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The Influence of Cardiac Sympathetics and Periods of Ischemic Insult upon Myocardial Blood Flow, Incidence of Arrhythmia, Electrical Activity and Myocardial Infarction During Coronary Occlusion

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THE INFLUENCE OF CARDIAC SYMPATHETICS AND PERIODS OF ISCHEMIC INSULT UPON MYOCARDIAL BLOOD FLOW, INCIDENCE OF ARRHYTHMIA, ELECTRICAL ACTIVITY AND MYOCARDIAL INFARCTION DURING CORONARY OCCLUSION

by

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BIOGRAPHY

Michael J. Barber, the son of Mr. and Mrs. Joseph W. Barber, was born on February 3, 1954, in Gary, Indiana. He attended elementary school in the Hobart, Indiana, public school system, and completed his secondary education in the Richmond Community school system. He graduated in 1972 from Richmond Senior High School, Richmond, Indiana.

In the fall of 1972, he entered Wabash College in Crawfordsville, Indiana. He graduated cum laude from Wabash in May of 1976 with a Bachelor of Arts degree in Biology.

The author began graduate studies at Loyola University of Chicago in July of 1976. He was a member of the Department of Physiology at the medical center campus, and completed his dissertation work under the direction of Dr. Walter C. Randall. During his graduate training the author was supported in part by Basic Science Fellowships, a Graduate Assistantship and a Schmitt Doctoral Fellowship from Loyola University.

The author is a member of Sigma Xi, and a student member of the American Physiological Society. The author has accepted a medical school appointment and will pursue an M.D. degree at Indiana University School of Medicine, Indianapolis, Indiana. He will continue research investigation in collaboration with Dr. Douglas P. Zipes at the Krannert Institute of Cardiology, Indiana University School of Medicine.

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CHAPTER I

INTRODUCTION

Previous studies have both supported and refuted the concept that chronically denervated dog hearts tolerate incidents of regional myocardial ischemia more effectively than do hearts with intact neural connections or hearts neurally decentralized immediately prior to occlusion. Reduction in infarct size and reduced incidence of arrhythmia following coronary artery occlusion in chronically denervated hearts has been reported, but contradictory evidence exists. The "protective effect" of chronic cardiac denervation was examined in the first section of this study utilizing the intrapericardial cardic denervation technique developed in this laboratory. Changes in regional myocardial blood flow and indices of myocardial injury (S-T segment elevation, arrhythmia) were examined in denervated and non-denervated dog hearts during periods of mid-left anterior descending coronary artery (LAD) occlusion. This was done to 1) determine if chronic cardiac denervation altered myocardial blood flow to the ventricles before and during LAD occlusion, 2) examine the concept that chronic cardiac denervation lessened local (endocardial and epicardial) injury as measured by regional S-T segment changes, and 3) determine if intrapericardial cardiac denervation lessened the incidence of arrhythmia during LAD occlusion.

Occlusion of a coronary artery for periods greater than 15-20 minutes causes irreversible damage to myocardial tissue in regions of

severe blood flow reduction. Permanent ligation of a coronary artery results in the development of myocardial necrosis in regions where myocardial blood flow (supply) is incapable of delivering adequate substrate quantities to meet cellular requirements (demand). Manipulations which augment the demand component of the supply:demand relationship increase the amount of cellular necrosis observed following coronary artery occlusion, while factors which decrease the demand component lessen ischemic injury. Reports of lowered metabolic demand in chronically denervated dog hearts precipitated the study of the minimal perfusion level (critical blood flow) necessary to sustain myocardial viability during coronary occlusion. The purpose of the second portion of this study was to 1) determine if a critical blood flow value could be identified and 2) ascertain if chronic cardiac denervation or augmented sympathetic activity altered the critical blood flow.

Recent evidence has shown coronary arterial vasospasm in the absence of severe vessel obstruction. While current (clinical) interest in vasospastic occlusion is high, there is relatively little information available on the effects of repetitive incidents of ischemia on the heart. Studies in this portion of the dissertation were designed to examine the effects of a previous ischemic insult upon the region of severe ischemia. The first model examined the effect of a short period of LAD occlusion followed by reperfusion on the subsequent infarct caused when a second permanent occlusion occurred. Analysis of infarct size and critical blood flow was performed to 1) determine if previous incidents of ischemia enhanced blood flow to the central ischemic zone, 2) determine whether the infarction process following LAD occlusion was

altered by the previous occlusion, and 3) ascertain whether the presence of low levels of stellate ganglion stimulation altered the damage. The second model of repeated occlusion utilized a series of four sequential LAD occlusions separated by short (3 min) or long (40-60 min) periods of reperfusion. Changes in blood flow, arrhythmia and electrical activity were examined in the severely ischemic zone to 1) determine if reperfusion (and time of reperfusion) altered myocardial blood flow distribution to the ischemic region, 2) determine if electrical abnormalities during successive occlusions were modified by varying the period of undisturbed reperfusion, and 3) determine if utilization of repeated occlusion models for study and quantitation of changes in arrhythmia were limited by a shifting "arrhythmia baseline".

CHAPTER II

LITERATURE REVIEW

A. The Supply:Demand Relationship of the Heart and Coronary Occlusion

Except under certain abnormal physiological conditions, myocardial blood flow, and the delivery of essential metabolic substrates to the myocardium remain closely coupled to myocardial metabolic demand (10, 12, 106, 160). The possibility of a close relationship between tissue metabolic activity (demand) and actual tissue blood flow delivery (supply) was first recognized in a series of elaborate experiments by Roy and Brown in 1879 (159). Visualizing the capillary circulation in the web of the hindlimb of the frog through a thin membrane made of calf's peritoneum, Roy and Brown were able to examine qualitative changes in capillary diameter and blood flow. They postulated that, following periods of temporary ischemia, the rapid blood flow seen in the ischemic tissue was due to an increase in flow necessary to re-establish normal tissue nutritive balance. This hyperemia was independent of neural control and related to tissue metabolic and nutritive demands.

These basic principles were extended to the heart primarily through studies by Shipley and Gregg (175) and Eckenhoff *et al.* (50). Eckenhoff *et al.* (175) examined left ventricular oxygen consumption as an index of cardiac metabolic activity. They attempted to correlate this parameter with other cardiac measurements during various types of physiological and artificial stresses. Utilizing a bubble flowmeter to

measure coronary blood flow in the LAD, they demonstrated a good correlation (r = .85) between left ventricular oxygen consumption and LAD blood flow. They also showed that during conditions where cardiac activity was altered, coronary blood flow was adjusted to meet and maintain increased or decreased demand of the heart for oxygen. Eckenhoff et al. examined several other parameters such as left ventricular work, coronary resistance, and cardiac output, and compared these to left ventricular oxygen consumption. They reported poor (r .50) but significant correlations of these parameters with oxygen consumption (50).

The results of Eckenhoff et al. (50) supported earlier work by Shipley and Gregg (175). Shipley and Gregg had examined relationships similar to those reported by Eckenhoff et al. (50) except during sympathetic nerve stimulation. Utilizing left or right stellate ganglion stimulation, Shipley and Gregg demonstrated an increase in left ventricular cardiac metabolism which was reflected by an increase in oxygen utilization and coronary blood flow. This increase in coronary blood flow and myocardial metabolism was in the absence of any reported change in aortic pressure or heart rate, and was attributed to an increased vigor of contraction during sympathetic stimulation. Unfortunately the authors did not monitor or quantitatively evaluate contractility directly, but only "visualized" the increase in this parameter. Also lacking in this report were the stimulus parameters utilized, making it impossible to evaluate the level of sympathetic nerve stimulation in this study.

Since those early studies (50, 175), the concept of a balanced supply:demand relationship in the normal heart has become firmly established (12, 22, 106, 160). Indeed, in subsequent studies (45, 164,

204) it appeared that the heart operated not only at a balanced supply:demand ratio, but at the minimal myocardial blood flow level necessary to maintain normal cardiac function. In a study by Downey (45), it was shown that small decreases in coronary blood flow significantly compromised left ventricular function as measured by isometric force gauges. When coronary blood flow was artifically increased above the control, autoperfused, blood flow level, myocardial function improved only slightly. Downey concluded that the match between blood flow requirements of the left ventricle and blood flow supply to the left ventricle was optimized, and regulatory mechanisms of the heart kept myocardial perfusion at levels which maintained contractile function at the "knee" of the contractile force-coronary blood flow curve (45). These data supported earlier reports by Weisberg et al. (204) and Sarnoff et al. (165).

The very delicate supply:demand relationship in the heart may become severely unbalanced in certain pathological instances, such as in the presence of compromised or interrupted blood flow. The ligation of a coronary vessel would result in such supply:demand imbalance, and limit blood supply to the tissue within the distribution of the affected artery. Compromised myocardial function and possible cellular death in the region of interrupted flow would be seen.

Experimental studies examining the effect of coronary artery occlusion on the function of the left ventricle have been performed. Orias (139) demonstrated that immediately following occlusion of the LAD, hypodynamic ventricular contractions were observed. These contractions were characterized by decreased systolic ventricular pressure development,

reduced cardiac output and decreased systemic (aortic) pressure. An initial decrease in diastolic pressure was observed, but with time an increase in diastolic left ventricular pressure and size were recorded. In addition to this increased left ventricular diastolic size, an increase in cardiac output, ventricular, and aortic systolic pressures was seen. Orias postulated a compensating function of the normal muscle unaffected by the occlusion. This was probably occurring through an increase in ventricular diastolic size and a rise in the initial diastolic tension of the normally perfused muscle (Frank-Starling mechanism).

Tennant and Wiggers (189), utilizing an optical kymograph developed by Otto Frank, repeated Orias' study (139) while recording changes in localized contractile function. Their tracings showed a rapid and progressive loss of localized contractile function within the zone of ischemia following LAD ligation. Less than one minute after occlusion, the ischemic zone of the left ventricle demonstrated asynergy and paradoxical motion. The myogram recording became completely inverted from the control, pre-occlusion tracing. Re-establishment of blood flow to the ischemic zone resulted in a rapid reversal of the myographic changes and a complete restoration of function provided the duration of occlusion was less than 23 minutes. Periods of LAD occlusion greater than 23 minutes resulted in incomplete recovery of ventricular function following reperfusion. Tennant and Wiggers (189) also demonstrated that the loss of function in the ischemic myocardium was not due to the inability of the muscle to be excited. Failure of the muscle to shorten was due to an enfeeblement of contraction (probably through insufficient oxygen supply), not to loss of excitability. Similar results concerning

the function (145, 188, 189, 190) and excitability (14, 145) of ischemic ventricular muscle have been reported.

The rapid loss of myocardial function has been attributed to an inadequate supply of oxygen and other essential substrates to the affected muscle, with subsequent alteration in the myocardial metabolic processes requiring a continuing nutrient supply (26, 101, 138). Unlike muscles which contract only intermittently, the heart has been shown to require a constant supply of high energy-phosphate compounds that must be delivered continuously to the contractile machinery (16). This high level of energy production and oxygen consumption required to sustain normal myocardial function was maintained by efficient pathways of oxidative metabolism, but the "price" of this efficiency was dependence upon the continuous delivery of oxygen. Interruption of coronary blood flow (myocardial oxygen supply) by occlusion of a coronary artery alters energy metabolism in the affected myocardium within a short period of time (16). Opie (138) demonstrated that the metabolic response of the myocardium in regions of reduced blood flow depended upon the amount of (collateral) blood flow reaching those oxygen limited tissues. Even in regions where blood flow was less than 10% of control, the pattern of metabolism, as defined by Opie, was predominantly oxidative. Over 90% of the ATP produced in these zones during ischemia was oxidatively produced, but the ATP production was only about 20% of that seen prior to ischemia (138).

The direct link between myocardial functional decay and high energy phosphate depletion in the ischemic region has not been found. Ventricular contractile force (139, 188, 189) and muscle shortening

(140, 190) decrease within approximately 10 seconds after coronary artery ligation while ATP and CP levels remain high (69). One possible explanation for this discrepancy would be that ATP directly utilized in maintaining myocardial function was regionally compartmentalized (69). Decreased oxygen delivery and reduced ATP production could rapidly deplete this readily available pool of "contractile" ATP while leaving other stores relatively unchanged. Although this has not been proven, it is an interesting and attractive theory.

Another important metabolic factor potentially linked to the rapid decay in contractile force following ischemia has been accumulation of intracellular hydrogen ions (102, 211) and a resultant intracellular acidosis. In 1969, Katz and Hecht (102) suggested a schema which involved a displacement of calcium ions binding to troponin during contraction. They postulated that hydrogen ion might decrease the number of effective calcium-troponin interactions, and therefore result in an impaired contractility and subsequent systolic bulging of the ischemic ventricle. Although, intracellular hydrogen ions accumulate very rapidly following coronary artery occlusion (92), it is still not known whether these hydrogen ions inhibit calcium-troponin interactions directly (102), or in some way alter calcium ion entry into the cell during the action potential, or modify some other phase of the cardiac calcium cycle (92, 195, 211).

Myocardial oxygen consumption (demand) may be altered by a number of physical and neurohumoral events (12, 19). Under normal circumstances, an increase in metabolic demand would be adequately compensated by an increased myocardial blood flow and substrate delivery through coronary

vasodilation. During coronary occlusion, an increased metabolic demand would aggravate and intensify the ischemic process (92, 117, 121). Changes in heart rate (19, 38, 134, 208), contractile state (19, 51, 117, 121), neural input (182, 200), and circulating catecholamine levels (137, 149) all have been shown to affect myocardial metabolic demand and influence compromised tissue either directly or indirectly.

B. Changes in Myocardial Blood Flow, S-T Segment and Electrical Activation as Indicators of Myocardial Injury

As discussed above, when the supply of oxygen and nutrients to the myocardium was interrupted, myocardial cellular function and vital cellular processes were compromized. If, as Tennant and Wiggers (189) demonstrated, coronary blood flow was re-established within a short time, myocardial function did not appear to be permanently impaired. On the other hand, when these investigators maintained LAD occlusion longer than 23 minutes, evidence for permanent functional alteration was seen (189). Therefore, ischemia produced during coronary occlusion appeared to consist of three distinct phases: 1) the initial ischemic event, 2) the completely reversible phase of ischemia in which, if reperfusion were performed, myocardial function returned, and 3) the irreversible phase of ischemia. In this final phase, actual cellular necrosis was believed to be occurring and reperfusion was unsuccessful in salvaging previously ischemic cells.

Subsequent meticulous studies by Jennings and co-workers (89, 90, 91, 93) have shown that this time sequence first described by Tennant and Wiggers (189) not only applied to cellular function, but also appeared to be applicable at the histologic level of cellular survival.

Ischemic, non-contracting cells were not dead, but only severely impaired following coronary artery ligation. Restoration of arterial flow after periods of ischemia up to 20 minutes resulted in full recovery of function (14, 189) and return of normal electrocardiographic parameters (93), together with absence of histologic evidence of injury (89, 93). Prolongation of the ischemic insult for periods greater than 20 minutes resulted in isolated patches of dead myocardium which were not restored to a viable condition even following reperfusion. Ischemic insults of increasing duration were shown to cause further irreversible cellular damage beginning with the subendocardial region of the myocardium and extending gradually to the epicardium (155, 156).

A number of criteria have been utilized to evaluate the severity of myocardial ischemia and/or subsequent necrosis following the occlusion of a coronary artery. Among the many techniques described, changes in myocardial blood flow (13, 98, 155, 157), depletion of myocardial enzyme activity (33, 132, 174, 176), alteration in S-T and T-Q segments of myocardial electrograms (4, 107, 121), and abnormalities of electrical activation (47, 161, 210) were used in this dissertation to evaluate the level of myocardial injury and insult.

Even with the tremendous amount of effort expended analyzing the supply:demand relationship of the heart, relatively little information exists describing the lower limits of myocardial blood flow, below which myocardial infarction occurs following abrupt occlusion of a coronary artery. From previous evidence that the functional (14, 189), structural (89, 90, 91) and metabolic (16, 26, 137) integrity of the myocardium are dependent upon adequate myocardial blood flow, it should follow that

myocardial blood flow level is a major determinant of whether the tissue ultimately survives or infarcts. In other words, a critical level of myocardial blood flow is necessary to maintain cellular integrity.

In 1975, Jennings et al. (91), using radiolabeled microspheres, defined ischemia on the basis of direct measurements of local flow as ranging from mild ischemia to severe ischemia. These authors described tissue as severely ischemic if myocardial blood flow was reduced to a value 10-15% of control myocardial blood flow levels. Moderately ischemic tissue was described as myocardium receiving 15-30% of the control flow, and mildly ischemic tissue was defined to have myocardial blood flow levels 30-50% of control. These flow ranges of ischemia were loosely associated with the extent of necrosis in the posterior papillary muscle of the canine heart (91).

Bishop et al. (13), in a study on conscious dogs, described four zones of histopathology following coronary artery occlusion. These zones were termed the normal, marginal, ischemic, and core (or central infarct) regions. Correlation of regional myocardial blood flow, as measured with radiolabeled microspheres, with each of these areas proved to be quite successful, as core regions demonstrated very low myocardial blood flow levels (2-11 ml/min/100 g) while myocardial blood flow in ischemic (12-38 ml/min/100 g) and marginal (40-50 ml/min/100 g) regions was higher. However, Bishop et al. (13) made no attempt to predict actual flow ranges or minimum flow values at which the myocardium developed infarction. Also, heart rate and hemodynamic parameters were not carefully observed or controlled in their animals, making animal to animal comparisons difficult.

In a report immediately following Bishop et al. (13), Rivas et al. (157) examined the actual relationship between myocardial blood flow and extent of myocardial infarction following circumflex coronary artery occlusion in the conscious dog. They reasoned, that if the energy requirements of the ischemic cell outstripped the energy production capability of the cell, cellular death should occur. Therefore, they attempted to determine the actual supply:demand relationship of the heart. Using radiolabeled microspheres (7-10 microns), Rivas et al. (157) found that the extent of infarction in any given tissue sample from the distribution of the circumflex artery was inversely related to the measured myocardial blood flow. When samples in the same blood flow range, but from different transmural myocardial layers, i.e. endocardial vs epicardial, were compared, the amount of infarction in the endocardial samples exceeded that in the epicardial samples. A definite relationship existed between the level of myocardial blood flow and the extent of subsequent myocardial infarction, but this relationship varied in different transmural layers (157).

Both Bishop (13) and Rivas (157) reported that with time, an increased level of "collateral blood flow" to the ischemic portion of the myocardium was present. These results have been supported by the work of Irvin and Cobb (88) and Jugdutt et al. (98), in conscious dogs, as well as by Smith et al. (179) in anesthetized dogs. On the other hand, numerous studies (60, 77, 82, 207) have failed to report an increase in collateral blood to the ischemic zone, and indeed some workers (82, 207) demonstrated an impairment of flow to the ischemic region. Two factors may explain these discrepancies. First, in each study the

criteria used to define ischemia were not substantially detailed. Laboratory to laboratory differences in the histologic and visual evaluation of ischemic tissue may have, to some extent, influenced the results. Second, in most reports where collateral blood flow to the ischemic area increased, these increases were modest (13, 88, 98, 179) and potentially not within the sensitivity of the microsphere technique (see critique of microsphere technique in the methods section of this dissertation).

Although histology of myocardial cells has remained the most accurate method of determining cellular necrosis, some problems exist. First, techniques of cellular histology have been shown to be time consuming and require expertise in preparing and analyzing the tissue. Second, analysis of the degree of necrosis in a given section of myocardium was arbitrary, not absolute, and subject to a large degree of variability. Third, in studies where myocardial infarction and myocardial blood flow were correlated, it was not possible to histologically examine the same tissue samples assayed for radioactive microsphere activity. Ideally, a technique to correlate cellular necrosis and myocardial blood flow levels should allow rapid evaluation of cellular viability, eliminate some of the arbitrary evaluation standards, and allow the same sample to be examined for both cellular damage and myocardial blood flow. This technique should not identify regions of infarction by gross visual examination only, as it has been shown that myocardium appearing normal to the naked eye, or during light microscopy may indeed show irreversible cellular damage with electron microscopy (91).

Nachlas and Shnitka (132) in 1963, expanding upon the work of Wachstein and Meisel (202), rapidly and accurately identified myocardial

infarcts in experimental hearts which had undergone at lease 4 hours of coronary artery occlusion. Following the occlusion of a coronary artery, succinic dehydrogenase enzyme activity is lost from the heart muscle within approximately 6 hours (202). Nachlas and Shnitka (132), developed a histochemical method for examining changes in dehydrogenase enzyme activity in cardiac muscle which utilized a tetrazolium salt, nitroblue tetrazolium (NBT). When placed in contact with the endogenous dehydrogenase enzymes of the myocardium, NBT is reduced from an almost colorless form to a rich blue formazan compound that is deposited in the cells. Macrohistochemical analysis of evolving and stable myocardial infarction had been described previously (135, 164) but the staining procedure utilized for these studies were complicated by the fact that the salts used to identify the infarct (sodium tellurite and triphenyltetrazolium chloride) were reduced very slowly. NBT did not require a long period of incubation to be reduced to its formazan product (20-40 minutes vs 3-8 hours with the other compounds). Additionally, the regions of healthy heart muscle were stained dark blue, and were easily differentiated from infarcted, unstained muscle (132). Further studies by Nachlas and Shnitka (132, 176) and later by Shatney et al. (174) and Schaper et al. (169, 170) demonstrated an excellent correlation between the regions of infarction as defined by the NBT staining technique, and actual histologic verification of cellular necrosis. This technique, therefore, offered a rapid, accurate and simple method to determine the presence of myocardial infarction, without the necessity of disrupting infarct orientation or geometry for histologic examination. The usefulness of this technique in conjunction with radiolabeled microspheres appeared excellent as

several studies have demonstrated the ability to obtain reasonable and repeatable values for infarct size with nitroblue tetrazolium (33, 95, 169, 174).

Techniques other than evaluation of myocardial blood flow and tissue necrosis have been used to assess myocardial injury following coronary artery occlusion. Abnormalities of electrical activation (47, 86, 121) and depolarization (47, 83, 161) in both the endocardial and epicardial regions of ischemia have been reported and utilized to evaluate ischemic damage.

Both unipolar and bipolar electrodes have been used to record electrical activity from cardiac muscle (83). Unipolar electrograms are recorded with one lead in contact with the cardiac muscle (active lead) and another lead (indifferent lead) remotely positioned. While unipolar leads are poorly suited for timing activation of the myocardium, they provide useful information concerning direction and time course of voltage changes. Unipolar electrodes are particularly useful for examination of the local S-T and T-Q segments of cardiac electrograms (83). Bipolar electrograms are used most frequently to time cardiac activation and activation sequences. Two active leads in close proximity are employed to quantitate onset and duration of activity in the area of interest. Used in conjunction with unipolar electrodes, a great deal of information may be obtained concerning regional myocardial activation and voltage changes.

In 1920, Pardee (141) noted that an elevation in the S-T segment region of the electrocardiogram was present in patients after acute myocardial infarction. Several post-mortem examinations of these patients

revealed the presence of marked myocardial necrosis, and Pardee concluded that S-T segment elevation indicated some of the physiologic changes occurring in the myocardium during the ischemia-infarction phenomenon (141). Later studies, using epicardial leads (9, 150, 213) have confirmed these observations.

Following ligation of a coronary artery in the dog, Rakita et al. (150), utilizing epicardial electrodes, showed S-T segment changes began to evolve within 60 seconds and tended to reach maximal levels by 5 to 7 minutes of occlusion. Exploration of the epicardial surface from the central region of ischemia to the visible peripheral boundary revealed a gradient of S-T elevation, with S-T segment abnormalities being great in the central region of ischemia and lesser at the periphery of the insult (150). Rakita et al. additionally placed small plunge electrodes intramurally and subendocardially to measure changes in S-T segment in these regions. They found that S-T segment elevation was definitely present in these areas, but appeared to be greater in the subendocardium than intramurally or subepicardially. In some experiments, on the periphery of the ischemic insult, S-T segment changes were recorded from the endocardial electrodes while epicardial recordings remained unchanged (150).

Since 1954, epicardial mapping and use of S-T segment elevation to quantitate ischemic injury has been extensively investigated. The precise mechanism causing S-T segment elevation remains unknown. Current theories predict an efflux of potassium ions from ischemic myocardium during an ischemic episode (86). This causes a partial depolarization of the ischemic cells, and results in a so called current of injury.

The diastolic injury current (T-Q depression) and systolic injury current (S-T elevation), when amplified by an AC preamplifier cannot be separated and are together called S-T segment elevation (86, 163, 201).

Braunwald and co-workers (4, 21, 105, 121) have examined the relationships between S-T segment alterations, myocardial CPK depletion and myocardial necrosis. They have shown that epicardial S-T segment elevation recorded 15 minutes after LAD ligation was a good predictor of loss of myocardial viability as determined 24 hours later by microscopic and biochemical analysis (105, 121). Unipolar epicardial mapping, they felt, could be used to predict and detect changes in the size of the ischemic region following various interventions (21, 105, 121).

The use of epicardial S-T segment change as a predictor of the size of the ischemic region has been challenged (84, 85, 86, 181). Holland and Brooks (85), using complex mathematical calculations in conjunction with the solid angle theorem first described by Sir Isaac Newton (137), analyzed relationships between the T-Q and S-T segments during ventricular ischemia in the pig heart. They concluded from their data that epicardial S-T segment alterations were indeed good indicators of ischemic injury, but use of this technique to estimate the size, shape and geometry of the ischemic area was severely limited. Accurate interpretation of the effect of pharmacologic interventions on infarct size by analysis of epicardial S-T segments was subject to many potentially misleading variables. S-T segment has appeared to correlate well with a number of variables indicative of myocardial ischemia and necrosis (19, 105, 121), and has been shown to be a good indicator of ischemic injury (84, 121, 163, 201), but the technique does have limi-

tations of which one must be aware (20, 84, 85, 86, 88, 179).

Acute myocardial ischemia produced by coronary artery occlusion has been shown to result not only in altered myocardial depolarization, but also has been demonstrated to change myocardial activation. Durrer et al. (47) described changes recorded from extracellular bipolar electrograms following coronary artery occlusion and verified the efficiency of this technique in sensing discrete alterations in the onset of tissue activation. Subsequent studies by Scherlag et al. (172, 173, 210), as well as Boineau and co-workers (15, 32), have further substantiated the bipolar electrogram as a sensitive indicator of myocardial activation.

Only one study currently exists in the literature attempting to correlate changes in bipolar electrograms with regional myocardial blood flow. Ruffy et al. (161) related the degree of regional myocardial ischemia quantitatively to a decreased amplitude of both regional endocardial and epicardial bipolar electrograms in the dog heart. Also noted was an increased duration of electrogram activation that was related to the amount of ischemia measured. Studies correlating myocardial necrosis to changes in myocardial activation following coronary artery occlusion are few (32).

C. Influence of Sympathetic Activation and Catecholamines on the Supply:Demand Relationship of the Heart

As mentioned in section A of this literature review, a number of factors may alter the supply:demand relationship present at any given time in the heart. The factors noted as having an effect on this balance were heart rate, contractile state of the heart, neural input to

the heart, and levels of circulating catecholamines influencing the heart. Changes in any one of these parameters could alter the supply:demand balance. Under normal physiologic conditions, this would present no immediate problem as compensation would occur quickly, but in the presence of coronary artery occlusion significant deletorious results might be manifested.

There is general agreement from both clinical and experimental studies that activation of the sympathetic nervous system, or increased levels of circulating catecholamines, exert a deletorious influence on the myocardium during periods of regional ischemia (75, 76, 119, 147). Raab and others (64, 65, 147, 148) have evolved the concept of a direct "oxygen wasting action" of catecholamines. While this proposal has not been universally accepted (111, 125, 134, 165), even an indirect augmentation of myocardial demand by sympathetically released catecholamines would be an important concept in defining the deletorious actions of sympathetic activation in the presence of coronary artery occlusion.

The effect of sympathetic nerve (catecholamine) stimulation on the supply:demand relationship of the heart may be broken down into two broad categories; direct and indirect effects. The direct effects of catecholamine stimulation are an augmentation of myocardial oxygen consumption (146, 148), and a modulation of coronary blood flow (12, 55, 185, 199). The indirect effects consist of an alteration of myocardial oxygen consumption or myocardial blood flow by changes in heart rate (38, 134, 205) or myocardial contractile state (19, 117). Separation of direct and indirect effects has been difficult.

During stimulation of the stellate ganglia, or during systemic

infusion of catecholamines, increases in the indirect determinants of supply and demand are seen. With supramaximal sympathetic nerve stimulation or catecholamine infusion, heart rate increased dramatically and ventricular contractile force was markedly augmented.

Changes in heart rate in the absence of sympathetic activation have been shown to drastically alter left ventricular myocardial blood flow (38, 134), but these changes in flow were in directions that could not be explained by the direct effect of heart rate. Increased heart rate increases left ventricular blood flow (38, 50, 134), while the direct effect due to extravascular compression (24, 106) would be a decreased level of left ventricular blood flow.

Khouri et al. (104), utilizing an electromagnetic flow probe on the main left coronary artery of the conscious dog, demonstrated that at peak isovolumic contraction, extravascular compression became so great that flow in the major coronary arteries actually reversed direction. This reversal resulted in brief periods of ischemia during systole (104). Tachycardia increased the systolic duration:cardiac cycle duration ratio so that the heart spent more time per minute in systole (12, 134). Since perfusion of the left ventricle occurred predominantly in diastole (104), left ventricular blood flow should decrease during tachycardia if no change in perfusion pressure gradient occurred. The increased left ventricular blood flow observed during tachycardia (38, 134) was attributed to a secondary increase in myocardial metabolism subsequent to the increase in heart rate (38, 50, 134).

Sympathetic activation has also been described to increase the "contractile state" of the heart. Rushmer (162) in his book on the

cardiovascular system, demonstrated that sympathetic stimulation accelerated heart rate, increased the rate of ventricular pressure development during systole, augmented ventricular ejection, increased peak systolic pressure, and heightened the aortic flow rate. All of these factors would tend to increase myocardial oxygen consumption (12, 19, 117). Conversely, sympathetic activation shortened the period of systolic ejection, accentuated ventricular relaxation and reduced diastolic size all of which would tend to decrease myocardial oxygen demand (12, 19, 117).

Wiggers and Katz (209) showed that at a given heart rate, stimulation of the left stellate ganglion resulted in an increased rate of systolic ejection, and a decreased systolic duration when heart rate was kept constant. This decreased systolic duration at constant cardiac cycle length resulted in a correspondingly increased duration of diastole. The increased duration of diastole accomplished two functions: first, it allowed for an increase in the filling time of the ventricle so that the augmented ventricular ejection was supplemented, and second, since perfusion in the left ventricle occurred predominantly during diastole, the period of myocardial perfusion increased. This prevented marked endocardial ischemia by allowing more time per beat for blood flow delivery.

The effects of sympathetic activation on heart rate and myocardial contractility are well documented (12, 162). Both of the above parameters directly influence the oxygen demand state of the heart (117), but should not be confused with the actual direct effects of sympathetic nerve stimulation on cellular metabolism, and coronary

vascular resistance mediated through changes in vascular smooth muscle.

Raab et al. (147, 148, 149), along with other investigators (28, 51, 64, 65, 68, 175), have described the influence of catecholamines on myocardial oxygen utilization as an excessive or "wasteful" use of oxygen. This conclusion was based upon observations where large infusions of catecholamines (28, 147, 149) or supramaximal sympathetic activation (51, 68, 149) markedly increased myocardial oxygen consumption, many times in the absence of a significant change in cardiac work. The oxygen "wasting" effect has been shown to be a dose dependent phenomenon and at low levels of catecholamines the effect was found to be very small (109, 166). Klocke et al. (198) in the isolated dog heart demonstrated that doses of catecholamines (1.0 micrograms or larger) injected into the coronary artery of the beating heart caused a marked increase in oxidative metabolism. Injection of graded doses of norepinephrine following arrest of the heart by potassium changed myocardial oxygen consumption only 15% at dose levels up to 10 micrograms. Low doses of norepinephrine (0.3 micrograms) augmented myocardial demand only 7% in the beating heart. Klocke et al. (109) concluded that while large doses of catecholamines increased oxygen consumption markedly in the beating heart, this increase was attributable mainly to an augmentation of contractile activity. The direct action of catecholamines on oxygen utilization by the myocardium was small. Sarnoff et al. (166), Sonnenblick et al. (183), and others (130, 171) have also proposed that the "oxygen wasting" effect probably was not due to direct augmentation of oxidative metabolism.

Sympathetic neuromediators have also been shown to have a direct

effect on the supply portion of the supply:demand relationship. Separation of the direct effects of sympathetic (norepinephrine) activation on the coronary vessels (alpha and beta-2 effects) from the indirect effects on myocardial metabolism (beta-1 effects) has been difficult and experiments have led to varying conclusions.

Supramaximal stimulation of cardiac sympathetic nerves or infusion of high doses of norepinephrine increase coronary blood flow (28, 51, 66, 68, 149, 175). This increase in blood flow presumably resulted largely from the indirect coronary dilator action associated with increased myocardial metabolism due to augmented heart rate and contractility. The predominant direct effect of sympathetic (norepinephrine) stimulation on coronary blood vessels has been described as a vasoconstrictor response (12, 55, 57, 185, 199). Also a direct vasodilator response during catecholamine activation (71, 108, 120, 125) may be attributed to the presence of coronary vascular beta-2 type receptors, but the functional significance has been questioned (71).

In a series of papers, Szentivanyi and Juhasz-Nagy (100, 185, 186, 187) identified three distinct types of cardiac sympathetic fibers. Two of these fiber types supplied the coronary vessels, operating as coronary vasoconstrictors (preganglionic adrenergic) or coronary vasodilators (preganglionic cholinergic). The third set of sympathetic fibers innervated the heart muscle itself (postganglionic adrenergic) and caused cardioacceleration. Szentivanyi and Juhasz-Nagy speculated upon the identity of these three sympathetic components based on the electrical stimulation parameters required to activate each (186).

Stimulations of low voltage (0.1-2 V) and frequency (1-6/sec)

elicited marked coronary vasoconstriction ranging up to 30%. These stimulus parameters did not change heart rate, blood pressure or oxygen consumption (100, 186). Reflex activation of the cardiac sympathetics by bilateral carotid occlusion also resulted in vasoconstriction (186, 187). On the other hand, stimulations with high voltage (5-20 V) and frequency (6-20/sec) excited cardioaccelerator fibers and resulted in an overall vasodilation of coronary vessels presumably due to metabolic mechanisms (100, 185). In a few dog preparations, but much more often in the cat (185), stimulation at low frequency and voltage resulted in coronary vasodilation. The authors concluded that the sympathetic vasodilator system was present in the dog but was variable and not nearly as prominent as the system in the cat (100, 185, 186).

Reports since Szentivanyi and Juhasz-Nagy have substantiated the presence of coronary vasoconstriction with sympathetic activation. Feigl (55) reported the presence of coronary vasoconstriction during stellate ganglion stimulation in dogs that had undergone beta blockade with propranolol. The vasoconstriction was abolished by alpha-adrenergic blockade with phenoxybenzamine. Feigl was unable to identify the vasodilation reported by Szentivanyi during sympathetic activation in any of his experiments. Berne, DeGeest and Levy (11) showed in paced, non-paced and fibrillating hearts that stellate ganglion stimulation resulted in an initial decrease in coronary flow (direct effect) followed by a subsequent increase in coronary flow. Granata et al. (66) reported similar results in the conscious, unanesthetized dog. These authors (11, 66) associated the increase in flow with secondary increases in myocardial metabolism.

McRaven et al. (125) reported that vasodilation associated with left cardiac nerve stimulation or intracoronary injection of norepinephrine was reversed to vasoconstriction in the presence of practolol. Practolol, a beta-1 adrenergic blocking agent, blocked the increases in heart rate, dP/dt, and systolic pressure observed during these manipulations and unmasked the alpha-adrenergic vasoconstrictor component. Phentolamine eliminated the vasoconstriction during nerve stimulation and norepinephrine infusion. The authors concluded that the direct effect of norepinephrine and sympathetic stimulation was a vasoconstrictor response, but this response was overidden by metabolic augmentation. A later paper by the same group (120) described a relative paucity of alpha-adrenergic receptors present in the coronary circulation when compared to skeletal muscle or cutaneous circulations. They inferred from these data (120) that perhaps metabolic vasodilation overwhelmed direct vasoconstriction because of this relatively low number of alpha receptors.

The presence of alpha-adrenergic receptors on the coronary vasculature intuitively may seem illogical. As stated earlier, the supply:demand relationship has been described to be a finely balanced state. Activation of coronary vascular alpha-adrenergic receptors by norepinephrine (18, 129, 199) or sympathetic stimulation (11, 100, 129) could cause vasoconstriction which would compete with the vasodilation associated with the augmented metabolic state induced by these manipulations. Indeed, several investigators have demonstrated the potential for this occurrence (18, 100, 129, 199). Vatner et al. (199), in conscious dogs, reported a coronary vasoconstrictor effect sufficiently intense to counteract completely the increased metabolic demand produced by an

intravenous bolus of 1.0 microgram/kg norepinephrine. In chloralose-anesthetized dogs, Mohrman and Feigl (129) demonstrated a competition between alpha-vasoconstriction and metabolic vasodilation. Utilizing graded doses of norepinephrine (intracoronary infusions at rates greater than 1.0 microgram/min), Mohrman and Feigl showed approximately 30% restriction of coronary flow.

Brandfonbrener et al. (18) in a similar study had demonstrated the importance of consideration of dose with respect to interpreting data obtained during norepinephrine administration. At low intracoronary infusion rates (approximately 3.7 micrograms/min), norepinephrine induced a coronary vasoconstrictor response and no change in heart rate or mean aortic pressure, but significantly reduced end diastolic pressure. Higher doses of norepinephrine (average infusion rate 10.1 micrograms/min) always induced vasodilation as well as increasing heart rate, end diastolic pressure and mean aortic pressure. Brandfonbrener et al. (18) concluded that initial resistance of the coronary vasculature, as well as the amount of norepinephrine infused into the system, played important roles in determing the overall response.

Coronary vasoconstriction with physiological levels of sympathetic activation has great clinical signifiance. If, as described by Vatner (199), Mohrman (129) and Brandfonbrener (18), coronary vasoconstriction can effectively compete with metabolic vasodilation, the phenomenon of coronary vasospasm takes on an important new dimension. Coronary artery spasm has been described as a real phenomenon (70, 122, 123) and has been attributed in some cases to alpha-receptor activation (70, 123). Vasoconstriction competing with vasodilation may limit availability of

oxygen to the myocardium and result in a supply:demand mismatch that leads to infarction. Increased metabolism via sympathetic activation, coupled with limited collateral blood flow delivery via vasoconstriction could cause an increase in infarction during instances of coronary occlusion.

D. Cardiac Denervation

The first utilization of cardiac denervation dates back to the work of Francois-Franck (61). Francois-Franck demonstrated experimentally that electrical excitation of the cephalic end of the cervical sympathetic chain resulted in a hypertension or hypotension. He concluded that the superior portion of the cervical sympathetics contained sensory fibers with both accelerator and depressor actions on the heart. Franck reasoned that these fibers were probably activated during times of stress or arousal and he extrapolated his experimental findings to explain the clinical phenomenon of chest pain (angina pectoris) during cardiac difficulty. He postulated that surgical transection of these sensory fibers would enhance a patient's condition by interrupting the activation pathway of this pain reflex. Franck concluded that extirpation of the cervical sympathetics would obtund the pain of angina pectoris by eliminating the pathways of the ascending sensory pain fibers.

The work of Franck was partially contradicted by J.N. Langley (113) who stated that sensory fibers from the heart passed, not by way of the cervical sympathetics, but through the stellate ganglia. He believed that the removal of the cervical sympathetics as a treatment for angina was an unfounded and incorrect conclusion. Langley explained that the apparent beneficial effect of cervical sympathectomy on angina

was of an indirect nature, and the result of the transection of motor, not sensory, nerve fibers.

Danielopolu (37) and Leriche (114) in the mid-1920's emphasized the deleterious effects of sympathectomy on normal cardiac function in animal and human hearts. They described a complete cessation of cardiac activity following stellatectomy, but by 1930 this concept had been completely discarded. Indeed, in 1931, Leriche (115, 116) examined canine hearts during coronary occlusion at various time intervals after the removal of the stellate ganglia. He found in hearts with comparable amounts of occluded coronary supply that the smallest amount of infarction was present in dogs that had been stellate ganglionectomized the longest period of time before coronary artery ligation. In a similar study five years later, Cox and Robertson (34) measured heart rate, respiratory rate, blood pressure and cardiac output in sympathectomized and non-sympathectomized dogs before and after coronary artery ligation. In these conscious animal studies, the sympathectomized dogs demonstrated no change in any of the above parameters after coronary artery ligation. Infarcts in sympathectomized hearts were smaller than in normally innervated hearts, and mortality following coronary ligation was reduced (10% mortality in sympathectomized dogs versus 50% mortality in control dogs) (34).

Since the work of Cox and Robertson (34), experimental reports have substantiated the concept that the denervated or sympathectomized heart performs adequately under normal physiological conditions (41), and may actually be at an advantage during certain pathophysiological circumstances, specifically during coronary occlusion. It has been

shown that the denervated heart operates at a disadvantage when compared to the normally innervated heart only when 1) stress is such that length-tension mechanisms can not fully compensate for the absence of neural and hormonal stimulations (42, 44) or 2) immediate (not gradual) increases in heart rate are required (43).

The physiological advantages exhibited by the denervated (sympathectomized) heart during myocardial ischemia have been the subject of previous investigation. Early studies (34, 37, 61, 115, 116) described an apparent "protective effect" of partial or total sympathetic cardiac denervation. Harris et al. (75) described a decrease in the incidence of ventricular fibrillation following surgical interruption of the sympathetic nerves to the heart immediately before occlusion of the LAD. Later studies (73, 76, 118) demonstrated that augmented sympathetic activity (73, 76) or infusion of catecholamines (118) increased the sensitivity of the heart to arrhythmia in the presence (76) or absence (73, 118) of coronary occlusion. These findings, coupled with the demonstration that coronary artery occlusion elicited local (113) and reflex (184) release of catecholamines, as well as increased levels of efferent sympathetic nerve activity (63, 119), supplied evidence that during the pathophysiologic state of coronary artery occlusion the innervated heart was at a disadvantage.

Harris' work (75) and studies by Ebert et al. (49), have shown that removal of sympathetic innervation to the heart immediately before coronary artery occlusion significantly reduced arrhythmia and mortality in dogs. Ebert et al. (49), Fowlis et al. (59) and Schaal et al. (167), demonstrated that chronic cardiac sympathectomy (49, 59) and

complete chronic cardiac denervation (49, 167) reduced arrhythmia and mortality during LAD occlusion to a greater extent than did acute denervation. Interestingly, complete chronic cardiac denervation by the mediastinal neural ablation method of Cooper et al. (30) resulted in shorter periods of arrhythmia, less arrhythmia, and lower incidents of ventricular fibrillation than did chronic sympathectomy alone (49). These studies supported the work of Cox and Robertson (34) and Leriche et al. (115, 116).

The protective effect of cardiac denervation with respect to arrhythmia following coronary artery occlusion has been well documented. In studies where cardiac denervation was performed immediately prior to coronary artery occlusion (49, 75), this protection was probably due to the interruption of a cardio-cardiac, or a cardio-adrenal medullary, reflex release of catecholamines. These data would support the conclusions of Francois-Franck (61) and Langley (113). On the other hand, the even more enhanced protection observed following chronic cardiac denervation (49, 59, 115, 116, 167) appeared to be related in some manner to the actual decrease of myocardial catecholamine levels observed in chronically denervated or sympathectomized hearts. Leriche (115, 116) showed greater protection during coronary artery occlusion in hearts denervated for the longest period of time. Cooper et al. (30, 31) showed conclusively that myocardial catecholamine depletion did not occur immediately after mediastinal neural ablation, i.e., acute denervation did not significantly change myocardial catecholamine levels, but was a gradual depletion process. This depletion required 60-72 hours to become complete. The protective effect of chronic cardiac sympathectomy

or denervation with respect to arrhythmia, may be at least in part due to the depletion of myocardial stores of catecholamines.

Many studies have placed great emphasis on the release of potassium ions from myocardial cells during coronary occlusion as the factor most responsible for arrhythmogenesis (27, 52, 72, 74). Vanderbeek and Ebert (196) demonstrated using the potassium releasing agent octylamine that chronically denervated hearts were more resistant to a rapid cellular efflux of potassium than were normally innervated or reserpinized hearts. Arrhythmia and ventricular fibrillation developed rapidly (within 6 minutes) in control hearts following intra-coronary (LAD) injection of octylamine, while arrhythmia only was seen in the reserpinized preparations. Chronically denervated hearts released similar levels of coronary sinus potassium following direct LAD injection of octylamine yet demonstrated no arrhythmia for the entire 30-minute observation period. The authors concluded that chronic cardiac denervation did not alter cellular membrane function as similar changes in coronary sinus potassium concentrations were seen in denervated and non-denervated hearts. They were not able to explain the mechanism but postulated a relationship between the release of potassium ions from myocardial cells, and a possible de-sensitization to this release in the absence of myocardial catecholamines.

The protective effect of cardiac denervation during coronary occlusion as indicated by a reduction in infarct size or an increase in long-term survival is not as well documented. Several studies have reported that cardiac denervation or sympathectomy reduced (34, 94, 95, 115, 116, 192) infarct size while other studies described no change in

infarct dimensions (49, 167, 214). Very early studies (34, 116) indicated complete removal of the cardiac sympathetics substantially reduced the amount of infarction following coronary artery ligation. Studies by Yodice (214), Ebert et al. (49) and Schaal et al. (167) reported cardiac denervation or sympathectomy did not reduce infarct size significantly from that seen in normally innervated hearts. These reports also demonstrated that overall survival of dogs following LAD occlusion was not significantly enhanced by partial or complete cardiac denervation over a long period of observation (49, 167, 214), even though acute arrhythmias following coronary artery occlusion were significantly reduced (49, 167). Even though denervation reduced the incidence of acute ventricular arrhythmias and fibrillation immediately following LAD ligation, over a period of 24-72 hours the denervated (sympathectomized) hearts became unable to meet systemic circulatory demands. The denervated heart did not fibrillate after LAD occlusion but simply went into failure at some later time.

Very recent reports have added more certainty to the question of denervation and infarct size (94, 95, 192). Jones et al. (94, 95) in 1978 utilized a "standardized" model of LAD occlusion to assess the effect of chronic cardiac sympathectomy and denervation on infarct size. In previous work (49, 115, 116, 167, 214), criteria for specific coronary arterial anatomy and distribution were not well defined. Inherent biological variability has been shown to exist between animals of the same species, and in these studies some degree of variability probably did exist. A large biological variability would hinder determination of a representative infarct size in small groups of animals and possibly

result in inconclusive or incorrect results. A second problem with the older studies (49, 115, 116, 167, 214) was the method by which infarct size was determined. Gross visual examination and qualitative estimation of the area of necrotic tissue could have potentially resulted in substantial errors in infarct size measurement. Jones et al. (94, 95) examined infarct size using the stain NBT. Infarct size was quantitated as a percent of total left ventricular weight. Using a standardized LAD occlusion model and NBT to determine infarct size, Jones found that chronically denervated dog hearts had an 80% smaller infarct size when compared to normally innervated control hearts (95). Chronic sympathectomy of the ventricles (94) also reduced infarct size but this reduction was not as great (approximately 60%) as that seen following complete denervation. Infarct size determinations were all made following six hours of LAD occlusion and during this time hemodynamic and heart rate parameters were not different in any of the groups.

Another factor that must be considered when analyzing the protective effect of cardiac denervation with respect to infarction is the size of the area of ischemic insult. Recent evidence (97, 177) has suggested that chronically denervated and sympathectomized hearts have reduced coronary collateral resistance when compared to the acutely denervated or normally innervated hearts. A decrease in coronary collateral resistance could result in an increase in coronary collateral blood flow and result in improved perfusion of the ischemic region. The reduction in infarct size seen in chronically denervated or sympathectomized hearts was in the presence of occlusion of the apical portion of the LAD (94, 95, 192). This protection has not been reported in studies

where mid-LAD (167) or high-LAD (49, 214) occlusion was used. The ability of the coronary collaterals to supply the ischemic myocardium in the denervated or sympathectomized heart may be limited. Ischemic areas of large size (mid-LAD occlusion or higher) may not demonstrate significant, or may demonstrate less significant, degrees of protection.

Chronic cardiac denervation and sympathectomy have also been shown to preserve functional parameters during the course of myocardial ischemia. In a preliminary report (194) and a recent publication (193), Thomas et al. utilizing the chronic cardiac denervation technique developed in this laboratory (153), compared loss of contractile function (as measured by Walton-Brodie strain gauge arches) in chronically denervated, acutely denervated and sham-denervated dog hearts. They demonstrated that under similar conditions of ischemic insult, control non-denervated hearts and acutely denervated hearts lost over 70% of the initial regional contractile force with occlusion of the apical portion of the LAD, while contractile force in chronically denervated hearts fell only 21.5%. In more recent experiments, Thomas (personal communication) has shown that chronically sympathectomized canine hearts maintain ischemic and systolic and end diastolic segment length and ventricular dP/dt levels at signi-ficantly higher levels than acutely denervated hearts during LAD occlusion.

Finally, chronic cardiac denervation (sympathectomy) may provide protection to the ischemic myocardium by altering myocardial oxygen consumption. It has been postulated that chronic cardiac denervation, and specifically the depletion of myocardial catecholamine stores following denervation, may lower myocardial oxygen demand, yet this has

not been conclusively demonstrated. Previous studies have reported increased (17, 212), decreased (8, 67, 96, 191) and unchanged (29, 36) levels of myocardial oxygen consumption following cardiac denervation. In other studies, measurements of myocardial blood flow have demonstrated similarly disturbing results, as increased (17, 46, 212), decreased (7, 8, 48, 67, 94, 96, 191) and unaltered (36) myocardial blood flow values have been reported following cardiac denervation. It has been shown that myocardial blood flow and myocardial oxygen consumption are closely correlated (10, 12, 107, 160) therefore decreases in myocardial blood flow in chronically denervated and sympathectomized hearts suggest a decrease in cardiac metabolism.

Differences in experimental design, control of hemodynamic variables, models of denervation, methods of myocardial blood flow determination and types of anesthesia make comparing and contrasting these studies difficult. Recent evidence though, utilizing the intrapericardial cardiac denervation model (153) performed in this dissertation, and the radiolabeled microsphere technique for myocardial blood flow determination has shown decreased levels of myocardial blood flow (7, 48, 94, 96, 191) and oxygen consumption (96, 191) in the denervated and sympathectomized hearts. These studies subjected denervated and non-denervated hearts to similar heart rate and blood pressure parameters, thereby allowing assessment of changes in myocardial blood flow to be made. Though the final answer concerning the effect of cardiac denervation on myocardial blood flow and oxygen consumption has not been reached, a decrease in these parameters following denervation would be an exciting and appealing result. Evidence for an "oxygen wasting action" of

catecholamines has been proposed (64, 65, 149). Verification of catecholamine depletion in the denervated heart would lend credence to the studies reporting decreased metabolic demand in these preparations (67, 96, 191).

CHAPTER III

METHODS

A. Chronic Surgical Procedure

Chronic cardiac denervation was attempted in 17 dogs and chronic ventricular sympathectomy was attempted in 10 dogs. Twelve dogs served as sham-operated controls.

Adult mongrel dogs (15-25 kg) were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and ventilated through a cuffed endotracheal tube with a positive pressure respirator (Bird Mark 7). Under aseptic conditions, a left thoracotomy at the level of the fourth intercostal space was performed. The right and left thoracic vagi, and the right and left ansae subclavia (anterior and posterior ansae) were isolated from surrounding tissue. Silk threads were placed loosely under each nerve to facilitate later identification. The pericardium was opened and the heart suspended in a cradle formed by securing the cut edges of the pericardium to the muscles of the chest. Walton-Brodie strain gauge arches were sutured to the left and right ventricles, and each nerve stimulated individually. Nerve stimulation was accomplished using a bipolar electrode and a Grass S9 stimulator which delivered square wave pulses of 5 msec duration and 7-8 volts at a rate of 10 (stellates) or 20 (vagi) pulses per second. Changes in heart rate, contractile force and lead II electrocardiographic configuration were

recorded on a Grass polygraph before, during, and after each stimulation.

Following control nerve stimulations, complete cardiac denervation was performed by the procedure described by Randall et al. (153). This technnique was utilized for four reasons. First, the intrapericardial cardiac denervation has been shown via rigorous testing in conscious and anesthetized dogs to be complete for the removal of both sympathetic and parasympathetic components to the heart. Second, the technique selectively eliminates neural input to the heart while leaving nerves intact to the other organs. Mediastinal neural ablation, stellate ganglionectomy or vagotomy are not selective for the heart. Third, in our hands the intrapericardial cardiac denervation procedure has been found to be a very safe procedure with a greater than 90% survival rate. Mediastinal neural ablation and total cardiac autotransplantation have associated with them a very high operative and post-operative mortality. Finally, the intrapericardial cardiac denervation affords the opportunity for the investigator to verify denervation at the time of the actual denervation, as well as after the actual procedure. We were able to insure completeness of denervation at the time of surgery, and again during the acute experiment.

Total cardiac denervation was performed as follows. The ventrolateral cardiac nerve was sectioned and the superior borders of the left and right atria were cleared of tissue. The adventitia was removed from the complete circumferences of the left superior pulmonary vein, main pulmonary artery, and superior vena cava. The azygous vein was ligated and transected at its point of entrance into the superior vena cava. This procedure has been shown to completely denervate the heart (153).

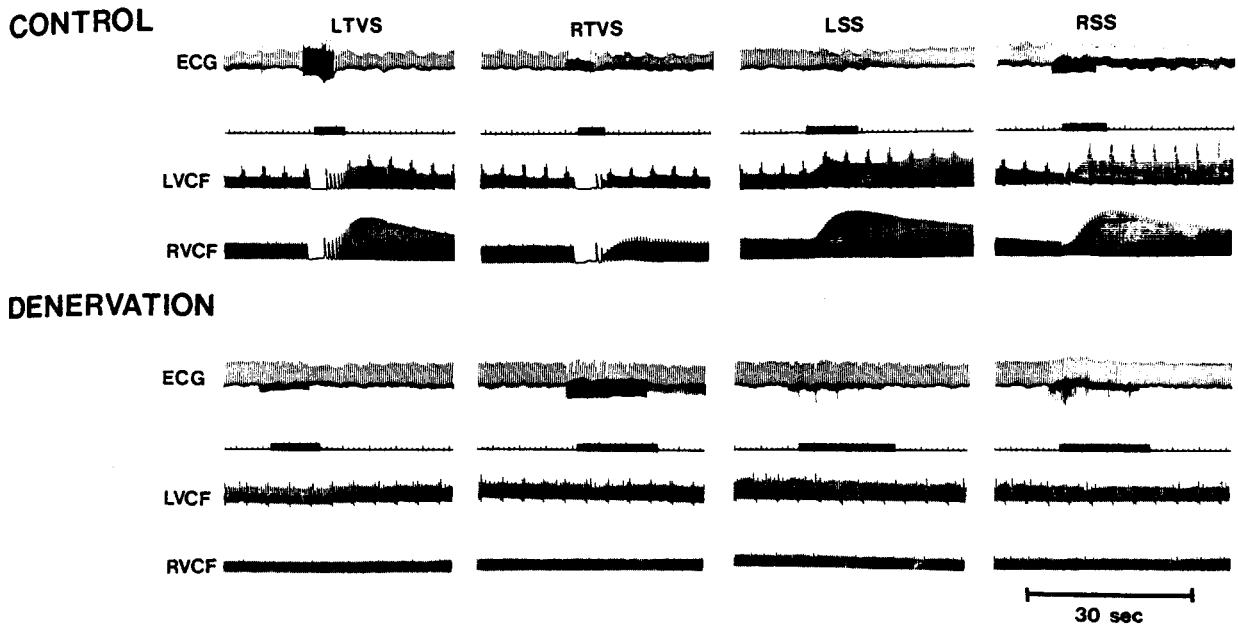
Figure 1 illustrates pre-operative and post-operative recordings of left ventricular (LVCF) and right ventricular (RVCF) contractile force, as well as a lead II electrocardiogram (ECG). The tracings were recorded before, during, and after direct nerve stimulations, and clearly demonstrate the abolition of heart rate and contractile force responses to sympathetic and parasympathetic nerve stimulation following the cardiac denervation procedure.

Ventricular sympathectomy was performed by modification of the above technique (153). The ventrolateral cardiac nerve was sectioned, and the adventitia removed from the left superior pulmonary vein and the pulmonary artery. The superior border of the left atrium was cleared of all neural and connective tissue, the fat pad between the pulmonary artery and aorta was sectioned, and the large nerves coursing along the lateral aspect of the pulmonary artery were transected. As per the complete denervation, pre-operative and post-operative ventricular contractile force recordings were examined. Figure 2 illustrates records similar to Figure 1, except post-operative vagal responses are seen while sympathetic contractile responses are ablated.

In the sham-operated animals, the chest was opened, the nerves isolated, the pericardium opened, and strain guages attached to the ventricles. Following stimulation of the isolated nerves, the heart was left undisturbed for 45 minutes to an hour. During this period, the epicardium was kept moist by occassional rinses with sterile saline. After this period, nerve stimulations were repeated. Pre-operative responses to direct nerve stimulation remained unaltered following sham manipulations.

FIGURE 1

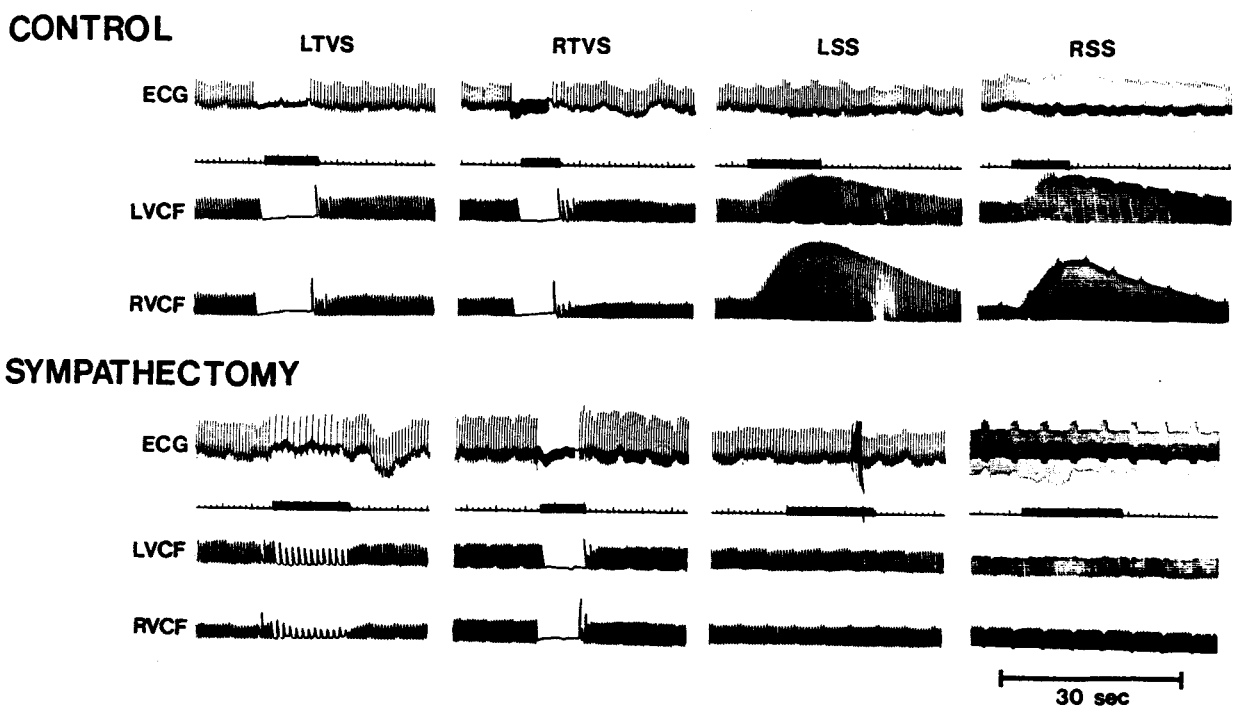
CONTRACTILE FORCE RESPONSES TO DIRECT NERVE STIMULATION
BEFORE AND AFTER COMPLETE CARDIAC DENERVATION



Intraoperative recordings of left (LVCF) and right (RVCF) ventricular contractile force responses before (CONTROL) and immediately after intrapericardial cardiac denervation (dog # DSTS-6). Note the marked asystole during both left (LVTS) and right (RTVS) thoracic vagal stimulation, and the pronounced augmentation in LVCF and RVCF during left (LSS) and right (RSS) sympathetic nerve stimulation before denervation. ECG = lead II electrocardiogram

FIGURE 2

CONTRACTILE FORCE RESPONSES TO DIRECT NER STIMULATION
BEFORE AND AFTER VENTRICULAR SYMPATHECTOMY



Intraoperative recordings of ventricular contractile force before (CONTROL) and immediately after intrapericardial ventricular sympathectomy (dog #SNSX-5). Figure labels are the same as described in Figure 1. Control, pre-sympathectomy responses are similar to those shown in Figure 1. Note the remaining parasympathetic responses during LTVS and RTVS, and the abolished response to LSS. Following ventricular sympathectomy, control heart rate was accelerated from 160 beats/min to 230 beats/min during RSS and a contractile response was noted. Pacing at 240 beats/min was initiated prior to the RSS tracing shown above, and the stimulation repeated. As can be seen in the RSS panel, during pacing no change in contractile force was seen following sympathectomy.

Upon conclusion of the surgical procedure, the chest of each animal was closed in layers and negative pressure breathing established. All dogs were treated post-operatively with antibiotic drugs for 3-5 days. None of the surgically prepared animals died due to post-operative complications. Two to four weeks after the initial surgery, dogs were anesthetized and acute surgical procedures, as described in Section B of these methods, performed. Prior to the actual execution of the acute experiment, completeness of the denervation or ventricular sympathectomy was thoroughly tested using direct nerve stimulation, tyramine injections (0.04 mg/kg, i.v.), and ventricular field stimulation (53). Contractile force responses of 5% or less during each manipulation were considered to reflect complete ventricular denervation (sympathectomy). Most animals demonstrated no response. If any test manipulation resulted in greater than a 5% response, the animal was excluded from this study. Of 17 complete cardiac denervations and 10 cardiac sympathectomies, 4 dogs were found to be incompletely prepared (2 in the complete denervation group and 2 in the ventricular sympathectomy series). At the end of the acute experiment, many of the animals had multiple transmural tissue samples taken from both the left and right ventricles. These tissue samples were rapidly frozen in liquid nitrogen and tissue norepinephrine content determined at a later date using the trihydroxyindole-fluorometric technique of Crout (35). (These data were generously supplied through the efforts of Dr. S.B. Jones, Department of Physiology, Loyola University Stritch School of Medicine). Analyses of tissue norepinephrine levels were performed in 11 chronically denervated hearts, 7 chronically sympathectomized hearts, and 6 sham-denervated hearts. The norepine-

phrine contents of the left and right ventricles for these three groups of hearts are shown in Table I. Tissue norepinephrine levels in the left and right ventricles of the sham-denervated hearts were similar to values previously reported in unoperated preparations (1, 103) and in sham-denervated preparations (30, 49). Complete cardiac denervation, as demonstrated by rigorous testing, resulted in significantly lower levels of ventricular norepinephrine when examined. These levels agreed with values previously reported for cardiac denervation (30, 49). Ventricular sympathectomy (again as demonstrated by stringent testing) significantly lowered tissue norepinephrine levels from values seen in sham-denervated animals, and these values were also significantly lower than seen in the denervated hearts. Data were therefore supplied for both physiological and functional evidence of denervation and sympathectomy.

B. Acute Surgical Procedures

Adult mongrel dogs of either sex weighing between 15 and 27 kg were anesthetized with sodium pentobarbital. A midline incision was made in the neck, the right and left cervical vagi isolated, the trachea cannulated, and animals ventilated 10-14 times/min with 40% oxygen using positive pressure ventilation (Bird Mark 7). Both femoral arteries and a femoral vein were isolated and catheterized with saline filled polyethylene tubing. One arterial catheter was connected to a Statham pressure transducer (P23Db) and Grass polygraph (Model 7) for continuous monitoring of arterial blood pressure. The other arterial catheter served as a site for blood withdrawal during radiolabeled microsphere injections. Additionally, arterial blood samples were obtained frequently through this catheter for analysis on a Corning 165 pH/blood gas analyzer.

TABLE 1

VENTRICULAR CATECHOLAMINE VALUES FOR DENERVATED, SYMPATHECTOMIZED AND SHAM-DENERVATED HEARTS

	Sham-Cardiac Denervation (N=6)	Complete Cardiac Denervation (N=12)	Ventricular Sympathectomy (N=7)
Left Ventricle	.744 ± .105	.035 ± .009*	.009 ± .004*
Right Ventricle	.740 ± .117	.036 ± .006*	.016 ± .002*

All values are expressed as micrograms norepinephrine/gram ventricular tissue (mean ± SEM). * = $p < .00001$ when compared to sham-denervated value.

Arterial blood gas levels, as well as pH and base deficit, were monitored. Ventilation was adjusted throughout the experiment to maintain arterial pO_2 at a level greater than 100 mmHg (maximum oxygen tension = 240 mmHg). Intravenous infusion of sodium bicarbonate via the femoral vein catheter was performed as needed to maintain arterial pH within a range of 7.35 - 7.47. Lactated Ringer's solution, muscle blocking agent (decamethonium, 0.2 mg/kg) and supplemental anesthetic were administered as required via the venous line.

A left thoracotomy at the level of the fifth intercostal space was performed, and the lungs gently retracted. The ansae subclavia of the right and left stellate ganglia were isolated and transected. The left and right cervical vagi were also transected at this time. These procedures should produce complete neural decentralization of the heart (128, 129, 152). In experiments described in Section A of Results utilizing surgically prepared totally denervated or sham-denervated dogs, neural decentralization was not performed. All other animals reported in this dissertation were neurally decentralized during the acute experiment.

The pericardium was opened via an incision parallel to the phrenic nerve. The left atrium was cannulated with a short polyvinyl-chloride catheter filled with heparinized saline. A bipolar pacing electrode was sewn to the left atrial appendage and all hearts were electrically paced at 150 beats/min. In preparations where spontaneous heart rate was greater than 150 beats/min, the heart was gently rotated and the region at the junction of the superior vena cava and right atrium exposed. The sino-atrial nodal and sulcus terminalis regions of

the heart were crushed and pacing from the left atrium initiated at 150 beats/min. All preparations were allowed to stablilize for 15-20 minutes before any measurements were made.

A small banjo type thermistor (YSI probe, Model 425) was secured to the surface of the left ventricular free wall and epicardial temperature monitored with a YSI telethermometer (Model 46). Rectal temperature was also monitored, and an infrared heating lamp utilized to keep epicardial and core temperature within a narrow range (37-39°C).

C. Myocardial Blood Flow Determination

Regional myocardial blood flow values were determined using radiolabeled carbonized spheres (microspheres) and techniques previously described (39).

Forty-five seconds prior to the injection of a well mixed species of microspheres (15 $\pm$ 2 microns), withdrawal of a reference blood sample (6.1 ml/min) was begun from the femoral artery catheter. This withdrawal was continued for two minutes after the injection of the microspheres. Microspheres were introduced into the left atrium via the left atrial cannula described above, and flushed with 5 ml of 0.9% saline over 5-10 seconds. Heart rate and hemodynamic conditions were monitored throughout the microsphere injection-saline flush-blood withdrawal protocol and remained stable or the blood flow determination was excluded. Four microsphere isotopes (strontium-85 or ruthenium-103; scandium-46; cerium-141; chromium-51) were utilized in most experiments, and the order of isotope injection was determined randomly immediately prior to the beginning of each experiment.

There are 24 distinct sequences in which four species of microspheres

can be injected. Each individual injection order was assigned a number ranging from 1-24, inclusive. At the beginning of the day, a number between 1 and 24 was drawn from a box and that number was used to determine the day's injection sequence. There were 89 successful experiments described in this report. To have achieved "perfect randomness", each injection sequence should have been used 3.7 times. There were two injection sequences utilized only twice (Ru-Cr-Sc-Ce; Cr-Ce-Ru-Sc), four injection sequences used five times (Ru-Sc-Ce-Cr; Sc-Ce-Cr-Ru; Cr-Ru-Sc-Ce; Sc-Ru-Ce-Cr), and one sequence used six times (Ce-Ru-Sc-Cr). The remaining 17 combinations were used in 3 or 4 experiments.

At the end of the acute experiment, all hearts were electrically fibrillated, removed from the chest, and washed thoroughly in cold tap water. In experiments where infarct size was determined (see Section F), the left ventricle was incubated in nitroblue tetrazolium, divided into infarcted and non-infarcted myocardium, weighed, and then placed in 20% formalin. In all other experiments, following the cold tap water rinse, hearts were fixed in 20% formalin solution for 12-24 hours. After this fixation period, hearts were again rinsed in cold tap water and multiple transmural tissue samples taken from both ventricles. These transmural tissue samples were divided into endocardial and epicardial portions, weighed, and placed in plastic tubes. Arterial blood obtained during each microsphere injection and distilled water used to rinse the glass withdrawal syringes were also placed in plastic tubes and capped. Tissue, blood, and rinse samples, along with pure reference isotope standards for each of the four isotopes used, were assayed for radioactivity at predetermined energy settings on a Searle

1185 Automatic Gamma Counter. Raw data were placed on paper tape and entered into a computer. Isotope-to-isotope interference correction coefficients were calculated and incorporated into a computer program (6) which converted radioactive counts to myocardial blood flow using the equation:

$$MBF = (C_t/TW) \times (RBW/C_b) \times 100$$

RMBF = Myocardial blood flow in ml/min/100 g

C_t = Tissue radioactivity in counts/min

TW = Tissue sample weight in g

RBW = Reference blood withdrawal rate in ml/min

C_b = Total radioactivity in the reference blood sample

Several previous investigators have established the validity of the tracer microsphere technique in the study of regional myocardial blood flow distribution (25, 39, 135, 157, 206). Although certain limitations of the technique must be recognized (2, 78, 25), microspheres remain a powerful tool in assessing changes in regional myocardial blood flow.

The techniques in this dissertation involving microsphere use adhered to several basic rules. First, repeated injections of the tracer microspheres in a given experiment had to result in reproducible values for flow distribution in areas unaffected by the LAD occlusion. The areas examined included several transmural tissue samples from various regions of the right ventricle, as well as at least three transmural tissue samples divided into endocardial and epicardial portions from left ventricular myocardium perfused by the circumflex coronary artery, the posterior descending coronary artery, and arteries

perfusing the septum uninvolved in the LAD occlusion. Second, as stated above, hemodynamic and heart rate parameters were continuously monitored. Any microsphere injection resulting in an "injection arrhythmia" was excluded from the data. Additionally, any injection resulting in a hypotensive reaction to the Tween 80 (127) was excluded. In only four experiments was the blood pressure response described by Millard et al. (127) observed. Third, in each experiment at least 4 million radiolabeled microspheres were injected. This insured that tissue samples of .5g or larger, with myocardial blood flow values greater than 20 ml/min/100g would have accurate blood flow values based on the criteria of a minimum of 400 microspheres per sample (25). In tissue samples less than 0.5g or with myocardial blood flow less than 20 ml/min/100g, an error of larger proportions has been described (25). It was anticipated that during ischemia the number of microspheres present in certain tissue samples would be less than the required 400 microspheres per sample. In each sample, repeated injections were examined for blood flow reproducibility in the samples containing low numbers of microspheres. Extreme variability in calculated blood flow resulted in the exclusion of samples that potentially had less than 400 microspheres. Fourth, apparent loss of microspheres from necrotic tissue has been described for infarcts over twelve hours old (99). Utilization of nitroblue tetrazolium to determine infarct size 6 hours after LAD occlusion eliminated the possibility of the acute loss of microspheres. Finally, 15 $\pm$ 2 micron microspheres were used in this study because very little shunting (less than 2% of the total number of injected microspheres) is reported for this size microsphere (54).

D. Local Unipolar and Bipolar Electrogram Recordings

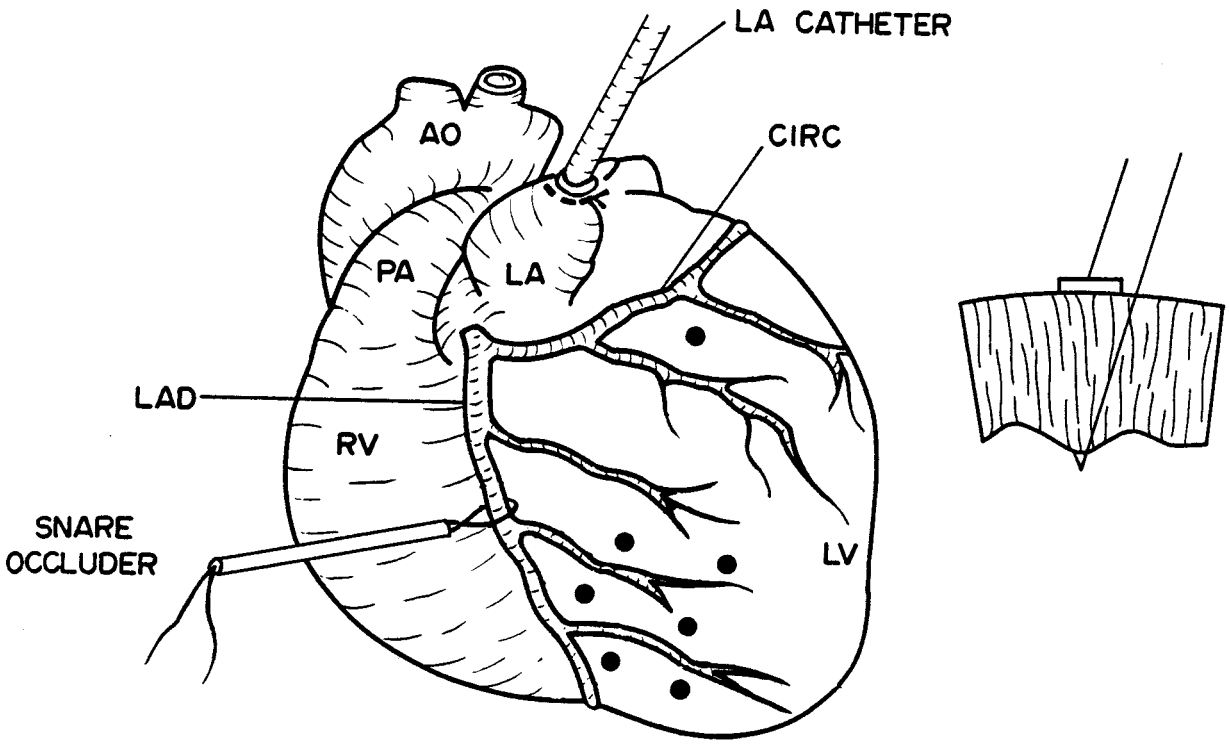
In several experiments changes in S-T segment elevation or myocardial activation were assessed. This was done through the use of unipolar or bipolar electrodes secured to the heart.

Unipolar electrograms (S-T segment) were recorded both endocardially and epicardially. Endocardial unipolar recording electrodes consisted of 25 gauge needles threaded with a single Teflon coated stainless steel wire (.005 inch diameter) bent at the tip to form a small hook. No insulation was stripped from the wire so the only point of contact with the myocardium was the cut end of the wire. The needles were inserted transmurally and withdrawn leaving the wires hooked in the endocardial muscle. Multiple endocardial sites were examined and the approximate locations shown in Figure 3. Epicardial unipolar electrodes were constructed of the same stainless steel wire embedded in the center of an acrylic plaque. The plaques were secured to the surface of the heart using a polycyanoacrylate ester (Eastman 910) (87). Epicardial and endocardial electrodes were paired transmurally (Figure 3) to record simultaneous endocardial and epicardial injury.

Each unipolar electrode lead was connected to a selector switch box which allowed rapid sampling of all sites. The electrograms were amplified with an AC preamplifier (time constant 0.1 sec) set to pass frequencies of .1 to 100 Hz. Utilization of an AC preamplifier did not allow separation of S-T segment elevation from T-Q segment depression in these recordings, therefore the combined S-T elevation - T-Q depression was termed S-T segment elevation in this study (see Figure 4). Signals were displayed on a Grass polygraph at a calibrated sensitivity of 5

FIGURE 3

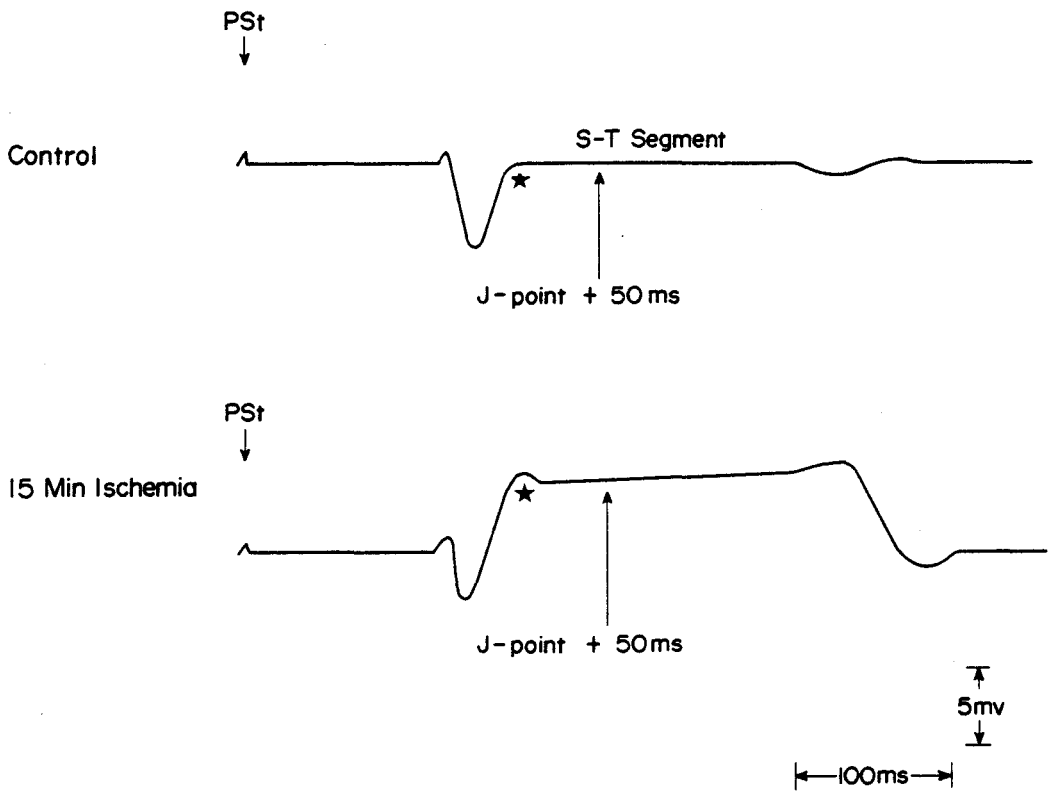
SCHEMATIC REPRESENTATION OF THE EXPERIMENTAL HEART



Schematic representation of the heart demonstrating the approximate location of the snare occluder and the paired unipolar electrode sampling sites (o). Also depicted is a representation of the actual transmural pairing of an epicardial plaque electrode and an endocardial plunge electrode. AO = aorta; CIRC = circumflex coronary artery; LAD = left anterior descending coronary artery; LA = left atrium; LV = left ventricle; PA = pulmonary artery; RV = right ventricle.

FIGURE 4

SCHEMATIC REPRESENTATION OF UNIPOLAR ELECTROGRAM



Schematic representation of an actual epicardial unipolar electrogram taken during one experiment reported in this dissertation. Control, pre-occlusion, electrograms often demonstrated small pacing artefacts (PSt) with distinct QRS complexes, isoelectric S-T segments and biphasic T-wave deflections. After a period of ischemia (15 min) the S-T segment in the ischemic regions was elevated, while those in non-ischemic areas demonstrated no change with occlusion. S-T elevation was measured at a point 50 ms from the J-point of the electrogram. The star on the traces denotes the location of the J-point.

mv/cm and a paper speed of 100 mm/sec. Measurement of S-T segment elevation was made 50 msec after the J-point of the electrogram (Figure 4) (87).

Bipolar recording electrodes (myocardial activation) consisted of 21-gauge needles threaded with two Teflon-coated stainless steel wires bent at the tip to form a hook. The needles were inserted subepicardially (approximately 2 mm into the heart muscle) and subendocardially (approximately 8 mm into the heart muscle) in normal myocardium and in myocardium that potentially would be made ischemic during coronary artery occlusion. Each electrogram was amplified by an AC preamplifier (time constant 0.1 sec) set to pass frequencies of 30 to 3,000 Hz. Signals were simultaneously displayed on a Tektronix storage oscilloscope (Model 5111) at a sweep speed of 200 mm/sec, and on a Grass polygraph at a paper speed of 100 mm/sec. Bipolar electrogram duration, amplitude and time-to-onset were examined (Figure 5).

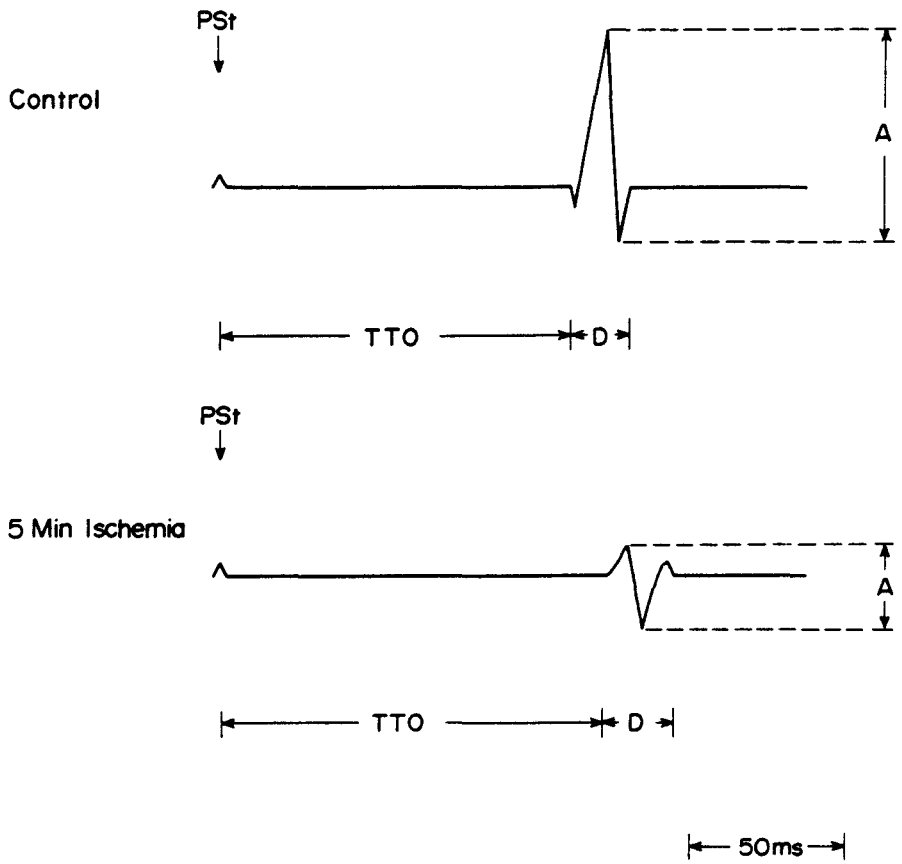
E. Left Anterior Descending Coronary Artery Occlusion and Cardiac Denervation

In all experiments described in this dissertation, coronary occlusion consisted of ligation of the left anterior descending coronary artery (LAD) at a level just above the second diagonal branch (Figure 3).

In 10 successfully denervated and 10 sham-denervated dog hearts, regional myocardial blood flow, S-T segment values, and arrhythmia were evaluated before and during a 20-minute LAD occlusion. These animals were prepared as described in Sections A and B of these methods. The LAD was dissected free from surrounding myocardium and a single piece of black silk placed around the artery. Both ends of the silk were passed

FIGURE 5

SCHEMATIC REPRESENTATION OF BIPOLAR ELECTROGRAM



Schematic representation of actual bipolar electrogram recording demonstrating the measured parameters of electrogram duration (D), amplitude (A) and time-to-onset (TTO) from the pacing stimulus (PSt). Shown are pre-occlusion (CONTROL) and post-occlusion tracings of an electrode in the ischemic zone.

through a piece of polyethylene tubing forming a snare that was used to occlude the artery. In each heart multiple endocardial and epicardial unipolar electrograms were recorded as described in Section D of the methods. Control, pre-occlusion measurements were made, and the LAD abruptly ligated. Fifteen minutes after LAD occlusion, changes in regional S-T segment elevation were evaluated and compared to the regional myocardial blood flow. Quantitation of arrhythmia during occlusion was accomplished simply by counting the number of premature ventricular beats from the onset of occlusion up to the subsequent release of the occlusion at 20 minutes. Incidents of ventricular tachycardia and ventricular fibrillation were also noted.

Following release of the occlusion, hearts were electrically fibrillated, removed from the chest, rinsed in cold tap water with the electrodes still in place, and fixed in formalin. After fixation, small myocardial tissue samples containing the electrode pairs were isolated, separated into endocardial and epicardial sections and processed for myocardial blood flow. The regional myocardial blood flow at each site was compared to the S-T segment elevation recorded at that site.

F. Left Anterior Descending Coronary Artery Occlusion and Infarct Size Determination

Marked variations in coronary artery anatomy may result in a wide variability when examining left ventricular infarct size following coronary artery occlusion. Therefore, it was considered necessary in this portion of the study to utilize a relatively standard model of LAD occlusion in order to compare left ventricular infarct size under varying conditions. If the LAD was found to 1) course along the interventricular sulcus, 2) have at least two diagonal branches above

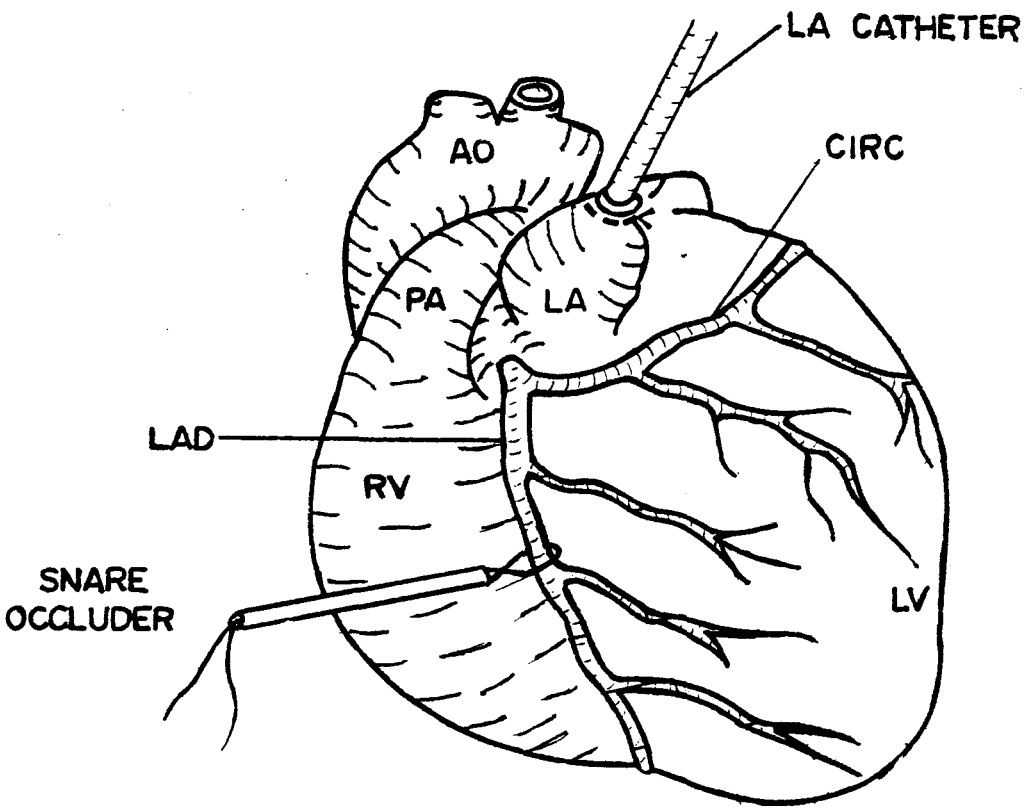
the apical branch, and 3) demonstrate no other noticeable abnormalities such as visible epicardial collateral channels or anastomoses with other coronary arteries, the LAD anatomy was considered "normal" and acceptable for an infarct size study. The artery was carefully dissected free from surrounding tissue above the second diagonal branch and two pieces of 2-0 silk were placed underneath the vessel. The free ends of the silk threads were passed through a short piece of polyvinylchloride tubing forming a snare similar to the one described above (Figure 6).

In a series of 37 dogs, experiments to define the role of the cardiac sympathetics on infarct size and regional myocardial blood flow were performed. In all dogs, regional myocardial blood flow was determined after at least 15 minutes of pacing at a rate of 150 beats/min to ensure relatively stable blood flow patterns. Following the initial microsphere injection, the LAD was abruptly ligated by pulling the snare occluder and clamping the tubing with a hemostat. Complete occlusion of the LAD was assured by visualizing the LAD inside the polyvinylchloride tubing itself. LAD occlusion was maintained in all preparations for at least six hours, during which time three subsequent microsphere injections were given at specified intervals.

The 37 dogs examined were divided into four groups. Group 1 control dogs (N = 16) were decentralized at the time of the acute experiment (Section B). These animals were considered to have no neural input to the heart throughout the occlusion protocol. Group 2 dogs (N = 5) were confirmed to have been chronically denervated and therefore without neural input to the heart for 2-4 weeks. Group 3 dogs (N = 8) were confirmed to have been chronically sympathectomized yet still

FIGURE 6

SITE OF LAD OCCLUSION AND "NORMAL" LAD ANATOMY
FOR AN INFARCT SIZE DETERMINATION



Schematic representation of the "normal" LAD coronary anatomy utilized in the infarct size studies. Symbols in this figure are the same as described in Figure 3.

demonstrated parasympathetic responses to direct vagal stimulation. Group 4 dogs (N = 8) were prepared in a manner similar to Group 1 animals, except at the time pacing was initiated, tonic left stellate nerve stimulation was also begun.

Tonic stellate nerve stimulation was accomplished using a bipolar electrode and a Grass SD9 stimulator. The stimulator was programmed to deliver square wave pulses (5 msec, 1-4 volts, 1-3 mamp) at a rate of 2-4 pulses/sec. This frequency has been described as the so called "tonic" firing rate of the cardiac sympathetics (23, 58, 197). The electrode was positioned to stimulate the peripheral section of the decentralized left ansae subclavia and was held in place by a magnetic base, flexible electrode holder. Stimulation at these parameters initially increased contractility slightly (10-15%) but contractility returned toward control within 5-10 minutes. Stimulus current was monitored throughout the entire LAD occlusion, and periodic examination of stimulus effectiveness (increased stimulus rate and/or current) demonstrated electrode contact throughout the entire 6 hours.

At the end of the 6 hours of occlusion, hearts were electrically fibrillated, removed from the chest, and washed in cold tap water. The right ventricle, atria, and valvular rings were dissected from the left ventricle and placed in formalin. The left ventricle was sliced into 6-10 cross-sectional rings of tissue approximately 0.7 cm in thickness. These tissue rings were quickly rinsed in cold tap water and placed in a solution of distilled water (288 ml), phosphate buffer (32 ml, pH = 7.4) and nitroblue tetrazolium (100 mg, Sigma Company) at 37°C for 7-12 minutes (95). Upon completion of the incubation, the tissue rings were

rinsed, carefully separated into infarcted (unstained), non-infarcted (stained) and mixed (patchy stain) tissue regions, and weighed. Infarct size expressed as a percent of left ventricular weight (unstained tissue weight + patchy stain tissue weight ÷ total weight of left ventricle x 100%), was determined in each heart. Following infarct size determination, stained, unstained, and patchy stained left ventricular tissues were placed in formalin overnight. Regional myocardial blood flow in each of the three regions was determined by the techniques described in Section C of the methods.

G. Repeated Occlusion of the Left Anterior Descending Coronary Artery

In another series of experiments, two groups of animals were subjected to a short period of LAD occlusion (5 min) followed by reperfusion (3 min) before permanent ligation of the LAD. These animals were prepared similarly to those in Section E and required "normal" LAD anatomy. The LAD of eight dogs was briefly occluded, reperfused, then permanently occluded in the absence of neural input (decentralized), and eight dogs were subjected to the identical protocol in the presence of left ansae subclavia stimulation. Regional myocardial blood flow was determined before occlusion, 2 minutes after the first LAD occlusion, 2 minutes after the second LAD occlusion, and 120 minutes after the second LAD occlusion. Six hours after the second LAD occlusion, the hearts were electrically fibrillated and infarct size determined as previously described. Regional myocardial blood flow in infarcted, patchy and normal myocardium was determined in these animals.

A number of animals did not demonstrate the "normal" LAD anatomy previously described and therefore were not included in the infarct size

studies. If the coronary anatomy was not aberant though, the animal was utilized in a second repeated occlusion study. In this protocol, short periods of LAD occlusion (5 min) were separated by brief (3 min) or prolonged (40-60 min) periods of reperfusion. Each animal was subjected to 4 short occlusions during which regional myocardial blood flow, endocardial and epicardial S-T segment, myocardial activation, and arrhythmia were evaluated in ischemic and normal regions. Animals were prepared as described in Section B, except coronary occlusion was performed by retracting the silk ligature and placing a small clip occluder on the artery. This insured that 1) the artery was minimally traumatized throughout the protocol, and 2) re-establishment of blood flow upon release of the occlusion was rapid. Eight dogs underwent the multiple occlusion protocol in the absence of neural input, and eight dogs received left sympathetic stimulation as previously described. Regional myocardial blood flow was determined after 2 minutes of ischemia. During each occlusion, S-T segment and myocardial activation measurements were taken before and immediately prior to the release of occlusion. Arrhythmia was quantitated as premature ventricular beats during the 5 minutes of occlusion. Episodes of ventricular tachycardia and ventricular fibrillation were also noted when present. At the end of the protocol, the LAD distal to the silk ligature was perfused with Pelikan Ink. The ink delineated the region of perfusion of the LAD, and allowed assessment of whether electrodes located in the ischemic region were positioned accurately. The heart was then electrically fibrillated, excised, washed thoroughly, and placed in formalin with the electrodes still in position. Myocardial blood flow was determined in tissue samples adjacent

to each electrode site by the techniques previously described.

Appendix I, found at the end of this document, contains a summary of all experimental groups utilized in this dissertation. Also included are the number of successful experiments as well as explanations for exclusion of animals from certain experimental groups.

H. Data Analysis

All results were expressed as mean plus or minus the standard error of the mean (± SEM). The statistical significance of differences was determined using the appropriate Student's t-test for paired or unpaired data, or the randomization test for matched paired data. When multiple group comparisons were made, a one-way or two-way analysis of variance was performed. When an analysis of variance demonstrated, a significance between some data group, a t-test, Scheffe test or least significant differences test was used to determine significance between individual means. Significance was considered to be present when a $p < .05$ was achieved. Statistical techniques were taken from Gilbert (62), and Snedecor and Cochran (180).

CHAPTER IV

RESULTS

A. Effect of Chronic Cardiac Denervation on Myocardial Blood Flow and S-T Segment Changes During Ischemia

For this series, 12 surgically denervated and 12 sham-denervated animals were prepared. At the time of the acute experiment, two of the animals in the denervated group had to be excluded due to incomplete denervation. Two of the sham-denervated dogs died with ventricular fibrillation during occlusion of the LAD and were excluded from the study. None of the denervated dogs had ventricular fibrillation during coronary occlusion.

During pacing (150 beats/min), mean aortic blood pressure and peak systolic aortic blood pressure in the denervated group were not different from those in the sham-denervated group either before or during coronary occlusion (Table 2). The double product (heart rate x peak systolic pressure) used as an index of cardiac work was not significantly different between the two groups.

Figure 7 shows left (LV) and right (RV) ventricular blood flow values for the two groups before coronary occlusion. In denervated hearts, left ventricular endocardial (endo) and epicardial (epi) blood flows were significantly lower than in sham-denervated hearts. Blood flows in the endocardium and epicardium of the right ventricle of the denervated group were not significantly different from those in the sham-denervated group.

TABLE 2

HEMODYNAMIC DATA BEFORE AND AFTER FIFTEEN MINUTES OF LAD OCCLUSION (EXPRESSED AS MEAN ± SEM)

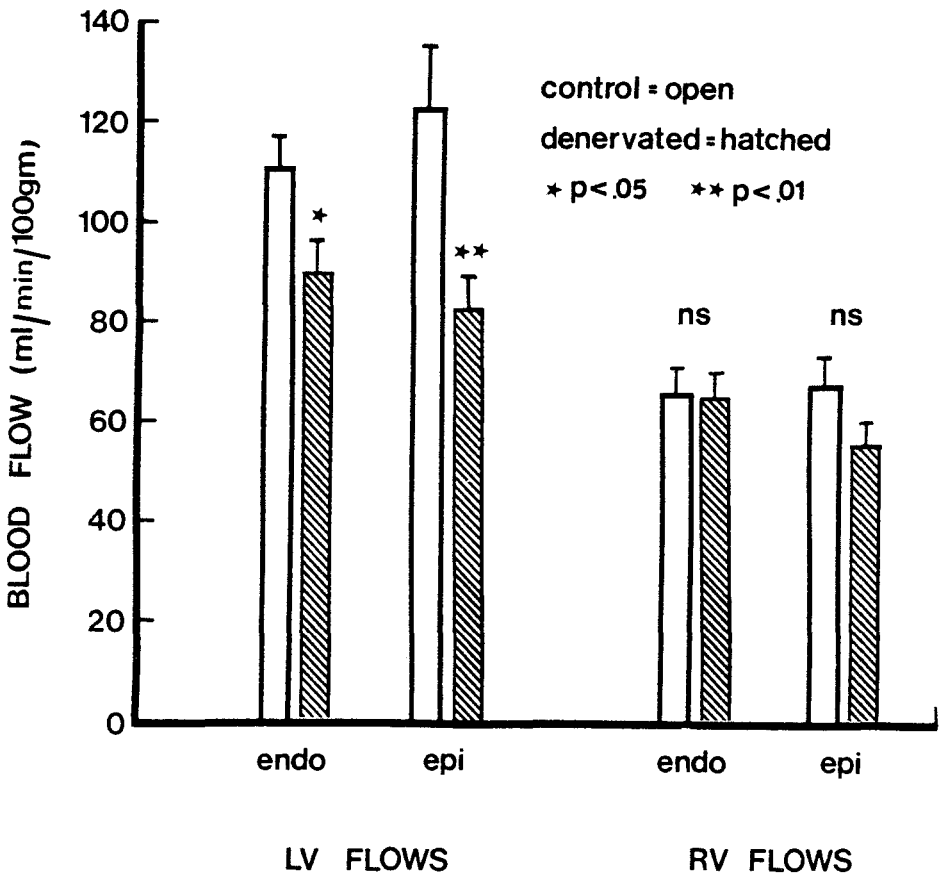
	Control*	Denervated*
Aortic blood pressure (mm Hg)		
Before occlusion	118 ± 4	115 ± 6
After occlusion	110 ± 4	112 ± 5
Peak systolic aortic pressure (mm Hg)		
Before occlusion	145 ± 5	144 ± 6
After occlusion	138 ± 4	141 ± 6
Double product (mm Hg)†		
Before occlusion	21,700 ± 740	21,500 ± 900
After occlusion	20,700 ± 590	21,100 ± 870

* All statistical comparisons of control versus denervated hearts and values before and after coronary occlusion yielded a probability (p) value of $p > 0.05$.

† Heart rate × peak systolic aortic pressure.

FIGURE 7

REGIONAL MYOCARDIAL BLOOD FLOW IN THE DENERVATED AND SHAM-DENERVATED VENTRICLES PRIOR TO OCCLUSION

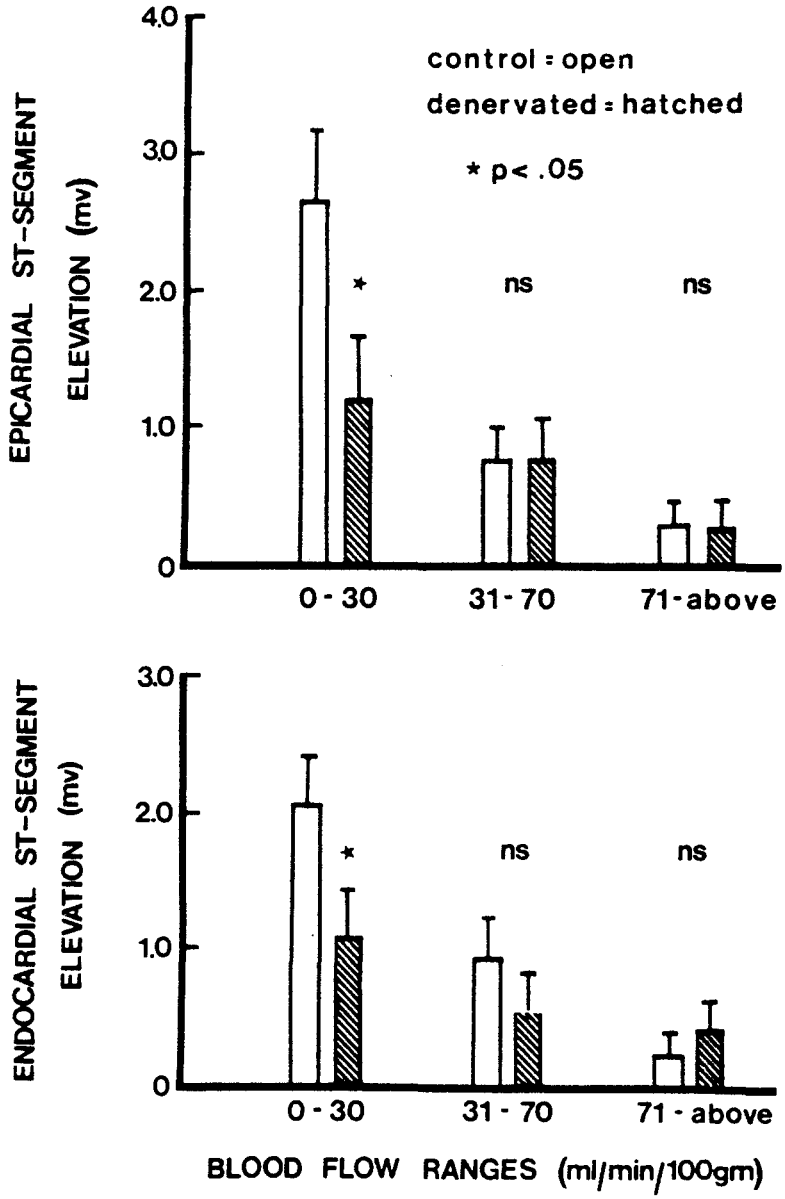


Regional myocardial blood flow in denervated (N = 10) and sham-denervated (N = 10) hearts prior to the complete occlusion of the LAD. Tissue samples from the right ventricle (RV) were taken from multiple regions of the RV free wall. Control left ventricular (LV) blood flow values were determined from samples perfused by the LAD, circumflex and posterior descending coronary arteries, as well as the ventricular septum. In all hearts comparable regions of the LV and RV were sampled. The apex was not used to determine control blood flow values. Statistical comparisons were made using unpaired t-tests.

The tissue samples in which both blood flow and S-T segments were measured at 15 minutes of occlusion were divided into three blood flow groups: 1) a zone of drastically reduced blood flow (0-30 ml/min/100g); 2) a zone of moderately reduced blood flow (31-70 ml/min/100g); and 3) a zone of relatively normal blood flow (greater than 70 ml/min/100g). Figure 8 shows the mean $\pm$ SEM for the S-T segment elevations of the epicardial and endocardial tissue sites in each blood flow range. Prior to occlusion of the LAD, endocardial S-T segment values averaged 0.5 $\pm$ 0.3 mv in denervated hearts and 0.3 $\pm$ 0.2 mv in sham-denervated hearts. Initial epicardial S-T segment values (0.5 $\pm$ 0.2 mv in sites sampled in denervated hearts and 0.5 $\pm$ 0.3 mv in sham-denervated hearts) were comparable to the pre-occlusion endocardial values. Following 15 minutes of LAD occlusion, S-T segment elevation from the pre-occlusion level was plotted as a function of the flow range of the tissue in which the electrode was embedded. Following LAD occlusion, there were no significant differences in S-T segment elevation between the denervated and sham-denervated hearts in blood flow zones 2 or 3. In the low blood flow region (zone 1) however, both epicardial and endocardial S-T segment elevations in the denervated sites were significantly less ($p < .05$) than those found in sham-denervated sites. In all tissue sites where S-T segment changes were measured, and regional myocardial blood flow was less than 30 ml/min/100g (zone 1), an average blood flow value for denervated and sham-denervated endocardial and epicardial tissue samples was obtained. The mean blood flow for the test sites sampled in the low flow zones of the two experimental groups was not significantly different either endocardially or epicardially as shown in Figure 9.

FIGURE 8

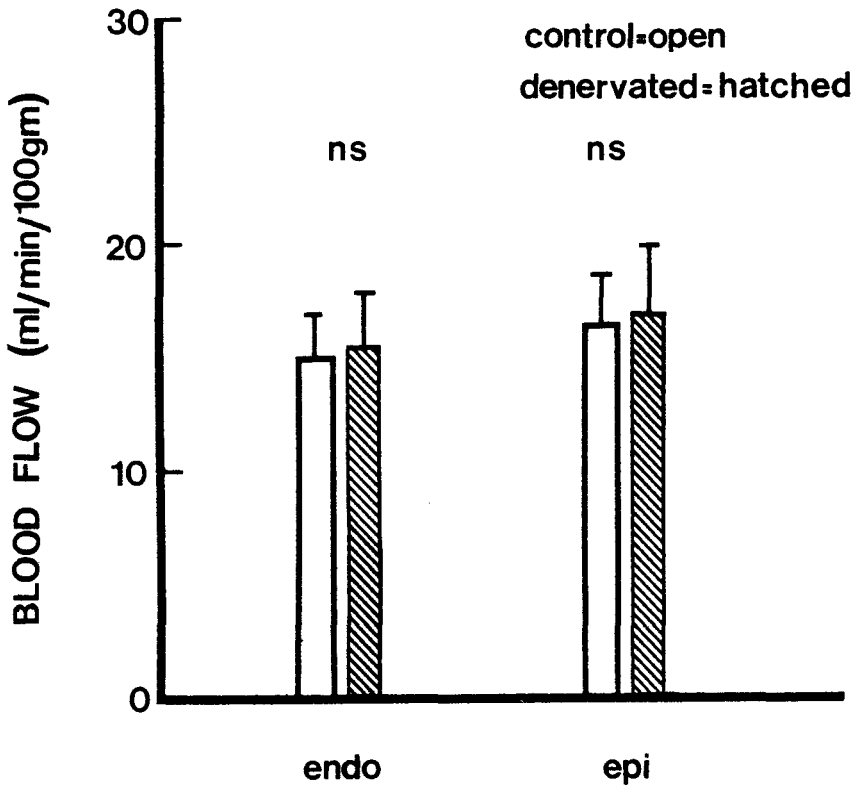
LOCAL ENDOCARDIAL AND EPICARDIAL S-T SEGMENT CHANGES
IN DENERVATED AND SHAM-DENERVATED LEFT VENTRICLES DURING LAD OCCLUSION



Regional S-T segment changes compared with the regional myocardial blood flow present at those sites. Blood flow ranges are in ml/min/100g, and correspond to the three zones described in the text. All S-T segment values are expressed as changes in mv from the pre-occlusion values. ns = not statistically significant as demonstrated by unpaired t-test.

FIGURE 9

MEAN BLOOD FLOW IN LOW FLOW LEFT VENTRICULAR SAMPLES



Mean blood flow for all S-T segment sample sites in which the measured myocardial blood flow was less than 30 ml/min/100g. Even though denervated hearts demonstrated less evidence of electrical injury (Figure 8), this difference was not explained on the basis of marked alteration in blood flow as measured by microspheres. ns = not significantly different as demonstrated by unpaired t-test.

This demonstrated that comparable sites from the low flow zones were sampled in both series of animals.

During each experiment, the number of premature ventricular beats that occurred during the 20 minutes of LAD occlusion were counted. The sham-denervated control group had a significantly greater number of premature ventricular beats than did the denervated animals during the 20-minute occlusion (89 $\pm$ 16 ventricular beats in control animals vs 4 $\pm$ 2 ventricular beats in denervated dogs; $p<.001$). In addition, as stated above, two of the sham-denervated dogs died in ventricular fibrillation before completion of the protocol (blood flow or S-T data for these animals were not included), while no denervated dogs succumbed during LAD occlusion.

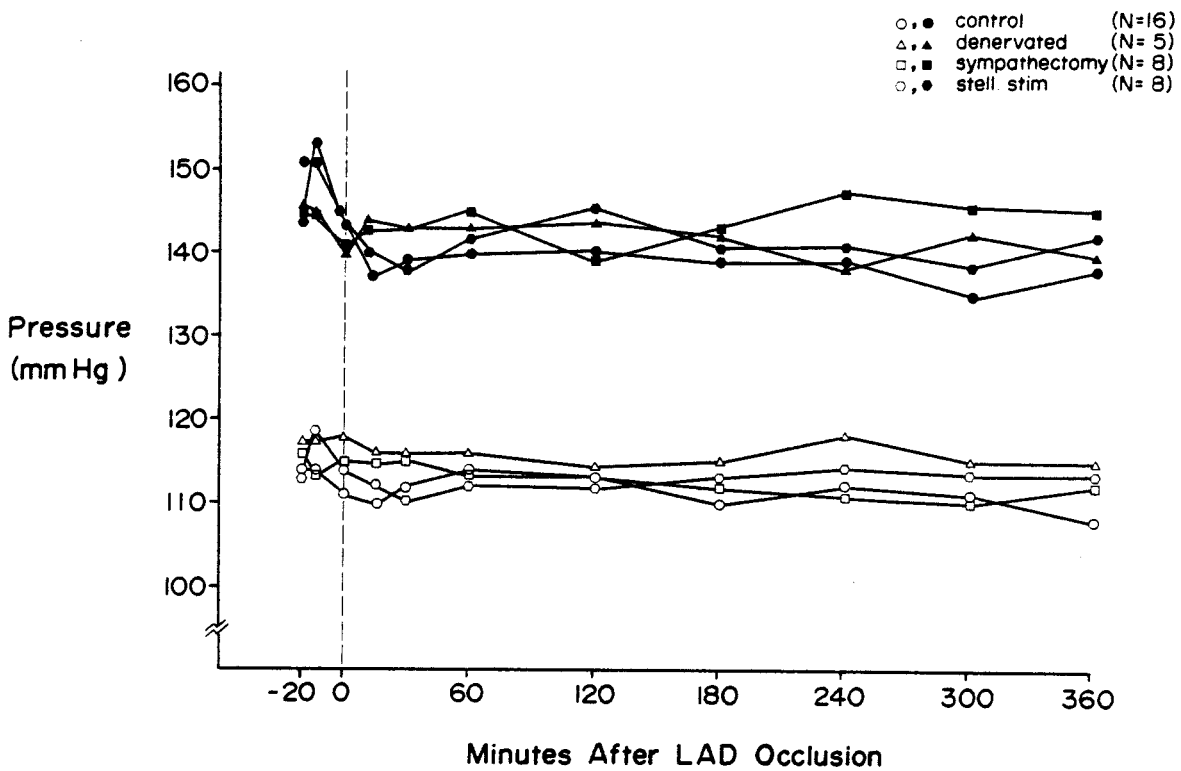
B. Myocardial Blood Flow and Infarction: Evidence for an Alteration in the Critical Blood Flow Following Denervation

Thirty-seven dogs survived 6 hours of LAD occlusion and were included in this portion of the study. These 37 animals consisted of the four groups described in section F of the methods. All animals maintained adequate hemodynamic parameters for the entire 6 hours (Figure 10). Mean arterial blood pressure and peak systolic pressure remained relatively stable in all four groups over the time measured.

Figure 11 shows actual raw traces from one of the 6-hour infarct experiments (dog #SNS-3). Prior to the injection of the first species of radiolabeled microspheres, measurement of arterial blood gases and arterial pH was made. If blood gases and pH were within the specified ranges (see methods), the pre-occlusion microsphere injection was given. All dogs demonstrating value(s) outside specified ranges were adjusted to values within the range by altering the respiratory rate and/or by

FIGURE 10

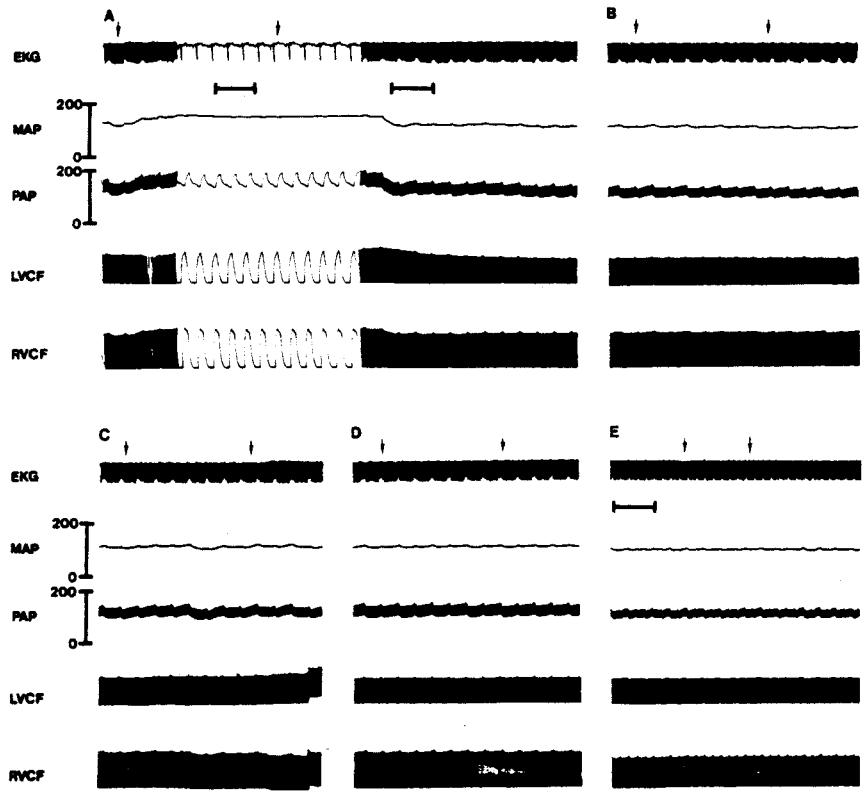
MEAN ARTERIAL AND PEAK SYSTOLIC PRESSURES
FOR INFARCT SIZE EXPERIMENTS



Hemodynamic parameters measured during the course of the infarct size experiments. Mean arterial pressure (open symbols) and peak systolic pressure (closed symbols) prior to and during 6 hours of LAD occlusion are shown for control, denervated, sympathectomized, and tonic sympathetic nerve stimulation (stell stim) preparations. Negative time values represent pre-occlusion measurements. The four groups are as described in the text. No significant differences as demonstrated by an analysis of variance with respect to either parameter were seen throughout the protocol.

FIGURE 11

EXPERIMENT FROM THE SIX-HOUR INFARCT PROTOCOL



Recordings of lead II electrocardiogram (EKG), mean arterial blood pressure (MAP), pulsatile arterial blood pressure (PAP), left (LVCF) and right (RVCF) ventricular contractile force during a single six-hour infarct size experiment (dog #SNS-3). All pressures are in mmHg. The response to the onset of left ansae subclavia stimulation (10 cycles/sec) is shown in panel A of this figure by the first arrow. The second arrow in panel A denotes a decrease in stimulus frequency to 3 cycles/sec. An obvious response to sympathetic activation is present. The fast trace in this panel has a time marker corresponding to 1 sec, while the remainder of this trace, as well as panels B, C, and D correspond to the second time marker representing 15 sec. Panels B-E were recorded during the onset of reference blood withdrawal (first arrow in these panels). Panel B was recorded prior to LAD occlusion (Cr-51 injection), while panels C, D, and E were recorded 2 (Sc-46), 15 (Ce-141) and 360 (Ru-103) minutes after LAD occlusion. The time bar in panel E signifies an interval of 30 sec.

infusing sodium bicarbonate intravenously. Fifteen minutes were allowed for equilibration to occur and repeat measurements of the arterial blood gases and pH made. If the new levels were adequate, the control injection of microspheres was given. Further adjustment was made when required and the experiment continued. Following LAD occlusion, arterial blood samples were drawn every 20 minutes for the first hour, and then approximately every 45 minutes for the remainder of the experiment. Adjustment of respiratory rate and arterial pH were made as required.

Table 3 shows the data for the infarct sizes of the four experimental groups. Infarct size as a percent of left ventricular weight was calculated by weighing tissue that was totally unstained or patchy stained, dividing this quantity by the weight of the total left ventricle, and multiplying by 100. Data in the table include the means $\pm$ SEM for left ventricular (LV) weight, infarct weight, infarct size as a percent of left ventricular weight, patchy tissue weight, and patchy infarct size as a percent of left ventricular weight.

Total left ventricular weight was not significantly different ($p > .05$) from the left ventricular weight of control dogs in the denervated, sympathectomized or sympathetic nerve stimulation groups. Infarct size was found to be significantly reduced in both the denervated ($p < .05$ when compared with control) and sympathectomized ($p < .05$ when compared with control) groups, while the tonic left stellate nerve stimulation dogs demonstrated a significantly greater degree of infarction ($p < .05$) when compared with control. Comparisons of infarct weights and patchy weights were not made.

Composition of the infarct as determined by quantitating patchy

TABLE 3

INFARCT SIZE DATA FOR ACUTELY DECENTRALIZED, CHRONICALLY DENERVATED AND SYMPATHETIC NERVE STIMULATION EXPERIMENTS

	LV Weight (g)	Infarct Weight (g)	Infarct Size (% LV)	Patchy Weight (g)	Patchy Size (% LV)	Transmural Infarction
Control (N = 16)	110.4 ± 4.8	24.0 ± 2.2	21.5 ± 1.8	1.3 ± .2	1.2 ± .2	9/16
Denervated (N = 5)	92.7 ± 6.9	12.1 ± 2.2	13.1 ± 2.4*	2.9 ± .3	3.1 ± .4*	0/5
Sympathectomized (N = 8)	91.9 ± 7.7	13.6 ± 1.6	15.1 ± 1.8*	2.1 ± .3	2.5 ± .5*	0/8
Tonic Stellate Stimulation (N=8)	99.4 ± 8.5	27.4 ± 2.3	28.0 ± 1.7*	1.3 ± .3	1.4 ± .4	8/8

An analysis of variance was performed to compare LV weight, infarct size, and patchy size between the four groups of animals. If significance was established, remaining comparisons were made using the test of least significant differences; * = $p < .05$ when compared to control.

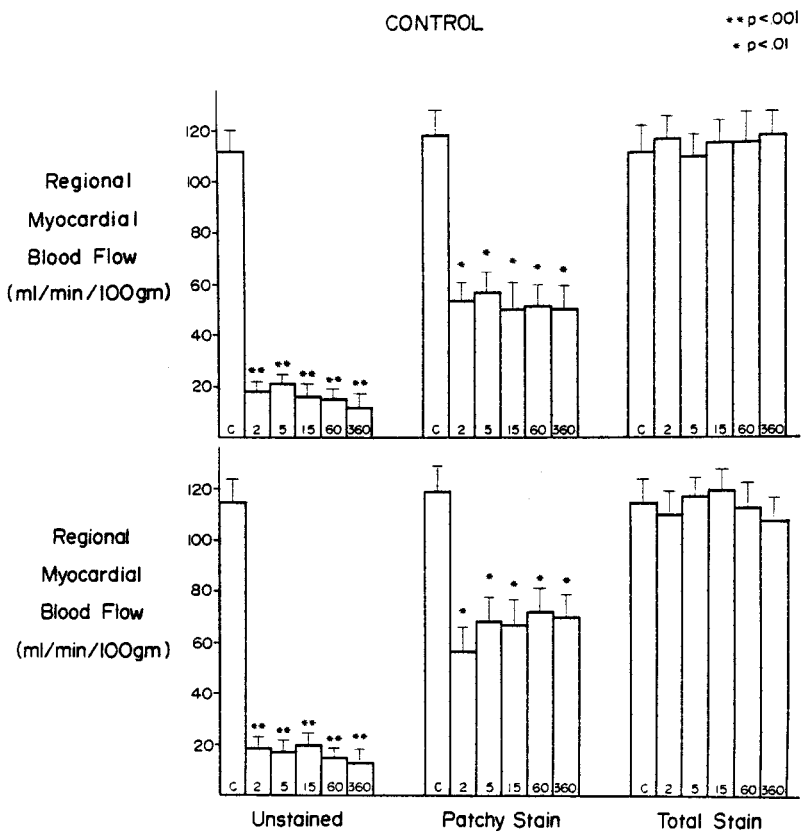
staining areas along the fringe of the infarct revealed significantly greater amounts of tissue demonstrating patchy stain in the denervated and sympathectomized groups. Infarcts from control, decentralized dogs were often transmural (9 of 16 animals), well circumscribed and demonstrated an easily defined central core of damage. Those hearts examined from the tonic stellate stimulation experiments demonstrated transmural infarction (8 of 8 animals), as well as a sharply defined zone of infarction. Infarcts from denervated and sympathectomized hearts generally showed a recognizable central core of damage (15 of 16 animals), but the infarct was surrounded laterally by a zone of patchiness. Also, epicardial sparing of at least 1 mm was present in all of these experiments, thus accounting for the absence of transmural infarction.

Left ventricular myocardial blood flow data for this study are shown in Figures 12-15. All four figures depict endocardial (top) and epicardial (bottom) blood flow before LAD occlusion (C) and at specific intervals following LAD occlusion. The numbers at the bottom of each bar represent the time in minutes after LAD occlusion that the values were obtained. Tissues from each heart were grouped according to their nitroblue tetrazolium staining characteristics into infarcted (all unstained tissue), partially infarcted (all patchy stained tissue) or normal (totally stained) myocardium.

Figure 12 shows the regional myocardial blood flow values for the endocardial and epicardial regions of the control group of animals. Pre-occlusion (C) endocardial blood flows were similar in all three zones. Average left ventricular endocardial blood flow before LAD occlusion was 113.8 $\pm$ 8.7 ml/min/100g. Two minutes after LAD occlusion,

FIGURE 12

REGIONAL MYOCARDIAL BLOOD FLOW IN TOTALLY INFARCTED, PARTIALLY INFARCTED AND NORMAL LEFT VENTRICLE OF ACUTELY DECENTRALIZED HEARTS



Left ventricular endocardial (top) and epicardial (bottom) blood flow in the three regions delineated by NBT staining. Myocardial blood flow was measured before LAD occlusion (C), and at 2, 5, 15, 60, and 360 minutes after LAD ligation (number at the bottom of each bar indicates the time after occlusion that blood flow was measured). Sixteen dogs were included in this portion of the study, and all sixteen dogs received microspheres at C and two minutes. Three different microsphere injection formats were followed after the two-minute determination such that six dogs received microspheres 15 and 360-minute post-occlusion, six dogs received microspheres 5 and 60-minute post-occlusion, and four dogs received microspheres at 5 and 15-minute post-occlusion. Therefore, data for time C and two minutes are compiled for sixteen animals, data for time 5 and 15 minutes are from ten dogs, and data for time 60 and 360 minutes are from six dogs.

blood flow in the infarct (unstained) region was less than 20% of the control myocardial blood flow of the area. Each of the four subsequent times examined throughout the period of LAD occlusion showed no significant change in flow from that seen at two minutes of occlusion. All post-occlusion values were significantly different ($p < .001$) from the pre-occlusion (C) value in the unstained tissue as shown by an analysis of variance. There was no evidence for a flow increase to the infarct (unstained) region. In the patchy staining region, endocardial blood flow was reduced approximately 50% at two minutes of occlusion. This was a significant reduction ($p < .01$) and remained at this level for the entire six-hour period. While measured flows in the unstained (infarcted) tissue varied from approximately zero in the very center of the infarct, up to a maximum 42 ml/min/100g on the edges of one infarct, within a given infarct the flow range was usually small (about 30 ml/min/100g). In contrast, patchy tissue, when present, demonstrated a much wider flow spread ranging from approximately 35 ml/min/100g up to about 85 ml/min/100g. Normal endocardium (total stain) appeared unaffected by occlusion as flow varied little throughout the period examined.

Epicardial blood flow changes in the three staining regions (lower portion of Figure 12) after LAD occlusion were similar to the endocardial changes. Average left ventricular epicardial blood flow before LAD occlusion was 115.1 ± 9.6 ml/min/100g. Infarcted epicardium exhibited very low blood flow at two minutes of occlusion, and flow appeared stable throughout the entire protocol (all values significantly lower than control; $p < .001$). The patchy region of infarction had flows approximately 50% of control two minutes after occlusion, and

though flow was higher at 5, 15, 60 and 360 minutes, this was not a significant change from the two-minute determination. All epicardial blood flow values in the patchy region after LAD occlusion were significantly different from control values in that region. Normal staining epicardium showed stable myocardial blood flow throughout the six hours.

In each heart, the mean myocardial blood flow, and the blood flow range were examined in the totally unstained (infarcted) region to determine a mean flow for the infarct and the maximum blood flow present in the infarct. As shown in the left hand portion of Table 4, in the control hearts, mean myocardial blood flow in the infarct region after two minutes of LAD occlusion was 18.0 $\pm$ 1.3 ml/min/100g. The two-minute point was chosen for two reasons 1) all 16 hearts received radiolabeled microspheres at this time, and 2) flow at 5, 15, 60 and 360 minutes did not differ from that determined at two minutes. The blood flow range observed in each infarct (right hand portion of Table 3) varied and was obtained by determining the high and low blood flow values calculated for each infarct. The maximum myocardial blood flow in each infarct was taken to represent the maximum level of blood flow delivery at which complete infarction of the myocardium occurred. The value at the bottom of the flow range column represents the mean $\pm$ SEM of the maximal flows observed in the 16 infarcts. This value (30.6 $\pm$ 1.6 ml/min/100g) was considered to be representative of the critical blood flow delivery. Myocardial blood flow levels above this value were able to salvage or maintain myocardial viability at the working conditions of this set of experiments.

Pre-occlusion left ventricular endocardial blood flow in the five

TABLE 4

AVERAGE AND MAXIMAL BLOOD FLOW IN INFARCTS OF ACUTELY DECENTRALIZED, CHRONICALLY DENERVATED AND TONIC SYMPATHETIC NERVE STIMULATION HEARTS

DOG#	FLOW IN INFARCT AT TWO MINUTES				BLOOD FLOW RANGE IN INFARCT			
	Control	Denervation	Sympathectomy	Nerve Stimulation	Control	Denervation	Sympathectomy	Nerve Stimulation
1	23.0	16.4	9.2	25.7	0 - 31	0 - 27	0 - 19	6 - 47
2	15.2	13.8	12.9	18.8	0 - 29	1 - 21	0 - 22	0 - 36
3	7.1	14.6	19.9	28.9	0 - 23	0 - 23	0 - 25	3 - 41
4	22.2	8.1	25.6	17.2	2 - 42	0 - 17	5 - 35	0 - 46
5	18.9	19.6	20.2	21.6	0 - 25	1 - 26	3 - 26	2 - 43
6	11.5	—	16.8	14.8	0 - 30	—	0 - 23	0 - 33
7	13.2	—	15.9	24.1	0 - 26	—	2 - 32	5 - 40
8	21.3	—	10.0	23.9	3 - 36	—	0 - 18	3 - 51
9	16.4	—	—	—	0 - 30	—	—	—
10	22.9	—	—	—	5 - 37	—	—	—
11	9.8	—	—	—	0 - 18	—	—	—
12	21.7	—	—	—	1 - 27	—	—	—
13	20.5	—	—	—	0 - 33	—	—	—
14	20.9	—	—	—	4 - 35	—	—	—
15	23.5	—	—	—	8 - 39	—	—	—
16	19.7	—	—	—	2 - 29	—	—	—
	18.0 ± 1.3	14.5 ± 1.9	16.3 ± 2.0	21.8 ± 1.7	30.6 ± 1.6	22.8 ± 1.8*	25.0 ± 2.1*	42.1 ± 2.1*

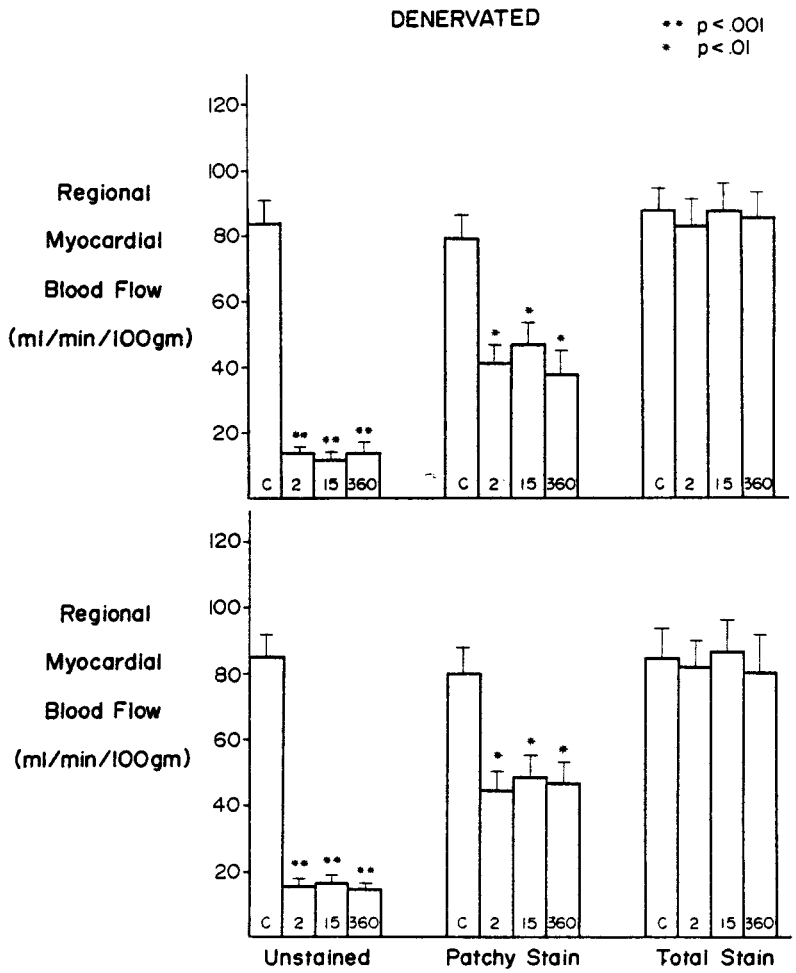
All values are expressed as ml/min/100g. All statistical comparisons were made to values from the control (acutely decentralized) group using a one-way analysis of variance and the test of least significant differences; * = p .05 from control.

denervated dogs (Figure 13) was 86.9 ± 7.1 ml/min/100g. This was significantly lower ($p<.05$) than the endocardial blood flow level seen in the control dogs and similar to the value reported for the 10 denervated hearts in section A of these results. Following LAD occlusion, flow measured in the infarct of the five denervated hearts fell to less than 20% of the pre-occlusion level and remained depressed for the entire 6 hours. Patchy stained tissue demonstrated endocardial blood flow values that were approximately 50% lower than the pre-occlusion flow and were unchanged at six hours. Normal endocardium remote from the occlusion site was unaffected by the LAD occlusion. Pre-occlusion epicardial blood flow in the denervated hearts was 84.2 ± 6.4 ml/min/100g, and after LAD occlusion, epicardial blood flows in each of the three regions of myocardiam were similar to those reported for the endocardium. The pre-occlusion epicardial flow in these denervated hearts was also significantly different from that observed in the control experiments ($p<.05$). The mean blood flow in the infarcts of the denervated hearts (Table 4) was lower than observed in the control hearts but the difference was not statistically significant. On the other hand, statistical analysis (least significant differences) of the critical blood flow value for the denervated hearts yielded a significant lowering ($p < .05$) of this value from the value determined in the control hearts. The microsphere technique, even with its limitations in the low flow range, is probably within the realm of its accuracy at these blood flow levels.

Figure 14 shows the endocardial and epicardial blood flow relationships in the ventricular sympathectomized dogs. Pre-occlusion endocardial (85.3 ± 8.0 ml/min/100g) and epicardial (81.9 ± 7.3 ml/min/100g)

FIGURE 13

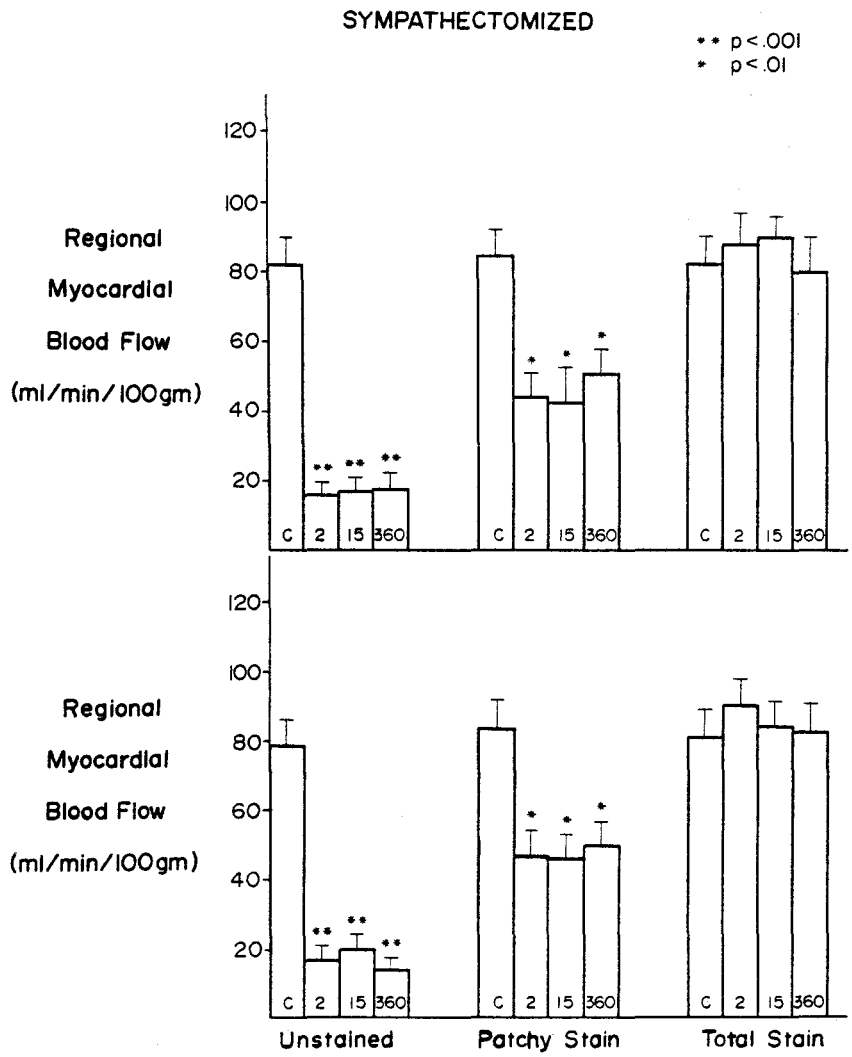
REGIONAL MYOCARDIAL BLOOD FLOW IN TOTALLY INFARCTED, PARTIALLY INFARCTED AND NORMAL LEFT VENTRICLE OF CHRONICALLY DENERVATED HEARTS



Left ventricular endocardial (top) and epicardial (bottom) blood flow in the three regions delineated by NBT staining. Myocardial blood flows were determined in the cardiac denervated hearts (N = 5) before (C) and 2, 15, and 360 minutes after LAD occlusion.

FIGURE 14

REGIONAL MYOCARDIAL BLOOD FLOW IN TOTALLY INFARCTED, PARTIALLY INFARCTED AND NORMAL LEFT VENTRICLE OF CHRONICALLY SYMPATHECTOMIZED HEARTS



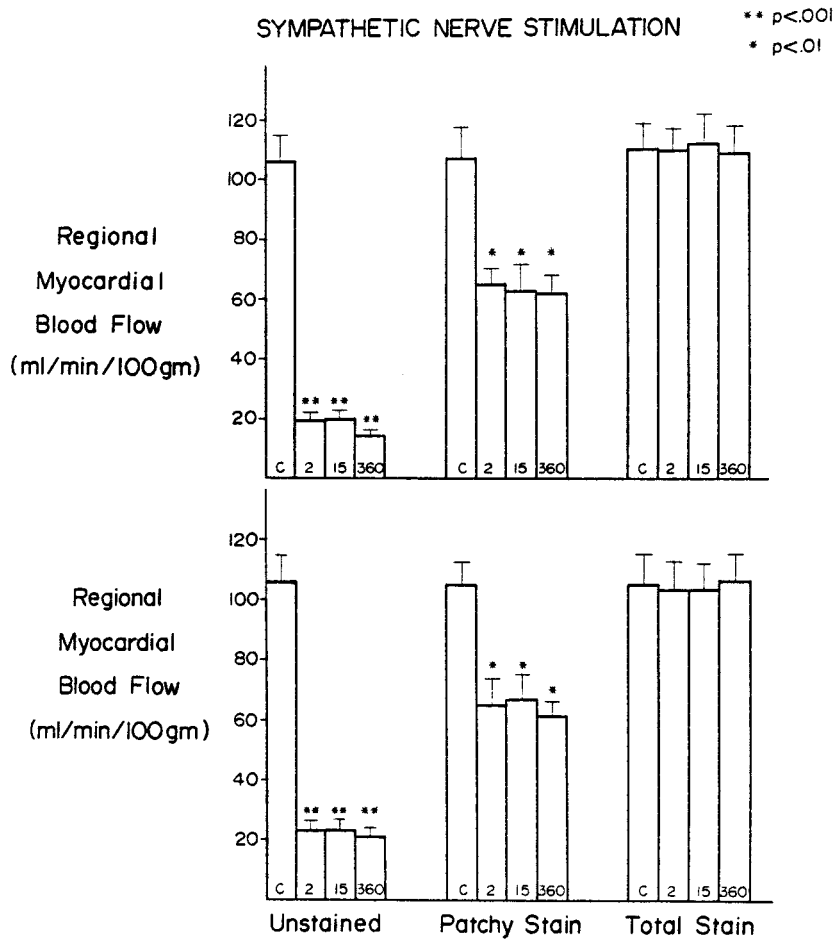
Left ventricular endocardial (top) and epicardial (bottom) blood flow in the three regions delineated by NBT staining. Blood flow was determined in ventricular sympathectomized hearts (N = 8) at the same time intervals described in Figure 13.

blood flow values were both significantly different from the control hearts ($p < .05$), but were not different from the values obtained in the denervated endocardium and epicardium. Following LAD ligation, mean myocardial blood flows in the infarcted endocardium and epicardium were 15.4 $\pm$ 1.7 ml/minl00g and 17.1 $\pm$ 2.0 ml/min/100g, respectively. The combined mean myocardial blood flow in the infarct at two minutes of LAD occlusion was 16.3 $\pm$ 2.0 ml/min/100g (Table 4). This value was not statistically different from the infarct flow value in control dogs. The critical blood flow found in the infarcted, sympathectomized left ventricle was 25.0 $\pm$ 2.1 ml/min/100g. This critical blood flow value was significantly lower ($p < .05$) than the control value and not different ($p > .05$) from the denervated group. Endocardial and epicardial blood flow in the patchy infarct and normal myocardium reflected relationships already described in the other two groups.

Regional endocardial and epicardial blood flows from the tonic sympathetic nerve stimulation experiments are depicted in Figure 15. Pre-occlusion endocardial (105.4 $\pm$ 8.9 ml/min/100g) and epicardial (105.2 $\pm$ 6.6 ml/min/100g) blood flow levels were not significantly different from pre-occlusion blood flow values in control dogs. Again, blood flow in the infarcted portions of these hearts was less than 20% of the pre-occlusion value, while blood flow in the patchy region was approximately 60% the pre-occlusion level. The mean blood flow in the infarcted myocardium of the stellate nerve stimulation experiments was 21.8 $\pm$ 1.7 ml/min/100g. This was not statistically a significant increase over the mean blood flow in the control infarct. The critical blood flow value was significantly elevated in the stellate nerve stimu-

FIGURE 15

REGIONAL MYOCARDIAL BLOOD FLOW IN TOTALLY INFARCTED, PARTIALLY INFARCTED AND NORMAL LEFT VENTRICLE OF TONIC SYMPATHETIC NERVE STIMULATION HEARTS



Left ventricular endocardial (top) and epicardial (bottom) blood flow in the three regions delineated by NBT staining. Myocardial blood flow was determined at time intervals described in Figure 13.

lation group (42.1 ± 2.1 ml/min/100g vs. 30.6 ± 1.6 ml/min/100g in the control animals; $p<.05$).

C. Effects of Previous Ischemic Insult on Infarct Size During LAD Occlusion in the Presence and Absence of Sympathetic Stimulation

Twenty experiments were attempted in this portion of the study. Sixteen successful experiments were completed and are included in this report. In these 16 successful experiments, the protocol consisted of a five-minute LAD occlusion, three minutes of reperfusion and 6 hours of LAD occlusion in the presence (N = 8) and absence (N = 8) of tonic sympathetic nerve stimulation. Two of the 10 dogs initially designated for the tonic sympathetic nerve stimulation study fibrillated within 10 seconds after the release of the first LAD occlusion and could not be electrically converted or hemodynamically stablized in time to continue the protocol and therefore were excluded. Two other dogs in the tonic nerve stimulation group fibrillated within 10 seconds after the release of the first LAD occlusion but rapid cardioversion (first attempt) was successful and the protocol continued. Two of the 10 dogs initially designated for the non-nerve stimulated group fibrillated immediately after the release of the first LAD occlusion and did not recover in time to continue the protocol.

All sixteen animals reported in this section maintained adequate hemodynamic and blood gas parameters for the entire 6 hours of the second LAD occlusion. Table 5 shows the data for the infarct sizes of these two experimental groups as well as data from the control animals in the previous section. Infarct size was calculated as described earlier with the total infarct size consisting of the totally unstained and patchy stained tissue.

TABLE 5

INFARCT SIZE DATA FOR ACUTELY DECENTRALIZED, REPEATED OCCLUSION AND REPEATED OCCLUSION-TONIC SYMPATHETIC NERVE STIMULATION EXPERIMENTS

	LV Weight (g)	Infarct Weight (g)	Infarct Size (% LV)	Patchy Weight (g)	Patchy Size (% LV)	Transmural Infarction
Control (N = 16)	110.4 ± 4.8	24.0 ± 2.2	21.5 ± 1.8	1.3 ± .2	1.2 ± .2	9/16
Repeated Occlusion (N = 8)	105.0 ± 11.2	24.6 ± 4.1	23.4 ± 1.2	5.9 ± .5	5.6 ± .7*	2/8
Repeated Occlusion - Tonic Stellate Stimulation (N = 8)	105.3 ± 6.7	29.4 ± 3.5	27.3 ± 2.0	2.0 ± .5	2.0 ± .5	5/8

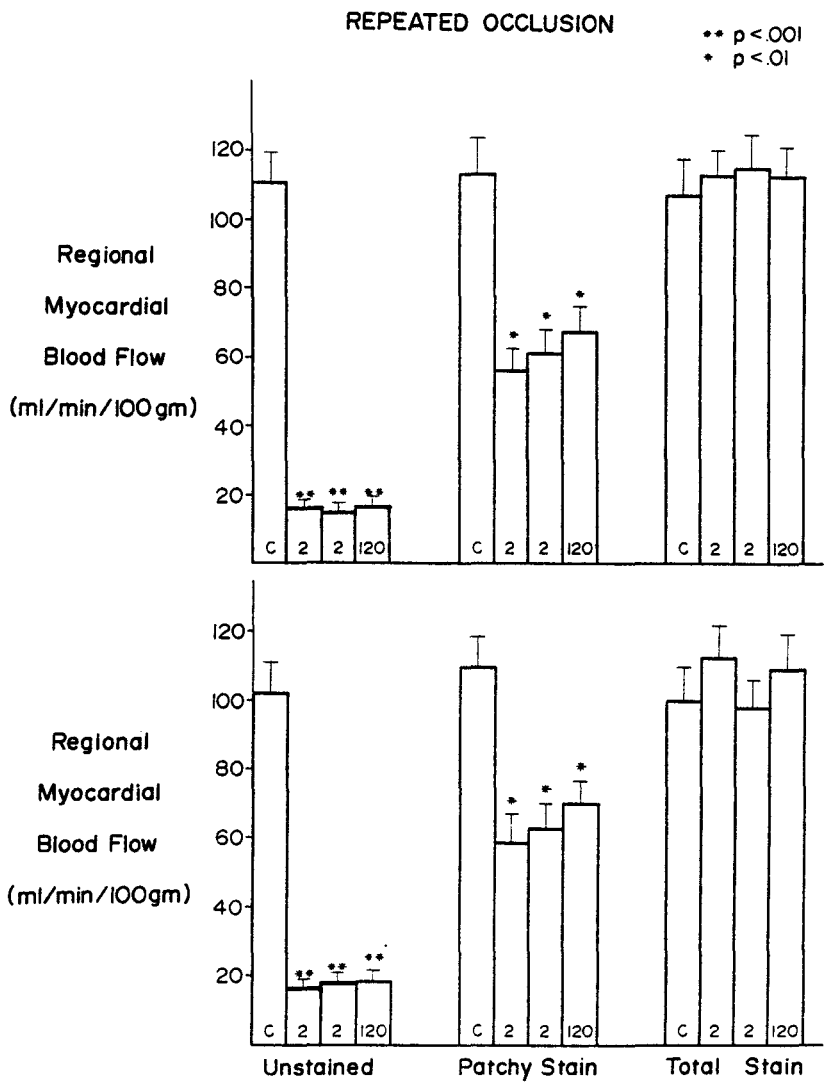
* = $p < .05$ when compared to control (acutely decentralized) hearts via a one-way analysis of variance and a test of least significant differences.

The total left ventricular weights of these three groups were not significantly different. Infarct size in the repeated occlusion group was not statistically altered from the control value, but the amount of patchy tissue present in the infarcts of the repeated occlusion group was almost five times larger than that seen in control ($p<.05$). Therefore, even though the total infarct size was unaltered, the repeated occlusion protocol resulted in a smaller core of total infarction and a larger region of partial (patchy) infarction. Also, in only two out of 8 repeated occlusion experiments was complete transmural infarction observed. Usually, the epicardial portion consisted of a region of patchy infarction instead of a well defined transmural infarct. Stellate stimulation in conjunction with repeated occlusion increased infarct size over that seen in either the control experiments or experiments with repeated occlusion only but this increase did not reach statistical significance. The size of the patchy area in the repeated occlusion-sympathetic nerve stimulation experiments was not significantly different from control. Transmural infarction was observed in 5 out of 8 experiments in which tonic sympathetic nerve stimulation was superimposed on repeated occlusion. The infarct size and patchy area size for the repeated occlusion-sympathetic nerve stimulation group were similar to those found under the conditions of sympathetic nerve stimulation alone (see Table 3).

Figure 16 demonstrates the endocardial (top) and epicardial (bottom) blood flow values in the three regions of NBT staining for the repeated occlusion experiments. Pre-occlusion left ventricular endocardial blood flow was 107.4 ± 7.2 ml/min/100g while epicardial flow was

FIGURE 16

REGIONAL MYOCARDIAL BLOOD FLOW IN TOTALLY INFARCTED, PARTIALLY INFARCTED AND NORMAL LEFT VENTRICLE OF REPEATED OCCLUSION HEARTS



Left ventricular endocardial (top) and epicardial (bottom) blood flow in the three regions of NBT staining. Myocardial blood flow in the repeated occlusion protocols was measured before LAD occlusion (C), two minutes after the first LAD occlusion (first data bar labeled 2), two minutes after the second LAD occlusion (second data bar labeled 2), and 120 minutes after the second LAD occlusion.

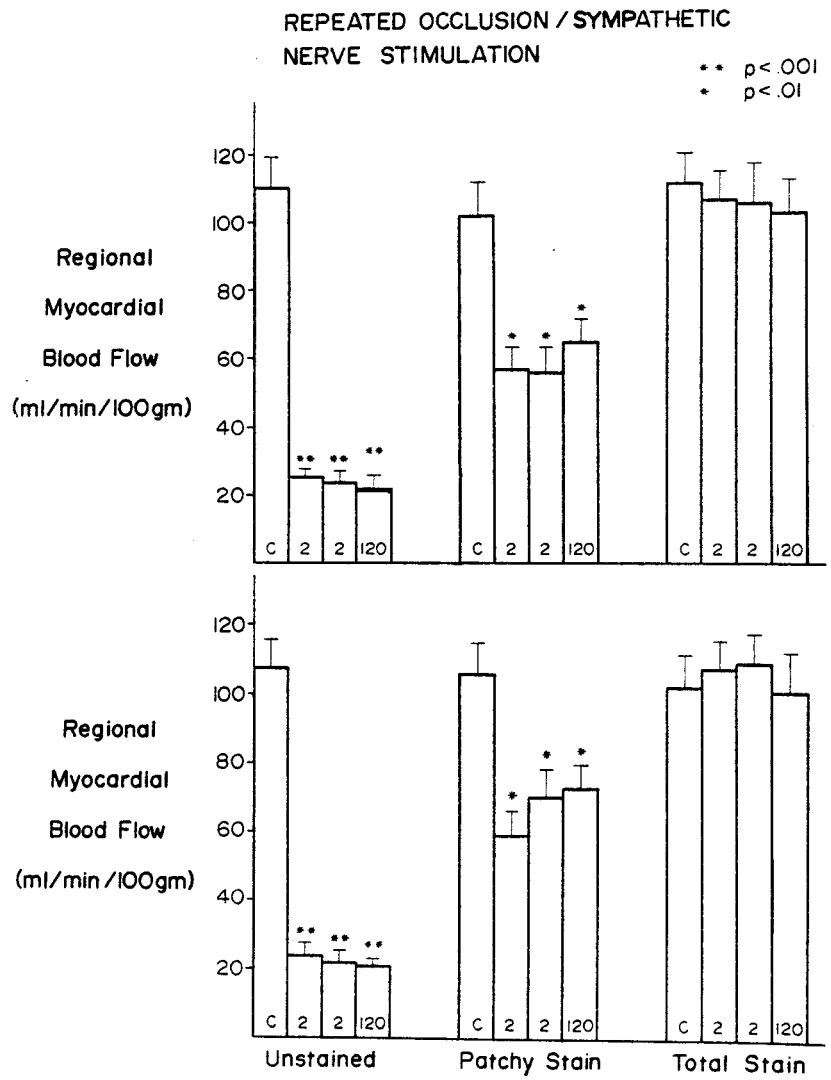
103.3 ± 6.5 ml/min/100g. During the first LAD occlusion, endocardial blood flow fell to 16.9 ± 2.5 ml/min/100g in the unstained region. This blood flow determination was made after two minutes of LAD occlusion. Following reperfusion and re-occlusion of the LAD, endocardial blood flows determined two minutes and 120 minutes after the second LAD occlusion were 15.2 ± 2.4 ml/min/100g and 17.1 ± 2.3 ml/min/100g, respectively, in the unstained tissue. These values were all significantly different from the pre-occlusion blood flow ($p < .001$). Blood flow in the totally infarcted (unstained) endocardium was not altered by the period of reperfusion, as flow at two minutes after the first LAD occlusion was not significantly different from that seen 2 or 120 minutes after the second LAD occlusion. Similar relations were seen in the epicardium.

Endocardial blood flow in the patchy stain region was reduced approximately 50% with the first LAD occlusion, was increased modestly at two minutes after the second LAD occlusion (approximately 6 ml/min/100g), and was even higher two hours into the second occlusion. Though similar results were seen in the epicardium, none of these changes proved to be statistically significant due to a wide variability. Normal staining endocardium and epicardium showed no change in blood flows at the four intervals assessed in this protocol.

Figure 17 demonstrates the blood flow data for the repeated occlusion-stellate nerve stimulation experiments. Endocardial and epicardial blood flow prior to LAD occlusion averaged 110.2 ± 7.2 ml/min/100g and 105.9 ± 8.1 ml/min/100g, respectively, for the left ventricle. These values were not significantly different from the pre-occlusion myocardial blood flow levels seen in the repeated occlusion

FIGURE 17

REGIONAL MYOCARDIAL BLOOD FLOW IN TOTALLY INFARCTED, PARTIALLY INFARCTED AND NORMAL LEFT VENTRICLE OF REPEATED OCCLUSION-TONIC SYMPATHETIC NERVE STIMULATION HEARTS



Left ventricular endocardial (top) and epicardial (bottom) blood flow in the three regions of NBT staining. Myocardial blood flow in the repeated occlusion-sympathetic nerve stimulation protocol was measured before LAD occlusion (C), two minutes after the first LAD occlusion (first data bar labeled 2), two minutes after the second LAD occlusion (second data bar labeled 2), and 120 minutes after the second LAD occlusion.

series. During the first LAD occlusion, endocardial and epicardial blood flow fell to levels approximately 25% of the pre-occlusion values in the unstained tissue. These levels were not altered during the second occlusion. In the patchy stain region, endocardial and epicardial flow fell to approximately 60% of the pre-occlusion level two minutes after the first LAD occlusion. During the second occlusion, endocardial blood flow was not different from the value determined during the first occlusion at either 2 minutes or 120 minutes. Epicardial blood flow was observed to be higher in the patchy staining tissue during the second occlusion but this was not found to be a significant increase. Endocardial and epicardial blood flows in the normal staining regions were stable.

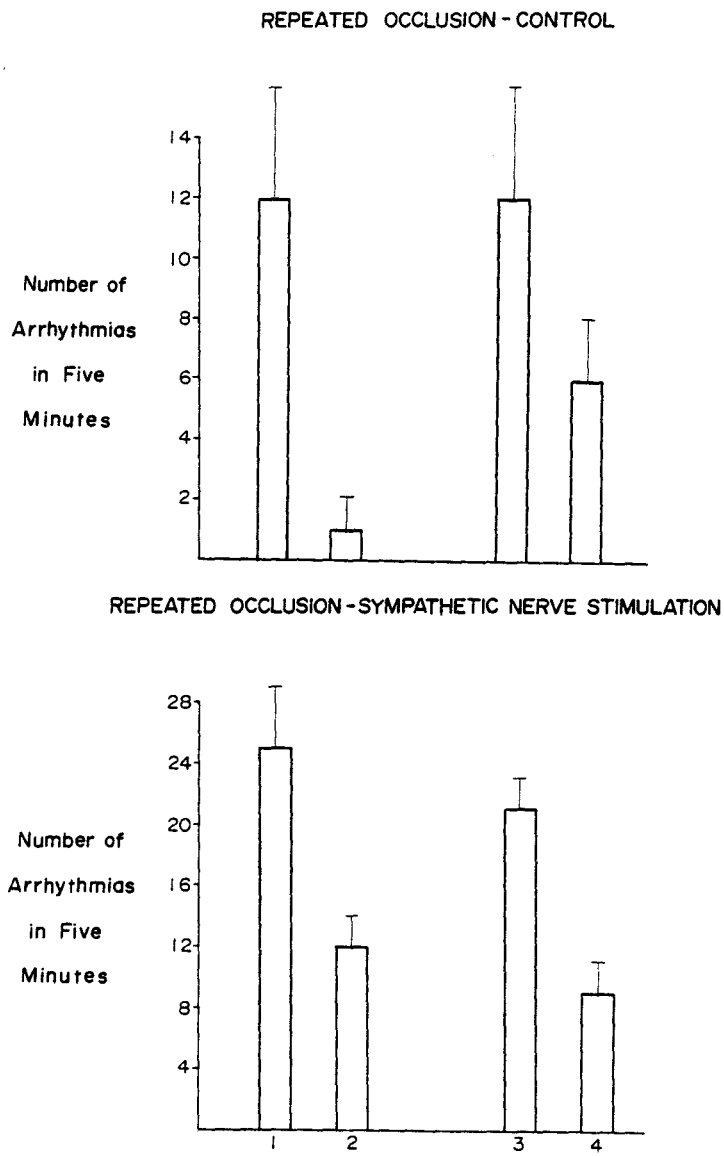
D. Effect of the Time Interval Between Repeated Brief LAD Occlusions Upon Arrhythmia, S-T Segment Elevation and Myocardial Blood Flow

Sixteen successful experiments were performed in this section of the study. Two groups of dogs were subjected to five minutes of LAD occlusion, three minutes of reperfusion, and five minutes of LAD occlusion. This was followed by 40-60 minutes of undisturbed reperfusion and then a repeat of the occlusion-reperfusion-occlusion protocol. One group of decentralized dogs (N = 8) received these repetitive occlusions in the absence of any neural activity, while the second group (N= 8) received tonic left sympathetic nerve stimulation. No animals succumbed to ventricular fibrillation during this portion of the study. All animals received radiolabeled microspheres two minutes into each occlusion.

Figure 18 shows the arrhythmia data for the dogs receiving no sympathetic nerve stimulation (top) and those receiving tonic sympathetic

FIGURE 18

ARRHYTHMIA IN THE PRESENCE AND ABSENCE OF SYMPATHETIC NERVE STIMULATION WITH FOUR SEQUENTIAL OCCLUSIONS



Number of PVCs occurring during each five-minute occlusion in the absence (top) and presence (bottom) of tonic sympathetic nerve stimulation. Each bar represents the mean ± SEM for eight dogs. The numbers at the bottom of the figure indicate the occlusion number for each set of experiments. Note change in scale along ordinate in the sympathetic nerve stimulation data.

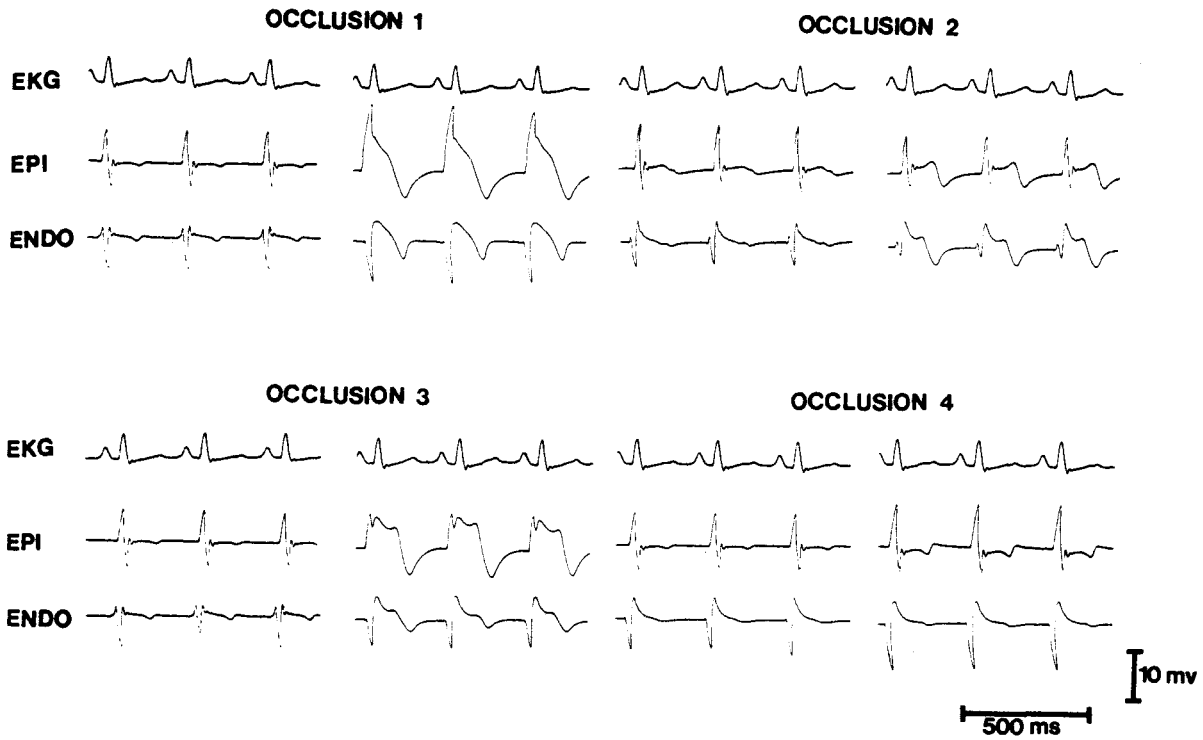
nerve stimulation (bottom). Prior to the first LAD occlusion, neither group demonstrated arrhythmia. During the first occlusion in the control group (top graph), the average number of premature ventricular complexes (PVCs) over five minutes of occlusion was 12 $\pm$ 3 with a range of 25 (5-30) PVCs. The second occlusion which followed after three minutes of reperfusion, demonstrated significantly less arrhythmia ($p < .001$), as only 1 $\pm$ 1 PVCs were seen over the entire five minutes of occlusion. The range in this set of data was 0 - 5 PVCs. Following 40-60 minutes of undisturbed reperfusion, LAD occlusion again resulted in a substantial number of premature ventricular complexes (12 $\pm$ 3; range 3 - 27). This number was not significantly different from the value obtained during occlusion #1. The fourth occlusion, which followed #3 after three minutes of reperfusion, demonstrated significantly less arrhythmia ($p < .01$) when compared to the third occlusion.

Similar directional results were seen in the sympathetic nerve stimulation experiments. Occlusion #1 in this set of experiments resulted in 25 $\pm$ 4 PVCs over the five minutes examined. This value was significantly elevated ($p < .01$) over the number observed in the absence of sympathetic nerve activation. A brief period of reperfusion appeared to return conditions toward those observed during occlusion #1, while occlusion #4 was similar to occlusion #2.

Evidence for abnormal electrical activation was examined in the endocardium and epicardium of the ischemic zone. Figure 19 depicts an actual experiment (dog #RO-3) where the effects of multiple LAD occlusions on endocardial (endo) and epicardial (epi) S-T segments were examined. In each case, the left hand traces of each pair represent the recordings

FIGURE 19

CHANGES IN ENDOCARDIAL AND EPICARDIAL S-T SEGMENT LEVELS DURING REPEATED OCCLUSION OF THE LAD



Simultaneous recordings of endocardial (endo) and epicardial (epi) unipolar electrograms, as well as the lead II electrocardiogram (EKG), during four sequential occlusions of the LAD. Each occlusion (Occlusion 1, etc.) depicts two traces, the first trace taken 10-15 seconds prior to the occlusion, and the second trace recorded immediately preceding the release of the given occlusion.

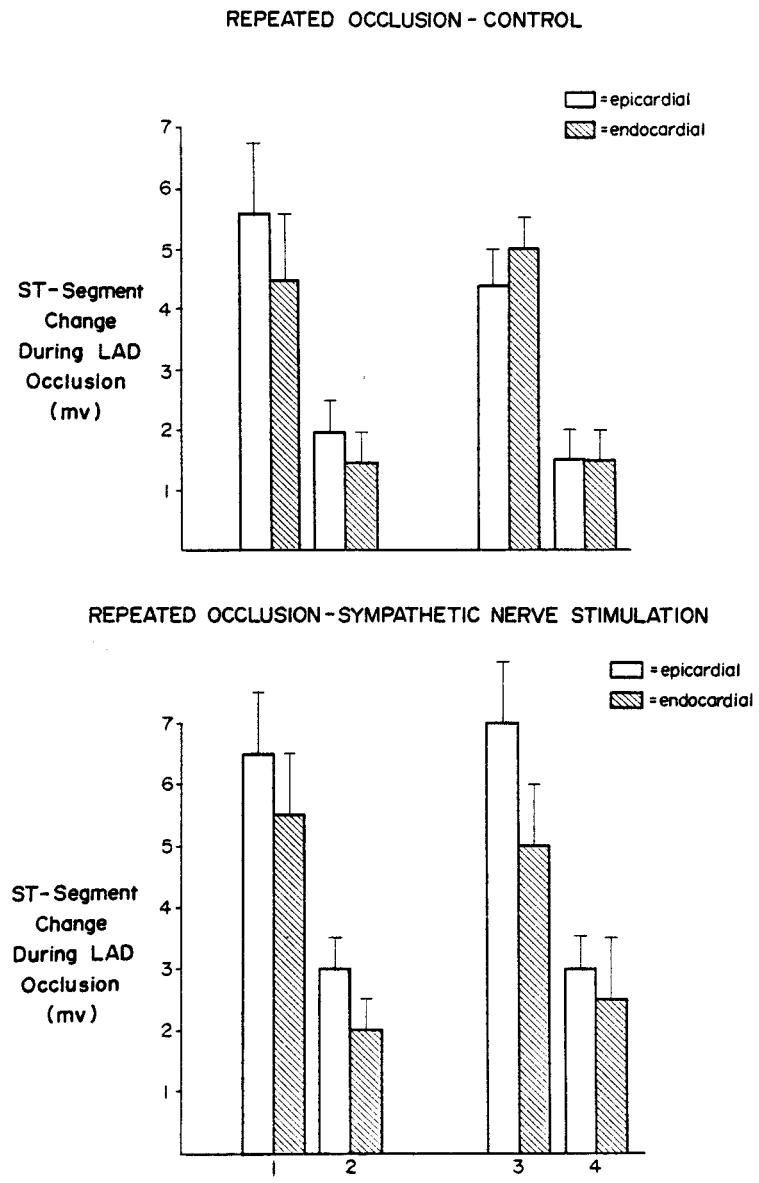
made immediately prior to the occlusion, while the second recordings were taken preceding release of the occlusion. In the figure, prior to occlusion #1, S-T segment alteration is present. After five minutes of LAD occlusion abnormal S-T segments are seen while the EKG appears normal. Upon release of the occlusion, S-T segment of the local electrograms returns rapidly toward the pre-occlusion level such that prior to occlusion #2, little evidence for injury is evident. Following the second five minutes of LAD occlusion, electrogram abnormalities are much less in both the endocardial and epicardial regions. Occlusions #3 and #4 demonstrate a similar sequence with marked S-T segment elevation present endocardially and epicardially during occlusion #3, but little evidence of injury present in occlusion #4.

Figure 20 shows the combined S-T segment (local unipolar electrogram) data for the control (top) and sympathetic nerve stimulation (bottom) groups. In each group, the endocardial and epicardial S-T segment values were evaluated before any occlusions were performed, and subtracted from the S-T elevation during each occlusion to give the S-T segment change plotted in the figure. In both groups of animals, significantly less ($p < .01$) S-T segment elevation was seen both endocardially and epicardially during occlusions #2 and #4 when compared with occlusions #1 and #3, respectively. S-T segment values in the repeated occlusion group and the sympathetic nerve stimulation group during occlusion #1 were not different though elevation tended to be higher in the nerve stimulation series during each occlusion.

Figure 21 demonstrates a representative experiment where two endocardial (BPI) and two epicardial (BPO) bipolar electrodes were

FIGURE 20

ENDOCARDIAL AND EPICARDIAL S-T SEGMENT DATA FOR REPEATED OCCLUSION AND REPEATED OCCLUSION-SYMPATHETIC NERVE STIMULATION HEARTS

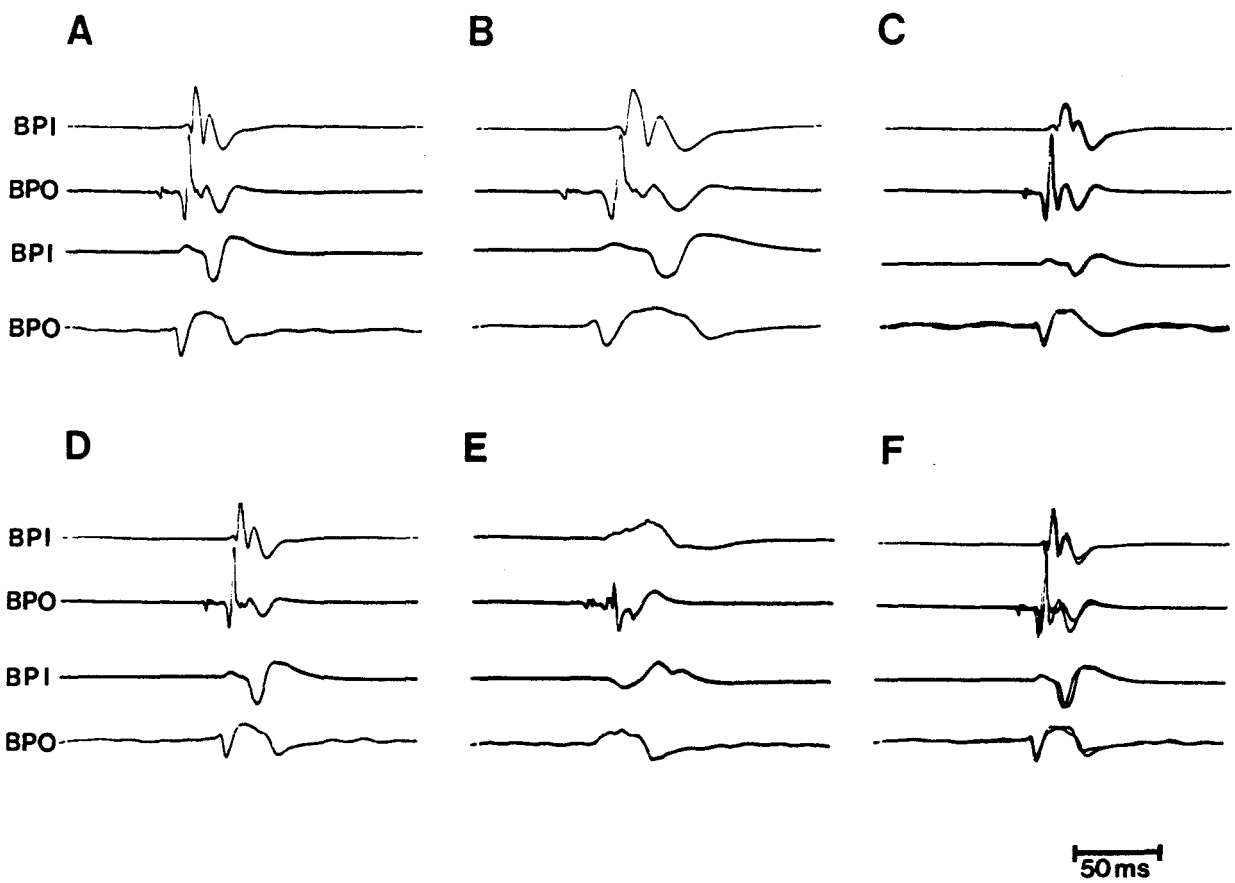


S-T segment changes in the endocardium and epicardium of the ischemic region in repeated occlusion (control) and repeated occlusion-sympathetic nerve stimulation experiments. Each bar represents the mean $\pm$ SEM for eight animals. All S-T segment values were measured as change in amplitude (mv) after five minutes of LAD occlusion.

FIGURE 21

ENDOCARDIAL AND EPICARDIAL BIPOLAR ELECTROGRAM TRACES

DURING SEQUENTIAL OCCLUSION OF THE LAD



Storage oscilloscope traces of two sets of transmurally paired bipolar endocardial (BPI) and epicardial (BPO) electrograms recorded from the ischemic zone. Prior to the first LAD occlusion (Panel A), each electrogram had a duration of activation of 45 ms, and a time-to-onset of 125 ms. Time-to-onset was determined by adding the oscilloscope trigger delay to the actual electrogram trace. Following five minutes of LAD occlusion (B), time-to-onset was altered 15 ms while duration increased 45 ms. Panel C depicts a control trace prior to occlusion #2 and a trace superimposed after five minutes of LAD occlusion #2. Panel D was recorded 50 minutes after the release of occlusion #2 and immediately preceding occlusion #3. Panel E shows changes in the bipolar electrogram after five minutes of LAD occlusion #3. Panel F depicts the electrogram configuration before and during occlusion #4 (double trace). Slight changes in the electrograms are seen.

paired transmurally in the region of potential ischemia and electrograms recorded during the four occlusions. Electrogram responses during coronary occlusions #1 and #3 are evident. During occlusion #1 (Panel B), there is considerable increase in the overall electrogram duration, slight increase in the time-to-onset, and little effect on the amplitude of both sets of electrograms (refer to Figure 5 in Methods). Similar responses are seen in Panel E during occlusion #3. Panels C and F represent oscilloscope traces taken immediately prior to occlusions #2 and #4 and recordings examined after five minutes of occlusion. It is evident that both endocardially and epicardially, little change in electrogram amplitude, time-to-onset or duration has occurred.

Prior to the onset of the repeated occlusion protocol, bipolar electrogram parameters (amplitude, time-to-onset, and duration) were measured. Following release of each five-minute occlusion, electrograms returned rapidly to the pre-occlusion values. No significant differences ($p < .05$) were seen in electrogram amplitude, duration or onset before any of the repeated occlusions when these parameters were compared to the pre-occlusion measurements.

Table 6 shows the mean $\pm$ SEM for the control (no occlusion) bipolar electrogram parameters recorded from the endocardium (endo) and epicardium (epi) of the two groups of animals. In both groups, no significant difference between the endocardial and epicardial values of a given parameter was found. Additionally, endocardial and epicardial parameters were not altered from the repeated occlusion values by the presence of tonic sympathetic nerve stimulation.

Within each group of animals, control (no occlusion) bipolar

TABLE 6

PRE-OCCLUSION VALUES FOR BIPOLAR ELECTROGRAM AMPLITUDE, TIME-TO-ONSET AND DURATION IN THE PRESENCE AND ABSENCE OF SYMPATHETIC NERVE STIMULATION

Experimental Group	Amplitude (mv)		Time-To-Onset (ms)		Duration (ms)	
	ENDO	EPI	ENDO	EPI	ENDO	EPI
Repeated Occlusion	10.1 ± 2.0 (5.5 - 21.0)	11.5 ± 1.8 (5.0 - 20.0)	133 ± 6 (105 - 165)	131 ± 7 (100 - 170)	43 ± 4 (30 - 65)	44 ± 4 (35 - 65)
Repeated Occlusion – Sympathetic Nerve Stimulation	11.0 ± 2.4 (3.5 - 23.5)	10.4 ± 1.7 (3.5 - 19.0)	118 ± 6 (100 - 145)	116 ± 5 (100 - 140)	37 ± 3 (25 - 55)	39 ± 3 (30 - 50)

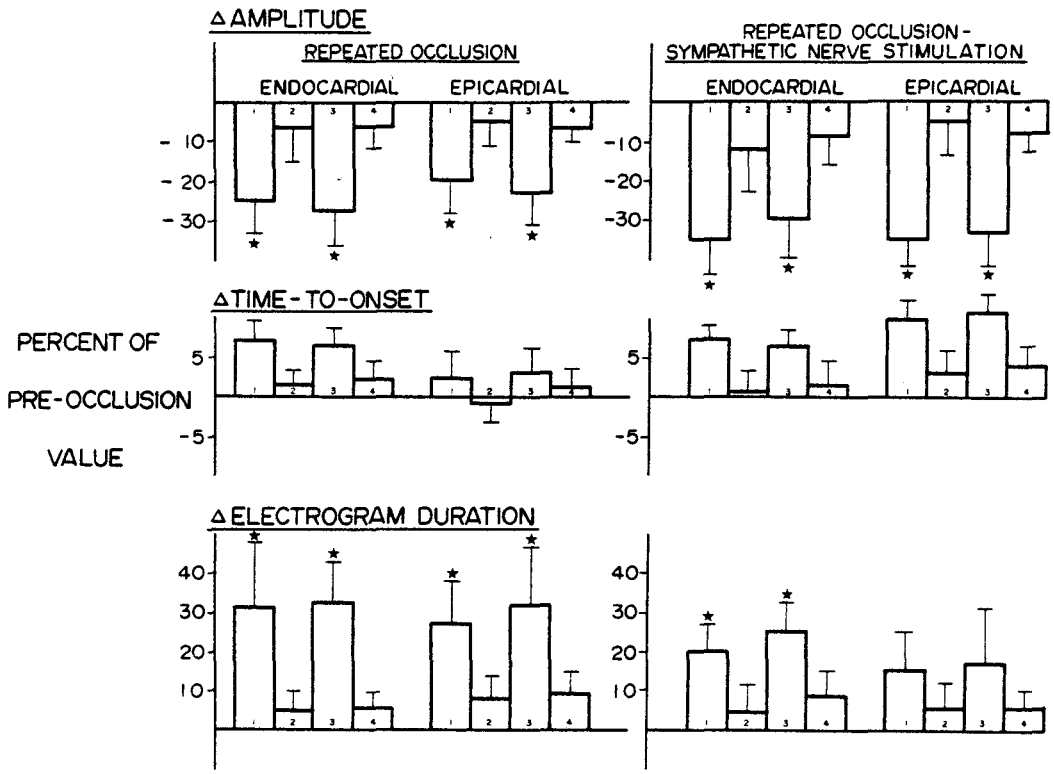
Upper values are mean ± SEM, while lower values in parenthesis are the range of measurements recorded. Data for amplitude, time-to-onset and duration were compared using a one-way analysis of variance.

electrogram parameters varied considerably. As shown in Table 6, the range (numbers in parenthesis) was quite large, and when statistical analysis was performed no significant differences of any type were found. To compensate for the extreme variability in the data, normalization of the electrogram parameters was performed. Figure 22 shows the percent changes from control in bipolar electrogram amplitude, time-to-onset, and duration during four sequential occlusions. Baseline in each graph represents the pre-occlusion value (100%). The left hand portion of the figure shows changes in the endocardial and epicardial bipolar electrogram parameters in the repeated occlusion group, while the right hand portion of the figure depicts the same changes in the endocardium and epicardium of the repeated occlusion-tonic sympathetic nerve stimulation experiments.

Three interesting results are depicted in this figure. First, abrupt occlusion of the LAD either in the presence or absence of tonic sympathetic nerve stimulation significantly alters endocardial and epicardial electrogram amplitude and duration. Time-to-onset is not significantly altered. Second, a short period of reperfusion (3 minutes between occlusions #1 and #2) followed by abrupt LAD occlusion results in small and insignificant changes in electrogram parameters from control, while a long period of reperfusion (40-60 minutes) followed by abrupt LAD occlusion again demonstrates significant alteration in electrogram amplitude and duration from the control value. Occlusion #1 and #3 were not found to be statistically different. Third, tonic sympathetic nerve stimulation did not change the responses to repeated occlusion as similar trends were observed in both groups of dogs.

FIGURE 22

NORMALIZED BIPOLAR ELECTROGRAM CHANGES IN AMPLITUDE, TIME-TO-ONSET AND DURATION IN THE PRESENCE AND ABSENCE OF SYMPATHETIC NERVE STIMULATION

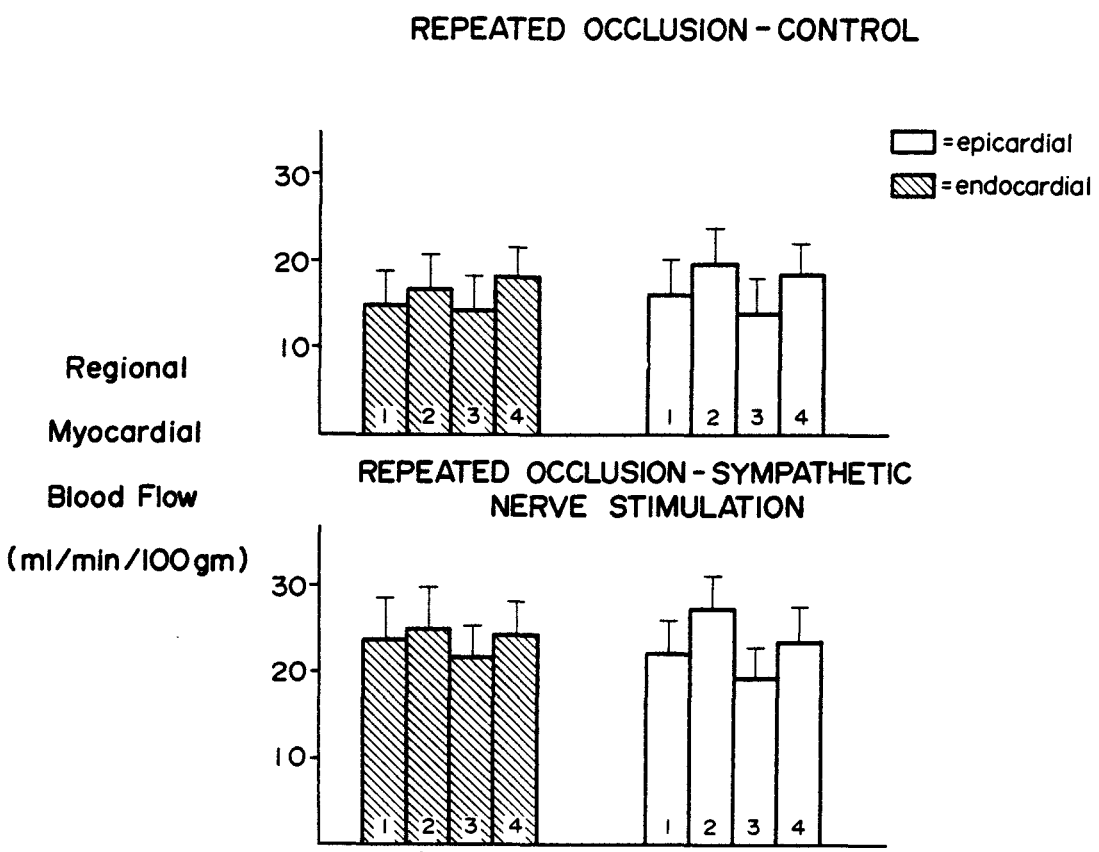


Numbers inside data bar signify the occlusion number. All data plotted as a mean ± SEM of the percent change from the pre-occlusion value.
* = $p < .05$ compared to the pre-occlusion control value.

The possibility that the lesser degree of local myocardial injury (as measured by unipolar and bipolar electrodes) seen during occlusions #2 and #4 was due to an increased myocardial blood flow to the electrode sites was examined. Regional endocardial and epicardial blood flow during each occlusion was calculated in tissue surrounding each electrode. The data (figure 23) do not support the theory that a significant increase in collateral, or overlap, blood flow was responsible for the decreased injury seen at these sites.

FIGURE 23

REGIONAL MYOCARDIAL BLOOD FLOW IN TISSUE WHERE ELECTRICAL INDICES OF INJURY WERE MEASURED DURING REPEATED OCCLUSION



Regional myocardial blood flow in endocardial and epicardial sites where either bipolar or unipolar electrodes were located. Each data bar represents the mean $\pm$ SEM for myocardial blood flow at the respective sites. Numbers at the bottom of each bar represent the occlusion number. One-way analysis of variance revealed no significant differences in endocardial or epicardial blood flows in either set of experiments.

CHAPTER V

DISCUSSION

A. Effect of Cardiac Denervation on Myocardial Blood Flow and S-T Segment Changes During Ischemia

The protective effect of chronic cardiac denervation during ischemia has been the subject of previous investigation (34, 49, 95, 167, 193). Reports by Ebert *et al.* (49) and Schaal *et al.* (167) described reduced arrhythmia, less evidence of electrocardiographic alteration, and lowered incidence of fibrillation during acute and chronic LAD ligation in dogs whose hearts had been previously denervated by the mediastinal neural ablation technique described by Cooper *et al.* (30).

Ebert *et al.* (49) examined anesthetized dogs for the development of rhythm disturbances. They found that 96% of the innervated dogs demonstrated some sort of severe arrhythmia within the first twenty minutes after occlusion of the LAD at a level just distal to its exit from the left main coronary artery. On the other hand, none of the chronic cardiac denervated animals had ventricular tachycardia or fibrillation in the first twenty minutes of LAD occlusion. Thirteen of twenty-five non-denervated animals succumbed to ventricular fibrillation within twenty minutes after the LAD was ligated.

Schaal *et al.* (167) examined similar relations in conscious, chronically cardiac denervated dogs and in sham-operated control dogs. Ventricular arrhythmias developed in all of the control animals, and

ventricular tachycardia was seen in nine out of ten dogs. None of the control dogs developed ventricular fibrillation within the observation period. Two of ten chronically denervated dogs demonstrated premature ventricular contractions, but none of the remaining animals showed arrhythmia of any sort following mid-LAD ligation. No dogs that had sustained cardiac denervation died following coronary artery occlusion.

The results reported in this study confirmed the above reports. Of the ten successful intrapericardial cardiac denervated dogs studied, only three dogs demonstrated any type of arrhythmia during the twenty-minute observation period after mid-LAD occlusion. The most severe arrhythmias were seen in dog #DSTS-10 where 17 isolated premature beats were observed. In contrast, two of the 12 sham-denervated animals originally prepared for the study were excluded because of the development of ventricular fibrillation at some point during the occlusion protocol. Of the ten control animals successfully analyzed for this report, all ten demonstrated significant numbers of premature ventricular beats during the twenty-minute occlusion period. The range of the data was quite high in this group, with dog #ISTS-11 having 33 premature beats (low value) while dog #ISTS-6 had 187 premature beats (high value). Eight out of ten dogs in the control group demonstrated ventricular tachycardia (at least three successive premature beats), while none of the cardiac denervated animals had tachycardia.

Harris et al. (72, 74) have hypothesized that the loss of potassium from ischemic myocardium results in a potassium gradient at the boundary of ischemic and normal cells. This boundary produced a zone of relative excitability which resulted in the subsequent arrhythmia observed during

occlusion. Vanderbeek and Ebert (196), utilized the agent octylamine to determine whether the rapid loss of potassium from the heart would result in ectopic activity or ventricular fibrillation in the denervated heart. Octylamine, a potassium releasing substance, when injected into the LAD of control hearts caused a rise in coronary sinus potassium, and all ten control hearts fibrillated within six minutes after the injection. Chronically denervated hearts exhibited marked ECG abnormalities, and similar rises in coronary sinus potassium after octylamine injection, but no actual rhythm disturbances of any type were observed. The authors concluded that chronic cardiac denervation did not alter membrane responses to octylamine as similar increases in coronary sinus potassium were seen in both groups. The mechanism by which protection occurred was postulated to be due to the catecholamine depletion resulting from chronic denervation (196).

Results from this section of the dissertation described a reduction in left ventricular myocardial blood flow two weeks after intrapericardial cardiac denervation (7). Myocardial blood flow has been shown to be a sensitive indicator of cardiac metabolic demand (12, 22, 106), and we interpreted this decreased regional myocardial blood flow in the left ventricle to reflect a decreased metabolic demand and probably a lowered myocardial oxygen consumption level. These results agreed in part with those described by Gregg et al. (67) in the conscious, resting dog following cardiac denervation by mediastinal neural ablation. The observations reported also agreed with similar determinations by Jones et al. (48, 94, 96) and Thomas et al. (191) in the sympathectomized left ventricle, but are contrary to observations from the work of other

authors (29, 36, 46). Thomas et al. (191) demonstrated that this lowered myocardial blood flow was in the presence of an unaltered oxygen extraction (A-V O_2 difference) in the left ventricle.

Coleman et al. (29) examined myocardial oxygen consumption in chronically denervated cat papillary muscles and in similar preparations that had not been denervated. They found no difference in oxygen requirements between the two groups. Daggett et al. (36) showed no change in myocardial oxygen consumption in the canine heart 6-12 weeks after cardiac autotransplantation when these hearts were compared to non-autotransplanted control hearts. Similar workloads in autotransplanted and control hearts resulted in similar levels of oxygen consumption.

The recent work of Drake et al. (46) has caused further separation of opinion as to the effects of cardiac denervation on myocardial blood flow and oxygen consumption. Drake and co-workers looked at these parameters in the dog heart before and after cardiac denervation. They reported (46) an increased left ventricular blood flow and oxygen consumption in the denervated hearts three weeks after mediastinal neural ablation. The discrepancies between Drake et al. (46) and the current study may be due to one or more of the following factors. First, the present studies were carried out under pentobarbital anesthesia while Drake et al. utilized chloralose anesthesia. Chloralose has been described to cause a general hyperreflexive condition which may cause an increase in circulating levels of catecholamines (5). These elevated levels of catecholamines would have stimulated cardiac metabolic processes by providing enhanced stimulation of the heart, thereby elevating myocardial blood flow levels and augmenting oxygen consumption. Second, in the

present study, completeness of denervation was thoroughly tested at the time of the acute experiment. Direct nerve stimulations were performed and changes in myocardial contractile force examined. Any animal demonstrating changes in inotropic or chronotropic parameters during nerve stimulation or tyramine testing was excluded from the study. Also, at the end of many experiments, analysis for tissue catecholamines was performed to determine if depletion had occurred. Drake et al. provided little evidence that the animals utilized in the reported study were completely denervated. In the Drake study, changes in heart rate following either atropine injection or startle reaction were used in conscious dogs to verify denervation. While these tests would possibly verify sinoatrial and atrioventricular nodal denervation, they would not necessarily indicate complete ventricular denervation. Indeed, Peiss et al. (142) demonstrated that in only one of eight dogs denervated by the mediastinal neural ablation technique described by Cooper (30) was complete evidence for denervation present. Seven other dogs showed varying degrees of cardiac activation in response to diencephalic stimulation or stimulation of the cardiac sympathetic nerves. Peiss et al. (142) concluded that mediastinal neural ablation cannot be assumed to be successful as a denervation technique without rigorous testing. Third, Drake et al. (46) utilized seven dogs in which after cardiac denervation, left ventricular blood flow went up in four dogs and down in three dogs. Utilization of the sign test for paired data demonstrated a very significant increase in blood flow after denervation. Use of a conventional paired t-test on the data did not reveal a difference.

It is interesting to note that in the present study denervation altered left ventricular blood flow yet had no effect on blood flow levels in the right ventricle. This is perplexing in that it has been shown in this laboratory that the right ventricle is very sensitive to direct nerve stimulation (3). Ventricular contractility and conduction are both markedly altered during sympathetic nerve stimulation. Furthermore, the right ventricle has been shown to have similar catecholamine levels as the left ventricle (1, 103). Though we have no data to explain these findings, it may be postulated that although the right ventricle is very sensitive to nerve stimulation, it may not be under as much "tone" as the left ventricle and consequently the metabolic requirements of the right ventricle (as reflected by myocardial blood flow) are not altered by removal of neural input. The right ventricle differs from the left ventricle in that it is a low pressure, volume pump with lower basic metabolic demands (205), and this may account for some of the apparent lack of effect of cardiac denervation on right ventricular blood flow.

Maroko and co-workers (121) have shown the extent and severity of acute epicardial S-T segment changes during coronary occlusion to be a good indicator of the ultimate tissue damage of the area. In this study, local epicardial and endocardial S-T segment changes were examined during coronary occlusion and utilized as an indicator of tissue damage. In areas of moderate and minimal flow reduction during coronary occlusion, the level of S-T segment change was not altered with denervation. In areas of severe blood flow compromise (30 ml/min/100g or less) there was less evidence of epicardial or endocardial damage in the denervated

heart. The significant difference in S-T segment elevation in the low blood flow zone could, theoretically, have been due to higher levels of blood flow in the sites comprising the denervated samples. Figure 9, however, clearly demonstrated this was not the case. The amount of injury in the denervated heart was less even at similar blood flow levels. The regional S-T segment data support observations made by Ebert et al. (49) where, using lead II electrocardiogram analysis, they noted less gross S-T segment abnormality in the denervated heart after occlusion; however, direct measurement of S-T segment from the epicardial and endocardial surfaces of the heart is a much more sensitive method to assess local tissue injury (121) and allows a more accurate quantitative evaluation to be made than would be possible from an examination of a lead II electrocardiogram.

Marked protection of the denervated myocardium during LAD occlusion in the form of reduced arrhythmia and S-T segment elevation was seen in this study. At similar work levels (double product), the denervated heart had a lowered left ventricular blood flow demand which reflected less need for the vital substrates, i.e., oxygen, and an increased efficiency (12, 22, 67). Also, the reduced level of S-T segment alteration seen both endocardially and epicardially in the denervated hearts as compared to the sham-denervated control hearts was in the presence of comparable absolute levels of myocardial blood flow. Denervation in our study lessened myocardial injury without a marked increase in blood flow to the area of ischemia, thus suggesting protection mediated by altered metabolic demand. The alteration in metabolic demand may alter the time course for the manifestation of injury as

indicated by the survival studies reported by Ebert (49).

B. Myocardial Blood Flow and Infarction: Evidence for an Alteration in the Critical Blood Flow Following Denervation

The protective effect of chronic cardiac denervation and sympathectomy with respect to arrhythmia during coronary occlusion has become well established (34, 49, 59, 75, 115, 116, 167, 196), as has the detrimental effect of sympathetic activation (75, 76, 119) during and following occlusion. However, this marked protection against early death due to arrhythmias following sympathectomy has not been universally supported (214). Studies examining the protective effect of chronic denervation and sympathectomy with respect to infarct size following coronary artery occlusion have been less convincing. Data supporting (34, 59, 95, 115, 116) and refuting (49, 167, 214) the concept exist. Also, some question as to the effect of denervation on long term survival following coronary artery occlusion has been raised (49, 214).

Much of the controversy concerning protection following chronic cardiac denervation or sympathectomy may be due to one or more of the following factors: 1) differing models of denervation, 2) varying amounts of coronary arterial insult utilized in various studies, and 3) inadequate hemodynamic monitoring during the period of infarction.

Investigators have utilized different models to effect denervation. Early workers simply removed the sympathetic chain to obtain sympathectomy (34, 115, 116, 214). While this technique was very effective in removing the sympathetic pathways to and from the heart, specific denervation of the heart was difficult. With the development of the mediastinal neural ablation technique (30), removal of sympathetic and parasympathetic

influences to the heart was possible (29, 30, 49, 67, 167). This technique was associated with a high degree of post-operative mortality and morbidity, as well as a potential to denervate structures other than the heart. Additionally, Peiss et al. (142) have questioned the ability to achieve complete cardiac denervation using mediastinal neural ablation, and suggested that only after rigorous testing can denervation be assumed. Autotransplantation of the heart (36, 212) insured global denervation of the myocardium, but is a difficult procedure with a high potential for operative and post-operative complications. The intrapericardial cardiac denervation technique developed in this laboratory (153) and used in this dissertation provided a simple, reliable, one-stage denervation technique while leaving innervation to other structures essentially intact (153). This technique and modifications of this technique have afforded some of the most recent evidence for protection during coronary occlusion (7, 48, 94, 95, 192, 193).

A potentially important factor playing a role in explaining discrepancies in the literature concerning the protective effect of denervation with respect to infarct size is the level of coronary artery occlusion utilized in a study. Studies which have occluded smaller regions of the coronary arterial circulation have reported the greatest amounts of protection following denervation.

Ebert et al. (49), ligated the LAD at its origin from the left main coronary artery. Even though deaths due to ventricular fibrillation were substantially reduced in the groups with denervated or sympathectomized hearts, the percentage of survivors 48-96 hours after LAD ligation was not different between control animals and chronic cardiac denervated

animals. Infarct size as measured by gross visual examination was not significantly altered by cardiac denervation (sympathectomy). Schaal et al. (167) and Yodice (214), ligated the LAD at approximately the midpoint of its distribution and saw no reduction in infarct size from control in either sympathectomized (214) or denervated (167) hearts. They also noted no overall enhancement of survival in their studies (167, 214). Cox and Robertson (34), on the other hand, reported reduced infarct size after mid-LAD ligation following chronic sympathectomy. Though none of the above studies (34, 49, 167, 214) gave actual infarct sizes, Cox and Robertson (34) did report the dimensions of the infarcts in 7 control dogs and 7 sympathectomized dogs. Rough calculations using their data showed that with a mid-LAD occlusion, these authors demonstrated approximately a 30-50% reduction in infarct size in the sympathectomized group. They also described a higher degree of long-term survival in stellatectomized animals.

Jones et al. (94, 95) and Thomas et al. (192) have shown marked protection of the denervated heart when the LAD was occluded immediately above its apical branch (low-LAD occlusion). These studies utilized a carefully defined (standardized) coronary anatomy, and the NBT technique to examine infarct size. This resulted in a very uniform ischemic insult and reproducible infarct sizes in all of the control, denervated and sympathectomized hearts. Denervated hearts demonstrated an 80% reduction in infarct size (95, 192), while sympathectomized hearts showed a 60% reduction in infarct size when compared to neurally intact, control hearts. The rigid standardization of the LAD anatomy, as well as the use of NBT, allowed these investigators to demonstrate marked

protection.

Results from the present study were obtained using techniques similar to Thomas et al. (192). A standardized model for the LAD coronary anatomy was used for all infarct size determinations such that grossly abnormal or aberrant coronary arteries were excluded. Only Thomas (192) and Jones (94, 95) have more rigidly controlled this important aspect which influences infarct size while examining the effect of cardiac denervation. Also in this study, as well as in the Thomas (192) and Jones (94, 95) studies, heart rate and hemodynamic variables were examined closely. Marked differences between these factors in the experimental groups could alter results dramatically, therefore animals not maintaining adequate blood pressure or blood gas parameters were excluded.

Utilizing a mid-LAD ligation, control infarct size in this study averaged 21.5% of the total left ventricular weight. This infarct size value was only slightly greater than reported by Jones et al. (95) with apical occlusion of the LAD. Jones et al. reported a 20.1% infarct size in neurally intact dogs and a 15.0% infarct size in acutely decentralized dogs. While the comparable values between the present study and the Jones' study would be 21.5% and 15.0%, these infarct sizes do not seem greatly different considering the substantially higher level of occlusion used in this study. Earlier studies (143, 174) using LAD anatomical criteria similar to Jones et al., described infarct sizes in the range of 12-15% in the neurally intact heart with apical LAD occlusion. The reasons for these reported differences in infarct size are not readily apparent.

Following denervation, infarct size in this report was reduced

approximately 40%, while after sympathectomy a 30% reduction was seen. These values agree in concept, but are smaller in magnitude than those shown by Jones et al. (94, 95). The data are close to the "calculated" reduction in infarct size seen by Cox and Robertson (34) following sympathectomy. The higher level of occlusion used in this study, and the lesser degree of protection observed support the concept of a limitation in the protective effect of denervation (sympathectomy). At higher levels of LAD occlusion the protection appeared to be less (48, 49, 167, 214).

The mechanism of protection in the denervated heart during coronary occlusion still remains to be described. At present, an apparent combination of increased collateral blood flow (94, 97, 177) and decreased metabolic demand (67, 96, 191) in the denervated or sympathectomized heart enables more tissue to remain viable during periods of coronary artery occlusion.

Direct evidence for decreased collateral resistance following ventricular sympathectomy has been reported (97, 177). Indirect evidence for decreased collateral resistance in sympathectomized hearts also has been presented. The indirect evidence is in the form of increased "collateral" blood flow to the central ischemic regions of the occluded LAD (94) and circumflex (48) coronary arteries. The central ischemic region, as defined by left atrial injection of the vital stain thioflavin-S during coronary arterial occlusion, demonstrated significantly higher flows in sympathectomized hearts.

While this increased level of blood flow reported by Jones et al. (48, 94) in the central ischemic region of the sympathectomized heart is

interesting, it still did not prevent some degree of infarction from occurring (94). The present study examined the blood flow in myocardium that actually infarcted after mid-LAD ligation. It was demonstrated that within the infarcted tissue of acutely decentralized, chronically denervated, chronically sympathectomized or tonically sympathetically stimulated hearts, myocardial blood flow both endocardially and epicardially was low. No evidence for increased collateral blood flow to the zones of total infarction during the ischemic period examined (2 min-6 hours) was seen in any of the experimental groups. These data conflict with several reports (13, 88, 98, 157, 179), but support other studies (60, 77, 82, 207).

Also in this study, no increase in the absolute level of perfusion to the totally infarcted region of the denervated or sympathectomized hearts was observed (Table 4). These data conflicted with a report by Jones et al. (94), where occlusion of the apical region of the LAD was performed, but agree with data by the same group (48) where circumflex occlusion was used. DuPont et al. (48) showed that after circumflex coronary artery occlusion absolute levels of myocardial blood flow in the central region of ischemia in the chronically sympathectomized hearts were not different from flow seen in the same region of control hearts. When these values were examined as a percent of the pre-occlusion flow, these investigators showed significantly higher perfusion in the sympathectomized hearts. A dramatic increase in perfusion to the severely ischemic region was not seen in this study as the mean blood flow in the infarct of control hearts was 15.7% of the pre-occlusion level, while mean blood flows in the infarcts of denervated and sympathectomized

hearts were 16.9% and 19.5% of the pre-occlusion value, respectively. Although denervated and sympathectomized hearts do demonstrate increased collateral or overlap blood flow to the central ischemic zone during apical LAD occlusion (48, 94), this increase is limited, and considerably less in the central ischemic zones of sympathectomized hearts when the area of insult increases.

In this study, chronic cardiac denervation and sympathectomy significantly altered the amount of partially infarcted tissue found in the infarct after LAD occlusion. Partially infarcted (patchy) tissue comprised approximately 5% of the total infarct in control, neurally decentralized hearts. This amount of patchy tissue was increased three and fivefold in sympathectomized and denervated hearts, respectively (Table 3). Chronic denervation (sympathectomy) decreased total infarct size following mid-LAD ligation, and increased the amount of marginally ischemic (patchy) tissue. Myocardial blood flow in the patchy stained tissue was reduced 50% from the pre-occlusion value in all groups of animals. The equal reduction in each group of animals means that more tissue in the denervated and sympathectomized hearts receives adequate perfusion to prevent total infarction and sustain at least some cellular viability. Also, more tissue in the denervated and sympathectomized heart was receiving approximately 50% of its original blood flow which would suggest increased perfusion. These data support the concept of Jones et al. (48, 94) that zone-II (their marginally ischemic zone) is more adequately perfused in the sympathectomized heart.

Average blood flows within the infarcts of the three experimental groups were not significantly different from the control group (Table

4). In many experiments, regions of the infarct demonstrated blood flow values equal to 0 ml/min/100g. While the measurement of zero flow must be attributed to low microsphere numbers present in the tissue sample plus calculated corrections for isotope-to-isotope interference, actual zero flow levels were probably not achieved. Computer computation of blood flow data yielded zero flow values which reflect very low, but probably not, zero blood flow. The critical blood flow, or the maximal level of myocardial blood flow at which total infarction was seen, was measured in each infarct and a mean critical blood flow for each group determined. Examination of the literature revealed four studies where regional myocardial blood flow and myocardial infarction had been carefully examined together (13, 98, 157, 207). In two of these studies (98, 207), histological evidence of necrosis was correlated with regional myocardial blood flow and the concept of a minimal or "critical" blood flow delivery examined. Jugdutt et al. (98) described regions which received myocardial blood flow of 40 ml/min/100g or less as having necrosis, while areas with greater than 50 ml/min/100g demonstrated no necrosis. White et al. (207) found that blood flows of less than 30 ml/min/100g resulted in large amounts of necrotic tissue, while blood flow levels ranging from 30-45 ml/min/100g were marginal and demonstrated only intermediate amounts of necrosis. After LAD ligation, when the initial blood flow was greater than 45 ml/min/100g, tissue necrosis was almost never observed.

If the pre-occlusion and normal zone blood flows in the experimental groups were representative of the myocardial oxygen demand, then the blood flow can be considered to be a rough index of myocardial

metabolic demand. Following coronary artery occlusion, it has been shown that contractile function of the ischemic myocardium deteriorates rapidly (188, 189, 190). The critical blood flow would provide an index of the amount of blood flow necessary to sustain non-contracting tissue. The value determined in this study for acutely decentralized hearts was 30.6 $\pm$ 1.6 ml/min/100g. This level of myocardial blood flow, above which at least partial tissue viability in hearts was maintained, agreed quite well with the values of white et al. (207) and Jugdutt et al. (98).

Factors that alter metabolic demand should alter not only the myocardial blood flow, but also the critical blood flow. Evidence for this was seen in the three experimental groups as both denervation and sympathectomy lowered myocardial blood flow prior to LAD occlusion, and also lowered the critical blood flow. Stellate nerve stimulation on the other hand, raised the critical blood flow value. The altered critical blood flow level could change the infarction process even in the presence of no change in collateral flow.

Kloner et al. (112) described a concept similar to this in studies examining the effect of propranolol during myocardial ischemia in the open chest dog. Several investigators have shown that propranolol reduces infarct size (21, 117, 121, 174), but the mechanism of action of this drug in delaying or preventing irreversible ischemic damage is unknown. One hypothesis describes an improved oxygen supply to the compromized tissue via an increased collateral blood flow. Kloner et al. (112) were unable to demonstrate such a phenomenon in canine hearts sustaining left circumflex coronary artery occlusion. They examined blood flow to the left ventricle 5 minutes after circumflex occlusion

before and after propranolol. The occlusions were short (7 min) and separated by at least 30 minutes of undisturbed reperfusion. No increase in collateral blood flow following propranolol was seen. They concluded the protection seen after propranolol may be due to a reduced "demand" component, such that even though perfusion to the ischemic region was reduced following propranolol, demand of the ischemic region was reduced even further. The net result is an improvement in the supply:demand relationship such that lesser amounts of myocardium outstrip available substrate supplies and a smaller infarct results.

Results from the present study support the concept of a modest increase in perfusion to the ischemic region, and reduced metabolic demand following chronic cardiac denervation and sympathectomy. These two aspects work in concert to lessen the amount of myocardium that becomes infarcted during periods of reduced coronary arterial perfusion. These data support other studies utilizing the intrapericardial denervation technique (48, 94, 95, 96, 97, 177, 191, 192).

Similar extrapolation may be used to explain the effect of tonic sympathetic nerve stimulation. During coronary occlusion, increased levels of catecholamines also have been implicated to cause an oxygen wasting action (64, 65, 146, 148).

Tonic sympathetic nerve stimulation significantly increased infarct size and critical blood flow levels, but did not alter myocardial blood flow to the normal zone when compared to the normal zone of the neurally decentralized hearts. At first this observation caused great concern. Sympathetic nerve stimulation has been shown to increase contractility of the ventricle (28, 133, 151), and substantially increase

myocardial blood flow (12, 51, 109). Augmented contractility (5-10%) during the initial phase of continued sympathetic nerve stimulation was seen in this study, but by 15 minutes of stimulation contractile force had returned toward control. No increase in blood flow was evident at this point. Several authors (55, 100, 186, 187) have described vasoconstriction of the coronary arteries at stimulus parameters identical to those used in this study. Additionally, Vatner et al. (199) and Feigl et al. (57, 130, 144) have demonstrated that at low (physiological) levels of adrenergic stimulation, alpha-receptor mediated vasoconstriction successfully competed with, or completely overrode, the metabolic vasodilatory response mediated by beta-1 adrenergic receptors.

The increased infarct size seen in this section during left sympathetic nerve stimulation was in the presence of an increased critical blood flow, but no evidence for increased myocardial blood flow levels was seen. Activation of coronary vascular alpha receptors by released norepinephrine could cause vasoconstriction which competed with the vasodilation normally associated with increased oxygen consumption. This altered steady-state (130, 199) of the supply:demand relationship would lead to more cells being inadequately perfused and, subsequently, a larger mass of necrotic tissue.

In contrast to the alpha vasoconstriction, norepinephrine released by sympathetic nerve stimulation might activate coronary vascular beta-2 receptors. Although these receptors have been demonstrated (40, 71, 109, 120, 125), they do not appear to be functionally innervated (71) and probably do not play a role during the sympathetic nerve stimulations.

C. Effects of Previous Ischemic Insult on Infarct Size During LAD Occlusion in the Presence and Absence of Sympathetic Stimulation

Recent documentation of complete vasospastic occlusion in human coronary arteries without apparent vessel disease (122, 123), and the demonstration of repeated incidents of brief myocardial ischemia in a human subject (158) makes study of the heart's responses to multiple ischemic episodes of particular interest. Several authors (70, 122, 123, 158), have implicated coronary artery vasospasm as a (the) cause of acute myocardial infarction. While an acceptable animal model to define the role of coronary vasospasm in the myocardial infarction process does not exist, it seemed important to determine how a previous episode of ischemia affected the degree of myocardial infarction observed when permanent vessel occlusion followed.

In this study, a short (5 min) occlusion of the LAD followed by 3 minutes of reperfusion did not significantly change the total amount of infarcted tissue (total infarction plus partial infarction) seen after a second occlusion of 6 hours duration (Table 5). The previous period of ischemia did increase the amount of patchy tissue found in the infarct especially along the epicardial border. The occurrence of transmural infarction was seen in only 25% of the repeated occlusion hearts, while hearts sustaining abrupt LAD occlusion showed transmural infarction in 56% of the cases.

A possible explanation for the increased area of patchiness in the subepicardium of the reperfused hearts may be derived from experiments reported by Schaper (168). During periods of brief myocardial ischemia, the tissue demand for oxygen temporarily exceeds the availability of oxygen resulting in the build-up of vasodilator metabolites (10, 12,

26). With the release of the occlusion, a reactive hyperemia is seen such that myocardial blood flow is substantially higher than the pre-occlusion value in the ischemic region (12, 79, 198). The dog heart normally contains a significant number of pre-existing subepicardial collateral vessels which dilate when subjected to hypoxic stimulation (168). Although the criteria for a normal LAD anatomy in this study required the absence of visible epicardial collaterals, it was not possible to eliminate the presence of subepicardial channels in any of the hearts. Subepicardial vessels present between the left circumflex coronary artery and the LAD may enlarge dramatically when pressure in the LAD is re-established with release of the first occlusion. This rapid increase in LAD pressure during reperfusion could accomplish in a matter of minutes the marked overstretching of collateral vessel walls that, as described by Schaper, normally takes many hours when the occlusion is maintained. Overstretching of the vessel wall results in a tremendous increase in vessel diameter, as well as the rupture of the internal elastic lamina. This acute increase in collateral vessel diameter would allow limited blood flow to become available to myocardium that normally would have infarcted had the reperfusion not been performed.

The actual levels of myocardial blood flow in the patchy staining endocardium and epicardium were not significantly different during the second LAD occlusion from the value measured during the first occlusion. In this set of experiments, myocardial blood flow 2 minutes into the second occlusion was higher (6.1 ml/min/100g in the endocardium and 4.3 ml/min/100g in the epicardium) than the blood flow seen in the respective layer during the first occlusion. At two hours, this increase in flow

was even greater (12.0 ml/min/100g in the endocardium and 12.2 ml/min/100g in the epicardium). The p value approached, but did not reach, significance in both regions when occlusion #1 blood flow values were compared to the values measured 120 minutes after occlusion #2. A wide variability in the data prevented significance from being obtained.

Even though myocardial blood flow was not statistically altered, some important aspects of these data must be discussed. First, all patchy staining epicardium or endocardium were combined to determine the blood flow values reported in the results. No attempt was made to separate patchy tissue that originally demonstrated flow below the critical blood flow level from patchy tissue with initial blood flow levels above the critical value. This technique of examining the samples may have masked significant change in flow to tissue that originally was critically insulted. Less observed transmural infarction in the repeated occlusion study compared to the non-repeated occlusion study indirectly supports this concept. Second, this study demonstrates that brief periods of ischemia do not increase the capacity of collateral blood flow enough to prevent infarction. Well defined cores of infarction were seen in the repeated occlusion hearts. Brief ischemia preceding complete occlusion does increase the region of tissue admixture, especially at the epicardial level. Salvaging of myocardium along the margin of the infarct following coronary artery occlusion by using various agents has been described (13, 21, 110, 117), and agrees with the concepts put forth in section B of this dissertation.

The addition of tonic sympathetic nerve activity during repeated occlusion reduced the salvage of ischemic tissue. Blood flow patterns

in the patchy endocardium and epicardium of the repeated occlusion-sympathetic nerve stimulation group were similar to those reported in the repeated occlusion study. Infarct size was larger in the repeated occlusion-sympathetic nerve stimulation group, but not significantly different from the animals with repeated occlusion alone ($p < .05$). Sympathetic activation increased the incidence of transmural infarction from 25% in the repeated occlusion animals to 63% in the repeated occlusion-sympathetic nerve stimulation group.

Release of norepinephrine during stellate stimulation increases myocardial metabolic demand (28, 51, 66) but again in this study, no increase in myocardial blood flow to the normal zone of the nerve stimulation dogs was seen. With repeated occlusion, the increase in flow occurring through the epicardial collaterals may not supply enough oxygen to offset the increased demand caused by the presence of the released norepinephrine. This results in less salvaging of myocardium along the margin of the infarct, i.e. less patchiness, and the occurrence of transmural infarction. The importance of even low levels of sympathetic activity (3 cycles/sec) is apparent.

D. Effect of Time Interval Between Repeated Brief LAD Occlusions Upon Arrhythmia, S-T Segment Elevation and Myocardial Blood Flow

After approximately two or three minutes of coronary artery occlusion, changes are noted in bipolar electrograms recorded both endocardially and epicardially in the ischemic region (47, 131, 161, 173, 210). Initially, a transient increase in conduction velocity (a decreased time-to-onset of the bipolar electrogram) is seen (172, 210), following which a loss of electrogram amplitude, an increase in electrogram duration and a delayed time-to-onset is observed (131, 161, 173). Also,

the appearance of one or more activation spikes following the initial major activation complex is seen (15, 131, 173, 210). Studies have also demonstrated epicardial alteration of the above parameters to be more severe than the endocardial changes (15, 32, 161).

Results from this study are, in part, consistent with previously reported data (131, 161, 173, 210). During the initial occlusion in either group of dogs a decrease in electrogram amplitude and an increase in electrogram duration were seen both endocardially and epicardially. Time-to-onset of the electrograms was not significantly increased during occlusion #1. No change from control values was seen in the time-to-onset in either the endocardium or epicardium of the repeated occlusion dogs or repeated occlusion-sympathetic nerve stimulation dogs. Unlike previous reports, there were no significant differences between the endocardial and epicardial parameters. In previous studies (32, 161, 173), multiple recording sites in each heart were utilized and each recording site included in determining the total N of the study. For example, Ruffy et al. (161) recorded 120 bipolar electrograms in 19 dogs and used, for statistical analysis, an N of 120. In this study, two endocardial and two epicardial recordings were analyzed from the ischemic zone of each heart and combined to give a mean value for endocardial and epicardial electrogram amplitude, duration, and time-to-onset. All statistics were performed on an N of 8 dogs in both the repeated occlusion and the repeated occlusion-sympathetic nerve stimulation groups.

In both groups of dogs, occlusion #1 and #3 resulted in significant decreases in electrogram amplitude, and significant increases in electrogram duration while no change from control was seen in these parameters

during occlusions #2 and #4.

Bipolar electrogram fractionation as described by Williams et al. (210), Murdock et al. (131), Scherlag et al. (173), and Boineau and Cox (15) was seen in this study, but was not precisely quantitated. Williams et al. (210) quantitated this fractionation of the electrogram, and found that the appearance of the marked desynchronization of activation coincided with the time of occurrence of ventricular arrhythmias. This desynchronized activity continued well past electrical systole and suggested the presence of extremely slow conduction or local fibrillation (210). Although the occurrence of desynchronized local electrical activity was not precisely quantitated in this study, several incidents were observed during occlusions #1 and #3 in both groups of dogs. Little evidence for desynchronized activity was seen during occlusions #2 and #4 in the repeated occlusion group.

The postulate that the presence of desynchronized activity is closely related to arrhythmia (15, 131, 173, 210), and the observation that the second and fourth occlusions of the experimental protocol resulted in very little electrogram fractionation, may explain the marked decrease in arrhythmia during these occlusions. It was originally postulated that the decrease in arrhythmia, S-T segment elevation and electrical abnormalities during occlusions #2 and #4 was due to enhanced blood flow to these regions during LAD ligation. Figure 23 did not support this hypothesis, as measured myocardial blood flow to the sites of electrical recording did not change significantly during any occlusion. An alternative hypothesis to explain the reduced incidence of electrical abnormality and arrhythmia during occlusions #2 and #4 had to be proposed.

During ischemia, potassium released from the ischemic cells (27, 72, 74, 154) may cause or contribute to changes in cellular excitability by creating a gradient of excitability from ischemic to normal myocardium (52, 73, 81). Ischemic muscle looses a large amount of potassium very rapidly (52, 81), and other work has suggested that release and accumulation of potassium in the extracellular space may be arrhythmogenic (27, 72, 196). Hill and Gettes (81) demonstrated a two to threefold increase in regional potassium ion concentration and activity in ischemic muscle following five minutes of LAD occlusion. Local bipolar electrograms located at the site of potassium measurement became progressively delayed and with time showed marked fractionation during the most rapid phase of cellular potassium loss. This phase usually occurred two to six minutes after the onset of LAD occlusion. Reperfusion would be expected to result in the rapid washout of potassium and the return of the extracellular potassium levels to control.

During occlusion #1 of the repeated occlusion protocol, a series of event similar to those described by Hill and Gettes (81) occurs. Reperfusion of the myocardium washes out the potassium lost from the cells and the excitability gradient is eliminated. The ischemic cells, though, recover function slowly (80, 141, 178) and metabolic processes may not return to control immediately (80, 112). It is probable that re-establishment of normal potassium levels in the ischemic cells also occurs slowly. The abrupt re-occlusion of the LAD after three minutes of reperfusion initiates the ischemic reactions in the cell, but the ischemic region may possess reduced levels of intracellular potassium (206) and therefore limited release of potassium during the second

occlusion occurs. Excitability gradients (52) would be less during this occlusion period and therefore reduced arrhythmia and bipolar electrogram abnormalities are expected. Several studies have implicated loss of intracellular potassium in the generation of S-T elevation (85, 163, 201), therefore decreased levels of extracellular potassium during the second occlusion may also explain the observed reduction in endocardial and epicardial S-T segment elevation. Long periods of reperfusion, i.e. forty to sixty minutes, would allow more time for the re-establishment of intracellular potassium levels, and would explain the similarity between phenomenon seen during occlusions #1 and #3.

This demonstration of lesser indices of injury during identical periods of occlusion was also seen in the presence of tonic sympathetic nerve stimulation. The recent report of Robertson et al. (158) describing a patient with multiple incidents of variant angina, S-T segment elevation and arrhythmia takes on even greater significance in light of these results. Repeated incidents of ischemia (vasospasm) closely associated in time may cause severe myocardial damage, but be manifest as only a relatively mild injury when examined by means of electrocardiography.

E. Speculation Upon Significance of Study

The data presented in this dissertation are of clinical interest, and potentially lend insight into the process of myocardial infarction during coronary occlusion. While the open-chest, anesthetized dog with no apparent evidence of cardiac disease may not be the exact model to mimic coronary disease in humans, some comments may be made.

First, it was apparent from sections A and B of the results and discussion that chronic, cardiac denervation or ventricular sympathectomy

afforded protection to the left ventricle during LAD occlusion. This protection to the left ventricle during LAD occlusion. This protection was in the form of reduced infarct size, lesser S-T segment elevation and fewer arrhythmias. Low levels of left ansae subclavia stimulation significantly increased left ventricular infarct size and arrhythmia. The recent speculations that coronary bypass surgery may actually denervate regions of the heart poses questions of 1) whether regional myocardial perfusion actually improves significantly after coronary bypass, 2) whether regional cardiac denervation (presumably in the regions of compromised myocardial blood flow) lowers metabolic demand such that substrate delivery is now adequate to support tissue demand, or 3) whether transection of the cardiac nerves prohibits increased levels of sympathetic neural activity from reaching the zone of compromised flow and accentuating ischemic damage. Experiments utilizing regional cardiac denervation techniques (i.e. selective denervation) will lend further insight into these questions. Results from this study would support concepts potentially involved in the answering of questions 2 and 3.

Secondly, data from this dissertation support the concept that tonic levels (3 cycles/sec) of sympathetic nerve activity is vasoconstrictor in nature, and competes with the metabolic vasodilation present during the activation. This observation merits particular interest in that levels of sympathetic activation of 3 cycles/sec have been recorded in animal experiments. Rarely does the sympathetic nervous system fire at rates of 10-20 cycles/sec, but these stimulation parameters are used routinely to effect supramaximal sympathetic stimulation. More intense

examination of the sympathetic nervous system and sympathetic neuromediators during myocardial infarction at "physiologic" levels of activation are called for. If levels of sympathetic activation from 1-5 cycles/sec do increase myocardial metabolism and vasoconstrict coronary arteries, re-evaluation of adrenergic blocking agents as therapy for myocardial ischemia may be in order. Experimental studies utilizing propranolol in some instances have shown this agent to decrease infarct size, while other studies could not demonstrate this protection. Prevailing levels of sympathetic tone and circulating catecholamines in various experimental models may, to some extent, account for the differences seen in these studies. Also, studies combining propranolol treatment with alpha-adrenergic blocking agents (phentolamine, phenoxybenzamine) may demonstrate more evidence for protection against myocardial infarction during coronary artery occlusion.

Finally, the importance of the knowledge of when repeated incidents of myocardial ischemia occur was demonstrated. Brief periods of LAD occlusion prior to permanent occlusion of the LAD resulted in no change in total infarct size but lesser evidence of total (core) infarction. Also, less evidence of damage as shown by electrical abnormalities (arrhythmia, S-T segment elevation, myocardial activation) was seen during the second occlusion. This is important in that clinically it is the electrical abnormalities that are first detected or monitored. Repeated incidents of ischemia may result in little or no evidence of electrical abnormalities, while large amounts of actual tissue damage (necrosis) may be occurring at the same time. Reports of documented repeated coronary vasospasm where some bouts of spasm showed little or

no change in the electrocardiographic parameters support the observations of this dissertation. It is therefore important to continue study into the role of coronary vasospasm on myocardial necrosis, and to realize that absence of electrocardiographic damage may not necessarily reflect the absence of actual tissue damage.

CHAPTER VI

SUMMARY

1. Under similar working conditions (double product), left ventricular myocardial blood flow is reduced following two weeks of complete cardiac denervation or ventricular sympathectomy when compared to the neurally intact or acutely decentralized left ventricle.

2. Following mid-LAD occlusion, the denervated heart demonstrates lesser indices of electrical injury (local endocardial and epicardial S-T segment elevation, arrhythmia, fibrillation) than the neurally intact heart.

3. Complete cardiac denervation and ventricular sympathectomy decrease infarct size following mid-LAD occlusion from the value observed in acutely decentralized hearts. This reduction in infarct size is not as great as was reported with ischemic insults of lesser severity.

4. Low levels of sympathetic nerve activity (3 impulses/sec) significantly increase left ventricular infarct size following mid-LAD occlusion.

5. The minimal (critical) blood flow necessary to sustain tissue viability following LAD occlusion is significantly lowered by chronic cardiac denervation and sympathectomy. Low levels of sympathetic nerve activity significantly raise the critical blood flow value.

6. A brief period of ischemia prior to permanent occlusion of

the LAD does not change total infarct size significantly in the absence of neural input. Severity of infarction is reduced, as the central core of total infarction is less, while a larger region of partial (patchy) infarction is seen.

7. A brief period of ischemia prior to permanent occlusion of the LAD in the presence of tonic sympathetic nerve stimulation does not alter total infarct size significantly from repeated occlusion in the absence of sympathetic nerve stimulation. A larger core of infarction is seen in the nerve stimulation study, and the region of partial (patchy) infarction is significantly less than the repeated occlusion group.

8. A brief period of reperfusion between two short (5 min) LAD occlusions significantly decreases the amount of injury seen during the second occlusion. Less arrhythmia, S-T segment elevation and myocardial activation abnormalities are seen during the second occlusion of a pair of occlusions separated by 3 min of undisturbed reperfusion.

9. A long period of reperfusion (40-60 min) between two short LAD occlusions results in reproducible levels of arrhythmia and electrical alteration.

10. No change in myocardial blood flow to the regions sampled for electrical abnormalities was seen in the repeated occlusion studies. Changes in flow therefore could not explain the observed lessening of injury during occlusions separated by only 3 min of reperfusion.

CHAPTER VII

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CHAPTER VIII

APPENDIX

Below is a summary of animals reported in the results section of the dissertation. Included in each summary are the animal groups, number of dogs attempted, number of successful experiments completed, procedure performed, and reasons for exclusion of animals. The procedure listed in each section is common to all experimental groups in that section.

A. Section A of Results

Group	Attempted	Successful	Procedure
Chronic Cardiac Denervation	12	10	Blood flow and S-T segment changes with LAD
Sham-Cardiac Denervation	12	10	Occlusion (Methods E)

- Two denervated dogs were excluded from the study due to incomplete denervation.
- Two sham-operated dogs were excluded from the study due to the development of ventricular fibrillation.

B. Section B of Results

Group	Attempted	Successful	Procedure
Chronic Cardiac Denervation	5	5	LAD occlusion for 6 hours - infarct size

Group	Attempted	Successful	Procedure
Chronic Ventricular Sympathectomy	10	8	Determination and serial myocardial blood flow
Acute Cardiac Decentralization	19	16	Measurements - evaluation of critical blood
Tonic Sympathetic Nerve Stimulation	11	8	Flow level (Methods F)

- Two chronic ventricular sympathectomy dogs were excluded due to incomplete denervation.
- Three acute cardiac decentralization dogs died in ventricular fibrillation and were excluded from the study.
- Three tonic sympathetic nerve stimulation dogs died in ventricular fibrillation and were excluded from the study.

C. Section C of Results

Group	Attempted	Successful	Procedure
Repeated Occlusion	10	8	5 min of LAD occlusion - 3 min reperfusion - 6 hr LAD occlusion - infarct size determination
Repeated Occlusion - Tonic Sympathetic Nerve Stimulation	10	8	And serial blood flow measurements - critical blood flow evaluation (Methods F)

APPROVAL SHEET

The dissertation submitted by Michael J. Barber has been read and approved by the following committee:

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The final copies have been examined by the Director of the dissertation and the signature which appears below certifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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