 ± 0.12	0.43 ± 0.08†	0.48 ± 0.15†	0.48 ± 0.15†	0.45 ± 0.07†	8

N1 = number of segments in each group at the initiation of the study; N2 = number of segments in each group at 1 week (18 of 34 dogs were killed at 24 hours). Abbreviations: EPI = epicardium, MID = midwall, ENDO = endocardium.

* Time after experimental coronary occlusion.

† Significantly different from control (Duncan's multiple range test, $P < 0.05$).

grossly determined infarct center ("infarct center") is included. Segments within class 1 and normal myocardium demonstrated no significant changes after LAD occlusion in either RMBF or blood flow distribution (as evaluated by the endocardial:epicardial flow ratio). In contrast, RMBF to the endocardial layer of class 2 segments was significantly reduced from 1.19 ± 0.08 ml/g per min in the control period to 0.86 ± 0.09 ml/g per min 5 minutes after occlusion (Table 3). A significant reduction in endocardial flow persisted through 1 week. This reduction was reflected in a concomitant decrease in transmural flow distribution, reflected by a reduction in the endocardial:epicardial flow ratio. No significant reduction in RMBF occurred in epicardial or midwall levels in these segments with a moderate degree of ischemic dysfunction. RMBF in segments with paradoxical systolic motion (class 3) was depressed transmurally 5 minutes after LAD occlusion (Table 3). Subsequent collateral flow development resulted in an increase in epicardial RMBF to near pre-occlusion control levels by 24 hours, whereas midwall endocardial and endo/epi flow remained depressed (endo/epi = 0.54 ± 0.08). A sequence of RMBF alterations similar to those of class 3 segments occurred in the infarct center; however, the severity of ischemia was greater (Table 3) and a significant increase in collateral flow did not occur in the epicardial layer.

Relationships between LV Regional Function and Regional Myocardial Blood Flow (Table 4)

Regional LV function was significantly correlated with RMBF for endocardial, midwall, and epicardial layers at each time period. The relationship

was found to be non-linear (Fig. 1). Regional LV function correlated at 5 minutes most strongly with endocardial flow ($r = 0.69$, $n = 143$) and less closely with epicardial flow ($r = 0.61$, $n = 143$). LV midwall and endocardial flows were consistently most strongly correlated with regional function through 1 week. As time after LAD occlusion increased, RMBF increased and the strength of the correlation between regional function and RMBF fell in the endocardium from 0.69 at 5 minutes to 0.60 at 24 hours, in the midwall from 0.69 to 0.59, and in the epicardium from 0.61 to 0.41. The fall in correlation coefficients expresses the discrepancy between increases in RMBF (Table 3) and the absence of correspondingly important increases in LV segmental function (Table 2). Since increases in epicardial RMBF occurred most rapidly, correlation coefficients between regional LV function and epicardial LV flow fell to a greater extent than those in mid-

TABLE 4 Histological Necrosis Expressed as Percent Segment Area

		Seg Nx	Total Nx
Class 1 $n = 24$	END	3.3 ± 0.9	
	MID	1.5 ± 0.7	3.7 ± 0.8
	EPI	5.6 ± 1.3	
Class 2 $n = 45$	END	27.2 ± 5.0	
	MID	18.8 ± 4.0	20.4 ± 3.9
	EPI	14.6 ± 3.2	
Class 3 $n = 53$	END	59.6 ± 5.0	
	MID	54.2 ± 4.9	51.2 ± 4.5
	EPI	42.4 ± 4.3	
Infarct center $n = 22$	END	93.3 ± 3.1	
	MID	87.7 ± 4.1	81.9 ± 4.3
	EPI	69.4 ± 6.7	

Abbreviations: Seg Nx = "segmental" myocardial necrosis; Total Nx = "total" segmental necrosis computed as a weighted average of endocardial, midwall, and epicardial values.

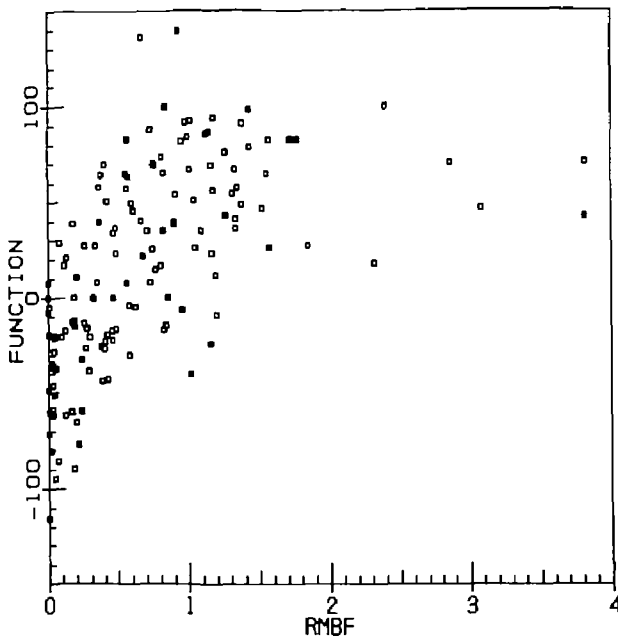


FIGURE 1 Regional left ventricular segmental function ("FUNCTION") is plotted against endocardial left ventricular myocardial blood flow ("RMBF" in ml/g per min) at 5 minutes post-occlusion. The relationship is nonlinear with a correlation coefficient of 0.69 ($n = 143$).

wall or in endocardial regions. At 1 week, flow-function correlations improved despite a reduction in the number of segments; thus, $r = 0.61, 0.66, 0.51$ for endocardial, midwall, and epicardial layers ($n = 142$).

Regional function of class 1 segments did not demonstrate a significant change between 8 hours ($\text{NET} = 74.6 \pm 5.9\%$) and 24 hours ($\text{NET} = 72.8 \pm 5.8\%$) after LAD occlusion. Class 2 segments did demonstrate an improvement in function during this time period (from $19.7 \pm 4.9\%$ to $32.6 \pm 6.7\%$). Class 3 segments demonstrated a significant reduction in the extent of paradoxical systolic thinning between 7 and 24 hours. The extent of functional recovery in segments with a moderate degree of functional recovery was significantly, although weakly, associated with a corresponding increase in RMBF ($P = 0.02$). No such relationship could be demonstrated for the most ischemic group 3 crystals ($P = 0.26$), suggesting that the observed decrease in the extent of paradoxical systolic wall thinning was a result of decreased segmental compliance.

Relationships between Segmental Necrosis RMBF and Regional Function (Fig. 2)

Degrees of histological necrosis in endocardial, midwall, and epicardial layers are summarized in Table 4. Endocardial necrosis was significantly more severe than that in midwall or epicardial layers for each functional class of segments. The weighted average of the three layers ("total necrosis") was greatest in the infarct center ($81.9 \pm 4.3\%$), next in functional class 3 segments ($51.2 \pm 4.5\%$),

next in areas of moderate functional loss ($20.4 \pm 3.9\%$), and least in segments with mild functional loss ($3.7 \pm 0.8\%$). Regional function was significantly correlated with histological necrosis at each time period function was measured (Fig. 2); the correlation coefficient ranged from -0.59 at 5 minutes after LAD occlusion to -0.78 at 24 hours after occlusion ($P < 0.001$ in both cases).

The extent of histological necrosis was also significantly correlated with RMBF for endocardial, midwall, and epicardial levels at each time period RMBF was measured after LAD occlusion. Correlations generally were higher in endocardial and midwall layers than epicardial layers. The correlation coefficient for endocardial flow at 5 minutes after occlusion vs. histological necrosis was -0.61 , whereas that for epicardium was -0.56 ($P < 0.0001$ in both cases). At 1 week, these relationships were -0.70 and -0.54 , respectively.

Apparent Microsphere Loss or Altered Microsphere Distribution

RMBF to the non-ischemic left ventricle is known to be relatively homogeneous; therefore one would expect control RMBF to be approximately equal in each class of tissue examined. However, a statistical analysis of the data presented in Table 3 discloses that significant differences exist in control flows, such that areas with the greatest ischemia have the lowest control RMBF values. The magnitude of differences in RMBF in the pre-occlusion period can be estimated by evaluating RMBF as percent LV posterior wall flow. Reductions in control RMBF, presumed to be artifactual, ranged from

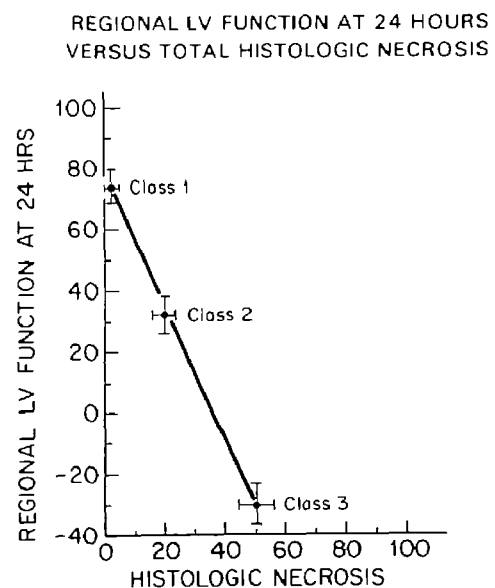


FIGURE 2 Regional left ventricular segmental function at 24 hours post-occlusion is plotted against the extent of total segmental histological necrosis determined by planimetry. The relationship is linear with a correlation coefficient of -0.78 .

25% in class 3 endocardial samples to 17% for class 1 segments.

Discussion

Regional LV myocardial ischemia and the resultant functional alterations and myocellular necrosis have been studied extensively. However, the interrelationships among these variables in the awake, unsedated dog hours to days after coronary occlusion have not been thoroughly evaluated. This study was designed to define these dynamic interrelationships. To avoid the disturbing effects of anesthesia and thoracotomy, we studied dogs in an awake, unsedated state. A sudden proximal occlusion of the LAD was produced and sequential measurements made of regional function with chronically implanted ultrasonic piezoelectric crystals (Roan et al., 1979) and RMBF with carbonized radioactive microspheres.

There are several important new findings in this study. First, we found that in the awake, unsedated dog, regional left ventricular function expressed as NET segmental systolic thickening is more sensitive to decreases in endocardial than epicardial flow. An examination of Table 3 thus discloses that, 5 minutes after LAD occlusion, regional function in class 2 segments is reduced to $35.3 \pm 2.2\%$ of control. RMBF, measured simultaneously, was reduced below control levels only in the endocardial layer where it fell from 1.19 ± 0.08 to 0.86 ± 0.09 ml/g per min. A marked segmental loss of function thus occurred in the absence of midwall or epicardial flow alterations. In those segments with paradoxical systolic thinning, on the other hand, ischemia occurred *transmurally*, although it was most severe in the endocardial layer. Reports from other investigators (Gallagher et al., 1980) are consistent with our findings. Vatner et al. (1979) measured only endocardial flow during gradual coronary occlusion and noted, as did we, a non-linear relationship between reductions in RMBF and corresponding functional loss. Relatively trivial (7%) reductions in RMBF produced significant reductions in regional function; however, a 94% reduction in the flow was required to produce a complete loss of active segmental shortening, whereas in our study a 71% decrease in flow occurred in class 3 segments 5 minutes after LAD occlusion. Vatner et al. (1979) measured endocardial segment length whereas we have measured regional systolic wall thickness, a difference which may account for the disparity in results.

Earlier work by other investigators using open-chest anesthetized dog or pig preparations to study the acute regional functional consequences of ischemia has yielded results similar to ours. In one study, the contractile force of deep and superficial myocardial fibers was examined in open-chest dogs. Contractile force fell in a linear fashion as coronary flow was reduced below levels determined by autoregulation (Downey, 1976). Contractile force devel-

oped by deep fibers may be lost before that of superficial fibers as coronary flow is reduced (Gallagher et al., 1978). For this reason, studies examining epicardial regional function in open-chest dogs during gradual coronary occlusion have demonstrated no loss of function with an approximate 25% reduction in coronary flow. In these studies, a 50% reduction in flow was required to demonstrate ischemic dysfunction and an increase in segment length (Banka et al., 1977). In the open-chest pig, a reduction in LAD flow produced a proportionate decrease in LV segmental shortening ($r = 0.77$) and systolic thickening ($r = 0.78$). Total calculated LV segmental work also correlated well with reductions in LAD flow ($r = 0.82$) (Stowe et al., 1978). These authors also noted that a moderate degree of hypoperfusion (50–75% reduction in LAD flow) caused a 70% greater reduction in the capacity of the segment to thicken than to shorten. Therefore, the measurement of regional performance by means of changes in regional LV wall thickness appears to be quite sensitive to reductions in coronary flow.

In addition to the characterization of the acute functional effects of ischemia, we have demonstrated that increases in regional myocardial blood flow after proximal occlusion of the LAD may have some effect on recovery of function. Thus, in class 2 segments, a significant correlation existed between changes in RMBF between 8 hours and 24 hours and corresponding changes in regional function ($P = 0.02$). In class 3 segments, on the other hand, the extent of reduction in paradoxical systolic wall thinning was not correlated with the corresponding RMBF increases ($P = 0.26$). This dissociation between RMBF and regional function in these paradoxical segments probably reflects the increased development of edema and hemorrhage in segments with the greatest degree of re-collateralization with a consequent loss of compliance and a decrease in paradoxical motion.

The pattern of changes in regional myocardial blood flow following coronary occlusion observed in the present study is consistent with those observed by other investigators. Endocardial flow reductions were more severe than those occurring in the epicardium (Becker et al., 1973). Collateral flow increased rapidly to ischemic myocardium, with close to a 2-fold augmentation by 24 hours after LAD occlusion. The greatest increases in collateral flow occurred in the epicardial layer. A rapid re-establishment of blood flow via collaterals has been observed by others between 20 seconds and 60 minutes after experimental coronary artery occlusion (Rivas et al., 1976). Preferential increases of collateral flow to the epicardium are a result of the epicardial location of canine myocardial collateral channels and of the decreased levels of transmural wall stress, forces which exert a compressive force on the coronary vasculature. Man has numerous smaller anastomoses throughout the left ventricular wall that are denser near the epicardial and endocardial sur-

faces. Some observed increases in collateral flow may represent a re-distribution of flow away from necrotic subendocardial layers to surviving epicardial ones (Hirzel et al., 1976). However, in the present study, because admixtures of normal and necrotic myocardium cannot be separated completely, it is impossible to assess whether the observed increases in flow are directed to viable or non-viable myocardium.

In the present study, we also determined the extent of myocellular necrosis in endocardial, mid-wall, and epicardial levels. The data demonstrate that regional function correlates with the corresponding extent of segmental necrosis (Fig. 2) and, in turn, that necrosis is correlated with regional myocardial blood flow. The marked loss of function without severe necrosis has been reported by us previously (Roan et al., 1979). The converse, however, is not true: severe degrees of segmental necrosis are invariably associated with nearly complete functional loss. These conclusions are supported by histological and angiographic data from humans. For example, absent or reduced wall motion was present in 63% of segments with a normal histological appearance (Hutchins et al., 1977), confirming and extending the findings of an earlier study (Baltaxe et al., 1974). In the present study, segmental LV necrosis was proportional to reductions in flow beyond 70–75% of normal posterior wall flow. Other investigators have established a linear correlation between necrosis and regional myocardial blood flow measured at 15 minutes ($r = 0.83$) and 2 hours after coronary occlusion (Irvin and Cobb, 1977).

We also found that endocardial necrosis was consistently more severe than midwall or epicardial necrosis. The increased susceptibility of the endocardium is attributable to both increased severity of ischemia and increased myocardial oxygen demand in the endocardium evidenced by increased lactate production (Griggs, 1979) and myocardial oxygen consumption (Weiss et al., 1978). Other investigators have noted increased endocardial necrosis in conscious (Rivas et al., 1976) and open-chest dogs (Reimer and Jennings, 1979).

The accuracy of regional blood flow determinations utilizing radioactive microspheres depends upon permanent trapping within the target organ, upon the absence of leaching of isotope and, since flows are expressed as volume per unit weight of myocardium, upon the maintenance of a constant tissue mass. Recent reports (Capurro et al., 1979) have noted discrepancies in pre-occlusion RMBF similar to those described in the present study. Apparent "loss" of microspheres may represent a decrease in microsphere density which may occur as a result of either actual migration of microspheres out of the target organ (Jugdutt et al., 1979) or an increase in ischemic tissue mass as a result of hemorrhage, edema, and inflammatory cellular infiltration (Reimer and Jennings, 1979). In our study, the significant increases in LV end-diastolic wall

thickness (Table 2) suggest that an augmentation in ischemic tissue mass after experimental coronary occlusion may play a role in the alteration of microsphere density. Other investigators (Jugdutt et al., 1979) suggest that approximately 40% of altered microsphere density is a result of increased tissue mass, while the remaining 60% is a consequence of actual microsphere loss. Leaching of isotope from microspheres over a 1-week period is trivial.

The artifactual alterations in microsphere density observed in this study have the effect of overestimating the magnitude of subsequent collateral flow changes. However, despite such overestimates, the observed increases in collateral flow were related largely to corresponding increases in LV regional function only in segments with a moderate degree of functional loss (class 2). Further, in many ischemic segments, flow reductions were so severe that a 25–30% underestimation of regional flow would have little physiological importance. Therefore, although the quantitative relationships between regional function and flow have been altered to a variable degree by alterations in microsphere density, the qualitative impact of our findings is unchanged. This conclusion is supported by the similarity of flow-function relationships measured just prior to sacrifice of the animals at 24 hours or 1 week to earlier determinations. Such flow-function relationships, obtained just prior to sacrifice of the animal, are unaffected by changes in microsphere density.

In summary, we have demonstrated in the present study that, after experimental coronary occlusion in the awake, unsedated dog, segmental LV systolic wall thickening is sensitive to small changes in endocardial flow 5 minutes after proximal LAD occlusion. Subsequent increases in collateral flow at 8 or 24 hours have little effect on LV regional function. Myocellular necrosis is most severe in the endocardium and directly proportional to the severity of ischemia as RMBF is reduced below 70–75% of flow to normally perfused areas of myocardium. LV regional function may be markedly depressed in the absence of advanced degrees of myocellular necrosis.

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