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CHANGES IN THE AREA AT RISK OVER TIME AND FOLLOWING
NICARDIPINE ADMINISTRATION IN THE DOG

A THESIS PRESENTED TO
THE SCHOOL OF GRADUATE STUDIES
THE UNIVERSITY OF OTTAWA

by

REENA SANDHU, B.Sc. (Hon. Biology)

In Partial Fulfillment of Requirements for the
Degree of Masters of Science

in the

Department of Physiology
Faculty of Medicine

George P. Biro, M.D., Ph.D.
Professor, Research Supervisor
Department of Physiology

Reena Sandhu
M.Sc. candidate



Reena Sandhu, Ottawa, Canada, 1990



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ABSTRACT

A major determinant of myocardial infarct size (IS) is the amount of myocardium at risk of infarcting (AAR). When determined in vivo, AAR size depends upon the size of the normal perfusion territory of the occluded bed and upon collateral flow. We tested the hypothesis that AAR size can change spontaneously (Group 2, n=12) or after therapy (Group 3, n=17). ^{99m}Tc macroaggregates (MAC) or Monastral Blue Dye (MBD) were used to define the AAR. In preliminary experiments, (Group 1, n=13) it was determined that MAC and MBD, when injected sequentially, delineate identical areas of myocardium. In Group 2, we determined the size of the AAR existing at 10 minutes post-occlusion using (MAC) and at 3 h using (MBD) in anesthetized dogs subjected to 3 h of LAD coronary artery occlusion. The protocol for Group 3, was identical to Group 2 except that Nicardipine infusion ($10\mu\text{g}/\text{kg}$ bolus, $8\mu\text{g}/\text{kg}/\text{min}$. for 165 minutes i.v.) began after MAC injection. After MBD, the hearts were excised, sliced, photographed and incubated in Nitro-Blue tetrazolium to identify the infarct. After rephotographing, the slices were autoradiographed. The two sets of photographs and autoradiographs were used for planimetry of the AAR as defined by MBD, infarct size, and AAR as defined by MAC respectively.

In all 3 groups there was a correlation between the AAR sizes as determined by the two techniques ($p < 0.01$). The slopes of each of the regression lines for Groups 1, 2 and 3 were 0.98, 1.05 and 0.86 respectively. Microsphere estimated collateral flow in Group 2 showed no change from 10-180 minutes post-occlusion and in group 3, only flow to the central epicardial region of the AAR showed a marginal increase over the same period. Infarct sizes in Group 3 were not significantly different from Group 2. Thus in this model, the AAR size does not change either spontaneously or following Nicardipine. Nicardipine also does not appear to substantially limit infarct size.

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ABBREVIATIONS

(In alphabetic order)

AAR	Area at Risk
AAR _{MAC}	Area at Risk as defined by ^{99m} Tc Macroaggregates
AAR _{MBD}	Area at Risk as defined by Monastral Blue Dye
AVAAR	Anatomical Vascular Area at Risk
BP	Blood Pressure
CEN	Central Region of the Area at Risk
CO	Cardiac Output
CVP	Central Venous Pressure
CxCA	Circumflex Coronary Artery
DAP	Diastolic Arterial Pressure
DLVP	Diastolic Left Ventricular Pressure
ECG	Electrocardiogram
HR	Heart Rate
I.D.	Internal Diameter
IS	Infarct Size
LADCA	Left Anterior Descending Coronary Artery
LAI	Lateral Adjacent Inner Margin of the Area at Risk
LAO	Lateral Adjacent Outer Margin of the Area at Risk
LAP	Left Atrial Pressure
LCCA	Left Common Coronary Artery
LV	Left Ventricular

LVEDP	Left Ventricular End Diastolic Pressure
LVFP	Left Ventricular Filling Pressure
MAC	^{99m} Tc Macroaggregates
MAP	Mean Arterial Pressure
MBD	Monastral Blue Dye
MI	Myocardial Infarction or Myocardial Infarct
NBT	Nitro-Blue Tetrazolium
NOR	Normal Myocardium
PAP	Pulmonary Arterial Pressure
PCWP	Pulmonary Capillary Wedge Pressure
PVAAR	Physiological Vascular Area at Risk
RAP	Right Atrial Pressure
RMBF	Regional Myocardial Blood Flow
SAP	Systolic Arterial Pressure
SEM	Standard Error of the Mean
SLVP	Left Ventricular Systolic Pressure
SPA	Septal Perforator Artery
SD	Standard Deviation of the Mean
SV	Stroke Volume
TPR	Total Peripheral Resistance
TTC	Triphenyl Tetrazolium

INTRODUCTION

PREAMBLE

Myocardial infarction (MI) is a major cause of death in industrialized societies, and in Canada and the United States alone, it has been estimated that approximately 1.5 million patients develop myocardial infarcts each year. Approximately half this number die each year from ischemic heart disease and its complications (Chu-Jeng Chiu, 1980).

In humans, it is known that several mechanisms interact in the pathogenesis of ischemic heart disease and MI. These include atherosclerosis, platelet adhesion, coronary thrombosis and coronary artery spasm. The relative importance of each of these factors and the interrelationships among them will not be discussed here, however, excellent reviews are by Anderson (1987) and Norris (1984).

1.1 INFARCT DEVELOPMENT

In the dog, as in the human, permanent coronary artery occlusion results in the development of an ischemic zone in which blood supply is insufficient to meet demand. The sequence initiated by the onset of ischemia begins with reversible cell changes, and unless interrupted, ends with irreversible cell changes, cell death and the development of a myocardial infarct (Braunwald et al., 1975).

In a classical series of experiments in the dog, Reimer and Jennings (1979) showed that the rate of cell death within the ischemic zone is not uniform, with irreversible injury initiated in the subendocardium,

progressing outward towards the epicardium with time. The progression of the infarct was, on average, greatest during the first 3 h of occlusion, with nearly all of the subendocardium and mid-myocardium being infarcted at this time. After 24 h of occlusion, the infarction process was nearly complete, with the infarct in some cases, being completely transmural.

This "wavefront" progression of necrosis beginning from the subendocardium is largely due to the fact that there is a definite gradient of residual blood flow to the ischemic zone progressively declining from the subepicardium to the subendocardium in the dog (Downey et al., 1973, Marcus et al., 1976, Russell et al., 1977). This residual flow comes from collaterals which are narrow, thin walled conduits that are an alternative to a major vascular conduit that has become non-functional i.e. obstructed. Thus, a collateral channel is a pathway that is recruited when the original vessel fails to permit normal flow. Whether or not flow occurs through collaterals in situations in which ischemia is not present is not known, however, it would appear unlikely in the absence of pressure gradients between vascular beds. Coronary collateral vessels can arise as a consequence of naturally occurring anastomoses between coronary arteries, between branches of the same coronary artery and also between coronary arteries and other systemic vessels (Cohen, 1985). The last type of connection gives rise to what is referred to as "non-coronary" collateral flow. Collaterals are extremely important in that they offer some metabolic support to delay or, in some cases prevent the death of myocytes (Cohen, 1985).

In the dog, the presence of collateral flow is usually sufficient to ensure some spontaneous subepicardial salvage which can often

represent as much as 10-20% of the area of myocardium at risk of infarcting (Yellon et al., 1981). Although collateral flow figures are not available for human MI, studies on human autopsy material reveal that the amount of spontaneous subepicardial salvage is similar to that seen in the dog (Chu-Jeng Chiu, 1980).

The greater rate and extent of necrosis in the subendocardium is also facilitated by the higher oxygen consumption of the subendocardium as compared to the subepicardium (Kirk et al., 1964, Whalen et al., 1973, Downey, 1984). This is due to the fact that the subendocardial fibers are subjected to higher wall tensions during systole (Mirsky, 1969, Sabbah et al, 1981, Downey, 1984). Lowe et al. (1983) has found that even in the absence of wall stress and blood flow gradients, a wavefront of cell death beginning from the subendocardial layers occurred. He suggested that the subendocardium was inherently less tolerant of ischemic insult. All of these factors combine to produce gradients of flow, injury and metabolism in the transmural plane. This gives rise to the development of a spatially identifiable transmural border zone of reversibly injured tissue in which the fate of the cells hangs in the balance and where progression to functional recovery or regression to necrosis depends upon the inequality between the demand and the supply of arterial blood.

In the lateral plane, some investigators have proposed that the boundary between the normal and ischemic tissue is sharp with the transition from normal to ischemic tissue being characterized by a sharp but irregular interface in flow and injury with severely ischemic tissue lying adjacent to well perfused tissue (Yellon et al, 1981, Murdock et

al., 1983, Janse, 1984). This view is supported by Okun et al. (1979) and Factor et al. (1978) who have shown by injecting latex of different colours into the coronary circulation of the isolated dog heart that capillaries derived from large adjacent arteries are discrete with no interconnections (See Figure 1.1a).

Others have proposed that the boundary between normal and ischemic tissue is not a discrete demarcation but rather a spatially identifiable zone of tissue characterized by intermediate values of blood flow and therefore ischemic injury. Such a border zone could exist if collateral flow was preferentially delivered to the lateral borders of the ischemic region. It has been reasoned that the anatomic center of the perfusion territory of the occluded artery would be farthest from the adjacent normal vessels which gives rise to the collaterals. According to Poiseuille's law, all other things being equal, the longer length of the collateral channel to the center would result in an increased vascular resistance and thus a diminished flow to the ischemic core (Cohen, 1985).

Another possibility that would also give rise to a lateral border zone is the existence of intramural anastomoses between adjacent coronary vessels and a complex pattern of interdigitation between adjacent arterial beds. Prezyklenk's study (1984) supports this hypothesis and has shown that capillary anastomoses between adjacent beds are abundant in the dog (20% between the Circumflex (CxCA) and the Left Anterior Descending Coronary Artery (LADCA)). This viewpoint is also supported by others (Becker et al., 1973, Jugdutt et al., 1979b). A series of biochemical, (Opie et al., 1976a-b, Hearse et al., 1977) histological, (Page et al., 1977, Buja et al., 1976), electrophysiological, (Kleber et

Figure 1.1 Lateral and Transmural Border Zones

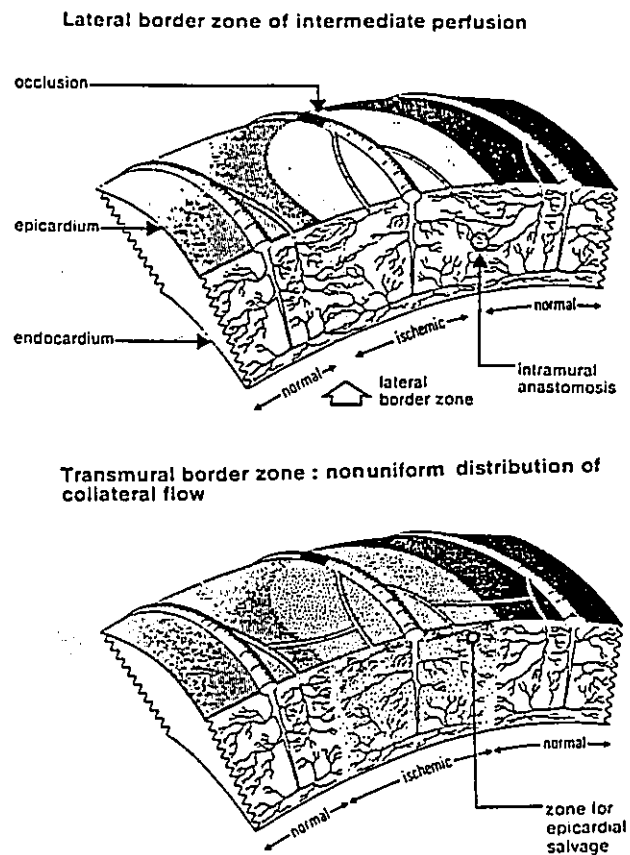


Figure 1.1 The mechanisms by which lateral and transmural border zones may arise. *A*: A lateral border zone may arise if intramural anastomoses between small vessels from adjacent vascular beds existed along with a complex pattern of interdigitation between beds. *B*: A transmural border zone is known to exist in the dog and arises due to a combination of factors. Firstly, collateral flow is preferentially delivered to the epicardium and secondly the endocardium has a greater energy requirement when compared to the epicardium. It has also been proposed that the endocardium is inherently less tolerant of ischemic insult. All of these factors may combine to produce a transmural border zone in which the epicardium is more likely to undergo natural salvage and be responsive to therapy. Taken from Hearse and Yellon, 1984

al., 1978) and a variety of mechanical observations (Theroux et al., 1974, Ross et al., 1976) have also been suggestive of a region of intermediate injury. In addition to this supporting evidence, many studies have demonstrated that the infarct that develops after coronary occlusion is smaller than the region at risk of infarcting with sparing of the lateral margins (Davenport et al., 1984, Reimer et al., 1979, Konyagi et al., 1982).

The reason for the discrepancies in the existence and characteristics of the lateral border zones are not clear. However, some investigators suggest that the lateral border zone of intermediate values for metabolites, enzymes, blood flows, ST segment elevations and systolic wall thickenings are a result of sampling artifact (i.e. a mixture of ischemic and normal cells) (Cohen 1985, Hearse and Yellon, 1984). Figure 1.1 shows how transmural and lateral border zones could arise.

Thus, the development of an infarct can be viewed as a growing mountain, the base of which is the subendocardium and the summit of which advances further and further towards the epicardium, reaching its ultimate height in about 24 hours. Both the maximum height and the slope and contour of the sides is determined by the nature of the transmural and lateral border zones (Figure 1.2) (Armiger, 1984).

1.2 INFARCT SIZE LIMITATION

Quantitative studies on human autopsy material have revealed that infarct size is correlated well with clinical course. Oliver (1984) and others (Chu-Jeng Chiu, 1980) have stated that an infarct involving more

Figure 1.2

The Process of Infarct Development

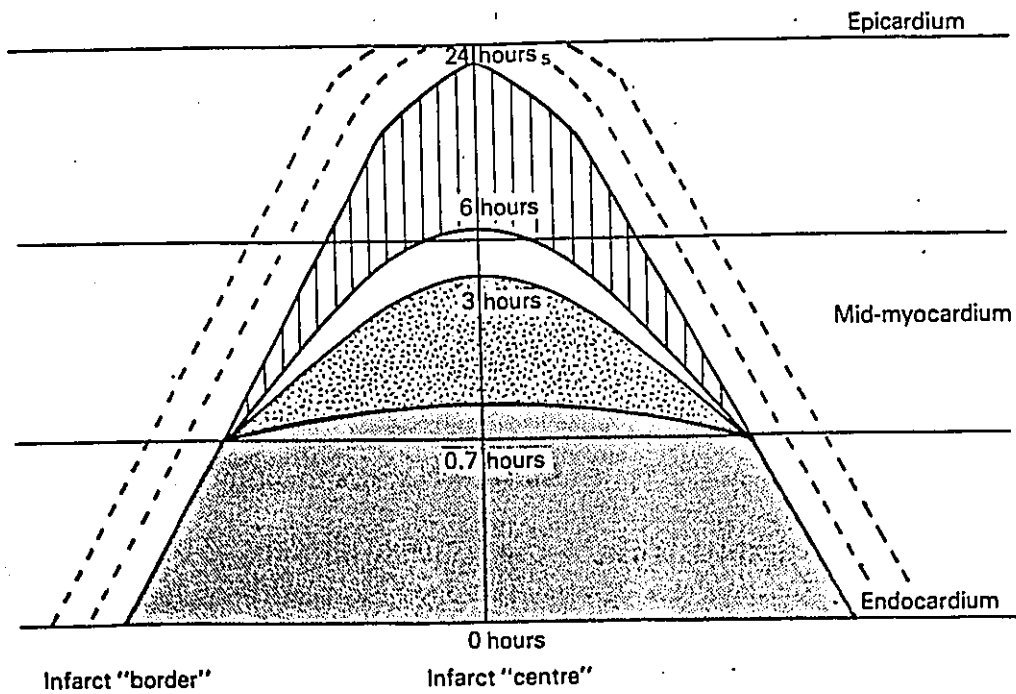


Figure 1.2 The development of an infarct can be viewed as a growing mountain, the base of which is the endocardium and the summit of which advances towards the epicardium with time. The ultimate height of the infarct and the contour of the sides depends upon the nature of the transmural and lateral border zones. Figure from Armiger, 1984

than 40% of the left ventricular mass often results in cardiogenic shock, severe congestive heart failure or fatal arrhythmias. It appears, that among clinicians, there is no doubt that interventions capable of limiting infarct size offer a great deal of promise in improving the prognosis of patients suffering large infarcts.

If one then considers the fact that infarction is not an all or none phenomenon, but a dynamic process in which the transmural and perhaps lateral margins of the ischemic region are more resistant to irreversible injury, one is left with the hope that infarct size limitation is a realistic possibility. Thus, if a therapeutic agent is applied early in the infarction process, and is capable of enhancing collateral flow or altering the energy demand or by directly protecting the myocytes from cell death, it seems quite plausible that it may influence the position of the border zones such that they lie closer to the subendocardium and the center of the ischemic core. This would then result in either a decrease in the total extent of necrosis or perhaps a decrease in the rate of necrosis. The latter possibility is not without value because if necrosis can be delayed this would buy time for secondary procedures such as balloon angioplasty or thrombolysis to be employed (Hearse and Yellon, 1984). Furthermore, it is documented (Cohen, 1985) that significant collateral growth begins approximately 24 h after occlusion, thus allowing for the possibility of natural salvage.

In the past two decades, numerous experimental and clinical studies have been conducted on as many as 50 infarct limiting agents. Many of these have reported encouraging results; however, none have been encouraging enough to be adopted for widespread and sustained clinical

use. Hearse and Yellon (1984) stated that definitive proof of substantial tissue salvage and thereby improvement in patient prognosis is lacking. There are probably two main factors which are responsible for the current sense of reservation about infarct size limitation. Firstly, there is a discrepancy between the "hope" generated by animal experiments and the "reality" of infarct size limitation as assessed in clinical studies. For example, Nifedipine, a calcium channel blocker, has been shown in countless animal experiments to be effective in limiting infarct size (Kloner et al. 1987). However, in the Nifedipine Angina Myocardial Infarction Study (NAMIS), Muller and coworkers (1984) were unable to demonstrate a reduction in the frequency or size of myocardial infarcts when patients were randomly given Nifedipine (80 mg/day) or placebo within 4.6 h after the onset of pain. Although the total mortality after 6 months did not differ between Nifedipine and placebo groups, the mortality was actually higher in the Nifedipine assigned group (7/89) when compared to the placebo assigned group (0/89). Secondly, there appears to be a general mistrust in the validity of animal experiments. This stems from the use of multiple models, widespread interspecies differences in collateral flow, (Maxwell et al., 1987a) various methodologies and endpoints used to answer the question "Does drug X limit infarct size?"

Reimer and Jennings (1984b) stated that in the dog model designed to evaluate the amount of tissue saved as a consequence of an intervention, an anatomic assessment of infarct size should be the primary endpoint. Although this may seem self evident, this has not been the case, as a variety of other endpoints have been used to identify potentially

beneficial effects of an intervention (See Table 1.1). Furthermore, the assessment of infarct size should ideally be made at a time when the infarction process is complete. If infarction is not complete, it becomes more difficult to determine whether the administration of a therapy has resulted in absolute myocardial salvage or has simply slowed the rate of progression of necrosis such that infarction is still inevitable but postponed until a later time. An excellent example of this is presented by Reimer and Jennings (1984a) who discussed a study in which flurbiprofen resulted in a significant reduction in infarct size when compared to control when infarct size was assessed at 6 h post-occlusion. However, if infarct size assessment was made at 24 h post-occlusion, no significant reduction in infarct size was seen.

It is also important that variations in the primary endpoint be controlled by measuring those variables which can influence the ultimate size of the evolving infarct. These variables include the two major determinants of infarct size, i.e. the size of the area at risk (AAR) and the amount of collateral flow received by this zone. Both of these factors are, to a large extent, determined by coronary anatomy which shows tremendous inter-individual variability.

In a study aimed at assessing the anatomy of the ventricular coronary arteries in the dog, Blair (1961) found that the dog is left coronary preponderant, with 92% of the dogs studied having a left common coronary artery (LCCA). Most commonly (55%) the LCCA branched into three arteries in roughly the same area; LADCA, CxCA and the Septal Perforator artery (SPA). In 36% of the cases however, the SPA was a branch of the LADCA. Since the SPA provides flow to the AV node, the bundle of His and the

Table 1.1

Endpoints Commonly used to Identify the Potentially
Beneficial Effects of an Intervention

Infarct size
Histologic assessments
Gross measurement (with or without dehydrogenase staining)
Indirect indices of infarct size <i>in vivo</i>
Enzyme release curves, ST segment and QRS mapping
Various imaging techniques
Echocardiography
Collateral blood flow
Myocardial contractile function
Ultrastructural changes
Function of subcellular organelles
Intact cells
Mitochondria
Sarcoplasmic reticulum
Sarcolemma
Lysosomes
Biochemical changes
High energy phosphates,
Ion gradients,
Catabolites
pH

Table 1.1 A number of different endpoints have been used to evaluate the beneficial effects of a therapy in attenuating the degree of ischemic injury. Reimer and Jennings (1984a) stated that in the dog model designed to evaluate the amount of tissue saved as a consequence of an intervention an anatomic assessment of infarct size should be the primary endpoint. Taken from Reimer and Jennings (1984a).

septal region, it is quite conceivable that in some dogs ligation of the LADCA may involve cessation of flow to these areas whereas in other cases it may not. Furthermore, Blair found considerable differences between dogs in the number of branches of the LADCA, with some animals having only 2 (17%) branches and others having as many as 6 (7%). There were also significant differences between animals in the number of anastomoses between coronary arteries; for example 25% of all dogs did not exhibit an anastomosis between the terminal branch of the LADCA and the posterior branch of the CxCA. Therefore it is quite obvious, that because of the differences in the branching pattern of the coronary arteries, the area of myocardium denied adequate perfusion when for example the LADCA is ligated "half way between the apex and the base" will be quite different in different animals. Furthermore, the amount of residual collateral flow received by this zone is dependent on the number of arterial anastomotic connections between coronary beds, which, as Blair has demonstrated, shows considerable inter-animal variability.

If however these variables (Collateral flow and AAR size) are measured, it is possible to normalize infarct sizes and thus detect therapeutic effects with greater confidence; for example, if the size of the AAR is known, it is possible to express infarct size as a percentage of this. In this way, individual differences in coronary anatomy, are largely controlled for. Furthermore, if infarct size is not normalized, false conclusions may be made about the therapeutic potential of an agent because these random selection differences are not accounted for. For example, if the treated group of animals has an inherently higher level of collateral flow and/or smaller area at risk, this may create the

appearance of lowered infarct sizes in treated animals and thereby lead to the erroneous conclusion that the drug limits infarct size. Finally, by measuring changes in these variables prior to and following therapy, it is possible to gain insight into a drug's mechanism of protection. This last concept is illustrated in Figure 1.3 which shows that if a therapy limits infarct size without changing collateral flow, the regression of subepicardial collateral flow on infarct size (as measured as a percentage of the AAR) would be similar to Figure 1.3a. when either pre- or post-therapy measures of collateral flow were used. That is to say that infarct size is smaller in the treated group for the same level of collateral flow. However, if the drug acts by increasing collateral flow to the subepicardium of the risk zone, the situation depicted in Figure 1.3b might exist (i.e. a downward shift of points when post-therapy collateral flow measurements were considered (Reimer and Jennings, 1984b).

Although in the last 15 years it has been standard practice to measure changes in collateral flow before and after drug administration, determination the AAR size has largely been regarded as a static variable used only to standardize infarct sizes. However, it is proposed that this too is an important variable that can be used to gain information about the mechanism of action of a therapeutic agent.

1.2.1 Area at Risk

The area at risk is the zone of hypoperfused tissue produced by the occlusion of a coronary artery. It is within the transmural and lateral margins of this zone that therapeutic agents may exert their infarct

Figure 1.3

The Possible Effects of an Infarct Size Limiting Therapy
on the Regression of Infarct Size on Collateral Flow

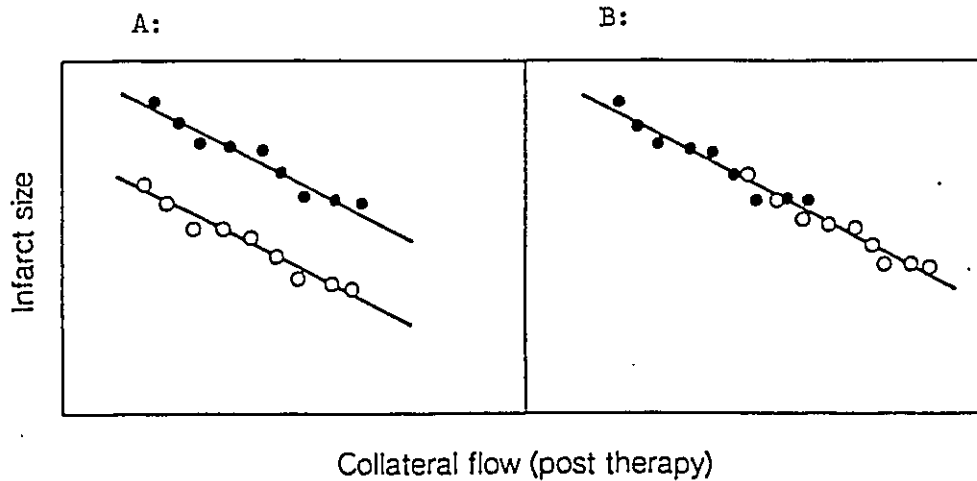


Figure 1.3 Theoretical results from protection which is flow independent A: versus protection which is flow dependent B:. In A the therapy has resulted in lower infarct sizes for any given level of collateral flow. In B infarct sizes are lower probably because collateral flow is higher. *Solid circles: control; open circles; treated.* Taken from Reimer and Jennings, 1984a.

limiting effects. It is now well recognized (for the reasons mentioned above) that infarct size must be expressed as a percentage of the area at risk in any study designed to compare infarct sizes in treated and untreated groups. In the past, this variable has been measured using multiple methodologies which cast some doubt on whether experimentalists were identifying the same area of myocardium (Cohen, 1985). This question is of considerable importance because of its importance in infarct size standardization; if the risk zones measured by the different methodologies are not the same, then it becomes difficult to compare infarct sizes with other studies in which the AAR has been measured using a different technique.

Presently, the most widely used methods to delineate AAR include:

1. Post-mortem injection of coloured dyes- This involves injection of two different coloured dyes into the isolated heart, one in the previously occluded artery at the site of occlusion, and the other into the remaining vasculature at equal pressure. The area at risk is thus circumscribed by the dye injected into the occluded artery (Lowe et al., 1978, Rivas et al., 1976, Ertl et al., 1982).
2. Post-mortem injection of radioopaque materials- This technique is similar to method 1 except that identification of the boundaries of the vascular beds is done on the basis of radiographs of the serial sections of the ventricle (Jugdutt et al., 1979a).
3. Pre-terminal dye injection- This involves injection of coloured dyes

into the left atrium or ventricle. Since dye injection is eventually fatal, this can only be done prior to the conclusion of the experiment. (Forman et al., 1985, Sandhu and Biro, 1989).

4. Injection of radioactively labelled particles- Like pre-terminal dyes these are also injected into the left atrium or ventricle. These can however, be injected at any point in the experiment. Delineation of the AAR is done on the basis of autoradiographic visualization of the area devoid of particles (Deboer et al., 1980, Endo et al., 1986, Ertl et al., 1982).

Positron emission tomography (PET), computerized tomography with fast scanners and nuclear magnetic resonance (NMR) have also been used to measure risk zones (Bishop, 1984). At present, the resolving power of these techniques is inadequate to produce precise and accurate quantitative assessments of the area at risk. However, it is possible that these techniques will find considerable applicability in this area in the future.

The first two methods define the area normally supplied by the occluded coronary artery i.e. the anatomical vascular area at risk (AVAAR). The main drawback of determining the AVAAR using these techniques is that it must be done post-mortem, and consequently, does not take into consideration physiological hemodynamic conditions.

Methods 3 and 4 define what is commonly referred to as the physiological vascular area at risk (PVAAR). These in vivo techniques are referred to as "physiological" because dye/sphere penetration is permitted into the anatomical area at risk where coronary and non-

coronary collateral flow is sufficient to permit its entry in detectable quantity. Therefore, the physiological risk region is potentially smaller than the anatomical risk region and thus represents the region which is severely ischemic and thus truly at risk of undergoing infarction (Ertl et al., 1982, Cobb et al., 1988). The PVAAR is a function of both the normal perfusion territory of an occluded artery and the level of collateral flow to this area.

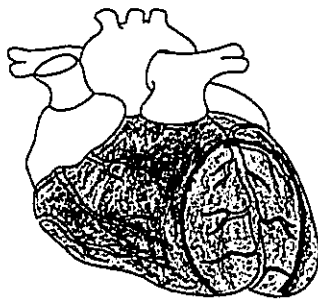
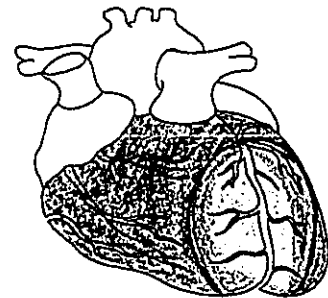
As recently as last year (Cobb et al., 1988) researchers have suggested that the PVAAR defined by the in vivo dye injection technique may not be appropriate because of the fact that the AAR can only be determined at the conclusion of the experiment. Therefore, any spontaneous or therapeutically induced change in collateral flow could change the size of the physiological risk region. Inherent in the idea that the size of the PVAAR can change is the notion that changes in collateral flow may occur and that these could shift the transmural and perhaps lateral border zones.

Figure 1.4 illustrates a potential consequence of measuring the PVAAR at the end of the experimental period. The top panel shows the area at risk measured by a pre-terminal dye technique in an untreated animal. The infarct size, as expressed as a percentage of the area at risk in this example, is 25%. The bottom panel shows an identical animal which had previously been treated by a drug which increases collateral flow to the risk zone and thereby decreases the size of the PVAAR from that which existed prior to drug administration. Even though this drug had no effect on infarct size, infarct size as expressed as a percentage of the area at risk is higher (In this case infarct size as a percentage of the

Figure 1.4

The Effect of a Change in the AAR on the Infarct/AAR ratio

A:

Untreated Animal**AAR Size=1000 mm****Infarct Size=250 mm
Infarct/AAR=0.25**

B:

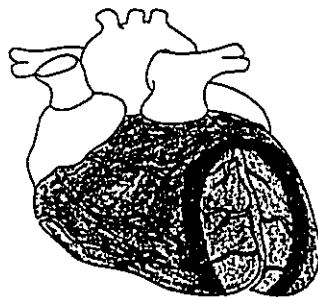
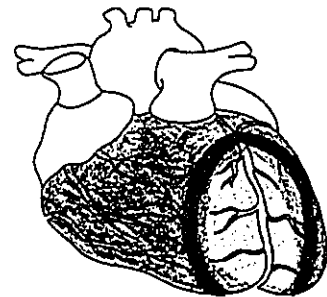
Treated Animal**AAR Size=750 mm****Infarct Size=250 mm
Infarct/AAR=0.33**

Figure 1.4 A: Hypothetical effect of a change in AAR size produced by a therapy which increases collateral flow and therefore decreases the size of the AAR as measured by *in vivo* dye injection. A: shows an untreated animal B: shows an animal which was treated with a therapy which decreased the size of the AAR but had no effect on the absolute amount of myocardium infarcted. Clearly, B will have a higher Infarct/AAR ratio because the therapy decreased AAR size.

area at risk=33%). If then, the benefit of a drug in reducing infarct size is evaluated by comparing the ratios of infarct size/area at risk in treated and untreated groups, it is quite obvious that the value of the drug may be underestimated or completely missed because the drug served to decrease the size of the area at risk to greater extent than its limited actual infarct size, thereby yielding a higher ratio. The converse of this would also be true, i.e. if the therapy decreased collateral flow, an increase in the size of the PVAAR would be seen, giving the misleading impression that infarct size reduction had been achieved. In an experimental setting in which an investigator would like to examine the degree of salvage achieved by a therapy, standardization of infarct sizes by expressing them as a percentage of the area at risk is essential, but this carries with it the problem of dealing with not only a numerator but also a denominator which is subject to change, thereby affecting the interpretation of the result.

However, this problem can be avoided if PVAAR is determined by autoradiographic visualization of microspheres or macroaggregates which can be administered at any time in the experimental period. Thus it is possible to determine the ischemic myocardium at risk in such a way as to reflect the physiological status of coronary blood flow after coronary artery occlusion but prior to the administration of drugs.

It seems reasonable to suggest that if an intervention is capable of increasing collateral flow and therefore reducing the size of the physiological area at risk from that which existed prior to the intervention, it has served a useful purpose because it has decreased the number of potentially necrotic cells. Therefore, any examination of the

therapeutic benefits of an infarct limiting agent should involve not only an analysis of its effect on the ratio of infarct size/area at risk but also an examination of its effect on the PVAAR. In this way, an investigator is better able to assess the therapeutic potential of an agent and also to gain insight into its mechanism of action.

1.3 TECHNIQUES EMPLOYED IN THIS STUDY

In the following sections the techniques used to measure PVAAR, infarct size and collateral flow in the current study will be described. Herein, the PVAAR will be referred to as simply the AAR as no measurement of the AVAAR was made.

1.3.1 Identification of the AAR using ^{99m}Tc Albumin Macroaggregates

These radioactively labelled macroaggregates of albumin (MAC) are commonly used in human lung scans (Mallinckrodt Product Information), and in most recent years, have also been used to identify the AAR (Deboer, 1980). Once injected, the macroaggregates become lodged in the circulation in proportion to the blood flow, usually on their first pass in the circulation. The average size of the macroaggregates is 20 μm , which results in their lodging in smaller arteriolar vessels downstream from the site of injection. The macroaggregates are biodegradable (biological half life = 10.8 hours) and are labelled with an isotope having a very short half-life (^{99m}Tc $t_{1/2}$ =6.02 hours) (Mallinckrodt Product Information). Because of the short half life of both the label

and the macroaggregates themselves, it has been suggested that the use of this method to identify area at risk size is reliable only for experiments of less than 12 hours duration (Deboer, 1980).

A potential drawback of this technique is that the macroaggregates may become eroded or fragmented while lodged in the circulation. These eroded or fragmented particles may then travel downstream from their point of lodging. Thus AAR size as measured by the autoradiography may be a function of the AAR at the time of injection, (from the macroaggregates permanently trapped) and also erosion or fragmentation of the macroaggregates significant enough to allow for their displacement. Another small disadvantage to this technique is that the MAC fog the X-ray film as a dark haze as opposed to discrete dots seen when ^{141}Ce microspheres are used for in vivo AAR identification (eg. Maxwell et al., 1987a). This may result in a small degree of error when circumscribing the AAR boundary.

1.3.2 Identification of the AAR using Monastral Blue Dye

Monastral Blue dye (MBD) is a stable suspension of copper phthalocyanine pigment in a saline solution. Electron microscopy studies have revealed that the Monastral Blue pigment consists of 2 components; rod shaped particles about 500 A wide and a dense granular amorphous material (Joris et al., 1982).

In the present study, MBD was used to identify the size of the AAR at the end of the occlusion period. In the last few years, MBD has been commonly used for this purpose, although initially it was introduced as a

technique to be used in vascular permeability studies when it was desired to label leaky vessels (Joris et al., 1982). The standard dose of MBD in vascular permeability studies is about 13X less than that used in the present study and, at this lower dose, is cleared from the circulation by the reticulo-endothelial system within a few hours of injection, with blue pigment remaining only in vessels which have become leaky. When this dye is used to identify the AAR, the animal is killed immediately after its injection and thus the MBD remains in the blood vessels and is not cleared by the reticulo-endothelial system.

1.3.3 Identification of Infarcted Myocardium Using Tetrazolium Staining

Nitro-blue tetrazolium (NBT) and triphenyl tetrazolium (TTC) are histochemical stains used to macroscopically identify areas of infarcted myocardium. Tetrazolium dyes stain (NBT-deep purple-blue and TTC-brick red) over non-infarcted myocardium whereas areas of necrosis fail to stain. The areas of necrosis delineated using this method are generally visually distinct and thus quantifiable.

In principle, the tetrazolium procedure is dependent upon the ability of tetrazolium salts to act as electron acceptors when normal dehydrogenase enzymes and appropriate cofactors are present (eg. NAD). Consequently, the tetrazolium salt will change its structure and precipitate over normal myocardium but since dehydrogenase mediated electron transport is dramatically reduced in infarcted myocardium, it will fail to precipitate over these areas (W. Schaper, 1984, Lie et al., 1974). W. Schaper (1984) suggests that discriminative staining (i.e.

absence of dye in infarcted areas but its presence in non-infarcted myocardium) could arise because of 3 different conditions:

1. absence of substrate for the dehydrogenase enzymes
2. absence of cofactors necessary for the dehydrogenase enzymes
3. absence of the dehydrogenases themselves

W. Schaper's studies support the notion that it is the absence of the NAD cofactor and not the absence of enzymes or substrates that is the limiting factor in the staining reaction. He arrived upon this conclusion after having demonstrated that the addition of NAD or NADH caused precipitation of dye over unstained infarcts and secondly that infarcted myocardium inherently showed very little NAD content. In further support of this idea, he demonstrated that infarct tissue, even up to 32 hours after coronary occlusion, had considerable dehydrogenase enzyme activity.

A decreased NAD content in infarcted myocardium was previously reported by Nunez (1974) who showed that this cofactor was split by glycohydrolase, an enzyme whose activity is disclosed in the damaged tissue. Nunez also postulated that some NAD hydrolysis occurred in the bloodstream after leakage of the cofactor out of the necrotic cells. Klein et al. (1981) proposed that the constantly falling intracellular pH of the ischemic myocardium was responsible for the activation of the NAD splitting glycohydrolase.

Regardless of the underlying mechanism, Fishbein (1981) has verified that myocardial infarct size as determined by the tetrazolium technique is

similar to that determined by electron microscopy. He demonstrated that this technique reliably quantified infarct sizes even after only 3 hours of occlusion.

In the present study, the tetrazolium technique was employed for gross histochemical evaluation of infarcted myocardium because of its confirmed reliability and the fact that it was much more economical and less time consuming when compared with conventional histology or ultrastructural methods.

1.3.4 Determination of Regional Myocardial Blood Flow using Radioactive Microspheres

Principles and Assumptions

Radioactive microspheres have provided a convenient method by which to sequentially assess blood flow to virtually all organs under a variety of experimental conditions. The principle behind their use in blood flow determination is similar to the macroaggregates; they lodge themselves in small peripheral vessels in proportion to the blood flow. Consequently, the fraction of the total radioactivity in any given organ (or organ sample) is proportional to the fraction of the cardiac output that it receives during the transit time of the microspheres (Heymann et al., 1977).

Thus, if one knows the rate of flow $F(k)$ and the radioactivity of any given organ $R(k)$ and, if one assumes that the ratio of flow to radioactivity is the same in all organs, then the rate of flow $F(u)$ to

any unknown organ can be determined by simply measuring the radioactivity of the organ $R(u)$ in question and calculating blood flow using the following equation:

$$F(u) = F(k) \times R(u)/R(k) \quad (1)$$

In order to avoid the errors associated with measuring organ flow directly, Makowski et al., (1968) introduced a method by which a "surrogate" organ was created. In this technique, arterial blood is withdrawn from the animal into a syringe at a constant, known rate before, during and after microsphere injection. The syringe withdrawal rate and radioactivity of this reference blood is noted and these values are substituted for $F(k)$ and $R(k)$ respectively in the above equation.

In order to utilize this method to accurately measure blood flow, the following criteria must be met

1. The microspheres must be uniformly mixed with the blood after injection, without streamlining of the microspheres so that the concentrations reaching all branch sites are the same.
2. A minimum of 384 microspheres must be present in each tissue sample analysed in order to avoid large sampling error.
3. The microspheres must be completely and permanently trapped in the vascular beds downstream from the injection site during their first pass.
4. Injection of the microspheres and withdrawal of the reference sample

should have no effect on the general circulation or local organ hemodynamics.

The main factors affecting the uniformity of mixing are the site of injection and the numbers of microspheres injected (Heymann et al., 1977). These authors suggest that when measuring the distribution of systemic blood flow, the microspheres should be injected into the left atrium in order to allow for maximal mixing prior to the first major arterial branch site. Based on counting statistics, they calculated that microsphere flows are reliable only if the tissue samples contain at least 384 microspheres. Since usually $1-3 \times 10^6$ microspheres are injected each time, this lower limit of microspheres does not present a problem in determining myocardial blood flows unless extremely small samples of tissue are taken. The extent of microsphere recirculation has been investigated by several investigators (Domenech et al., 1969, Fortuin et al., 1971, Buckberg et al., 1971, Fan et al., 1979, Marshall et al., 1976). These studies demonstrated negligible (<2%) recovery of 15 μ m microspheres in the coronary sinus following left atrial microsphere injection although during conditions of vasodilation large numbers of 9 μ m microspheres were recovered in the coronary sinus. Heymann et al. (1977) found no significant failure of entrapment of 15 μ m microspheres in fetal and neonatal lambs under a variety of experimental conditions. In one study in the dog, (Consigny et al. 1982) involving injection of 9 and 15 μ m microspheres directly into the coronary arteries, it was found that 98.3% of all microspheres escaping from the myocardium and lodging in the lungs had diameters of 10.3 μ m and that the maximum diameter

observed was 12.5 μm further supporting the idea that smaller diameter microspheres have a greater potential for non-entrapment. The absence of significant hemodynamic effects produced by microsphere injection were confirmed by Fortuin et al. (1971) and Falsetti et al. (1975) who found that injections of $>12 \times 10^6$ microspheres at one time had very little hemodynamic effect, thus it appears that the safety margin is appreciable.

A complete review of the studies concerning the principles, assumptions and limitations of the microsphere technique will not be given here, however, two very thorough reviews are given by Rudolph and Heymann (1977) and Leblanc (1983).

Comparison of Microsphere Method of Organ Flow determination with other Methods

The accuracy of the microsphere method for measuring the distribution of flows within individual organs has been tested by comparing blood flows determined using diffusible indicators with those determined using microspheres (Tripp et al., 1977, Schanzenbacher et al., 1980, Delaney et al., 1964). In dogs, gastric blood flow was measured using the clearance of radioactive ^{42}K with that measured using ^{35}Na -labelled 16-20 μm glass microspheres (Delaney et al., 1964). They first tested the validity of using the ^{42}K clearance technique for determining gastric blood flow by simultaneous determinations of total gastric flow by collection of venous effluent and injection of ^{42}K . The average difference between the latter techniques was 5.4%. The distribution of microspheres was closely

correlated with the distribution of ^{42}K . When comparisons of are made between blood flows determined using radioactive iodoantipyrine or ^{86}Rb with those determined using microspheres, close correlations were found in some tissues but not in others. Bassingthwaighte et al. (1987) compared blood flows in rabbits as determined by $16.5\ \mu\text{m}$ ^{141}Ce and ^{103}Ru -labelled microspheres with those determined using 2-iododesmethylimipramine (IDMI), a new "molecular microsphere" whose high lipid solubility permits it to be delivered to tissue in proportion to flow and whose binding in tissue prevents rapid washout. He found high correlations between the two techniques ($0.87 < r < 0.96$). However, microsphere deposition tended to be lower than IDMI deposition at low flows and higher at high flows. Bassingthwaighte concluded that microspheres were adequate for estimating regional flows but suffer from some systematic error due to a bias at bifurcations toward entering branches with higher flow and secondarily towards entering those branches which are straighter.

Extremely close correlations were found when microsphere determined blood flows were compared with the same flows measured using more direct methods such as electromagnetic flow probes, and collection of venous effluent (Heymann et al., 1977). Since microsphere determined flows were closely related to those determined using the more direct methods i.e. electromagnetic flow probes and collection of venous effluent, it is likely that differences in microsphere and diffusible indicator determined flows were a result of inaccuracies in the indicator techniques. For example, extraction of ^{86}Rb from the blood by the tissue depends upon the rate of blood flow through the capillary bed, capillary

surface area, the permeability of the capillary and cellular membranes and also on the rate of ionic backflux (Cohen, 1985). Thus blood flow determinations by this technique are a function of a number of factors, some of which can be altered by a variety of experimental conditions. In contrast, the microsphere technique, when appropriately applied and when all of the necessary conditions are satisfied, can be quite accurate in determining flow under a variety of experimental conditions (Heymann et al., 1977). Maxwell et al. (1987b) addressed the specific question of the adequacy of the microsphere method to determine the low flows delivered by collaterals. They found that the microsphere method did not fail to estimate all components of collateral flow.

Physical Characteristics of the Microspheres and Size Selection

The microspheres are made of an inert plastic with the isotope being incorporated into the microsphere itself, not merely coated on the surface. The specific gravity of the microsphere is 1.30, similar to the 1.05 of whole blood. Consequently, the distribution of the microspheres in the circulation approximates that of the red cell. The size of the microspheres used in this study is 15 μm . This size was selected because studies by Domenech et al. (1969) and others (Phibbs et al., 1970) have found that larger diameter microspheres (50 μm) tend to be concentrated centripetally in the myocardium (i.e. greater numbers in the epicardium as compared to the endocardium) whereas microspheres of sizes smaller than 15 μm tend to be more or less evenly distributed. Secondly, as

mentioned above, microsphere sizes smaller than 15 μm have been seen to show an increased potential for non-entrapment and therefore recirculation, and thus were also unsatisfactory. Thus it appeared that microspheres of 15 μm would be most desirable as they possess the evenness of distribution exhibited by the smaller microspheres, and are also completely trapped in the circulation.

1.4 COLLATERAL FLOW TO THE ISCHEMIC MYOCARDIUM

Radioactive microspheres have been used extensively to follow changes in perfusion to the ischemic myocardium. Using microspheres it has been determined that, following coronary artery occlusion in the dog, blood flow in the territory of the occluded artery ranges from 10-30% of normal flow (Cohen, 1985). Although it has been accepted that flow increases after occlusion, the exact time course of the increase is not entirely clear (See Table 1.2). Most agree that during the first few minutes of occlusion and after 24 h flow to the ischemic zone increases, (Davenport et al., 1984, Marcus et al., 1976, Jugdutt et al., 1979a) there is no unanimous vote on what happens to blood flow after the first few minutes to 6 h post-occlusion. This lack of agreement may, in part, be attributed to the limitations imposed by the radioactive microsphere technique.

Although unlike the various clearance techniques, the microsphere technique allows for the evaluation of flows from multiple areas of interest, the samples must be large (0.5-1 gm) in order to get the minimum number of microspheres (Approximately 400) in any given sample (Heymann et al., 1977) and, as a result, do not allow for the spatial

resolution necessary to adequately evaluate flows within the border zones (Cohen, 1985). A common problem associated with analysing flow within the ischemic myocardium is that of contamination of AAR tissue with normally perfused tissue. Since blood flow in the normal zone usually increases following an occlusion (because the remaining heart muscle is doing the work for the approximately 25% of the heart muscle rendered non-functional) and because it is virtually impossible to exclude some normal tissue in the sample, this may be wrongly interpreted as an increase in flow to the ischemic zone.

Another common problem associated with blood flow determination within the AAR is that of sampling. While some investigators define the perfusion territory with dye injection (either post-mortem or in vivo), others define it using post-mortem arteriography, while others define it on the basis of the surface distribution of the occluded vessel (Cohen, 1985). Thus, if these techniques are identifying different areas of myocardium, and it is on this basis that tissue sampling is done, it becomes obvious that this may in part, be responsible for some of the disagreement in answering the question of what happens to blood flow within the ischemic myocardium

In table 1.2, a few studies report decreases in flow between 1 and 6 h. Grayson et al. (1968) suggested that these were related to vasospasm of adjacent normal coronary arteries supplying collateral vessels, however, this observation was not supported by Rees and Redding (1968). It may be that decreases in collateral flow occurred due to the swelling of myocardial and/or endothelial cells which resulted in increased vascular resistance and a diminished blood flow to the AAR (Nayler, 1987

Table 1.2

Collateral Flow during the First Six Hours of Coronary Artery Occlusion in the Dog

Author and Year	Technique used to measure flow	Identification of Perfusion territory	Experimental Design	Results
Marcus et al. 1976	Radioactive microspheres	Surface Distribution of the Occluded Artery	CxCA artery ligation in the open chested dog with microsphere injection at 20 sec, 5 min. and 1 hr.	Flow 20 sec < 5 min < 60 min.
Bishop et al. 1976	Radioactive microspheres	Surface Distribution of the Occluded Artery	LADCA or CxCA occlusion in the conscious dog with microsphere injection at 10 min., 2 hrs, and 6 hrs	No change in flow over the 6 hr period
Rivas et al. 1976	Radioactive microspheres	Injection of Evan's Blue Dye into the Left Main Coronary Artery post-mortem	CxCA occlusion in the closed chested anesthetized dog with microsphere injection at 45 secs, 2 hrs, and 6 hrs	Flow 45 sec.< 2 hrs but no change between 2 and 6 hrs
Jugdutt et al. 1979b	Radioactive microspheres	Post-mortem coronary arteriography	CxCA occlusion in the conscious dog with microsphere injection at 20 secs. and 1 hr	Flow 20 sec.< 1 hr
Jugdutt et al. 1979c	Radioactive microspheres	Post-mortem coronary arteriography	CxCA occlusion in the conscious and anesthetized open chested dog with microsphere injection at 20 secs, 5 mins, 1 hr and 6 hrs	Flow 20 sec.<5min. but no change between 1 and 6 hrs in either anesthetized or conscious dogs
Davenport et al. 1984	Radioactive microspheres	Injection of Evan's Blue Dye into the previously occluded LAD-CA artery post-mortem	LADCA occlusion in the open chested anesthetized dog with microsphere injection at 5 mins, 20 mins, and 4 hrs	Flow 5 min.< 20 min < 4 hrs
Rees et al. 1968 ^a 1968 ^b	¹³³ Xe clearance	-	LADCA occlusion in the anesthetized dog with flow measurements taken every half-hour for 6 hrs	Flow decreased in the first few mins but then increased over the next 2 hrs. From 2 to 6 hrs flow decreased again
Marshall et al. 1974	¹³³ Xe clearance	-	LADCA occlusion in the anesthetized closed chested dog flow measurements made every half-hour for 4 hrs	Flow falls during the first half-hour and remains unchanged for the duration of the 4 hr observation period.
Grayson et al. 1968	Thermo-electric method	-	LADCA occlusion in the anesthetized closed chested dog. Measurements made from a few mins post occlusion to 6 hrs	Flow falls to near zero levels from a few minutes post occlusion to 6 hrs

Table 1.2 Summary of studies in which collateral flow was measured during the first 6 h of occlusion in the untreated dog. From these studies it is apparent that there is a lack of agreement on what happens to collateral flow up to 6 h after occlusion.

Kloner et al., 1974).

Most studies report no change in flow during this time period, and, this would appear, at least theoretically, to be the most likely. It has been stated that following an occlusion all collaterals are fully recruited and maximally dilated (Cohen, 1985). It is also documented that growth of the collateral vessels allowing for further increases in their vasodilating capacity does not occur until at least 24 hours after occlusion (Cohen, 1985). Therefore, it appears improbable that, in the absence of change in other hemodynamic parameters, an increase in collateral flow and/or the advancement of the lateral perfusion interface would occur over the 1 to 6 h period.

1.5 CALCIUM CHANNEL BLOCKERS

The calcium channel blockers are a group of drugs which have been studied extensively for their ability to limit myocardial infarct size. In addition, their antiarrhythmic and vasodilatory properties have also received considerable attention in the treatment of other cardiovascular disorders (Fleckenstein, 1983). The purpose of following sections is to describe their mechanism of action, the rationale for their use in myocardial infarct size limitation studies and finally to explain why Nicardipine, a member of this group, was chosen as an agent to analyse changes in AAR, collateral flow and infarct size.

1.5.1 How they work

Ionized calcium plays an essential role in the function of cardiac

tissue and vascular smooth muscle. When a myocyte depolarizes, Ca^{2+} enters the cell across the sarcolemma through the slow channels and causes the intracellular Ca^{2+} concentration to rise above 10^{-7} M. This then removes the inhibitory influence of the troponin-tropomyosin complex on the actin filaments, thereby permitting the actin and myosin filaments to slide past one another in an ATP dependent process which then results in contraction. Thus, the Ca^{2+} entering the cell during the action potential plays a pivotal role in excitation-contraction coupling. However, evidence suggests that the amount of Ca^{2+} entering the cell across the sarcolemma during an action potential is insufficient to cause contraction directly, but that it does trigger the release of Ca^{2+} from intracellular stores in a process termed " Ca^{2+} -activated- Ca^{2+} release" (Stone et al., 1980).

Like cardiac cells, the extent of contraction of vascular smooth muscle cells depends upon transmembrane Ca^{2+} entry. However, unlike cardiac muscle, vascular smooth muscle cells also require transmembrane Ca^{2+} entry for the maintenance of a basal level of intrinsic tone (Stone et al. 1980, Weiner, 1988).

There appear to be two types of channels that regulate calcium movement across myocardial and vascular smooth muscle cells. The first type of channel is activated by membrane depolarization which causes a change in the voltage across the membrane and consequently causes the calcium channels to open, thereby permitting extracellular Ca^{2+} entry, resulting in muscle contraction. The second type of channel is regulated by binding of norepinephrine or other agonists to α receptors which

subsequently regulates release of Ca^{2+} from intracellular stores. Calcium channel blockers mainly interfere with calcium entry through the voltage sensitive slow channels (POCS) although high concentrations of certain calcium channel blockers can compete with norepinephrine at the α -adrenergic receptor site (receptor-operated channels (ROCS)) (Weiner, 1988).

Evidence has accumulated to suggest that the actions of calcium channel blockers on vascular smooth muscle are organ selective; typically, blockers appear to have a greater affinity for coronary, cerebral, femoral and occasionally mesenteric vascular beds (Godfraind, 1983) than for renal beds. The reasons for this selectivity is somewhat uncertain. Godfraind (1987) suggested that there are two fundamental factors that account for this selectivity of action. Firstly, the blockers can only be effective inhibitors of tissue activation if tissue activation depends upon significant entry of extracellular Ca^{2+} . Some vessels can be induced to contract in the absence of extracellular Ca^{2+} and vessels from different tissues respond differently to the same extracellular Ca^{2+} concentration and thus vary in their response to the presence of Ca^{2+} blockers. A second factor is that Ca^{2+} channels probably do not constitute a single homogeneous population in different tissues or even within the same tissue. In consequence, the affinity of different calcium channels for different blockers may vary. At therapeutic doses, these drugs do not appear to have a significant effect on other physiological functions although Ca^{2+} ions play an essential role in a variety of other cellular processes including contractile process of skeletal muscle, intestinal motility, glandular secretion and release of

neurotransmitters (Hurst, 1982).

1.5.2 Actions on Cardiovascular Function

Ca^{2+} plays a profound role in the electrophysiological properties of specialized cardiac tissue (i.e. automaticity of the S.A. and A.V. nodes, the velocity of conduction, rhythmicity and excitability of the Purkinje fibers), mechanical aspects of cardiac function and in the level of contraction (and therefore resistance) of the systemic and coronary blood vessels. It is therefore not surprising that calcium channel blockers produce dramatic alterations in cardiovascular function.

Principally, (but definitely not exclusively) their actions on cardiovascular hemodynamics are: coronary artery dilation, peripheral arterial dilation and a negative inotropic effect. Both coronary and peripheral arterial dilation are achieved by an inhibition of both Ca^{2+} dependent vascular tone and spastic contractions. The reduction in cardiac contractility is due to a reduction in transmembrane calcium influx during the cell's action potential. The net hemodynamic effects of these agents vary depending upon the relative selectivity of the particular drug for each of the hemodynamic actions and also upon the dosage administered. In addition, these drugs may bring about reflex mechanisms which may contribute to or perhaps be the primary determinant of the overall hemodynamic effect (Stone et al., 1980). For example, Nifedipine has been shown to decrease total peripheral resistance (TPR) and therefore blood pressure. The lowering in blood pressure has been known to bring about reflex tachycardia and a positive inotropic effect

(Sorkin, 1987).

1.5.3 Actions on the Ischemic Myocardium

Fleckenstein (1980) and others (Nayler, 1980, Kloner et al. 1987) have argued that calcium channel blockers could have a direct effect in protecting the ischemic myocardium because they could prevent the intracellular Ca^{2+} overload which accompanies irreversible injury. At present, the critical event causing the transition of cells from a stage of reversible to irreversible injury is unknown; however, it is possible, as Fleckenstein suggests, that it is due to sarcolemmal disruption caused by an increase in cytosolic Ca^{2+} . The potential relationships between ATP depletion, Ca^{2+} influx and sarcolemmal damage are shown in Figure 1.5. This concept, proposed by Jennings and Reimer (1981), suggests that the level of cellular ATP declines because its utilization exceeds the capacity of the cell to produce high energy phosphates. Intracellular Ca^{2+} [Ca^{2+}_i] may increase because of increased membrane permeability of Ca^{2+} or because of inadequate ATP to maintain efflux across the plasma membrane or sequestration by the sarcoplasmic reticulum. The increased [Ca^{2+}_i] may accelerate ATP depletion through Ca^{2+} activated ATPases. Sarcolemmal damage is known to occur when ATP levels fall to less than 8% of control. The mechanisms of damage are unknown but, one possible mechanism is activation of endogeneous sarcolemmal phospholipases by [Ca^{2+}_i] or depressed phosphorylation of the membrane proteins which normally inhibit endogeneous phospholipase activity of the sarcolemma. Sarcolemmal damage may also be caused by other mechanisms not directly linked to Ca^{2+} overload such as release of

Figure 1.5

Possible Mechanism by Which Ischemia Could Result in Cell Death

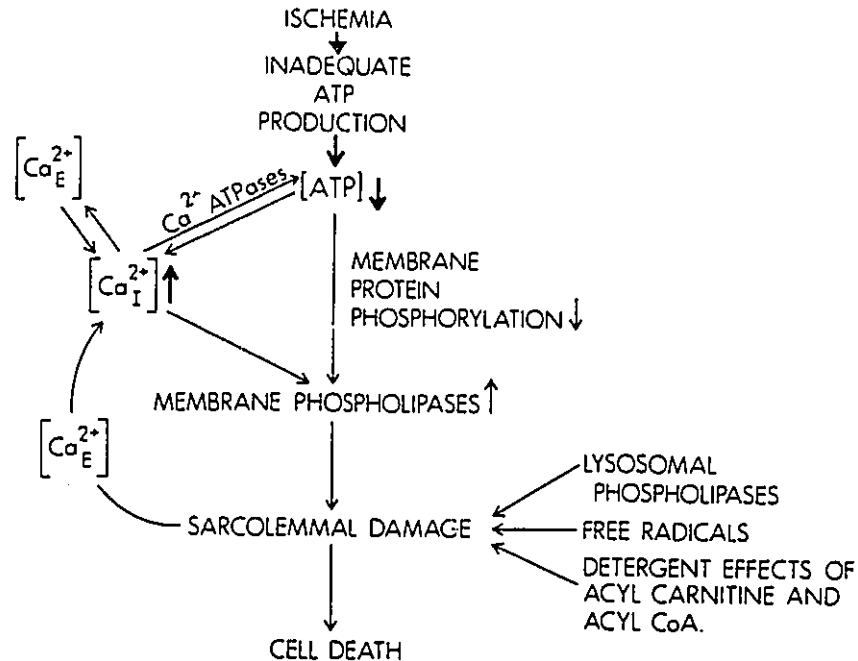


Figure 1.5 A summary of The potential relationships between ATP depletion, Ca^{2+} influx and sarcolemmal damage. The level of ATP declines because its utilization exceeds the capacity of the cell to produce it. Intracellular Ca^{2+} may increase because of an increased permeability to Ca^{2+} or because of inadequate ATP to maintain efflux across the plasma membrane or sequestration by the sarcoplasmic reticulum. As intracellular Ca^{2+} rises, ATP depletion is accelerated because of activation of Ca^{2+} dependent ATPases. The high intracellular Ca^{2+} may also act to activate endogenous sarcolemmal phospholipases and/or depress phosphorylation of proteins in the membrane which normally inhibit these phospholipases. Sarcolemmal damage probably occurs through a variety of mechanisms, some of which are not directly linked to intracellular Ca^{2+} overloading. However, once sarcolemmal damage has occurred, severe disturbance of ionic homeostasis and cell death are inevitable. Taken from Hurst, 1985.

phospholipases by lysosomes, free radicals or the detergent actions of acylcarnitine of acylCoA (Reimer and Jennings, 1981).

Although some suggest (Poole-Wilson, 1984) that calcium overload is not the critical event in lethality, one reason for its emphasis as a mediator in cell death may be that no situation has ever been described in which cells accumulate massive amounts of calcium and yet survive. Secondly, as already partially described above, cytosolic calcium levels affect almost all cellular processes including contraction (Ringer, 1883), the stability of membranes, the function of enzymes, and mitochondrial production of ATP (Poole-Wilson, 1984). Considering Ca^{2+} ubiquitous role, it is quite conceivable that intracellular Ca^{2+} overload is an important mediator in irreversible injury, and that irreversibility could be prevented or delayed by the early administration of calcium channel blockers. However, if the blocker is applied after cells have undergone sustained membrane damage, blockers are ineffective in preventing intracellular Ca^{2+} accumulation because they can only prevent Ca^{2+} entry through the slow channels and are consequently ineffective in preventing uncontrolled entry of Ca^{2+} through leaks in the sarcolemmal membrane. Numerous experimental studies have shown that Ca^{2+} channel blockers are unable to limit infarct size when administration is delayed beyond the first few hours after occlusion (Kloner, 1987).

It is documented (Nayler, 1987) that during postischemic reperfusion, ischemic myocardial cells accumulate massive amount of Ca^{2+} . Numerous studies have shown that calcium channel blockers are ineffective in decreasing the extent of "reperfusion injury" which occurs as a result of Ca^{2+} overloading although they do slow the rate of Ca^{2+} gain (Nayler,

1987). As mentioned above, this is primarily due to the fact, that calcium channel blockers cannot prevent uncontrolled Ca^{2+} entry through a disrupted sarcolemma. In fact, W. Schaper (1984) has argued that reversibly injured cells do not become "more injured" following reperfusion although reperfusion can accelerate destruction in cells which have sustained membrane damage sufficient to permit the entry of large amounts of Ca^{2+} upon reflow. He suggests that reperfusion serves only to amplify the differences between irreversibly and reversibly injured cells and does not lead to death in those cells, which at the time of reperfusion, are only reversibly injured.

1.5.4 Rationale for use in Myocardial Infarct Size Limitation

The use of these drugs to salvage ischemic myocardium stems from their inhibition of calcium ion flux across cell membranes in myocardial cells and in vascular smooth muscle (Kloner, 1987). The possible mechanisms by which this effect achieves myocardial preservation is described in Table 1.3.

1.5.5 Specific Studies using Calcium Channel Blockers

The calcium channel blockers are an extremely heterogeneous group of drugs, quite varied in chemical structure and selectivity of action (Godfraind, 1987). Some members of this family that have been most extensively investigated under both experimental and clinical situations include Verapamil, Nifedipine, Diltiazem, Tiapamil, D600, Nisoldipine,

Table 1.3 Possible Mechanisms for Myocardial Preservation
with Calcium Channel Blocking Agents

1. Increased Myocardial Oxygen Supply to the Area at Risk
 - A. Relieve Coronary Vasospasm and Constriction-Results in coronary arterial dilation and subsequent improvement in collateral flow
2. Decreased Myocardial Oxygen Demand
 - A. Reduced Heart Rate
 - B. Reduced Preload
 - C. Reduced Afterload
 - D. Reduced Contractility
3. Prevent intracellular calcium overload (See Figure 1.5.3)
4. Alter Myocardial Energy Metabolism?-Involves the inhibition of lypolysis and a shift to carbohydrate metabolism which is more energy efficient. This effect remains to be verified¹.

(Adapted from Chu-Jeng Chiu, 1980)

¹ The importance of this last point is somewhat questionable because during ischemia oxidative phosphorylation via the Krebs cycle is dramatically reduced and consequently glycolysis is a major source of ATP. Therefore, the contribution of lipids to ATP generation is already quite low in the ischemic condition.

FR7534, Bepridil and most recently Nicardipine (Weiner, 1988). At present, no consensus has yet emerged concerning the efficacy of these agents in limiting infarct size as both negative and positive results have appeared in the literature.

1.5.6 Effects of Calcium Channel Blockers on Collateral Flow

Equally unclear are the effects of the various calcium channel blockers on collateral flow to the ischemic myocardium as studies have yielded conflicting results. A proposed mechanism of action for most of the calcium channel blockers is vasodilation, suggesting improved perfusion to the risk zone via the collaterals. However if the collaterals are fully dilated within the first few minutes after occlusion and if subsequent development of collaterals allowing for further vasodilation does not occur until 24 h after occlusion (Cohen, 1985) it may be that the calcium channel blockers are ineffective in decreasing the resistance of these conduits.

Studies have shown that arteriolar vascular smooth muscle is far more sensitive to the vasodilating action of calcium channel blockers than that of the venous system (Weiner, 1988). In addition, the calcium channel blockers have been shown to induce vasodilation of proximal coronary arteries which could assume therapeutic significance in patients with ischemic heart disease in whom atherosclerotic obstruction or spastic contractions may compromise the lumen of the artery. The overall effect of administration of a calcium channel blocker is difficult to predict and depends upon the extent to which these drugs alter the

vascular resistances at each level of the vascular tree. Since the calcium channel blockers are a fairly heterogeneous group of compounds, it is probable that the magnitude of the dilation that they produce in each of these vessels varies depending upon the blocker in question.

It is known that the resistance of native intramural coronary collaterals is extremely sensitive to changes in extra-vascular compressive forces. Therefore, it may be that one mechanism whereby calcium channel blockers exert their beneficial effects by decreasing extra-vascular compression (i.e. through their actions on decreasing heart rate, contractility) and consequently increase collateral flow and decrease myocardial O₂ demand. Any intervention capable of increasing perfusion via the collateral vessels and decreasing oxygen demand would be beneficial as this would decrease the inequality between the supply and demand of blood thereby decrease the extent and rate of necrosis (Cohen, 1985).

1.5.7 Nicardipine

Nicardipine hydrochloride, a relatively new addition to the calcium channel blocker group of drugs, is a dihydropyridine derivative which was first introduced as a cerebral vasodilator, but was later discovered to have potent coronary vasodilating effects. (Sorkin, 1987). In isolated canine coronary arteries, Nicardipine has been found to be more potent than Nifedipine, Verapamil and Diltiazem in relaxing potassium and 3,4-diaminopyridine induced contractions (Takenaka et al., 1985).

Experiments in anesthetized dogs have revealed that Nicardipine decreases TPR in a dose dependent manner, but that it is highly selective in its

vasodilating action (Bograni et al., 1985). Bograni found that the vasodilating effects of Nicardipine were greater on the coronary and cerebral vessels than on mesenteric and femoral vessels. As a consequence of this decrease in TPR, Nicardipine produced dose dependent decreases in mean arterial pressure (MAP). The hypotensive effect of Nicardipine is almost the same as Nifedipine and 10x greater than either those of Verapamil or Diltiazem (Endo et al., 1986). Nicardipine has also been shown to reduce preload (Alps et al., 1983b). Reductions in preload and afterload would serve to benefit ischemic myocardium as this would reduce left ventricular wall stress resulting in a decrease in both myocardial oxygen demand and extravascular compression of the native collateral (improved supply of arterial blood). Its direct vasodilatory actions may also improve perfusion of the collaterals. Finally, as a calcium channel blocker, Nicardipine may also directly protect the ischemic myocardium by inhibiting calcium uptake by myocytes thereby preventing the deleterious effects of calcium overloading (Hashimoto et al., 1985, Sorokin, 1987). A significant drawback of some calcium channel blockers investigated in the past for infarct size limitation is that they have significant negative inotropic effects which then serves to compromise ventricular pumping performance. This could, quite conceivably, be detrimental to a patient undergoing infarction. Nicardipine produces no discernible negative inotropic effect except at extremely high doses (Bograni, 1985). Thus Nicardipine would be expected to reduce the workload of the heart without compromising cardiac function.

However, Nicardipine administration is usually accompanied by reflex

increases in heart rate and cardiac output because of the induced fall in blood pressure (Silke et al., 1984, Takenaka et al., 1985, Sorkin, 1987). Therefore, it is possible that if these increases in cardiac output and heart rate are persistent and substantial, this may increase oxygen demand to the ischemic myocardium and increase extravascular compression of the collaterals. A second drawback may be that its hypotensive effects would result in a decrease in coronary perfusion pressure, which would, if acting alone, serve to decrease flow to the ischemic region and thus produce an even greater inequality between demand and supply of coronary blood. Furthermore, the use of Nicardipine may produce "coronary steal". This phenomenon refers to the arteriolar vasodilation in normal myocardium which would serve to redistribute blood away from the ischemic myocardium.

Thus the ultimate effect of Nicardipine on the ischemic myocardium may be complex and involve the interaction of many factors and is consequently not predictable. Nicardipine has not been shown to affect interatrial, atrioventricular or interventricular conduction (Takenaka et al., 1985, Nakaya et al., 1982), which lends promise to its use as an infarct limiting agent. Table 1.4 provides a summary of all published studies investigating the use of Nicardipine as an infarct size limiting agent. Given the generally rosy picture presented by the data in this table, Nicardipine appeared to be a suitable candidate for a study assessing the effects of an anti-infarct agent on ultimate infarct size, changes in AAR and collateral flow.

Table 1.4

Experimental Studies of Nicardipine on Myocardial Infarct Size Limitation

Study/ Year	Model	Duration of Ischemia	Reper- fusion	Assesment of Infarct Size or Ischemia	Ischemia or Infarct Size Reduction	Coronary Collateral Flow	Time of Start of Therapy	Comments
Alps et al. 1983a	anesthetized baboon	6 hours	none	Tetrazolium staining	+	Not measured	1 month pre-occlusion, 5 mins and 1 hr post-occlusion	
Alps et al. 1983b	anesthetized dog	3 months	none	Tetrazolium staining	+	Not measured	1 month pre-occlusion, 1 hr post-occlusion	
Endo et al. 1986	anesthetized dog	6 hours	none	Tetrazolium staining	+	Not measured	15 mins pre-occlusion, 15 mins post-occlusion, 3 hrs post-occlusion	Efficacious treatment in pre- and early post-occlusion groups only
Berdeaux et al. 1985	anesthetized dog	2 hours	none	Electrophy- siological observations	-	Increase to endocardium only	Single doses at 50 and 100 mins post-occlusion	
Hashimoto et al. 1985	anesthetized dog	2 hours	none	Electrophy- siological observations	+	No change to severely ischemic tissue, increase to mildly ischemic tissue	0.3 to 100 µg/kg Nicardipine at varying intervals post-occlusion	

Table 1.4 Summary of studies investigating the effects of Nicardipine on myocardial infarct size limitation. Note that 4/5 studies report significant reductions in infarct size or the degree of ischemia.

1.6 OBJECTIVE

The purpose of this investigation is to answer the following questions:

1. Do the ^{99m}Tc macroaggregates and the Monastral Blue Dye identify the same area of myocardium when injected sequentially?.
2. Does the size of the AAR change spontaneously in untreated dogs subjected to three hours of coronary artery occlusion?
3. Does Nicardipine change the size of the AAR and/or the Infarct/AAR ratio?
4. What happens to Collateral Flow within the AAR in untreated animals and those administered Nicardipine?

METHODS

2.1 ANIMALS

Mongrel dogs of either sex weighing between 14-31 kg were used for this study. The dogs were obtained primarily from local pounds, but some were also obtained from breeders (13%). In all experimental groups there were approximately equal numbers of animals from the two sources. The dogs were kept in a 12 hour light/12 hour dark cycle at 22 °C and a relative humidity of 45%. Each day, the dogs were exercised for approximately 90 minutes in an outdoor runway. Eighteen hours prior to the planned start of surgery, food but not water was withheld.

A total of 64 dogs were used for these experiments; of these, 5 dogs were rejected because of technical difficulties (i.e ventricular fibrillation and anesthetic overdose) associated with the experiment. These are not reported here.

2.2 ANESTHETIC

Animals were initially pre-medicated with Pre-Mix ((0.1 ml/kg) made from 2 mls of Demerol (6.67 mg/kg) and 1 ml of Acepromazine (0.33 mg/kg)) by intramuscular injection and subsequently anesthetized with Somnotol (26 mg/kg) intravenously. Supplemental anesthetic was given when needed to maintain the required level of anesthesia. Corneal reflex and toe pinch reflex were used as guides to the level of anesthesia. No muscle relaxants were used in these studies.

2.3 EXPERIMENTAL PREPARATION AND MEASUREMENTS

The dogs were intubated with a cuffed Bush endotracheal tube (internal diameter (i.d) 9-12 mm, depending on animal size) and ventilated by a Penlon Volume Ventilator (Model No. AV-500) on a Boyle Anesthetic Machine (The British Oxygen Co.) delivering a mixture of 20% nitrous oxide in pure oxygen at a rate of 12 breaths/minute with a tidal volume between 350-600 mls. Blood samples for measurement of arterial pH, pO₂ and pCO₂ were obtained prior to, 10 minutes following and where applicable, every half hour subsequent to coronary artery occlusion. Measurements were made using a blood gas analyzer (Instrumentation Laboratories, Model No. 513). Measurements on any given sample were completed within 3 minutes of obtaining it. By adjusting tidal volume the pH, pO₂, and pCO₂ were maintained between 7.2-7.3, 160-225 mm Hg and 33-39 mm Hg respectively.

A Swan Ganz catheter (American Edwards Laboratories, i.d. 0.91 mm) was placed in the left jugular vein for the purpose of monitoring the following pressures: right atrial (RAP), pulmonary arterial (PAP), pulmonary capillary wedge (PCWP) and for determination of cardiac output (CO). A No. 7 Cardix catheter (Cordis, i.d. 0.38 mm) was placed in the right carotid artery and advanced into the left ventricular cavity for recording left ventricular pressures (SLVP and DLVP). A catheter was placed in the right jugular vein and was used for intravenous infusion of saline (12-15 ml/kg/hour). A catheter was placed in the femoral artery and was advanced into the lower abdominal aorta for the purpose of

recording arterial systolic and diastolic pressures (SAP and DAP) and for obtaining blood samples. A catheter was also placed in the femoral vein and was used for drug administration. Unless otherwise indicated, all catheter internal diameters were 1.50 mm.

A left thoracotomy through the fourth or fifth intercostal space was performed and hemostasis was achieved by cautery. All blood vessels too large to be cauterized were tied at both ends using No. 2 vascular silk and out. The left lung was retracted and a Levine Feeding tube (i.d. 0.91 mm, length 20 cm) was inserted into a branch of the pulmonary vein and advanced into the left atrium for the purpose of injecting microspheres, MAC, MBD and for measuring left atrial pressure (LAP). The pericardium was subsequently opened along the phrenic nerve and a No. 2 vascular silk ligature placed around the LADCA at a point usually distal to the first diagonal branch. After allowing approximately 20 minutes to establish a stable hemodynamic state, baseline measurements of RAP, PAP, PCWP, LAP, SLVP, DLVP, arterial pressures, CO and heart rate (HR) were determined. CO was measured by the thermodilution method using a cardiac output computer (American Edwards Laboratory, Model No. 9520A). All pressures were referenced to mid-chest level and were measured using a Gould Statham transducer (Model No. P23Db). The output of the transducers was amplified by a low level D.C. preamplifier (Model No. 7P1F) and recorded on a four channel Grass recorder.

Mean LV and arterial blood pressures (MLVP and MAP) were obtained by electronic averaging of the signal. HR was determined by counting cardiac

cycles on the arterial pressure tracing over a 20 second period. The transducers were calibrated prior to, periodically during, and after each experiment. Lead II of the ECG was continually recorded throughout the experiment.

2.4 EXPERIMENTAL GROUPS

The protocol followed for the 3 experimental groups is described below and summarized in Figure 2.1.

Group 1- Microspheres were injected five minutes prior to and at 10 minutes following coronary artery occlusion in order to determine myocardial blood flow at these times. Immediately after the second microsphere injection, MAC were injected followed by MBD in order to delineate AAR 10 minutes post-occlusion by both indicators.

Group 2-(Control) The protocol was similar to Group 1 except that the coronary artery was tied for 3 h and the MBD was injected at the end of the occlusion period. In addition, a third microsphere was injected 3 h after occlusion to ascertain the level of flow to the normal and ischemic myocardium at this time.

Group 3-(Nicardipine Treated) The protocol was identical to Group 2 except that after AAR determination by MAC a bolus dose of Nicardipine (10 ug/kg)

constant infusion of 8 ug/kg/hr (0.33ml/min) over the remainder of the 3 h occlusion period.

Hemodynamic measurements were made just prior to the second microsphere injection in all groups. In groups 2 and 3, these measurements were subsequently made every half hour throughout the experiment. If ventricular fibrillation occurred during the course of the experiment, a defibrillator was used (Hewlett Packard, Model No. 42110-A). Defibrillation was attempted in 3 dogs. All of these attempts were unsuccessful. Any data obtained from these animals were discarded.

Following MBD injection at the end of the 3 h occlusion period, KCl was administered into the left atrium until ventricular standstill occurred. The heart was subsequently excised, rinsed in saline 5-6 times to remove excess blood and patted dry. Using a scalpel blade (no.10) a slit was made along the LADCA extending from the apex to the base of the heart. This served as a point of reference for alignment of the heart slices in the autoradiography procedure. The heart was then sliced using an electric meat slicer (Rival Electric Co.) into 6 mm thick transverse sections from apex to base and the slices placed into individually labelled dishes. After trimming off the right ventricle and any visible fat, the heart slices were used to determine AAR as defined by MAC and MBD injections and also for determination of infarct size and Regional Myocardial Blood Flow (RMBF).

2.5 DRUG PREPARATION

15 mg of Nicardipine was generously supplied by Syntex Co., Palo Alto, California. This was dissolved in 3 mls DMAC (dimethylacetamide, BDH Chemical Co.) and made up to a final volume of 15 mls with sterile saline. The stock solution (1mg/ml) was stored in sterile evacuated blood collection tubes (additive free) at 4°C until needed.

2.6 MEASUREMENTS AND TISSUE PREPARATION

2.6.1 ^{99m}Tc Macroaggregates

These were used to determine the extent of the AAR at 10 minutes post-occlusion.

Preparation

One million albumin MACs (3M Co.,) were labelled with ^{99m}Tc (0.35 mCi/kg) and subsequently made up to a volume of 9 mls with sterile saline (0.9%). During the labelling reaction, care was taken to ensure minimal exposure of the reaction vial to air or any type of oxidant as this has been shown to adversely affect the quality of the preparation (Mallinckrodt Co., Product Information). Because of the short half life

of the ^{99m}Tc label (6 h), the MACs were prepared immediately prior to injection.

Labelling efficiency was periodically assessed by thin layer chromatography by the following procedure:

A small drop of the MAC preparation was placed 1 cm from the bottom of an 8 x 1 cm chromatographic strip. Once the spot was completely dry, the strip was placed using forceps into a scintillation vial containing acetone to a depth of 0.5 cm. After the solvent had travelled to the top of the strip (approx. 5 minutes) the strip was removed from the vial and cut at a distance approximately 2 cm from the top. Both pieces were placed in separate tubes for counting using a dose calibrator. The following formula was used to calculate the % of free ^{99m}Tc :

$$\frac{\text{AT} - \text{B}}{(\text{AT} + \text{AB})} \times 100$$

% Bound = 100% - % Free

where AT = Activity of the top 2 cm piece

AB = Activity of the bottom piece

B = Background

Labelling efficiency was between 90% and 97%.

The MAC suspension was then injected into the left atrium at 10 minutes post-occlusion. This was followed by a 10 ml flush of warm saline. Prior to injection, the MACs were swirled gently¹ to prevent aggregation and this lack of aggregation was confirmed periodically by microscopic inspection.

Autoradiography was performed by placing the heart slices in KODAK X-omatic x-ray cassettes with sensitive medium intensifying screens. High speed CHROMEX x-ray film (Dupont) was placed in the cassette and exposed for 4 hours² at 4°C (to prevent tissue distortion or shrinkage). The film was developed by hand using KODAK GBX developer and fixer.

Once developed, the heart slices were oriented and appropriately aligned on top of the autoradiographs and the inner and outer outlines of the left ventricle were traced. The heart slices were now either autoradiographed for a second time or processed for RMBF.

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¹ Rough or excessive agitation of the ^{99m}Tc MAC preparation may result in fragmentation or erosion of the albumin particles (Mallinckrodt Co. Product Information).

² A series of experiments with longer exposure times (5,8,10,18 hours) revealed no significant increase in the degree of fogging produced. Therefore, it was decided to incubate for the shorter period.

Only ^{99m}Tc labelled MACs and not radiolabelled microspheres trapped in the circulation fogged the x-ray film to any appreciable extent. This was due to the fact that the MACs contained approximately 2000X the amount of radioactivity contained in the microspheres. The extent of x-ray film fogging produced by radiolabelled microspheres was assessed by allowing some heart slices to sit for 72 hours. At this time only 0.024% of ^{99m}Tc originally injected remains in the sample. Since the isotopes with which the microspheres are labelled have much longer half-lives (65-127 days), almost all of the radioactivity contained in the microspheres is still present at this time. At this point the film was exposed for periods varying from 4-18 hours but these second set of autoradiographs revealed virtually no fogging. However, when the microspheres were new and therefore more radioactive, a small degree of background fogging was seen in the second set of autoradiographs after 18 hours of exposure. However, if the second exposure time was reduced to 4 hours no fogging even with new microspheres, was ever seen. Since exposure times of greater than 4 hours did not significantly increase the degree of fogging produced by the ^{99m}Tc , the first exposure was reduced to 4 hours.

2.6.2 Monastral Blue

In order to determine the AAR at the end of the occlusion period, Monastral Blue ([0.5 ml/kg], 25.8% copper phthalocyanine pigment, Heubach Co., Newark, NJ) was administered over a two minute period in the left atrium. This resulted in the blue staining of all myocardium not supplied by the occluded LADCA. In the heart slices, the AAR is delineated by its absence of stain.

In order to assess planimetrically the size of the AAR as defined by the Monastral Blue, the heart slices were immersed in saline prior to autoradiography and their basal surfaces photographed under Tungsten light using a Nikon camera fitted with a macro lens at a distance of 38 cm. Subsequently, a fine scalpel blade was used to outline the lateral and if present transmural borders of the AAR.

2.6.3 Identification Of The Infarct

Nitro-Blue tetrazolium (2,2'-di-p-nitrophenyl-5,5'-diphenyl-3,3'-[3,3'-dimethoxy-4,4'-biphenylene] ditetrazolium chloride), Sigma Chemical Co.) was used to identify the zone of necrosis. The stain forms a purple-blue precipitate over normal tissue, whereas areas of necrosis fail to stain. (Fishbein, 1984)

Preparation of Nitro-Blue Tetrazolium

For each experiment, 600 mls of Nitro Blue Tetrazolium (0.5 mg/ml) solution was freshly prepared in a dark bottle (Nitro blue is light sensitive) by first combining 57 mls (0.2 M) and 243 mls (0.2 M) of sodium phosphate mono- and di-basic stock solutions respectively. The preparation was never used more than 24 hours after preparation as this may affect the quality of the preparation. Nitro-blue tetrazolium (300 mg) and pyrogen free water was then added to the buffer to bring up the volume to 600 mls. If necessary, pH was adjusted to 7.40 using sodium hydroxide. One hour prior to use, the solution was stirred and prewarmed to a temperature of 37°C.

Infarct Visualization

Immediately following the first set of photographs the heart slices were incubated for 15 minutes in a second set of dishes containing prewarmed Nitro-blue tetrazolium. During the incubation period, the dishes were covered with a heating pad at low setting to maintain dish temperature at approximately 37°C. At the end of the incubation period, the heart slices were placed in 4% formalin which, not only fixes the tissue, but also enhances the colour contrast developed. The heart slices were then rephotographed, and the photographs used for planimetric assessment of the size of the infarct zone. The heart slices were then

placed in appropriately labelled ziplock plastic bags and autoradiographed.

2.6.4 Planimetry

Autoradiographs, MBD determined AAR and Infarct photographs were analyzed using an IBAS-1 (Interactive Image Based Analysis System with IBAS-1 standard software (Version 5.41)) to determine the area occupied by the left ventricle, area at risk and infarct. The outlines of the AAR in the photographs and autoradiographs were done on the basis of the presence or absence of macroaggregate fogging or blue stain. Wherever macroaggregates or blue stain were clearly visible, the area was excluded from the AAR. Each measurement was taken using three or more replicates, with the coefficient of variance for any measurement not exceeding 3%. Inter-observer error, as measured in two animals, was less than 1%. AAR size was determined by dividing AAR size of a slice by the area occupied by the left ventricle in the same slice. The values for the sequential slices were then added to provide the total risk zone for each heart. Infarct size was determined by dividing the size of the infarct in a slice by the area at risk of the same slice as determined by both photography and autoradiography. Once again infarct sizes for sequential slices were summed and the total infarct size for each animal determined. Since the autoradiography procedure resulted in a small degree of left ventricular compression and therefore flattening, the autoradiographs revealed a

greater left ventricular area and therefore area at risk size. Therefore, a correction factor was applied to normalize infarct size to this artifactually larger area at risk size. This "squish factor" was determined by dividing the left ventricular area measured in the autoradiographs by the left ventricular area measured in the photographs for each slice. This factor was then applied to all autoradiograph AAR measurements. Implicit in the application of this squish factor is that the area at risk is compressed to the same degree as the rest of the left ventricle. All risk zone and infarct areas are expressed as percentages of the left ventricle and area at risk respectively.

2.7 REGIONAL MYOCARDIAL BLOOD FLOW

RMBF was measured in each animal by injecting 1.2 million microspheres prior to and 10 minutes after LADCA occlusion. In groups 2 and 3 an additional microsphere was injected 180 minutes after occlusion.

How Supplied

The microspheres are supplied in 20 ml suspensions (Dupont-NEN, Montreal) each containing 100 mg of microspheres labelled with 1 mCi of isotope. This represents approximately 3.04×10^7 microspheres. The suspending medium consists of a 10% dextran solution and 0.01% Tween 80 (polyoxy ethylene sorbitan mono-oleate, Fisher Scientific Co.). The

purpose of the dextran is to increase the viscosity of the suspending medium in order to retard the settling of the microspheres. The Tween 80 is necessary to overcome the tendency of the microspheres to form clumps, thereby allowing it to remain as a homogeneous dispersion.

The microspheres used were labelled with either ^{113}Sn , ^{85}Sr , ^{46}Sc , or ^{57}Co . The microsphere labels were chosen such that the primary energy peaks of the gamma spectra of each isotope did not overlap and secondly so that there were relatively few counts outside the isotope's primary energy peak. Nuclide specificity was periodically monitored by analyzing the gamma spectra of most new batches delivered and looking for extraneous peaks. None were noted.

Preparation

The microsphere suspension was vortexed for 5 minutes and 20 l ml aliquots were subsequently removed. Each aliquot contained approximately 1.5 million microspheres. Nineteen mls of 0.9% sterile saline and 0.01% Tween 80 were then added to each aliquot. Once in this form, the microspheres were ready for injection. The microspheres were stored at 4°C or frozen. The freezing did not appear to alter the structure or leach characteristics of the microspheres and thus would be the storage method of choice as it eliminates bacterial or fungal growth in the suspension medium.

The degree of isotope leaching from the microspheres was periodically determined by vortexing a 20 ml aliquot of microspheres for 3 minutes and withdrawing a 1 ml sample of the microsphere suspension. The remaining 19 mls were centrifuged at 1100 g's for 15 minutes. A 1 ml sample of supernatant was then withdrawn from this aliquot. Both the microsphere sample and the non-microsphere supernatant was then counted on a gamma spectrometer and the total counts per minute for both tubes compared. The non microsphere supernatant radioactivity was never greater than 0.5% of the radioactivity of the microsphere sample.

Experimental Protocol

The microspheres were vortexed for 10 minutes prior to injection. Subsequently, 17 mls (1.2 million microspheres) of the microsphere suspension was injected into the left atrium over 20 seconds followed by a 10 ml flush of warm saline. Five seconds before microsphere injection, arterial blood withdrawal was begun from the femoral artery at a constant rate of 9 mls/minute over 2 minutes using a dual infusion withdrawal pump (Harvard Apparatus Co.). The reference withdrawal catheter was filled with 1 ml heparinized saline (500 U/ml). The reference bloods were subsequently placed in 50 ml sterile tubes containing 5 mls of isocitrate anticoagulant.

Preparation of the Anticoagulant

Citric acid (0.04 M) was completely dissolved in 80 mls of pyrogen free water and sodium citrate (0.08 M) was subsequently added. Dextrose (0.12 M) was then added to the solution and the final volume was brought up to 100 mls with pyrogen free water.

Tissue Preparation

After photography and autoradiography, transmural slices from the non-ischemic (normal) myocardium, lateral adjacent outer and inner margins of the AAR (LAO and LAI respectively) and central ischemic zone (CEN) were divided into epicardial and endocardial layers as shown in Figure 2.2. Identification of the CEN and LAI regions was done on the basis of the AAR delineations of the autoradiographs.

Approximately similar sized samples were taken and weighed (weight range 0.5-0.9 grams) and microsphere content was determined by counting radioactivity in a 5 channel LKB Compu-Gamma spectrometer (Model No. 1282). All samples were counted on the same day. The window settings of the spectrometer were adjusted for optimal counting on each isotope's main photopeak. The window settings for each of the isotopes used is shown in Appendix Figure 1.

Blood flow was then calculated according to equation 1 as reviewed in Section 1.3.4 using a Lotus 1-2-3 spreadsheet.

Figure 2.2

Sectioning of Myocardium for Regional
Myocardial Blood Flow Analysis

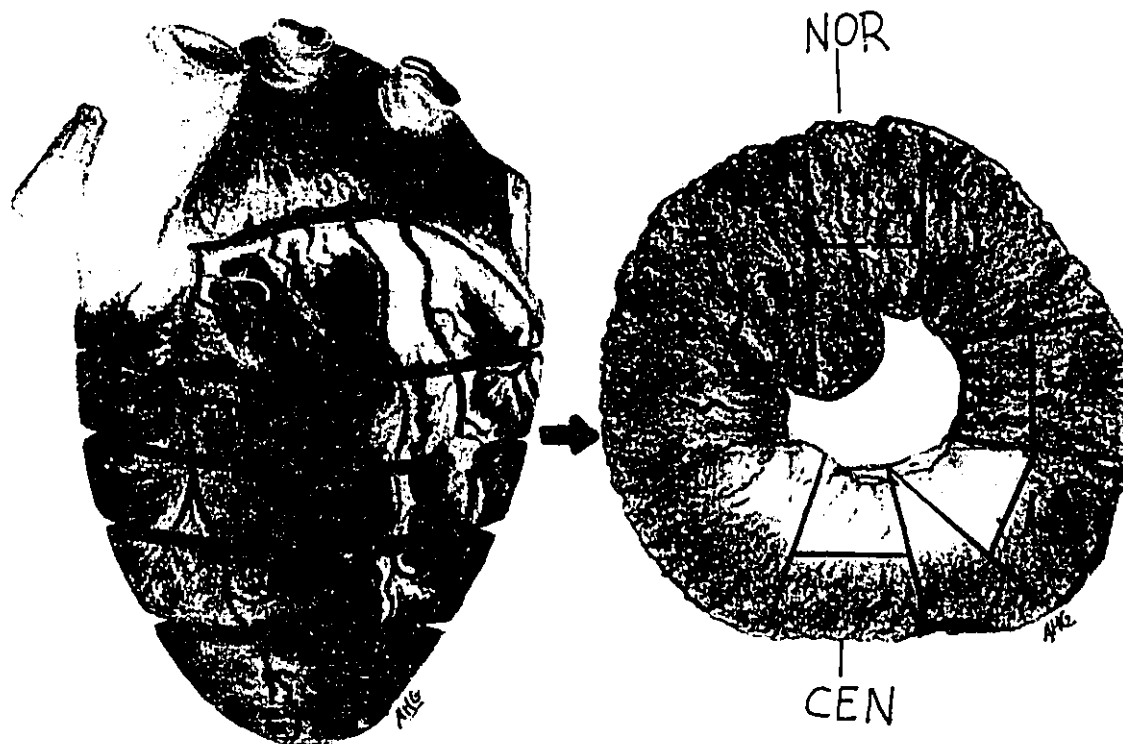


Figure 2.2 Sectioning of myocardium for RMBF. Sections were taken from the normal myocardium (NOR), lateral adjacent outer (LAO) and lateral adjacent inner margin of the AAR (LAI) and central region (CEN) of the AAR. Each of these samples were subsequently divided into epicardial and endocardial halves.

2.8 STATISTICS

Unless otherwise indicated all values are expressed as Mean \pm SEM. Hemodynamic data were analyzed using one-way ANOVA for Repeated Measures (Statistical Analysis Software (SAS) PROC GLM). Slopes of regression lines were compared to one another using the t-distribution to test for significant differences. A one-way ANOVA was also used to examine the blood flow data from the three groups followed by the Tukey's HSD test for multiple comparison when analysis of variance yielded significant differences (SAS, PROC GLM). Statistical significance was defined as a p value of less than 0.05.

RESULTS

3.1 HEMODYNAMICS

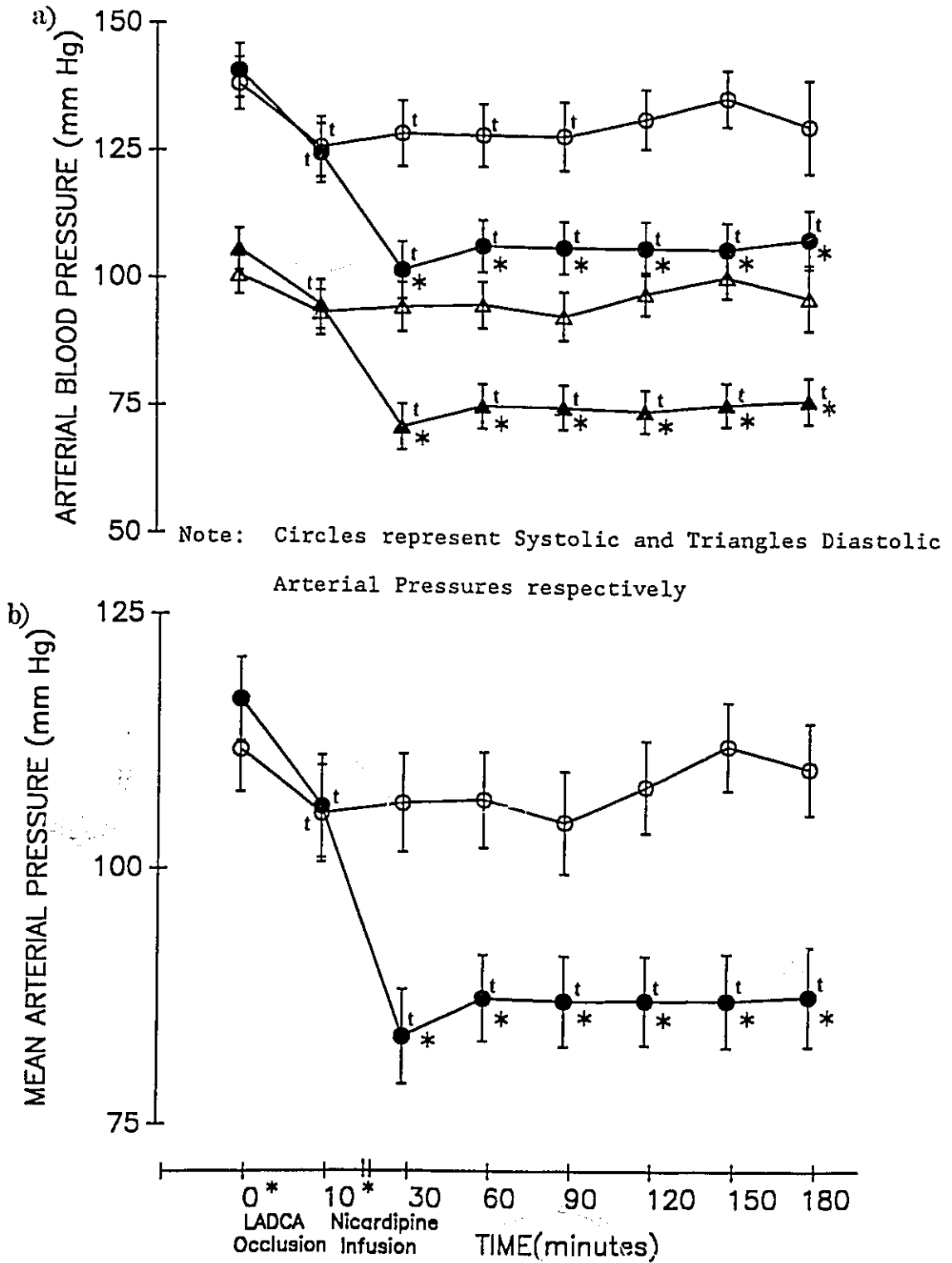
Groups 1, 2 and 3 did not differ from one another in any hemodynamic parameter measured prior to occlusion or 10 minutes after occlusion (i.e. prior to Nicardipine administration in group 3). A summary of hemodynamic variables measured in all groups prior to and 10 minutes following occlusion are given in Appendix table 1.

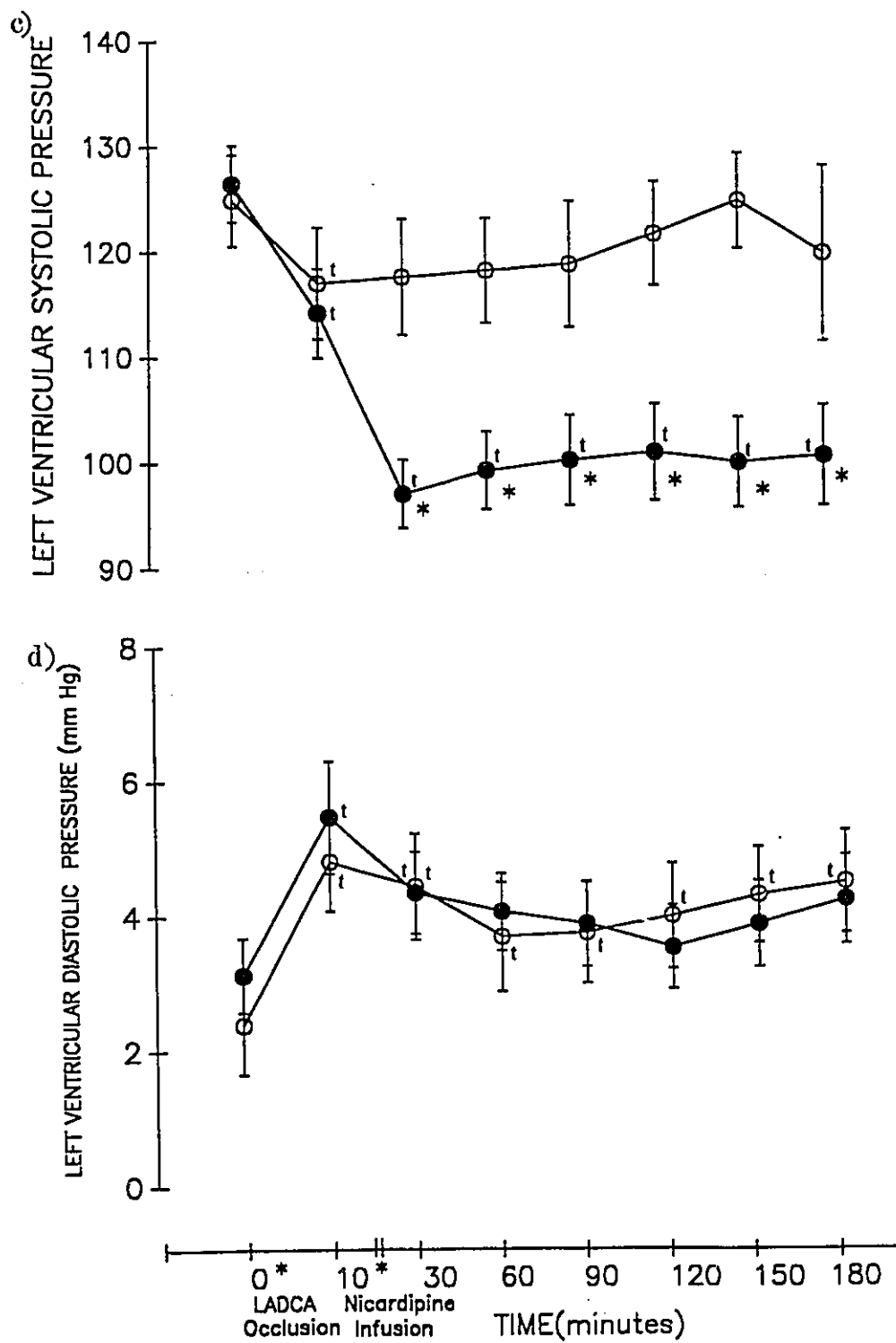
3.1.1 Effects of Three Hours of LADCA Occlusion

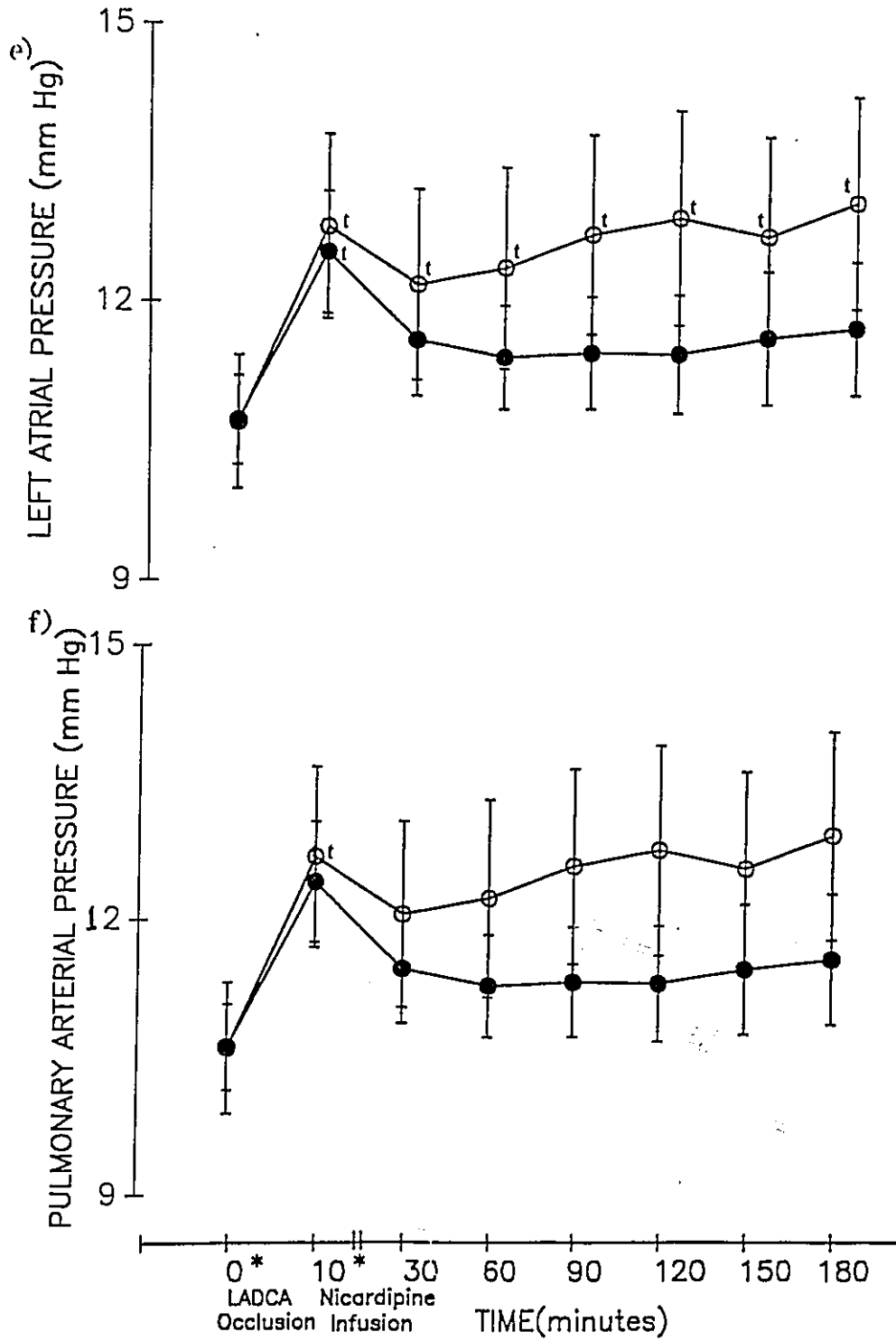
The hemodynamic effects of three hours of LADCA occlusion are shown in Figure 3.1. All comparisons made below refer to contrasts made between baseline pre-occlusion measurements and those made subsequently after the occlusion in the same group.

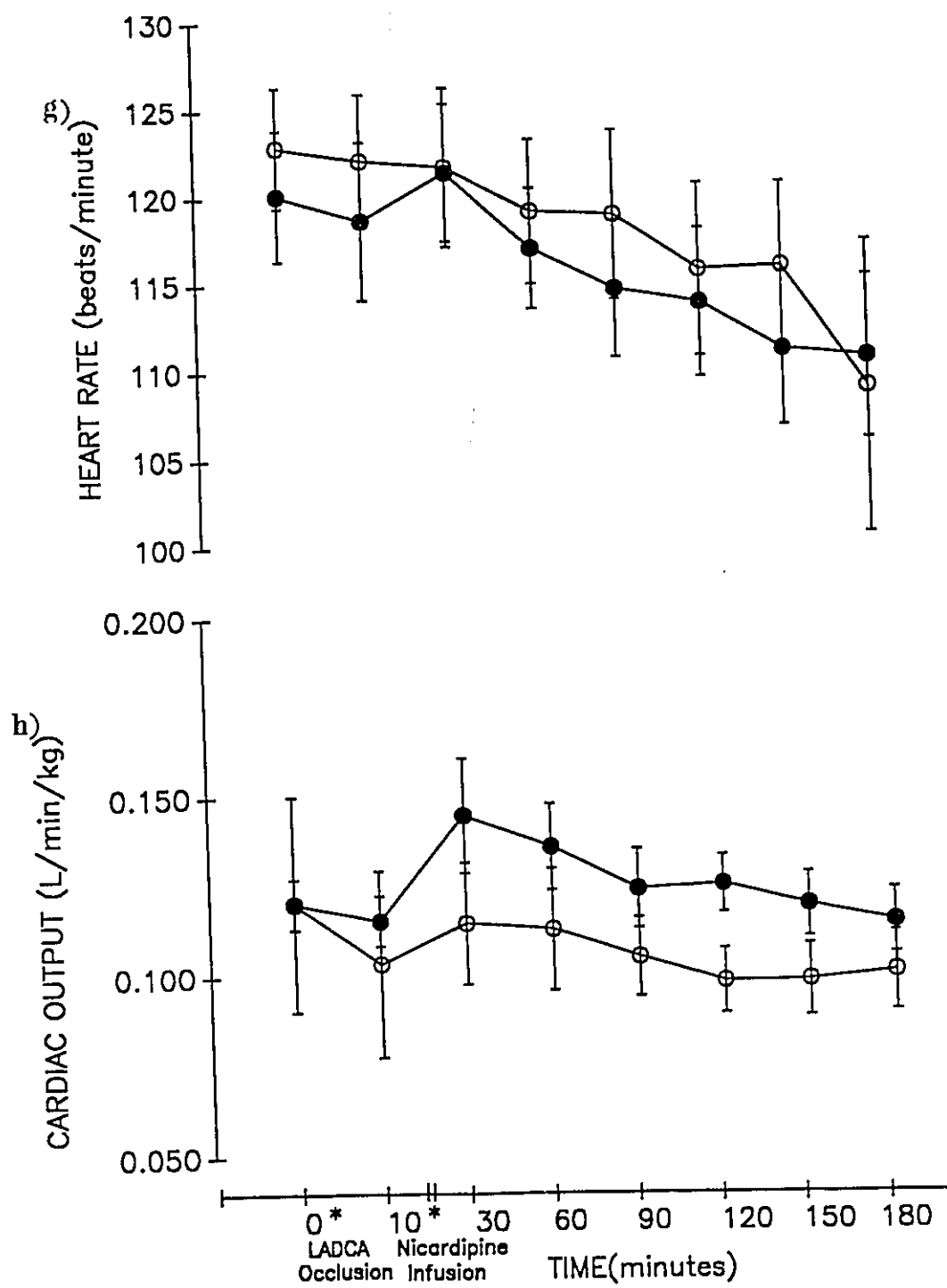
LADCA occlusion produced significant drops in the group means of AP, SAP and SLVP 10 minutes after the occlusion in both groups 2 and 3. In group 2, DAP was also significantly lower than baseline. The magnitude of the decrease in these four parameters at this time was 7-16 mm Hg. In group 2 the significant drop in SAP was maintained until 90 minutes post-occlusion, at which point the pressure began to increase toward pre-ligation values. DAP and SLVP also showed a similar trend towards increasing values throughout the remainder of the occlusion period in this group. LADCA occlusion produced significant increases in LAP and DLVP in both groups 2 and 3 at 10 minutes post-ligation. In group 2, a

Figure 3.1 Hemodynamic Changes Following LADCA Occlusion in Untreated and Nicardipine Treated Dogs









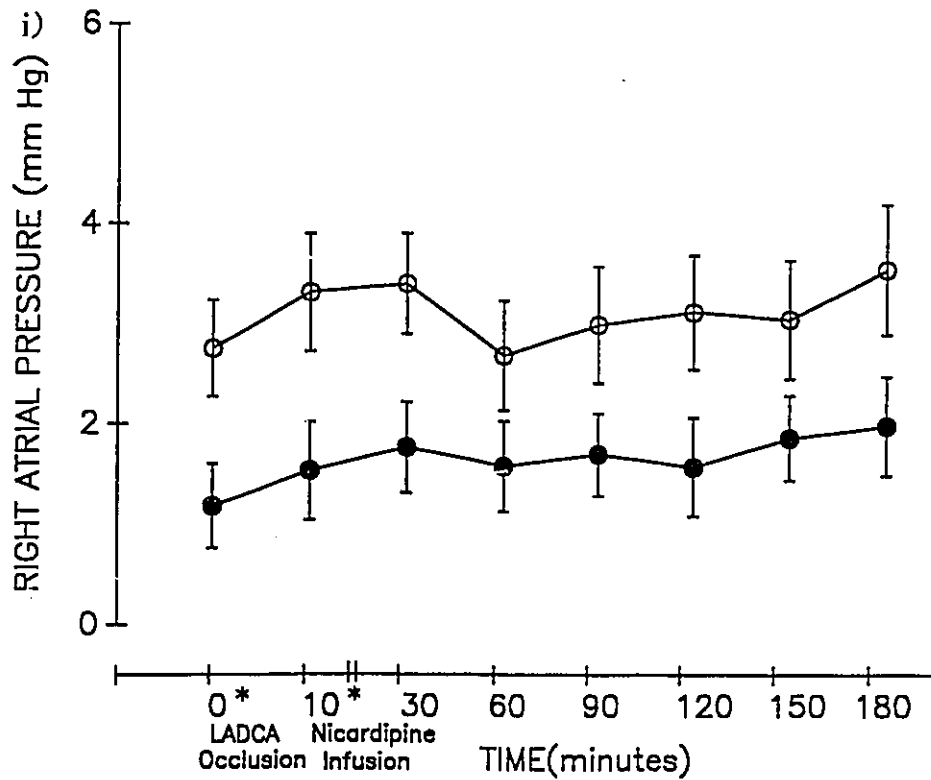


Figure 3.1 The Hemodynamic Changes Following LADCA Occlusion in the untreated and Nicardipine Treated Groups. Open symbols represent untreated animals (n=12), and the closed symbols represent the Nicardipine treated animals (n=17). Bars indicate \pm SEM.

*p<0.05 as compared to the value of the untreated animals at the same time.

p<0.05 when compared to the baseline control value in the same group

significant increase in PAP was also seen at 10 minutes after the occlusion. At this time, the increases were between 1.5-3 mm Hg. The significant increases in LAP and DLVP were maintained throughout the 180 minutes of occlusion in group 2. Group 2 animals showed no significant change in HR, CO and RAP at any time during the occlusion period. However, HR showed a decreasing trend throughout the 180 minutes of occlusion.

3.1.2 Effects of Nicardipine

The hemodynamic effects of Nicardipine are shown in Figure 3.1

3.1.2.1 Effects on Arterial Blood Pressures

Nicardipine administration produced significant falls in MAP, SAP, and DAP when compared to either baseline control values within the same group or when compared to the group 2 value at the same time; these effects persisted over the entire occlusion period. The mean drop in systolic and diastolic pressures from pre-occlusion to 180 minutes post-occlusion were 34 mm Hg and 30 mm Hg respectively. This represents a 24% and 28% decrement in pressure from pre-ligation values. In group 2, the mean fall in systolic and diastolic pressures over this same period were 9 mm Hg (6% of baseline) and 5 mm Hg (5% of baseline) respectively.

3.1.2.2 Effect on Left Heart Pressures

Nicardipine administration produced a significant decrease in SLVP which persisted throughout the 180 minutes of occlusion. The drop in SLVP was 25 mm Hg (17% compared to baseline). Group 3 animals showed no change in LAP or PAP when compared to baseline. DLVP was also not significantly higher than baseline at any time after 30 minutes of occlusion. In contrast, untreated animals showed significant increases in LAP at all post-occlusion times when comparisons were made to baseline.

3.1.2.3 Effect on Right Heart Pressures

Group 3 showed consistently lower RAP at all times when comparisons are made to the value for group 2 at the same time, however, RAP was not significantly different from baseline.

3.1.2.4 Heart Rate and Cardiac Output

Like group 2, heart rate declined throughout the 180 minutes of occlusion, although there were no significant differences in HR between the two groups at any measurement time. Cardiac output was consistently higher in the group 3 animals, although this was not significant because of the small and unequal sample size.

3.2 AREA AT RISK MEASUREMENTS

3.2.1 Are the Two techniques delineating the same area?

In all three experimental groups there was a significant and high correlation between the ^{99m}Tc macroaggregates and Monastral Blue dye estimates of AAR size ($p < 0.01$) (Figure 3.2). The Y-intercepts for all 3 groups were close to 0 (-0.19, 0.16, and 0.93 for groups 1, 2, and 3 respectively). A comparison of slopes revealed that the slopes of each of the three regression lines was not significantly different from the other indicating that neither technique was consistently over-estimating the other.

In order to ascertain whether the size of the AAR may influence the accuracy of the estimate, all AAR's were subdivided into categories of $>10\%$ of LV area and $>20\%$ of LV area (Table 3.1). Paired t-tests comparing the two estimates of AAR size within each group in all animals (Table 3.2, Category 1) revealed no significant differences. Unpaired t-tests were used in categories 2 and 3 to compare within group differences in AAR size determination following the application of exclusion criteria to eliminate dogs with AAR sizes of less than 10% and 20% of the LV area. There were no significant differences within any of the experimental groups in any of these categories (See Table 3.2). A one-way ANOVA was used to compare the AAR sizes determined by the same technique across the three experimental groups (Table 3.3). This analysis also yielded no

Figure 3.2 THE RELATIONSHIP BETWEEN AAR SIZES AS MEASURED BY THE TWO TECHNIQUES

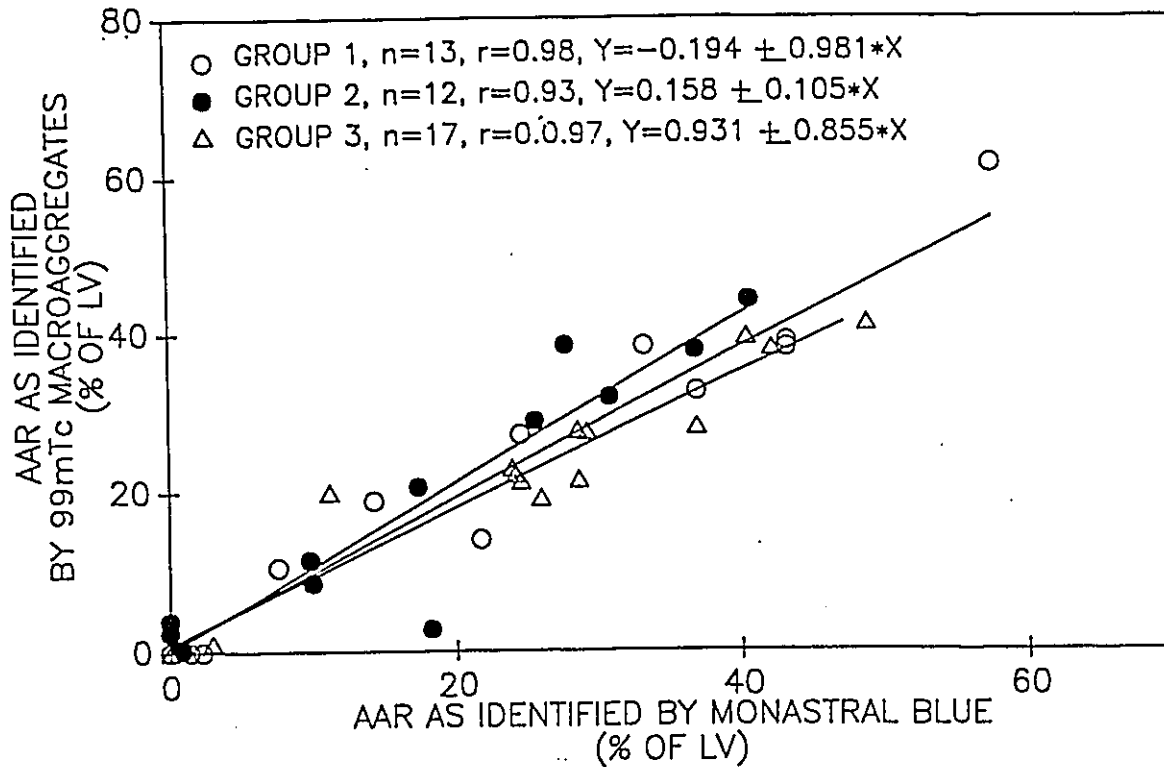


Figure 3.2 The relationship between the AAR size as determined by ^{99m}Tc macroaggregate and Monastral Blue dye injections. For all three groups the correlation was significant ($p < 0.01$). The slopes of the regression lines as given on the Figure for all three groups were not significantly different from one another indicating that neither technique was consistently overestimating the other.

Table 3.1

Do the Two Techniques Identify the Same Area When the AAR is Large?

CATEGORY	METHOD	GROUP 1	GROUP 2 (Control)	GROUP 3 (Nicardipine)
Mean AAR Size (All dogs) (1)	MAC	24.58±5.31 (19.13)	19.28±4.69 (16.26)	21.43±4.01 (16.54)
	MBD	25.23±5.27 (19.06)	18.15±4.14 (14.34)	21.64±3.96 (16.34)
Mean AAR Size (Only dogs with AAR>10%) (2)	MAC	35.22±4.20 (13.30)	28.17±3.32 (8.79)	30.40±2.93 (10.14)
	MBD	31.96±4.43 (15.04)	30.47±4.29 (11.32)	30.26±3.02 (10.40)
Mean AAR Size (Only dogs with AAR>20%) (3)	MAC	37.85±4.06 (11.47)	32.35±2.81 (6.21)	32.16±2.56 (8.50)
	MBD	39.44±4.06 (10.75)	33.64±3.40 (8.33)	31.19±3.13 (10.37)

Table 3.1 This table summarizes the effect of an application of exclusion criteria to eliminate dogs with AAR sizes <10% and <20% of the LV area on the ability of the two techniques to delineate similar areas of myocardium. Regardless of whether all animals or only animals with large AAR sizes are considered, both techniques identify similar areas of myocardium in all three groups. Note that the values for SD are given in brackets.

Table 3.2

T-test Table: Comparison of AAR Sizes Determined by the Two Techniques Within Groups (AAR_{MAC} vs. AAR_{MBD})

Category	1	2	3
	All Animals Paired t-test	Only Animals with AAR>10% Unpaired t-test	Only Animals with AAR>20% Unpaired t-test
Group 1			
T value	0.47	0.50	0.28
df	11	17	13
p	0.66	0.63	0.79
Group 2			
T value	0.79	0.43	0.29
df	12	12	9
p	0.45	0.68	0.78
Group 3			
T value	0.10	0.34	0.24
df	16	22	20
p	0.92	0.79	0.82

Table 3.2 This table shows the results from paired and unpaired t-test comparisons of AAR sizes as estimated by the two techniques within any given group. Exclusion criteria were applied to eliminate dogs with AAR sizes <10% and <20% of the LV area in order to determine if the size of the AAR had an influence on the ability of the two estimates to delineate the same area of myocardium. There were no significant differences between the MAC and MBD estimations of AAR size within the same group in any of the three groups in any category. Note that paired t-tests could only be used to compare within group AAR sizes for the first category because the use of exclusion criteria resulted in an unequal number of observations in each subsequent category.

Table 3.3

ANOVA Table: Comparison of AAR Sizes as Determined by the Same
Technique between the Three Experimental Groups

Category	All Animals		Only Animals with AAR>10%		Only Animals with AAR>20%	
	MAC	MBD	MAC	MBD	MAC	MBD
F	0.30	0.56	0.06	0.90	1.40	0.98
df(N)	2	2	2	2	2	2
df(within)	39	39	26	25	21	21
Total SS	11856.1	11214.3	4011.7	3224.4	2409.2	1971.3
Between SS	178.5	314.3	17.2	215.7	293.3	168.8
Within SS	11677.6	10900.1	3994.6	3008.7	2115.9	1802.5
Mean Variance	301.3	278.8	154.2	118.9	97.5	81.1
Variance of Means	7.1	12.6	0.9	13.0	17.9	10.4
P	0.70	0.57	0.95	0.42	0.26	0.39

Table 3.3 This table summarizes the results of a one-way ANOVA comparing AAR sizes as estimated by the same technique between the three experimental groups, when all animals and only animals with AAR sizes >10% or >20% of the LV area were considered. There were no significant differences between the AAR sizes as determined by MAC or MBD across the three experimental groups.

significant differences in any of the 3 categories. Table 3.4 shows the number of animals in each group having small AAR sizes. Of the 42 animals used in the 3 groups, 14-19% had no AAR as delineated by either technique, 31-33% had AAR sizes of less than 10% of the LV area and 43% had AAR sizes measuring less than 20% of the LV area. The percentage of dogs having small AAR sizes (i.e. <20%) was greatest in Group 2 (50-58%) and least in Group 3 (35.3%).

Frequency histograms showing the percentage difference between the AAR measurements obtained using the two techniques ($AAR_{MBD} - AAR_{MAC}$) in each slice analysed for all groups is given in Figures 3.3-3.5. Grouped frequency tables for data appearing in these figures is given in Appendix Tables 2, 3 and 4. In approximately 94% of all slices in Group 1, the percentage difference in the AAR estimation by the two techniques was less than $\pm 12\%$. In 47% of all observations in this group the MBD estimated a higher AAR size, whereas in 36% of all cases the MAC estimation of AAR size was higher. In group 2, 84% of all slices had differences between the two measurements of less than $\pm 12\%$. In this group, the MBD revealed a higher AAR size in 28% of all cases. In 39% of all cases the MAC estimated a higher AAR. In group 3, 93% of all observations had measurement differences of less than $\pm 12\%$. In 40% of all observations the MBD determination of AAR size was higher and in 25% of the cases the MAC gave a higher AAR size. In 17%, 33% and 34% of all slices in Groups 1, 2 and 3 respectively, both techniques yielded an AAR of 0. In summary, it does not appear that the dispersion of data in

Table 3.4

Number of Animals in Each Group having Small AAR Sizes

	TOTAL (n)	METHOD	AAR=0 (Frequency)	AAR<10% (Cumulative Frequency)	AAR<20% (Cumulative Frequency)
GROUP 1					
	13	MAC	3 (23.07)	3 (23.07)	6 (46.15)
		MBD	1 (7.69)	4 (30.77)	5 (38.46)
GROUP 2					
	12	MAC	1 (8.33)	5 (41.60)	6 (50.00)
		MBD	1 (8.33)	5 (41.60)	7 (58.30)
GROUP 3					
	17	MAC	4 (23.53)	5 (29.41)	6 (35.30)
		MBD	4 (23.53)	5 (29.41)	6 (35.30)
TOTAL	42	MAC	6 (14.29)	14 (33.33)	18 (42.90)
		MBD	8 (19.04)	13 (30.95)	18 (42.90)

Table 3.4 The number of animals in each group and in all of the groups having no AAR, AARs of <10% and <20% of the LV area. 43% of all animals used in this study has AAR sizes that were less than 20% of the LV area. Group 2 had the highest proportion of animals with AAR sizes less than 20% of the LV area and group 3 had the lowest. Note: The values given in brackets indicate the number of animals in the category as a percentage of the total number of animals in the group.

Figure 3.3

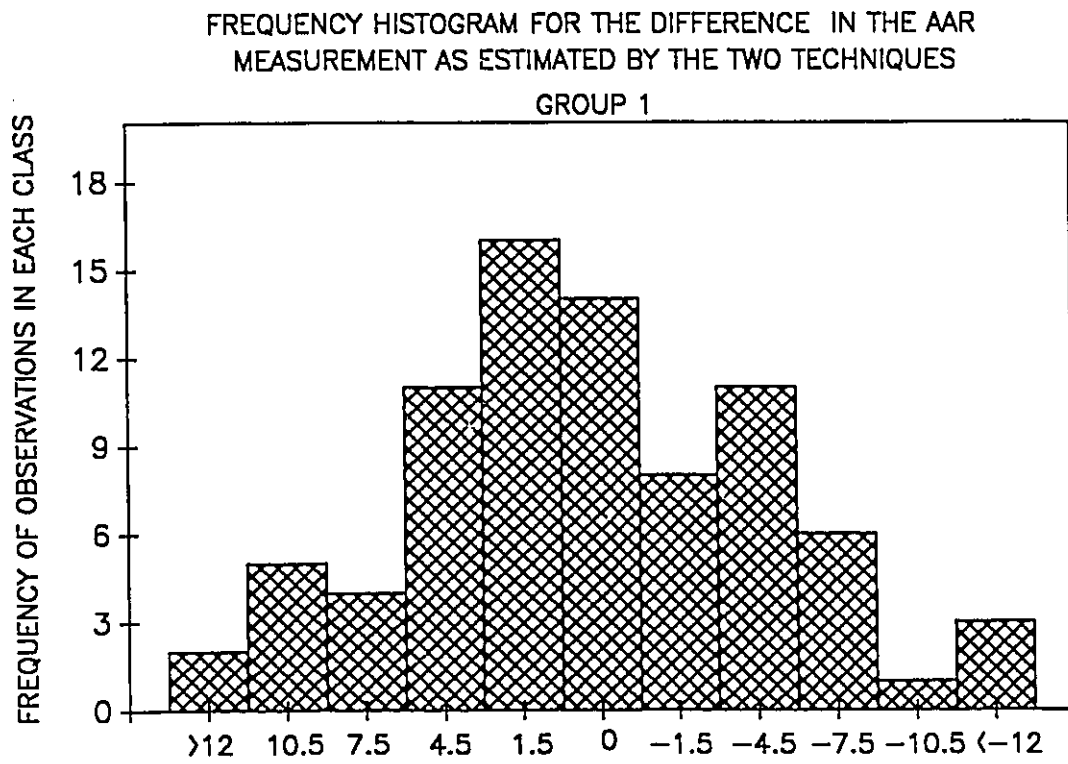


Figure 3.4

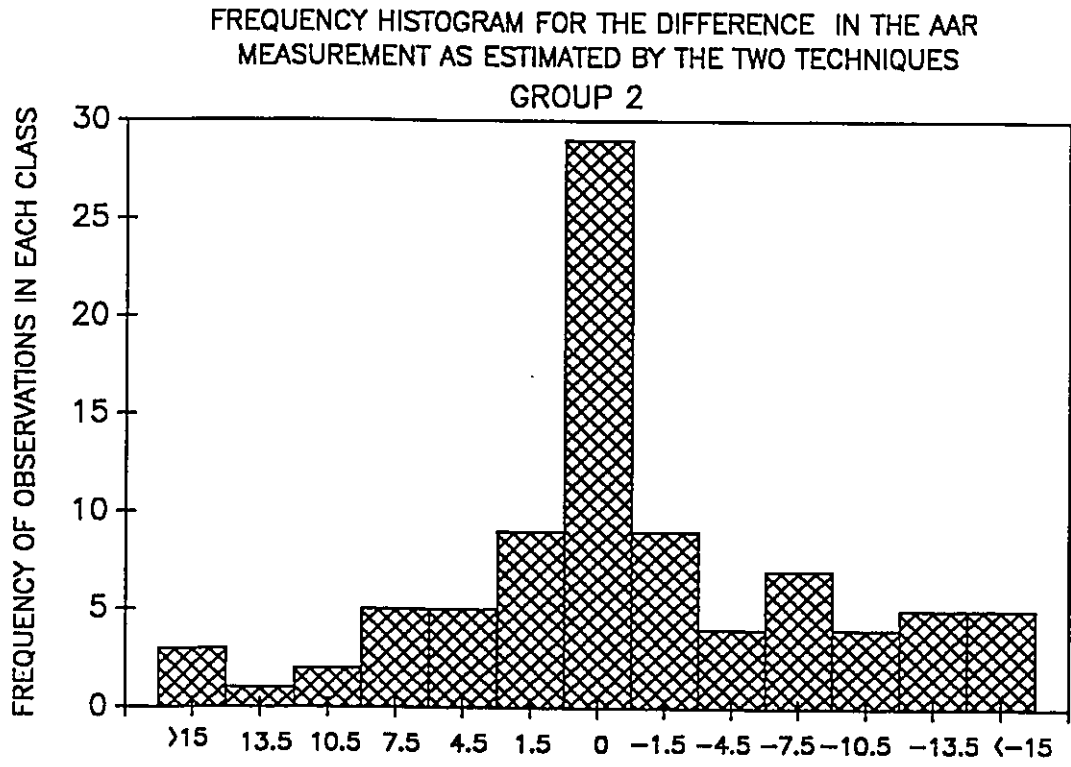


Figure 3.5

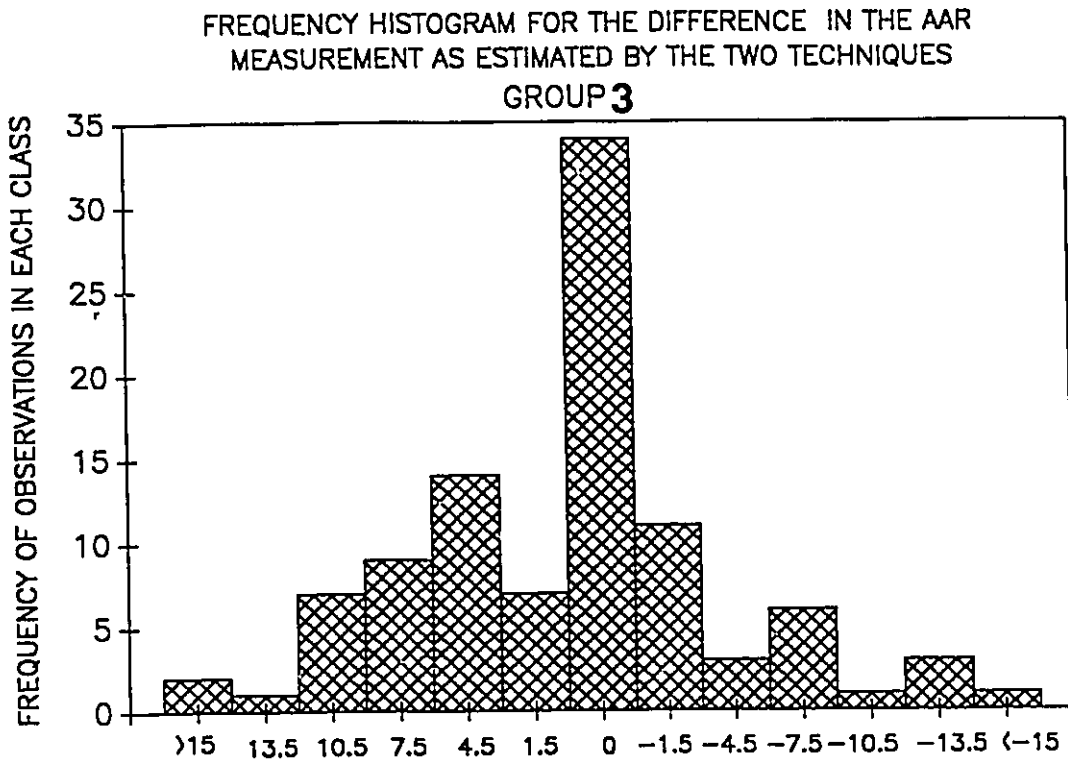


Figure 3.3-3.5 The frequency histograms show the percentage difference in the AAR measurement as estimated by the two techniques ($AAR_{MBD} - AAR_{MAC}$) in each slice analysed. In all three groups, the difference between the two AAR estimates was less than $\pm 12\%$ in approximately 90% of all slices analysed. Given on the X-axis is the midpoint of each class interval. The size of each class interval was chosen as 3 units.

either group 2 or 3 measurements is greater than that seen in group 1, nor does it appear that one of the techniques is consistent in its overestimation.

3.3 SPATIAL CHARACTERISTICS OF THE AAR AND AREA INFARCTED

The spatial characteristics of the AAR and area infarcted for all groups are shown in Figures 3.6-3.8.

The size and location of the in vivo AAR is, in part, a function of the perfusion territory of the occluded bed. In the dog, the LADCA supplies an increasing fraction of the cardiac circumference proceeding from the base to the apex (Reimer et al., 1981). In all 3 experimental groups in the present study, the size of the AAR was greatest at the apex (Approx. 40% of the LV slice area) and declined towards the base (Approx. 12% of the LV slice area) (See Figures 3.6-3.8). Regardless of slice location, both MAC and MBD estimated approximately the same area. In both groups 2 and 3, apical slices had the highest proportion of LV slice area infarcted (Approx. 20%) and the base had the least (Approx. 3%). Please note that all animals were included in this analysis (i.e. including those without visible AARs or infarcts).

Figure 3.6

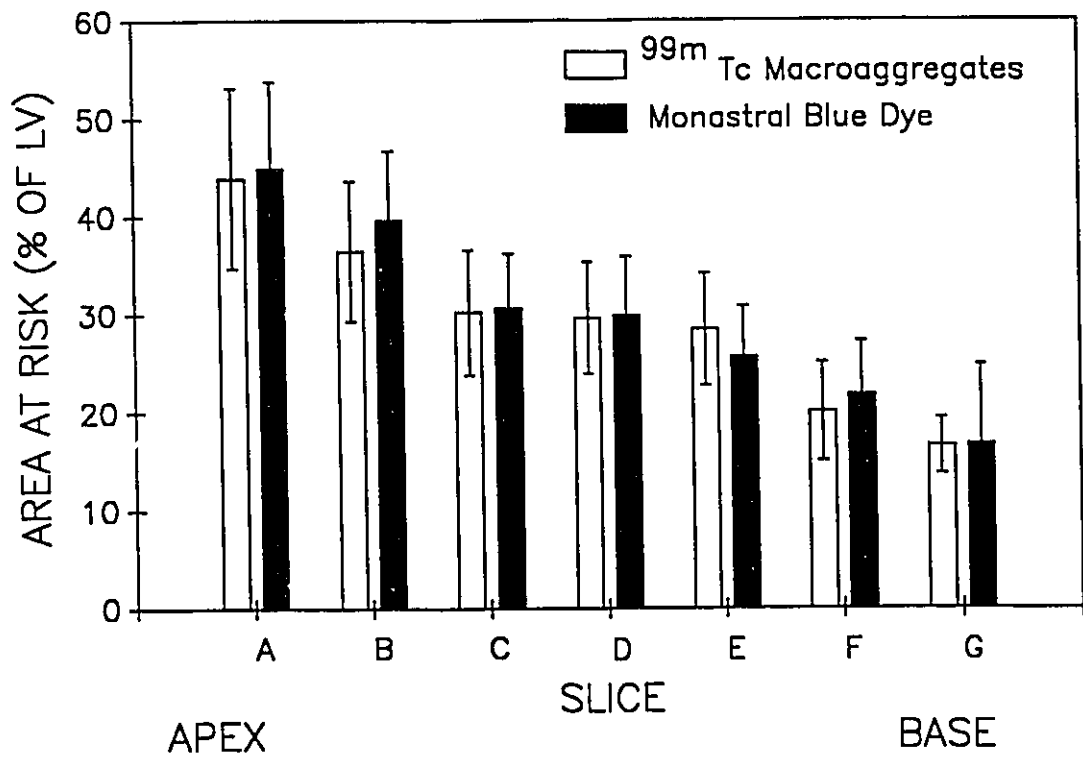
SPATIAL CHARACTERISTICS OF THE AAR
GROUP 1

Figure 3.7

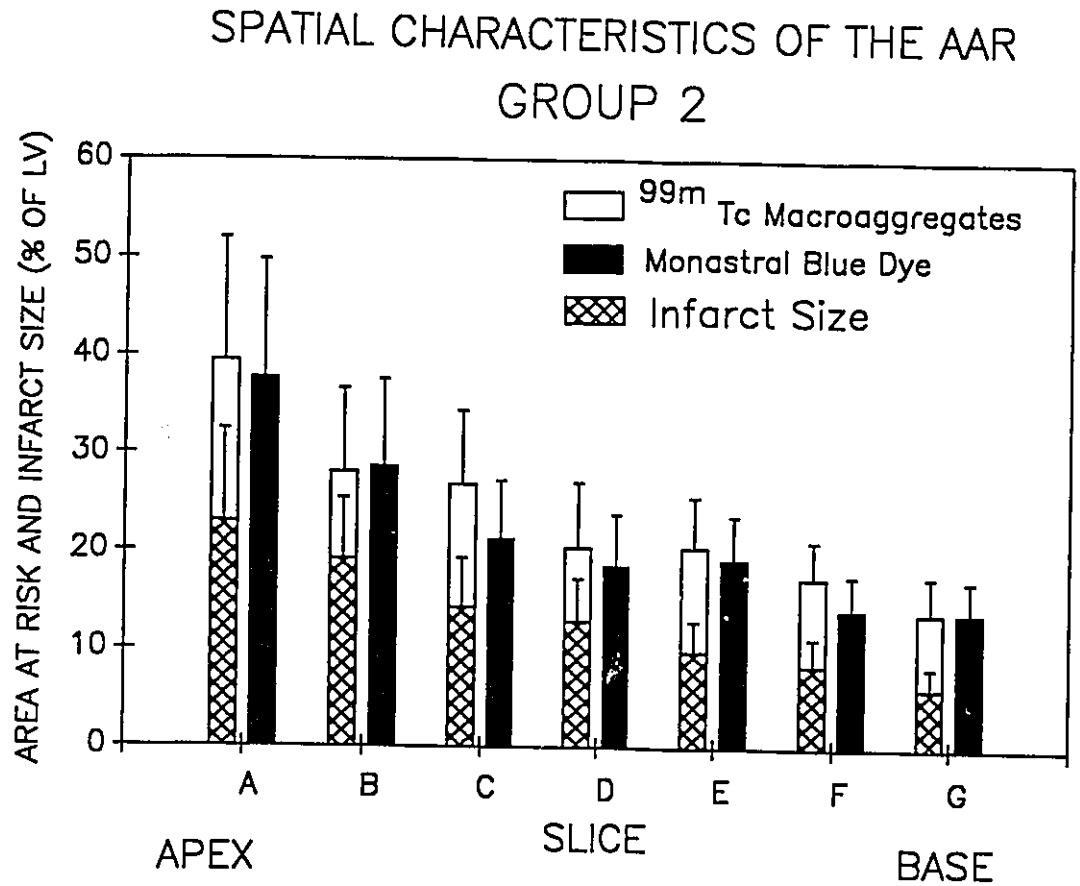
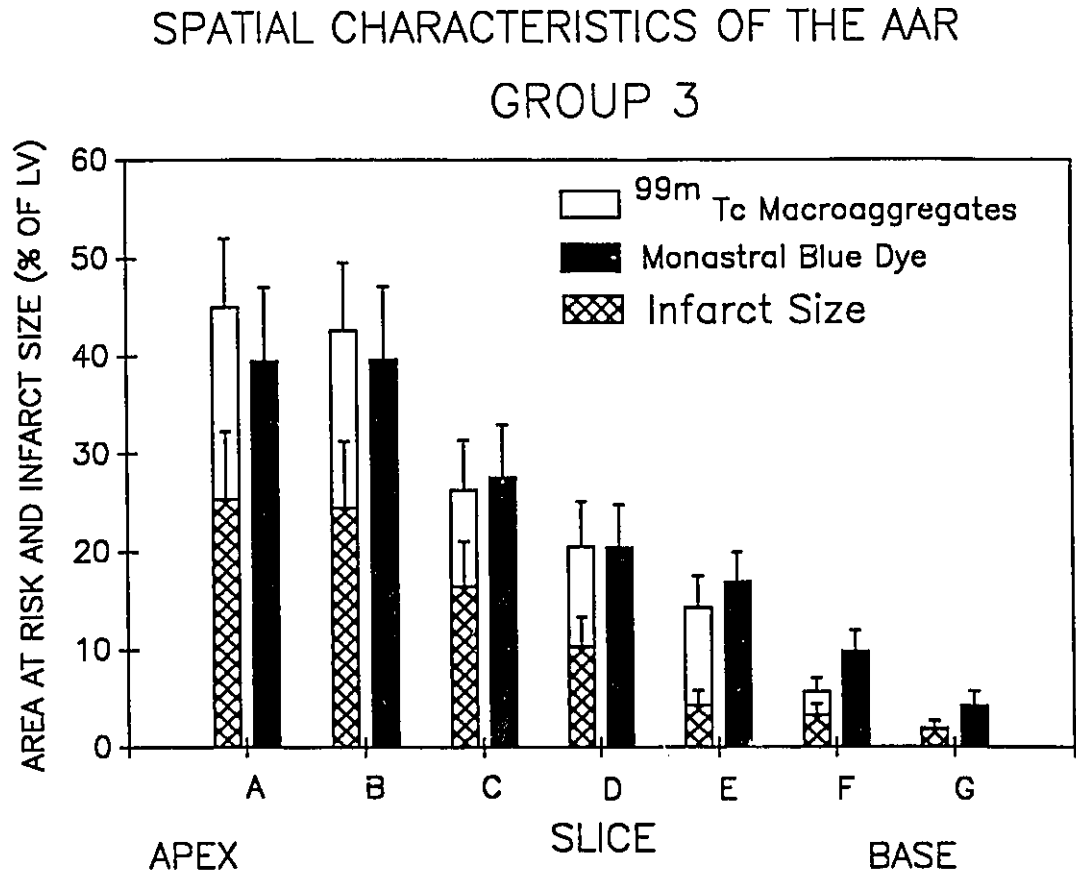


Figure 3.8



Figures 3.6-3.8 These graphs show the percentage of each slice occupied by the AAR in all three groups and the area occupied by the infarct in groups 2 and 3. In all three groups, the AAR was greatest at the apex (Approx. 40%) and declined towards the base (Approx 10-15%). Note that both techniques were estimating approximately the same AAR in each of the different locations. Infarct sizes, in both groups, were also greatest at the apex (Approx. 20%) and declined towards the base (Approx. 3%).

3.4 GROSS MACROSCOPIC OBSERVATIONS OF THE AREA AT RISK AND AREA INFARCTED

Figure 3.9 shows 3 examples of photographs and autoradiographs of the same slice. In example 1, the photograph shows no accumulation of blue dye and thus all of the LV area in this slice is at risk. Similarly, the autoradiograph shows no "fogging" resulting from the presence of macroaggregates and is thus also completely at risk. The infarct photograph shows that 88% of this slice underwent necrosis with marginal epicardial salvage. In example 2 both the autoradiographs and the photographs reveal an AAR which has a sharp but irregular lateral border zone and no visibly identifiable transmural border zone (as depicted by the absence of blue colour or macroaggregate fogging within the epicardium of the AAR). In this slice, AAR measured 83% of the LV area for the autoradiographs and 84% of the LV area for the photographs. The infarct photo reveals that most of the AAR is infarcted (Approx 82%) with no epicardial salvage. Here, the boundary of the infarcted tissue was also sharp but irregular.

Compared to the above examples, the level of collateral flow is greater in the example 3 slice resulting in the accumulation of both macroaggregates and blue dye in the transmural plane. In this slice, the AAR measured 24% and 20% of the LV area in the autoradiographs and photographs, respectively. Although in this particular case the difference between the two AAR estimations was only 4%, in some other

Figure 3.9

Gross Macroscopic Observations of the AAR and Area Infarcted

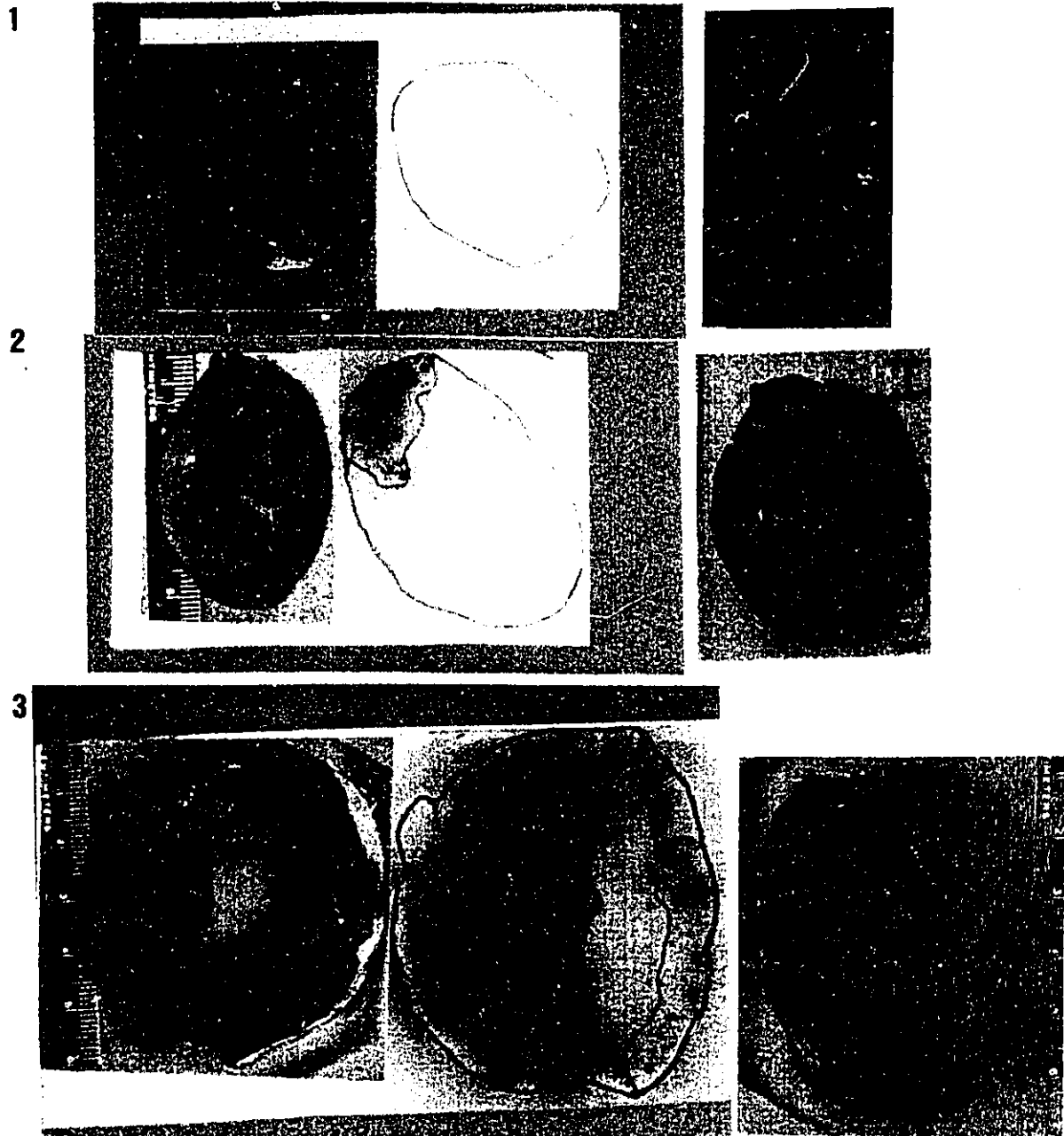


Figure 3.9 Photographs and Autoradiographs of the same slice showing the area infarcted and the AAR as delineated by MAC and MBD. Please note that the magnification for the AAR photographs, autoradiographs and the infarct photographs were different.

cases in which collateral flow was high, delineation of the AAR became difficult and somewhat more subjective. The photograph shows that the infarct was confined to the endocardium. In some animals, the infarct did not appear as a confluent area, but rather as islands of infarcted tissue interspersed among non-infarcted tissue.

3.5 THE RELATIONSHIP BETWEEN INFARCT SIZE AND AAR

In controls, 42% of all animals failed to infarct, compared with 29% in the Nicardipine treated group (Table 3.5). Unpaired t-tests were used to compare infarct sizes between control and Nicardipine treated groups. When all animals were considered, Nicardipine treated animals did not have infarct sizes which were significantly different from control animals (See Table 3.6). In order to determine whether the effectiveness of Nicardipine in limiting infarct size was influenced by the size of the AAR, exclusion criteria were applied to eliminate dogs with small AAR sizes. When only dogs with large AAR sizes were considered (i.e. >20%), group 3 had a smaller mean infarct size than group 2 but this was not significant. When all animals without infarcts were eliminated from the analysis, the Nicardipine treated animals had a mean Inf/AAR size which is 12% lower than the controls. The $\text{inf/AAR}_{\text{MBD}}$ ratio was significantly lower in Nicardipine treated dogs at the 0.10 level (See table 3.6).

The relationship between AAR and infarct size is given in Figures 3.10 and 3.11. Myocardial infarct area was significantly and positively

Table 3.5

Is the Effectiveness of Nicardipine in Limiting Infarct Size
Dependent on the Size of the Area at Risk?

CATEGORY	METHOD	GROUP 2 (Control)	GROUP 3 (Nicardipine)
No. dogs with Infarct=0		5/12 (41.7%)	5/17 (29.41%)
Mean Infarct Size (All dogs) (1)	MAC	36.08±9.87 (35.69)	40.70±8.07 (33.34)
	MBD	39.05±0.52 (38.05)	35.22±7.14 (29.54)
Mean Infarct Size (Only dogs with AAR>10%) (2)	MAC	54.72±11.23 (29.71)	55.89±7.90 (26.20)
	MBD	60.69±11.54	49.94±6.63
Mean Infarct Size (Only dogs with AAR>20%) (3)	MAC	63.90±8.64 (21.26)	60.07±8.26 (26.12)
	MBD	67.85±8.88 (19.86)	53.62±6.45 (20.39)
Mean Infarct Size (Only dogs with Infarcts) (4)	MAC	61.68±7.67 (21.82)	54.27±7.40 (26.69)
	MBD	66.85±8.88 (21.85)	47.00±6.78 (24.25)

Table 3.5 This table shows mean infarct sizes in untreated and Nicardipine treated groups when all dogs, only dogs with infarcts and only dogs with AAR sizes >10% and >20% of the LV area were considered. There were no significant differences between untreated and Nicardipine treated animals in any of the categories considered although when all animals without infarcts are excluded from the analysis, Nicardipine treated dogs had lower infarct sizes and this approached statistical significance ($0.10 > p > 0.05$). Note that the values for SD are given in brackets.

Unpaired T-test Table: Comparison of Infarct Sizes between
Control and Nicardipine Treated Animals

	All Animals		Only animals with AAR>10%		Only animals with AAR>20%		Only animals with Infarcts	
	MAC	MBD	MAC	MBD	MAC	MBD	MAC	MBD
T value	0.36	0.29	0.08	0.82	0.28	1.24	0.64	1.80
df	26	26	16	16	14	14	17	17
p	0.80	0.77	0.94	0.43	0.80	0.23	0.64	0.09

Table 3.6 Results from unpaired t-tests comparing infarct sizes between the two groups when all animals, only animals, only animals with infarcts and only animals with AAR sizes >10% and >20% of the LV area were considered. Infarct Sizes in Nicardipine treated animals were not significantly different from controls in any of the categories considered ($p>0.05$). Note: One of the Nicardipine treated dogs was not used in the infarct analysis because of technical difficulties with the infarct photographs.

Figure 3.10

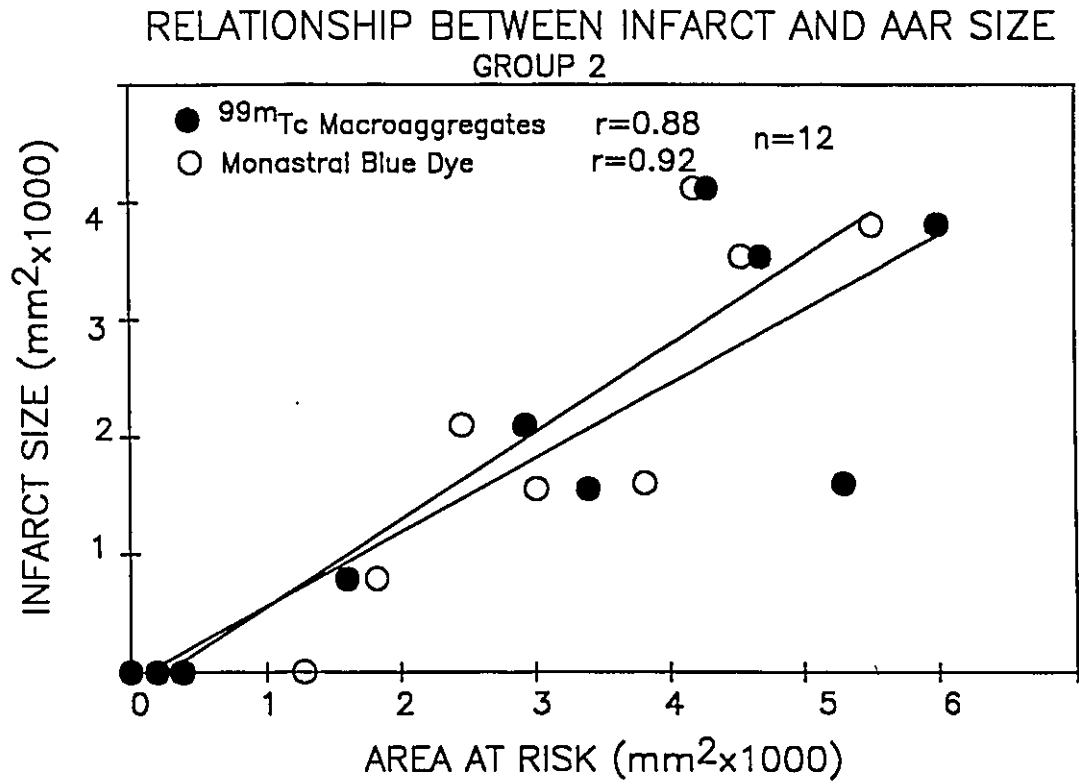


Figure 3.11

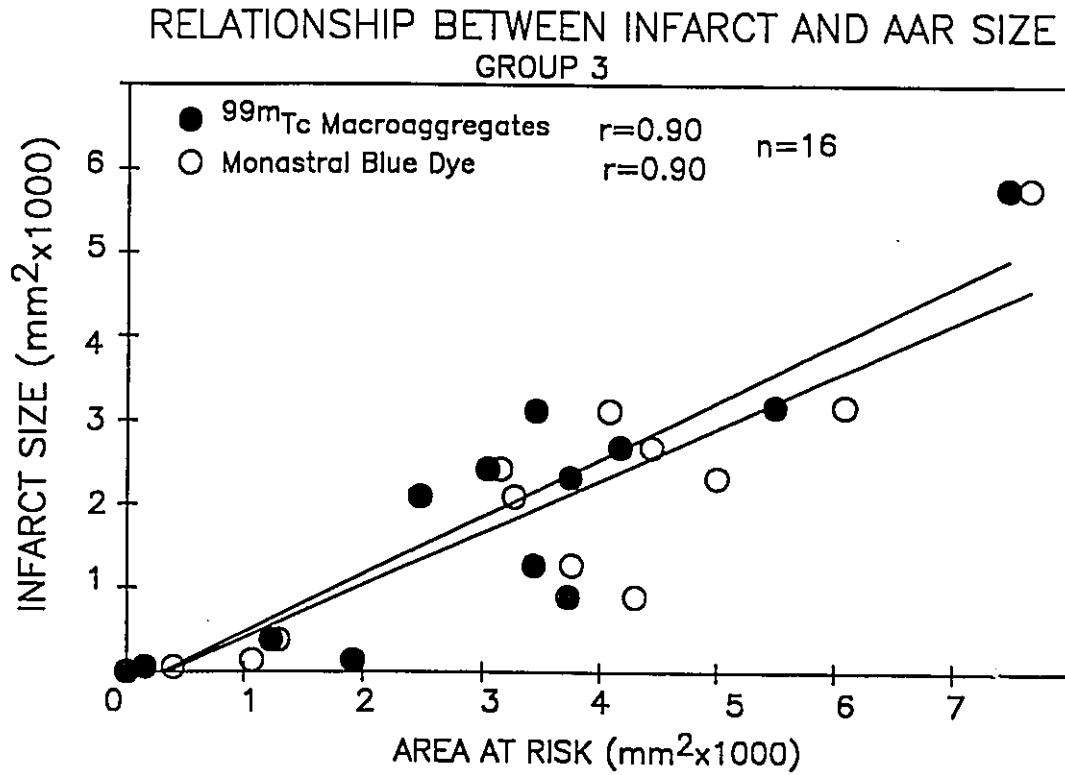


Figure 3.10 and 3.11 The relationship between the total area infarcted ($\text{mm}^2 \times 1000$) and the area at risk of infarcting ($\text{mm}^2 \times 1000$) as estimated by the two techniques in untreated and Nicardipine treated animals. The correlation between infarcted area and AAR was significant for both AAR estimations in both groups ($p < 0.04$).

correlated to the area occupied by the AAR in both groups 2 and 3 ($p < 0.05$). All AAR and infarct sizes obtained in each of the animals used in this study are presented in Appendix Table 5.

3.6 THE RELATIONSHIP BETWEEN INFARCT SIZE AND COLLATERAL FLOW

In both groups 2 and 3 a significant ($p < 0.05$) negative correlation existed between infarct size and collateral flow to the epicardium of the LAI of the AAR (Figure 3.12). Only dogs which underwent infarction could be used for this correlation. Note that the points of the Nicardipine regression line are shifted downward relative to Group 2 suggesting that for the same level of collateral flow Nicardipine treated animals had lower infarct sizes when only dogs with infarcts are considered. The slopes of the two regression lines were not significantly different from one another.

3.7 BLOOD FLOW RESULTS

All blood flow values are expressed as a percentage of pre-occlusion flow in the same section. Groups 1 and 2 did not differ from each other in flows measured prior to occlusion and 10 minutes after occlusion. Consequently, the flow values at each of these times was pooled for the two groups. A one-way ANOVA was used to compare blood flow values obtained in untreated and Nicardipine treated animals at the same time.

Figure 3.12

Relationship between Infarct Size and Collateral Flow
to the Epicardium of the Lateral Inner Margin of the AAR

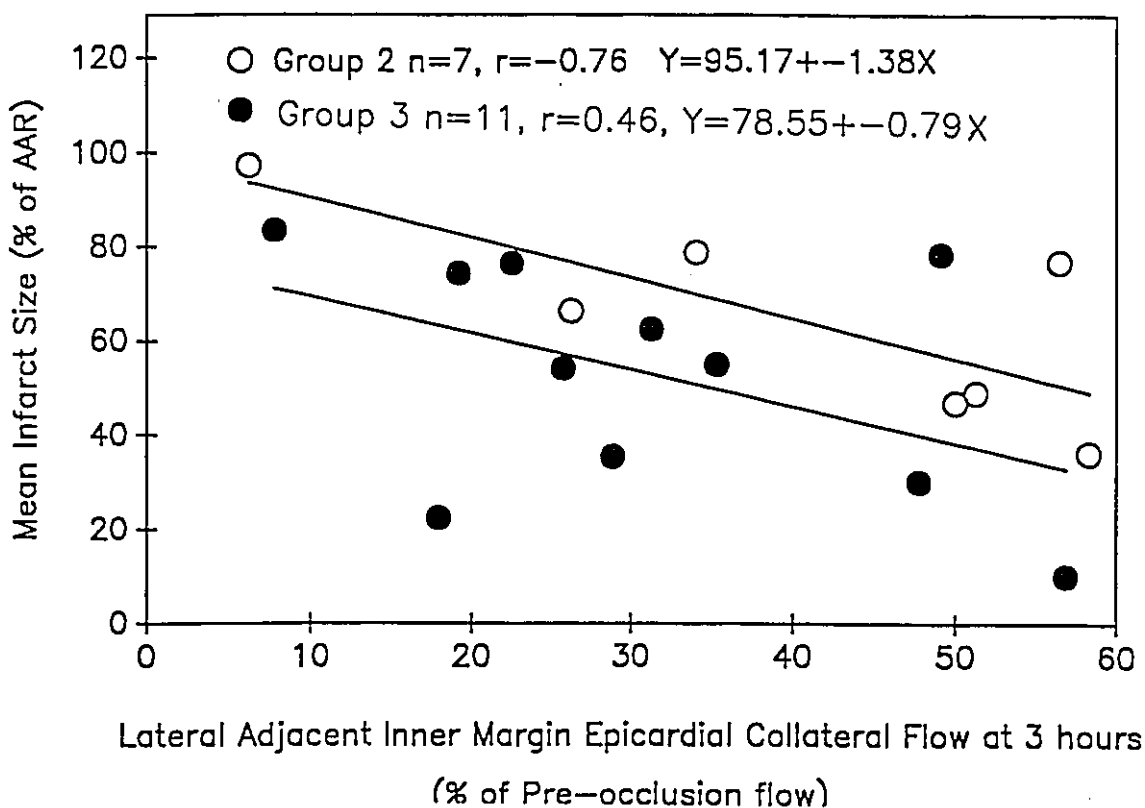


Figure 3.12 Linear Regression showing the relationship between infarct size and collateral flow to the epicardium of the LAI of the AAR at 3 hours post-occlusion. Both correlation coefficients were significantly different from 0 ($p < 0.05$). Note that all dogs without infarcts were excluded from this analysis.

A one-way ANOVA was also used to compare blood flow values within a group across time. Tukey's Studentized Range Test was used to determine where the significant differences lie.

3.7.1 Blood Flow to the Normal Myocardium

Figure 3.13 shows that in groups 2 and 3, blood flow to the normal section increased from pre-occlusion to 10 minutes after occlusion and increased further from 10 to 180 minutes post-occlusion. In both groups, the increases in flow from pre-occlusion to 10 minutes post-occlusion and from 10 to 180 minutes post-occlusion lacked significance. When compared at the same time, flows in group 2 did not differ significantly from group 3 at either 10 or 180 minutes after occlusion.

In the LAO section blood flow values in both groups were lower than those in the normal section at both post-occlusion times. In both groups, blood flow increased significantly from 10 to 180 minutes post-occlusion when compared to baseline in the same group. However, group 2 and 3 animals did not differ from one another in this section at both post-occlusion times.

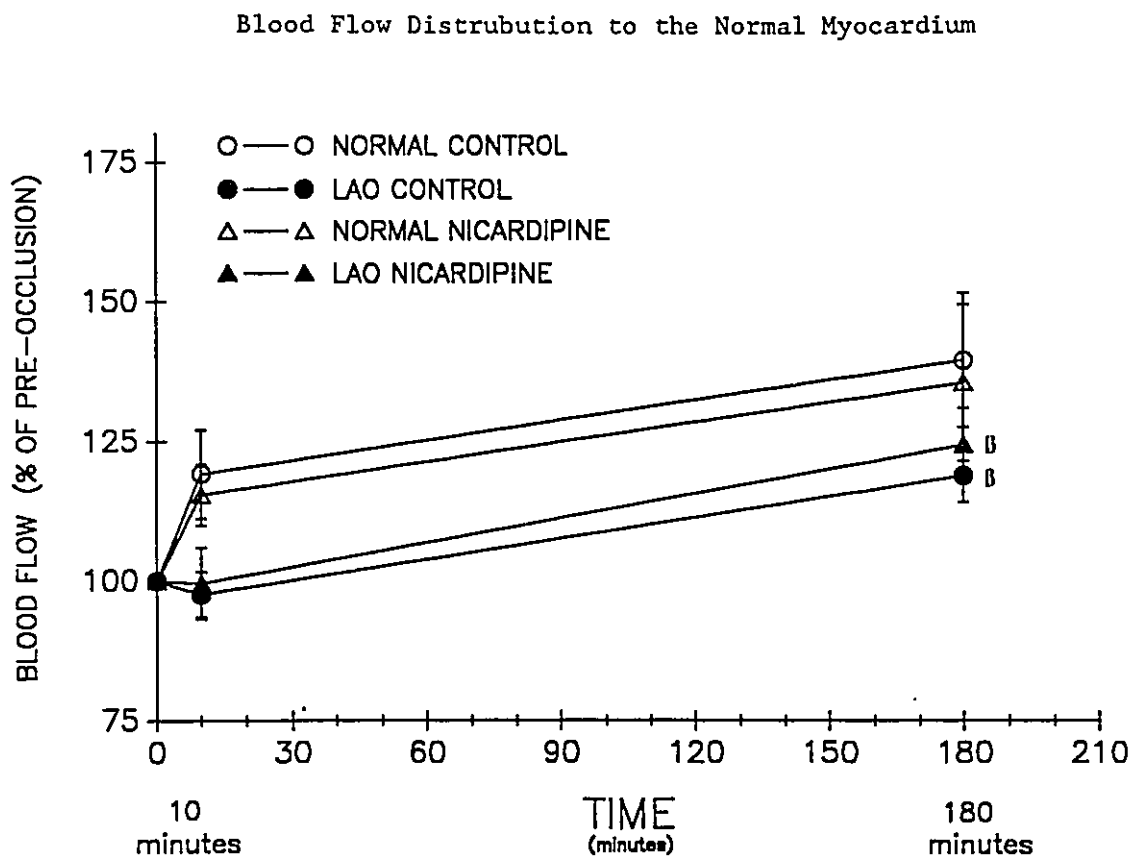


Figure 3.13 Blood Flow Distribution to sections of tissue taken from the normal myocardium in untreated and Nicardipine treated groups.

ⓑ indicates significantly different when compared to 10 minutes post-occlusion ($p < 0.05$). In the NOR section none of the 180 minute post-occlusion flows in either control or Nicardipine treated groups were significantly higher than the corresponding 10 minute flow in the same group. However, in the LAO sections flow increased significantly from 10 to 180 minutes post-occlusion in both groups. At both 10 and 180 minutes, Nicardipine treated animals were not significantly different from controls in either NOR or LAO sections.

3.7.2 Blood Flow within the AAR (These results are shown in Figure 3.14)

Within the AAR at 10 minutes post-occlusion blood flow values dropped to about 14-34% of pre-occlusion values in both groups 2 and 3. In both of these groups, the decline in flow to the LAI was less than that in the CEN region. Group 2 animals did not differ significantly from those of group 3 in either the LAI or CEN region flows at this time, although flows in group 3 were numerically lower in the CEN region. In group 2, flow to the LAI and CEN regions did not change significantly from 10 to 180 minutes post-occlusion. In group 3 flow to the LAI at 180 minutes post-occlusion was not different from the corresponding 10 minute value although flow to the CEN region increased. At 3 hours flow to the LAI was not significantly different between Nicardipine treated and untreated animals although, at this time, Nicardipine treated animals showed lower flows to the CEN region, and this was marginally significant ($p=0.052$). Comparisons made against the 10 minute flows in the Nicardipine treated group must, however, be made with some caution as only 9 of the 17 dogs in this group received a 10 minute microsphere injection and of these 3 had no AAR and thus no CEN or LAI samples for blood flow analysis. Thus the total number of observations in the 10 minute AAR sections of the Nicardipine group was reduced.

3.7.3 Transmural Distribution of Blood Flow

The transmural distribution of blood flow within normal myocardium and myocardium which is part of the AAR is given in Table 3.7. Within the normal myocardial sections (NOR and LAO) there were no differences between groups 2 and 3 at either 10 minutes or 3 hours post-occlusion. At both 10 minutes and 3 hours post-occlusion, epicardial flow was quantitatively but not significantly higher than endocardial flow in both of the groups.

Within the AAR, (CEN and LAI regions) the flow to the epicardium was numerically higher than that of the corresponding endocardial sections at both post-occlusion times in both groups. At 10 minutes post-occlusion, group 2 animals were not significantly different from group 3 animals in either the CEN or LAI sections although flows in the CEN region were lower in group 3. In group 2, mean flow to the CEN epicardium and endocardium increased 22% and 24% respectively from 10 to 180 minutes. In group 3 the increases in epicardial and endocardial flow over the same period were 50% and 29% respectively. Only flow to the CEN epicardium in group 3 increased significantly from the corresponding 10 minute flow value. In the LAI region there were no significant changes in flow from 10 to 180 minutes post-occlusion in either group 2 or group 3. However, a word of caution must be given to comparisons made using the 10 minutes mean flow value in group 3 because of the small number of observations at that time. When comparisons are made between the groups at 180 minutes

Transmural Distribution of Blood Flow within
the Normal and Ischemic Myocardium

Region	Time (min)	Control		Nicardipine	
		Epi	Endo	Epi	Endo
Normal	10	130.2±15.2	108.4±3.5	120.4±8.3	110.5±7.1
	180	149.6±17.5	129.2±4.6 β	154.0±20.3	117.0±6.0
LAO	10	100.0±5.0	102.0±6.1	100.7±9.1	98.6±9.4
	180	125.6±6.4 β	121.5±6.8 β	133.09±10.3 β	116.6±8.2
Central AAR	10	26.3±3.7	21.0±4.0	14.9±2.88	14.0±3.4
	180	34.1±2.5	17.2±2.5	22.4±2.9 β *	17.4±2.5
LAI	10	29.9±3.4	27.8±5.4	40.5±6.8	27.6±6.1
	180	36.1±3.5	28.8±2.8	30.8±3.5	21.2±2.4

Table 3.7 The transmural distribution in blood flow to the normal myocardium and myocardium within the AAR at 10 and 180 minutes post-occlusion in groups 2 and 3. In all sections taken from the normal myocardium blood flow increased over the 180 minute occlusion period to both the endocardium and the epicardium. Within the AAR, a definite transmural gradient in collateral flow was seen, with epicardial flow being consistently higher than endocardial flow in both groups at both post-occlusion times. β indicates significantly different when compared to the 10 minute value in the same group. * indicates significantly different when compared to the control value at the same time.

post-occlusion, none of mean flows for any section are significantly different except for the CEN epicardium which is significantly lower in group 3.

3.8 Summary of Results

1. In all three experimental groups, AAR size as estimated by the ^{99m}Tc macroaggregates and the Monastral Blue dye were highly correlated and neither technique appeared to consistently overestimate the other.
2. Infarct and AAR size were correlated in each of the Control and Nicardipine treated groups.
3. When all animals or when only animals with large AAR sizes were considered, mean infarct sizes in Nicardipine treated animals were not significantly different from controls. When all animals without infarcts were removed from the analysis, Nicardipine treated animals had an approximately 12% lower infarct size, and this approached statistical significance ($0.10 > p > 0.05$).
4. a. There were no differences between Nicardipine treated and untreated animals in flow to the normal myocardium at either 10 or 180 minutes.
b. Blood flow to the normally perfused myocardium increased over the 3 hour occlusion period in both control and Nicardipine treated groups.

The increases in flow from 10-180 minutes were significant only in the LAO section in both groups.

c. In untreated animals there was no change in flow from 10 minutes to 3 hours post-occlusion in either the CEN or LAI regions of the AAR.

5. There were no significant differences between Nicardipine treated and untreated animals in flow to any section of the AAR at 10 minutes post-occlusion or the LAI section at 3 hours post-occlusion.

6. In the CEN epicardium of group 3, flow increased from 10 to 180 minutes post-occlusion although, the flow the CEN epicardium was lower than the corresponding value for the group 2 animals. However, even prior to the commencement of Nicardipine administration, group 2 animals had higher flows to this region.

7. Infarct size and Collateral flow were negatively correlated in Control and Nicardipine treated groups. When only dogs with infarcts were considered, the regression line for the Nicardipine treated dogs was displaced downward. The slopes of the two regression lines were not statistically different.

DISCUSSION

4.1 HEMODYNAMICS

4.1.1 Hemodynamic Effects of Three Hours of LADCA Occlusion in Untreated

Animals

Statistically significant changes in cardiovascular function occurred during the first 10 minutes of LADCA occlusion: Systolic and diastolic arterial blood pressures dropped 7-11%, CO fell about 10%, and all measures of left ventricular filling pressure (LVFP) (LAP, and DLVP) increased. LAP was significantly higher than baseline (pre-occlusion) at all post-occlusion times. Changes in all of these parameters can be explained by an apparent reduction in left ventricular pumping ability imparted by the ischemic attack. During the next 170 minutes comparatively minor changes occurred; HR continued to decline, PAP and LAP continued to increase and systolic and diastolic arterial pressures showed relatively minor increases towards pre-ligation values.

Relative to baseline, Central Venous Pressure (CVP) did not change significantly over the 180 minutes of occlusion. Analysis of this parameter alone would indicate that the balance between venous return and the ability of the heart to pump blood out of the right atrium is maintained; however, CVP should not be expected to be, and has not been found to be an index of LV function. Forrester et al. (1971) found that in cases of human MI, measurement of CVP was grossly inadequate in

predicting the degree of LV functional impairment; by the time blood dams up to the CVP catheter tip, pulmonary vascular congestion and edema are usually well established. LAP has been stated to be a more direct hemodynamic determinant of pulmonary congestion and pulmonary edema (Ross, 1975). Ross also found that PCWP was a reliable indicator of LAP. In patients with AMI, Ross found that PCWP correlated well to the degree of LV functional impairment. He also noted that as patients recovered from AMI, initially elevated PCWP returned to normal.

Meerbaum (1974), Lowe et al. (1978), and Forman et al. (1985) found similar trends in hemodynamic variables in untreated dogs subjected to coronary occlusion over the same period.

4.1.2 Effects of Nicardipine

Our study revealed that Nicardipine administration produced striking hypotensive effects, a finding that has been confirmed in a host of animal and human models including acute myocardial infarction, angina pectoris, essential hypertension, congestive heart failure and also in healthy volunteers (Sorkin, 1987).

In anesthetized open chested dogs subjected to LADCA occlusion Alps et al. (1983b) found that in doses similar to those used in the present study, Nicardipine lowered SAP and DAP from pre-occlusion to 2 h after occlusion by 26% and 32% respectively. In the present study, the drops in SAP and DAP over the same period were quite comparable to those found

by Alps et al. (25% and 30% drops in SAP and DAP respectively). Endo et al. (1986) in an experimental regime and dosage identical to ours found that dogs in which Nicardipine infusion had begun 15 minutes prior to coronary occlusion showed a significant time trend towards decreasing MAP over the 6 h occlusion period; this trend was not seen if infusion was suspended until 15 minutes after occlusion although analysis of data revealed that this later group had consistently lower MAP than control dogs. Hashimoto et al. (1985) also found that Nicardipine (at a dosage of 10ug/kg i.v., comparable to the bolus dose given in our study) produced a significant drop in MAP of 26% in the anesthetized dog. This significant fall in MAP is also quite similar to the drop in MAP seen in the present study between 10 minutes post-occlusion (prior to Nicardipine infusion) and 30 minutes post-occlusion (after the 10ug/kg bolus and 15 minutes after the 8 ug/kg/hr had begun).

Alps et al. (1983a) found that anesthetized baboons subjected to 6 h of coronary artery occlusion, with Nicardipine infusion beginning one hour after occlusion, (10ug/kg bolus, 2ug/kg/15 min for 5 h) had systolic and diastolic blood pressures 10-15 mm Hg lower than untreated animals and that these effects persisted until the end of the occlusion period. He also found that the decreases in blood pressure produced by Nicardipine were greater than those of either Verapamil or Diltiazem.

Since Nicardipine treated animals had cardiac outputs which were consistently higher than untreated controls, a fall in BP must be the result of a fall in TPR brought about by its systemic vasodilator

effects. More direct evidence of Nicardipine's vasodilatory actions are given in section 1.5.7. The decrease in MAP reduced afterload and thus would have had both beneficial and detrimental effects on the ischemic myocardium as previously discussed in Section 1.5.7.

Dose related increases in CO following Nicardipine administration with simultaneous reductions in afterload were seen in healthy volunteers, patients with coronary artery disease, hypertension and also in patients with left ventricular failure (Sorkin, 1987). Significant increases in CO were noted following Nicardipine infusion in anesthetized open-chested dogs by Takenaka et al. (1981) and Bograni et al. (1985)

The increases in CO produced by Nicardipine may be a reflex response to its primary action, the fall in BP caused by the dilation of peripheral resistance vessels. Takenaka et al. (1981) found that the dose related increases in HR following Nicardipine infusion were completely abolished after section of the vagus and carotid sinus nerves and beta adrenergic blockade by propranolol, indicating that the tachycardia was of reflex origin. In the current study the higher CO seen in the Nicardipine treated group was due to an increase in stroke volume and not an increase in HR. In models similar to ours, Endo et al. (1986) reported no significant changes in HR following Nicardipine infusion. Alps et al. (1983b) in a similar model and dosage regime showed that dogs treated with Nicardipine one hour post-occlusion had higher heart rates than untreated controls but that these were not significant.

The Nicardipine treated animals showed consistently lower LAP when compared to their untreated counterparts at all post-treatment times. In control animals this parameter was significantly higher than baseline, but in Nicardipine treated animals there were no significant differences when compared to baseline. However, when comparisons of LAP are made between the two groups at the same time, there were no significant differences.

In this study, there were no differences in DLVP between the two groups, which would, at first glance, indicate that there were no differences in preload among the control and Nicardipine treated groups. However, in AMI, LV diastolic pressure may not be an adequate reflection of diastolic volume because of ventricular compliance is known to be increased early on in the infarction process (Forrester, 1972). Changes in global compliance occurring following an infarct depend upon the size of the infarct and upon the degree of the regional compliance change associated with any given infarct. It is therefore possible that the compliance changes occurring in the Nicardipine treated animals were different from those in the untreated group, and, in the literature there appears no evidence to discredit this possibility. Therefore, interpretation of comparisons made between DLVP in Nicardipine treated and untreated animals becomes difficult because changes in compliance may have been different in these two groups.

However, the non-significant increases in LAP when compared to baseline in the treated group may perhaps indicate a lower preload in this group. Alps et al.(1983b) reported that Nicardipine treated dogs had significantly lower LVEDP when compared to control animals and that these reductions in LVEDP were maintained during the 2 hour observation period. Once again, interpretation of this observation is difficult because of the possibility of differential compliance changes between the two groups. However, Kishi et al. (1984) in a study on the effects of Nicardipine on essential hypertension and Feldman et al. (1987) in a study on the dose related effects of Nicardipine on patients undergoing percutaneous transluminal angioplasty did not find significant changes in left ventricular filling pressure after Nicardipine administration. Therefore, it is not clear from these studies whether Nicardipine is acting as a specific vasodilator on arterioles or if it may also be acting as a venodilator as well.

In summary, the primary effects of Nicardipine appear to be peripheral vasodilation leading to reflex increases in cardiac pumping activity; CO increased while MAP was reduced. The magnitude of the blood pressure drop was therefore less than the drop in TPR. The improvement in cardiac pumping ability was probably due to a reduction in afterload and may also have been related to reflex mediated enhancement of sympathetic tone. It is not clear whether Nicardipine reduced preload since comparisons of LAP between treated and untreated groups at all post-treatment times yielded no significant differences. However, if

comparisons are made to baseline, untreated animals showed significant elevations in these parameters at all post-occlusion times whereas Nicardipine treated animals did not.

4.2 AREA AT RISK RESULTS

The canine coronary occlusion model has been used extensively in studies assessing the effectiveness of pharmacological agents on infarct size limitation. It has long been recognized that the size of the AAR is a major determinant of infarct size, and therefore needs to be standardized. By expressing infarct size as a percentage of the AAR individual differences in coronary anatomy resulting in differences in the amount of myocardium denied adequate perfusion are controlled for. In the past, a variety of isolated heart and in vivo methods have been used to delineate the AAR; these are described in section 1.2.1. Unlike the isolated heart techniques, the in vivo techniques define an AAR which is dependent upon both the normal perfusion territory of the occluded bed and upon collateral flow to this zone. It is felt that the in vivo techniques are more "physiological" because they take into consideration the hemodynamic conditions existing in the animal and are more reflective of the amount of myocardium that is actually at risk of infarction (Ertl et al, 1982, Cobb et al, 1988). Ertl et al. (1982) found that when risk areas were measured using isolated heart and in vivo techniques in the same animal, the extent of necrosis was more closely defined by the in

vivo techniques and that the ratio of necrosis to AAR was always higher when the in vivo technique was used.

Until now, it is largely unknown whether MBD and MAC, two techniques commonly used to define AAR, delineate similar areas of myocardium. Results from group 1 in which the MAC and MBD were injected sequentially reveal a high correlation and a slope of 0.98.

In the past, a major concern surrounding the use of the in vivo MBD technique to estimate AAR size was the problem that this technique could only be used at the end of the occlusion period. Therefore, it was thought possible that the size of the AAR at this time was different from that which existed just after occlusion because of a change in collateral flow to the border zones (Cobb et al. 1988, Jugdutt, et al., 1979b). The MAC technique of AAR estimation allows this problem to be largely circumvented because the size of the AAR can be estimated just after the occlusion and is thus reflective of the status of the coronary circulation at this time. Results from group 2, in which MAC and MBD were used to identify the AAR at 10 minutes and 3 h respectively show that the AAR does not change spontaneously over this time. Since the MBD staining technique offers the advantages of minimizing experimenter exposure to radioactivity and is also much cheaper and easier to use compared to MAC, it may be preferable to use this technique in short-term studies in which collateral flow to risk zone is not altered by therapy.

In a study by Ertl et al. (1982), AAR size was measured at 10 minutes and 6 h post-occlusion using MAC and an intravenous injection of Thioflavin S respectively. Thioflavin S is a fluorescent vital stain for endothelium. In untreated animals, it was found that the AAR sizes were similar using these two techniques, a finding coincident with ours. However, Kloner et al. (1975) reported that Thioflavin S binds to albumin when the two were mixed in vitro and stains poorly when the bound dye is injected intravenously. Therefore, Thioflavin S staining depends not only on blood flow but also upon how much free stain there is in the plasma. In Ertl's study, the MAC and thioflavin S were used in combination; since the macroaggregates are aggregated particles of albumin, it is entirely possible that in vivo interactions of Thioflavin S and the albumin particles occurred. Therefore, determination of AAR by one technique may not be completely independent of the other. Since the MBD is a particulate dye, which, when used in in vivo determinations of AAR stays within the vasculature, its distribution is not influenced by the presence of the albumin macroaggregates or other protein within the plasma.

Results from the Nicardipine treated group of animals revealed that the size of the AAR measured 3 h post-occlusion using MBD (after 165 minutes of Nicardipine infusion) was not significantly different from the AAR estimated prior to Nicardipine administration using MAC. Analysis of this observation would suggest that the marginal changes in collateral flow produced by Nicardipine over this period are insufficient to produce

a change in AAR size. Ertl et al. (1982) reported that following the infusion of angiotensin converting enzyme inhibitor, which apparently increased collateral flow dramatically, a change in AAR size between 10 minutes and 6 h was seen.

The frequency histograms of the percentage difference between the AAR measurements estimated by the two techniques ($AAR_{MBD} - AAR_{MAC}$) showed that a high proportion of all slices (about 90%) had differences between the two measurements of less than 12% in all three groups. The histograms appeared to be normally distributed indicating that neither technique was consistently overestimating the other in any of the 3 groups.

A comparison of AAR sizes as estimated by the same technique across the 3 experimental groups ranged from 19-25% of the LV using MAC and 18-25% of the LV using MBD when all dogs were considered. None of these AAR sizes were significantly different. In an LADCA occlusion model similar to ours, Tumas et al. (1984), Ribero (1982), Deboer et al. (1980) and Endo et al. (1986) found that using MAC, the AAR was not significantly different between treated and untreated groups of animals. Lo (1984) found a similar result using MBD to measure AAR size in a model in which dogs were subjected to 3 hours of occlusion followed by 3 hours of reperfusion.

The observation that AAR sizes are usually not different in treated and untreated groups tends to suggest that the average size of the perfusion territory in a group is not significantly different.

However, this in no way suggests that AAR determination is unnecessary; by controlling for differences in coronary anatomy within any given dog, it is possible to determine the therapeutic potential of a drug with greater confidence. Deboer et al. (1980) in a study assessing the effects of the calcium channel blocker Verapamil on infarct size limitation found that the variances for the comparison of infarct sizes between treated and untreated groups was much higher when infarct sizes were expressed as a percentage of the AAR than when they were expressed as a percentage of the LV. Mean AAR sizes in our studies are in the range of those reported in the studies listed above, with mean AAR size produced by distal occlusion averaging about 25% of the LV. However, such comparisons are difficult because of differences in experimental protocols and random selection differences which may have resulted in a preponderance of dogs having high or low risk areas in any given group.

4.3 SPATIAL CHARACTERISTICS OF AAR AND AREA INFARCTED

The LADCA gives off between one and five diagonal branches and supplies an increasing circumference of left ventricular myocardium proceeding from the base to the apex (Blair 1961). In all three experimental groups the LADCA appeared to contribute little to the perfusion of the base of the heart but perfused a large portion of the apex. Reimer (1981) in a study assessing the effect of coronary occlusion site on ischemic bed size found that distal LADCA occlusion

produced an apical area at risk (as measured by post-mortem dye injection) of approximately 65% of the slice. The size of the AAR produced by this occlusion also progressively declined proceeding towards the base. The larger AAR sizes reported in Reimer's study as compared to those in the current study are probably related to three factors: their estimation of AAR size was based solely on the normal perfusion territory of the occluded LADCA and did not take into consideration collateral flow. Secondly, the anatomical location of the occlusion may have been different from that in our study. Thirdly random selection differences may have resulted in differences coronary anatomy and thus the amount of myocardium at risk.

Since the size of the AAR is a major determinant of infarct size, it is not surprising that the slices with the greatest AAR also have the greatest amount of myocardium infarcted. Jugdutt et al. (1979c) in a study in which the circumflex artery was occluded in dogs found that slices having the largest AAR also had the largest infarct sizes. However, since the circumflex artery contributes much to the perfusion of the base but little to the perfusion of the apex, Jugdutt and coworkers found that both infarct and AAR regions taper towards the apex of the LV with the bulk of the infarct and risk area being in the basal three slices.

4.4 RELATIONSHIP BETWEEN INFARCT SIZE AND AAR

In both untreated and Nicardipine treated animals there was a significant correlation between the AAR and the area occupied by the infarct when either MAC or MBD estimates of AAR size are used. Jugdutt et al. (1979c) also reported a strong correlation between infarct and AAR size, an observation also reported by Lowe et al. (1978) and Konyagi et al. (1982). However, Jugdutt and coworkers (1979c) found that dogs had no infarcts when the AAR was less than 20 grams or approximately 20% of the LV. In Jugdutt's study, in vitro estimates of AAR size were used. Bishop (1984), Yellon (1983) and W. Schaper (1978) have shown that infarction does occur in cases in which the risk zone is considerably less than 20% of the LV. In the present study, infarction did occur when AAR size was less than 20%, although incidences were rare (7%). Although the failure of small risk regions to infarct when in vitro measures of AAR are used is not entirely clear, it may be that with a decreasing size of the risk region, there is an increasing relative surface area to mass thus providing a greater opportunity for collaterals to penetrate (W. Schaper, 1984).

4.5 THE EFFECT OF NICARDIPINE ON INFARCT SIZE

When all dogs were considered, Nicardipine treated animals did not have infarct sizes which were significantly different from controls. When all animals without infarcts were excluded from the analysis, infarct sizes were lower in Nicardipine treated animals and this approached statistical significance ($0.10 > p > 0.05$). When exclusion criteria were applied eliminating all dogs with small AAR sizes (<20% of LV), Nicardipine treated animals also did not have significantly lower infarct sizes when compared to controls, indicating that the size of the AAR was not a major factor in determining the effectiveness of Nicardipine in limiting infarct size. Regardless of whether all animals, only animals with infarcts, or only animals with large hypoperfused zones were considered, Nicardipine treated animals generally had lower infarcts than their untreated counterparts although this was not significant.

Endo et al. (1986) found that dogs treated with Nicardipine 15 minutes after coronary artery occlusion had significantly smaller infarcts than the untreated controls when infarct size was assessed at 6 h. He also found that the effectiveness of Nicardipine in limiting infarct size depended upon the initial size of the AAR (as assessed by MAC), with dogs having large AAR sizes (>30% of LV) being less protected than dogs with small AAR sizes. He explained that this was likely due to the higher level of collateral flow in dogs with small AAR sizes allowing

for more drug penetration. Since these parameters are interdependent, it is impossible to determine the relative contribution of collateral flow, quantity of Nicardipine received by the AAR and the size of the anatomical AAR on Endo's observation that dogs with small AAR sizes were more protected.

The value of a drug which is capable of limiting infarct size only when the extent of the AAR is small is somewhat questionable in its applicability to the human condition. The objective of limiting infarct size is the hope that following infarction there will be fewer deaths and a lesser impairment of ventricular function. During the early period after a human infarct, the chief cause of death is ventricular arrhythmia. It is well documented that the extent of tissue damage is strongly correlated to incidences of ventricular arrhythmias when the area of infarction is large, but that when infarcts are small or moderate in size lethal arrhythmias are not common and not predictable (Oliver, 1984). Thus infarct size limitation is most beneficial in circumstances where the size of the infarct is potentially very large (i.e. in a situation in which the AAR is large). However, Nicardipine, according to Endo's work, appears to have the opposite effect i.e. it limits potentially small infarcts to even smaller ones while not substantially limiting the size of potentially large infarcts.

Alps et al. (1983a) in a study in anesthetized baboons subjected to 6 h of coronary artery occlusion found significantly smaller infarcts in Nicardipine treated animals when compared to controls ($44 \pm 4\%$ of AAR vs.

110±6% of AAR). The AAR was measured using a post-mortem technique which delineates the anatomical perfusion territory of the occluded bed and does not take into account collateral flow to the risk zone. However, I faced a little uncertainty in interpreting these results because in the untreated animals, the area occupied by the infarct extended beyond the anatomical perfusion territory of the occluded bed in nearly all cases. An explanation of this interesting phenomenon was not given.

Thus, although our results tend to show that Nicardipine has a marginally beneficial effect, our results are not as striking as those of Endo et al. (1986) or Alps (1983a). The reason for this is not entirely clear but one possibility may be that planimetry of the sometimes small and patchy infarcts seen at 3 h are subject to a greater degree of technical error than those present at 6 h (see discussion of methodological considerations). This error would then increase the variability of the infarct size measurements, and thus decrease the probability of detecting a significant difference between the two groups. Another possibility is that since our collateral flow data show that Nicardipine treated animals had lower levels of collateral flow to the CEN region of the AAR at 10 minutes post occlusion, chance randomization of animals with initially low collateral flow to the treatment group may have occurred leading to less beneficial effect on infarct size. The converse is also possible with chance randomization of animals with high collateral flow in the treatment groups in the studies by Alps et al. (1983b) and Endo et al. (1986) thereby leading to an overly enthusiastic

conclusion about Nicardipine's infarct size limiting abilities. Since collateral flow was not measured in either of these studies, one cannot eliminate this possibility. Reimer (1985) found an apparent reduction in infarct size of approximately 30% in an analysis based on percent infarction of the vascular risk region; however, consideration of ischemic blood flows revealed that chance randomization of animals with initially high collateral flow to the treatment group had occurred and consequently that the reduction in infarct size could not be attributed solely to the treatment effects. This study underscores the fact that infarct size depends upon not only the size of the AAR but also upon the amount of collateral flow received by this zone.

4.6 THE RELATIONSHIP BETWEEN INFARCT SIZE AND COLLATERAL FLOW

In the present study there was a significant inverse correlation between percent necrosis of the risk zone and collateral flow to the epicardium of the LAI of the AAR measured 3 hours after occlusion indicating that blood flow through the native collaterals is a determinant of the extent of infarction within the risk zone. Bobb et al. (1948) in an attempt to establish the effects of collaterals on infarct size, cauterized the edges of the distribution area of the LADCA. In these studies, it was first confirmed that these 3-5 mm wide and approximately 1 mm deep burns did not cause infarction in dogs that did

not undergo LADCA ligation. All dogs surviving the LADCA ligation and associated cauterization had completely transmural infarcts when measured 24 hours post-occlusion. In contrast, of the 8 control dogs that underwent LADCA occlusion and no cauterization, 2 had no infarcts, 4 had infarcts smaller than the risk region and 2 had large, almost transmural infarcts. Bobb and coworkers consequently suggested that epicardial collaterals were important determinants of infarct size.

More direct evidence of the importance of coronary collateral blood flow on infarct size was given in a retrospective study by Muiria et al. (1987) who showed that in dogs subjected to 24 or 48 h of permanent coronary artery occlusion, there was a strong inverse correlation between infarct size (% of AAR) and normalized collateral flow to the subepicardium of the risk zone measured 2 minutes after occlusion. Normalization was done by expressing flow as a percent of the flow to the non-ischemic tissue.

In a similar study by Cohen (1973) in which dogs were subjected to one week of coronary artery occlusion this result was also confirmed. Cohen found that necrosis only occurred when endocardial flow measured immediately after occlusion was reduced by about 50%. Below this level there was a significant inverse correlation between infarct size and normalized endocardial flow to the ischemic area (AAR size was not assessed in this study). He found that infarct size was more strongly correlated to normalized epicardial flow ($r=0.86$) than to normalized endocardial flow ($r=0.80$). Some investigators have found that

correlations between blood flow and infarct size are better when only measures of endocardial (Bishop et al., 1976, Patterson et al. 1982) or epicardial (Reimer et al. (1979) collateral flow were analysed although Jugdutt et al. (1979c) found significant correlations regardless of the transmural location of the collateral flow measurement. Although not presented in the current study, infarct size showed a negative correlation coefficient to endocardial flow in both groups, but the correlation coefficient was higher when epicardial flow was considered. Waltier (1986) also found good correlations between infarct size and collateral flow in a dog model involving 2 h occlusion and 30 minute reperfusion. Collateral flow appears to be related to infarct sizes regardless of when it is measured during the infarct extension process. In studies by Cohen (1973) Muiria (1987) and Jugdutt (1979c) collateral flow measured less than 20 minutes after coronary artery occlusion were taken for the regression analysis although others have reported high correlations between infarct size and collateral flow measured as late as 24-96 hours after occlusion (Bishop et al., 1976, Patterson et al., 1982).

The inverse correlation between infarct size and collateral flow is consistent with the studies of Konyagi et al. (1982) who demonstrated that infarct size as measured 2 h post-occlusion is greater in the subendocardium of the AAR than the subepicardium. Thus, within the AAR there are areas which are at greater risk than others and thus more likely to infarct. This is due, to a large extent, to differences in

collateral flow to these regions. In Konyagi's study collateral flow to the myocardium destined to infarct fell by 86% of pre-occlusion flows and by only 34% in areas in which infarction did not occur. The importance of collateral flow in defining the extent of infarction has also been offered as an explanation for the differences in infarct sizes among different species. In the pig, 3 h of permanent coronary artery occlusion results in almost completely transmural infarcts (in pigs which survive this procedure) (Cohen, 1985). This is due to the fact that, unlike the dog, the pig has no preponderance of epicardial collaterals which would render this area more tolerant to ischemic attack and consequently the pig undergoes infarction much faster (Hearse et al. 1986, Cohen, 1984, Maxwell et al., 1987a).

Hearse and Yellon (1984) identified critical levels of collateral flow necessary to permit total sparing of myocardium following coronary occlusion. They found that areas of myocardium undergoing flow reductions of 80% or more were likely to infarct within the first 1 or 2 hours of occlusion and in those with flow reductions between 60-80% were likely to infarct between 6-12 hours of occlusion. Cohen et al. (1974) and Hearse and Yellon (1984) suggest that areas in which flow reductions are less than 50% are those most likely to be spared. In our studies no dogs with lateral epicardial flows of >60% of the pre-occlusion value showed infarcts.

Thus the level of irreversible damage that occurs following coronary artery occlusion in different regions of any given heart,

between hearts and between different species is closely correlated with the level of collateral flow to the ischemic bed.

An interesting observation in our study is that the regression line for the Nicardipine treated animals (when only animals with infarcts were considered) was shifted downwards when compared to the untreated animals. This would tend to suggest that for any given level of collateral flow, infarct sizes were lower for this group. Analysis of this observation alone supports the notion that Nicardipine's beneficial action on the ischemic myocardium were not entirely flow dependent (Reimer et al, 1984) and consequently perhaps more related to decreases in O₂ demand rather than to an increase in O₂ supply (Reimer and Jennings, 1984b).

4.7 METHODOLOGICAL CONSIDERATIONS

4.7.1 Area at Risk Determination

All methods used to define the in vivo AAR depend upon the visual definition of areas with or without label and thus involve some degree of subjectivity. The ease with which the AAR can be delineated most objectively in either the autoradiographs or the photographs (which reveal the AAR as identified by MAC and the MBD respectively) depends upon the level of collateral flow. When collateral flow is low, the lateral boundaries of the AAR are clearly defined and in sharp contrast to the normal myocardium; with myocardium part of the AAR either showing

a lack of fogging produced by the presence of macroaggregates or a lack of blue stain. In these situations, I have found that autoradiographs and outlines of the AAR as delineated by the MBD are superimposable and differences between the two AAR measurements can be largely attributed to variability associated with planimetric assessment. However, when collateral flow is high, more subjectivity is involved in demarcating the border of the risk zone, especially in the transmural plane because this now involves differential degrees of stain intensity and autoradiographic fogging. In such cases, questions like "how blue is blue enough to exclude this tissue from the AAR?" do arise. Ertl et al., (1982) found when using the macroaggregates and the thioflavin S techniques to delineate the AAR, that broad areas of intermediate fluorescence or radioactivity were not present and that the boundaries of the AAR were always easily discernible. This observation was not supported in the current study. Inherent in the idea that the size of the AAR can change is the notion that there are gradients of flow in the transmural and perhaps lateral planes, with collateral flow increasing towards the epicardium and maybe towards the lateral boundaries of the occluded bed. Although the MAC and MBD techniques can not be used to accurately quantify blood flow, they do lodge in the circulation in proportion to the blood flow. Thus it is reasonable that the border zones would receive proportionately more particles of MAC and MBD with fogging and stain intensity progressively decreasing to areas of lower flow (the ischemic core). In such cases, somewhat subjective decisions about the

radioactivity and blue colour cutoffs which separate AAR from non-AAR must be made. I wonder therefore, how Ertl and coworkers (1982) were able to find a change in AAR size from 10 minutes to 6 h post-occlusion when it was stated that the boundaries were always sharp and clearly discernible. Ertl's finding that collateral flow did change significantly over this period such that the lateral borders received greater flow than previously seen would suggest that the intensity of staining towards the lateral boundaries had increased and was thus not "all or none".

4.7.2 Infarct Size Determination

Fishbein (1981) has reported that infarct sizes can be quantified reliably using the tetrazolium staining technique after 3 hours of LADCA occlusion in the dog. I found that although the staining procedure produced a sharp and clear demarcation of infarcted tissue when the area infarcted was large and confluent, the size of the infarct was not so easily quantifiable when the area was small and consisting of islands of necrotic tissue separated by small intervening regions of normal tissue. Fishbein (1981) found that evidence of necrosis determined histologically was not as well correlated with that determined using tetrazolium staining when infarcts were patchy. However, he found that this was primarily related to technical difficulties associated with planimetry in cases in which multiple, small, irregular areas were to be

measured rather than the inability of the tetrazolium staining technique to identify necrotic tissue. It may be therefore, that infarct sizes as measured at 3 h post-occlusion were more variable than if infarct size were measured at a time when infarcts became more confluent.

4.7.3 Regional Myocardial Blood Flow

4.8 REGIONAL MYOCARDIAL BLOOD FLOW

4.8.1 Blood Flow to the Normal Myocardium

In untreated and Nicardipine treated animals blood flow to the normal section of myocardium increased from pre-occlusion to 10 minutes post-occlusion quantitatively but not significantly. In both of these groups flow proceeded to increase further from 10 to 180 minutes and this increase was not significant. These increases in flow to the normal myocardium can be explained by an increased workload coupled with autoregulatory changes in blood flow to these regions. The increased workload is associated with the fact that the remainder of the heart is now responsible for maintaining pumping function when approximately 25% of the left ventricle is rendered non-functional by the ischemic attack. Increases in flow to the normal myocardium following coronary artery occlusion have also been found by Savage et al. (1981). Analysis of the data revealed that flow measured at 15 minutes and at 48 h post-occlusion

was 130% and 149% of pre-occlusion flow respectively and that the increases in flow from pre-occlusion to 15 minutes post occlusion and those from 15 minutes to 48 h post-occlusion were significant. Quantitatively; these results are similar to those found in the present study.

In the LAO region, blood flows are lower than those of the normal region of myocardium and show no change from pre-occlusion to 10 minutes post-occlusion but increase significantly from 10 to 180 minutes in both Nicardipine treated and untreated groups. The observation that the flow profile to this region is similar to the normal region but that flows are generally lower may be explained by two postulates; either that the transition zone from normal to ischemic tissue is relatively broad in the lateral plane with large areas of myocardium receiving intermediate levels of flow or that the lateral border zone is sharp but irregular and that these intermediate levels of flow in the LAO are artifactual resulting from the unavoidable contamination of the LAO tissue sections by tissue from within the AAR. As discussed in section 1.1, it is impossible to exclude this possibility. In fact, it is probably unlikely that the nature of the lateral border zone can be determined using the microspheres in the numbers used in blood flow studies ($1-2 \times 10^6$) because the sample size required for blood flow analysis using this number of microspheres is relatively large (0.5-1 gm) (See section 1.4). Therefore, if the lateral border zone is as narrow as that suggested by Murdock (1983) (<3 mm), the microsphere technique employing only 1 or 2

million microspheres would not have an adequate resolving power to determine if this exists.

There were no significant differences between Nicardipine treated and untreated animals at 10 minutes post-occlusion in either the Normal or LAO regions indicating that prior to Nicardipine administration, flow to the normal myocardium was similar in these two groups. At 180 minutes post-occlusion, flow to both of these normal myocardial regions was also not different between treated and untreated animals indicating that Nicardipine did not improve perfusion to the normal myocardium. This was probably related to the significant reductions in MAP and consequently coronary perfusion pressure following Nicardipine administration. The observations that blood flow to the normal myocardium was not different between the two groups may indicate the operation of autoregulation.

4.8.2 Blood Flow to the AAR

4.8.2.1 Untreated dogs

LADCA occlusion led to significant and extensive reductions in coronary flow to all sections of myocardium within the AAR. 10 minutes after the occlusion, residual collateral flow to the AAR was between 15-30% of pre-occlusion flow. The distribution of this flow, was however, not uniform, there being a declining gradient from the subepicardium the

subendocardium in both the CEN and LAI regions at both 10 and 180 minutes. The existence of a transmural gradient in flow has been verified by many others (Downey et al., 1973, Cohen, 1985, Hearse, 1984) and is due to the preponderance of subepicardial collaterals in the dog heart. A gradient of flow also existed in the lateral plane, with LAI flows being higher than CEN region flows at both 10 and 180 minutes. The mechanisms by which a gradient can arise in the lateral plane has been discussed in sections 1.1 and 4.8.1. All flows within the risk region at 10 minutes and 180 minutes post-occlusion are quite comparable to those reported in the literature (Fukunami et al., 1987, Hearse, 1984, Cohen, 1985). Our results also indicate that flow to the risk zone does not change between 10 and 180 minutes. As previously discussed in section 1.4, there are some discrepancies over this point. Most researchers agree that collateral flow increases from 0-5 minutes following occlusion but there is some uncertainty as to what happens between 5 minutes and 6 h. While some investigators report increases others report decreases while still others report no change (See Table 1.2). Cohen has stated that collaterals become fully recruited and maximally dilated in the first few minutes after occlusion and subsequent further growth of collaterals does not occur until 24 hours after occlusion (Cohen 1985). If this is true, then no change in flow over this period appears to be the most likely in the absence of any major hemodynamic changes.

4.8.2.2 Effects of Nicardipine on Collateral Flow

Nicardipine administration did not result in an improvement in collateral flow to any tissue section within the AAR except the central epicardium in which there was a marginal increase. As in untreated dogs, a transmural and lateral gradient was present at both 10 and 180 minutes post-occlusion. At 10 minutes post-occlusion, there were no significant differences in collateral flow between Nicardipine treated and untreated dogs although Nicardipine treated dogs had lower flows in the CEN region when compared to their untreated counterpart. At 180 minutes although flow to the CEN epicardium increased from its corresponding 10 minute flow value, it was significantly lower than the value for the untreated controls at the same time. The difference between the two groups at 3 h in this section may have been due to random section differences in the groups which may have resulted in dogs with initially low collateral flows to the ischemic core to be assigned to the Nicardipine treated group (See section 4.5).

Hashimoto et al. (1985) found that in anesthetized, open-chested dogs, Nicardipine at a dose of 10 ug/kg was not able to increase collateral flow to "severely" ischemic tissue although an increase in flow to "mildly" ischemic tissue was seen. An interesting point in this study was that pre-treatment blood flow in the "mildly" ischemic tissue was 47% of the pre-occlusion value. Since the mildly ischemic tissue section was transmural, this represents an average of epicardial and

endocardial flow to this region. This value is higher than that of the current study (34% of pre-occlusion) and also higher than control post-occlusion flows reported in the literature (10-35%). Also of note was that the size of the AAR was not measured in this study. Tissue sectioning was done on the basis of the surface distribution of the occluded bed. When both of these factors are taken into consideration, it is quite possible that these mildly ischemic areas were contaminated with normal myocardium. Since Hashimoto et al. (1985) reported that blood flow to the normal myocardium increased significantly following Nicardipine administration, increases in flow to the mildly ischemic region may simply be a reflection of the increases in flow to the neighbouring normal tissue. Berdeaux et al. (1985) found that in the anesthetized, open chested dog subjected to 2 h of coronary occlusion, Nicardipine (10 ug/kg) significantly increased flow to the endocardium of the ischemic region but was not able to improve epicardial perfusion. Endo et al. (1986) reported that personal communications with Takenaka revealed that Nicardipine increased collateral flow to the endocardium by 90% but that epicardial perfusion was only marginally improved.

An intervention capable of selectively improving endocardial collateral perfusion is probably not as beneficial as one capable of increasing epicardial collateral perfusion in its ability to limit infarct size. It is well established that due to a higher oxygen demand and a lower collateral flow, the endocardium is first to undergo necrosis and that infarction passes as a "wavefront" from the endocardium to the

epicardium. Since in the ischemic zone of the dog, endocardial flows are approximately 15% of the pre-occlusion value, even a 100% increase in flow to 30% of pre-occlusion is unlikely to result in complete endocardial salvage, although it may be enough to slow the rate of progression of injury.

Thus the effects of Nicardipine on collateral flow to the ischemic myocardium are not entirely clear from our studies or those reported by Hashimoto et al. (1985) although Berdeaux et al. (1986) reported a significant increase. However, Berdeaux was not able to demonstrate that Nicardipine produced significant beneficial effects on the degree of ischemic injury as assessed by epicardial-ST segment elevation. Hashimoto et al. (1985) also did not find that Nicardipine (30 ug/kg) had any significant effect in attenuating the degree of electrophysiological evidence of the magnitude of ischemic injury following permanent coronary occlusion although a beneficial effect was observed at higher doses.

Since Nicardipine administration did not result in a substantial improvement in collateral flow within all areas of the risk zone, the marginally beneficial effects of Nicardipine on infarct size limitation were probably related to a reduction in afterload and perhaps a reduction in preload which would then result in a reduction in O₂ demand. Afterload reductions have been confirmed by most others investigating the effects of Nicardipine on infarct size limitation (Hashimoto et al. (1985), Berdeaux et al. (1986), Alps et al. (1983 a and b) and preload reductions have been found by Alps et al. (1983b). Although not investigated, another possibility is that part of Nicardipine's

beneficial effect may be related to direct protection of ischemic myocardium by preventing intracellular Ca^{2+} accumulation and its deleterious effects (Nayler, 1987).

4.9 RELATIONSHIP BETWEEN AAR SIZE AND COLLATERAL FLOW

In untreated animals, the finding that collateral flow did not change significantly from 10 minutes to 3 hours in any portion of the AAR supported the finding that the size of the AAR also did not change over this period. This finding was also confirmed by Ertl (1982) who found no significant change in either AAR size or collateral flow from 15 min to 6 h post-occlusion value.

In Nicardipine treated animals, collateral flow did not change from 10 to 180 minutes following occlusion in any of the LAI sections of the AAR or to the central endocardium. However, blood flow to the central epicardium increased over this period from 15% to 22% of pre-occlusion. This increase in flow did not result in a change in the AAR size. Ertl et al., (1982) found that angiotensin converting enzyme inhibitor was capable of changing AAR size from 10 minutes to 6 h. Analysis of data revealed that this treatment was not capable of changing flow to the central region of the risk zone but increased flow to both epicardial and endocardial lateral margins of the risk zone by about 62%. The authors stated that this increase in flow was responsible for producing a change in AAR size.

Thus, whether or not an intervention is capable of changing the size of the AAR depends upon how substantially it can change collateral flow. If changes in collateral flow are small, it is unlikely that a change in AAR size will be seen. However, if as in Ertl's study, large increases in collateral flow occur, the size of the AAR measured after the flow increase can be significantly smaller than that seen prior to the increase. It has not been determined if agents capable of decreasing collateral flow to the risk zone (such as adenosine) which would "steal" blood away from the ischemic zone and redistribute it to the normal myocardium are able to increase the size of the risk zone. Although more studies need to be done, measurement of physiological AAR sizes prior to and following the administration of a therapy may be useful in elucidating its mechanism of action. By coupling these observations with an estimate of the anatomical perfusion territory of the occluded bed in the same animal it is possible to get an idea of firstly, the initial level of collateral flow and secondly how much benefit the therapy has had. For example, if the anatomical perfusion territory of any given animal measured 1000 arbitrary units (a.u.) and the physiological AAR measures 800 a.u., it could be reasoned that 200 a.u. of the occluded bed is receiving adequate perfusion and thus is not likely to infarct regardless of the therapy. If then, a therapy is applied which improves collateral flow, the AAR measured after the therapy may be smaller than that assessed prior to the application of the therapy resulting in further potential salvage.

Inherent in the idea that changes in AAR size may be used to ascertain changes in collateral flow is the notion that AAR size determination can be used as a quantitative measure of changes in collateral flow. However, as previously discussed, the degree of subjectivity in assessing AAR size is somewhat increased when collateral flow to the risk zone is high. Although AAR sizes have been measured in almost all infarct size limitation studies, researchers have either not recognized or failed to report the degree of subjectivity involved in AAR measurements. Therefore, in order to utilize the AAR assessment more quantitatively, especially if it is desired to assess changes in AAR size, it may be worthwhile to couple the in vivo AAR measurements with densitometric or colorimetric analyses to determine differences in density of "fogging" or staining resulting from different levels of collateral flow.

4.9 SUMMARY

Sequential injections of MAC and MBD revealed that these techniques identify similar areas of myocardium. When MAC and MBD techniques were used to identify the size of the AAR at 10 and 180 minutes post-occlusion respectively, it was found that the size of the AAR did not change spontaneously. This result was supported by collateral flow data which suggested that over this time period, collateral flow to the risk zone also did not change. Nicardipine administration also did not change

the size of the AAR from 10 to 180 minutes post-occlusion. In these dogs, collateral flow did not change from 10 to 180 minutes in any region of the LAI or to the central endocardium although central epicardial flow showed a marginal increase. This increase in flow was however insufficient to produce a change in AAR size.

When only dogs with infarcts were considered, Nicardipine treated animals had lower infarct sizes than untreated controls, and this approached statistical significance ($0.10 > p > 0.05$). This marginal benefit was probably related to a decrease in O_2 demand produced by the significant reductions in afterload and may also be related to preload reductions.

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APPENDIX

MISCELLANEOUS EXPERIMENTS

1. HEMODYNAMIC EFFECTS OF TWEEN 80

Occasionally during microsphere injection, the arterial blood pressure would drop precipitously, sometimes to as low as 60 mm Hg. However, within a few seconds the blood pressure would recover but never to pre-occlusion values. In order to determine the reason for these drops, the following experiment was conducted in one dog.

3-20 ml aliquots of microspheres labelled with 3 different isotopes (See section 2.6) were centrifuged and the supernatant removed. The microspheres were resuspended in sterile saline and placed in sterile glass tubes. This microsphere "washing" was repeated twice. 17 mls of the solutions listed below were then injected into the pulmonary vein at 10 minute intervals and changes in blood pressure and HR noted. The order of the injections was as follows:

1. Microsphere 1 (without Tween) in a glass tube
2. Microsphere 2 (without Tween) in a glass tube
3. Microsphere 3 (without Tween) in a glass tube
4. Sterile saline with 0.02% Tween in a glass tube
5. Sterile saline with 0.02% Tween in a plastic tube

Injections 1-3 did not produce a decrease in blood pressure or HR during or soon after injection. However, both 4 and 5 produced MAP drops of approximately 15 mm Hg and declines in HR of about 10 beats/minute during injection. It was therefore concluded that the presence of Tween 80 was responsible for the hemodynamic alterations seen.

Drops in blood pressure accompanying microsphere injection occurred in 4 separate animals; in two animals in each of groups 1 and 2. All data from these experiments were discarded. A microscopic inspection of a few drops of the microsphere suspension injected into one of the excluded dogs revealed that the sample was contaminated with mold. All data reported in this thesis is from dogs which did not exhibit any major hemodynamic alteration accompanying microsphere injection.

1.1 Discussion of the Hemodynamic Effects of Tween 80

In order to obtain meaningful information using the radioactive microsphere technique it is necessary that microsphere injection produce no significant hemodynamic effects. In dogs, up to 21.6×10^6 microspheres have been injected without apparent adverse effects (Falsetti et. al., 1975). In the current study, injection of approximately 1.2×10^6 microspheres produced no significant hemodynamic effects in 60 of the 64 animals studied. In the remaining 4 dogs, blood pressure and HR declined simultaneously with microsphere injection. It is believed that the hemodynamic perturbations seen in these animals were not due to the presence of the microspheres themselves but rather the presence of other

materials in the microsphere suspending media. In the above study (See Appendix section 1.) we found that injection of sterile saline with 0.02% Tween 80 (a detergent used to inhibit microsphere aggregation) produced significant drops in HR and MAP. Flain et. al. (1978) also found significant drops in HR when Tween 80 and saline alone were injected into rats. It is also possible that the presence of 10% dextran in the suspending media (used to retard microsphere settling) may also have contributed to the hemodynamic alterations seen in these dogs. Voorhees (1951) showed that 10% dextran has significant effects on blood pressure, heart rate, peak left ventricular systolic pressure and end diastolic pressure, producing significant hypotensive effects in rats. Either of these two factors or perhaps a third, the presence of mold in the suspending media, may have contributed to the hemodynamic alterations seen. However, it is not possible to ascribe the relative contributions of each of these three factors to the declines in HR and MAP in these 4 dogs.

2. DEVELOPMENT OF TECHNIQUES

2.1 Area at Risk Determination

The Monastral Blue and ^{99m}Tc macroaggregate injection techniques for AAR identification were selected after having first experimented with a variety of other AAR identification techniques. These are described below:

In Vivo

1. Monastral Blue (3% phthalocyanine pigment, Sigma Chemical Co., (3 ml/kg)) - (n=8) This dye was injected into the pulmonary vein at the end of the experimental period. However, it's use was abandoned because of inadequate visual contrast between normal and ischemic tissue.
2. Evans Blue (Sigma Chemical Co.) - (n=1) Used in a manner similar to 1. It's use was abandoned for the same reason.
3. Monastral Blue and Triphenyl Tetrazolium(TTC)-(n=1) Just prior to the end of the occlusion period, the LADCA was cannulated (i.d.1.14 mm, length 7 cm) at a point immediately distal to the site of occlusion. To identify both the area at risk and infarct zone, Monastral blue was injected into the pulmonary vein while TTC was simultaneously injected into the LADCA. The use of this technique was abandoned because of

technical difficulties associated with *in vivo* LADCA cannulation and secondly but more importantly because it was felt that the area at risk as defined in this way is not "physiological" (See Section 1.2.1).

Isolated Heart

1. (n=1) After excision and rinsing the heart, a cannula (i.d. 5.28 mm, length 12 cm) was placed in the aorta and secured in place by a purse string suture (No. 0 vascular silk). A second cannula (i.d. 1.14 mm, length 7 cm) was placed in the LADCA immediately distal to the site of occlusion and secured in place using a No. 2 vascular silk. The aorta and the LADCA were then simultaneously perfused with Monastral Blue (3% Sigma) and TTC respectively. The technique was abandoned for the same reasons as 3. above.

2.2 Infarct Identification

1. (TTC) - (n=6) The mechanism of action is similar to that of Nitro-blue tetrazolium (Schaper, 1984). However, the TTC forms a brick red precipitate over normal myocardium. It's use was abandoned because it was felt that Nitro-blue produced a better contrast between normal and infarcted myocardium.

Planimetric data from these proceeding experiments (Section 2.8.1) was not included in any experimental group reported.

Baseline (Pre-Occlusion) and 10 minute post-occlusion
Hemodynamic Parameters in Groups 1, 2 and 3

PARAMETER

		GROUP 1 (n=13)	GROUP 2 (n=12)	GROUP 3 (n=17)
HR	0	132.9 ±5.70	122.82±3.45	120.06±3.75
	10	130.33±6.50	122.06±3.82	118.59±4.53
MAP	0	115.17±5.10	111.71±4.17	116.65±4.10
	10	109.33±6.07	105.41±4.17	106.12±5.04
SAP	0	139.92±5.88	138.06±5.18	140.59±5.30
	10	130.67±6.96	125.47±5.95	124.24±5.83
DAP	0	101.25±5.81	100.47±3.72	105.53±4.06
	10	97.50±6.53	92.94±4.37	94.47±4.06
CO	0	-	0.122±0.031	0.120±0.007
	10	-	0.103±0.026	0.115±0.007
SLVP	0	130.43±6.60	124.94±4.35	126.53±3.64
	10	122.57±8.08	117.06±5.29	114.24±4.21
DLVP	0	2.57±0.69	2.38±0.73	3.11±0.56
	10	3.86±0.71	4.81±0.73	5.47±0.83
LAP	0	4.45±0.34	4.69±0.72	4.71±0.48
	10	6.73±0.47	6.81±1.00	6.53±0.66
RAP	0	-	2.75±0.48	1.18±0.48
	10	-	3.31±0.59	1.53±0.49
PAP	0	-	12.81±0.56	11.77±0.42
	10	-	13.75±0.62	12.35±0.49

Table 1 Pre-occlusion and 10 minute post-occlusion hemodynamic parameters in all three experimental groups. There were no significant differences between the groups for any of the hemodynamic parameters listed. RAP and PAP were not determined in group 1 because a Swan Ganz catheter was not inserted. Due to problems with the cardiac output machine, this was also not determined for group 1.

Table 2

Grouped Frequency Table for Data in Figure 3.3

Group 1

Class Interval	Class Frequency (f)	Relative Class Frequency (f/n*100)	Cumulative Frequency (F)	Relative Cumulative Frequency (F/n*100)
>12	2	2.5	2	2.5
9-12	5	6.2	7	8.6
6-9	4	4.9	11	13.6
3-6	11	13.6	22	27.2
0-3	16	19.8	38	46.9
0	14	17.3	52	64.2
0--3	8	9.9	60	74.1
-3--6	11	13.6	71	87.7
-6--9	6	7.4	77	95.1
-9--12	1	1.2	78	96.3
<-12	3	3.7	81	100.0

n=81

Table 2 Grouped frequency table showing the percentage difference in the AAR measurements as estimated by the two techniques (AAR_{MBD} vs. AAR_{MAC}) in each slice analysed for group 1. Note that in approximately 95% of all cases, the difference between the two techniques was $< \pm 12\%$.

Table 3

Grouped Frequency Table for Data in Figure 3.4

Group 2

Class Interval	Frequency (f)	Relative Frequency (f/n*100)	Cumulative Frequency (F)	Relative Cumulative Frequency (F/n*100)
>15	3	3.4	3	3.4
12-15	1	1.1	4	4.6
9-12	2	2.3	6	6.8
6-9	5	5.7	11	12.5
3-6	5	5.7	16	18.2
0-3	9	10.2	25	28.4
0	29	33.0	54	61.3
0--3	9	10.2	63	72.0
-3--6	4	4.6	67	76.1
-6--9	7	8.0	74	84.0
-9--12	4	4.6	78	89.0
-12--15	5	5.7	83	94.0
<-15	5	5.7	88	100.0

n=88

Table 3 Grouped frequency table showing the percentage difference in the AAR measurements as estimated by the two techniques (AAR_{MBD} vs. AAR_{MAC}) in each slice analysed for group 2. Note that in approximately 85% of all cases the difference between the two techniques was $\leq \pm 12\%$.

Table 4

Grouped Frequency Table for Data In Figure 3.5

Group 3

Class Interval	Class Frequency (f)	Relative Class Frequency (f/n*100)	Cumulative Frequency (F)	Relative Cumulative Frequency (F/n*100)
>15	2	2.0	2	2.0
12-15	1	1.0	3	3.0
9-12	7	7.1	10	10.1
6-9	9	9.1	19	19.2
3-6	14	14.1	33	33.3
0-3	7	7.1	40	40.4
0	34	34.3	74	74.7
0--3	11	11.1	85	85.9
-3--6	3	3.0	88	88.9
-6--9	6	6.1	94	94.9
-9--12	1	1.0	95	96.0
-12--15	3	3.0	98	99.0
<-15	1	1.0	99	100.0

n=99

Table 4 Grouped frequency table showing the percentage difference in the AAR measurements as estimated by the two techniques (AAR_{MBD} vs. AAR_{MAC} in each slice analysed for group 3. Note that in approximately 95% of all cases the difference between the two techniques was $< \pm 12\%$.

Table 5 AAR and Infarct Sizes in All Animals in All Groups

GROUP #	DOG #	AREA AT RISK MAC	AREA AT RISK MBD	INFARCT SIZE MAC	INFARCT SIZE MBD
1	16	13.99	21.77		
	17	61.60	57.58		
	18	0	0		
	28	0	1.36		
	29	38.35	33.32		
	30	38.89	43.18		
	31	38.09	43.16		
	32	32.59	36.97		
	33	39.41	42.17		
	34	27.16	24.59		
	36	18.90	14.23		
37	0	2.23			
39	10.55	7.55			
2	19	37.80	36.88	96.11	98.53
	20	20.63	17.31	71.81	85.58
	21	31.88	30.89	75.52	77.96
	22	2.44	0	0	0
	23	28.86	25.58	45.87	51.74
	24	11.49	9.77	0	0
	25	2.76	18.21	0	0
	26	8.66	9.89	49.93	43.71
	27	44.21	40.64	63.47	69.05
	35	0.33	0.85	0	0
	40	38.48	27.74	30.29	42.01
	41	3.93	0	0	0
	3	N1	21.50	28.71	61.82
N2		41.24	48.76	90.45	76.49
N3		27.51	29.28	64.27	60.38
N4		27.72	28.62	79.53	77.03
N5		22.13	24.21	37.05	33.87
N6		28.10	36.96	84.40	64.15
N7		22.93	24.02	30.91	29.51
N8		53.15	26.01	0	0
N9		0	0	0	0
N10		21.33	24.62	24.01	20.82
N11		0	0	0	0
N12		19.99	11.11	7.28	13.11
N13		0	0	0	0
N14		0	0	0	0
N15		1.15	2.95	36.55	14.25
N16		39.49	40.44	77.34	75.51
N17		38.08	42.15	57.73	52.16

Table 5 AAR and Infarct sizes in all groups. AAR and infarct sizes are expressed as a percentage of the LV area and AAR respectively.

Figure 1

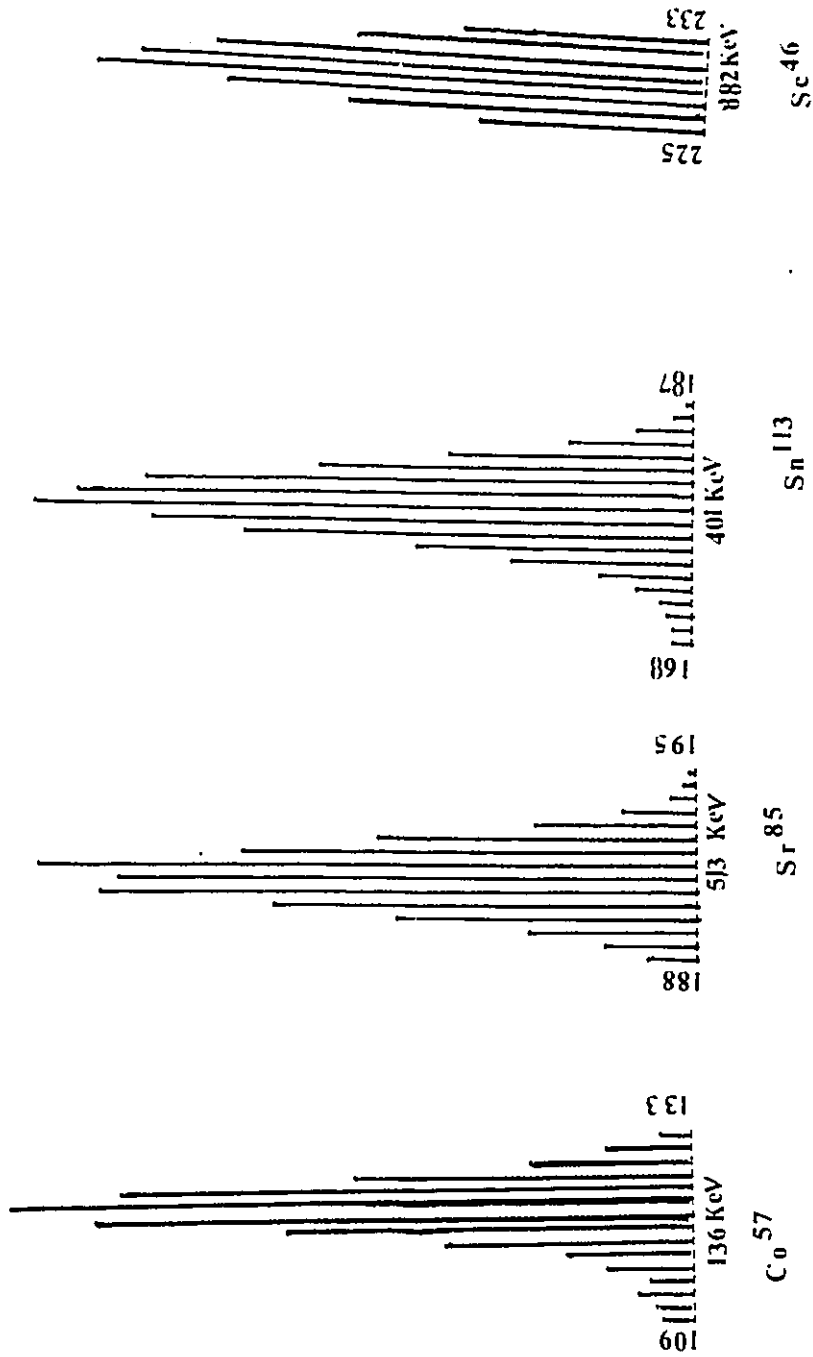


Figure 1 Gamma spectrometer window selection for the 4 isotopes used in this study. These windows were selected such that there were few counts outside of the isotopes main photopeak and minimal overlap of counts from the different isotopes in any given window.