The Use of a Radioiodinated Fatty Acid Analogue in the Study of Myocardial Ischemia and Infarction

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ABSTRACT

The purpose of this study was to utilize 15-p-^{123}I-phenylpentadecanoic acid (^{123}IPPA) and 15-p-^{123}I-β-methyl iodophenylpentadecanoic acid (B^{123}IPPA) in the assessment of myocardial metabolism and perfusion following ischemia and infarction.

Canine models of global ischemia, regional ischemia and infarction were used. Time-activity curve analysis was performed on the first two models using 15-p-^{123}I-iodophenylpentadecanoic acid to determine changes in metabolic status of the myocardium. Reported are the half-life values $\pm$ SD. B^{123}IPPA, ^{201}Tl and a histochemical method (tetrazolium staining) were used in the latter model to assess perfusion defects, including ischemic risk zone.

Significant differences in the elimination rate (mins) of ^{123}IPPA metabolic by-products for the early and late phases of the time-activity curves of the global ischemia model in the lateral wall (early and late phase) and apical wall (late phase) were observed with Iso-osmolar (Tyers) solution. There were no significant differences for either the Iso-osmolar + superoxide dismutase (SOD) or Iso-osmolar + allopurinol. Also there was no significant difference in $t_{\frac{1}{2}}$ values between the three areas of the left ventricle (apex, septal wall and lateral wall) within the control group.

Results with the regional ischemia model indicate an increase in early phase $t_{\frac{1}{2}}$ values representing ^{123}IPPA washout, between control and 14 days

post-operative animals in both the lateral wall (22±10 to 60±107 min - $p>0.05$) and the apical wall (23±10 to 37±33 min - $p>0.05$) and a decrease in ^{123}IPPA washout in the septal wall (22±14 to 14±7 min - $p>0.05$). An increase in $t_{1/2}$ between control and 14 days was seen in the late phase post-operatively in the lateral wall (34±12 to 71±50 min - $p>0.05$), the apex (44±8 to 115±104 min - $p>0.05$) and the septal wall (34±2 to 626±1325 min - $p>0.05$).

The results of the myocardial infarction model indicated a greater degree of correlation between the size of perfusion defects estimated by a histochemical method (TTZ staining) vs. B^{123}IPPA ($r = 0.65$, $p<0.005$), than vs. ^{201}Tl ($r = 0.49$, $p<0.005$).

The following conclusions can be made on the basis of the results obtained. The utilization of iodinated free fatty acids within each area of the left ventricle (lateral wall, apical wall and septal wall) demonstrate similar rates of ^{123}IPPA washout. The effect of reversible global ischemia on myocardial washout of 15-^{123}IPPA may be modified by the addition of SOD or allopurinol to the control iso-osmolar cardioplegic solution. The imaging agent ^{123}IPPA may be a useful indicator of metabolic status of the myocardium, revealing changes in washout rates during the period of developing regional ischemia. Lastly, the utilization of B^{123}IPPA does not appear to be effective in determining the size of perfusion defects, or in identifying the ischemic risk zone because of border demarcation problems.

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Abbreviations and Formulae

ANOVA	-	Analysis of Variance
$B^{123}IPPA$	-	Beta-methyl-iodophenylpentadecanoic acid
BMIPP	-	Beta-methyl iodinated pentadecanoic acid
CAD	-	Coronary Artery Disease
CI	-	Cardiac Index
CK-MB%	-	The % of Creatine kinase in the form of the MB iso-enzyme
^{11}CPA	-	^{11}Carbon-palmitic acid
CVD	-	Cardiovascular Disease
Cs	-	Cesium
CPI	-	^{99m}Tc-hexakis (carbomethoxyisopropyl-isonitrile) technetium (I)
Cl	-	Chlorine
DMIPP	-	Dimethyl iodinated pentadecanoic acid
dp/dt	-	First Derivative of Left Ventricular Pressure
DMIVN	-	Dimethyl-^{125}I-18-nonadecanoic acid
DCM	-	Dilated cardiomyopathy
ECG	-	Electrocardiography
EF	-	Ejection Fraction
FFA	-	Free Fatty Acids
γ	-	Gamma
HCM	-	Hypertrophic cardiomyopathy
HR	-	Heart Rate
HDA	-	Hexadecanoic acid
HSA	-	Human serum albumin
IPP	-	Iodinated phenylpentadecanoic acid
$^{123,131}I$	-	Iodine
$^{123}IPPA$	-	Iodinated phenylpentadecanoic acid
IPI	-	^{99m}Tc-hexakis (isopropyl-isonitrile) technetium (I)
IHD	-	Ischemic Heart Disease
IHA	-	Hexadecanoic acid
KI	-	Potassium iodide
KeV	-	Kiloelectron volts

LVP	-	Left Ventricular Systolic Pressure
LAD	-	Left Anterior Descending Coronary Artery
LCX	-	Left Circumflex Coronary Artery
MVO_2	-	Oxygen extraction
MUGA	-	Multiuser gated angiography
MIBI	-	^{99m}Tc-hexakis (2-methoxyisobutyl-isonitrile) technetium (I)
mCi	-	Millicuries
M.W.	-	Molecular Weight
PCTA	-	Percutaneous transluminal angioplasty
PP	-	Pyrophosphate
R_f	-	Regional front
RP-30	-	^{99m}Tc-methoxy-isobutyl-isonitrile
RMBF	-	Regional Myocardial Blood Flow
SI	-	Stroke Index
SWI	-	Stroke Work Index
SBP	-	Systolic Blood Pressure
SVR	-	Systemic Vascular Resistance
SPECT	-	Single Photon Emission Computed Tomography
Sb	-	Antimony
T-A	-	Time-Activity
TBI	-	^{99m}Tc-hexakis-(t-butyl-isonitrile) technetium (I)
^{99m}Tc	-	Technetium
^{201}Tl	-	Thallium
$t_{½}$	-	Half-lives
Te	-	Tellurium
TTZ	-	Tetrazolium
TPT	-	Triphenyltetrazolium
WHO	-	World Health Organization
Xe	-	Xenon

(i) Body Surface Area

$$BSA\ (m^2) = \frac{K(wt)^{2/3}}{10^4}$$

wt ≡ weight

(Constant) K = 11.2 (dog)

(ii) Cardiac Index

$$CI = \frac{MCO}{BSA} \quad (L/min/m^2)$$

MCO ≡ Mean Cardiac Output

(iii) Stroke Index

$$SI = \frac{CI\ (1000)}{HR} \quad (mL/beat/m^2)$$

HR ≡ Heart rate

(iv) Systemic Vascular Resistance

$$SVR = \frac{(MBP - RAP)}{(\ MCO\ )}\ 80 \quad (dynes \cdot sec/cm^5)$$

MBP ≡ Mean Blood Pressure
RAP ≡ Right Atrial Pressure

(v) Ejection Fraction

$$EF = \frac{SV}{EDV} \times 100$$

SV ≡ Stroke volume
EDV ≡ End-diastolic volume

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I dedicate this thesis to my family, especially to my father.

TO

MY FATHER AND MOTHER

BROTHER AND SISTER

"There is no wealth but Life". John Ruskin: Unto this Last, 1862.

"You cannot find a medicine for life when once man is dead".

Chrysippus: 550 B.C.

INTRODUCTION

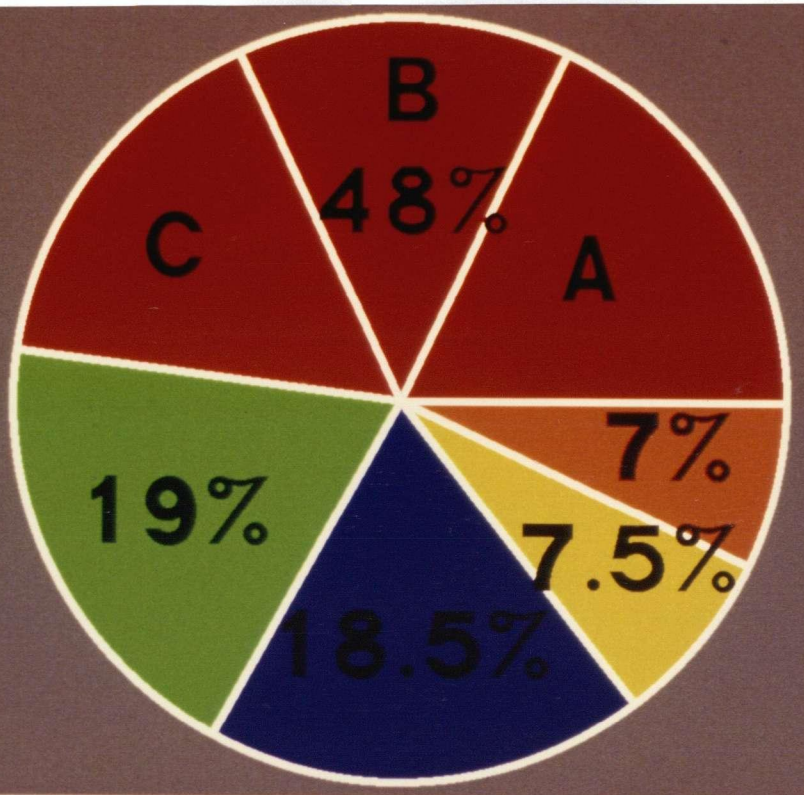
Myocardial ischemia continues to plague many people in most developed countries of the world (WHO statistics, 1986). Cardiovascular disease (CVD) was the primary cause of death in Canada in 1986 (Figure 1, WHO statistics, 1986). It has been reported that 4.3 billion dollars is lost by the Canadian economy per year as a result of cardiovascular disease (B.C. Heart Foundation Fact Letter, 1987). Clearly, CVD is a major problem that must be addressed. A major clinical goal in CVD is its identification prior to its presentation as myocardial infarction, or irreversible myocardial damage. Currently, diagnosis of myocardial ischemia is based on electrocardiography, elevations of specific serum enzymes, angiography and patient history (angina pectoris) (Braunwald, 1982). However, the diagnosis remains inconclusive with these factors, only becoming conclusive subsequent to myocardial damage. Califf et al. (1988) have developed an angina score based on the cause of angina, frequency of angina and ECG changes to grade the severity of CAD. The score also incorporates the patient's age, sex, coronary anatomy and left ventricular function as independent variables. However, calculation of the angina score, which can range from 0 to ≥ 9, will only identify those patients who may require aggressive therapy and will not identify those patients before angina appears. Early diagnosis may prevent some of the deaths associated with myocardial ischemia.

Since the diagnosis of myocardial ischemia remains inconclusive, development of alternative diagnostic modalities, which will identify myocardial ischemia early in its pathogenesis, are needed. Nuclear medicine may serve this need. However, no radiopharmaceutical exists at present which possesses all of the desired characteristics necessary for optimal

Figure 1 - Schematic from WHO, showing causes of death in developed countries (1986).

I.H.D. - Ischemic Heart Disease

C.V.D. - Cerebro-Vascular Disease



Diseases of the circulatory system
A – I.H.D.
B – C.V.D.
C – Other diseases of C.S.

Neoplasms

Other and ill–defined causes

Respiratory diseases

myocardial imaging. Thallium-201, having been considered the most suitable radiopharmaceutical for clinical application, is utilized in cardiac centres as an accepted non-invasive test to evaluate myocardial perfusion. However, ^{201}Tl does have some drawbacks, including γ energy, which falls below the optimal range of commercially available imaging systems, a long physical half-life and a high total body radiation exposure to the patient (Zaret, 1977). Alternative imaging agents are presently being developed and evaluated for optimum assessment of myocardial ischemia and infarction. Two such groups of compounds are radiolabeled free fatty acids and isonitriles.

The use of radioactive tracers to study the cardiovascular system dates back to the 1920's (Blumgart and Weiss, 1927). The use of radioiodinated fatty acids as myocardial imaging agents was first developed by Evans and colleagues in 1965. Evans utilized ^{131}I-oleic acid to demonstrate the possibility of photoscanning the hearts of mongrel dogs. The authors chose free fatty acids because they are an important energy substate for the myocardium (Bing et al., 1956). However, a problem with the ^{131}I-labeled fatty acids was a γ energy of 364 KeV falling above the optimal range of the imaging systems of that time (decreasing diagnostic quality of the images obtained). Another problem associated with this early work by Evans was the double-bond saturation technique used to iodinate the oleic acid. The products of this procedure exhibited a reduced myocardial extraction efficiency and a lower rate of blood clearance, relative to the non-iodinated parent compound (Poe et al., 1973). In order to alleviate this problem, Poe et al. (1975) developed a radioiodine exchange-type process, yielding a terminally-labeled product which retained the

characteristics of the parent compound. In addressing the problem of an ideal radiopharmaceutical suitable for gamma imaging Poe et al. (1976b) proposed the use of ^{123}I because of its more ideal imaging properties (Tables 1 & 2).

The technique of using these iodinated fatty acids in myocardial imaging has been established by Poe and his colleagues. The application of this technique to diagnose myocardial ischemia and infarction, however, remains an area of active research.

Table 1. Imaging Properties of the "IDEAL" Radiopharmaceutical

Gamma Energy (KeV)	100-200
Extraction Efficiency (%)	100
Physical Half-Life (min)	30-60
Biological Half-Life (min)	short
Total Body Exposure (mrad/Ci)	low

Table 2. Various physical characteristics of ^{201}-Thallium, ^{123}IFFA and the ideal radiopharmaceutical

	^{201}Tl	IDEAL	^{123}I-FFA
Gamma Energy (KeV)	80	100-200	159
Extraction Efficiency (%)	87	100	78
Physical Half-Life	72 hrs	30-60 min	13.3 hrs
Biological Half-Life	7 hrs	short	0.5 hrs
Total Body Exposure(mrads)	210	low	30

II LITERATURE REVIEW

(1) MYOCARDIAL METABOLISM

In view of the clinical use of these iodinated fatty acids, a brief review of myocardial metabolism is necessary. Cardiac muscle has the ability to maintain a variety of energy stores. However, it has long been appreciated that the myocardium preferentially utilizes free fatty acids above all others (Bing, 1956).

(a) Fatty Acids - The metabolic process of fatty acid oxidation is well-documented and may be divided into various steps including:

i) membrane transport;

ii) activation and acyl transfer to carnitine;

iii) beta-oxidation

(Idell-Wenger et al., 1978).

i) Free Fatty acids are removed from the blood by the myocardium. While in the blood, the fatty acids may exist in two forms: as free fatty acids bound to albumin, or as triglycerides (Lehninger, 1982). The majority of serum fatty acids are bound to albumin (Idell-Wenger et al., 1978 and Goodman, 1958). Following release from albumin, the transport of the FFA through the cell membrane occurs via passive diffusion (Idell-Wenger et al., 1978).

An equilibrium, however, is maintained between plasma and cellular pools by an energy-dependent process (Opie, 1968).

This equilibrium-maintaining extraction process is controlled by a number of factors, including the albumin:FFA molar ratio, metabolic status of the tissue, concentration of the substrate in plasma, availability of alternative substrates, mechanical activity of the heart, rate of oxidative respiration and plasma levels of certain hormones (Neely, 1972).

The other form of the fatty acid within the blood is as a triglyceride. The former is composed of a glycerol backbone with three fatty acid molecules attached to it. Triglycerides are the major storage form of fatty acids in cells, but are not normally found in membranes (Lehninger, 1982).

ii) Following diffusion through the cell membrane, FFA's are transformed into fatty acyl-CoA esters (Lehninger, 1982). This step occurs on the outer mitochondrial membrane and is enzymatically catalyzed (Idell-Wenger et al., 1978) and allows the fatty acid to continue through the three-step transport process, in which fatty acid moves from the outer mitochondrial membrane into the mitochondrial matrix.

iii) There are three known pathways of fatty acid oxidation of which only beta-oxidation predominates in the body (Antony and

Landau, 1968). Beta-oxidation occurs within the matrix of the mitochondrion. The sequence of beta-oxidation has been known since the early 1900's. Knoop (1904) demonstrated the successive removal of 2-carbon fragments (as acetyl-CoA) from the long chain fatty acid. Each successive removal of acetyl CoA results in the loss of hydrogen atoms from the fatty acid (through the action of dehydrogenases), which subsequently enter the electron transport chain also located in the mitochondria (Lehninger, 1982). The product is the original fatty acid less 2 carbon atoms, which can then re-enter the beta-oxidation sequence.

Fatty acid oxidation is not limited to the mitochondria, having been shown to also occur in peroxisomes (Christensen et al., 1986). These authors used hepatocytes from fasted rats to show that peroxisomal fatty acid oxidation plays an important role in chain-shortening of very long chain fatty acids, ie. C_{22} and longer. These findings have been confirmed by Yamada et al. (1987) who, again using isolated rat hepatocytes, showed that peroxisomal beta-oxidation is involved in the chain-shortening of omega-phenyl fatty acids. The products of the peroxisomal beta-oxidation sequence, with fewer carbon atoms, were then able to enter the mitochondria and undergo mitochondrial beta-oxidation with subsequent generation of ATP through coupled processes. Several important differences exist between peroxisomal and

mitochondrial beta-oxidation including individual enzymes, cofactor requirements, coupling to energy production and specificity for fatty acids of different chain lengths (Wanders et al., 1987). Much of the work reported on peroxisomal beta-oxidation confirms the presence of these organelles within the liver. However, other studies have also shown their existence in other tissues including the myocardium (Connock and Perry, 1983). Therefore, when examining beta-oxidation of free fatty acids within the myocardium, one must refer to both components, ie. a mitochondrial and a peroxisomal sequence.

(b) Glucose - It is known that the myocardium preferentially utilizes fatty acids over glucose as a metabolic fuel (Bing, 1956; Evans et al., 1962). The glycolytic cycle must be inhibited in some way for this to occur. Randle et al. (1964), Neely et al. (1969), and Neely (1972) have demonstrated that glucose transport is inhibited by fatty acids or ketone bodies. Opie (1972) has also reported on the inhibition of glycolysis by free fatty acids.

(c) Fatty acids and energy production - The end result of fatty acid oxidation is the production of acetyl-CoA units. The latter can then enter the citric acid cycle via a condensation reaction with oxaloacetate to form citrate (Lehninger, 1982). The latter continues on through the citric acid cycle, producing reducing equivalents and CO_2. The electrons from the reducing

equivalents are transferred to the electron transport chain within the inner mitochondrial membrane, which transports them to oxygen. The energy liberated from these electrons cascading down this chain is coupled to the synthesis of ATP from ADP and phosphate in a process called oxidative phosphorylation.

(2) MYOCARDIAL ISCHEMIA AND INFARCTION

Ischemia is defined as a deficiency of blood due to functional constriction or actual obstruction of a blood vessel, leading to a reduction in the delivery of oxygen to that particular tissue (Dorlands, 1988). Pathological myocardial ischemia elicits a series of progressive subcellular and cellular changes which affect many of the cell's normal metabolic processes. It is a dynamic process, which can have varying effects, including:

a) cellular alterations;
b) electrophysiological alterations;
c) functional alterations;
d) metabolic alterations.

a) Cellular Alterations - Ischemia is a dynamic, heterogeneous process characterized by a continuum of stages sometimes leading to irreversible injury (Trump, 1982). Although the effects of ischemia on cell homeostasis are many, an increase in cytosolic Ca^{++} is very important. The latter

leads to an increase in Ca^{++}-calmodulin complexes, which alters the cell's cytoskeleton in a number of ways, including cell shape, organelle orientation and alters particles within the membranes (Trump, 1982). The increased number of Ca^{++}-calmodulin complexes also activates phospholipases, which leads to hydrolysis of membrane phospholipids which, in turn, will cause cell membrane damage and mitochondrial membrane damage (Trump, 1982). The latter may affect the transport of fatty acids and their release.

b) Electrophysiological Alterations - Under normal resting conditions, heart rate may be within a range of 60-80 beats/min (Guyton, 1986). The P wave is produced by excitation of atrial muscle, with the right activated prior to the left atrium. The interval between the P wave and the QRS complex represents the time taken for the action potential to travel from the SAN (sinoatrial node), through the AVN (atrioventricular node) and the Purkinge system. The QRS complex represents the excitation of the ventricles and the T wave is the repolarization of the ventricles.

Electrophysiological evidence of ischemia is lacking in specificity, ie. one cannot diagnose ischemia based on ECG evidence alone. It has been demonstrated on many occasions that S-T segment elevation often precedes coronary artery ligation (Opie, 1972; Coraboeuf et al., 1976). Sampson et al. (1960) have attributed this elevation in the S-T segment to a flow of current towards the ischemic area, which is surrounded by normal tissue. Therefore, the ECG will be deflected in the positive direction during the S-T segment. The exact mechanism of this phenomenon is still controversial. Other changes include T-Q depression (Fozzard and Makielski,

1985), shortening of action potential duration, slowed intramyocardial conduction and dispersion of refractory currents (Corr and Sobel, 1979). The cellular events underlying these electrophysiological changes remain inconclusive, but these are believed to be multifactorial.

c) Functional Alterations - The functional consequences of ischemia on the myocardium have been examined extensively. Vatner (1980) demonstrated that myocardial dysfunction is correlated with a reduction in blood flow in the conscious dog. Echocardiographic assessments demonstrated that indices of left ventricular function, including regional wall thickening, ejection fraction and end-systolic pressure/area ratio decreased (Akaishi et al., 1985). These authors also demonstrated a fall in cardiac output, left ventricular stroke work and $^{dp}/_{dt}$ following severe stenosis of the myocardial vessels. The cause of this dysfunction continues to be a controversial issue. Some believe that the fall in tissue ATP levels is the cause (Braunwald et al., 1982; Swain et al., 1982), while others believe that dysfunction associated with global ischemia is due to oxygen free radicals (Myers et al., 1986). Becker et al., (1986) have demonstrated that the ischemic myocardium possesses significant functional capacity and therefore deficient levels of ATP may not be the ultimate cause of the dysfunction. Currently, many authors believe that oxygen free radicals are very important in discerning the underlying cause of any ventricular dysfunction associated with ischemia.

d) Metabolic Alterations - Many studies have been performed examining the effects of ischemia on cellular metabolic activity. The

oxidation of fatty acids is one pathway which is dramatically affected by an ischemic episode. Early work by Evans (1964) demonstrated that subsequent to an ischemic episode, fatty acids are diverted away from beta-oxidation and converted to the storage form of fatty acids (triglycerides), which are then deposited in the liver and adipose tissue. The effects on mitochondria and free fatty acid oxidation will also be discussed under the radiopharmaceuticals section of this thesis.

The metabolic consequences of ischemia result from a fall in oxygen tension. Since the myocardium is dependent upon aerobic metabolism, a fall in oxygen tension will give rise to an increase in anaerobic glycolysis, in an attempt to generate high energy phosphates. Associated with this shift in metabolism to the anaerobic state is an accumulation of end-products of metabolism, such as lactate, hydrogen ion, purine bases and adenosine, leading to a fall in myocardial pH. In response to a limited supply of energy, hormones are released which stimulate cAMP synthesis within plasma membranes of adipocytes (Trump, 1984). This induces a lipolytic cascade which results in activation of lipases which hydrolyze triglycerides to fatty acids and glycerol. The latter is released into plasma for transport to the liver and subsequent use in the formation of glucose (Lehninger, 1982). Shug et al. (1973) reported that free fatty acids accumulate in the mitochondrial fraction of myocardial cells subjected to ischemia because of a reduced utilization. Concomitant with this increased concentration of intracellular free fatty acid is an accumulation of long-chain acyl-CoA and long-chain acylcarnitine esters (Subramanian et al., 1987). These esters have been shown to inhibit a number of enzyme systems including the mitochondrial adenine nucleotide translocator, an enzyme involved in the

translocation of ATP and ADP across the inner membrane of the mitochondria (Shug et al. (1975), Paulson et al. (1984), Paulson et al. (1986), Shug et al. (1987)). Thus, hearts exposed to this increased concentration of free fatty acids during ischemia may show enhanced deterioration of metabolic function with subsequent limited energy production. Also affected by ischemia are the processes of lipid and protein metabolism (Reimer and Jennings, 1986). Thus, ischemia will alter many aspects of the cell's normal metabolic machinery, along with fatty acid metabolism.

e) Experimental Models of Ischemia - There are a large number of available parameters which can be utilized to assess myocardial ischemia. In view of the many physiological changes associated with ischemia, the proper choice of a representative system will be dependent upon the purpose of the study. The experimental models used in this study include:

i) Global Ischemia in situ - Global ischemia involves the production of ischemia throughout the heart. Animals are placed on cardiopulmonary bypass, with oxygenator support, caval occlusion and left atrial venting. An aortic cross-clamp is positioned effectively eliminating coronary flow, producing global ischemia. Cardiac surgical procedures rely on this technique to obtain a quiescent, bloodless field in which the surgeon can work. Many studies have been completed validating this model in the examination of the metabolic and functional consequences of temporary total ischemia (Conti et al., 1984; Rousou et al., 1986). This approach can also be used to assess the effectiveness of cardioplegic solutions in myocardial protection (Catinella et al., 1984).

ii) Regional Ischemia <u>in situ</u> - Regional ischemia differs from the first in a number of ways (Schaper, 1979):

(a) collateral circulation;

(b) mechanical stress of one portion of myocardium (non-ischemic) compared to the ischemic portion;

(c) lipid accumulation;

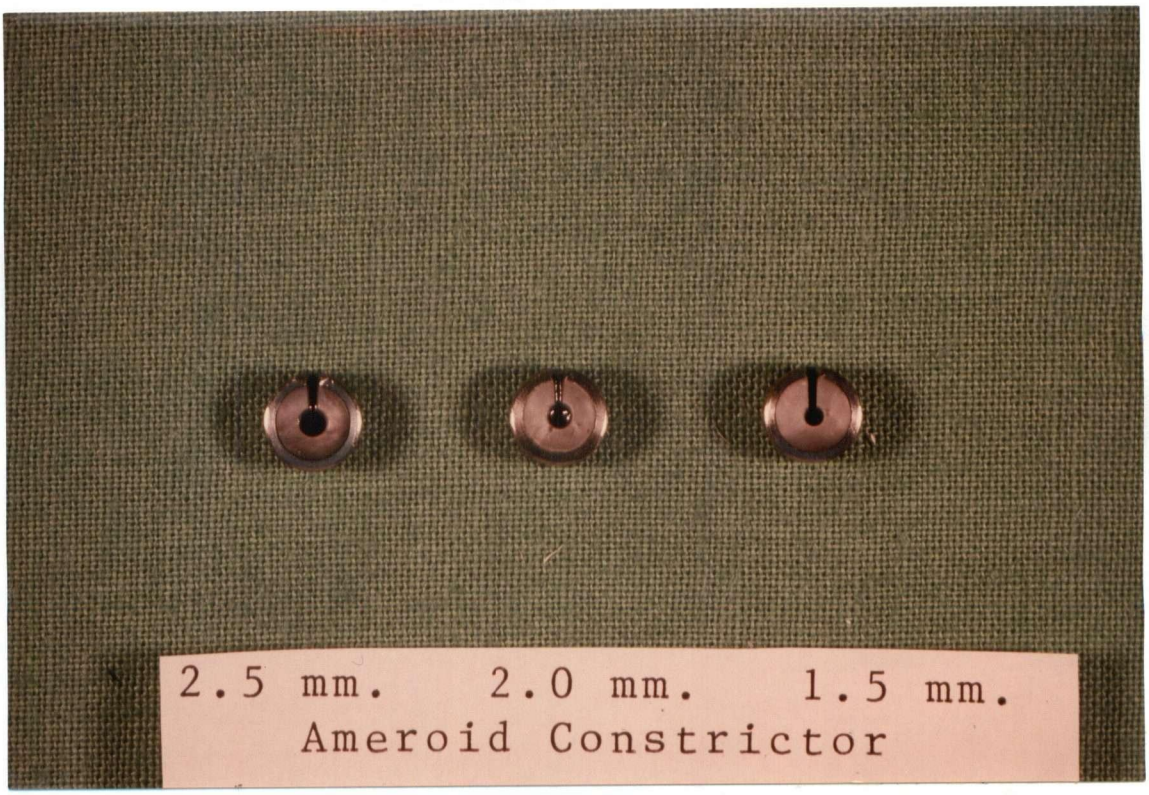
(d) cell death accompanied by extravasation of blood cells and proteins.

The process of coronary artery disease involves the progressive closure of a coronary artery usually by the development of an atherosclerotic plaque. A reliable experimental model, therefore, should produce a gradual coronary occlusion over a known time period, so that various ischemia-related parameters can be assessed. One method, developed in the late 1950's by Litvak and Vineberg, utilizes a casein plastic, Ameroid, which has been cured in formalin to harden it (Figure 2A). The ameroid constrictor is composed of the ameroid, which is sheathed by a metal jacket. There is a central lumen, which communicates with the outer surface by a narrow arm (Figure 2B). The constrictor can be placed onto the coronary artery by way of this arm. The material will slowly engorge with fluid and progressively swell and pressure will be directed towards the central lumen. Thus, the coronary artery will be gradually stenosed, mimicking the progression of coronary artery disease. Vineberg and Mahonti (1960) demonstrated that this technique is an excellent model of gradual coronary occlusion with death of the animals occurring when 50% or more of the lumen of the two left coronary arteries was blocked.

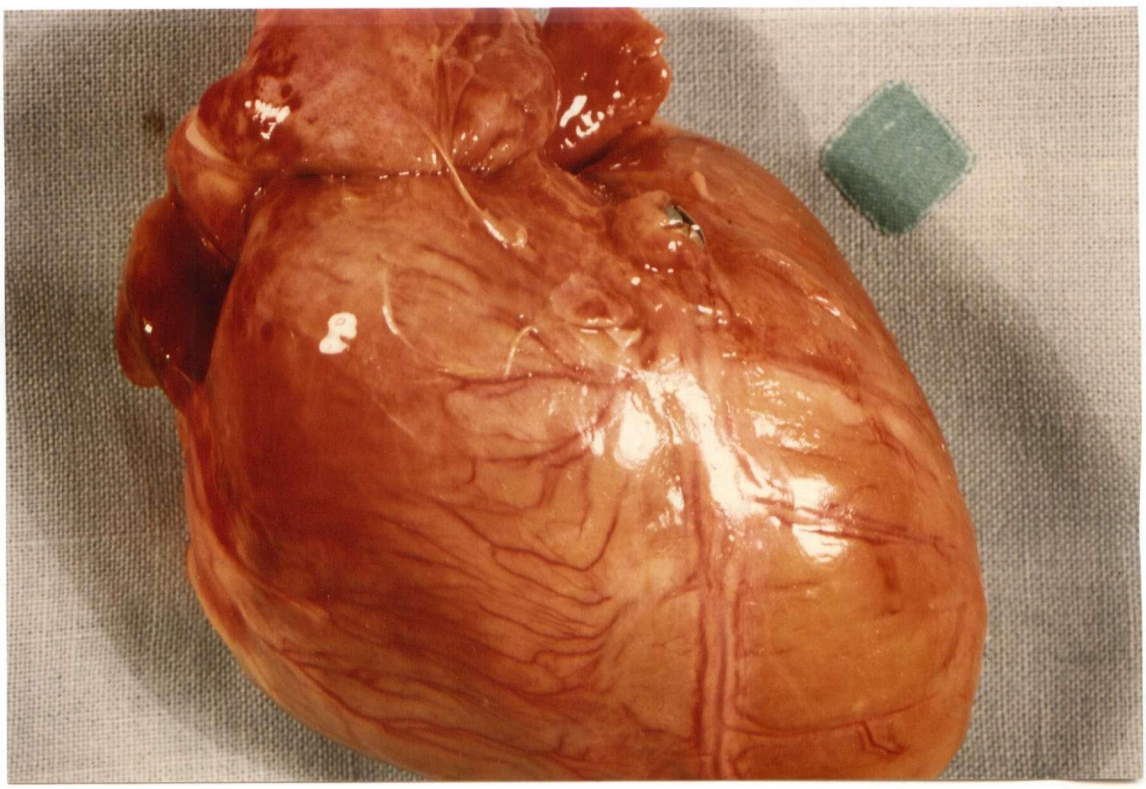
Figure 2 (A) - Various sizes of ameroid constrictors available.

(B) - Ameroid constrictor in place on LAD coronary artery - Heart has been excised following 14 days of gradual coronary occlusion to demonstrate collateral circulation.

(A)



(B)



iii) Myocardial Infarction in situ - Myocardial infarction is an inevitable outcome of untreated coronary artery disease. Gradually, the lesion from CAD will grow inwards and result in complete blockage of that artery. The infarction in this study was produced following a thoracotomy and dissection of the coronary artery. Utilizing a dexon suture, the coronary artery was ligated, producing an acute loss of blood flow to the regional myocardial bed supplied by that artery. Myocardial infarction (ie. a heart attack) may vary in its outcome. With small infarcts, the remaining viable myocardium can continue to function, such that hemodynamic status is relatively well maintained. However, in many cases it is fatal (See Figure 1, p.2).

(3) RADIOPHARMACEUTICALS IN THE CLINICAL DIAGNOSIS OF MYOCARDIAL INFARCTION AND ISCHEMIA.

(a) Technetium (^{99m}Tc)

(i) ^{99m}Tc-pyrophosphate

One approach to imaging myocardial infarction is the use of infarct-avid agents, such as ^{99m}Tc pyrophosphate. ^{99m}-Technetium (^{99m}Tc), as ^{99m}Tc stannous pyrophosphate, was first used to image acute infarcted human myocardium by Parkey and his coworkers in 1974. Of 23 patients, thirteen had positive ^{99m}Tc scans, following planar gamma imaging - eleven of these patients were scanned 3-5 days following infarction and two, 7-10 days after infarction. Localization of infarction by ^{99m}Tc scan and ECG correlated well. These authors believed that pyrophosphate was

incorporated into the hydroxyapatite crystalline structure found in the mitochondria of irreversibly damaged myocardium.

Since this study, a number of investigators have attempted to size myocardial infarctions under experimental conditions using ^{99m}Tc PP and SPECT. Keyes et al. (1978) were the first to attempt this 3-dimensional representation of the myocardium using 15 mCi of ^{99m}Tc pyrophosphate. Infarctions were produced by ligation of the LAD and LCX arteries and imaging performed 60-90 minutes later. The main problems reported by Keyes and his co-workers related to attenuation and image resolution. The correlation between the histochemical infarct size (tetrazolium staining) and SPECT infarct size, both measured in grams, was good ($r = 0.85$), even though the latter problems did exist. In a similar study, Caldwell et al. (1984) reported a high degree of correlation ($r = 0.92$) between in vivo defect volume by SPECT and in vitro defect volume, by well-counting of myocardial samples. The authors defined defect volume as that volume of left ventricle which fell below 2 or more standard deviations of counts from normal myocardium. If ^{201}Tl was used with this technique, the correlation between these two assessments of defect volume was $r = 0.70$. Although correlations were deemed appropriate, more accurate borders were needed in order to differentiate infarcted tissue from ischemic tissue.

Kaul et al. (1985) addressed this issue, utilizing ^{99m}Tc-labeled microspheres, SPECT and macroautoradiography. These authors concluded that assessments of perfusion defects, including the area at risk, is difficult to determine in the apical region. The average correlation on a slice-by-slice comparison was $r = 0.88$ with

the basal slices showing the highest degree of correlation and the apical slices showing the worst. It must be noted that these studies were performed in excised hearts.

With the evident success of this infarct-avid agent in the experimental setting, Holman et al. (1982) took it to the next level - the clinical setting. In 20 patients with acute myocardial infarction, ^{99m}Tc pyrophosphate and ^{99m}Tc-labeled RBC's were used to quantitate infarct size, both by planar imaging and SPECT. Infarct size assessments were then correlated with patient prognosis. Results showed that 85% of patients with infarcts of >40g had complications during the 17.8 month follow-up period. Of the patients with infarcts of <40g, only 29% had complications. This study also indicated that two-dimensional imaging cannot be used to assess the size of perfusion defects because of problems with edge detection and boundary overlap. Anatomical location of the infarction also limits the usefulness of planar imaging with only anterior infarcts being seen. SPECT imaging proved to be more accurate with better delineation of borders and better able to detect both interior and anterior infarcts. In a similar study, Corbett et al. (1984) assessed infarct size via planar imaging and SPECT with and without blood pool overlay to determine its sensitivity. Blood pool overlay refers to the activity within the chambers of the heart. It was found that SPECT with blood pool overlay was significantly better than SPECT or planar imaging, alone, at determining myocardial infarct site and size, especially in the sensitivity for detection of non-transmural infarcts. A number of other studies have validated the use of SPECT as a tool for infarct

quantification - Jansen et al. (1985) ^{99m}Tc-PP, - Nohara et al. (1984) - ^{201}Tl and SPECT.

Socher et al. (1987) have demonstrated a net retention of ^{99m}Tc-PP approaching zero within normal myocardium versus >30% in reperfused myocardium. It appeared that the extent of ^{99m}Tc uptake was dependent upon the degree of irreversibility of myocardial damage. These authors further suggested and demonstrated a more accurate assessment of myocardial tissue damage using a dual tracer technique. Although appearing to be the optimal agent for assessment of perfusion in clinical nuclear medicine, based on its radionuclidic properties, ^{99m}Tc has yet to replace ^{201}Tl in the assessment of cardiac ischemia. ^{99m}Tc-PP is also a non-physiological agent, thereby limiting its potential.

(ii) ^{99m}Tc-Isonitriles

The more optimal gamma energy (140 KeV) of ^{99m}Tc, its half-life of 6 hours and its ease of generation has led to the search for an imaging agent which is taken up by normal myocardium. One possible ^{99m}Tc-based myocardial imaging agent, which has received a great deal of attention recently, is the labeled isonitrile. In 1981, Deutsch and coworkers screened a large number of cationic ^{99m}Tc complexes for myocardial uptake in dogs and rats. Nineteen cationic complexes investigated were divided into 5 groups, including the dimethylarsines, diphenylarsino-ethanes, diphenylphosphino-ethylphosphines, monothioglycerols and diphenylphosphinoethyl amines. Of these groups, only one, the dimethylarsines, exhibited detectable

myocardial uptake upon imaging. Even among the 4 halogen species within the group, variability existed in terms of image quality and biological distribution. The bromo and fluoro complexes yielded the best images. However, the danger and difficulty of working with hydrofluoric acid favors further investigation of the former compounds. Distribution studies revealed an average heart uptake of 0.022 % dose/g for dimethylarsine compared with 0.038% dose/g for ^{201}Tl. Although the uptake was lower, myocardial images were of good quality with dimethylarsine. In later studies, the chloro derivative appeared to be the best of the halogens (Ketring et al., 1983).

Jones et al. (1984) state that none of these agents, thus far, have shown any success in imaging human myocardium, although animal studies have shown tremendous promise. Therefore, these authors developed an alternative class of ^{99m}Tc complexes - hexakis (alkyl isonitrile) technetium (I) cations the difference being that in the latter, the isonitrile ligand is monodentate, while in the work of Deutsch and colleagues it was a bidentate ligand. Biodistribution studies in mice revealed marked liver and skeletal muscle uptake and some myocardial uptake (1.22±0.12% of the injected dose) at 5 mins. post-injection. The lipophilicity was also shown to affect uptake with increased lipophilicity being associated with increased uptake. Although normal myocardium was being imaged, the mechanism of the uptake of these agents was still unclear and there were no studies involving human myocardium.

Holman et al. (1984) utilized ^{99m}Tc hexakis (t-butyl isonitrile) technetium (I) (TBI) for imaging 4 normal persons and 2

patients with CAD. The study demonstrated very good quality images obtained by both planar and tomographic scanning. This agent, however, may not be suitable for clinical use, because of very high uptake in the lung, which decreases the quality of the image in the first hour post-injection (Gerundini, 1986). At times $\geq$ 1 hr, liver and spleen uptake obscures the apical wall of the image. Another problem was the low radiochemical yield (30-60%) as reported by Holman et al. (1984).

In order to better understand the uptake mechanism of these agents, Sands et al. (1986) examined the kinetics of TBI and ^{99m}Tc-hexakis (isopropylisonitrile) technetium (I) (IPI), using neonatal rat myocytes and human erythrocytes. It was found that the uptake was unaffected by ouabain or KCl, so that it was probably unrelated to the Na^+/K^+ATPase system. Sands et al. (1986) suggested that lipophilicity was involved in myocardial uptake just as Jones et al. (1984) had shown previously. Maublant et al. (1988) have demonstrated that in cultures of myocardial cells of newborn rats ^{99m}Tc-hexakis-2-methoxyisobutylisonitrile-Technetium (^{99m}Tc-MIBI) shows slower uptake and is relatively insensitive to metabolic inhibitors, such as cyanide, iodoacetate and ouabain.

(^{99m}Tc)MIBI uptake was also found to be insensitive to changes in pH. Piwnica-Worms et al. (1988) have found that hexakis (carbomethoxy-isopropylisonitrile) technetium (I) (^{99m}Tc-CPI) uptake is also insensitive to several metabolic inhibitors, but was temperature-sensitive. Clearly, this agent, like ^{99m}Tc pyrophosphate, is a non-physiological tracer and thus would be useful

as a tracer for tissue perfusion studies but would yield no metabolic information. It was also found that extracellular alkalinization (pH ~ 8.5) partially inhibited ^{99m}Tc-CPI uptake, while intracellular pH changes had no effect. Although the uptake mechanism is still not clear, data are still becoming available which will answer some of the questions which remain (Zanelli et al., 1988).

Another isonitrile which has shown excellent myocardial uptake and imaging potential is ^{99m}Tc-methoxy-isobutyl isonitrile, or RP-30 (Maddahi et al., 1986). Background-to-target ratios were shown to be much improved for RP-30 over TBI. Normal endocardial and epicardial motion as well as normal myocardial wall thickening were seen with gated planar imaging and RP-30. In a recent clinical study, Tatum et al. (1988) utilized RP-30 and ^{201}Tl to identify the perfusion defect in a patient suffering from acute myocardial infarction. Nine days post-infarction, the patient underwent a stress test using ^{201}Tl, demonstrating an area of decreased activity, but no evidence of ischemia. The following day, the patient underwent a stress test using RP-30. The results indicated a small myocardial infarct, seen both on the resting and post-exercise scans. A defect, however, was seen only on the post-exercise scan representing an area of ischemia. Therefore, the isonitriles, unlike ^{201}Tl, may provide a means of identifying ischemic areas around the borders of a myocardial infarction.

(b) Thallium - 201

(i) Historical Perspectives - The utilization of radioisotopic studies of the cardiovascular system dates back to the 1920's. Blumgart and Weiss, in 1927, described the use of radium C in determining the velocity of blood flow through the pulmonary circulation in man. With the advent of radioisotopes, many saw the possibility of utilizing these agents to trace pathways and localize various physiological elements, such as ^{45}K (Greenberg et al., 1938) and ^{42}K (Joseph et al., 1939). These authors utilized these agents to study the predominant pathways of absorption and biodistribution in various organ systems. Love et al. (1954) reported that the use of ^{42}K may be unsuitable for studying myocardial circulation, because of the need for large amounts of injected carrier K. In view of this, Carr et al. (1962) examined the utilization of ^{86}Rb as an agent which would show a reduced uptake in areas of myocardial infarction. Thus, the feasibility and possibility of using radioisotopic studies of the myocardium was established. Attention now turned to the development and refinement of more suitable tracers.

It was not until 1970, with the realization that available potassium isotopic analogs had emissions which were too high, did Kawana et al. (1970) suggest the use of the thallous ion, Tl^{+}, for radionuclide imaging. Lebowitz et al. (1974) demonstrated that ^{201}Tl may be a useful agent in myocardial scanning. Studies using goats demonstrated high concentrations of the tracer within the kidney, liver and heart. In many other species, including rabbits, dogs and mice, no

adverse reactions to the tracer were found. Its physical characteristics and good resolution with standard gamma imaging systems made ^{201}Tl an acceptable means of non-invasive testing of patients suspected of having IHD (Botvinick et al., 1980).

ii) Radionuclidic Properties - Much of the work on the chemistry of thallium lies in the field of organometallic chemistry. Thallium is a metallic element, found in group IIIA of the periodic table, with an atomic number of 81. It has a M.W. of 204.39 and has two stable valences +1 and +3 (Kemp, 1980). It is similar to potassium and can substitute for K^+ in the activation of pyruvate kinase (Strauss et al., 1975). Twenty isotopic forms of Tl exist, with atomic masses ranging from 191 to 210.

iii) Clinical Application and Experimental Evaluation - $^{201}Tl_{81}$, as a radioisotope, has a half-life of 73 hrs and decays by orbital electron capture and is utilized as a myocardial imaging agent (Lebowitz et al., 1975). Although utilized in many cardiac centres as the standard myocardial perfusion imaging agent, over the past 10-15 years of clinical use, ^{201}Tl has shown some downfalls. Zaret (1977) discussed the problem of a $t_{\frac{1}{2}}$ = 72 hrs, which precludes any sequential imaging (Maublant et al., 1981), and also its energy of 89 KeV was not optimal for commercially available gamma imaging systems. Attenuation was also found to be of considerable importance. Keyes et al. (1978) and Burdine (1979) both report on poor resolution of myocardial planar images, due to attenuation by other tissues of the thoraco-abdominal

cavity. Another area of concern was that ^{201}Tl is a non-physiological imaging agent thus would yield little information on the metabolic status of the tissue (Gewirtz, 1978). In view of the fact that washout rates of ^{201}Tl within normal and ischemic myocardium were unaffected by regional myocardial blood flow and both areas exhibited similar washout rates, ^{201}Tl was not able to yield any information which would distinguish between these two areas (Gewirtz, 1978 and Leppo et al., 1980).

Another disadvantage of ^{201}Tl was its lack of specificity (Wackers et al., 1980). The use of ^{201}Tl did not distinguish between recent and old myocardial infarction or ischemic and necrotized tissue. The utilization of ^{201}Tl to determine the size of myocardial infarction continues to be investigated. A major problem associated with this technique is redistribution (Beller et al., 1980). Redistribution was first reported by Pohost et al. (1977). These authors demonstrated redistribution of ^{201}Tl into ischemic myocardium during transient coronary occlusion in dogs and following exercise-stress tests in man. Beller et al. (1980), in clinical studies, demonstrated that defect areas (cold spots) on initial scans resolved over time. Thus, an apparent infarct may represent an area of ischemia, which resolved itself over time, as the result of increased collateral circulation and decreased myocardial oxygen demand. Massie et al. (1984) have utilized this observation to identify areas of acute myocardial infarction, by repeating the ^{201}Tl study after 3 to 4 hours. The problem, however, is assessing the significance of this redistribution phenomenon. Tatum et al. (1988) describe a case-study

in which the information obtained following a ^{201}Tl redistribution study was inaccurate, defining a lesion much larger than that defined by (^{99m}Tc) isonitrile. Tatum et al. (1988) also report that both in vivo and in vitro studies have demonstrated an accelerated efflux of ^{201}Tl from the infarcted myocardium.

Clearly, problems associated with ^{201}Tl called for further research into the development of a more ideal radiopharmaceutical (Table 1). One of the more promising substances which possess more ideal characteristics for optimal myocardial imaging are iodinated free fatty acids. The use of iodinated free fatty acids, however, remains in its early stages of clinical and experimental assessment.

(c) Iodine-123, 131

(i) Historical Perspectives of Iodinated Fatty Acids - The preferential utilization under aerobic conditions of free fatty acids by the heart has been appreciated since the 1950's (Bing, 1956). In order to assess the importance of fatty acids as an energy pool, Evans et al. (1963) examined the extraction and fate of ^{14}C-labeled palmitic acid in an isolated rat heart model. The inability of ^{14}C-labeled palmitic acid to accurately assess both the myocardial extraction and triglyceride storage involved in fatty acid metabolism resulted in attempts by Evans and co-workers to utilize ^{131}I, a gamma-emitting isotope, labeled to the fatty acid (Evans et al., 1964 a,b). This radioisotope allowed both myocardial extraction and incorporation of fatty acids into the triglyceride form to be

assessed. Evans and his co-authors recognized the diagnostic potential of these labeled fatty acids for the purpose of photoscanning the heart. Problems remained with the ^{131}I label, which limited its usefulness as a clinical imaging agent. These included a large radiation dose to the patient because of the β emissions and a large residual exposure of activity and because of its extremely long half-life (~193 hrs - average life 279 hrs) (Myers, 1974) (Table 3). Therefore, work was needed to identify the availability of suitable radionuclides for <u>in vivo</u> use and refinements in the labeling procedures to obtain greater purity of the final products.

Table 3 Physical Properties of ^{123}I, ^{125}I and ^{131}I (Myers et al., 1974) - clinically used radioiodides. (Radionuclide Transformations, ICRP Publication 38, 1983. ed. F.D. Sowby, Pergammon Press, N.Y., N.Y.)

	^{123}I	^{125}I	^{131}I
Half-life	13 hrs.	1344 hrs.	193 hrs.
Modes of decay	EC	EC	β
Beta Particles	none	none	90.4% 192 KeV average
γ energy	158 KeV	none (X-rays 27 & 35 keV)	363 keV
Other photons	γ ~ 3% 274-530 keV	none	γ ~ 6.8% 637 keV ~ 1.6% 723 keV

The decreased enthusiasm over iodine as a radionuclide over the next decade prompted the development of alternative imaging agents for non-invasive assessment of the cardiovascular system. These agents included ^{129}Cs (Yano et al., 1970), ^{43}K (Hurley et al., 1971), $^{13}N\text{-}NH_4$ (Hunter & Monahan, 1971), and ^{81}Ru (Martin et al., 1974). Many workers continued to use the ^{14}C label (Antony and Landau, 1968). Thallium, as discussed earlier, was also being actively explored as an indicator of myocardial perfusion. It was not until 1975 that Poe and co-workers utilized ^{131}I in the terminal position of hexadecanoic acid (HDA) and found that extraction percentages compared favourably with the naturally occurring compounds (77±11% for HDA compared to 70±7.5% for stearic acid and 61±7.8% for oleic acids labeled with ^{11}C).

ii) ^{123}Iodine (^{123}I)

1) Production of ^{123}I.

There exist 29 radioisotopes of Iodine which can be used in medicine today (Mani, 1985). All are produced in reactors and involve any one of five possible neutron-induced reactions, including (n,γ), (n,ρ), (n,α), (n,γ) followed by decay, and lastly, (n,fission). Of the 29 radioisotopes of iodine available, ^{123}I has the physical properties which satisfies the requirements of an ideal radiopharmaceutical for in vivo medical use (Myers et al., 1962). These early clinical studies on in vivo medical use of the ^{123}I label centered around thyroid uptake and scanning. The ^{123}I was first produced by the Sb^{121} $(He^4, 2n)I^{123}$ reaction with a 30 MeV beam.

This resulted in contamination with high-energy γ-rays from ^{124}I. The technical difficulties associated with this method necessitated the development of alternative reactions, including ^{122}Te $(\alpha,3n)^{123}Xe$, (Sodd and Blue, 1968) and $^{122}Te(^{3}He, 2n)^{123}Xe \xrightarrow{\beta+} {}^{123}I$, (Sodd et al., 1969), which produced carrier-free iodide ion by the decay of ^{123}Xe. In order to obtain high yields of ^{123}I, Lambrecht and Wolf (1972) examined the use of the $^{122}Te(^{4}He,3n)^{123}Xe \longrightarrow {}^{123}$I generator and found a yield purity of 99.8% ^{123}I, with $\leq$0.2% contaminating ^{125}I. The presence of this impurity, which increased radiation dose to the patient, led to the development of an alternative reaction by Fusco et al. (1972), in which it was reported that virtually all radioactive contaminants were eliminated. These authors utilized the $^{127}I(p,5n)^{123}Xe$ reaction, resulting in 3.0 mCi/μA/hr. of activity and only 0.1% ^{125}I impurity and no ^{124}I. However, the proton beam energy required for this reaction is >50 MeV, which is not readily available from most cyclotrons.

Currently, there are 4 main types of nuclear reactions suitable for preparing ^{123}I: the $^{127}I(p,5n)^{123}Xe(EC, \beta+)^{123}I(70MeV)$, $^{124}Te(p,2n)$ ^{123}I (25-40MeV) and, lastly, ^{124}Xe $(p,2n)$ ^{123}Cs $(\beta+)$ ^{123}Xe (EC, $\beta+$) ^{123}I (25-40 MeV), (Machulla et al., 1986) and a final sequence involving both a direct reaction $^{124}Xe(p,2p) \longrightarrow {}^{123}I$ (75%) and an indirect reaction ^{124}Xe $(p,pn)^{123}Xe \longrightarrow {}^{123}I$ (25%) (AECL, Vancouver, B.C., Canada). This latter method was the preparation of ^{123}I used for this experimental endeavour.

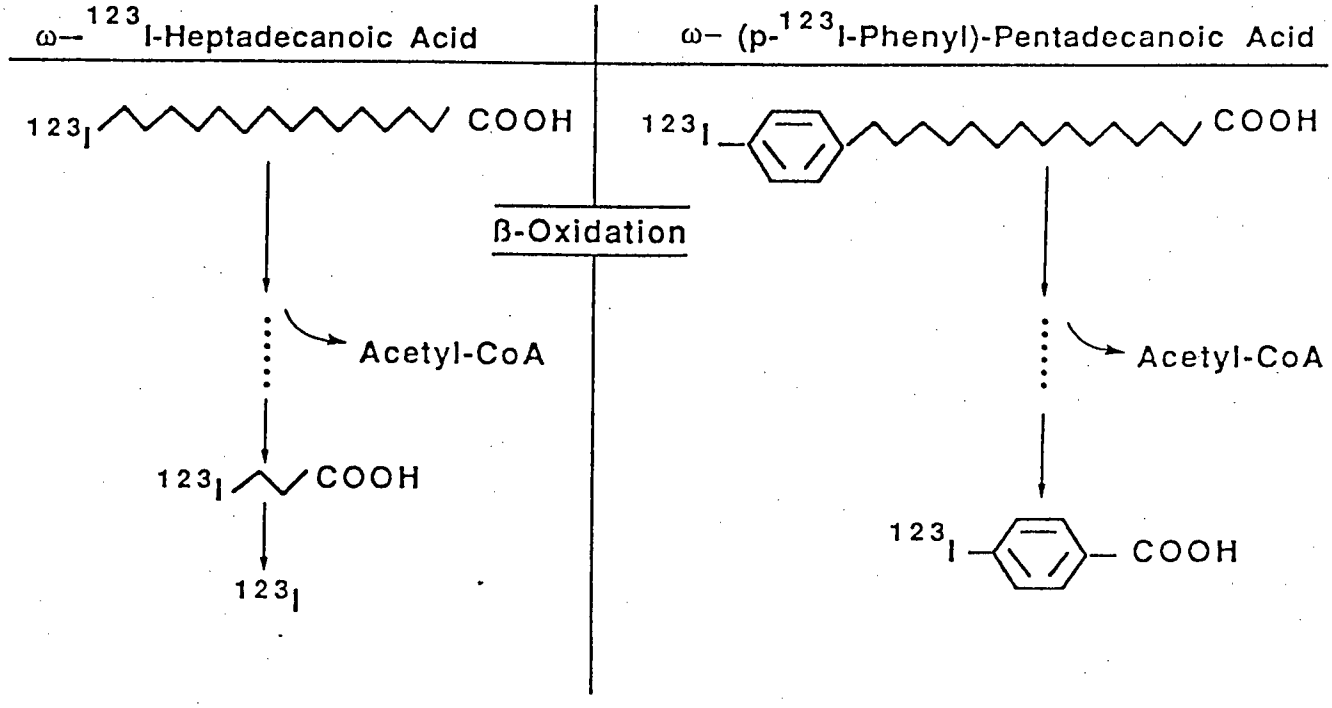
The availability and purity of ^{123}I is necessary as the thrust of much of the research today is into the development of a simple and quick labeling kit, which will yield a pure product.

2) Fatty Acid Iodination Procedures.

Early attempts at iodination involved iodine monochloride addition to a double bond (Evans et al., 1965) or iodide replacement at the terminal position in omega-bromo-(long chain)-fatty acids (Robinson et al., 1974). It became apparent from these studies that terminally iodinated fatty acid analogs resulted in higher levels of activity within the tissue when compared to images obtained from unsaturated fatty acids. The reason for such a discrepancy was determined by Poe et al. (1975) and reconfirmed by Machulla et al. (1980). It was established that myocardial extraction of terminally-iodinated fatty acids was 45% higher than that of fatty acids labeled by iodination across the double bond. Thus, a larger amount of the free fatty acid was being removed from the circulation facilitating gamma scintigraphy.

Although image intensity was increased by labeling the fatty acid at the terminal position, Robinson and Lee (1974) had concerns regarding the possibility that recirculation of free iodide may be limiting the accuracy of myocardial images. Machulla et al. (1978), demonstrated that the highest uptake of omega fatty acid occurred when carbon chain length was 17 carbon units. They also noted a very high level of background radioactivity, due to free iodide recirculating within the blood stream following beta-oxidation of the fatty acids (Figure 3). In order to eliminate this background problem, the preparation of iodinated-^{123}I-phenylpentadecanoic acid (IPPA) for in vivo metabolic studies was first described by Machulla et al. (1980) and Machulla and Dutschka (1981b). In their preparation of IPPA, the authors utilized nucleophilic aromatic substitution of iodine

Figure 3 - Catabolic pathways of ^{123}IPPA and ^{123}IHA.


ω-^{123}I-Heptadecanoic Acid
ω- (p-^{123}I-Phenyl)-Pentadecanoic Acid
^{123}I
COOH
ß-Oxidation
Acetyl-CoA
^{123}I
COOH
^{123}I
^{123}I
COOH
Acetyl-CoA
^{123}I
COOH

onto the phenyl ring. The result was the production of 70% para substituent and 30% ortho substituent in a radiochemical yield of 70%. High pressure liquid chromatography was then used to separate and purify each of the isomeric forms of the labeled fatty acid. The para-IPPA was then dissolved in 4% HSA for administration into the test animals. Machulla chose the para isomer because of its greater uptake by the myocardium. This preparation method was found to suffer from a number of problems, including the formation of an isomeric mixture (70% para, 30% ortho), the requirement of complicated HPLC procedures to isolate the para form, and, lastly, the loss of 29% of ^{123}I label at the end of the preparation.

In an attempt to alleviate these problems, associated with the nucleophilic aromatic substitution method, Eisenhut (1982) devised a fast and simple synthesis of p-^{123}IPPA from pure, unlabeled p-IPPA by iodine-isotope exchange. The procedure involved the preparation of isomeric 15-iodophenyl-pentadecanoic acid, with subsequent isolation of the para derivative, using recrystallization from hexane. The product was then radioiodinated by iodine-isotope exchange.

The reaction conditions included a temperature of 170°C for 30 minutes, yielding 95% para isomer. Studies of the reaction at various temperatures demonstrated highest radiochemical yield at 170°C, with decreasing yields at 140°C and 102°C (95%, 78% and 50% yields, respectively). The authors then examined scintigrams of rabbit heart, liver and background areas of interest, noting differences between ^{123}IHA and ^{123}IPPA. The latter demonstrated increased heart uptake and reduced liver uptake and background activity compared to ^{123}IHA.

However, this preparative method results in a relatively low specific activity.

Therefore, alternative means of preparation were developed, including triazene decomposition (Goodman et al., 1982), destannylation (Franke et al., 1983), boron replacement (Kabalka et al., 1981), and thallium replacement (Kulkarni and Parkey, 1982). The latter represents an important component of the iodination procedures used for the present study.

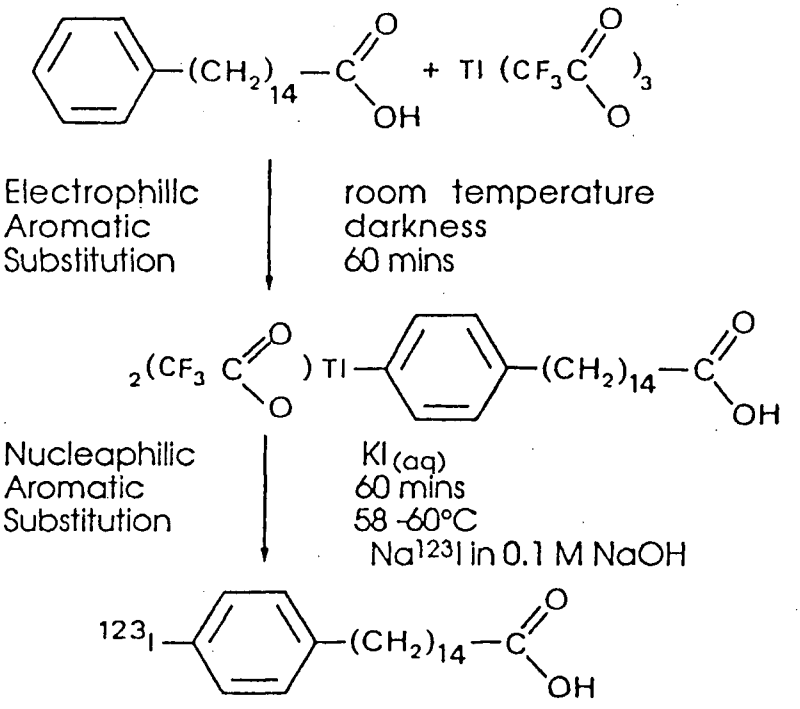
In order to acquire faster and more efficient labeling techniques, thallation was introduced as a component of aromatic electrophilic substitution in 1934 (Gilman and Abbott, 1934). These early reactions, however, were limited in use by the poor electrophilicity of the reagent used. In 1969(a), McKillop and co-workers developed an alternative reagent for the thallation of aromatic compounds which also induced some isomeric specificity into the procedure. The preference for the para position on the phenyl ring by iodine was believed to be due to the steric bulk of the thallium molecule. These thallated aromatic compounds were found to be very effective intermediates for the formation of substituted aromatic compounds through the cleavage of the C-Tl bond. McKillop et al. (1969b) later used this reagent to develop a high-yield, one-step synthesis of aryl iodides. These authors found that treating the aryl thallium intermediates with aqueous KI resulted in the formation of the corresponding aromatic iodides. The reaction was reported to be complete in a few minutes at room temperature. Thus, an extremely simple synthesis of aromatic iodides was developed.

The reaction sequence shown in Figure 4 describes the preparation of 15-p-^{123}I-iodophenylpentadecanoic acid utilized in this study (Dougan et al., 1986a,b).

The orientation of the incoming Tl electrophile could be influenced by the nature of the aromatic nuclear substituents (McKillop et al., 1971) and by the temperature of the reaction mixture (Eisenhut, 1982). The latter authors also demonstrated that the concentration of base could alter the radioiodination yield, which was maximal with 0.1 M NaOH.

Machulla et al. (1981a,b) used electrophilic aromatic substitution to generate IPPA with isomeric yields of 70% para and 30% ortho. Kulkarni and Parkey (1983) found that combining thallation with iodination resulted in yields of 99% para and 1% ortho. They reasoned that the steric hindrance associated with thallation severely limits ortho substitution, while promoting para substitution. Radiochemical yields were still low, however, with values between 50 and 70%. Therefore, efforts continue to develop a labeling method which is more efficient, allowing greater radiochemical yields.

Figure 4 - Aromatic electrophilic and nucleophilic substitution reaction.


Electrophillc
Aromatic
Substitution
room temperature
darkness
60 mins
Nucleaphilic
Aromatic
Substitution
KI(aq)
60 mins
58 -60°C
Na123I in 0.1 M NaOH
OH
OH
OH

iii) Applications - Clinical and Experimental

a) Iodinated hexadecenoic acid

As mentioned earlier, Poe and coworkers (1975) re-emphasized the potential usefulness of iodinated fatty acids for diagnostic imaging. These authors made a comparative evaluation of long-chain fatty acids labeled by ^{11}C in the carboxyl position, ^{131}I addition by a double bond saturation technique and, lastly, ^{131}I substitution at the terminal position. Canine hearts were utilized to examine the extraction efficiencies of each of these products. The ^{11}C compounds were stearic acid and oleic acid. The fatty acids labeled by the double bond saturation technique included oleic, linoleic and linolenic acids. The terminally labeled acid was hexadecenoic acid.

Direct coronary artery injection of the fatty acids resulted in the generation of time-activity curves using a sodium-iodide gamma scintillation detector and flat-head collimator. Biphasic myocardial washout curves indicated that terminal iodination yielded an end-product which was extracted by the myocardium at an efficiency similar to that of the parent compound. These authors also concluded that the iodine-label protruding from the double bond sterically alters the normal metabolic cascade associated with free fatty acid oxidation, such that extraction efficiency was much reduced (77 $\pm$ 11% for IHA compared to 33 $\pm$ 5.2% for iodinated saturated oleic acid).

In view of the optimal physical characteristics of the ^{123}I label, Poe et al. (1977) undertook a clinical study utilizing 16-^{123}I-iodo-9-hexadecenoic acid (IHA). Twenty-one patients with a mean age of 60, undergoing cardiac catheterization, were imaged and

washout rates calculated. A number of views, including left and right anterior oblique, anterior and lateral, were assessed. The study demonstrated reduced IHA uptake in various regions of the myocardium in asymptomatic angina pectoris patients at rest. Definite deficiencies in tracer uptake were seen in 4 angina pectoris patients who had no infarction or any regional wall abnormalities. Robinson and Lee (1975) reported on the high extraction efficiency of 16-iodo-9-hexadecenoic acid (~70%) and believed its metabolic behaviour reflected that of a true analog of a physiologic, unsaturated fatty acid. Both experimental and clinical studies demonstrated the feasibility of myocardial imaging with ^{123}IHA (Poe et al., 1976a and Poe et al., 1977). Van der Wall et al. (1980) compared the imaging properties of ^{123}I-16-iodo-9-hexadecenoic acid and thallium-201. These authors studied twelve patients with documented coronary artery disease - 8 with acute MI and 4 with unstable angina. The results revealed similar tracer distribution between ^{201}Tl and ^{123}IHA, with the latter demonstrating increased hepatic activity. Van der Wall et al. (1981) studied the value of ^{131}IHA in determining regional myocardial metabolism in a canine model. They found that $t_{\frac{1}{2}}$ values were longer in ischemic segments as compared to normal control values. It has also been found that myocardial uptake of this agent and ^{201}Tl showed a good correlation (Westera et al., 1980). In view of the more optimal physical characteristics of the ^{123}I label, Demaison et al. (1984) utilized this same fatty acid with a ^{123}I tag for intracoronary injection in an isolated rat heart perfused with Kreb's solution. These authors demonstrated changes in fatty acid metabolism induced by the presence or absence of glucose.

Some years earlier, in 1978, Machulla et al. demonstrated that the chain length and site of labeling of a fatty acid would affect its uptake by the myocardium. These authors found that myocardial extraction of omega-halogenated fatty acids with a chain length of 15 carbon atoms or higher occurred more readily than that of alpha-halogenated fatty acids of similar length. The authors performed comparative kinetic studies of accumulation and washout from the myocardium of mice demonstrating a high background level of radioactivity due to free iodide release. The effects of fatty acid chain length on myocardial extraction was examined by Otto et al. (1981). Experiments using six omega-halogenated fatty acids (n = 10, 12, 15, 18, 21, 26), labeled with ^{123}I, showed that chain length influences both uptake and residence time within rat myocardium. Chain lengths of $n \leq 15$ were found suitable for study of beta-oxidation, while $n \geq 19$ were well-suited for myocardial perfusion imaging because of longer residence times within the myocardium.

Currently, nonadecenoic fatty acids are being investigated as potential perfusion imaging agents. Ambrose et al. (1987b,c) have examined the effects of methyl-branching on myocardial retention of terminally iodovinyl substituted fatty acids and have shown that significant differences exist in the metabolic behavior of these agents. Goodman et al. (1987) have demonstrated greater heart uptake, slower myocardial release and higher heart:blood ratios for (E)-19-[^{125}I]iodo-3,3-dimethyl-18-nonadecenoic acid (DMIVN). The latter has been used by Som et al. (1988) to examine the effects of hypertension and verapamil on fatty acid kinetics in rats.

b) Iodinated hexa/heptadecanoic acid

The influence of chain length prompted examination into the possible use of longer chain fatty acids ($n \geq 15$), such as hexa/heptadecanoic acid. Van der Wall et al. (1981) examined the myocardial distribution of ^{125}I-hexadecenoic acid and ^{131}I-hexadecanoic acid. It was reported that myocardial uptake of hexadecenoic acid was 40% lower than hexadecanoic acid. Thus, the latter may have greater scintigraphic value.

The problem of high background activity was addressed by Freundlieb (1978), whose method was utilized by Vyska (1979a,b) at the Institute of Nuclear Medicine, Nuclear Research Center, in the Federal Republic of Germany. These authors assessed myocardial fatty acid metabolism using omega-halogenated-(^{123}I)-hepta-decanoic acid in 20 normal persons, 35 coronary heart disease patients and 3 patients with old myocardial infarctions. The background subtraction technique of injecting a 300μCi bolus of Na^{123}I 20 minutes following the routine scan was employed. The activity released by the myocardium was determined from the background-corrected time-activity curves. Infarcted zones were clearly visible as cold spots and ischemic regions identified as having prolonged $t_{\frac{1}{2}}$ values. The authors concluded that HDA was an excellent radiopharmaceutical capable of non-invasively differentiating normal from pathological myocardium.

Many clinical studies followed this work in an attempt to identify fatty acid (iodinated hexadecanoic acid) metabolic aberrations in various cardiac abnormalities. Freundlieb et al. (1980) examined $t_{\frac{1}{2}}$ values in normal volunteers, patients suffering from CAD and, lastly,

patients with congestive heart failure. These authors were able to identify areas of infarction and ischemia by a reduction in tracer uptake. Examination of $t_{\frac{1}{2}}$ values, however, revealed no significant differences between these three groups. Similar findings were reported by Dudczak et al. (1982a). Van der Wall et al. (1981a) assessed the $t_{\frac{1}{2}}$ values of 30 patients who had suffered a myocardial infarction of less than one week's duration. Washout rates obtained from mono-exponential time-activity curves of infarcted regions revealed significantly lower values compared to non-infarcted areas (16.8 $\pm$ 3.5 compared with 34.8 $\pm$ 7.7 min, respectively). These authors attributed this phenomenon to rapid influx and outflow of tissue fluids within inflamed myocardium and/or an increase in contractility of the subepicardium to compensate for non-functional subendocardium, thus increasing oxygen requirements and utilization of FFA by the subepicardium. The prolonged washout rates associated with reversible ischemia were explained by a shift in metabolic substrate from fatty acids to glucose in 30 patients suffering from stable angina pectoris (Van der Wall et al., 1981b).

Van der Wall et al. (1983) examined the washout rates of ^{123}I-hexadecanoic acid in patients with unstable angina pectoris and found similar results. Washout rates in the ischemic segments (perfusion defects) were significantly prolonged when compared to normally perfused areas (45.4 $\pm$ 4.8 compared with 29.1 $\pm$ 3.6 min, respectively). The prolonged washout rates were reported to be due to myocardial ischemia, which diminishes fatty acid uptake, reduces beta-oxidation and increases triglyceride formation. Freundlieb et al. (1982) examined myocardial washout rates of ^{123}I-hexadecanoic acid in

patients before and after cardiac surgery for coronary artery grafting. Results were variable, in that, after revascularization, $t_{½}$ values increased, decreased or were unchanged. In a similar study, Fridrich et al. (1986) examined the tracer kinetics of ^{123}I-hepta-decanoic acid in patients following aortocoronary bypass grafting compared to non-grafted patients. These authors also assessed ventricular function and correlated it with myocardial metabolism of HDA. It was found that normal function (EF > 50% at rest) was indicative of normal metabolism. Visser et al. (1985) utilized iodo-hepta-decanoic acid to assess myocardial metabolism in patients following successful thrombolysis for myocardial infarction. Beckurts et al. (1985) utilized this agent to examine the kinetics of fatty acid metabolism in normal and diabetic rats. Kuikka et al. (1988) assessed HDA kinetics in patients with non-insulin-dependent diabetes. Villavecchia et al. (1985) examined washout of ^{123}I-hepta-decanoic acid in normal subjects and proposed its usefulness for monitoring myocardial metabolism following the administration of drugs which may cause myocardial toxicity.

Speculation as to the validity of measured myocardial elimination rate (mins) of hepta-decanoic acid were raised by Visser et al. (1985). To this point, many investigators believed that washout rates reflected the process of beta-oxidation. Visser and his colleagues, however, demonstrated that the washout rates of the fatty acid may not be representing beta-oxidation, since five minutes after IV injection, 69% of the radioactivity was present as free iodine, 24% as lipid and 7% as unchanged fatty acid. Thus, washout rates may represent washout

of free iodide. Visser et al. (1986), however, demonstrated that distinct patterns of distribution and elimination rate (mins) of this tracer may allow its use in the study of myocardial metabolism following various metabolic interventions. Luthy et al. (1988) have demonstrated that de-iodination of HDA is representative of oxidative fatty acid metabolism.

The validity of myocardial washout rates of ^{123}I-hepta-decanoic acid, and what it represents, however, continues to be questioned. Schon et al. (1986) believed that the half-time of HDA was not a quantitative assessment of beta-oxidation, since the kinetics differed from those of ^{11}CPA. Also, HDA washout was prolonged with increased MVO_2, which is opposite to what should actually occur. The ratio of the size (y-intercept on T-A curve) of the early phase over the size of the late phase may be a better indicator of myocardial metabolism of HDA (Schon et al., 1986). These authors demonstrated a direct correlation between this ratio and MVO_2. Stoddart et al. (1987) demonstrated the variability of $t_{½}$ values in patients suffering from acute myocardial infarction, who had undergone an HDA scan and a MUGA scan (Multi user-gated-angiographic-EF) within 6 days of infarction. The authors concluded that improvements in imaging and background subtraction techniques are needed before this information becomes useful. Similar conclusions were reported by Railton et al. (1987) as a test for CAD and by Gavin et al. (1987) as an assessment of percutaneous transluminal angioplasty.

c) Iodinated Phenylpentadecanoic acid

The problem of rapid metabolic washout of straight chain fatty acid imaging agents was addressed by Machulla et al. (1980). The authors proposed an alteration in the fatty acid carrier, such that high iodine background activity would be minimized. Machulla and coworkers chose omega-p-^{123}I-phenylpentadecanoic acid (^{123}IPPA) as the imaging agent based on work by Knoop (1904) and Dakin (1909). The result of adding a phenyl group, to which the iodine can bind, was a substantial decrease in free radioiodide release. In mice, it was demonstrated that 4% of injected radioactivity accumulated in tissue with a heart/blood ratio of 20. In rabbits, the image/background ratio was 2 at 20-30 min post-injection. Eisenhut (1982) demonstrated higher uptake and lower background activity with ^{123}IPPA in the rabbit heart, when compared to iodo-hexadecanoic acid. The catabolic end product of ^{123}IPPA metabolism was identified as iodobenzoic acid, which was known to be excreted like hippuric acid. This effectively eliminated any residual ^{123}I from the circulation and limited the background activity. Reske et al. (1984b,c) also examined the differences between ^{123}IPPA and the physiological agent ^{14}C-palmitic acid. In a Langendorff rat heart model, the authors measured rates of production of $^{14}CO_2$ and ^{123}I-benzoic acid. The correlation coefficient between the two by-products was $r = 0.87$. Similar results were reported by Eckhardt et al. (1984). Therefore, although ^{123}IPPA is not a physiological agent, it does appear to adequately reflect the metabolism of fatty acids.

In order to examine the pharmacokinetics of ^{123}IPPA, Daus et al. (1981) utilized mouse myocardium and demonstrated effective extraction. The authors also showed that a portion of the fatty acid was incorporated into the triglyceride pool. Reske et al. (1982a) examined myocardial uptake and washout of ^{123}IPPA from rat myocardium. A maximal uptake of 17% dose/g tissue occurred at 2 minutes post-injection. The absolute uptake of both iodo-hexadecanoic acid and ^{123}IPPA was similar; however, the relative uptake within ischemic myocardial segments was greater for the straight chain analogue than for the phenylated derivative (Westera et al., 1983). The authors believed that two uptake mechanisms existed - passive diffusion and a carrier-mediated diffusion. They hypothesized that the latter becomes less active as ischemia occurs.

The maximal uptake by the myocardium reported by Reske et al. (1982a) was followed by a two component washout curve. Most of the ^{123}IPPA was present as myocardial lipids with <1% dose/g present as free ^{123}IPPA. This finding was also reported by Fuchs et al. (1982). The major catabolic product found in the outflow perfusate of the Langendorff preparation was ^{123}I-benzoic acid (Ercan et al., 1983). Schmitz et al. (1984) utilized a gas-liquid chromatographic-mass spectrometric analysis method to characterize the metabolites formed in the lipid extract of the perfusate as it was removed from the perfused rat heart. Three major metabolites were identified, including omega-p-iodo-phenylpropionic acid, omega-p-iodo-phenylpropenoic acid and, lastly, p-iodobenzoic acid.

Time-activity curves, derived from the washout rates of ^{123}IPPA, following intracoronary administration in patients with valvular heart disease using planar gamma imaging, exhibited a vascular spike at 0-10 seconds, which was followed by a multicomponent washout (Reske et al., 1982c,d). The curve could be divided into 3 slopes exhibiting $t_{½}$ values of 0.24-0.5 min, 6-10 min and 40-60 min. Similar findings were reported by Reske et al. (1984a) in which patients with CAD were studied. Analysis of blood radioactivity at 1-5 minutes post-injection revealed that 50-80% of the activity was in the form of ^{123}I-hippuric acid and ^{123}I-benzoic acid. Reske et al. (1982a,d) also reported that 25-30% of ^{123}IPPA was extracted by normally perfused myocardium at every blood passage. Kulkarni et al. (1985) obtained clear myocardial images from 4 to 40 min following the injection of ^{123}IPPA at rest and after peak exercise in normal volunteers with no adverse effects.

Following this work, many studies were performed, examining the potential of ^{123}IPPA in analyzing changes in fatty acid washout in various pathological states. In patients suffering from CAD, Dudczak et al. (1982b) assessed myocardial washout rates of ^{123}I-HDA and ^{123}IPPA. They found that both agents exhibited biexponential curves and that halothane anesthesia prolonged $t_{½}$ values. In a later study, Dudczak et al. (1983a) found $t_{½}$ values of 69.3 min, which were in accordance with the values reported for patients with CAD from his earlier work. The para isomer was also shown to have a decreased myocardial/background ratio relative to the ortho isomer (1.50 vs. 1.96, respectively). Machulla et al. (1986), however, reported

decreased myocardial uptake for the ortho isomer. Kaiser et al. (1988) have demonstrated that o-^{123}IPPA is retained in human myocardium with long retention times relative to p-^{123}IPPA. A number of other studies have been performed examining fatty acid washout rates in patients with CAD, including Vyska et al. (1987), Davies et al. (1987) and Reske et al. (1987).

Patients suffering from cardiomyopathy have also been assessed using this type of analysis. Dudczak et al. (1982b) demonstrated extremely prolonged $t_{½}$ values in such patients showing biexponential labeled-fatty acid washout curves. These cardiomyopathic patients exhibited gross, irregular activity, with fatty acid washout rates of 43-150 min (Dudczak et al., 1983b). Knapp et al. (1987) examined fatty acid myocardial extraction in cardiomyopathy patients and 4 normal subjects. The cardiomyopathy patients were subdivided into dilated cardiomyopathy (DCM) (n = 9) and hypertrophic cardiomyopathy (HCM) (n = 11). While undergoing fasting ergometry, patients were injected with ^{123}IPPA IV. The patients suffering from DCM had extraction fractions (EF) 10% below normal and in the HCM group, EF values were 50% below normal in the septum and 12% below normal in the posterolateral wall.

Animal studies assessing myocardial washout rates of ^{123}IPPA have produced similar findings as described above. Dudczak et al. (1982b) found biexponential washout curves for ^{123}I-HDA and ^{123}IPPA in calves and prolonged washout rates following coronary artery occlusion. Reske et al. (1988) examined ^{123}IPPA uptake in a coronary occlusion (40 min)/reperfusion (30 min) Langendorff swine heart model. Cardiac ^{123}IPPA uptake was found to be preserved in viable tissue

following reversible ischemia. Clearly, ^{123}IPPA shows potential as a myocardial imaging agent capable of assessing metabolism as well as flow.

Since perfusion directly influences ^{123}IPPA availability, the relationship between myocardial blood flow and uptake of ^{123}IPPA must be addressed (Reske et al., 1984d). Regional myocardial blood flow (RMBF) was found to be 90-120ml/min/100g in control animals with a total cardiac uptake of ^{123}IPPA of 4.5-6.0% of the injected dose. In order to determine the relationship between the two, correlation analysis was performed. It revealed a close correlation ($r = 0.94$) between RMBF and ^{123}IPPA uptake under control conditions and as well as under conditions of acute ischemia where RMBF = 20-50 ml/min/100g. At increased flow rates, such as those seen with pacing, these authors found only a moderate increase of ^{123}IPPA uptake. It was also noted that a plateau or upper limit existed at 150-170 ml/min/100g and that exceeding this level resulted in a fall in ^{123}IPPA uptake. Schoen et al. (1984) reported similar findings with limited uptake at higher flow rates. Caldwell et al. (1987) determined whether ^{123}IPPA uptake at high flow rates could reflect accurately RMBF. The authors compared ^{123}IPPA uptake and microsphere deposition during treadmill exercise in 6 chronically instrumented dogs. While the animals were running, the coronary artery was occluded and the agents injected into the left atrium. It was found that ^{123}IPPA could be used to accurately assess RMBF.

An interesting finding with major implications is the inhibition of ^{123}IPPA or fatty acid metabolism by lactate (Eckardt et al., 1984).

Reske et al. (1986) also reported that lactate in high concentrations decreased fatty acid oxidation in canine hearts and Langendorff rat hearts. Duwel et al. (1987) examined the effects of sodium lactate infusion before scintigraphy in patients suffering from CAD. Similar $t_{1/2}$ patterns were shown for the lactate intervention group and ischemia group, ie. inhibition of fatty acid oxidation. Duwel et al. (1988) continued this work and found that not only does lactate inhibit FFA oxidation under ischemic conditions, but it also does so under normal conditions. This could have particularly profound effects during anaerobic states or ischemic conditions.

In view of its apparent success in the assessment of myocardial metabolism, some investigators have attempted to assess cardiac geometry using ^{123}IPPA and SPECT imaging. Reske et al. (1982d) obtained quality images, visualizing myocardial infarcts as areas of reduced ^{123}IPPA uptake. Examination of kinetics revealed marked prolongation of $t_{1/2}$ values within infarcted dogs as compared to control animals (202 $\pm$ 65 compared to 41.5 $\pm$ 7.1 min, respectively). No attempt was made in this study to quantitate the myocardial infarction in terms of tissue volumes.

Rellas et al. (1983) conducted a similar experiment in which uptake and washout of ^{123}IPPA under control conditions and after permanent and temporary coronary artery occlusion in dogs were measured. Infarcted myocardial segments with fixed LAD closure exhibited reduced uptake. In dogs with temporary occlusion followed by reperfusion, myocardial uptake was only slightly decreased compared to normal myocardial segments. The zones of infarction estimated by

examining ^{123}IPPA kinetics correlated closely with zones of infarction demarcated by tetrazolium staining. It was noted, however, that there was a rapid initial washout from the myocardium which was probably due to beta oxidation. Kiess et al. (1987) also could differentiate between areas of infarction and of stunned, but viable, myocardium by ^{123}IPPA washout rates. Schwaiger et al. (1987) examined the metabolic consequences of short periods (30 min) of myocardial ischemia. The outcome was an increase in the relative uptake of ^{18}F-deoxyglucose, even though normal myocardial blood flow was reinstituted.

Pippin et al. (1987) demonstrated that, in patients with CAD, ^{123}IPPA is more sensitive than thallium in the detection of reversible abnormalities, but was equivalent to thallium for detection of coronary artery stenosis.

d) Miscellaneous Fatty Acids

Straight-chain fatty acids and the iodophenyl fatty acids have been shown to suffer from rapid initial washout, which decreases image quality over time. Thus, the benefits of increasing myocardial residence time can be appreciated when an attempt is made to assess perfusion defects, in particular, myocardial infarction. Such an assessment may have important clinical significance and influence patient management and therapeutic interventions. The utilization of radionuclides to assess myocardial perfusion defects has been attempted by many groups. Conventional imaging systems (ie. planar imaging) have attempted to compress the 3-dimensional image of the heart onto a 2-dimensional plane. Keyes et al. (1978) believed that to accurately

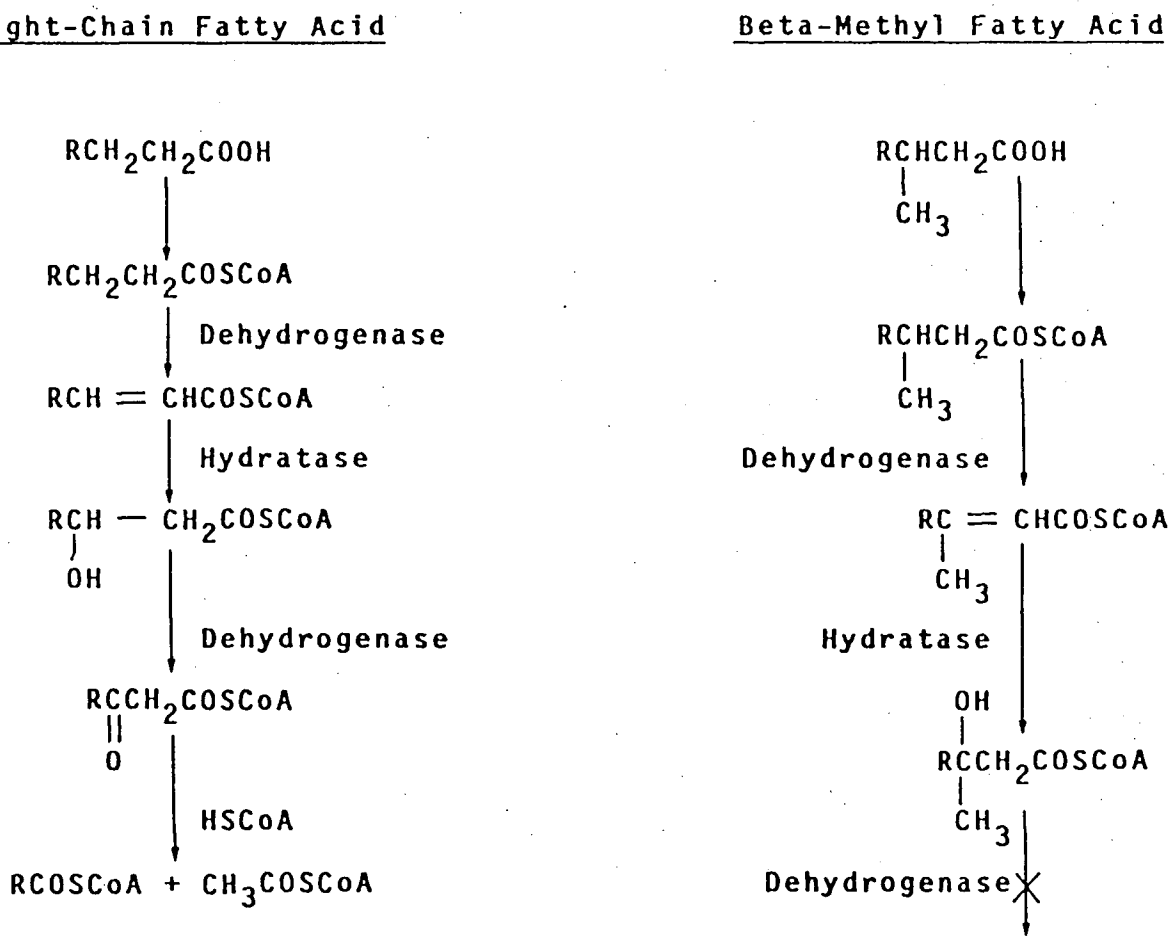
quantify these perfusion defects, a method was required which would display 3-dimensional information. Results from their work demonstrated the location of the myocardial infarction in all three planes (x, y and z). The utilization of SPECT offered distinct advantages over conventional planar imaging when attempting to quantitate perfusion defects (Caldwell et al., 1984).

One method of radiopharmaceutical development, (proposed 34 years ago in 1954), which would aid in perfusion imaging, was metabolic trapping, (Gallagher et al., 1978). By substituting a fluorine atom for a hydroxyl group at carbon 2 of deoxyglucose, Gallagher showed a reduction in tissue washout rates. One attempt at metabolic trapping was performed by Goodman et al. (1982). The introduction of a tellurium heteroatom onto carbon 6 of 15-p-iodophenylpentadecanoic acid blocked beta-oxidation and increased the myocardial residence time. This agent also demonstrated rapid myocardial uptake and low in vivo deiodination. A similar study using a new tellurium-iodinated-phenylpentadecanoic acid was performed showing minimal deiodination and prolonged retention times in dogs and cats (Goodman et al., 1985). However, problems with toxicity and stability of the tellurium heteroatom in the presence of oxygen has dramatically limited its clinical usefulness (Machulla et al., 1986). Efforts are continuing towards the development of a tellurium fatty acid analogue for perfusion studies (Srivastava et al., 1987).

Alternatively, Livni et al. (1982) hypothesized that a labeled straight-chain fatty acid that is partially metabolized and trapped within the myocardium by introducing a methyl group at the beta carbon

may offer an ideal perfusion imaging agent. In their scheme, beta-methyl[^{11}C]-heptadecanoic acid was utilized with the beta-oxidation sequence being halted prior to the formation of the corresponding beta-keto-acyl CoA (Figure 5).

Figure 5 - Metabolic trapping scheme


Straight-Chain Fatty Acid
Beta-Methyl Fatty Acid
RCH2CH2COOH
RCH2CH2COSCoA
Dehydrogenase
RCH = CHCOSCoA
Hydratase
RCH — CH2COSCoA
OH
Dehydrogenase
RCCH2COSCoA
O
HSCoA
RCOSCoA + CH3COSCoA
RCHCH2COOH
CH3
RCHCH2COSCoA
CH3
Dehydrogenase
RC = CHCOSCoA
CH3
Hydratase
OH
RCCH2COSCoA
CH3
Dehydrogenase

The radioactivity of this agent in both rats and dogs showed very little decrease over time (2.3% dose/g tissue at 5 minutes to 2.7% dose/g tissue at 60 minutes). Thus, the fatty acid was extracted but metabolism halted with the radioactive label remaining within the myocardium. The mechanism of myocardial retention was also suggested to be steric and/or chemical inhibition from the methyl group (Goodman et al., 1984 and Livni et al., 1985). Abendschein et al. (1984) also utilized ^{11}C-beta-methyl-heptadecanoic acid in open-chest dogs following LCX marginal branch occlusion and reperfusion. Extraction fractions were calculated (25 $\pm$ 5% in normal and 45 $\pm$ 15% in ischemic zones) and it was noted that 5% of the extracted agent was oxidized in normal zones, while <1% was oxidized within ischemic zones. Blocking the beta-oxidation sequence results in minor ventricular dysfunction, including decreased LVSP and % systolic wall thickening (Wijns et al., 1985). Metabolic alterations also ensued, including decreased MVO_2, a 60% decrease in FFA utilization and an 18% increase in glucose and lactate usage (Wijns et al., 1985). The latter would increase the myocardial retention of fatty acids and increase their time spent within the myocardium, thereby improving image quality when scanning.

Since Goodman et al. (1982) first suggested the potential value of phenylpentadecanoic acid with beta-methylation, some investigators have extended this work. Miller et al. (1985) examined the metabolic activity in a normal zone (posterior wall) and border and central ischemic zones of canine myocardium following LAD occlusion/reperfusion and scanning of closed chest dogs. The metabolic behaviour of normal,

border ischemic and central ischemic (partially infarcted) zones differed. The latter two areas were significantly different from normal zones, with $t_{½}$ values within the ischemic zones being significantly prolonged relative to the control zones (115.6 ± 29 compared to 86.5 ± 3 min, respectively). Knapp et al. (1986a,b) report that methyl substitution considerably increased the myocardial half-lives in fasted rats: iodophenylpentadecanoic acid (IPP), 5-10 min; beta-methyl-iodophenylpentadecanoic acid (BMIPP), 30-45 min; dimethyl-iodophenylpentadecanoic acid (DMIPP), 6-7 hrs. De Grado et al. (1987), in a working isolated rat heart model, demonstrated higher myocardial retention times for beta-methyl-IPPA compared to IPPA.

In order to further understand these differences in myocardial retention, Ambrose et al. (1987a) examined subcellular distribution patterns of these agents. The dimethyl analogue was found predominantly in the mitochondria and microsomes (34% and 38%), while IPP showed little accumulation within these organelles (18% and 15%). In an earlier study, Otto et al. (1985a) reported an accumulation of 13.4 ± 1.7% and 8.3 ± 1.1% of IPP within mitochondria and microsomes, respectively. The beta-methyl analogue, however, showed 13.7 ± 0.3% and 3.7 ± 0.2% accumulation, respectively, with retention of activity over time. Dudczak et al. (1986) assessed the metabolic behavior of B^{123}IPPA in patients suffering from CAD and acute MI. From plasma and urine samples, benzoic and hippuric acids were identified as the major metabolic byproducts using TLC.

In order to determine the clinical usefulness of such an agent, investigators have compared the currently used perfusion imaging agent,

^{201}Tl with the beta-methyl-fatty acid. Okano et al. (1987) measured uptake and release of B^{125}IPPA, ^{125}IPPA and ^{201}Tl in cultured myocytes from mouse embryo. These values were compared to intracellular ATP content after metabolic inhibition by cyanide (CN) or 2-deoxyglucose (2-DG). The latter decreased B^{125}IPPA uptake although it did not affect its release. CN had no effect on uptake or release of B^{125}IPPA. The correlation of B^{125}IPPA with intracellular [ATP] was high with $r = 0.89$ ($p<0.05$). The correlation of ^{201}Tl to [ATP], however, was poor with an $r = 0.53$ (pNS). Thus, fatty acids offer the distinct advantage of being an indicator of both myocardial metabolism and perfusion.

In canine studies examining the differences of beta-methyl fatty acids and ^{201}Tl, Kairento et al. (1988) reported that both agents are suitable for detecting infarction (seen as decreased activity within the infarcted tissue). The ^{201}Tl consistently overestimated the size of the perfusion defect. Redistribution was quoted as another inaccuracy with the use of ^{201}Tl. Fischmann et al. (1988) examined the possible benefits of combined fatty acid and thallium imaging 7 days after acute ligation of LAD in dogs. These authors found that in zones of decreased perfusion, fatty acid accumulation occurred in 7 of 7 animals. Therefore, it appears that within areas of decreased RMBF, fatty acids continue to be taken up by the myocardium.

Although animal studies seem to indicate the usefulness of combining fatty acid with thallium for imaging of myocardial infarction, clinical studies were needed to optimise its usefulness.

Patients suffering from severe myocardial ischemia (12 pts - unstable angina (n = 2) and acute MI, who underwent thrombolysis with transluminal percutaneous angioplasty (n = 10)) were imaged using thallium and fatty acid (Strauss et al., 1987). From these studies, variability in uptake was noted based on the degree of coronary stenosis. Three patterns emerged: FFA uptake >>Tl in severe stenosis with some flow or good collateral circulation (n = 6); FFA = Tl in totally occluded or severe stenosis with minimal flow; and FFA < Tl in reperfused myocardium supplied by vessels with marked residual stenosis but with normal ^{201}Tl distribution at rest (n = 4). Thus, both FFA and Tl used in combination may indeed supply more information than if the agents are used singly.

With the discovery of metabolic degradation of mono-methylated fatty acids, it was postulated that dimethylated analogues may be better able to remain within the myocardium (Machulla et al., 1986; Knapp et al., 1986b). Myocardial retention times were reported for non-methylated, monomethylated and dimethylated-IPPA analogues, with the latter showing the longest times within the myocardium of rats. High heart/blood ratios were reported for all three agents; however, the 4,4-DMIPP was shown to have the highest heart uptake and best heart/blood ratio. These findings vary, however, since 3,3-DMIPP has also been shown to have the longest retention time in rats.

Investigations continue in an attempt to identify the ideal imaging agent. Jones et al. (1988) injected 1-[^{11}C]-3,3-dimethyl-heptadecanoic acid in fasted rats. The agent showed moderate uptake with considerable washout of activity from the myocardium over the

initial 30 minute post-injection period. In a canine study, uptake was again low with major localization within the lung. These data suggest that dimethyl substitution at the beta-position may be inadequate, due to deficiencies in myocardial uptake and retention. Demaison et al. (1988), using 16-(^{123}I)-iodo-3,3-methylhexadecanoic acid injected directly into the coronary artery of isolated rat hearts, demonstrated that mono-beta, di-alpha and di-beta-methylated iodinated fatty acids are suitable agents for studying cellular uptake because of their relatively low oxidation rates. Other perfusion imaging agents being investigated are the iodo-vinyl long chain fatty acids, (Ambrose et al., 1987b,c; Som et al., 1988). However, there is currently a lack of an ideal imaging agent.

(4) ISCHEMIA AND CARDIOPLEGIA

Ischemia-induced changes have been implicated in the post-operative ventricular dysfunction associated with coronary artery disease (CAD) (Jamieson et al., 1983). The subcellular and cellular changes associated with ischemia are diverse, affecting transmembrane ion balance, mitochondrial activity, ATP production and oxidative metabolism (Trump, 1982). In order to mitigate these diverse changes, cardioplegic techniques rely on conservation of high energy stores (rapid diastolic arrest), slowing of metabolic rate (hypothermia) and combatting the deleterious subcellular and cellular consequences of ischemia through the use of protective additives.

There are two basic cardioplegic formulations: 1) those which mimic extracellular ion concentrations; and 2) those with intracellular ion concentrations (Hearse et al., 1986). The former can be either crystalloid potassium solution or blood-based. Most extracellular solutions contain Na^+ and Ca^{++}. The intracellular solutions contain no Na^+ or Ca^{++}. Each type of solution has advantages and disadvantages which must be considered when a choice between them is made. Experience with the solution by the surgeon will usually determine the choice of which solution is used.

An important aspect of myocardial protection which must be addressed is reperfusion injury. Two critical factors involved in the latter phenomenon is the accumulation of Ca^{++} and the generation of oxygen free radicals (Shen and Jennings, 1972; and Guarnieri, 1980). Shen and Jennings have reported on the explosive uptake of Ca^{++}, on reperfusion following 40

mins. of ischemia, with an almost 10-fold increase above normal tissue Ca^{++} levels. Indeed, many researchers have attempted to control this large influx of Ca^{++} into the tissues following reperfusion by the addition of calcium entry blockers, such as verapamil, nifedipine or diltiazem, and have achieved some degree of success. These agents block calcium receptor sites, thus limiting the amount of this ion which enters the cells. Although appearing beneficial, altering Ca^{++} movements within the tissue may also be dangerous, since Ca^{++} ions mediate membrane excitation and contraction. Therefore, calcium entry blockers are not without risks.

Guarnieri (1980) proposed that the etiology of reperfusion injury was related to re-oxygenation of the myocardium. It is now believed that myocardial damage associated with reperfusion injury is produced by cytotoxic metabolites of oxygen, which include superoxide anion, hydrogen peroxide and free hydroxy radical (Subramanian et al., 1987). The effects of these oxygen free radicals are widespread. They cause alterations in membrane phospholipids by peroxidation, causing an increased release of malondialdehyde (Subramanian, et al., 1987), myocardial edema because of enhanced membrane permeability (Guarnieri, et al., 1980), decreased mitochondrial activity and increased cellular permeability (Fox, et al., 1985).

Cardioplegia provides a vehicle through which protective agents, which can counteract some of these changes, can be administered and assessed. The solution utilized in this study was iso-osmolar Tyers solution, which was first prepared by Tyers et al. (1974). In 10 canine experiments, 60 minutes of hypothermic ischemic arrest using coronary perfusion with iso-osmolar

solution resulted in normal function and only mild perivascular edema on histopathological assessments. The solution was first used clinically by Tyers et al. (1977) and showed only moderate protection. Of recent interest is the inclusion of enzymes to scavenge or block cytotoxic oxygen metabolites (McCord, 1985). Studies have been performed utilizing allopurinol (which blocks the generation of hydroxyl radicals as a result of inhibition of xanthine oxidase), superoxide dismutase (which breaks down superoxide anion) and catalase (which reduces H_2O_2) in an attempt to minimize the damage induced by oxygen free radicals (Ambrosio et al., (1987), Das et al., (1987a,b), Godin et al., (1986), Myers et al., (1986)). A great deal of literature is currently available which examines the functional consequences of global ischemia. A number of studies are available which examine the fate of free fatty acids, the primary energy-generating substates of the myocardium, following global ischemia, and the cardioprotective benefits of L-carnitine derivatives (Paulson et al. (1986), Regitz et al. (1987), Ferrari et al. (1988)). However, the precise mechanism of these cardioprotective effects are still unknown.

The field of cardioplegia is a dynamic one with many controversies still being debated. These include the benefits of oxygenated vs. non-oxygenated solutions (Tabayashi et al., (1988), de Wit et al., (1988), Ledingham et al., (1988)), blood vs. crystalloid solutions (Khuri et al., 1988), the provision of various additives and their potential benefits (Greenfield et al., 1988), and a number of procedural questions, such as rate and method of delivery of solution, as well as the optimal temperature (Rousou et al., 1988).

III PURPOSE

As has been illustrated, the prevalence of cardiovascular disease in today's society necessitates research into the reduction of mortality rates and the improvement of assessment techniques. Both diagnostic tests and evaluation of current techniques are required. Current diagnostic tests, aimed at assessing myocardial ischemia and infarction in clinical nuclear medicine, lack an ideal radiopharmaceutical. Iodinated (^{123}I) free fatty acids may satisfy the optimal requirements for the ideal imaging agent for myocardial ischemia and infarction (both as a diagnostic tool and method of evaluation). Therefore, the objectives of this thesis are three-fold:

1) to evaluate the effect of reversible global ischemia in a canine model on myocardial metabolism of 15-p-(^{123}I)-iodophenyl-pentadecanoic acid ($^{123}IPPA$);
2) to assess the longitudinal effect of progressive regional ischemia on myocardial metabolism of $^{123}IPPA$ in a canine model; and
3) to utilize $^{201}Thallium$ and Beta-methyl-$^{123}IPPA$ ($B^{123}IPPA$) for assessing perfusion defects and to determine their ability to identify the ischemic risk zone in a canine model.

IV EXPERIMENTAL MATERIALS AND METHODS

(1) Global Ischemia

(a) Experimental Model of Cardioplegia

Adult dogs (17-28 kg) were fasted overnight and anaesthetized with sodium pentobarbital (25 mg/kg) IV. Supplemental sodium pentobarbital was given as necessary throughout the procedure. The dogs were intubated and maintained on a pressure-limited ventilator (Mark-7 Respirator - Bird Corporation). A standard median sternotomy was performed. A catheter was placed into the right internal mammary artery to monitor blood pressure. Polyethylene catheters were inserted into the left atrium and right atrium to monitor atrial pressures. A 7-French Swan-Ganz thermodilution catheter was inserted into the pulmonary artery. The catheters were attached to standard transducers to monitor pressure. The ECG, heart rate and pressures were displayed on a twelve-channel oscilloscope with concurrent digital readout and recording capabilities (SE Laboratories, England; John Fortin Mfg. Ltd., Canada). Rectal and myocardial temperatures were monitored continuously. The animals were placed on cardiopulmonary bypass (CPB), with oxygenator support, caval occlusion and left atrial venting. The dogs received 0.5 mg/kg heparin prior to initiation of CPB. The CPB pump was primed with donor dog blood.

(b) Protocol

Following cardiac cannulation, control measurements were recorded. These included heart rate, arterial blood pressure, pulmonary

artery pressure and left and right atrial pressures. Cardiac outputs were measured using a cardiac output computer and cold saline injections into the right atrium. The heart was subjected to two hours of ischemic arrest at a myocardial temperature of 15-19°C. Cardioplegic solution was administered intermittently to maintain myocardial temperature at the desired range. Acid-base status was assessed by periodic blood-gas analysis. Systemic temperature was maintained at 30°C by the CPB unit.

Three groups of experiments were performed. Cardioplegia was initiated with iso-osmolar solution (310 mOsm/L, K^+ 25 meq/L, Mg^{+2} 3 meq/L, Ca^{+2} 2 meq/L and Na^+ 144 meq/L). In Group A (n = 5) iso-osmolar solution was utilized without additions. In Group B (n = 7), superoxide dismutase (SOD - Carlsberg Biotechnology, Copenhagen, Denmark) was administered at 6.5 mg/kg. dissolved in saline. One third of the total dose (110-182 mg) was added to the final delivery of cardioplegia and the remaining dose administered systemically IV during the first five minutes of reperfusion. In Group C (n = 7) allopurinol (Wellcome Foundation Ltd., London, England) was administered at 40 mg/kg with the majority (95% of total dose) being added systemically IV and the remainder (5% of total dose) to the final infusion of cardioplegic solution. Cardioplegic solution temperature was maintained at 8-10°C and infused through an aortic cannula connected to a CPB circuit pump.

The final infusion of cardioplegia was administered twenty minutes prior to removal of the aortic cross-clamp and reperfusion. Systemic rewarming was also initiated at the final infusion of cardioplegic solution. Following removal of the aortic cross-clamp and release of caval occlusion, the heart was defibrillated if necessary. Throughout the

cross-clamp period, aortic pressure was maintained above 60 mmHg through the infusion of cardioplegic solution at a flow rate of 100mL/kg/min. Periodic blood-gas analysis was performed during the post-CPB period and sodium bicarbonate was administered, if required. Hemodynamic assessments were performed thirty minutes after weaning from CPB.

(c) Preparation of 15-p-^{123}I-iodophenylpentadecanoic acid (^{123}IPPA)

(i) Radioiodine. - Radioiodine (^{123}I)NaI was obtained through the ^{124}Xe (p,2p) ^{123}I (26MeV) direct reaction and ^{124}Xe (p,pn) ^{123}Xe ^{123}I (26MeV) indirect reaction (Atomic Energy of Canada Ltd., Vancouver, B.C.) with a radionuclide purity greater than 99.9% at calibration time.

(ii) Production ^{123}IPPA - ^{123}IPPA was produced by a method similar to that of Kulkarni and Parkey (1982). Thallation was carried out in a 1.0 ml. reactivial using 0.75 mg. PPA and 3.0 mg. thallium tris trifluoroacetate [$Tl(TFA)_3$] dissolved in 225 μL trifluoroacetic acid (Chien et al., 1983). The mixture was allowed to proceed at room temperature for one hour. To the in situ parathallium complex was added (^{123}I)NaI (10mCi) and 3μL KI solution (6 mg/ml in distilled H_2O). This was allowed to react for one hour at 58-60°C and resulted in iodo-dethallation forming ^{123}IPPA.

(iii) Hexane Extraction - The ^{123}IPPA was extracted from the reaction mixture into 1 ml. of hexane and washed serially three more times (1ml hexane/extraction). The activity was measured in a well-counter throughout the procedure with the majority (>80%) occurring in the initial two vacutainers.

(iv) Purification - Purification of the ^{123}IPPA was completed using a small SiO_2 column (Waters SiO_2 Sep-Pak PIN 51900) and elution with 2.5% methanol in methylene chloride. The ^{123}IPPA fractions were collected and the solvent evaporated.

(v) Preparation of ^{123}IPPA for Injection - Following evaporation of the solvent using a nitrogen stream, the ^{123}IPPA was dissolved in 200μl 99% ethanol and suspended with sonication in 3 mls. 6.0% human serum albumin. The product was filtered using a 0.22μm millipore filter to remove any contaminant particulate matter prior to injection.

(vi) Quality Control - Thin layer chromatography was carried out on silica gel plates (Baker flex 1B2-F) with 2.5% methanol in methylene chloride as the solvent. The origin was spotted with 10-20μls methanol. Ten μls of IPPA (quality control) was then applied to the origin and subsequently the sheets were developed. The R_f value for ^{123}IPPA is 0.6 with the iodide remaining at the origin and any impurity travelling with the solvent front. Two impurities which were encountered included benzoic acid (at solvent front) and free ^{123}I (R_f = 0.0).

(d) Electron Microscopy

(i) Tissue Collection - Punch biopsies of the left ventricle were taken for electron microscopic examination prior to sacrifice, providing ultrastructural evidence of any ischemic changes. The samples were immediately placed in cold glutaraldehyde and refrigerated for 12-15 hrs (overnight). The glutaraldehyde-fixed samples were then transferred to a 0.1M cacodylate buffer solution, in which the sample can be stored for several years.

(ii) Processing - Following the completion of the series, glutaraldehyde-fixed specimens were cut into 1-2 mm cubes, with an unused razor blade. These cubes were fixed in 1% osmic acid for 1½ hours at 4°C. The samples were then washed 3 times with distilled water at 5 minute intervals. The sample was then stained with 5% aqueous uranyl acetate for 15 minutes at 37°C, followed by rinsing with distilled water and dehydration in increasing concentrations of alcohol and finally propylene oxide. The final mixture for dehydration was 50% propylene oxide and 50% effapoxy mixture, in which the sample was incubated, closed to air, for 3½ hours and then exposed overnight to air. The samples were then embedded in fresh 100% effapoxy mixture in Beem capsules. The Beem capsules were incubated overnight at 37°C and at 60°C for 48 hours.

(iii) Sectioning - Using a microtome, 1 μm thick sections were cut from the Beem capsule and stained with 1% toluidine blue in 1% borax for 30 seconds on a hot plate. These sections were placed on slides and examined under light microscopy. A suitable section was chosen and that block was then cut into thin sections (60-100 nm) using an ultramicrotome. These sections were placed on grids and stained with Reynolds lead citrate for 8 minutes in a covered Petri dish containing NaOH pellets. The final sections were then examined under the electron microscope.

(e) Light Microscopy

Following sacrifice, the hearts were excised and samples of the right ventricle, septum and left ventricle taken for light microscopic examination. Small sections of tissue were cut with a scalpel and then

placed into tissue sample boxes and passed through a Histomatic Tissue Processor. This machine automatically passes the sample through a series of dehydration steps in progressively increasing concentrations of alcohol, followed by clearing in xylol and finally impregnation and embedding in wax. The blocks were then cut into thick sections using a microtome and placed onto a slide. The slides were then stained with hematoxylin and eosin, which stained nuclei blue because of their basophilic nature and cytoplasm and connective tissue shades of pink.

(f) Data Acquisition

(i) Imaging - The animals were fasted overnight and anaesthetized with sodium pentobarbital (25 mg/kg). Supplemental sodium pentobarbital was given if necessary. Planar gamma imaging was performed with a Picker International Data Mo^{+m}-Mobile camera (fitted with a low energy all-purpose collimator) and a mobile computer terminal (ADAC-Cam II). The anterior view of the dog was imaged following the injection of 3-5 mCi of ^{123}IPPA. The scans were performed one week pre-operatively (control) and two and one-half hours post-operatively. Images were collected on a 128 x 128 x 8 matrix over a 30 minute period, commencing immediately after injection. Frames were collected every five seconds over the first 2 minutes and every 1 minute from 2 to 30 minutes.

(ii) Blood-Gas Analysis - Blood gases were assessed using a Corning 175 Automatic pH/Blood Gas System (Corning Medical, Medfield, MA). Blood samples were injected into the system through the intake port. The system measured pH, pCO_2, pO_2 and barometric pressure directly. The

system also calculated bicarbonate, base excess, oxygen content and oxygen saturation based on the measured values listed previously. A printer, which is incorporated into the machine, recorded the sample data.

(g) Data Analysis

(i) Image Analysis - Image analysis was performed on an ADAC Laboratories Computer (Cam II). The five second frames were summed into one minute images. Areas of interest were created around the superior vena cava (background), lateral wall, apical wall, septal wall, and the total heart. These compressed data were utilized for numerical data manipulations. The data were normalized to peak counts and the background (SVC) was subtracted. These numerical data were then transferred to an IBM-PC. Time-activity curves were generated using a program supplied by RS/1 (Research System/1: BBN Software Products Corp., Cambridge, MA). This same program was utilized to analyze the curves using a biexponential least squares fit with curve peeling, resulting in a best fit line and equation for each curve. ^{123}IPPA half-lives ($t_{½}$) were calculated from the slopes of the lines.

(ii) Statistical Analysis - Hemodynamic assessments are reported as mean $\pm$ SD and paired Student T-Tests were used to test for any significant differences between control and post-operative values.

^{123}IPPA half-lives ($t_{½}$) are reported as mean $\pm$ SD and a Wilk-Shapiro test for normality was performed on the percent change between control and post-operative values. Changes in $t_{½}$ between groups were then subjected to an F-test for testing the homogeneity of the variance. If the data were

homogenous, a pooled variance estimate T-test was applied. If the data were not homogenous, a separate variance estimate T-test was applied. Testing the data with a Mann-Whitney test produced similar statistical results. Changes between control and post-operative values of $t_{\frac{1}{2}}$ within groups were assessed using a Student Paired T-test. Control measurements of $t_{\frac{1}{2}}$ were pooled for each individual area (lateral wall, apical wall, septal wall) and any differences were analyzed for by ANOVA.

(2) Regional Ischemia

(a) Experimental Model of Regional Ischemia

The study group consisted of twelve canine experiments. Adult dogs (17-33 kg) were fasted overnight and anaesthetized with sodium pentobarbital (25 mg/kg) IV. Supplemental sodium pentobarbital was given as necessary. The dogs were intubated and maintained on a pressure-limited ventilator (Mark-7 Respirator-Bird Corporation). The heart was exposed via a fourth intercostal space thoracotomy. The ECG and heart rate were displayed on a 12-channel oscilloscope with concurrent digital readout and recording capabilities (SE Laboratories, England; John Fortin Mfg., Ltd., Canada). Approximately 1.0-2.0 cm of the left anterior descending coronary artery was dissected out distal to the bifurcation with the left circumflex coronary artery. Vessiloops were utilized to extend the artery and an ameroid constrictor (Three Points Products, Montreal, Quebec, Canada) of appropriate size was positioned. Atrial pacing wires were implanted and directed to a subcutaneous pocket lying at the base of the neck of the animal. The

pericardium was closed following 30 minutes or when the animal was stabilized. The incision was then closed and the animals returned to the holding facilities. The animals were given Derapen, a penicillin, IM, once a day for 3 days post-operatively. The animals were sacrificed two weeks post-operatively, immediately after the final scan.

(b) Metabolic Assessment

Metabolic blood analysis was performed on venous blood samples acquired pre-operatively and 24 hrs, 5 days, 7 days and 14 days post-operatively. Samples were apportioned into appropriate vials for analysis. A Dupont Automatic Clinical Analyzer (ACA) (Dupont Co., Clinical Systems Division, Wilmingdon, DE) was used to quantitate lactate levels. Lactate dehydrogenase and creatine kinase activity in blood samples were analyzed on a Cobas Bio spectrophotometric Analyzer. Each enzyme has a specific test pack, ie. lactate - LA pack, LDH - Beckman Dri-STAT LDL Reagent pack and CK - Beckman Dri-STAT optimized CK-NAC Reagent pack. To determine lactate levels, the appropriate analytical test pack and sample are loaded into the ACA. This machine automatically advances the pack through the test procedure and prints out the results. Lactate dehydrogenase and CK determinations require a minor amount of operator preparation. Using the appropriate packs, the reagents must be prepared. The sample tray can then be loaded appropriately. The appropriate test key is selected and the parameter list will be printed out.

(c) Preparation of 15-p-^{123}I-iodophenylpentadecanoic acid (^{123}IPPA)

(i) Radioiodine - Method the same as in Global Ischemia section.

(ii) Production ^{123}IPPA - Method the same as in Global Ischemia section.

(iii) Hexane Extraction - Method the same as in Global Ischemia section.

(iv) Purification - Method the same as in Global Ischemia section.

(v) Preparation of ^{123}IPPA for Injection - Method the same as in Global Ischemia section.

(vi) Quality Control - Method the same as in Global Ischemia section.

(d) Data Acquisition

(i) Imaging - Imaging protocol the same as in Global Ischemia Section. The scans, however, were performed one week pre-operatively and at 6 hrs., 5 days, 7 days and 14 days post-operatively. The latter two time assessments were performed with atrial pacing. The pacing wires were exposed from the subcutaneous pocket. A Medtronic pacing unit (Medtronic Medical Inc., Los Angeles, Ca) was used. Hearts were paced at a rate of 185 beats/min.

(ii) Blood-Gas Analysis - Blood gas analysis was performed on a Beckman model 175 Automatic pH/Blood Gas System (Corning Medical, Medfield, MA) as described in Global Ischemia Model earlier.

(e) Electron Microscopy

(i) Tissue Collection - Punch biopsies of the regional myocardial bed were taken prior to sacrifice for electron microscopic examination, providing ultrastructural evidence of any ischemic changes. A biopsy was taken from the non-ischemic area proximal to the ameroid constrictor, as a control specimen, and a specimen also taken from the ischemic area of the left ventricle distal to the ameroid constrictor. Remaining procedures were identical to those described in the Global Ischemia section.

(ii) Processing - As reported in Global Ischemia section.

(iii) Sectioning - As reported in Global Ischemia section.

(f) Light Microscopy

Following sacrifice, the hearts were excised and samples of the regional myocardial bed taken within the ischemic area and the non-ischemic area. Methods the same as that reported in Global Ischemia section.

(g) Data Analysis

(i) ^{123}IPPA Image Analysis - see section E(i) of Global Ischemia Model.

(ii) Statistical Analysis - Statistical analysis of the metabolic data and assessment of ^{123}IPPA washout rates were performed

using a multivariate repeated measures design to designate differences between mean values of the pre-operative and post-operative time intervals. Data were expressed as mean ± SD. Linear regression analysis was performed to examine the correlation between any of the metabolic parameters and ^{123}IPPA washout with the functional data over the progression of ischemia.

(3) Myocardial Infarction Model

(a) Experimental Representation of Myocardial Infarction

Nine adult dogs (15-23 kg) were fasted overnight and anaesthetized with sodium pentobarbital (25 mg/kg) IV. Supplemental sodium pentobarbital was given as necessary. The dogs were intubated and maintained on a pressure-limited ventilator (Mark-7 Respirator - Bird Corporation). The electrocardiograph was displayed on a twelve-channel oscilloscope with concurrent digital readout and recording capabilities (SE Laboratories, England; John Fortin Mfg., Ltd., Canada). The heart was exposed *via* a fourth intercostal space thoracotomy. The femoral artery was dissected clear and cannulated to allow monitoring of arterial pressure throughout the procedure. The femoral vein was cannulated to obtain blood samples at various intervals during the procedure.

The left anterior descending coronary artery was dissected clear at a point approximately 3 cm from the bifurcation of the LAD from the main left coronary artery. Prior to ligation of the LAD, the dogs were placed on 100% oxygen for 15 minutes, given 50 mg. Xylocaine, IV, 0.5 gm Calcium gluconate, IV and ½ ampule of sodium bicarbonate, IV. The LAD was ligated completely using a 2-0 dexon suture and the dogs allowed to stabilize for 30 minutes

prior to closing the incision. The dogs were then weaned off the respirator.

(b) Metabolic Assessments

Metabolic assessments of venous blood samples included lactate dehydrogenase, creatine kinase and CK-MB isoenzyme determinations. Blood was withdrawn pre-operatively, pre-infarction, ½-hour post-infarction, 2 hours post-infarction and immediately prior to sacrifice. Determinations were made as described in section (B) of Regional Ischemia.

(c) Preparation of Beta-Methyl-15-p-^{123}I-iodophenyl pentadecanoic Acid ($B^{123}IPPA$)

(i) Radioiodine - identical to section C(i) of Global Ischemia.

(ii) Production of $B^{123}IPPA$ - identical to section C(ii) of Global ischemia, except for substitution of beta-methyl-PPA for PPA.

(iii) Hexane Extraction - identical to section C(iii) of Global Ischemia.

(iv) Purification - identical to section C(iv) of Global Ischemia.

(v) Preparation of $B^{123}IPPA$ for Injection - identical to section C(v) of Global Ischemia.

(vi) Quality Control - identical to section C(vi) of Global Ischemia.

(d) Data Acquisition

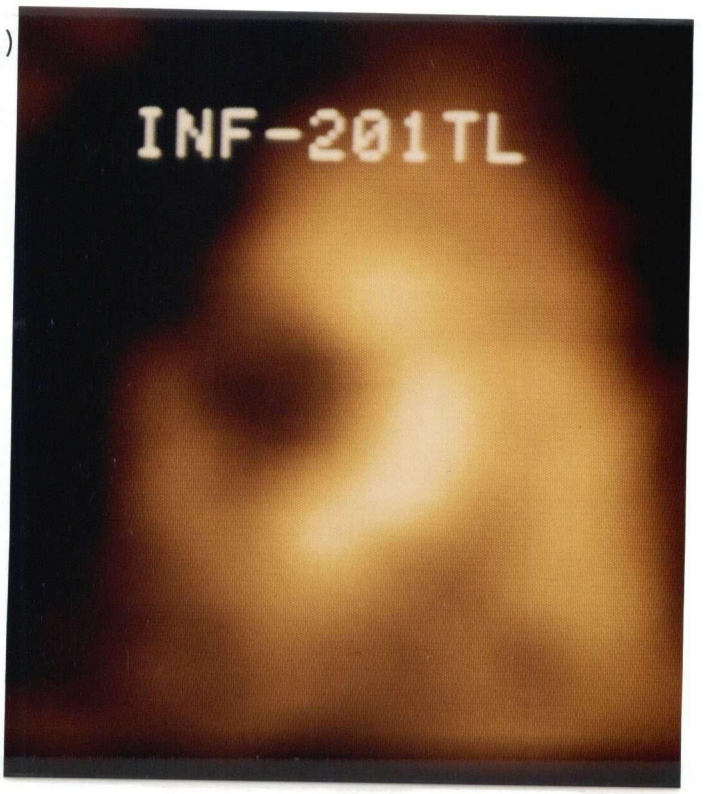
(i) Imaging - At 6 hours post-infarction, single-photon emission computerized tomography (SPECT) was performed using a Siemans Rota Camera (Siemans GammaSonics Inc., Des Plaines, Illinois). In 7 of 9 dogs, in situ scanning was commenced immediately following the injection of 2 mCi of ^{201}Thallium-chloride (Dupont). The dual camera heads rotated at 6° increments taking 30 serial images over a total scanning time of 20 minutes. Immediately following the ^{201}Tl scan, 3-5 mCi of B^{123}IPPA was injected and a similar scan performed (Figure 6).

Images were collected on a 64 x 64 x 8 matrix and recorded for 40 seconds at each angle. In the final two animals, each dog was imaged with only one of the radionuclides and similar analysis carried out. Thus, images were obtained for ^{201}Tl alone, as well as B^{123}IPPA alone. The animals were sacrificed following the imaging procedure.

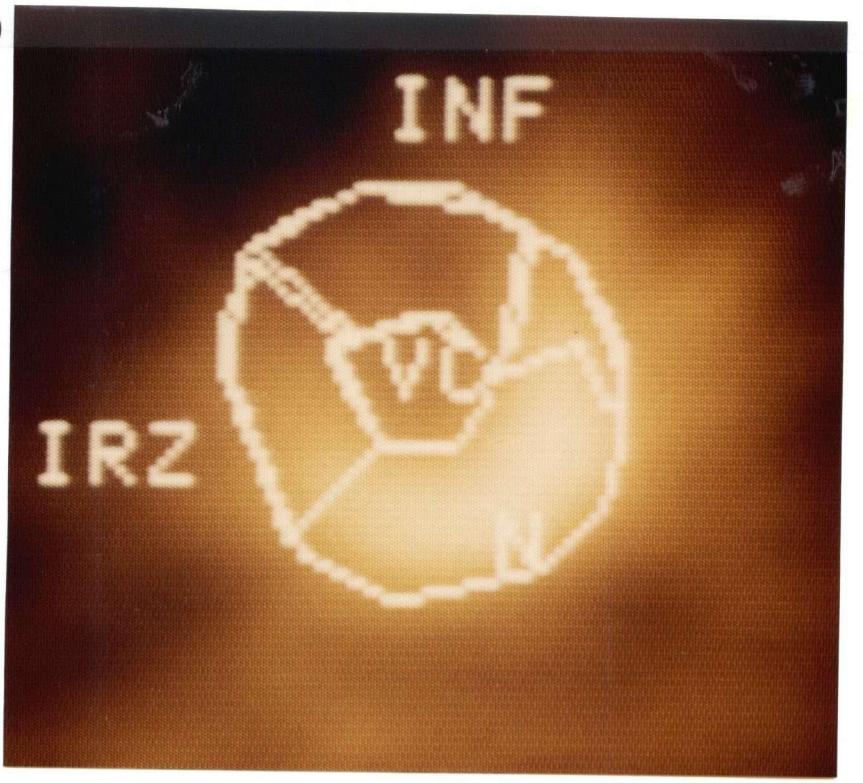
Figure 6 (A) - ^{201}Thallium SPECT-scan in dog (anterior projection at 6 hours post-infarction), showing the ventricular cavity as a black hole surrounded by a ring of activity. The infarct appears as an area of decreased activity within the ring.

(B) - B^{123}IPPA SPECT-scan in same dog as above (anterior projection at 6 hours post-infarction), showing the infarct (INF), ventricular cavity (VC), normal myocardium (N) and the ischemic risk zones (IRZ).

(A)

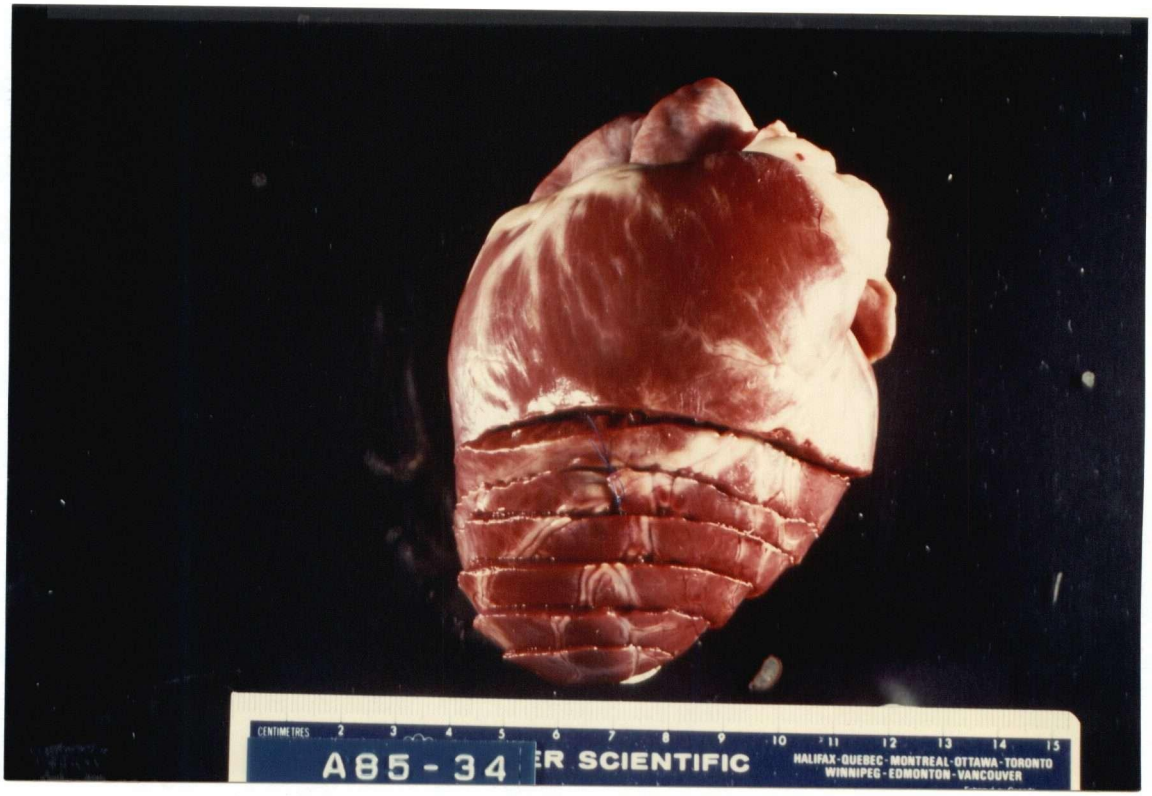


(B)



(ii) Histochemical Assessment - Immediately after the animals were sacrificed, the hearts were excised, weighed and sliced transversely (to the level of the mitral valve) into 10 mm. sections, using an electric meat slicer. The number of slices varied from 4 to 9 (Figure 7). Each of the slices was weighed and placed into a bath containing 0.2 M phosphate buffer (pH 7.4), 0.4 %wt/vol triphenyl tetrazolium, and 0.04%wt/vol nitroblue tetrazolium. These latter two agents were obtained from Sigma Chemicals Inc., (St. Louis, MO). The slices remained in the bath for 20 minutes at 37°C. This technique allowed for visualization of infarcted myocardium (Hearse, 1976, Caldwell et al., 1984).

Figure 7 - Heart showing ligation and section cutting technique.

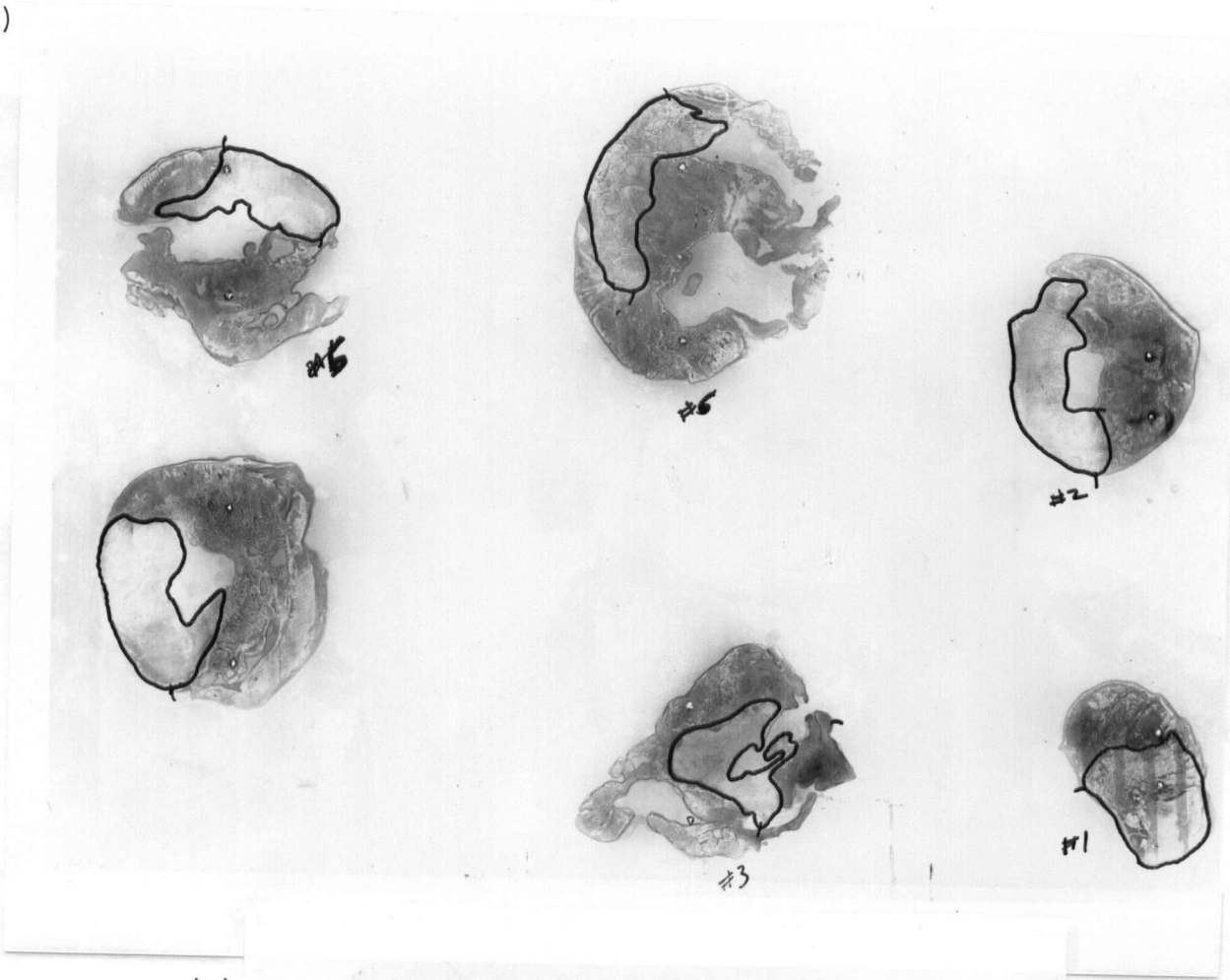

CENTIMETRES 2 3 4 5 6 7 8 9 10 11 12 13 14 15
A85-34
R SCIENTIFIC
HALIFAX-QUEBEC-MONTREAL-OTTAWA-TORONTO
WINNIPEG-EDMONTON-VANCOUVER

(iii) Autoradiography - In the final two experiments, in which imaging was completed with ^{201}Tl alone and $B^{123}IPPA$ alone, ischemic risk zones were determined by a macroautoradiographic technique. The stained, intact slices were placed between two acrylic sheets and the position marked. The slices were frozen at -70°C for 20 minutes. A sheet of X-ray film was then interposed between one acrylic sheet and the slices. The whole apparatus was placed in a light-proof box and stored at -20°C for 24 hours. The films were removed at the end of this time period and developed using an automatic processor. A sheet of clear acetate paper was placed over the marked slices and the individual slices drawn manually including the infarct borders. The perfused areas on the macroautoradiographs appeared as radiodense areas, while unperfused regions appeared opaque. The clear acetate tracings were superimposed onto the macroautoradiographs and ischemic risk zones seen, ie. that area of underperfusion between the infarct border and the radiodense areas (Figure 8). Both the acetate tracings and macroautoradiographs were quantitated using planimetry. Planimetric quantification was completed by one observer twice with a three month interval between and by a second observer once.

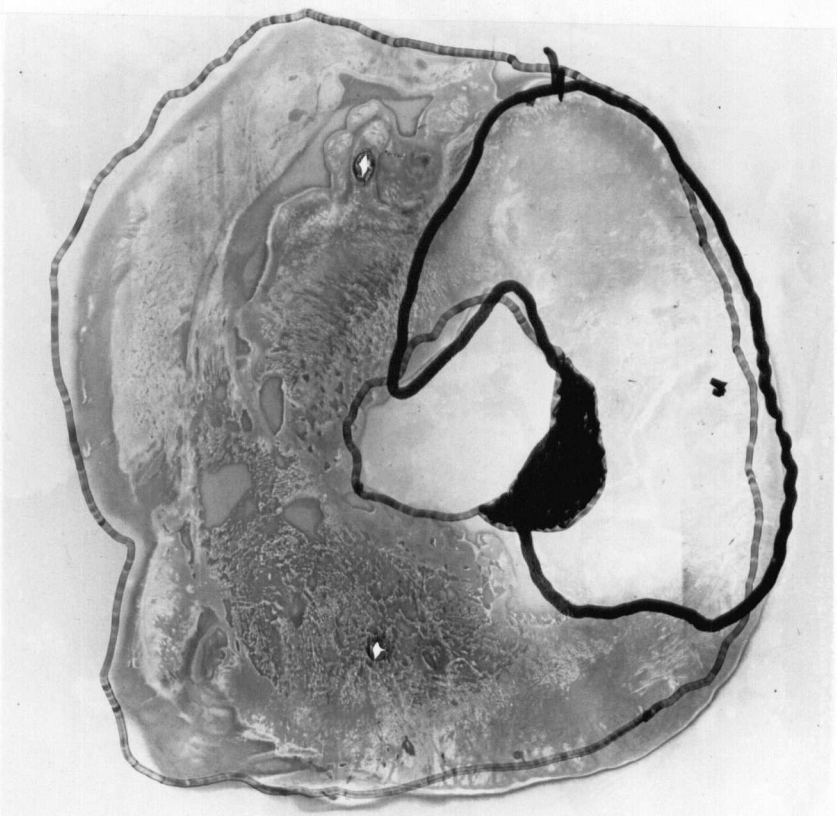
Figure 8 (A) - The findings of the autoradiographic technique utilized in the present study are shown. Perfusion in the slices of heart from one experiment with the slice tracings overlaid onto the autoradiograph, showing borders of the slices and infarcts, is seen.

(B) - A closer view of the clear acetate paper overlaid onto one slice of the autoradiograph showing demarcation of the infarction and the ischemic risk zone.

(A)



(B)



(iv) Histology - Following macroautoradiography, the slices were removed from the apparatus and infarcted tissue carefully dissected out and weighed. Samples were then dissected from the stained area (viable tissue) and infarcted region and placed into formalin for subsequent histological staining and examination. The tissues were then placed into tissue sample boxes and passed through a Histomatic Tissue Processor. This machine automatically passes the sample through a series of dehydration steps in progressively increasing concentrations of alcohol, followed by clearing in xylol and finally impregnation and embedding in wax. The blocks were then cut into thick sections using a microtome and placed onto a slide. The slides were then stained with hematoxylin and eosin, which stained nuclei blue because of their basophilic nature and cytoplasm and connective tissue shades of pink.

(e) Data Analysis

(i) B^{123}IPPA Image analysis - Image analysis was performed on an ADAC Systems Laboratories computer (Cam II). Standard and oblique reconstruction was completed by filtered back projection. Thirty computer-reconstructed slices were obtained beginning from the apical region and travelling towards the base. Each single short-axis image represented a thickness of 0.53 cm in real space. Therefore, 2 short-axis images corresponded to a single 1 cm myocardial slice. Total heart length was checked for accuracy between the excised heart and the computer-calculated length. Images were analyzed quantitatively from apex to base ending at the level of the mitral valve. Quantification was completed by calculating the

total number of pixels within each area of interest, i.e. the slice, the infarct, the ventricular cavity and the ischemic risk zone. Then, based on known pixel numbers, percentages could be calculated. As was the case for planimetric quantification, computer quantification was completed by one observer twice with a 3 month period between and by a second observer once.

(ii) Statistical Analysis - Inter-observer and intra-observer differences were calculated for the computer-generated and histochemical observations using the UBC Statistical Package, Genlin and performing an ANOVA (Kaul et al., 1985). Data were expressed as a mean $\pm$SD. Linear regression analysis as well as the correlation coefficient were used to determine the more accurate method of assessing perfusion defects including the ischemic risk zone.

V RESULTS

(1) Global Ischemia

(a) Analysis of Time-Activity Curves

Table 4 reports the mean values of the elimination rate ($t_{1/2}$ in mins) of ^{123}IPPA in the lateral wall, apical wall and septal wall of the left ventricle for the early and late phases of the T-A curve. The curve is bi-exponential and it is hypothesized that the early phase (first portion of the curve) represents beta-oxidation, while the second component (or late phase) represents storage of the fatty acids as triglycerides. A normalized curve, as shown by Figure 9, was utilized to calculate the $t_{1/2}$ values listed in Table 4 and is graphically represented in Figure 10. Curves were normalized to peak counts at the 2 min time interval. Elimination rate (mins) of ^{123}IPPA is shown by Figure 10 and Table 4 (early phase and late phase). An increase in $t_{1/2}$ was observed post-operatively in the lateral wall only for group A (Figure 10(A)). The septal wall and the apical wall for group A showed no significant change post-operatively. Group B showed a decreasing trend in the lateral wall and septal wall, with no change in the apical wall. The final group, C, illustrates a decreasing trend in $t_{1/2}$ values for the lateral wall and apical wall, with little change in the septal wall.

Figure 10(B) and Table 4 illustrate the effect of various cardioplegic techniques on the late phase of ^{123}IPPA washout. The $t_{1/2}$ is increased for Group A in all three areas of the left ventricle ie. the lateral wall, septal wall and apical wall (Figure 10(B)). Increasing trends are shown for group B in the lateral wall, septal wall and the apical wall. In group C, a slight decrease in the lateral wall and increases in the apical wall and septal wall were seen.

Figure 9 - Time-Activity curves for ^{123}IPPA washout from the myocardium (normalized to 2 min peak counts) (A) pre-operatively and (B) post-operatively, following 2 hrs of global ischemia.

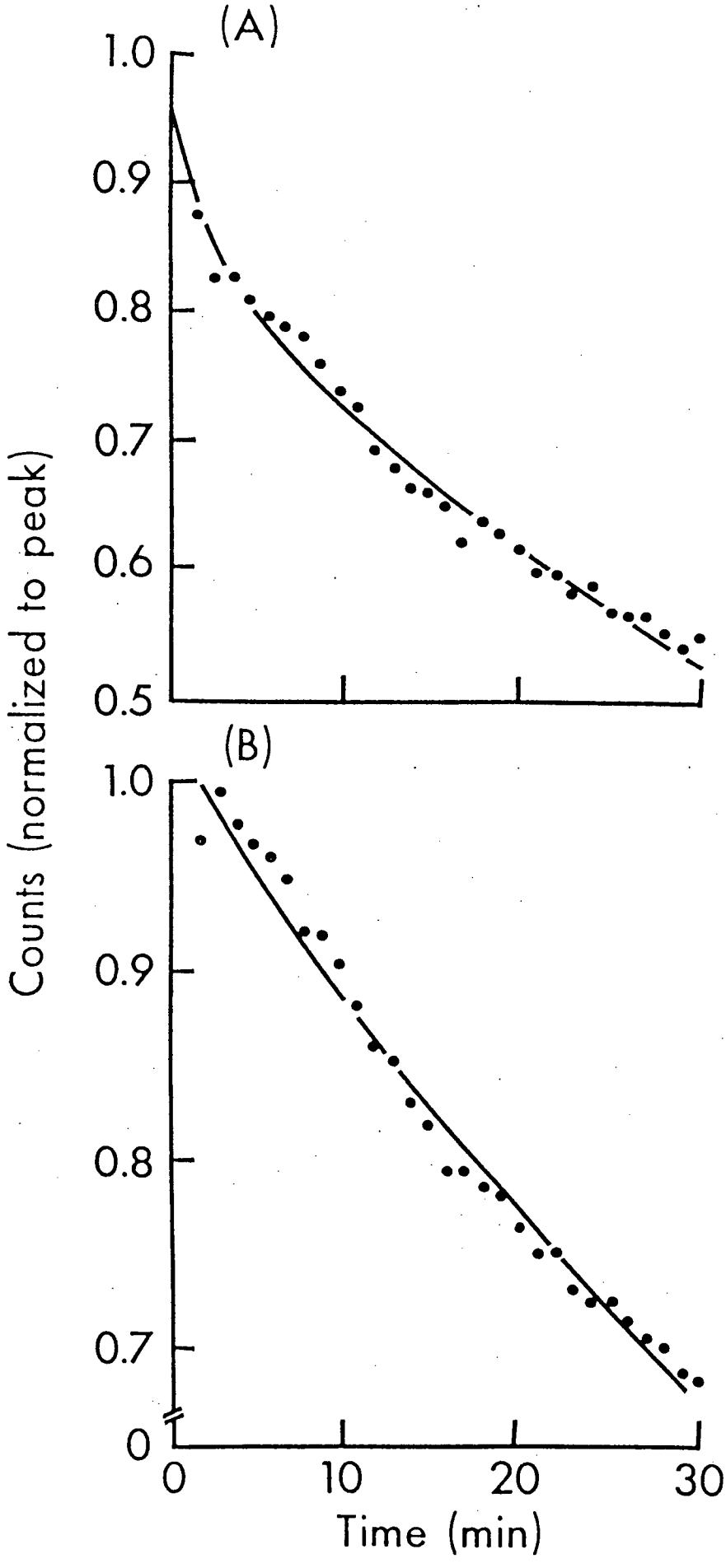

(A)
1.0
0.9
0.8
0.7
0.6
0.5
(B)
1.0
0.9
0.8
0.7
0
Counts (normalized to peak)
0
10
20
30
Time (min)

Figure 10 - Washout rate ($t_{½}$ in min) of ^{123}IPPA for the (A) early phase and (B) late phase of the T-A curve.

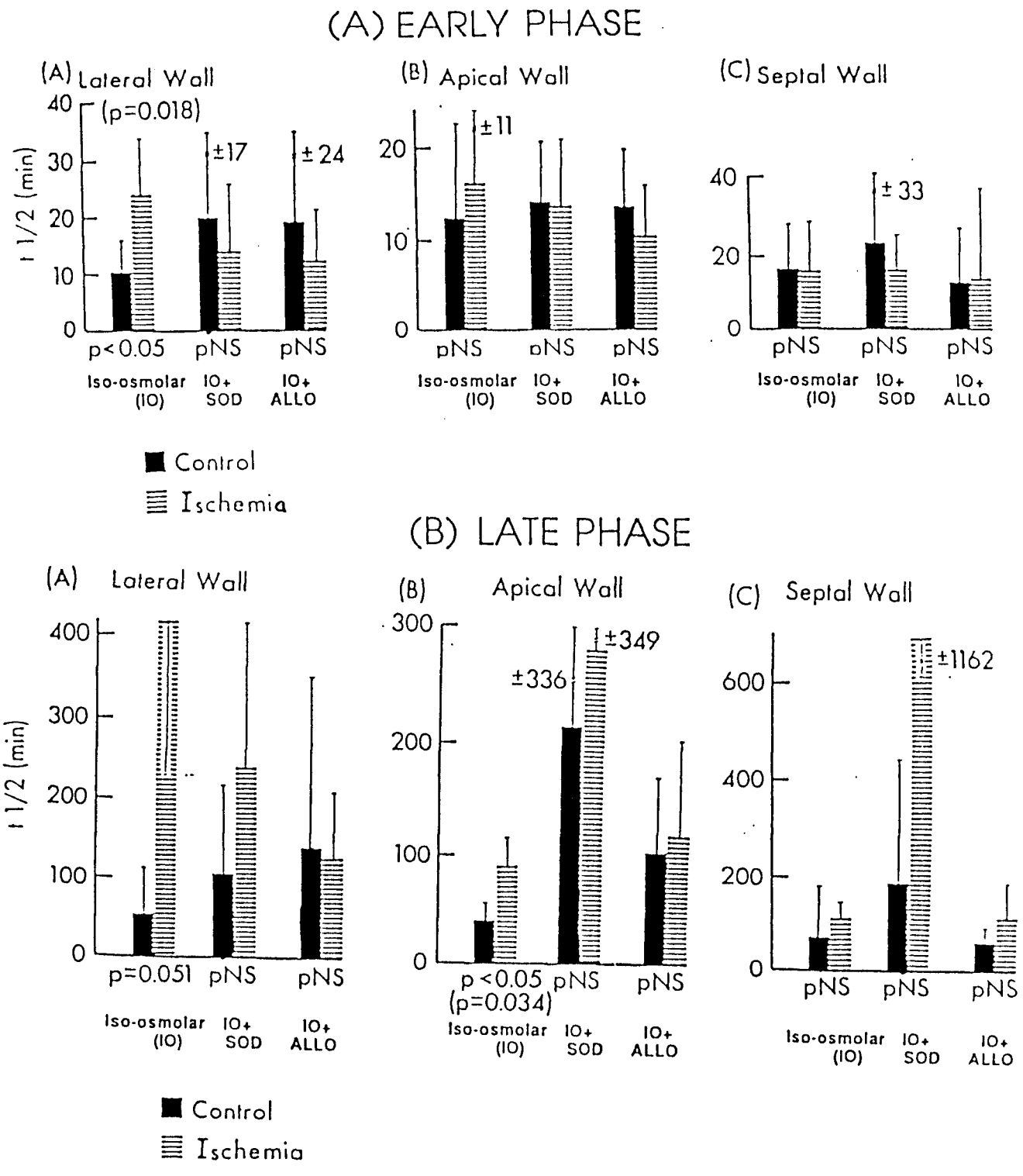

(A) EARLY PHASE
(A) Lateral Wall
(B) Apical Wall
(C) Septal Wall
t 1/2 (min)
40
30
20
10
0
(p=0.018)
±17
±24
±11
±33
p<0.05
pNS
Iso-osmolar (IO)
IO+ SOD
IO+ ALLO
Control
Ischemia
(B) LATE PHASE
(A) Lateral Wall
(B) Apical Wall
(C) Septal Wall
400
300
200
100
0
600
±336
±349
±1162
p=0.051
p<0.05
(p=0.034)

Table 4 Washout rate ($t_{½}$ in mins ± SD) of 15-p-iodophenyl Pentadecanoic Acid at control and post-operative time intervals examined by T-A curve analysis. Myocardial regions included the lateral wall, apical wall and septal wall.

Group A - Tyers' Iso-osmolar Solution (IO)
Group B - IO + SOD
Group C - IO + Allopurinol

	Group A		Group B		Group C	
	Control	Post-operative	Control	Post-operative	Control	Post-operative
EARLY PHASE						
Lateral Wall	11± 6	24±10(p<0.05)	20±17	14±12	19±24	13± 9
Apex	13±10	16±15	14± 7	14± 7	14± 6	11± 5
Septal Wall	16±11	16±13	24±34	17± 9	13±14	15±23
LATE PHASE						
Lateral Wall	56±60	416±238(p=0.05)	111±101	240± 270	146±215	132±85
Apex	39±13	89± 26(p<0.05)	217±336	281± 344	108± 62	122±80
Septal Wall	72±96	109± 46	194±272	699±1162	69± 16	116±95

Figure 11(A) shows the mean control washout rate ($t_{½}$ in min) for the early phase in the lateral wall, apex and septal wall of the left ventricle. Figure 11(B) depicts the situation found for the late phase of the IPPA metabolic time-activity curve.

Figure 11 - Washout rate ($t_{½}$ in min) assessed pre-operatively for the (A) early phase and (B) late phase of the T-A curves.

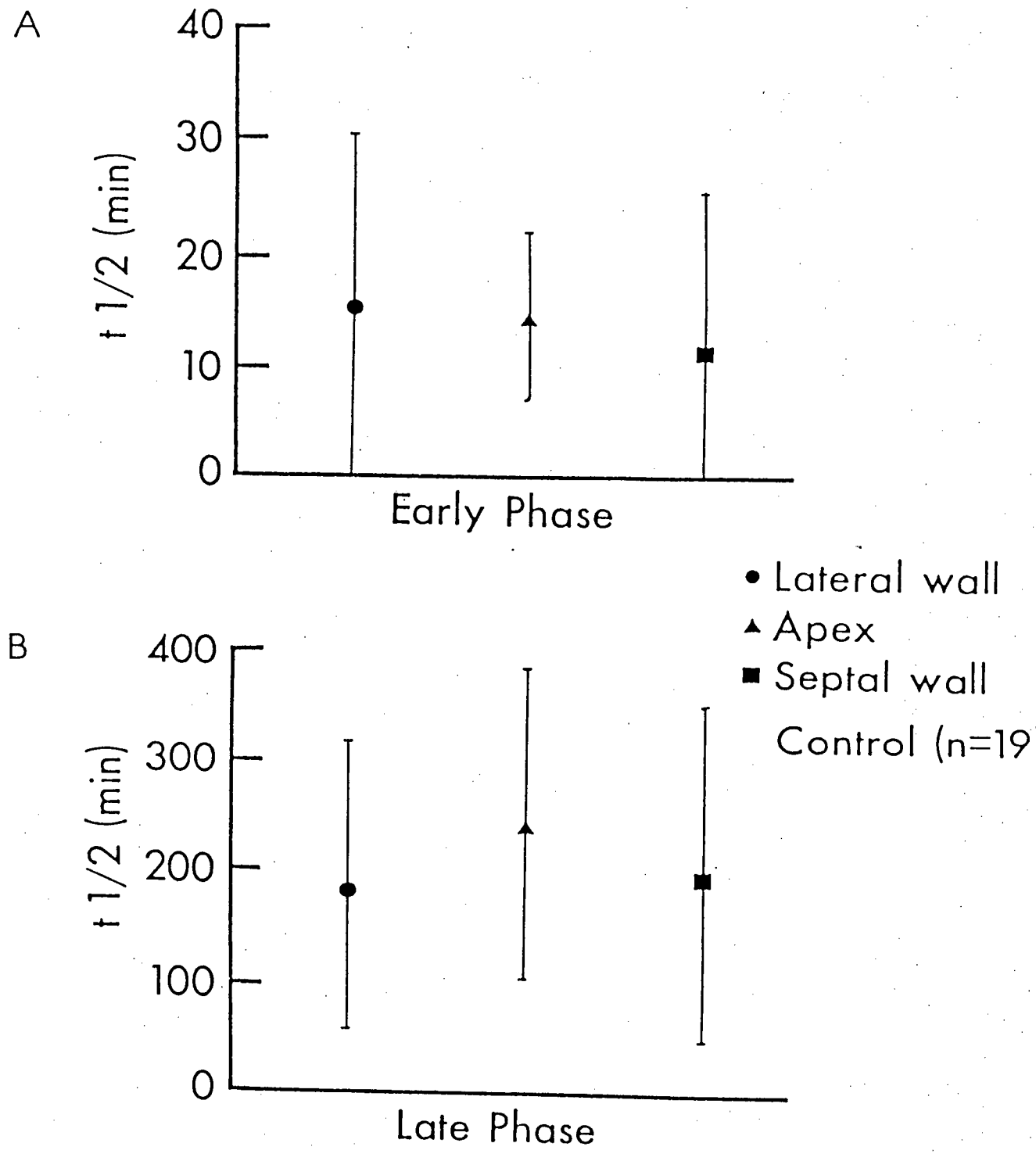

A
40
30
20
10
0
t 1/2 (min)
Early Phase
B
400
300
200
100
0
t 1/2 (min)
Late Phase
• Lateral wall
▲ Apex
■ Septal wall
Control (n=19)

Table 5 Measured and calculated parameters of cardiac hemodynamic status (CI ≡ Cardiac index, SI ≡ Stroke index, SWI ≡ Stroke work index, SVR ≡Systemic vascular resistance, SBP ≡ Systolic blood pressure, EF ≡ Ejection fraction). Parameters were assessed at 2-time intervals, including "pre"-cardiopulmonary bypass (as a control assessment) and "post"-cardiopulmonary bypass (as a post-operative assessment).

	Time	CI	SI	SWI	SVR	HR	SBP	EF
Group A	Pre-CBP	1.9±0.84	15±1	20±1	5153± 713	126±5	121±11	73±5
	Post-CBP	2.6±0.3	22±3	22±3	3079± 582 ($p<0.05$)	125±4	104±6	71±8
Group B	Pre-CBP	2.6±0.3	19±2	24±3	3906± 394	135±4	127±8	80±2
	Post-CBP	3.0±0.4	19±2	23±3	3517± 584	152±7 ($p<0.05$)	131±9	77±5
Group C	Pre-CBP	2.5±0.3	18±2	25±3	4579± 605	143±7	143±9	78±4
	Post-CBP	2.6±0.2	16±1	21±3	4023± 511	158±7	141±7	79±4

(b) Hemodynamics

Cardiac index (CI) was unchanged in groups A, B and C (Table 5).

Stroke Index (SI) also remained unchanged in all three groups. There was no change in left ventricular stroke work index (SWI) in groups A, B and C. Ejection fraction (EF) was unchanged in group A, group B and group C. Systemic vascular resistance (SVR) decreased in group A, but remained unchanged in groups B and C. Heart rate (HR) was unchanged in group A and group C, but increased in group B. Systolic blood pressure (SBP) was unchanged in all three groups.

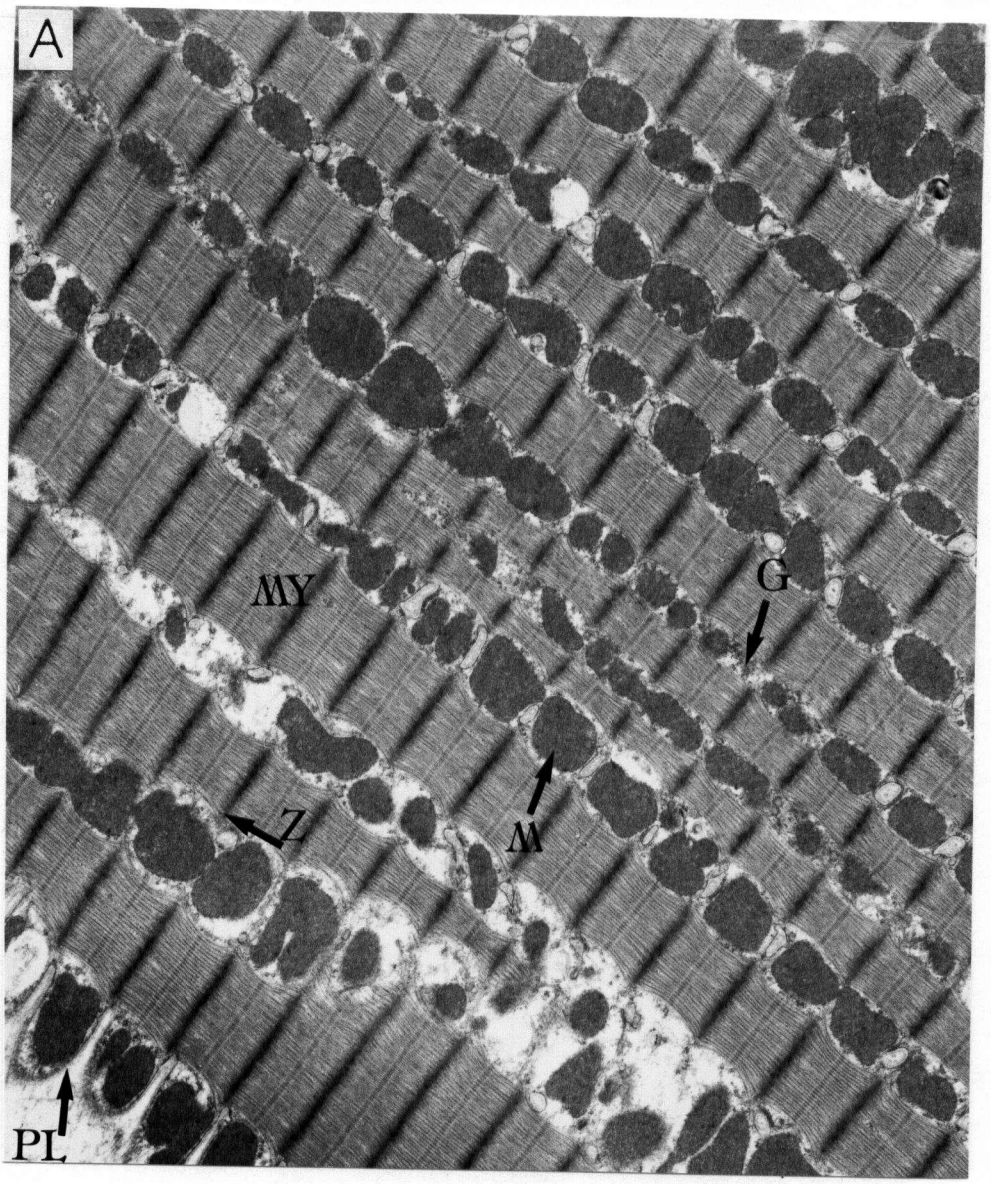
(c) Histology

(i) Electron Microscopy - Ultrastructural changes associated with each of the cardioplegic solutions are demonstrated in Figure 12. All three groups show minimal ultrastructural changes in myofibrillar integrity, mitochondrial swelling, interstitial and intracellular edema, vacuolation of the sarcoplasmic reticulum and disruption of the mitochondrial cristae.

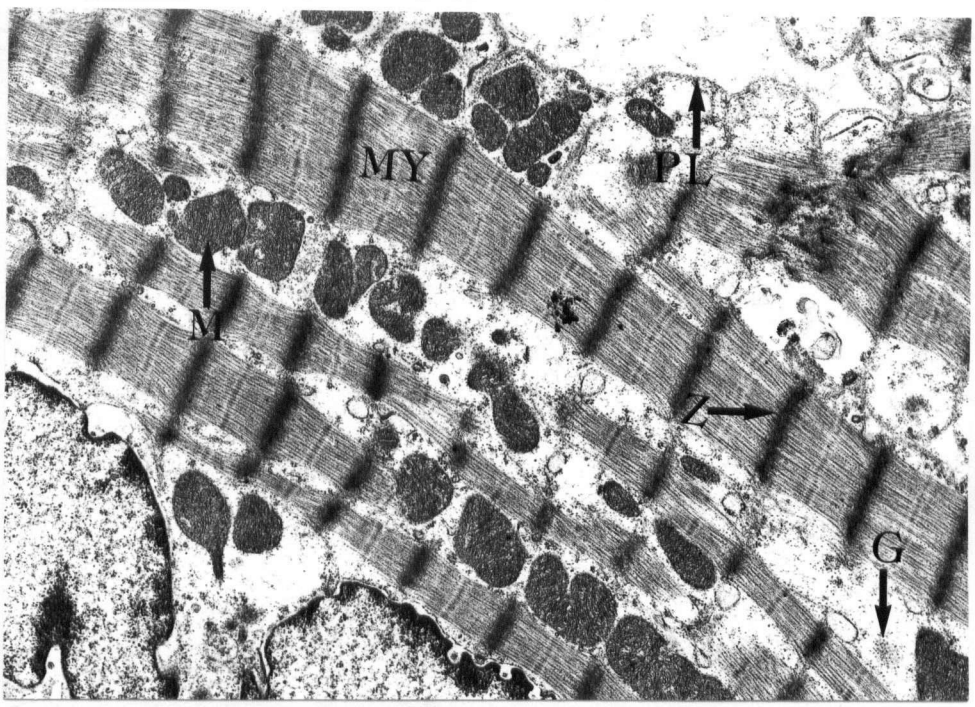
Figure 12 - Electron microscopic evidence of ischemic changes at the ultrastructural levels.

(A) normal tissue (X 8550).
(B) (i) Iso-osmolar (Tyer's) solution (X 8550).
(ii) IO + SOD (X 8550).
(iii) IO + Allopurinol (X 8550).

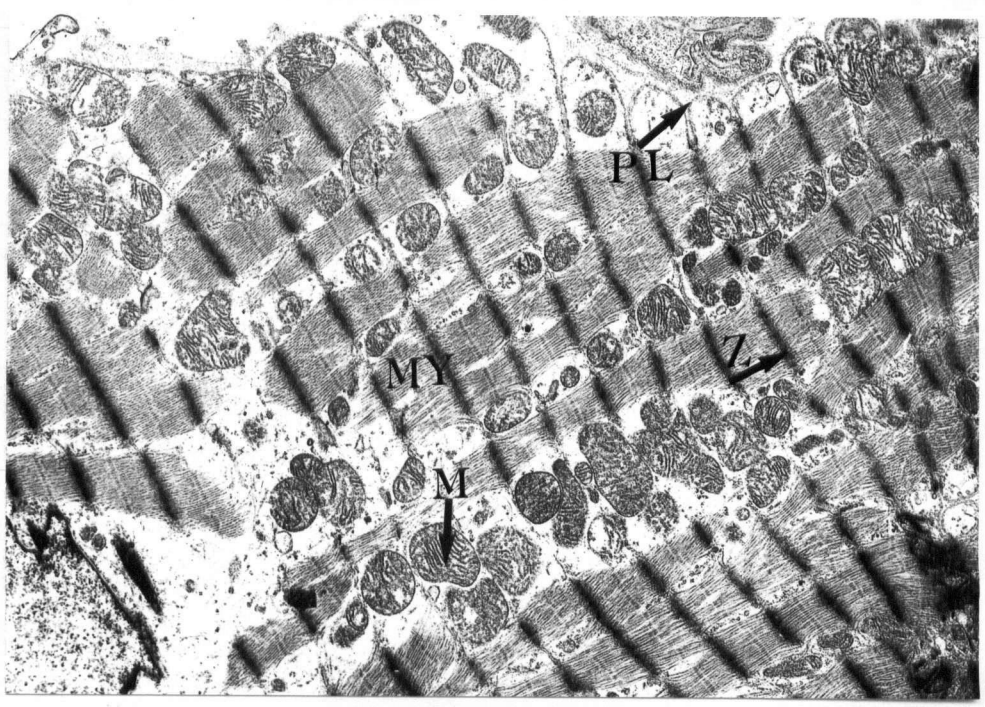
MY ≡ myofibril
PL ≡ plasma membrane
Z ≡ Z-line
G ≡ glycogen
M ≡ mitochondria


A
MY
G
Z
M
PL

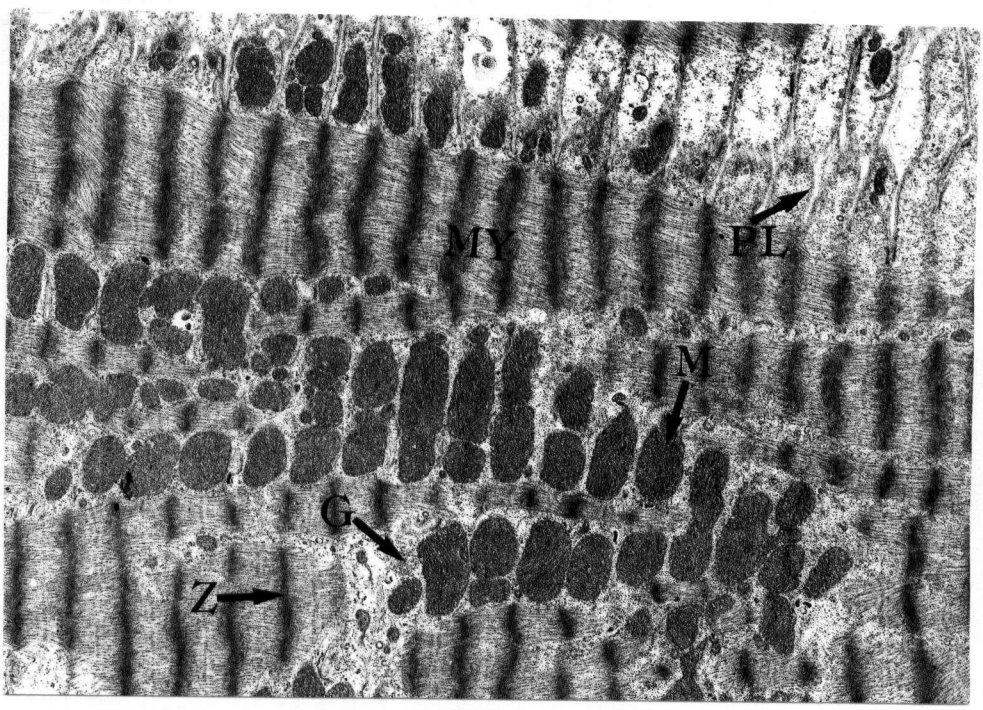
B(i)



B(ii)



B(iii)



(ii) Light Microscopy - Gross cellular changes found in all three specimens include edema and cellular infiltration (Figure 13). No distinction could be made between the three cardioplegic solutions based on gross cellular anatomy.

Figure 13 (A) - Control specimen showing macroscopic anatomy of the myocardium (X 63).

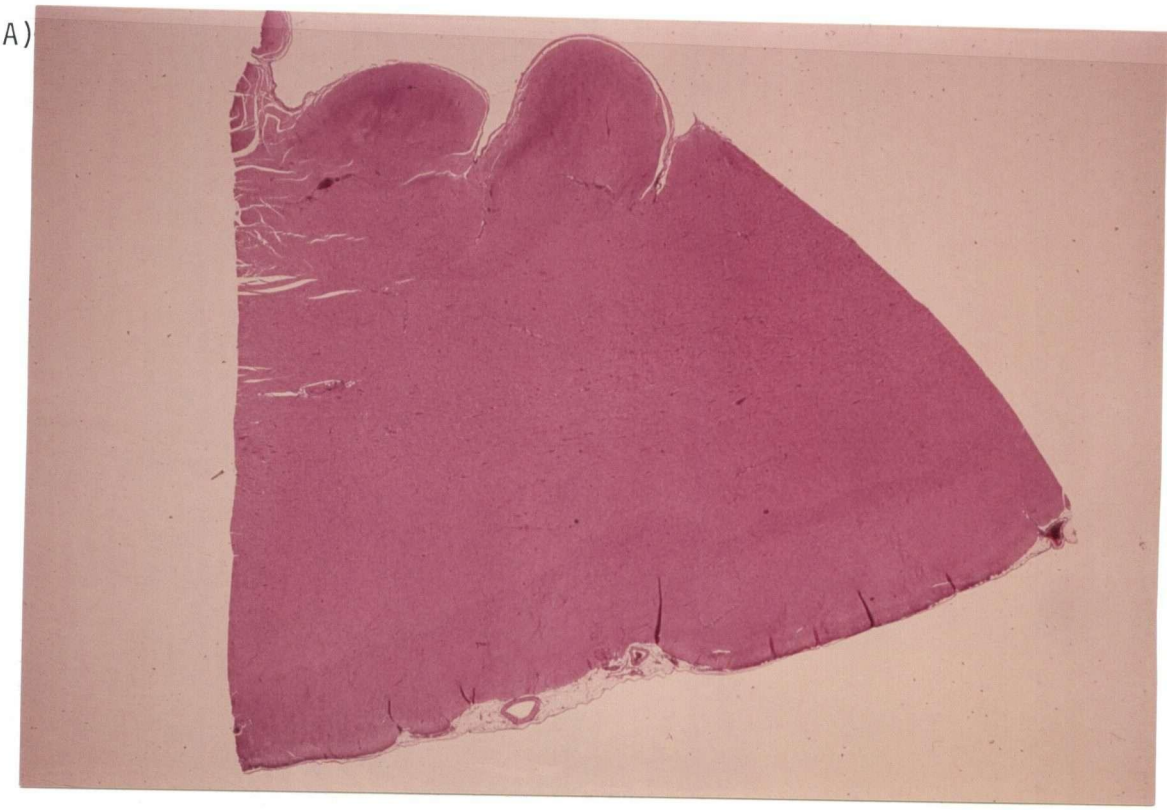
(B) - Ischemic specimen showing gross changes at the cellular level.

(i) Iso-osmolar (Tyer's) solution (X 96).

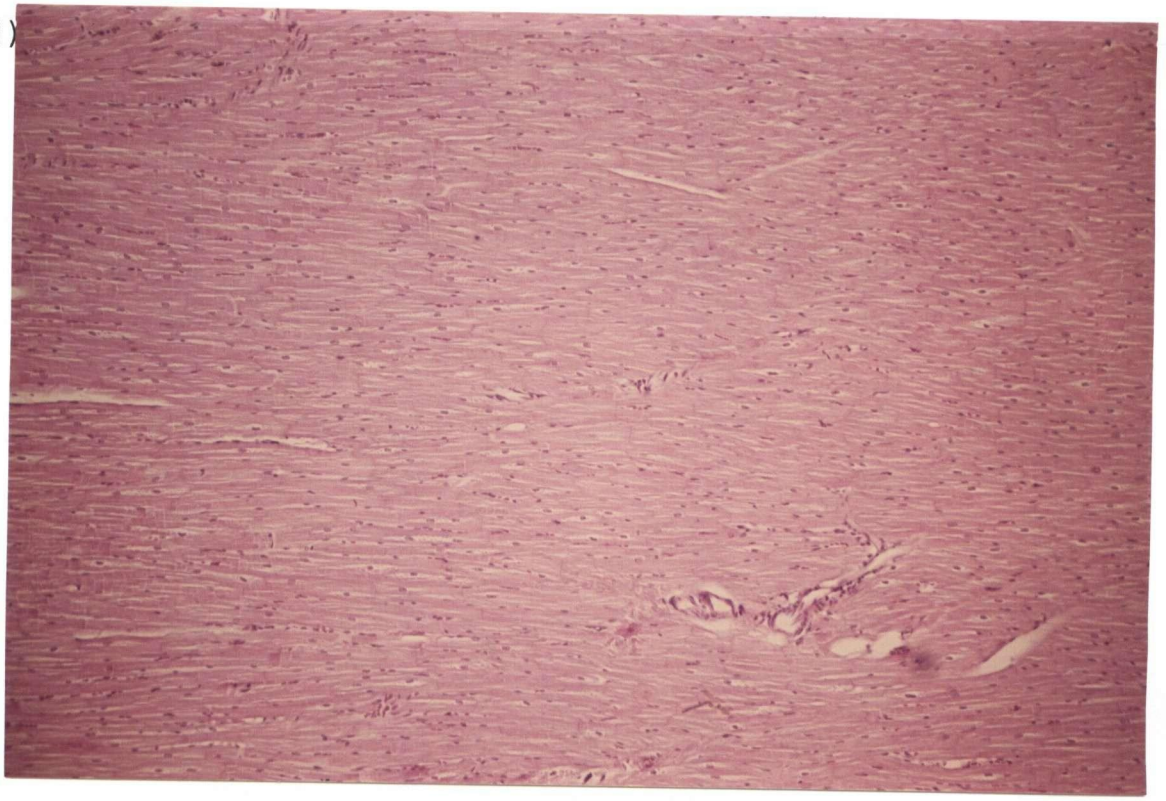
(ii) IO + SOD (X 96).

(iii) IO + Allopurinol (X 96).

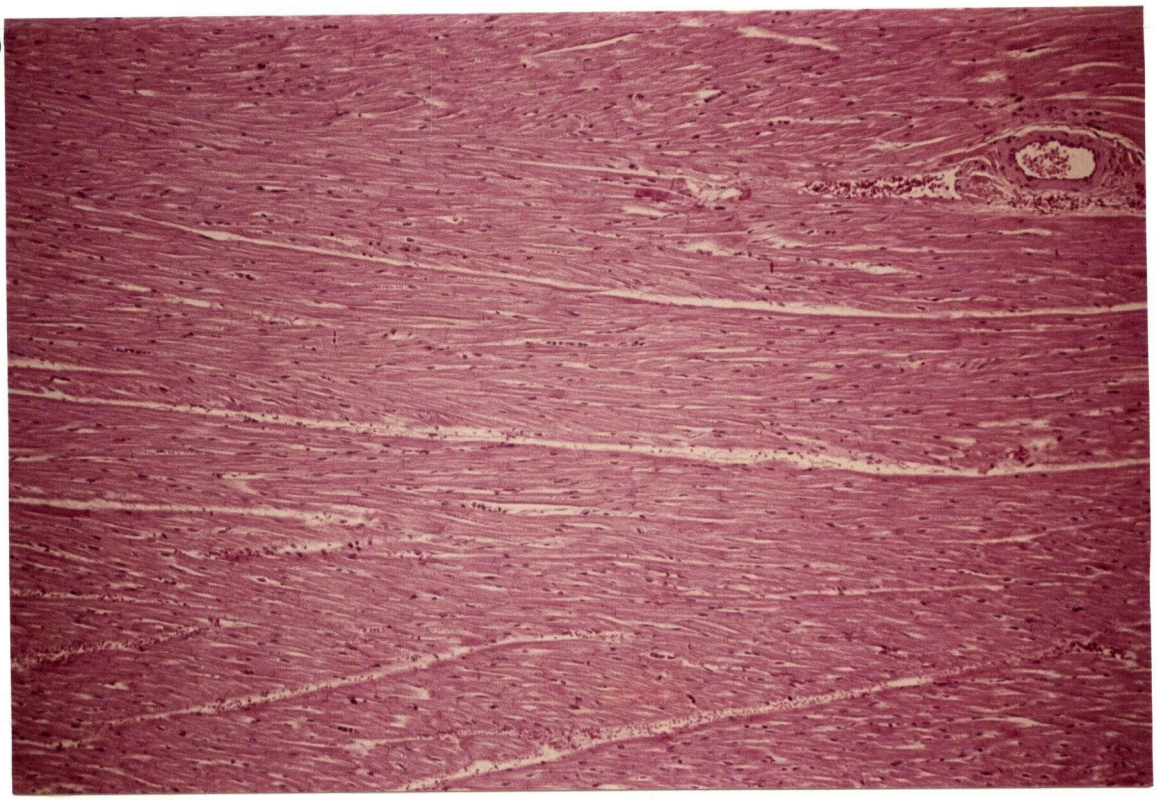
(A)



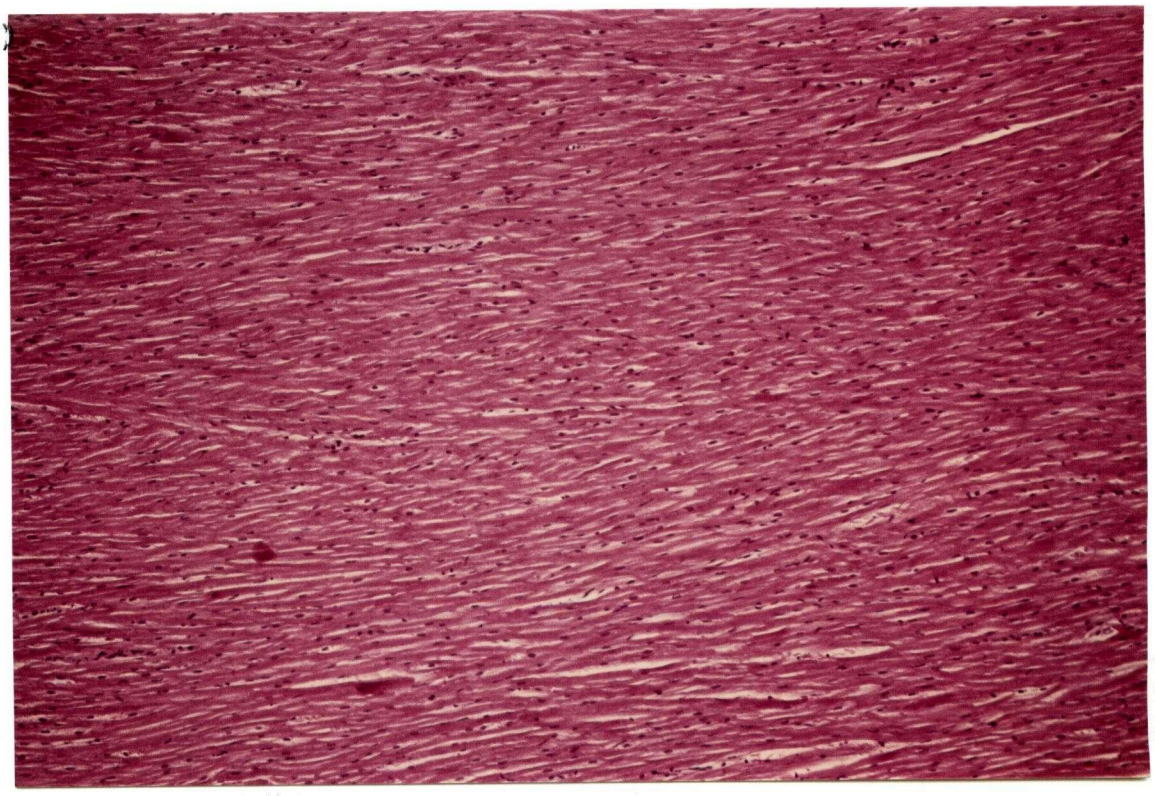
B(i)



B(ii)



B(iii)



(2) Regional Ischemia

The placement of an ameroid constrictor on the left anterior descending coronary artery was completed on 12 dogs. Seven dogs, which comprised the study group, survived to 14 days. The remaining animals did not survive to the completion of the experiment because of infarction and thus were excluded from the study.

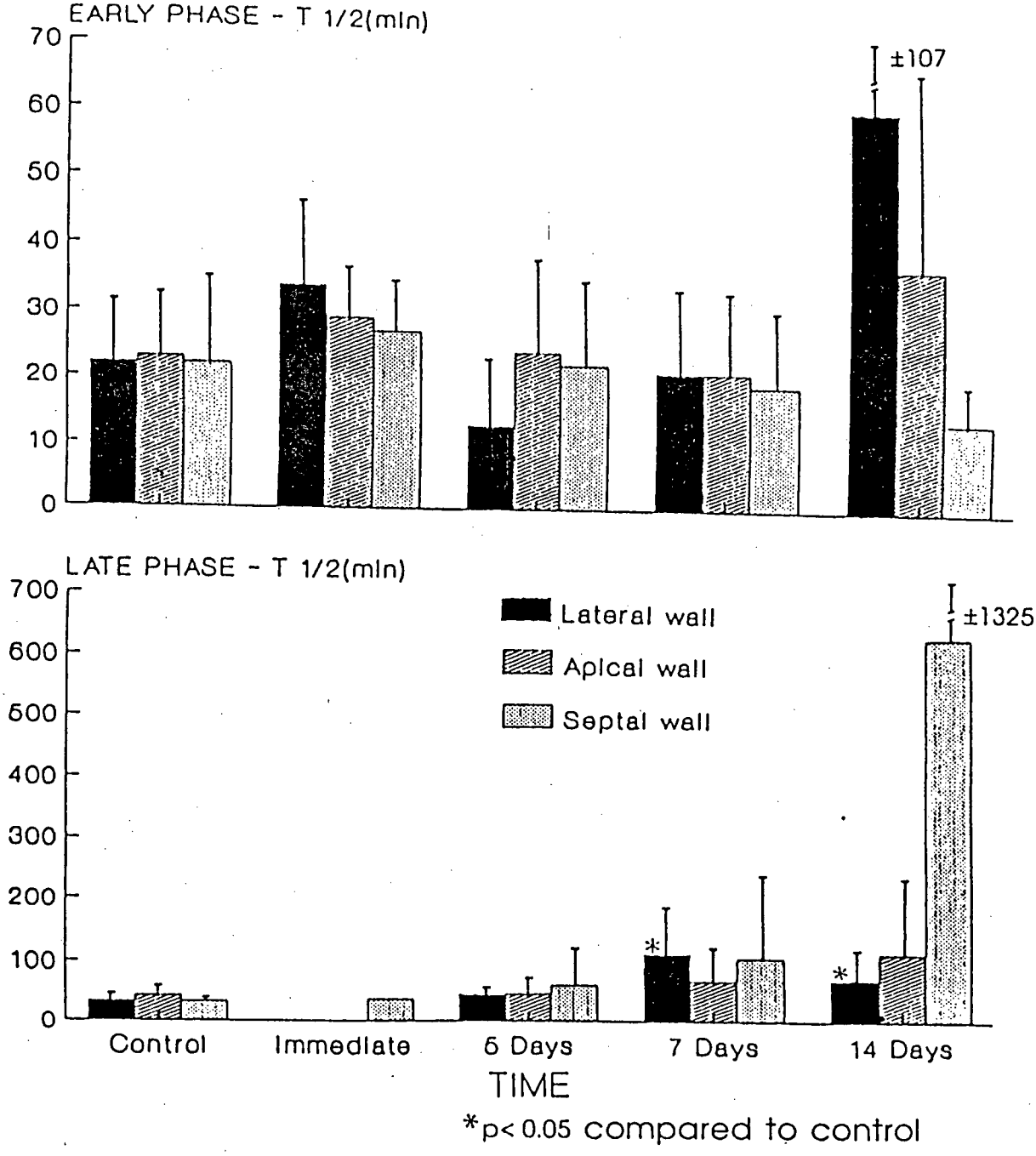
(a) Analysis of ^{123}IPPA curves

Regional myocardial washout of ^{123}IPPA (Table 6 and Figure 14(A)) for the early phase of the time-activity curves showed no significant changes; however, trends indicated decreased washout in the ischemic segments (lateral wall and apical wall) as shown by increased $t_{\frac{1}{2}}$ values. Similarly increased $t_{\frac{1}{2}}$ values were observed for the late phase in the ischemic segments as well (Figure 14(B)). The non-ischemic segment (septal wall) showed no statistically significant change in $t_{\frac{1}{2}}$ for the early phase (Figure 14(A)). The late phase showed an increase which was not statistically significant (Figure 14(B)). Results obtained with one study animal led to the large mean and standard deviation observed and if this animal was excluded, the value was 85 ± 40-$p > 0.05$. The animal showed a large $t_{\frac{1}{2}}$ value indicating little washout of the fatty acid.

Figure 14 (A) - Regional myocardial washout rate of ^{123}IPPA ($t_{½}$ in min) for the early phase of the T-A curve.

(B) - Regional myocardial washout rate ($t_{½}$ in min) of ^{123}IPPA for the late phase of the T-A curve.

IPPA ANAYLSIS



*p< 0.05 compared to control

Table 6 ^{123}IPPA washout for both the early and late phases of the T-A curves over 14 days of developing ischemia. Areas examined were the lateral, apical and septal wall of the left ventricle.

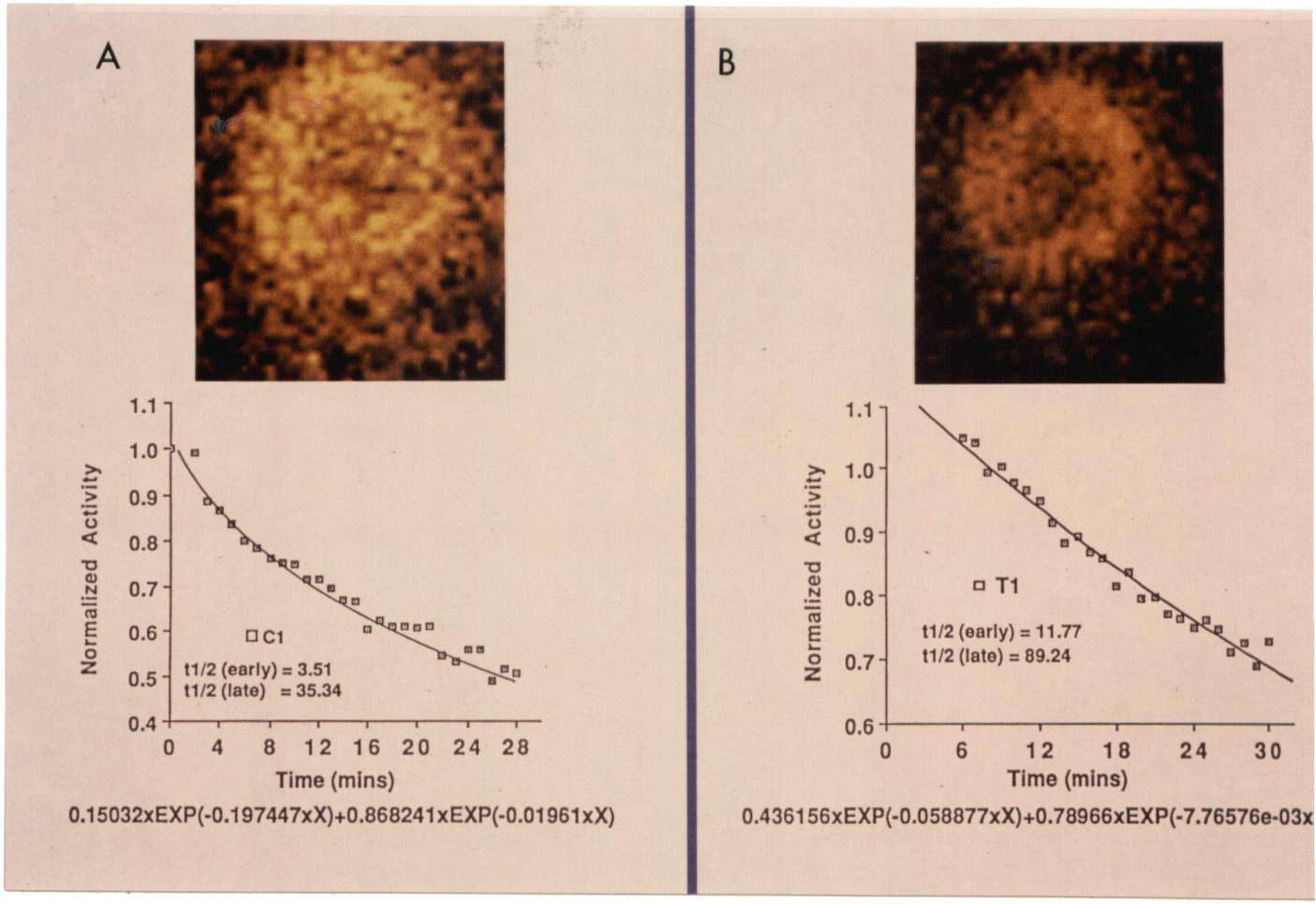
* large standard deviation is due to one dog.

	CONTROL	6 HRS POST-OPERATIVELY	5 DAYS	7 DAYS	14 DAYS
Early Phase ($t_{½}$ in min)					
Lateral Wall	22±10	34±14	13±11	21±13	60±107
Apex	23±10	29± 7	24±15	21±12	37± 33
Septal Wall	22±14	27± 8	22±14	19±12	14± 7
Late Phase ($t_{½}$ in min)					
Lateral Wall	34±12	---	45±12	114±9 ($p<0.05$)	71± 50 ($p<0.05$)
Apex	44± 8	---	47±23	69±49	115± 104
Septal Wall	34± 2	38±28	62±63	107±146	626±1325*

Control and post-operative scintigrams at 14 days with associated time-activity curves and values are shown in Figures 15(A) and (B).

Figure 15 (A) - Control scintigram in dog injected with 15-p-^{123}I-iodophenyl pentadecanoic acid (anterior view) with T-A curve and $t_{\frac{1}{2}}$ values. (C1).

(B) - Post-operative scintigram in dog injected with 15-p-^{123}I-iodophenyl pentadecanoic acid (anterior view) at 14 days with T-A curve and $t_{\frac{1}{2}}$ values. (T1).


A
Normalized Activity
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0
4
8
12
16
20
24
28
Time (mins)
C1
t1/2 (early) = 3.51
t1/2 (late) = 35.34
0.15032xEXP(-0.197447xX)+0.868241xEXP(-0.01961xX)
B
Normalized Activity
1.1
1.0
0.9
0.8
0.7
0.6
0
6
12
18
24
30
Time (mins)
T1
t1/2 (early) = 11.77
t1/2 (late) = 89.24
0.436156xEXP(-0.058877xX)+0.78966xEXP(-7.76576e-03x

(b) Metabolic Assessments

Metabolic data (Table 7) showed no statistically significant changes in any of the parameters except for CK values, in which the immediate post-operative value of 6333.4±1728 was significantly increased ($p<0.002$), but returned to control levels at 5 days (Table 7 and Figure 16(A)). Lactate dehydrogenase levels remained unchanged throughout the 14 days, (Table 7 and Figure 16(A)). Levels of the isoenzyme CK-MB rose significantly immediately following surgery and were normal at 14 days post-operatively (Figure 16(B)). Lactate levels showed no significant changes throughout the experiment (Table 7 and Figure 17).

(c) Hemodynamic Assessments (Table 7)

There was no significant change in heart rate throughout the experiment. Pacing resulted in a statistically significant increase in heart rate ($p<0.001$), during the assessment of myocardial metabolism using ^{123}IPPA at both seven days and 14 days post-operatively.

Figure 16 (A) - Lactate dehydrogenase and CK levels pre- and post-operatively.

(B) - CK-MB% assessed at control and post-operative time intervals.

Enzymatic Analysis

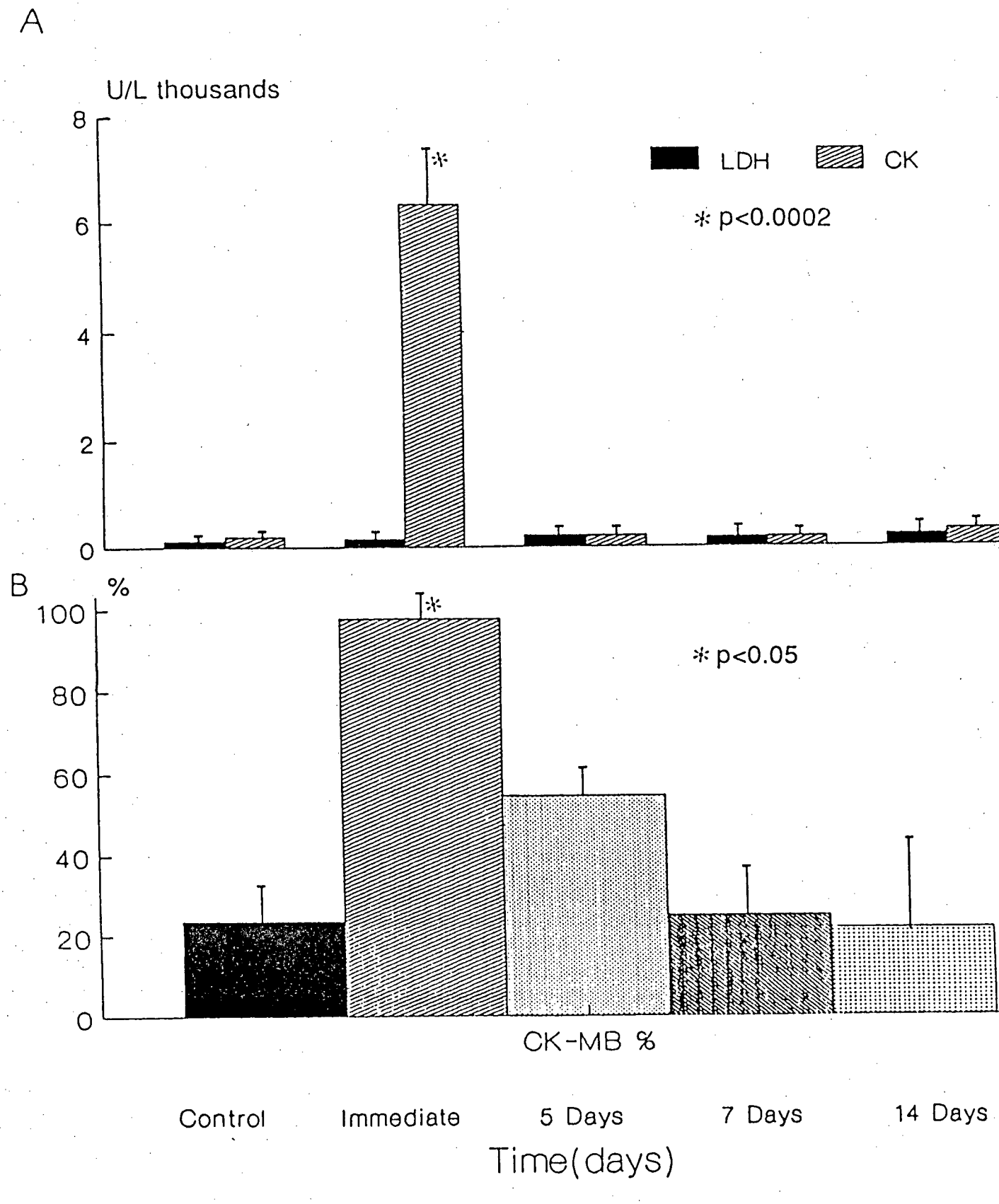


Figure 17 - Lactate assessed at pre- and post-operative time intervals.

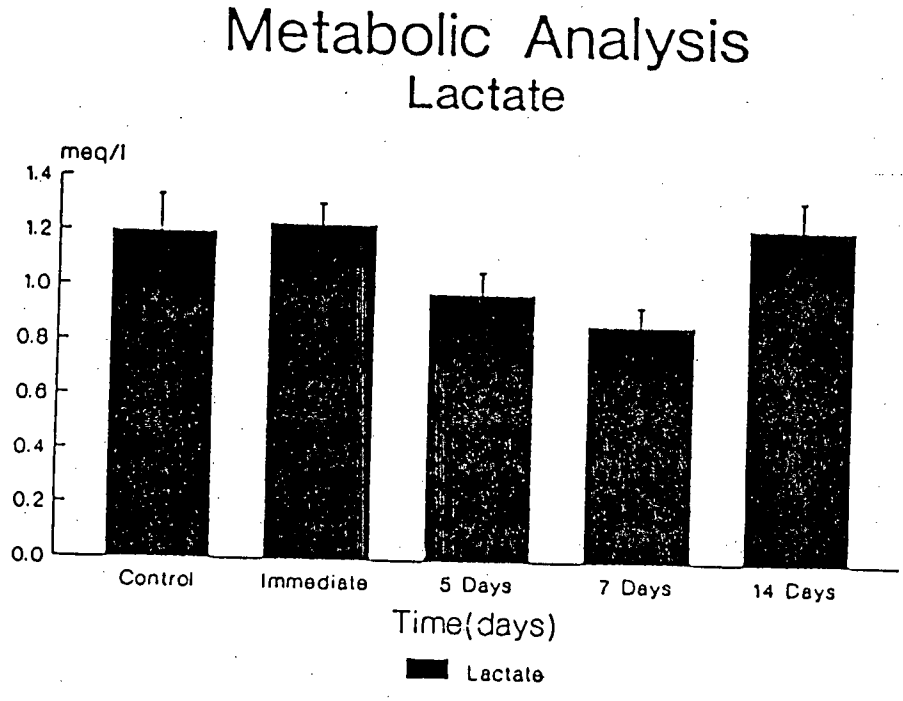

Metabolic Analysis
Lactate
meq/l
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Control
Immediate
5 Days
7 Days
14 Days
Time(days)
Lactate

Table 7. Hemodynamic and blood analyses at various time periods during gradual coronary occlusion over a 14 day period.

* paced to a rate of 185 beats/minute.

VARIABLE	CONTROL	IMMEDIATE	5 DAYS	7 DAYS (paced HR)	14 DAYS (paced HR)
Pulse beats/min)	120±36	135±19	127±29	123±22 * (185) ($p<0.001$)	136±24 * (185) ($p<0.001$)
pH	7.36±0.04	7.34±0.03	7.38±0.02	7.38±0.03	7.36±0.04
pCO_2 mmHg	43±7	41±6	42±3	41.43±2.5	41±4
pO_2 mmHg	51±14	37±9	43±14	37.43±7.9	54±11
HCO_3 mmol/l	24±1.8	22±1.5	25.1±1.8	24.29±0.95	23.00±2.5
Lactate meq/l	1.20±0.74	1.23±0.54	0.98±0.27	0.87±0.22	1.23±0.57
LD U/l	125±103	160±56	212±167	175±114	213±146
CK U/l	206±204	6333±1728 ($p<0.002$)	220±79	183±66	321±332

(d) Histology

Post-mortem examination of the left anterior descending coronary artery revealed patency in all seven animals of the study group (Figure 18(B and C)). Ameroid constriction, however, did result in severe closure at the 14 day interval (Figure 18(E)). All five animals which had died showed evidence of myocardial infarction as assessed by tetrazolium staining and complete closure of the ameroid constrictor (Figure 18(D)). Figure 18(A) shows a normal coronary artery with normal patency.

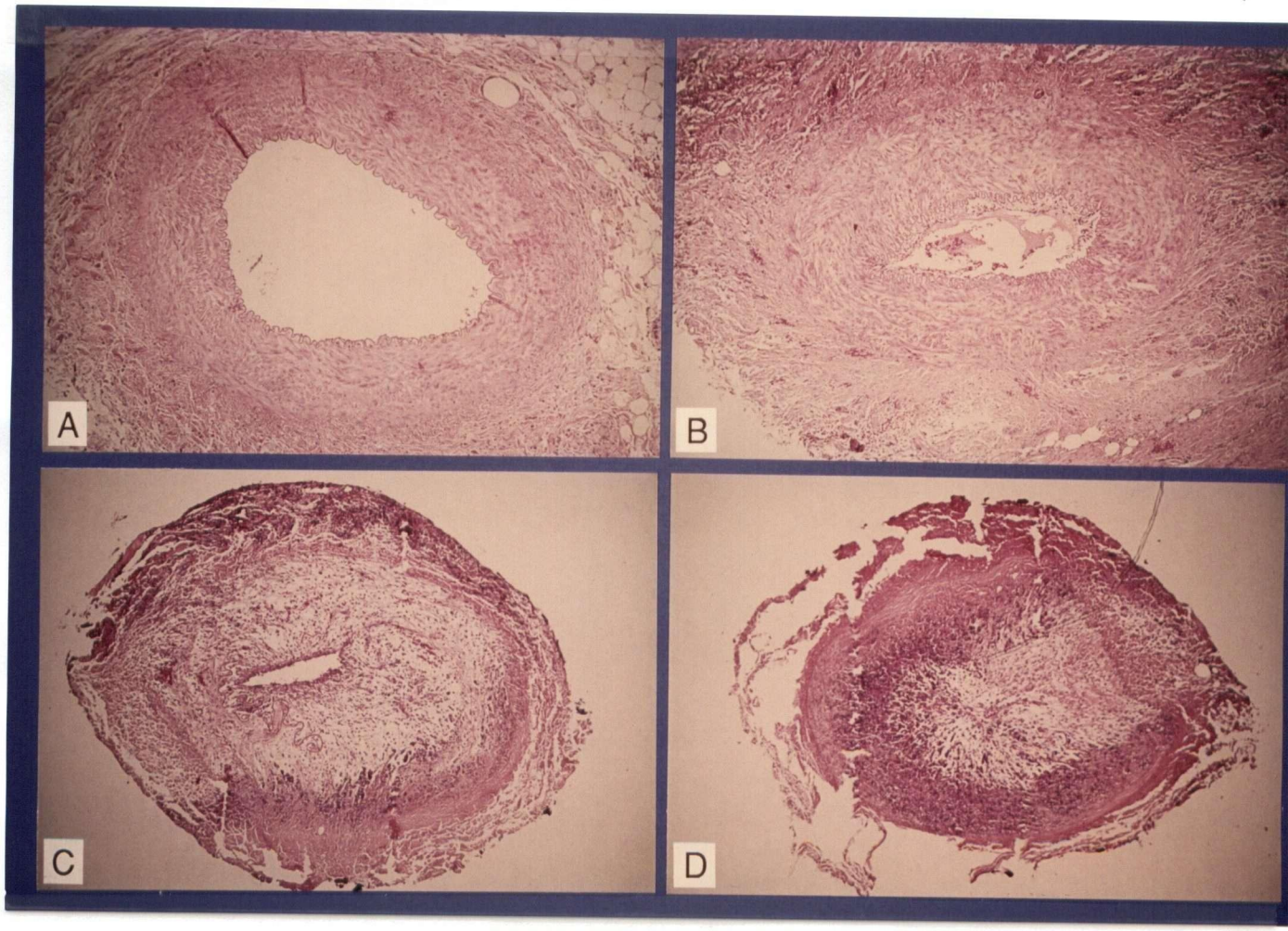
Figure 18 (A) - Microscopic assessment of LAD coronary artery showing normal diameter with no constriction (X 96).

(B) - Assessment of LAD coronary artery in one experiment at 14 days of constriction (X 96).

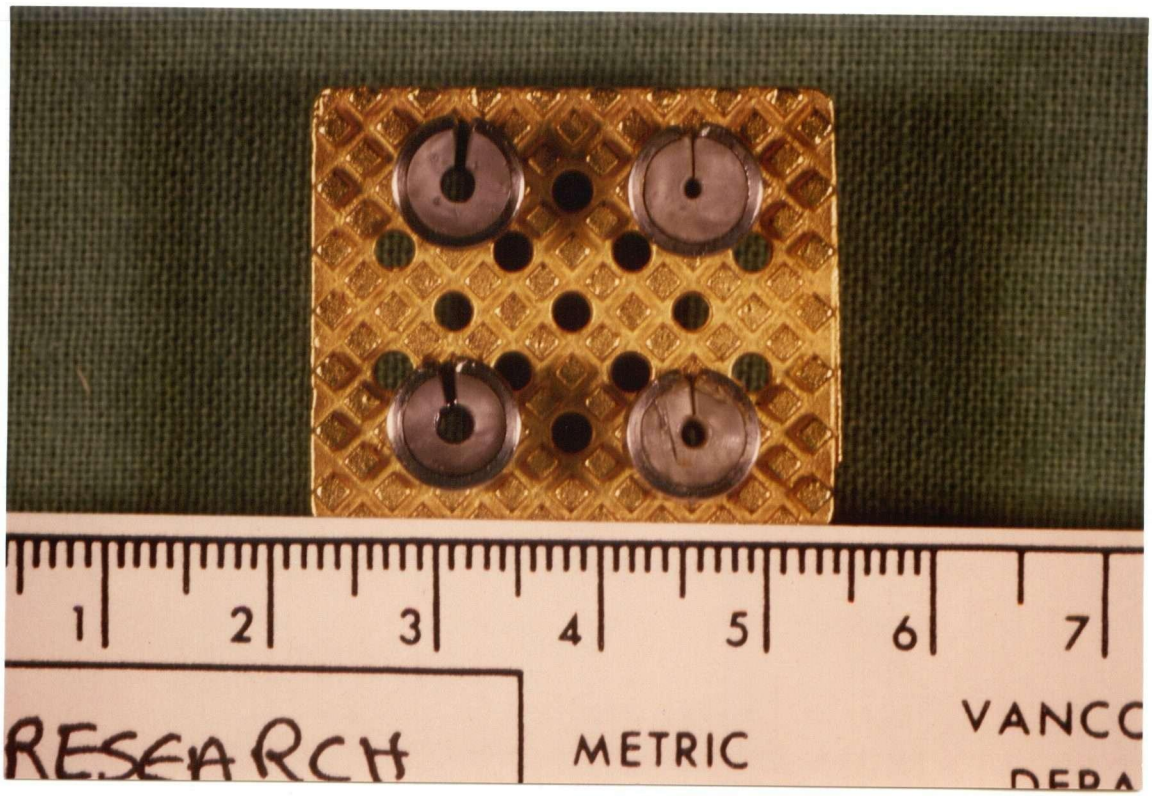
(C) - Different degree of closure in another animal in the study (X 96).

(D) - Microscopic assessment of LAD coronary artery showing complete closures of vessel from animal which collapsed from myocardial infarction (X 96).

(E) - Ameroid constrictors - at 0 days and after 14 days *in situ*.



(E)



(i) Electron microscopic studies of the regional myocardial bed showed ischemic changes at 14 days post-operatively, (Figures 19(B) and (D)). Gross changes included edema and myofilament clumping. Mitochondria appeared grossly swollen and irregular in shape, with thickened cristae. It must also be noted that the ischemia ranged from mild (Figure 19(B)) to severe (Figure 19(D)) within this study. Normal myocardial ultrastructure is seen in Figure 19(A and C).

(ii) Light microscopy revealed evidence of ischemia as shown in Figure 20. Damage was restricted to gross changes, such as edema.

Figure 19 - Electron microscopic assessment of normal myocardial tissue, (A) Dog 1, (C) Dog 2 (X 8550).
- Ultrastructural changes of myocardial tissue showing evidence of ischemia, (B) Dog 1, (D) Dog 2 (X 8550).

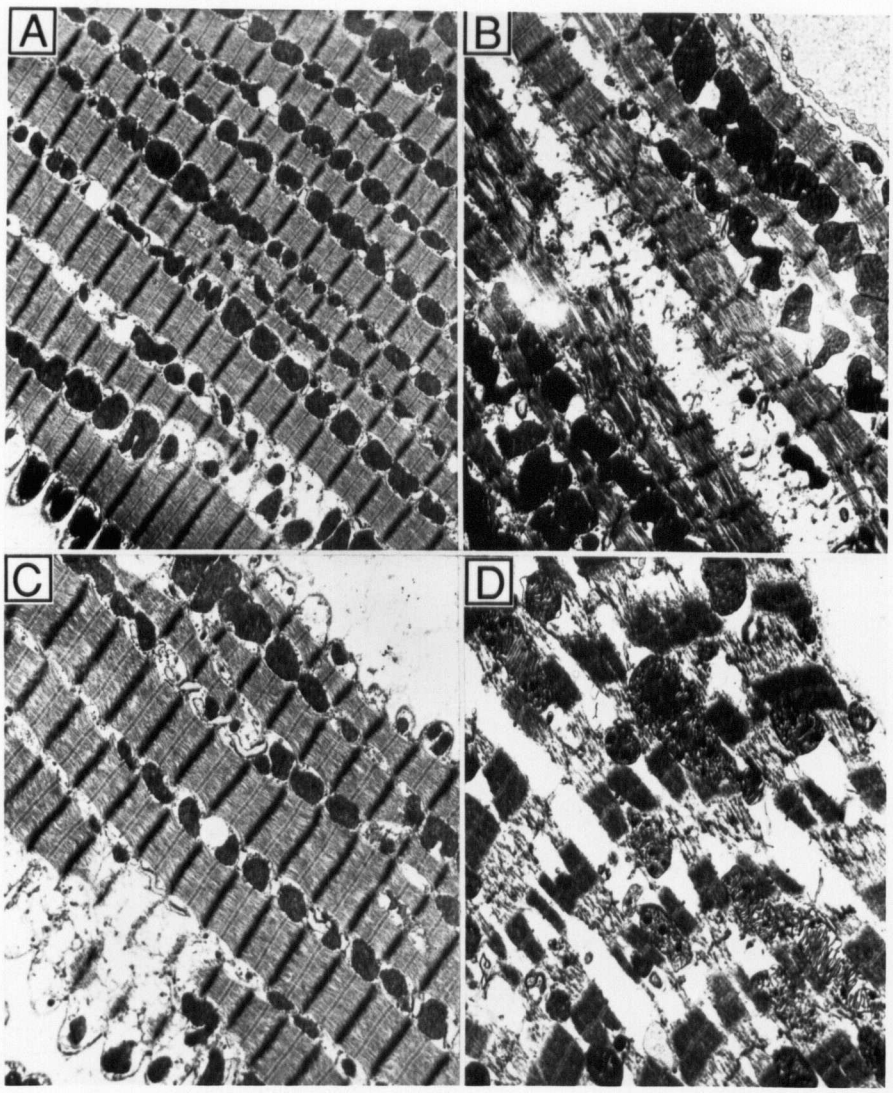
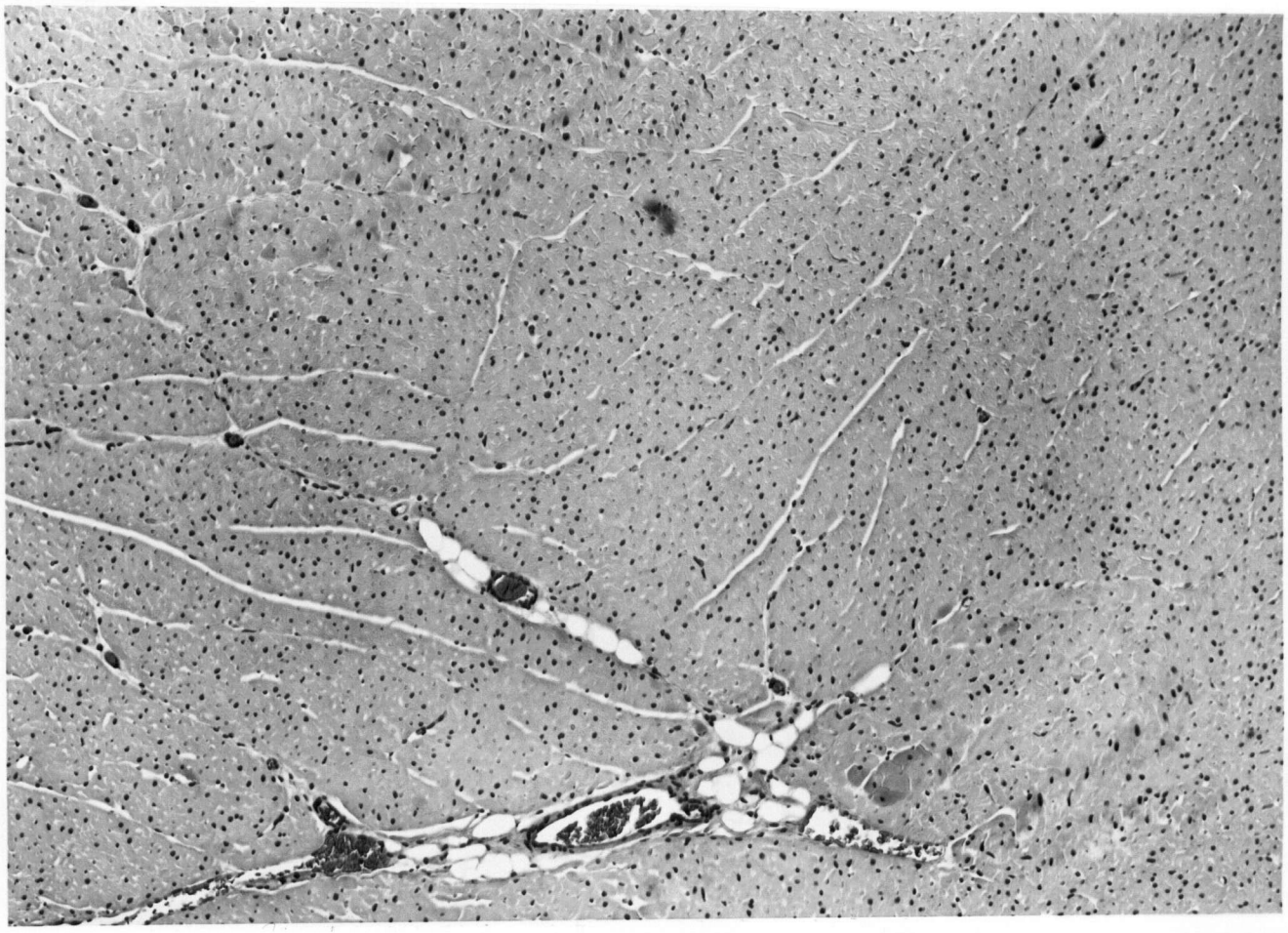
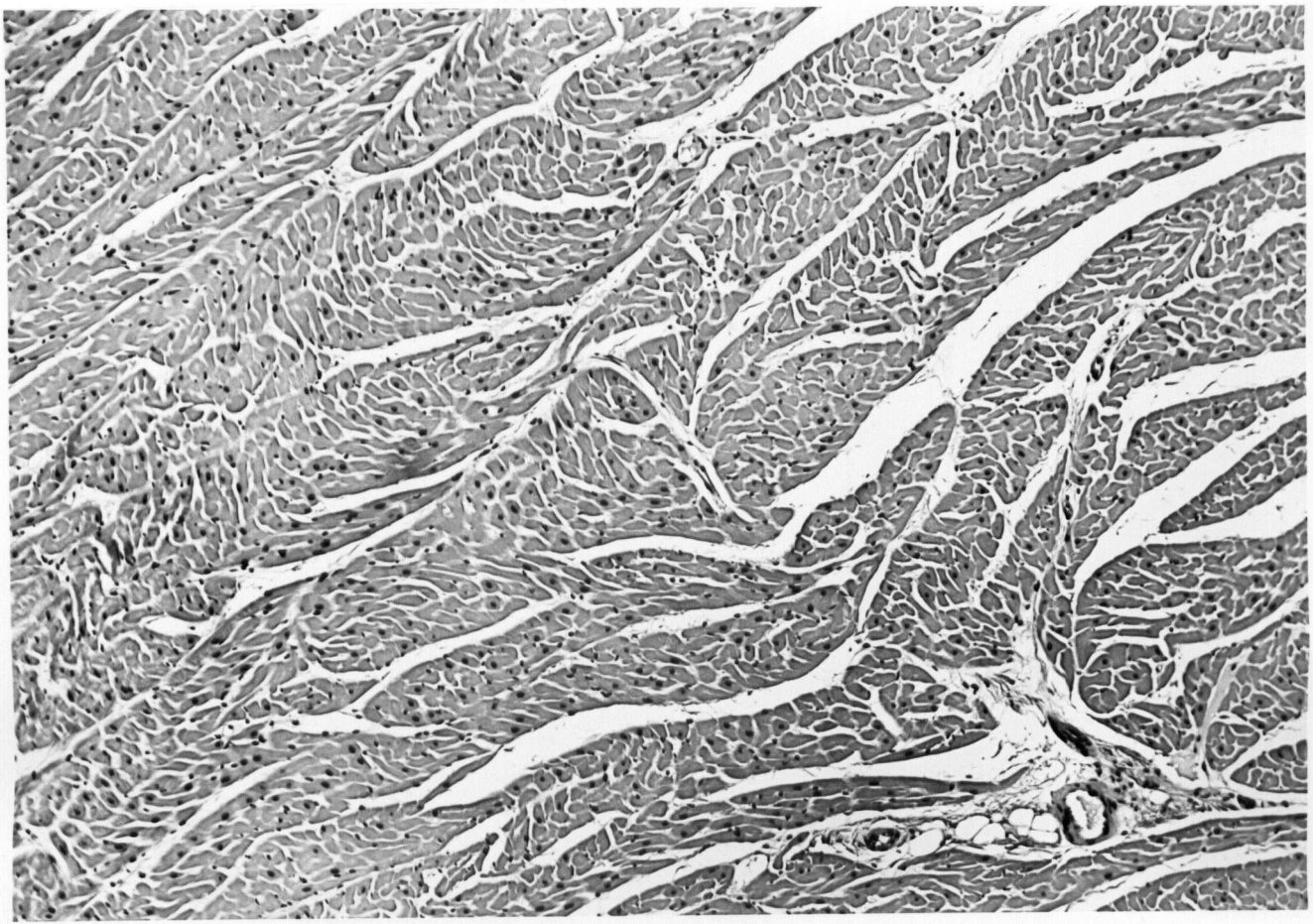

A
B
C
D

Figure 20 - Light microscopic changes produced by ischemia at 14 days in two different animals (A) Dog 1, and (B) Dog 2 (X 265).

(A)



(B)

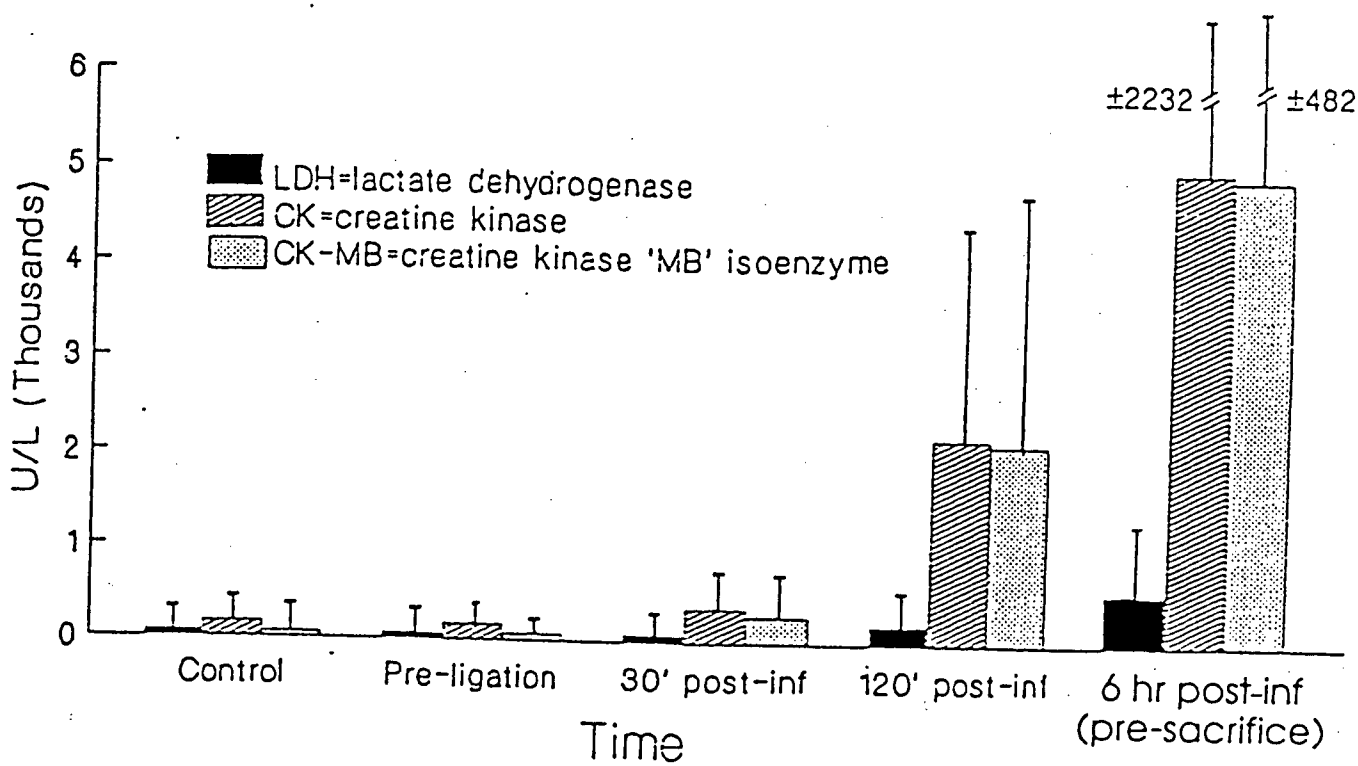


(3) Myocardial Infarction Model

(a) Metabolic Assessments

Venous concentrations of lactate dehydrogenase (LDH), creatine kinase (CK) and creatine-kinase-MB isoenzyme (CK-MB) are depicted in Figure 21. LDH increased from a control value of 74.56 ± 25.30 U/L to a pre-sacrifice value of 548.60 ± 684.20 U/L ($p<0.05$). CK values increased from 160.33 ± 46.44 U/L to 5030.60 ± 2232 U/L ($p<0.05$). The control and pre-sacrifice values for CK-MB were 318.50 ± 151.1 U/L and 829.87 ± 81.38 U/L, respectively ($p<0.05$).

Figure 21 - Venous concentrations of lactate dehydrogenase, creatine kinase and creatine kinase-MB isoenzyme at pre- and post-operative intervals.


Venous
LDH,CK,CK-MB
U/L (Thousands)
6
5
4
3
2
1
0
LDH=lactate dehydrogenase
CK=creatine kinase
CK-MB=creatine kinase 'MB' isoenzyme
±2232
±4820
Control
Pre-ligation
30' post-inf
120' post-inf
6 hr post-inf
(pre-sacrifice)
Time

(b) Analysis of Perfusion Defect Assessments

Figure 22 shows the clear demarcation of the perfusion defect with TTZ staining. Table 8 lists the planimetric infarct quantification for each of the three methods calculated as % of total slice size. Also listed are the ratios of $B^{123}IPPA$ to ^{201}Tl for each perfusion defect assessment. (Mean ratio = 1.01 ± 1.25).

Figure 22 - Staining of a myocardial infarction using a stain combining nitroblue tetrazolium and triphenyl tetrazolium. The staining of formazans within viable tissue can be seen as dark tissue, while the infarcted region remains pale.

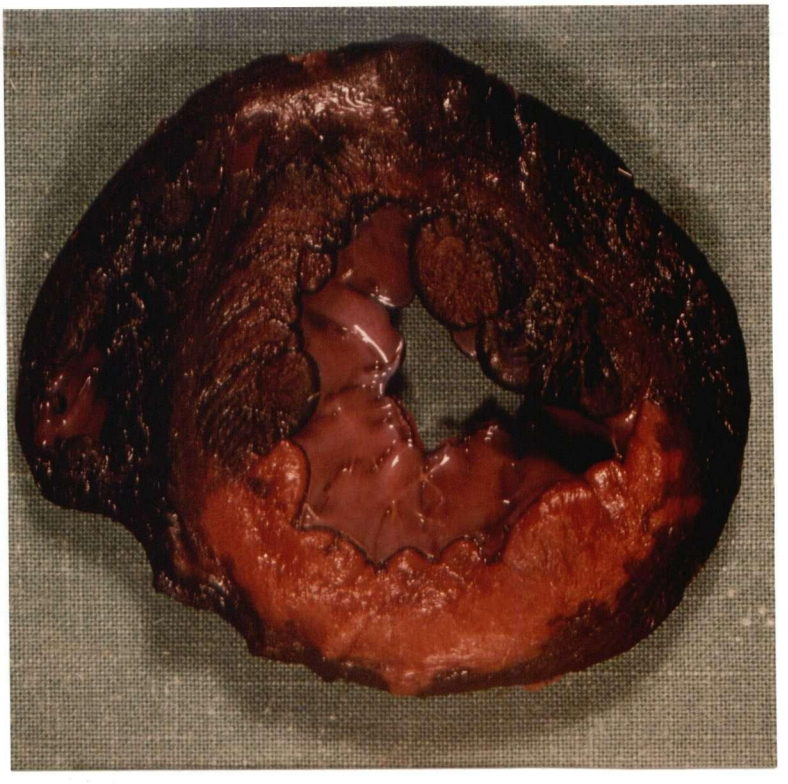


Table 8 - Perfusion defect size calculated as % of total slice size for each of the three methods - histochemical (TTZ staining), $B^{123}IPPA$ and ^{201}Tl.

Dog	Slice	(Histochemical) Mean of 2 Observer (%)	($B^{123}IPPA$) Mean of 2 Observer (%)	(^{201}Tl) Mean of 2 Observer (%)	Ratios $\left(\frac{B^{123}IPPA}{^{201}Tl}\right)$
1	1	27.4	30.8	19.4	1.59
1	2	32.6	15.9	10.8	1.47
1	3	28.8	7.0	10.2	0.69
1	4	21.2	11.2	23.8	0.47
1	5	23.9	15.2	34.3	0.44
1	6	0	12.3	40.1	0.31
2	1	9.6	45.8	49.5	0.93
2	2	10.4	30.7	49.1	0.63
2	3	9.1	12.0	23.8	0.51
2	4	4.2	9.9	31.6	0.31
2	5	9.3	20.0	5.3	3.77
2	6	14.3	8.2	0.0	0.0
3	1	42.1	38.1	28.9	1.32
3	2	32.7	31.4	25.4	1.22
3	3	38.5	17.8	21.1	0.84
3	4	36.7	7.1	24.9	0.29
3	5	27.3	9.1	17.0	0.54
3	6	19.8	5.1	20.1	0.25
4	1	2.3	2.8	0	0.0
4	2	0	0	0	0.0
4	3	0	0	0	0.0
4	4	3.9	0	4.5	0.0
4	5	7.4	0	23.2	0.0
4	6	0	0	37.2	0.0
5	1	29.7	12.8	34.2	0.37
5	2	19.8	13.0	28.5	0.46
5	3	21.6	13.9	10.4	1.34
5	4	22.5	32.8	17.0	1.93
5	5	7.9	39.3	14.4	2.73
6	1	24.7	31.5	24.4	1.29
6	2	20.4	23.4	25.8	0.91
6	3	27.2	10.5	11.4	0.92
6	4	13.5	22.9	0.0	0.0
6	5	14.1	31.3	6.7	4.67

Table 8 (continued)

Dog	Slice	(Histochemical) Mean of 2 Observer (%)	(B^{123}IPPA) Mean of 2 Observer (%)	(^{201}Tl) Mean of 2 Observer (%)	Ratios $\left(\frac{B^{123}IPPA}{^{201}Tl}\right)$
7	1	41.3	27.4	33.6	0.82
7	2	23.1	11.4	21.5	0.53
7	3	15.5	4.6	3.6	1.28
7	4	11.7	17.9	3.2	5.59
				mean ratio -	1.01±1.25
8	1	48.5		34.6	
8	2	38.0		50.2	
8	3	47.2		36.5	
8	4	40.6		39.1	
8	5	38.1		37.4	
8	6	41.7		32.5	
8	7	41.3		5.8	
9	1	20.3	24.8		
9	2	16.9	10.1		
9	3	10.4	0.0		
9	4	0	25.5		
9	5	0	16.9		
9	6	0	7.6		

The correlation between each of the methods is depicted by Figure 23. The actual infarct size, as assessed by tetrazolium staining, is compared to $B^{123}IPPA$ ($r=0.65$) ($p<0.005$) and ^{201}Tl ($r=0.49$) ($p<0.005$).

Figure 24 demonstrates the relationship between $B^{123}IPPA$ and ^{201}Tl ($r=0.54$ $p<0.005$).

The overall ratio for $B^{123}IPPA/^{201}Tl$ is 1.01 ± 1.25.

Figure 23 - Relationship between estimates of infarct size by the histochemical method (TTZ staining) vs. (A) $B^{123}IPPA$ and (B) ^{201}Tl

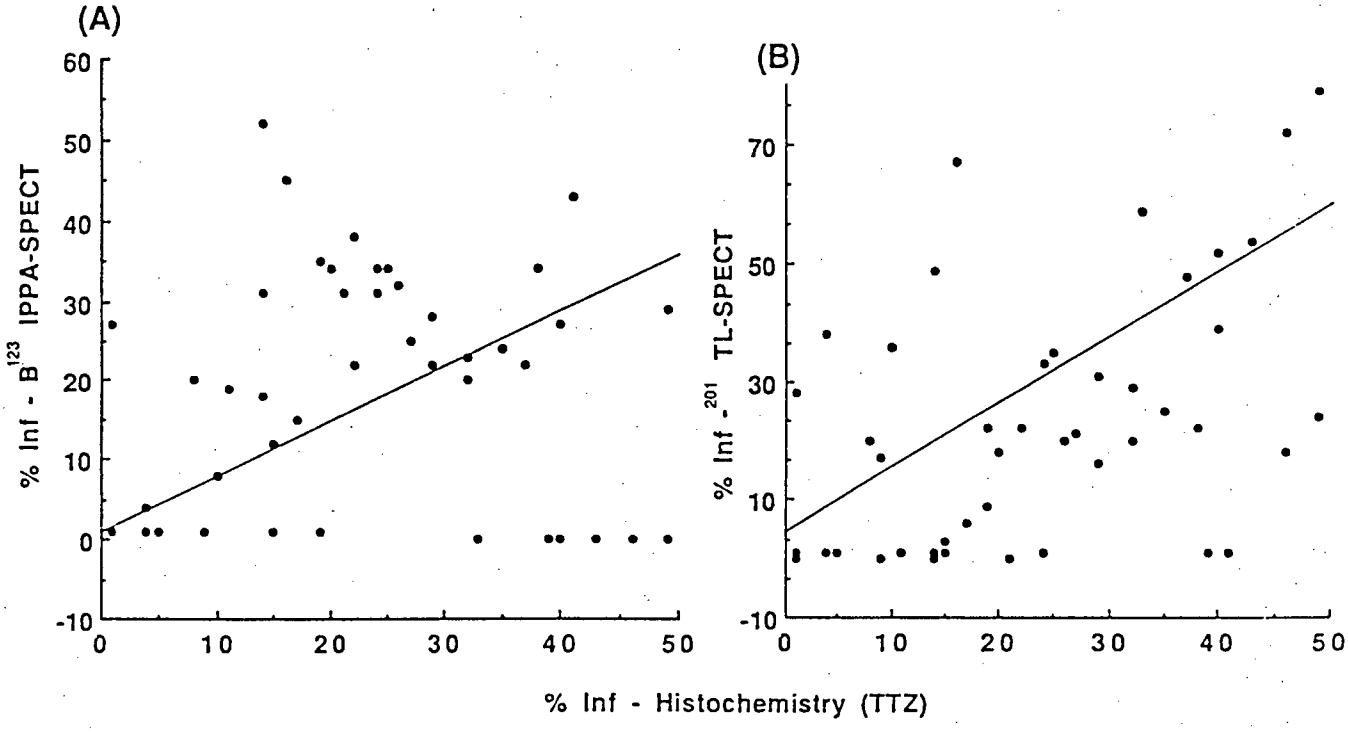
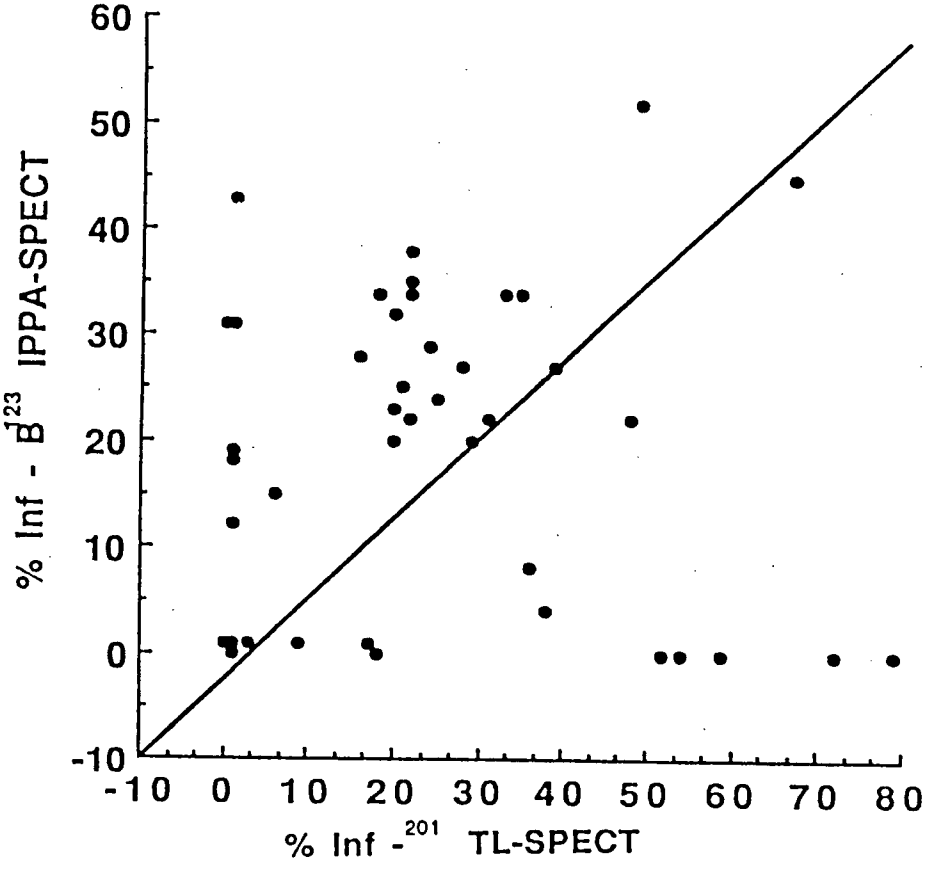

(A)
% Inf - B^{123} IPPA-SPECT
60
50
40
30
20
10
0
-10
0
10
20
30
40
50
(B)
% Inf - ^{201} TL-SPECT
70
50
30
10
-10
0
10
20
30
40
50
% Inf - Histochemistry (TTZ)

Figure 24 - Graph of $B^{123}IPPA$ *vs*. ^{201}Tl showing the relationship between the estimation of perfusion defect size by these two imaging agents.


60
50
40
30
20
10
0
-10
-10 0 10 20 30 40 50 60 70 80
% Inf - I^{123} IPPA-SPECT
% Inf - ^{201} TL-SPECT

(c) Autoradiographic assessment of IRZ

Table 9 lists the planimetric quantitation of the ischemic risk zone as well as the ischemic risk zone assessed by $B^{123}IPPA$ and ^{201}Tl.

Table 9 - Autoradiographic and scintigraphic assessment of ischemic risk zone calculated as % of total slice size.

Dog-slice	Autoradiography	$B^{123}IPPA$	^{201}Tl
8-1	0.0	-	24.9
8-2	67.5	-	16.5
8-3	69.7	-	0.0
8-4	56.3	-	0.0
8-5	60.4	-	0.0
8-6	48.3	-	0.0
8-7	49.1	-	0.0
9-1	0.0	9.5	-
9-2	0.0	0.0	-
9-3	0.0	18.7	-
9-4	0.0	0.0	-
9-5	0.0	19.2	-
9-6	0.0	23.2	-

(d) Histology

Light microscopy was used to examine gross cellular changes often associated with myocardial infarction (Figures 25(A) normal and (B) infarcted tissue). The most evident finding is tissue edema.

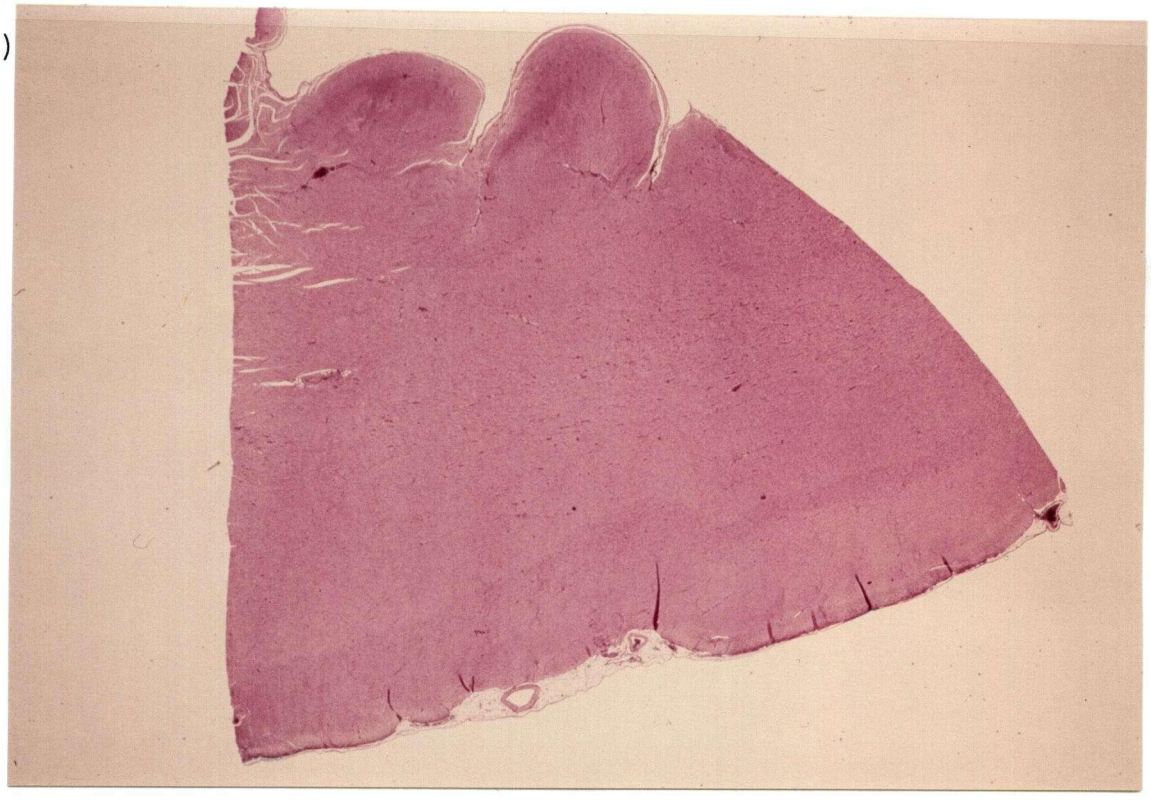
(e) Inter-observer and Intra-observer Variance

There was marginal inter-observer variance expressed by ANOVA and Duncan multiple range tests. Analysis showed two homogenous subsets in which $p>0.05$. Intra-observer variance was large demonstrating significant differences at $p<0.05$.

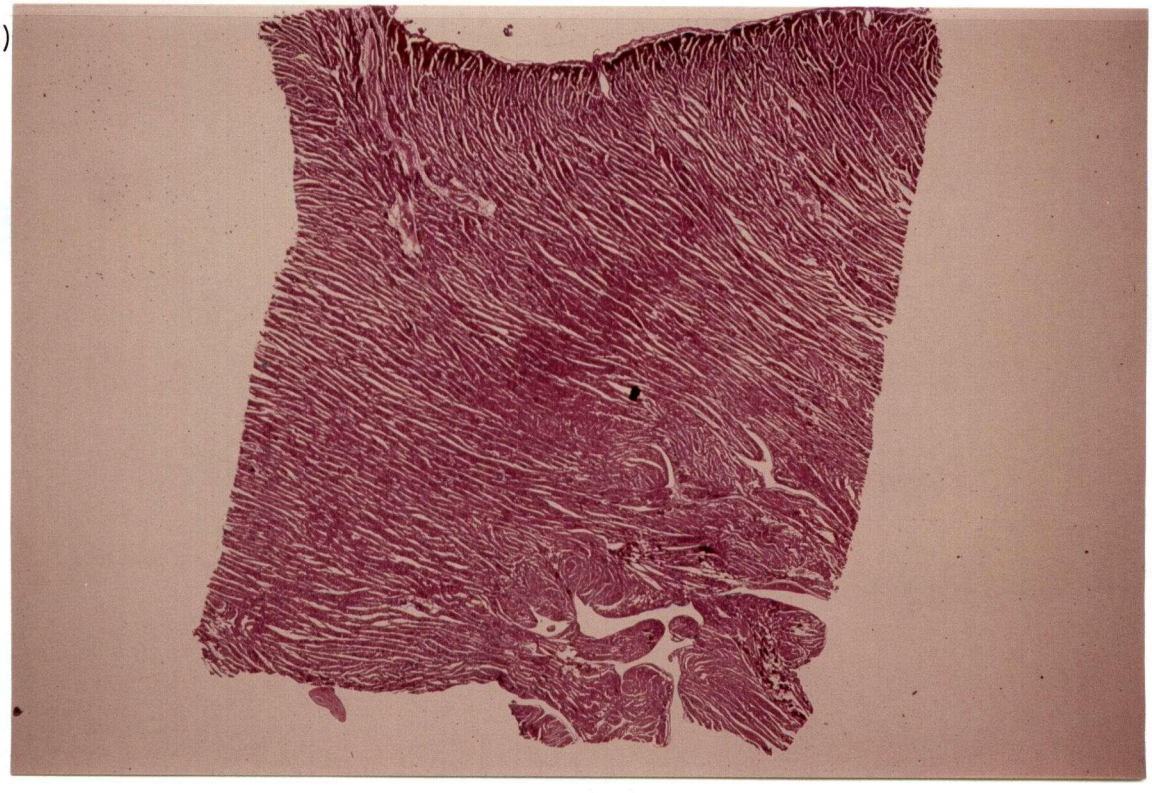
Figure 25 (A) - Light microscopic assessment of normal canine myocardium (X 63).

(B) - Gross cellular changes following acute myocardial infarction (X 63).

(A)



(B)



VI DISCUSSION

(a) Global Ischemia

The hemodynamic results of this study indicate little protection afforded by the addition of allopurinol or SOD to the cardioplegic solution. Results indicate that group A (iso-osmolar cardioplegic solution) is similar to group B (IO + SOD) and group C (IO + allopurinol). Myers et al. (1986), using a model of global ischemia in isolated rabbit hearts, reported marginal functional protection afforded by allopurinol added to the cardioplegic solution and no protection afforded by SOD included in the cardioplegic solution. A number of studies have been performed (Stewart et al., 1985; and Godin et al., 1986) in which pretreatment of the animals with allopurinol demonstrated preservation of left ventricular function as assessed by hemodynamic measurements and ultrastructural preservation, respectively. Thus, the ineffectiveness of allopurinol and SOD in preserving left ventricular function in this study may be a reflection of the failure to pretreat the animals.

All hemodynamic measurements were made only 30 minutes post-operatively. It is important to note that left ventricular function shortly following CPB may consistently be depressed (Swanson et al., 1983). In order to better assess the effects of the interventions, measurements should have been repeated after several hours or ideally 24-48 hours post-reperfusion.

The report of favorable preservation of mitochondrial ultrastructure and function by Godin et al. (1986), using allopurinol pretreatment, as well as the reported protection of mitochondrial oxidative phosphorylation using

SOD and catalase in the cardioplegic solution by Shlafer et al. (1982), suggests continued fatty acid metabolism. The assessment of fatty acid metabolism by radiolabeled fatty acids may provide a useful tool for indicating mitochondrial integrity and deserves future consideration as such.

The utilization of hypothermic cardioplegic arrest has become increasingly popular over the past 25 years (Roberts, 1987). Metabolism of ^{123}IPPA in the left ventricular walls of hearts subjected to reversible global ischemia is shown in Table 4 and Figure 10. Examination of trends indicates that the iso-osmolar solution was less effective in maintaining myocardial fatty acid metabolism, compared to the solutions supplemented with SOD and allopurinol. Hemodynamically, all groups showed similar results. In a study examining hemodynamics and metabolism at similar times, Fridrich et al. (1986) demonstrated that normal hemodynamics are indicative of normal metabolism. In this study, hemodynamics were assessed 30 minutes after weaning from CPB, compared with metabolism which was assessed 3 hours post CPB. This could have a bearing on our results. During the ischemic period, anaerobic glycolysis is the dominant mechanism of energy production (Conti and Kao, 1983). However, following reperfusion, there may exist a mixed population of cells, some functioning aerobically, while the majority maintain an anaerobic state, as demonstrated by Opie et al. (1973). These authors demonstrated increased utilization of glucose within the ischemic zone, following coronary artery ligation over a two hour interval. We found a trend of increasing functional indices during the initial post-bypass period for the iso-osmolar group (Table 5) although only SVR was significant at $p<0.05$. It must be noted, however, that systemic vascular resistance was

decreased to a greater extent in group A, compared to groups B and C (Table 5). Thus, the trend towards improved function of group A could simply reflect this fall in SVR. Indeed, ejection fraction remained relatively unchanged in the Tyers' group (73 ± 5 to 71 ± 8)($p>0.05$), indicating no change in function (Table 5). Groups B and C show no significant change in function post-operatively.

The dramatic fall in SVR seen in group A is interesting. Although we were not examining the consequences of global ischemia on SVR, this result is noteworthy. The fall in SVR will influence afterload and thus will indirectly affect variables dependent upon the latter, such as cardiac index and stroke index. The fall in resistance may lead to an increased cardiac output, which will in turn increase cardiac index (noting the formula for CI). Similarly, a rise in CI will affect SI, since the latter is dependent upon the former. The question of why the SVR decreased so dramatically in group A is unknown. Examining the formula, one notices that many variables may influence its value. Although the exact cause is unknown, our main concern was not with the consequences of global ischemia on SVR, but on metabolism. Similarly, the increase in HR for group B cannot be accounted for, but in view of the large number of variables which will affect it the answer may be very complex.

The metabolic assessment was performed three hours following weaning from bypass. As already mentioned, reperfusion, as well as ischemia, may lead to a mixed population of cells, ie. variable degrees of ischemic effects. The lateral wall plays a major role in left ventricular function and showed significantly increased $t_{\frac{1}{2}}$ values [11 ± 6 to 24 ± 10 ($p<0.05$) and 56 ± 60 to 417 ± 238 ($p = 0.051$)] for the early and late phases,

respectively, (Table 4) in the iso-osmolar group. This implies impairment of fatty acid oxidation. Although ejection fraction appeared to be unchanged immediately following bypass, assessment of function and metabolism several (ideally 24-48) hours after bypass may have provided a more meaningful comparison.

The ability of SOD and allopurinol to abolish this ischemia-related shift in the beta-oxidation phase of metabolism is questionable. It is known that global ischemia causes a shift from aerobic to anaerobic metabolism (Opie, 1976a,b), as evidenced by a decrease in oxidative phosphorylation, thus decreasing fatty acid metabolism, since both processes are coupled. Fatty acid metabolism may also be affected by oxygen-derived free-radical-induced hydrolysis of membranes and mitochondrial membrane damage (Trump, 1982). These events may lead to impairment of fatty acid metabolism as reflected in decreased myocardial washout rates of the fatty acid. The results of this study demonstrate that only the lateral wall in group A (Tyers iso-osmolar solution) is significantly altered, compared to the two other cardioplegic formulations, as assessed by the early phase of ^{123}IPPA washout (Figure 10). Thus, it would appear that the cardioplegic solutions (groups B and C), aimed at preventing oxygen cytotoxicity, may have proven beneficial for maintenance of myocardial fatty acid oxidation. However, the large standard deviations obtained in this study may have masked any changes in $t_{1/2}$ in the other solutions.

Therefore the fact that only the iso-osmolar group showed a significant increase of $t_{1/2}$ for the lateral wall may or may not signify poor myocardial preservation. The remaining groups may or may not protect the myocardium well; however, again the SD values are large and may mask significant values.

Variability of the results of this study may be explained by work completed by Fox et al. (1985) and Lerch (1986). These authors reported that washout rates of those fatty acids which back-diffuse or are not metabolized could affect the washout curves and, thus, account for the variability in $t_{1/2}$ values. They also found that supply of the tracer to the myocardium and washout of the tracer by the coronary circulation may influence the results. These factors were not taken into account in this study.

Late phase pharmacokinetics reflect either the incorporation of fatty acids as triglycerides and phospholipids (Van der Wall, 1985) or the storage and decay of the isotope (Visser et al., 1986). The importance of this energy store is relatively secondary to the clinically more relevant beta-oxidation phase. Control myocardial washout rates ($t_{1/2}$) are greater in the late phase, relative to the early phase, indicating a slower utilization of this triglyceride and phospholipid pool. Van der Wall et al. (1981) report $t_{1/2}$ values in excess of 100 min in this phase. Variability in our results may reflect a scanning period of only 30 minutes, which would account for only the beginning of this late phase. Many of the studies examining myocardial metabolism now extend the scanning time to 60-90 minutes (Schon et al., 1986). All walls demonstrated an increase in $t_{1/2}$ for the late phase, between control and post-operative assessments. However, only values for the lateral wall and apical wall for the iso-osmolar group were statistically significantly increased ($p<0.05$) (Figure 10). These prolonged $t_{1/2}$ values, following the ischemic interval, may imply impairment in the release of triglycerides and phospholipids from this secondary energy pool, or could reflect an increase in the esterification of intracellular

free fatty acids, thus reducing fatty acid availability for beta-oxidation, as postulated by Opie et al. (1973). The standard iso-osmolar solution and the cardioplegic solutions supplemented with SOD and allopurinol showed similar effects on the late phase of ^{123}IPPA metabolism, thus implying that all three solutions result in an increase in the content of this lipid pool and, by implication, poor protection as assessed by comparing pre-operative to post-operative t½ values.

Teoh et al. (1988) have demonstrated that oxidation of fatty acids and glucose by the myocardium is impaired after cardioplegic arrest for coronary bypass grafting. Nineteen patients were used to examine the effect of arterial lactate concentrations on the metabolism of fatty acids and also on ventricular function since lactate can be used as an energy substitute. Patient criteria were: stable exertional angina pectoris, double- or triple-vessel CAD, and LVEF>30%. In randomized groups, patients within the low lactate concentration (0 mmol/L) group received an intravenous infusion of 150 mls/hr of normal saline perioperatively, while the patients who were in the high lactate group received 150 mls/hr of Lactated Ringers solution (24.3 mmol/L lactate). Lactate ^{14}C was used to assess myocardial lactate metabolism. The group which received the perioperative infusion with high lactate showed improved functional recovery compared to the group infused with low lactate levels which may support the hypothesis that lactate may be the preferred substrate after cardioplegic arrest.

This shift away from free fatty acid metabolism, following cardioplegic arrest, may also explain the increases in t½ seen in this study. Lactate levels were not measured in the study, so confirmation of lactate metabolism before and after cardioplegic arrest was not possible.

Regional heterogeneity of the various areas of the heart was also addressed in this study (Figure 11). The results demonstrate no significant difference in normal canine myocardial washout rates of the various areas of the left ventricle although, again, large standard deviations were noted. This validates the use of control areas and experimental areas within a single heart, since the areas demonstrate similar washout rates.

(b) Regional Ischemia Model

The lateral wall and apical wall within the left ventricle showed significant increases in $t_{\frac{1}{2}}$ values after 14 days of gradual coronary occlusion, suggesting impairment of oxidative free fatty acid metabolism (Table 6). These results concur with experimental and clinical studies, which have demonstrated decreased fatty acid washout rates within ischemic myocardium (Reske et al., 1982 a,b; Dudczak et al., 1982a,b; Van der Wall et al., 1983). Ischemia was confirmed by electron microscopic examination of a control specimen and an ischemic area within the regional myocardial bed after 14 days of gradual coronary occlusion (Figure 19). Ultrastructural ischemic changes are similar to those reported by Reimer and Jennings (1987), ie. edema, myofilament clumping, mitochondrial swelling with irregularities in their shape and cristae thickening. Variability between animals, as to extent of ischemia, could be observed. The flocculent densities, or amorphous specks, found within the mitochondria are representative of a certain degree of ischemia. However, not all animals manifested this change; thus, clearly some discrepancy exists as to the level or degree of ischemic injury between animals.

A study by Railton et al. (1987) suggests that imaging with iodinated aliphatic fatty acids (e.g. ^{123}I-heptadecanoic acid) may not be of sufficient sensitivity or specificity to be used for routine clinical assessment of cardiac patients. These authors reported that 40% of patients with angina pectoris and a positive exercise electrocardiogram could not be reliably diagnosed using this imaging agent. However, the terminally-labeled 17-carbon straight-chain fatty acid used (^{123}I-HDA) required background subtraction and this procedure may have decreased its sensitivity.

Recently, studies have questioned the interpretation of myocardial washout rates in terms of the mechanism of washout. It is reported that the early elimination phase of the T-A curve, assumed to represent beta-oxidation, is actually a reflection of the diffusion of the free iodine from the myocyte into the bloodstream (Visser et al., 1985). These authors suggest the decrease in myocardial washout rates demonstrated under ischemic conditions may be the result of a decreased washout of radioiodide. However, radioiodinated heptadecanoic acid, subsequent to catabolism, releases free radioiodide, which is free to recirculate. Machulla et al. (1980) in order to prevent the release of radioiodide following injection of a labeled fatty acid, used radioiodinated omega-phenylpentadecanoic acid in a murine model. Reske et al. (1985a,c) examined the kinetics of tissue concentrations of labeled catabolites of ^{123}IPPA and found a rapid oxidation of IPPA and subsequent washout of ^{123}IPPA catabolites from the myocardium. Therefore, the $t_{1/2}$ values should not be influenced by recirculating iodine since the iodide will be removed from the body still attached to the phenyl ring. The washout of radiolabeled fatty acids will require further investigation before it becomes completely understood.

The phenylated derivative of pentadecanoic acid used in this study results in the release of ^{123}I-benzoic acid into the peripheral circulation, where it can be further metabolized by the liver and kidneys to ^{123}I-hippuric acid and finally excreted in the urine (Machulla et al., 1980 and Reske et al., 1982a,d). The myocardial images obtained allowed clear demarcation of left ventricular walls as reported by Reske et al. (1982a,d).

The prolonged $t_{\frac{1}{2}}$ values in both early and late phases of the time-activity curves (Table 6 and Figure 14) of the ischemic segments at 14 days may be attributed to a reduction in oxygen supply induced by a decrease in coronary diameter by way of the ameroid constrictor. Atrial pacing induced an additional stress by increasing oxygen demand, thus removing any benefit of compensatory flow via the development of collateral circulation within the ischemic bed (Brazier et al., 1975; Schell et al., 1979; Hornby et al., 1976). The results obtained in this study are in agreement with those of Van der Wall et al. (1981), in which a group of patients with coronary artery disease were exercised and scanned with HDA. The ischemic regions showed significantly increased $t_{\frac{1}{2}}$ values, while the control group (normal volunteers), at rest, had normal washout rates. In the present study, atrial pacing resulted in significantly increased heart rates and the ischemic regions showed increased $t_{\frac{1}{2}}$ values. Hansen et al. (1988) demonstrated that exercise-induced myocardial ischemia results in altered ^{123}IPPA uptake, reduced washout, or both. These results are consistent with the longitudinal study performed here and also with a report published by Kennedy et al. (1986). The latter demonstrated dramatically reduced ^{123}IPPA washout in patients with severe CHD compared to normal volunteers.

Schon et al. (1986), reports that the so-called beta-oxidation phase is influenced by increasing MVO_2 and augmented cardiac work. If these two factors were to influence washout rates as expected, an increase in fatty acid washout should be seen. The increased availability of oxygen and increased workload should result in an increased utilization of the fatty acid, thus increasing washout rates. However, the effects are such that myocardial washout rates decrease. This would suggest that $t_{½}$ of this early phase is not a reflection of fatty acid metabolism. However, since HDA and ^{123}IPPA oxidation result in different catabolic end-products, studies comparing the effect of MVO_2 and cardiac work on ^{123}IPPA metabolism are needed to distinguish any differences from the physiologically occurring palmitic acid labeled with ^{11}C. Schon has stated that the sizes (ie. the Y-intercept of the T-A curve) of the early and late phases are valid measures of fatty acid metabolism and may provide a more valid comparison. The debate, however, centres around what these so-called elimination phases represent.

The influence of ischemia, by way of regional myocardial blood flow and various interventions, on the oxidation of ^{123}IPPA was also examined by Reske et al. (1986). These authors demonstrated that increasing concentrations of lactate decreased fatty acid washout, resulting in significantly increased $t_{½}$ values. Similar findings were reported by Duwel et al. (1988) in which fasted patients were loaded with sodium lactate (3mM/kg) prior to ^{123}I-HDA scintigraphy. The half-life values increased and uptake levels decreased following the lactate intervention. Both lactate and exercise demonstrated similar scintigraphic findings suggesting that lactate will inhibit FFA oxidation under normal, as well as ischemic,

conditions (Duwel et al., 1988). Perfusion was also identified as a major determinant of cardiac ^{123}IPPA uptake. The changes in $t_{1/2}$ values reported in this study may be the result of a reduction in blood flow, thus reducing oxygen supply, but not an increase in lactate, an end-product of ischemic metabolism. The latter was shown not to significantly increase in this study (Table 7).

A number of concerns are apparent in this study. The degree of closure and rate of swelling (Figure 18) of the ameroid constrictor could not be controlled, leading to variable degrees of ischemia in experimental animals as mentioned earlier. The electron micrographs showed variable degrees of ischemic damage (Figure 19) and this discrepancy in the extent of ischemia may account for the variability seen in $t_{1/2}$ values. The large standard deviations associated with assessment of $t_{1/2}$ may also be due to the way in which animals in the study were grouped. Washout rates ($t_{1/2}$) were grouped according to the time of assessment. However, as mentioned earlier, rate of swelling could not be controlled. Therefore, the radius of the central lumen of one constrictor at day five, for example, could have been reduced by 50%, while in another constrictor, the reduction in radius could have been 80%. However, both $t_{1/2}$ assessments were performed on the same day and thus were placed into the same group. If the assessments of $t_{1/2}$ were categorized as to degree of ischemia and not time, the standard deviations may have been reduced. That is, if we were able to inject microspheres, as discussed earlier, and quantitate blood flow reduction, categories based on degree of ischemia might have been recorded. Thus, all $t_{1/2}$ values at 50% reduction in blood flow, for example, would have been assessment 1, 60% assessment 2, and so on. This might alleviate some of the statistical

problems we experienced (large standard deviations) in the analysis of the data. The assessment of regional myocardial bloodflow by a microsphere technique was proposed. However, injecting these microspheres at each time interval would necessitate an atrial cannula be maintained for 14 days. The problems associated with this resulted in its exclusion from the study. We do realize that this difficulty must be overcome if this research is to move forward. Five of twelve animals died as a result of complete closure of the constrictor, causing myocardial infarction prior to the final 14 days assessment period, showing the variability of this technique.

The different responses of the septal wall and the remaining two walls (Table 6, Figure 14) may be explained by the coronary anatomy of the dog. Donald and Essex (1954) report that the septal branch of the LAD supplies 70 to 75% of the interventricular septum. The septal branch (ramus septalis), however, may originate in a number of different locations (Miller, 1964). This could explain the difference between the septum and other two walls, since the ameroid constrictor may have been placed distally or proximally to the ramus septalis. Thus, this area of the ventricle may or may not have been affected by the reduction in blood flow through the LAD distal to the constrictor. Fluoroscopy studies of the coronary anatomy might have alleviated this problem.

Schelbert (1986) states that a number of factors will influence the slope of the tissue washout curve. These include the volume of distribution of ^{123}IPPA in tissue, back diffusion, recirculation and flow-dependent removal of ^{123}IPPA and its metabolic by-products. Although these factors were not considered in this study, they have been addressed in the literature. Reske et al. (1984d) calculated that 4.5 - 6.0% of the injected

dose of ^{123}IPPA was taken up by the myocardium. In 1985(a), Reske and coworkers demonstrated that a number of other tissues extracted ^{123}IPPA, but at significantly slower rates. These tissues included lung, liver, kidney, spleen and skeletal muscle. Therefore, although the majority of the fatty acid metabolism may occur elsewhere in the body, the effect of recirculation is minimal due to the fact that the ^{123}I-benzoic acid produced is removed from the circulation by the kidneys and liver and subsequently eliminated from the body in the urine. The use of the phenylated derivative thus avoided the problem of recirculation (Machulla et al., 1980). Since occlusion of the coronary artery was incomplete and extensive collateral circulation was present, removal of metabolic by-products could be assumed to have continued (Lerch, 1986). The final parameter not addressed was back-diffusion of unmetabolized ^{123}IPPA (Fox et al., 1985). This may also have contributed to variability in $t_{\frac{1}{2}}$ values.

In spite of these shortcomings, the results of this study indicate that 15-p-^{123}I-iodophenyl-pentadecanoic acid may be utilized to examine disturbances in regional myocardial metabolism caused by ischemia.

(c) Myocardial Infarction Model

Radionuclide techniques aimed at quantifying myocardial infarction still lack an ideal radiopharmaceutical. In this experimental endeavour, the utilization of a beta-methylated fatty acid, chosen for its reduced metabolic rate and increased intramyocardial residence time (Livini et al., 1982), has shown little effectiveness for assessing the size of myocardial perfusion defects. In comparison to a histochemical assessment of perfusion defect size, our results show a low degree of correlation for the perfusion

defects estimated by the iodinated fatty acid analogue ($r = 0.65$-$p<0.005$) and by ^{201}Tl ($r = 0.49$-$p<0.005$). Both agents suffer from border demarcation problems which will greatly limit their usefulness in such a manner.

Significant post-infarction increases of creatine kinase and CK-MB enzymes in this study confirmed the presence of myocardial infarction (Figure 21). The increase in CK and CK-MB enzymes following myocardial infarction has been known since the 1960's (Dreyfus et al., 1960) and has been confirmed more recently by Sylven (1980) and Heyndrick et al. (1986).

Although the correlation between histochemical assessment of infarct size and the assessment by $B^{123}IPPA$ was higher, relative to that of ^{201}Tl, the values were still low, compared to those reported in the literature for the scintigraphic assessment of infarct size (Kaul et al., 1985; and Wolfe et al., 1985). These low correlation values may be explained by a decrease in tracer availability because of metabolic degradation as suggested by Dudczak et al. (1986), as well as Ambrose et al. (1987). These authors reported decreased myocardial retention times within the myocardium of a beta-methylated agent. Dudczak et al. (1986) generated mono- and bi-exponential washout curves in 19 patients using a beta-methylated fatty acid. Myocardial washout was bi-exponential in 11 of 19 patients with a mean $t_{½}$ = 13.8 min for the early phase and mean $t_{½}$ = 187 min for the late phase. In 8 of 19 patients, washout was mono-exponential with $t_{½}$ = 218 min. Similarly, Ambrose et al. (1987a) reported that following IV administration of beta-methyl-iodophenyl-pentadecanoic acid in rats, there were differences in heart retention times and subcellular distribution. Subcellular distribution was also examined by Otto et al. (1985a). These authors measured washout times for iodophenyl-pentadecanoic

acid (IPP), beta-methyl-IPP and beta-di-methyl-IPP and found significant increases in washout times for the latter two agents compared to IPP, 5-10 min, 30-45 min, and 6-7 hours, respectively. The mechanism by which catabolism of the mono-methyl-substituted agent occurs is a complex pathway involving a number of additional steps prior to entry into the beta-oxidation pathway (Dudczak et al., 1986). Scintigraphic images of sufficient quality , however, could still be obtained up to 120 minutes after injection of the beta-methyl-IPP, as reported by Dudczak et al. (1986). Although some metabolic degradation of our fatty acid may have taken place, our scan time of 20 min would be associated with minimal loss of tracer from the myocardium.

Of greater concern with this study, and many other studies in this field, is border demarcation on the tomographic images, especially at the apical region, following LAD occlusion. Caldwell et al. (1984) reports that, in myocardial infarcts at the apical region, it was necessary to occasionally estimate the location of the apex from a subjective visual impression. Such an approach appears to be inadequate, in view of our intra-observer differences (Table 8).

Caldwell et al. (1984) also stated that, if the defects were assessed as volumes, the tomographic estimates would be higher than actual values. This was attributed to blood pool activity within the ventricular cavity. In order to exclude this problem from our study, blood pool-endocardial boundaries on the tomogram were defined and subsequently the central cavity subtracted from each slice image. This was difficult since endocardial, as well as epicardial borders, were not as well-defined as may have been possible because of problems with resolution. This problem with border

clarity may have contributed to error within the study and influenced our interpretation of image geometry. In view of the heterogenous geometry often associated with myocardial infarction, difficulties in placing accurate borders around such an area do exist. In order to compensate for this, Prigent et al. (1987) utilized maximum count circumferential profile analysis of ^{201}Tl SPECT images to quantify infarct size using a number of derived algorithms. These authors demonstrated high linear correlation values with these methods, which were independent of edge detection. Refinements in volume determination by SPECT indicates that this technique may be an accurate diagnostic tool (Halama and Henkin, 1986; Mortelmans et al., 1986). Halama and Henkin (1986) reported on the use of edge enhancement and volume projection to estimate volumes by SPECT with good correlation. Mortelmans et al. (1986) have described a new thresholding method, which increases the accuracy of edge detection and thus increases the accuracy of volume determination by SPECT. These techniques required computer programs which were either too expensive, or unavailable, so that the only method available to us, ie. a subjective assessment with areas-of-interest drawn manually, was utilized in this study.

Although SPECT appears to offer many advantages, there remain some technical problems which require further investigation before this can become a fully acceptable means of quantitatively assessing perfusion defects. Another possible source of error may have been the utilization of filters in the reconstruction process (Jaszczak, 1988). Although beneficial for image quality, the masking out of any levels of activity may limit the usefulness of this technique. The images in this study were reconstructed from a 64 x 64 matrix. It was felt, at the time, that this

was appropriate; however, a 128 x 128 matrix may have improved image quality. Thus, inappropriate matrix size may have contributed to the error in this portion of the study.

Clearly, there are a number of technical factors associated with SPECT, which were not addressed in this study and thus may have contributed to the low correlation values. However, it must be kept in mind that when dealing with mm. or cm. precision, any small variations in image fuzziness could be transmitted into very substantial errors. It is clear that the method utilized to assess the size of perfusion defects in this study was inappropriate.

As mentioned earlier, the utilization of both imaging agents together may allow for a better assessment of the ischemic risk zone and give some indication of irreversibly injured myocardium. Strauss et al. (1987) demonstrated variable uptake patterns when utilizing ^{201}Tl and beta-methyl-p-^{123}I-iodophenylpentadecanoic acid. It was found that in areas supplied by vessels which were totally occluded or severely stenosed, FFA uptake was equal to ^{201}Tl uptake. In areas in which the vessel was severely stenosed, but had some flow or good collaterals, FFA uptake was greater than that of ^{201}Tl. In zones of reperfused myocardium, FFA uptake was less than ^{201}Tl. Therefore, it is clear that the utilization of both agents may differentiate between various zones of ischemia within the myocardium. Fischmann et al. (1988) also examined the role of combined fatty acid and thallium imaging in the assessment of myocardial ischemia and infarction. Following acute LAD occlusion in mongrel dogs, both ^{201}Tl and ^{123}I-para-iodophenyl-9-methyl heptadecanoic acid were injected and scans performed. The authors observed a relative excess of the fatty acid in the

infarcted area in 7 of 7 dogs. Therefore, it appears that the infarcted area had taken up the fatty acid, presumably via collateral circulation. Thus, both ^{201}Tl and fatty acid would provide information as to the extent of the infarction and the degree of ischemia. In this study, however, B^{123}IPPA/^{201}Tl ratios indicated similar estimations of perfusion defect size (mean ratio 1.01±1.25), indicating no advantage of using one over the other or of using both together.

Kairento et al. (1988) evaluated ^{201}Tl and ^{123}I-p-14-iodophenyl-Beta-methyl-tetradecanoic acid with SPECT in a canine model of myocardial infarction. Results indicated that both agents showed decreased activity within areas of infarction. It was also shown that ^{201}Tl overestimated the size of the damaged myocardium relative to the fatty acid imaging agent. Within the zone of infarction, however, in view of the well-developed collateral circulation in the dog, various degrees of ischemia may exist. Thus, fatty acid uptake within these cells may also vary, thereby influencing our SPECT image. This was also shown to be the case in Kairento's work. Therefore, this type of residual activity within the infarcted area could lead to image fuzziness and thus give rise to possible error.

In an interesting study by Maublant et al. (1988), beating ventricular cells from 3 day old newborn rats were cultured and incubated with 37 kBq (1μCi) of ^{201}Tl. The effect of extracellular pH on intracellular [^{201}Tl] was investigated. At 20 min, intracellular [^{201}Tl] was higher at pH 8 than at pH 6, inferring some alteration in the removal of ^{201}Tl from the cell as pH increases. This may indicate that there may be less ^{201}Tl accumulation within cells due to acidosis, which is indicative of a decreased pH.

Our data do not support the choice of the beta-methyl-FFA over ^{201}Tl as a marker of tissue perfusion. Although physical characteristics would indicate its superiority, FFA imaging still requires some further technical and developmental refinement before accurate quantitative assessments of myocardial perfusion defects can be made.

VII SUMMARY AND CONCLUSIONS

The purpose of this study was to utilize 15-p-^{123}I-phenyl-pentadecanoic acid and 15-p-beta-methyl-iodo(^{123}I)phenylpentadecanoic acid in the assessment of myocardial metabolism and perfusion following ischemia and infarction.

We can conclude from this study:

(1) In a model of global ischemia, we found that myocardial washout of 15-^{123}IPPA may be modified by the addition of SOD or allopurinol to the control cardioplegic solution (Tyers iso-osmolar solution). Iso-osmolar solution does not appear as effective in maintaining FFA washout as do solutions supplemented with SOD or allopurinol. It is important to mention that the dosing regime and time of administration may modify the extent of protection by these two agents against oxygen cytotoxicity.

(2) In a model of regional ischemia, ^{123}IPPA may be a useful indicator of metabolic status of the myocardium. During the period of developing ischemia, biochemical derangements in metabolic pathways may be occurring prior to any functional impairment. Since the primary energy substrates for the myocardium are free fatty acids, early ischemic injury may be manifested as altered washout rates (utilization) of these fuels. Changes in regional washout rates of 15-^{123}IPPA were demonstrated at 14 days of slowly developing regional ischemia.

(3) The effectiveness of the beta-methyl fatty acid analogue in determining the size of perfusion defects is minimal. Problems remain in image clarity and border demarcation.

(4) The use of iodinated free fatty acids as a non-invasive, in vivo diagnostic tool may represent a means of early assessment of myocardial ischemia. It may also provide a means of assessing the effectiveness of various cardioplegic techniques in order to achieve optimal myocardial preservation.

(5) The zone at risk associated with myocardial infarction must be assessed, such that early therapeutic interventions may salvage this myocardium; however, $B^{123}IPPA$ appears inadequate for such an assessment.

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IX APPENDICES

Appendix I - Histochemical assessment (TTZ staining) of perfusion defect size using planimetric quantification.

0 Dog-Slice	1 thick(cm)	Total Slice 2 vol(cm3)-1st observer	Total Slice 3 vol(cm3)-2nd observations by 1st observer	Total Slice 4 vol(cm3)-2nd observer
1 1-1	1.0	2.60	2.95	3.20
2 1-2	1.0	7.00	7.25	7.05
3 1-3	1.2	12.80	13.50	13.50
4 1-4	1.0	20.80	21.76	21.12
5 1-5	1.0	18.56	18.24	18.32
6 1-6	1.2	28.90	28.70	28.80
7 2-1	1.0	5.08	5.12	5.56
8 2-2	1.0	12.12	12.30	12.96
9 2-3	1.0	10.25	10.10	10.35
10 2-4	1.0	23.12	23.36	23.44
11 2-5	1.0	16.80	17.44	16.88
12 2-6	1.0	22.82	22.54	22.61
13 3-1	1.0	1.80	1.68	1.80
14 3-2	1.0	8.65	8.60	8.90
15 3-3	1.0	16.75	16.95	16.80
16 3-4	1.0	28.63	28.49	28.63
17 3-5	1.2	33.80	33.60	34.00
18 3-6	1.0	32.40	32.56	32.48
19 4-1	1.0	5.45	4.90	5.10
20 4-2	0.3	5.28	4.75	5.55
21 4-3	1.0	8.47	8.33	8.82
22 4-4	1.0	19.74	19.39	20.72
23 4-5	1.0	16.80	16.40	16.88
24 4-6	1.0	17.52	17.76	18.08
25 5-1	1.0	9.54	9.12	9.12
26 5-2	1.0	11.69	12.39	12.25
27 5-3	1.0	21.77	21.77	23.34
28 5-4	1.0	24.50	23.59	23.80
29 5-5	1.0	23.04	25.56	23.22
30 6-1	1.0	16.32	16.96	16.72
31 6-2	1.0	19.60	19.52	19.84
32 6-3	1.0	22.24	22.24	22.88
33 6-4	1.0	25.76	23.80	24.22
34 6-5	1.0	25.20	25.12	25.76
35 7-1	1.0	18.88	19.60	19.76
36 7-2	1.0	36.24	39.04	36.80
37 7-3	1.2	32.10	31.40	31.40
38 7-4	1.0	25.84	25.52	24.24
39 8-1	1.0	4.00	4.10	3.45
40 8-2	1.0	9.84	10.38	9.90

continued.....

Appendix I (continued)

0 Dog-Slice	1 thick(cm)	Total Slice 2 vol(cm3)-1st observer	Total Slice 3 vol(cm3)-2nd observations by 1st observer	Total Slice 4 vol(cm3)-2nd observer
41 8-3	1.0	15.84	15.24	16.08
42 8-4	1.0	17.52	18.12	18.60
43 8-5	1.0	13.35	14.30	13.25
44 8-6	1.0	18.06	18.00	17.70
45 8-7	1.0	17.70	19.02	15.78
46 9-1	1.0	4.45	4.95	3.95
47 9-2	1.0	6.40	7.85	7.10
48 9-3	1.0	11.90	12.50	12.30
49 9-4	1.0	16.14	18.06	16.80
50 9-5	1.0	9.80	11.25	10.10
51 9-6	1.0	13.44	14.94	13.08

Appendix I (continued)

0 Dog-Slice	5 inf-vol(cm3)-1st observer	6 inf-vol(cm3)-2nd observations by 1st observer	7 inf-vol(cm3)-2nd observer
1 1-1	0.95	0.75	0.65
2 1-2	2.40	2.35	2.20
3 1-3	3.60	3.90	4.00
4 1-4	4.96	4.16	4.40
5 1-5	4.32	4.48	4.40
6 1-6	0.00	0.00	0.00
7 2-1	0.76	0.28	0.48
8 2-2	1.62	0.66	1.62
9 2-3	0.95	1.00	0.85
10 2-4	0.64	1.28	1.04
11 2-5	1.36	1.92	1.52
12 2-6	3.22	5.60	0.91
13 3-1	0.69	0.78	0.75
14 3-2	2.65	3.00	2.90
15 3-3	6.45	6.45	6.55
16 3-4	10.43	10.43	10.64
17 3-5	9.50	9.10	9.10
18 3-6	5.68	7.04	6.64
19 4-1	0.00	0.25	0.10
20 4-2	0.00	0.00	0.00
21 4-3	0.00	0.00	0.00
22 4-4	0.70	0.70	0.98
23 4-5	0.48	2.64	0.56
24 4-6	0.00	0.00	0.00
25 5-1	2.94	2.88	2.46
26 5-2	2.17	2.66	2.38
27 5-3	5.60	4.41	4.41
28 5-4	5.25	5.53	5.39
29 5-5	1.56	2.22	1.92
30 6-1	3.68	4.48	4.24
31 6-2	3.52	4.56	4.00
32 6-3	5.60	6.72	6.00
33 6-4	3.22	3.29	3.50
34 6-5	3.52	4.00	3.20
35 7-1	7.60	8.48	8.00
36 7-2	7.52	10.32	8.24
37 7-3	5.10	4.80	4.90
38 7-4	2.48	3.12	3.28
39 8-1	1.50	2.65	1.50
40 8-2	4.74	2.94	3.72
		Continued.....	...

Appendix I (continued)

0 Dog-Slice	5 inf-vol(cm3)-1st observer	6 inf-vol(cm3)-2nd observations by 1st observer	7 inf-vol(cm3)-2nd observer
41 8-3	7.08	7.62	7.56
42 8-4	5.58	9.06	7.44
43 8-5	5.15	5.55	4.90
44 8-6	7.50	8.34	6.60
45 8-7	7.92	7.20	6.54
46 9-1	0.60	1.35	0.80
47 9-2	1.25	1.35	1.00
48 9-3	0.95	1.60	1.30
49 9-4	0.00	0.00	0.00
50 9-5	0.00	0.00	0.00
51 9-6	0.00	0.00	0.00

Appendix II - Scintigraphic assessment of perfusion defect using $B^{123}IPPA$ and ^{201}Tl

	$B^{123}IFFA$ (cols 1-3)			^{201}-TI (cols 4-6)		
D-S-A	1 1st observer	2 2nd observations by 1st observer	3 2nd observer	4 1st observer	5 2nd observations by 1st observer	6 2nd observer
1-1 s	54.25	63.19	149.19	49.81	62.31	161.50
v			17.44			19.44
i	18.00	63.19		10.38	12.81	30.94
r					33.69	
1-2 s	84.19	139.81	140.00	106.56	94.31	149.19
v		21.31	14.81			31.63
i	19.75	33.69		25.88	19.06	
r		29.82			29.,44	24.56
1-3 s	108.50	130.63	163.56	111.75	129.44	171.50
v		24.13	15.56		16.00	25.63
i	32.69	34.00		38.44	32.13	
r		15.13			43.63	29.38
1-4 s	118.81	109.94	187.69	171.06	178.31	160.31
v		17.69	15.81		25.19	23.25
i	15.13	14.00		47.75	52.25	
r					34.00	
1-5 s	205.69	116.94	152.31	198.50	185.75	188.19
v		20.81	16.06		24.38	25.19
i	68.00			67.63	60.25	
r		19.06			20.00	
1-6 s	222.56	137.69	168.88	216.25	227.63	185.63
v		22.88	15.81		27.19	19.00
i	72.56			69.89	68.38	
r			14.19		38.94	
1-7 s	218.81	180.75	152.63	187.44	170.00	203.75
v		33.31	20.75		38.88	25.00
i	51.50			48.25	44.50	
r			71.31			
1-8 s	252.06	149.69	146.38	219.75		186.1
v		25.69	14.94			27.38
i	73.13		72.94	60.00		
r						
1-9 s		109.06	151.38			197.81
v		26.31	13.19			32.88
i		22.00	117.19			
r		27.44				61.75

continued.....

Appendix II (continued)

	$B^{123}IPPA$ (cols 1-3)			^{201}-Tl (cols 4-6)		
D-S-A	1 1st observer	2 2nd observations by 1st observer	3 2nd observer	4 1st observer	5 2nd observations by 1st observer	6 2nd observer
2-1 s	53.81	45.75	26.88	82.00	94.88	143.88
v					15.75	18.06
i	23.56	32.62		54.31	40.38	50.00
r	52.81	32.62		79.88	26.44	26.05
2-2 s	93.38	84.44	79.56	106.69	125.31	133.56
v		13.81	7.63	13.00	22.31	24.19
i	47.81	30.31	39.25	45.44	42.44	36.31
r	37.38	20.69	20.69	27.44	31.59	
2-3 s	101.88	86.19	128.13	125.19	128.94	139.75
v	10.31	16.94	12.63	16.19	20.56	21.31
i	6.75	15.19	21.19	38.19	134.75	40.00
r	76.69	30.75	61.75	75.44	19.56	
2-4 s	112.69	103.50	138.38	132.63	116.81	144.81
v	11.88	15.88	12.63	19.38	22.31	13.94
i	3.12	20.56	22.31	41.94	32.94	
r	67.88	15.69	34.06	72.81		77.44
2-5 s	113.56	112.25	144.63	122.31	115.00	136.13
v	10.13	20.38	16.00	13.94	16.31	13.31
i		30.69		17.06	19.44	
r	23.56		55.63	91.25		10.94
2-6 s	114.31	106.88	137.94	126.25	115.06	127.06
v	8.38	17.69	12.00	14.31	15.38	14.38
i				1.81	35.38	20.38
r	23.69	24.94		26.06		
2-7 s	122.94	96.00	136.19	99.38	117.00	153.00
v	9.69	13.25	12.44	10.75	14.52	14.38
i				0.75	30.15	36.00
r			53.44			
2-8 s		107.69	130.69			120.38
v		18.44	11.38			13.63
i		20.06				33.69
r			27.75			
3-1 s	47.38	75.26	58.19	62.19	118.44	184.13
v			5.62		17.19	19.69
i	13.25	36.19	25.13	14.06	51.50	32.63
r	34.13		16.06	27.06		76.56
3-2 s	54.19	90.62	201.56	59.81	162.56	190.94
v		9.44	16.88		25.19	32.13
i	10.50	37.94	65.50	16.81	31.75	61.88
r	27.38	17.19	39.44	14.50	50.56	18.63
			Continued	.....		

Appendix II (continued)

	$B^{123}IPPA$ (cols 1-3)			^{201}-Tl (cols 4-6)		
D-S-A	1 1st observer	2 2nd observations by 1st observer	3 2nd observer	4 1st observer	5 2nd observations by 1st observer	6 2nd observer
3-3 s	64.00	98.50	106.25	92.00	125.31	191.31
v		17.19	13.50		20.38	19.94
i	16.44	25.56	39.13	35.25	43.94	71.63
r	29.50	31.12	25.88	28.75	18.00	33.31
3-4 s	116.63	114.94	210.08	141.44	165.44	206.13
v	11.56	14.06	29.19	17.63	27.75	85.63
i	22.44	29.38	82.50	57.94	19.38	
r	54.69	35.00	35.13	34.44	31.94	30.88
3-5 s	171.38	118.75	168.31	157.69	191.44	191.44
v	13.31	17.00	18.13	17.69	29.69	24.13
i	39.19	30.00	41.31	26.38	12.31	23.44
r	54.31	35.00	31.69	63.06	48.00	44.13
3-6 s	135.19	126.19	170.38	189.29	120.38	172.31
v	11.81	19.88	12.88	24.88	24.75	17.13
i	37.50			19.44	33.19	
r	37.81	33.63	26.00	79.63	48.00	75.44
3-7 s	167.00	121.31	145.88	187.69	134.74	131.19
v	16.81	25.56	17.38	21.06	27.13	21.69
i				13.31	36.31	
r	75.69	20.63		73.75	12.31	
3-8 s		109.88	158.00		125.75	149.19
v		16.38	9.69		21.19	20.50
i					22.69	
r			31.94			19.88
4-1 s	49.88	56.31	92.38	55.25	52.00	52.88
v		8.94	14.19			
i		9.19				
r			11.94	11.81		
4-2 s	32.44	77.14	91.38	59.19	65.31	63.06
v		14.81	10.31			
i						
r				12.63		
4-3 s	76.63	77.69	95.06	83.19	72.31	63.75
v	8.56	17.00	11.75		4.63	
i						
r		14.13	8.19			
			Continued.	.....		

Appendix II (continued)

	$B^{123}IPPA$ (cols 1-3)			^{201}-Tl (cols 4-6)		
D-S-A	1 1st observer	2 2nd observations by 1st observer	3 2nd observer	4 1st observer	5 2nd observations by 1st observer	6 2nd observer
4-4 s	86.31	73.25	102.19	81.44	92.25	71.81
v	10.44	16.44	11.81	5.69	7.13	1.44
i						
r			25.88	14.75		
4-5 s	117.19	96.56	112.56	101.88	100.00	102.94
v	14.81	16.13	10.54	6.69	9.86	5.13
i						
r				22.81		
4-6 s	101.38	107.13	90.62	117.19	102.38	94.06
v	13.88	5.88	7.06	7.81	11.06	8.12
i						
r	16.44		19.38	61.63		
4-7 s	115.13	51.38	71.56	124.88	98.673	106.44
v	11.44	5.94	5.88	9.44	15.31	10.06
i					23.25	
r	32.50			31.06		44.94
4-8 s		42.06	46.69		98.69	110.56
v		6.19	7.44		11.13	10.00
i						
r						51.63
4-9 s		45.06	54.25		94.81	145.50
v					12.94	13.31
i						34.94
r						41.88
5-1 s	50.19	84.56	151.63	30.88	124.88	151.13
v		16.88	14.30		18.00	21.69
i	11.25	13.81		6.00	37.75	59.81
r	38.38	39.88		7.94	23.69	
5-2 s	51.81	117.31	177.63	49.06	181.63	166.00
v	5.62	24.56	15.94		25.50	19.63
i	15.13	11.81		8.19	55.00	71,63
r	33.50	51.38		13.88		
5-3 s	40.69	130.44	177.81	49.50	186.38	157.81
v	3.25	25.75	13.78		24.13	21.94
i	9.06	11.88		9.75	43.38	62.38
r		73.44		18.31		
			Continued			

Appendix II (continued)

	$B^{123}IPPA$ (cols 1-3			^{201}-Tl (cols 4-6)		
D-S-A	1 1st observer	2 2nd observations by 1st observer	3 2nd observer	4 1st observer	5 2nd observations by 1st observer	6 2nd observer
5-3 s	76.94	113.31	151.63	59.37	165.44	151.25
v	6.31	24.19	18.00		17.63	19.19
i	25.94			12.44	38.88	51.25
r	12.50	33.38		25.88		
5-5 s	89.81	120.63	155.88	111.13	128.63	137.94
v	7.81	24.44	19.50	13.31	17.19	17.63
i	15.81			18.88	38.88	
r	22.13	19.25	20.25	32.69	23.81	41.69
5-6 s	118.25	126.44	149.13	72.19	132.50	152.00
v	13.69	25.69	13.50		16.75	15.31
i	19.25		47.31	26.50	26.19	
r	35.13	21.69	31.56	22.31		39.88
5-7 s		138.81	135.50		122.00	190.56
v		19.44	13.75		17.25	17.69
i		22.38	85.25		30.63	
r		45.69				54.06
5-8 s		94.88	166.56		142.94	190.56
v		15.75	22.88		12.25	17.69
i		16.31	26.56		39.94	
r		18.13	55.19			54.06
5-9 s		75.63	135.50		146.56	169.50
v		12.31	13.75		12.63	19.75
i		24.94	85.25		31.69	
r						40.94
6-1 s	30.19	100.75	179.56	43.25	140.19	173.88
v		24.31	22.63		29.19	36.44
i	9.13	28.12	65.75		58.88	45.63
r	8.81	12.38	24.25		62.31	76.25
6-2 s	61.38	101.56	125.69	140.19	141.38	169.69
v	5.13	18.88	16.25	11.50	29.06	13.94
i	19.25	15.13	33.13	18.19	32.63	49.00
r	15.13	25.44			44.50	49.50
6-3 s	110.31	73.38	136.81	102.81	174.75	165.74
v	10.69	13.75	12.94	13.13	34.56	18.81
i	31.25	15.13	33.31	17.19	43.00	35.94
r	14.31				22.81	17.88

Continued .....

Appendix II (continued)

	$B^{123}IPPA$ (cols 1-3			^{201}-Tl (cols 4-6)		
D-S-A	1 1st observer	2 2nd observations by 1st observer	3 2nd observer	4 1st observer	5 2nd observations by 1st observer	6 2nd observer
6-4 s	110.44	86.00	133.50	129.88	162.81	159.19
v	9.37	17.88	8.63	15.00	23.13	14.25
i	17.44	13.63			38.00	43.06
r	19.81		20.56			
6-5 s	111.69	100.06	114.38	114.63	152.00	155.19
v	7.13	18.44	8.50	14.81	28.38	16.06
i	11.25				34.88	
r	16.56					33.00
6-6 s	118.56	113.63	128.63	113.31	127.31	121.50
v	9.88	14.38	9.31	15.19	18.63	16.25
i						
r		27.81	16.38			
6-7 s	124.06	113.69	143.00	113.94	114.63	150.88
v	9.81	14.81	9.37	14.31	14.81	11.38
i		16.94	21.75			
r		29.94	23.38			
6-8 s		119.06	128.44		129.88	140.38
v		14.88	9.25		15.00	14.94
i		40.00	36.88			
r		25.19				
6-9 s		117.50	147.25		102.81	136.63
v		17.44	12.75		13.13	13.25
i		44.00	56.88		17.19	
r		21.56	30.19			15.50
7-1 s	43.38	80.50	153.56	45.13	130.88	187.06
v		13.88	21.38		22.00	30.50
i	18.25		62.81		35.75	127.81
r		27.56	39.44		42.69	
7-2 s	103.81	100.50	136.69	36.13	189.69	185.25
v	16.75	23.56	19.94		36.00	27.88
i	18.44	21.56		7.50	44.13	93.50
r	15.94	32.38			28.93	15.75
7-3 s	121.50	111.19	156.19	111.19	194.13	188.38
v	22.63	22.63	12.25	13.88	25.63	18.69
i	18.81	23.00		11.13	39.69	39.63
r	22.13	31.25				35.75

Continued .....

Appendix II (continued)

	$B^{123}IPPA$ (cols 1-3			^{201}-Tl (cols 4-6)		
D-S-A	1 1st observer	2 2nd observations by 1st observer	3 2nd observer	4 1st observer	5 2nd observations by 1st observer	6 2nd observer
7-4 s	149.13	114.50	149.31	136.38	183.06	167.44
v	17.56	25.38	19.31	15.38	29.06	22.44
i	13.25				25.50	34.13
r	17.13					
7-5 s	151.25	131.56	147.44	141.38	154.94	212.13
v	16.06	27.44	17.44	12.88	26.06	12.06
i	23.94				14.31	
r						31.88
7-6 s	157.19	130.19	175.19	166.13	141.38	216.63
v	22.25	30.00	21.25	18.81	12.88	16.44
i	8.19			14.69		
r			30.44			
7-7 s		149.13	196.38		136.38	144.69
v		17.56	21.13		15.38	12.19
i		13.25	37.94		10.13	
r		17.13				
7-8 s		121.50	142.88		111.19	159.75
v		22.63	21.13		13.88	14.31
i		18.81	27.44		11.13	
r		22.13	30.88			
8-1 s				43.50	151.81	192.06
v					29.13	27.56
i					70.56	81.63
r					32.50	38.88
8-2 s				72.38	151.81	142.75
v					29.13	14.13
i				56.57	62.88	63.44
r					28.69	
8-3 s				118.69	172.56	116.94
v				20.81	27.75	15.81
i				69.56	76.88	34.63
r				24.75	19.19	

Continued .....

Appendix II (continued)

	$B^{123}IPPA$ (cols 1-3			^{201}-Tl (cols 4-6)		
D-S-A	1 1st observer	2 2nd observations by 1st observer	3 2nd observer	4 1st observer	5 2nd observations by 1st observer	6 2nd observer
8-4 s				151.81	131.00	155.00
v				29.13	18.75	16.00
i				70.56	19.63	49.31
r				32.50	23.26	11.25
8-5 s				151.81	100.13	133.88
v				29.13	13.25	12.50
i				62.88	19.75	
r				28.69		55.88
8-6 s				172.56	131.44	183.00
v				27.75	13.19	11.81
i				76.88	27.63	44.88
r						
8-7 s				131.00	159.25	146.31
v				18.75	16.81	12.44
i				19.63	49.50	33.44
r				23.26		
8-8 s					149.69	137.69
v					19.31	10.56
i					54.00	24.63
r						
9-1 s	59.63	91.06	219.31			
v		21.06	19.25			
i	17.75	26.44	81.50			
r	17.13	28.06				
9-2 s	100.56	98.38	115.81			
v	13.00	19.88	12.94			
i	26.56					
r	19.69	35.62	28.75			
9-3 s	134.31	127.81	128.81			
v	18.81	18.00	12.81			
i						
r	28.19	50.50				
9-4 s	132.69	124.75	129.88			
v	10.06	24.44	17.25			
i						
r	27.75	37.00				
			Continued	.....		

Appendix II (continued)

	$B^{123}IPPA$ (cols 1-3			^{201}-Tl (cols 4-6)		
D-S-A	1 1st observer	2 2nd observations by 1st observer	3 2nd observer	4 1st observer	5 2nd observations by 1st observer	6 2nd observer
9-5 s	115.75	114.06	127.13			
v	14.38	15.69	15.38			
i						
r	41.50	20.00				
9-6 s	134.88	122.25	124.88			
v	12.88	16.13	14.13			
i						
r		34.44	41.50			
9-7 s		98.31	118.19			
v		8.75	14.00			
i		30.88	42.06			
r						
9-8 s		100.75	121.44			
v		10.94	12.13			
i		30.94	47,44			
r						
9-9 s		99.69	98.00			
v		9.44	10.69			
i		33.06	44.25			
r		28.88				

Appendix III - $B^{123}IPPA/^{201}Tl$ ratios for each experiment and corresponding area-of-interest

D-S-A	7 1st Observer FA/TL	8 2nd Observer FA/TL
1-1 s	0.49	0.98
v		0.91
i	3.19	2.66
r		
1-2 s	1.09	1.01
v	0.97	0.65
i	2.32	
r		1.35
1-3 s	0.86	0.87
v	0.04	0.64
i	1.73	
r		1.42
1-4 s	0.75	0.93
v	0.74	0.75
i		
r		
1-5 s	0.89	0.74
v	0.77	0.59
i		
r		
1-6 s	0.96	0.88
v	0.73	0.82
i		
r		
1-7 s	1.16	0.92
v	1.22	0.63
i		
r		
1-8 s	1.06	0.82
v	0.77	0.59
i		
r		
1-9 s	0.72	0.85
v	0.85	0.48
i	0.41	
r		0.23

D-S-A	7 1st Observer FA/TL	8 2nd Observer FA/TL
2-1 s	0.48	0.19
v		
i	0.81	
r	1.23	
2-2 s	0.67	0.60
v	0.62	0.32
i	0.71	1.08
r	0.66	
2-3 s	0.67	0.92
v	0.82	0.59
i	0.11	0.53
r	1.57	
2-4 s	0.89	0.96
v	0.71	0.91
i	0.62	
r		0.91
2-5 s	0.98	1.06
v	1.24	1.20
i	1.58	
r		5.09
2-6 s	0.93	1.09
v	1.15	0.83
i		
r		
2-7 s	0.82	
v	0.91	
i		
r		
2-8 s		
v		
i		
r		
3-1 s	0.61	0.32
v		0.29
i	0.70	0.77
r		0.21

Appendix III (continued)

D-S-A	7 1st Observer FA/TL	8 2nd Observer FA/TL
3-2 s	0.56	1.06
v	0.37	0.53
i	1.19	1.06
r	0.34	2.12
3-3 s	0.79	0.56
v	0.84	0.68
i	0.58	0.55
r	1.73	0.78
3-4 s	0.69	1.01
v	0.51	0.34
i	1.52	
r	1.10	1.38
3-5 s	0.62	0.88
v	0.57	0.75
i	2.44	1.76
r	0.73	0.71
3-6 s	1.05	0.99
v	0.80	0.75
i		
r	0.70	0.34
3-7 s	0.90	1.11
v	0.94	0.80
i		
r	1.68	
3-8 s	0.87	1.06
v	0.77	0.47
i		
r		
4-1 s	1.08	1.74
v		
i		
r		
4-2 s	1.18	1.45
v		
i		
r		
4-3 s	1.07	1.49
v	3.67	
i		
r		

D-S-A	7 1st Observer FA/TL	8 2nd Observer FA/TL
4-4 s	0.79	1.42
v	2.31	8.20
i		
r		
4-5 s	0.97	1.09
v	1.63	2.04
i		
r		
4-6 s	1.05	0.96
v	0.53	0.87
i		
r		
4-7 s	0.52	0.67
v	0.39	0.58
i		
r		
4-8 s	0.43	0.42
v	0.56	0.74
i		
r		
4-9 s	0.48	0.37
v		
i		
r		
5-1 s	0.68	1.00
v	0.94	0.67
i	0.37	
r	1.68	
5-2 s	0.65	1.07
v	0.96	0.81
i	0.21	
r		
5-3 s	0.70	1.13
v	1.07	0.63
i	0.27	
r		
5-4 s	0.68	1.00
v	1.37	0.94
i		
r		

Appendix III (continued)

D-S-A	7 1st Observer FA/TL	8 2nd Observer FA/TL
5-5 s	0.94	1.13
v	1.42	1.10
i		
r	0.81	0.49
5-6 s	0.95	0.98
v	1.53	0.88
i		
r		0.79
5-7 s	1.14	0.71
v	1.13	0.78
i	0.73	
r		
5-8 s	0.66	0.87
v	1.29	1.29
i	0.41	
r		1.02
5-9 s	0.52	0.80
v	0.97	0.70
i	0.79	
r		
6-1 s	0.72	1.03
v	0.83	0.62
i	0.48	1.44
r	0.20	0.32
6-2 s	0.72	0.74
v	0.65	1.17
i	0.46	0.68
r	0.57	
6-3 s	0.42	0.82
v	0.40	0.69
i	0.35	0.93
r		
6-4 s	0.53	0.84
v	0.77	0.61
i	0.36	
r		

D-S-A	7 1st Observer FA/TL	8 2nd Observer FA/TL
6-5 s	0.66	0.74
v	0.65	0.53
i		
r		
6-6 s	0.89	1.06
v	0.77	0.57
i		
r		
6-7 s	0.99	0.95
v	1.00	0.82
i		
r		
6-8 s	0.92	0.91
v	0.99	0.62
i		
r		
6-9 s	1.14	1.08
v	1.32	0.96
i	2.56	
r		1.95
7-1 s	1.14	0.82
v	1.20	0.70
i	1.56	0.49
r		
7-2 s	0.62	0.74
v	0.63	0.72
i		
r	0.65	
7-3 s	0.53	0.83
v	0.65	0.66
i	0.49	
r	1.12	
7-4 s	0.57	0.89
v	0.88	0.86
i	0.58	
r		

Appendix III (continued)

D-S-A	7 1st Observer FA/TL	8 2nd Observer FA/TL
7-5 s	0.85	0.70
v	1.05	1.45
i		
r		
7-6 s	0.92	0.81
v	2.33	1.29
i		
r		
7-7 s	1.09	1.36
v	1.14	1.73
i	1.31	
r		
7-8 s	1.09	0.89
v	1.63	1.48
i	1.69	
r		
8-1 s		
v		
i		
r		
8-2 s		
v		
i		
r		
8-3 s		
v		
i		
r		
8-4 s		
v		
i		
r		
8-5 s		
v		
i		
r		

Appendix IV - Infarcted tissue as percent of entire slice expressed for each of the three techniques of perfusion defect assessment, including histochemistry (TTZ staining), $B^{123}IPPA$ and ^{201}Tl.

Dog-Slice	1 % Inf 1st observer	2 % Inf 2nd observations by 1st observer	3 % Inf 2nd observer	4 $B^{123}IPPA$ 1st observer
1-1	36.54	25.42	20.31	33.24
1-2	34.29	32.41	31.21	23.46
1-3	28.13	28.89	29.63	21.04
1-4	23.85	19.12	20.83	33.06
1-5	23.28	24.56	24.02	32.60
1-6	0.00	0.00	0.00	26.47
2-1	14.96	5.47	8.63	43.78
2-2	13.37	5.37	12.50	51.20
2-3	9.27	9.90	8.21	7.37
2-4	2.77	5.48	4.44	3.10
2-5	8.11	11.01	9.00	0.00
2-6	14.11	24.84	4.02	0.00
3-1	38.33	46.43	41.67	27.96
3-2	30.64	34.88	32.58	19.38
3-3	38.51	38.05	38.99	25.69
3-4	36.43	36.61	37.16	21.36
3-5	28.11	27.08	26.76	27.25
3-6	17.53	21.62	20.44	0.00
4-1	0.00	5.10	1.96	0.00
4-2	0.00	0.00	0.00	0.00
4-3	0.00	0.00	0.00	0.00
4-4	3.55	3.61	4.73	0.00
4-5	2.86	16.10	3.32	0.00
4-6	0.00	0.00	0.00	0.00
5-1	30.82	31.58	26.97	22.42
5-2	18.56	21.47	19.43	32.76
5-3	25.72	20.26	18.90	24.19
5-4	21.43	23.44	22.65	36.73
5-5	6.77	8.68	8.27	19.28
6-1	22.55	26.42	25.36	30.24
6-2	17.96	23.36	20.16	34.24
6-3	25.18	30.22	26.22	31.37
6-4	12.50	13.82	14.45	17.26
6-5	13.97	15.92	12.42	10.76
7-1	40.25	43.27	40.49	42.06
7-2	20.75	26.43	22.39	21.12
7-3	15.89	15.28	15.61	13.91
7-4	9.60	12.23	13.53	17.71
		Continued.....	....	

Appendix IV (continued)

Dog-Slice	1 % Inf 1st observer	2 % Inf 2nd observations by 1st observer	3 % Inf 2nd observer	4 B^{123}IPPA 1st observer
8-1	37.50	64.63	43.48	
8-2	48.17	28.32	37.58	
8-3	44.70	50.00	47.01	
8-4	31.85	50.00	40.00	
8-5	38.58	38.81	36.98	
8-6	41.53	46.33	37.27	
8-7	44.75	37.85	41.44	
9-1	13.48	27.27	20.25	29.76
9-2	19.53	17.20	14.08	30.35
9-3	7.98	12.80	10.57	0.00
9-4	0.00	0.00	0.00	0.00
9-5	0.00	0.00	0.00	0.00
9-6	0.00	0.00	0.00	0.00

Appendix IV (continued)

Dog-Slice	5 $B^{123}IPPA$ observation by 1st observer	6 $B^{123}IPPA$ 2nd observer	7 TL 1st observer	8 TL 2nd observation by 1st	9 TL 2nd observer observer
1-1	18.5	40.6	20.84	16.0	21.4
1-2	24.2	0.0	24.28	8.0	0.0
1-3	0.0	0.0	30.48	0.0	0.0
1-4	0.0	0.0	34.07	37.4	0.0
1-5	12.9	0.0	32.31	47.8	22.8
1-6	0.0	70.5	26.59	45.9	48.3
2-1	54.1	39.6	66.23	45.5	36.7
2-2	22.8	18.0	48.49	82.7	16.1
2-3	17.0	0.0	34.96	27.6	8.7
2-4	11.7	0.0	37.03	29.4	28.4
2-5	35.1	24.8	15.74	0.0	0.0
2-6	24.5	0.0	1.62	0.0	0.0
3-1	48.2	38.2	22.61	34.9	29.2
3-2	30.2	49.5	28.10	26.1	22.1
3-3	14.4	13.4	38.31	17.5	7.3
3-4	0.0	0.0	46.80	27.8	0.0
3-5	0.0	0.0	15.05	30.2	5.1
3-6	0.0	15.4	7.99	34.2	18.2
4-1	8.4	0.0	0.0	0.0	0.0
4-2	0.0	0.0	0.0	0.0	0.0
4-3	0.0	0.0	0.0	0.0	0.0
4-4	0.0	0.0	0.0	13.4	0.0
4-5	0.0	0.0	0.0	54.5	15.0
4-6	0.0	0.0	0.0	59.4	52.3
5-1	16.0	0.0	19.44	35.3	47.7
5-2	6.1	0.0	16.69	26.5	42.4
5-3	0.0	17.4	19.70	11.5	0.0
5-4	19.5	42.1	20.95	30.0	0.0
5-5	38.8	59.8	19.30	23.9	0.0
6-1	27.2	37.1	0.0	41.0	32.3
6-2	22.5	13.4	21.3	28.9	27.1
6-3	0.0	0.0	19.17	15.0	0.0
6-4	28.1	23.2	0.0	0.0	0.0
6-5	36.8	46.2	0.0	20.21	0.0

Continued..

Appendix IV (continued)

Dog-Slice	5 $B^{123}IPPA$ observation by 1st observer	6 $B^{123}IPPA$ 2nd observer	7 TL 1st observer	8 TL 2nd observation by 1st	9 TL 2nd observer observer
7-1	62.63	0.00	0.00	45.21	23.36
7-2	0.00	0.00	20.75	32.83	23.54
7-3	26.93	0.00	5.10	26.02	0.00
7-4	0.00	0.00	0.00	16.56	0.00
8-1			0..0	54.4	49.5
8-2			78.12	37.5	35.0
8-3			71.07	21.1	15.3
8-4			57.53	34.8	25.0
8-5			51.26	41.4	19.4
8-6			53.09	31.3	13.0
8-7			17.49	0.0	0.0
9-1	17.8	26.9			
9-2	0.0	0.0			
9-3	0.0	0.0			
9-4	39.5	41.9			
9-5	20.0	30.7			
9-6	0.0	22.8			

Appendix V - Autoradiographic assessment of the ischemic risk zone for dogs 8 and 9 using planimetric quantitation.

Dog-Slice	1 slice vol(cm3)-1st observer	2 slice vol(cm3)- 2nd observations by 1st observer	3 slice vol(cm3)-2nd observer
8-1	3.55	3.95	3.45
8-2	10.44	10.14	9.48
8-3	15.36	15.66	15.84
8-4	16.44	17.04	17.64
8-5	11.80	13.25	13.35
8-6	16.80	17.64	17.40
8-7	22.14	19.80	19.38
9-1	3.75	3.65	3.50
9-2	7.40	7.15	7.00
9-3	11.35	11.45	11.50
9-4	17.34	17.28	16.92
9-5	10.10	10.10	10.00
9-6	13.26	13.32	13.38

Dog-Slice	4 IRZ (cm3) - 1st observer	5 IRZ (cm3) - 2nd observations by 1st observer	6 IRZ (cm3) - 2nd observer
8-1	3.55	3.95	3.45
8-2	6.54	6.84	6.60
8-3	10.74	10.92	10.44
8-4	8.82	9.60	9.78
8-5	8.30	8.00	7.45
8-6	8.10	8.52	8.16
8-7	9.53	9.71	8.97
9-1	0.00	0.00	0.00
9-2	0.00	0.00	0.00
9-3	0.00	0.00	0.00
9-4	0.00	0.00	0.00
9-5	0.00	0.00	0.00
9-6	0.00	0.00	0.00