

SEROTONIN ANTAGONISM IN PRIMATE EXPERIMENTAL MYOCARDIAL INFARCTION

Craig Gordon Hartford

**A Dissertation submitted to the Faculty of Medicine,
University of the Witwatersrand, Johannesburg,
for the Degree of Master of Science.**

August 1992

DECLARATION

I declare that this dissertation is my own, unaided work, submitted for the degree of Master of Science in the Department of Medical Physiology, University of the Witwatersrand, Johannesburg. It has not been previously submitted as a requirement for any degree or examination at this or any other university.

.....07.....day of.....October....., 19..92

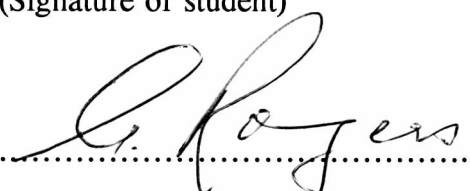
..........

(CRAIG GORDON HARTFORD)

I certify that the study contained in this dissertation has the approval of the Animal Ethics Screening Committee, clearance certificate number 90/15/5, and clearance for the use of radioactive isotopes (See Appendices 7.2 and 7.3).

.......... Date..07/10/92

(Signature of student)

.......... Date..7 OCT 1992

(Signature of supervisor)

ABSTRACT

Serotonin (5-Hydroxytryptamine, 5-HT) mediates vasoconstriction and vasodilation in the normal coronary circulation of various animal species. In the presence of coronary artery disease serotonin may inhibit coronary collateral formation and stimulate predominantly vasoconstriction. This study tested the effect of ketanserin, a selective 5-HT₂ receptor antagonist and platelet aggregation inhibitor, on ischaemic myocardium blood flow and coronary collateral formation following coronary artery occlusion in primates.

Fifteen baboons were subjected to left-anterior descending coronary artery ligation and thrombus formation. Haemodynamics and regional myocardial blood flows (microsphere technique) were measured before, 45 min after and one week after coronary artery occlusion.

There was no significant difference in the haemodynamic measurements, gross infarct size or infarct-ischaemic zone myocardial blood flow in the experimental group (ketanserin 1 mg/kg daily, n = 9) compared with the control group (injectable water, n = 6). In both groups there was a similar significant increase in the blood flow ratio of infarct-ischaemic:normal myocardium blood flow at one week compared with shortly after coronary occlusion. Thus selective 5-HT₂ receptor blockade has neither an adverse nor a protective effect on the myocardial infarct following acute thrombotic coronary occlusion in baboons.

TABLE OF CONTENTS

Declaration	(i)
Abstract	(ii)
Table of Contents	(iii)
List of Tables	(viii)
List of Figures	(ix)
List of Abbreviations	(xi)
Publications	(xiii)
Acknowledgements	(xiv)
Preface	(xv)
Chapter	Page
1. <u>INTRODUCTION</u>	(xvi)
2. <u>LITERATURE REVIEW</u>	1
2.1 Myocardial infarction and myocardial ischaemia	1
2.1.1 Definitions	1
2.1.2 Myocardial infarction in the baboon model	2
2.1.2.1 The baboon's normal regional myocardial blood flow	2
2.1.2.2 Distribution of the baboon myocardial infarction	2
2.1.3 The pathogenesis of myocardial infarction	4

2.1.3.1	Myocardial oxygen supply	4
2.1.3.2	Myocardial oxygen demand	6
2.1.3.3	The concept of infarct size limitation	7
2.2	The coronary collateral circulation	8
2.2.1	Introduction	8
2.2.2	Stimulation of newly developed collaterals	9
2.2.3	Animal models and collateral anatomy	10
2.2.4	The functional significance of coronary collaterals	11
2.3	Serotonin and the cardiovascular system	14
2.3.1	Introduction: the serotonin molecule	14
2.3.2	Serotonin receptor theory and serotonin antagonism	17
2.3.2.1	Serotonin receptor theory	17
2.3.2.2	Serotonin antagonism	19
2.3.3	Serotonin and the vasculature	24
2.3.3.1	Introduction	24
2.3.3.2	Serotonin and the peripheral vasculature	25
2.3.3.3	The mechanism of serotonin's vascular action	31
2.3.3.4	Serotonin and the coronary circulation	32
(i)	Coronary artery responses to serotonin	32
(ii)	Serotonin's interaction with the vascular endothelium	34
(iii)	Conclusion	36

Chapter	Page
2.4 <u>Methods</u> of regional and collateral myocardial blood flow measurement	37
2.4.1 Techniques of collateral circulation measurement	37
2.4.2 The radioactive microsphere technique	39
2.4.2.1 Historical background	39
2.4.2.2 Technical points pertaining to the use of microspheres	40
2.4.2.3 Technical points pertaining to the administration of microspheres	43
2.4.2.4 Calculation of blood flow	45
(i) Method of tissue sample counting	45
(ii) Analysis of counts	46
(iii) Spectral distribution stripping	49
(iv) Validity of the radioactive microsphere technique	52
2.5 <u>Experimental production</u> of myocardial infarction	56
2.5.1 Obstruction of coronary arteries	56
2.5.2 Coronary artery ligation	58
2.5.3 Additional methods of producing coronary occlusion	59
2.5.4 Methods of experimental arterial thrombosis	60
2.6 <u>Methods</u> of determining infarct size	62

Chapter	Page
3. <u>METHODS</u>	65
3.1 Experimental model	65
3.2 Procedure	65
3.3 Instrumentation	67
3.4 Regional myocardial blood flow	70
3.5 Calibration and validation	79
3.6 Calculations	80
3.7 Statistics	82
4. <u>RESULTS</u>	83
4.1 Blood chemistry	83
4.2 Histology	83
4.3 Haemodynamics	89
4.4 Regional blood flows	94
5. <u>DISCUSSION</u>	100
5.1 Haemodynamics	100
5.2 Ketanserin and regional blood flow	101

Chapter	Page
5.3 Ketanserin and infarct size	102
5.4 Microsphere loss	103
5.5 Serotonin antagonism	105
5.6 Conclusion	107
 6. <u>REFERENCES</u>	 108
 7. <u>APPENDIX</u>	 126
 7.1 Note of acceptance of paper for publication	 127
7.2 Animal ethics clearance certificates	128
7.3 Clearance for use of radioactive isotopes certificate	129

LIST OF TABLES

Table	Page
2.1 5-HT ₂ receptor responses to serotonin in various species.....	21
2.2 Energy range schematic skeleton for the calculation of regional blood flows.....	50
2.3 Q-Basic computer program for spectral distribution stripping calculation.....	55
4.1 Blood chemistry values of <i>Papio ursinus</i> baboons in captivity.....	84
4.2 Haemodynamic variables after ketanserin administration and coronary occlusion.....	90
4.3 Haemodynamic variables after ketanserin administration and coronary occlusion.....	91
4.4 Effect of coronary artery occlusion and ketanserin administration on regional myocardial blood flow.....	97

LIST OF FIGURES

Figure	Page
2.1 The molecular structure of serotonin.....	16
2.2 Structural formulae of serotonin 5-HT ₂ receptor antagonists.....	23
2.3 The normal and the pathological in vivo vascular responses to an intra-arterial serotonin infusion.....	28
2.4 Serotonin's vascular modes of action.....	30
3.1 Schematic illustration of study design sequence.....	72
3.2 Schematic illustration of instrumentation.....	74
3.3 Schematic illustration of coronary artery ligation and thrombus induction.....	76
3.4 Schematic illustration of myocardial tissue sectioning technique.....	78

LIST OF FIGURES (continued)

Figure	Page
4.1 Light microscope sections of baboon coronary artery thrombi.....	86
4.2 Light microscope sections of baboon myocardial infarction.....	88
4.3 Baboon heart coronary angiography plates.....	93
4.4 Infarct size (mean $\pm$ SEM) determined from ventricle myocardial weights.....	96
4.5 Ratio (mean $\pm$ SEM) of ventricle infarct-ischaemic myocardium to normal myocardium blood flow before and after coronary occlusion.....	99

LIST OF ABBREVIATIONS

5-HT	Five-hydroxytryptamine
BP	Blood pressure
CFV	Cyclic flow variation
cm	Centimetre
cpm	Counts per minute
CO	Cardiac output
CVR	Coronary vascular resistance
DP	Diastolic pressure
dp/dt	Peak left ventricular pressure development rate
eg.	For example
fℓ	Femtolitre
g	Grams
h	Hour
H ₁	Histamine-1
H ₂ O	Water
Hb	Haemoglobin
Hct	Haematocrit
Hg	Mercury
HR	Heart rate
ie.	Id est
IHD	Ischaemic heart disease
kg	Kilograms
ℓ	Litre
LAD	Left anterior descending coronary artery
LAP	Left atrial pressure
LV	Left ventricle
MAP	Mean arterial pressure
MI	Myocardial infarction
min	Minutes

mm	Millimetre
mm Hg	Millimetres of Mercury
n	Sample number
pg	Picograms
PP	Pulse Pressure
p <	Probability value less than
p >	Probability value greater than
s	Second
SD	Standard deviation
SEM	Standard error of the mean
SP	Systolic pressure
SV	Stroke volume
SVR	Systemic vascular resistance
u	International unit
μ	Micron
μ g	Microgram
μ m	Micrometre
viz.	Videlicet
%	Percentage
$^{\circ}$ C	Degrees celsius

Much of the work in support of this dissertation has been presented and published in the proceedings of meetings.

- 1) *HARTFORD CG, MARCOS E, ROGERS GG, ROSENDORFF C (1990)*
The effect of selective 5-HT₂ receptor antagonism on regional myocardial blood flow following acute myocardial infarction in the baboon. (abstract), *South African Journal of Science*, 86:544-545. (Paper presented at the meeting of the Physiology Society of Southern Africa, Cape Town 1990).
- 2) *HARTFORD CG, MARCOS E, ROGERS GG, ROSENDORFF C (1992)*
Coronary collateral formation following acute myocardial infarction in baboons. (Paper presented to the African Association of Physiological Sciences, affiliated to the International Union of Physiological Sciences, Kenya, September 1992).
- 3) *HARTFORD CG, ROGERS GG, MARCOS EF, ROSENDORFF C (1992)*
The influence of ketanserin on regional myocardial blood flow following myocardial infarction in baboons. *Journal of Cardiovascular Pharmacology*,
In press: See Appendix 7.1.

ACKNOWLEDGEMENTS

I gratefully acknowledge the assistance of my supervisor, Professor G. Rogers, who's tutoring has enabled me not only to grasp the essential principles of sound biological and scientific experimentation, but also to apply these physical principles appropriately in the field of medical research.

I appreciate also the technical assistance of Ms. E. Marcos, and the help of Ms. L. Richards.

I am indebted to my mother, Colleen, for her enduring support, encouragement and kindness throughout all of my studies.

PREFACE

My interest in the field of myocardial ischaemia developed after the completion of my undergraduate medical training, during my term as a medical intern. I noticed then that a large number of patients with myocardial ischaemia were suffering from repeated myocardial infarction, despite invasive therapeutic interventions such as coronary artery bypass surgery or coronary artery balloon angioplasty. The possibility of an effective non-invasive cardioprotective pharmacological intervention, for the prevention and management of ischaemic heart disease, has led to my investigation of the effects of serotonin on the coronary collateral circulation under conditions of primate experimental myocardial ischaemia.

CHAPTER 1: INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT), a vasoactive monoamine found and stored in platelets, has an adverse effect on the regional blood flow following peripheral arterial ligation or thrombosis (Nevelsteen *et al.* 1984; Blackshear *et al.* 1985; Orlandi, 1986). Arterial thrombotic occlusion reduces the subsequent formation of a peripheral collateral circulation in cats (Imhoff, 1961; Blackshear *et al.* 1985); peripheral arterial collateral formation and/or function is inhibited by serotonin in the cat, dog, rat and rabbit (Hollenberg, 1987); and the 5-HT₂ receptor antagonist, ketanserin, inhibits serotonin-induced femoral artery constriction in dogs (Blackshear *et al.* 1985) and improves post-thrombotic peripheral collateral function in cats (Nevelsteen *et al.* 1984).

The normal in vivo response to serotonin is one of large artery vasoconstriction and microvascular vasodilation with a net increase in blood flow (Houston and Vanhoutte, 1986). However, in pathology such as atherosclerosis, the response to serotonin is predominantly vasoconstriction with a net reduction in blood flow (Heistad, 1984; Blackshear *et al.* 1985; Orlandi, 1986). This effect may be due to increased vascular myocyte exposure to serotonin following endothelial damage (Cocks and Angus, 1983; Lamping *et al.* 1985; Vanhoutte, 1986) or increased local serotonin concentrations following platelet aggregation in thrombi at the sites of arterial occlusion (Houston and Vanhoutte, 1986). Activation of endothelial 5-HT₁-like receptors leads to coronary artery vasodilation while stimulation of myocyte 5-HT₂ and a minority of 5-HT₁-like receptors produces vasoconstriction (Blackshear *et al.* 1985; Bradley *et al.* 1986;

Golino *et al.* 1991). 5-HT₂ receptors are also found on platelets, where, in addition to propagating platelet aggregation, they facilitate platelet release of serotonin and other vasoactive substances (Houston and Vanhoutte, 1986; Vanhoutte, 1986).

Coronary arterial collaterals are known to develop in response to acute and chronic myocardial ischemia (Fujita *et al.* 1988). Serotonin is a vasoactive agent in coronary arteries, mediating vasoconstriction, vasodilation and cyclic flow variations (Ashton *et al.* 1986; Bush, 1987; Chu and Cobb, 1987;). The aim of this study was to determine whether selective 5-HT₂ receptor blockade improves regional myocardial blood flow following coronary artery occlusion and thrombosis.

CHAPTER 2: LITERATURE REVIEW

2.1 MYOCARDIAL ISCHAEMIA AND MYOCARDIAL INFARCTION.

2.1.1 DEFINITIONS.

Myocardial *ischaemia* refers to cellular oxygen deprivation (hypoxia), nutrient deprivation and the accumulation of waste products resulting from decreased myocardial perfusion of blood. The term *ischaemic heart disease* defines a disease spectrum of broad etiology, with the common factor being an imbalance of oxygen demand and oxygen supply, leading to ischaemic cell changes, and is usually the result of atherosclerosis. *Myocardial infarction* is a localised area of complete ischaemic cell necrosis (cell death), and more commonly arises from vascular thrombosis superimposed on atheromatous coronary plaques. Myocardial infarction is predominantly a disease of the left ventricle and interventricular septum, but the area which is damaged may extend into the right ventricle or even the atria if large. Some of the clinical consequences of ischaemic heart disease include decreased myocardial contractility with a decrease in systolic cardiac performance, diastolic dysfunction (ventricular filling abnormalities) and irregularities of heart rhythm.

2.1.2 MYOCARDIAL INFARCTION IN THE BABOON MODEL.

2.1.2.1 THE BABOON'S NORMAL REGIONAL MYOCARDIAL BLOOD FLOW.

In the experiment of Lubbe *et al.* (1974) it was found that there was an equal distribution of regional blood flow to the left ventricular epicardium and endocardium prior to coronary occlusion, but in the experiment of Lavallee *et al.* (1984) regional myocardial blood flow was found to be distributed primarily to the endocardium in conscious baboons (prior to myocardial infarction). The latter finding has also been noted in conscious chair-trained baboons by Vatner *et al.* (1987). King *et al.* (1985) showed that the degree of spatial heterogeneity of local myocardial flows in awake baboons is similar to that of the anaesthetized animals; that the degree of myocardial blood flow heterogeneity is marked, and that this pattern of regional flow heterogeneity tended to remain stable with time: They did not comment on endocardial versus epicardial blood flows.

2.1.2.2 DISTRIBUTION OF THE BABOON MYOCARDIAL INFARCTION.

The transmural distribution of a myocardial infarction has been compared in different animal species. Flameng *et al.* (1986) noted that, following left anterior descending coronary artery ligation, dogs developed a predominantly subendocardial infarct whereas that of baboons was primarily subepicardial (human infarcts are usually

transmural or subendocardial). In opposition to this Geary *et al.* (1983) found that baboons evolved sub-endocardial and mid-myocardial infarcts, rather than subepicardial infarcts. Flameng *et al.* (1986) has also demonstrated that the baboon infarct size, when expressed as a percentage of the perfusion bed of the occluded coronary artery, is greater than that found in dogs. This fact may be related to the higher myocardial metabolic demand of a baboon heart (as judged by the higher heart rates and blood pressures found in baboons, when compared with dogs).

Barker *et al.* (1980) reported that, in both baboons and monkeys, infarcts following coronary occlusion were transmural (i.e. all of epi-, mid- and endocardial). Lubbe *et al.* (1974), Lavalley *et al.* (1984), Heyndrickx *et al.* (1985) and Ruf *et al.* (1986) have noted that reductions of blood flow in the epi-, mid- and endo- cardial thirds of myocardial infarcts following coronary occlusion in baboons were similar, pointing towards the development of mainly transmural infarcts in the baboon model. In addition, Crozatier *et al.* (1978), Geary *et al.* (1981) and Vatner *et al.* (1987) have histologically confirmed the presence of transmural infarction in baboons, although the above-mentioned Vatner study also showed a prominent rim of both endocardial and epicardial infarct sparing. In any of the above cases mentioned, the site of infarct produced following coronary occlusion in the baboon appears to mimic more closely the site found in humans, than other animal species do.

2.1.3 THE PATHOGENESIS OF MYOCARDIAL INFARCTION.

(Maroko *et al.* 1971; Van der Werf, 1980; Francis and Cohn, 1984; Katz, 1983; Henderson and Marcus, 1984).

An imbalance between myocardial oxygen supply and demand results in myocardial ischaemia and/or infarction: Pharmacological interventions which reduce the myocardial oxygen demand or increase the oxygen supply may therefore affect infarct size and improve heart function following an infarct (Maroko and Braunwald, 1975).

2.1.3.1 MYOCARDIAL OXYGEN SUPPLY.

Myocardial oxygen supply is dependent on coronary arterial blood flow which in turn is influenced by perfusion pressure, aortic blood flow, coronary systolic and diastolic vascular resistances (neurogenic and autoregulatory controls) and the presence of a functioning coronary collateral circulation, as follows:

1) Coronary perfusion pressure (the difference between proximal coronary artery pressure and coronary sinus pressure): Since pressure in the large coronary arteries branching from the ascending aorta is identical to aortic pressure and, since at the venous side coronary sinus pressure equals right atrial pressure, the coronary perfusion pressure may be taken as the difference between aortic pressure (diastolic) and right atrial pressure.

2) Myocardial extravascular resistance (systolic compression of coronary vessels): The tension generated during ventricular systole in the myocardium is enough to collapse subendocardial vessels and to severely restrict flow to the subepicardial myocardium. The degree of this flow-limiting tension is dependent on the pressure developed by the left ventricle, the radius of the ventricle and the ventricular wall thickness (the Laplace equation), as well as on myocardial contractility and heart rate (since heart rate influences the relative lengths of systole and diastole and therefore the duration of myocardial vascular occlusion).

3) Diastolic coronary resistance: Is mainly determined by the arteriolar diameter, which is controlled in the major part by local metabolic factors (the local partial oxygen pressure being the most important regulatory factor of these). Metabolic autoregulation is dominant over neural and humoral regulation. The known metabolic mediators of coronary artery vasodilation include adenosine, potassium ions, hydrogen ions, various prostaglandins, histamine and bradykinin.

4) Autoregulation: Although an autoregulatory capacity (the inherent ability of blood vessels to compensate for excessive changes in intra-luminal blood pressure) exists in the coronary circulation, it is usually coupled with metabolic regulation, and tends to be more effective in the subendocardial than in the subepicardial layers of the left ventricle.

5) Neural control: The direct effects of sympathetic stimulation produce modest

coronary constriction while the indirect effects (an increase in left ventricular myocardial oxygen consumption) lead to a drop in coronary vascular resistance. Vagal stimulation may occasionally produce indirect coronary arteriolar constriction because of the associated decline in myocardial oxygen consumption (stimulation of myocardial M_2 receptors). However, the usual net effect of both sympathetic or parasympathetic stimulation is an increase in coronary blood flow.

6) Humoral control: The major circulating humoral agents which influence coronary tone are catecholamines, angiotensin-II and thyroid hormone. More recently, the humoral substances acetylcholine, serotonin and thrombin have been found to be important modulators of coronary vascular tone.

2.1.3.2 MYOCARDIAL OXYGEN DEMAND.

Cardiac muscle utilises predominantly fatty acids and lactate as substrates for oxidation. The energy derived from the breakdown of these products is stored in the form of adenosine triphosphate (ATP). The heart metabolises as a predominantly aerobic organ, but even under conditions of adequate oxygen availability lactate is consumed by cardiac muscle. As a result the coronary sinus lactate concentration is usually less than that of arterial blood.

Myocardial oxygen demand is governed primarily by the intracardiac systolic pressure (myocardial wall stress or tension), the degree and rate of myocardial fibre shortening

(inotropic state or contractility) and heart rate (the duration of the systolic period). Basal myocardial metabolic rate, external cardiac work (force times distance) and energy of activation also influence myocardial oxygen demand to a lesser degree. Clinically the product of aortic systolic pressure (which is nearly the same as left ventricular systolic pressure) and heart rate, the so-called rate-pressure product, is often used as an indicator of myocardial oxygen demand. However, other factors such as an increased myocardial contractility may place a higher value on oxygen demand than that calculated using the rate-pressure product. It has been noted recently that infarct size in baboons is determined by the size of the perfusion bed of the occluded coronary artery and the duration of that occlusion rather, than by the rate-pressure product or by collateral blood flow (Flameng *et al.* 1990). Since myocardial oxygen demand influences the production of numerous vasoactive metabolites, this demand must play an important role in the regulation of coronary blood flow.

2.1.3.3 THE CONCEPT OF INFARCT SIZE LIMITATION.

Interventions which decrease myocardial oxygen demand or increase myocardial oxygen supply have been shown to reduce the extent of acute ischaemic injury (Rogers *et al.* 1982; Heyndrickx *et al.* 1985). Any intervention therefore which, through manipulation of the above-mentioned factors that control coronary blood flow increases the coronary blood flow, may limit the size of an infarct. A major portion of the jeopardised myocardium within the territory of an occluded coronary artery is preserved by low blood flow perfusion. This low-flow perfusion gains access to the affected arterial

system by vascular collaterals: Yet, with prolonged occlusion, even this low-flow area succumbs to infarction. The area encapsulating the severely ischaemic core of an area of regional ischaemia, and separating it from the surrounding normal tissue, consists of a zone of intermediate flow and intermediate cell injury, and is known as the "border zone": The existence of the border zone implies that a wavefront of slowly progressing infarction is associated with prolonged ischaemia and/or with a recession of the collateral blood flow. Thus potentially, infarct size may be limitable by the pharmacological manipulation of the development of vascular collaterals and/or by a change in the resistance to blood flow of those collaterals supplying an ischaemic region.

2.2 THE CORONARY COLLATERAL CIRCULATION.

2.2.1 INTRODUCTION

A coronary collateral channel is an initially unused vascular pathway that becomes functional after failure of the existing coronary circulation to permit normal blood flows, such as occurs in the presence of ischaemic heart disease. The collateral network, which varies in different species, may consist of either a single or an interconnecting system of channels and is either pre-existing or newly-developed. The anatomical presence of collaterals has been researched and confirmed in the canine, porcine, human and non-human primate myocardium, however the functional significance of these channels is still controversial.

2.2.2 STIMULATION OF NEWLY DEVELOPED COLLATERALS.

Theories of the mechanisms contributing towards collateral vessel growth include both biochemical factors (hormones from mesoderm-derived heart cells and platelets) and physical factors which induce a mitogenic response in the ischaemic myocardial microcirculation's endothelial cells (Schaper *et al.* 1988): The ischaemic myocardium produces biochemical signals, the different types of which are as yet uncertain, but fibroblast growth factors (Montesano *et al.* 1990), platelet derived growth factors and tumor derived growth factors may all be involved. These biochemical signals trigger cellular transformation and mitosis in pre-existing and newly-developing collateral vessels. The extracellular matrix (collagen) is also implicated as a solid-phase inducer of capillary morphogenesis.

Schaper *et al.* (1978), using an experimental model of coronary occlusion in the dog, concluded that the determinants of coronary collateral blood flow include the coronary perfusion pressure, the degree of extravascular compression of underperfused myocardium by myocardial contraction, the size of the area-at-risk and the genetic factors mentioned above. Thus, physical forces, which include the gradient of pressure along inter-connecting arteriolar channels, changes in tangential wall stress (eg. as a consequence of vessel diameter changes and therefore changes in vessel wall tension) and increments in viscous drag and shear stress following increased blood flow velocity, are also implicated in the moulding of collateral growth and in the generation of mitogenic biochemical signals.

2.2.3 ANIMAL MODELS AND COLLATERAL ANATOMY.

The capacity to develop a coronary collateral circulation varies among and within species: Dogs show a well developed collateral circulation distributed primarily in the epicardium, compared with the less prominent collateral circulation which is found in pigs and baboons and which is situated primarily in the subendocardium and transmurally (Crozatier *et al.* 1978; Verani, 1983; Flameng *et al.* 1990).

Although the dog has frequently been used as an experimental model for coronary occlusion/myocardial infarction and the collateral circulation, the main advantage of the baboon model is its phylogenetic similarity to man. The coronary artery anatomy of baboons mimics more closely than other species do that of the coronary artery distribution of man (Flameng *et al.* 1986). Weisse *et al.* (1976) found that the heart weight to body weight ratio in baboons (mean 5.2 grams per kilogram) is similar to that found in man (mean 5.3 grams per kilogram) and less than that of dogs, but that the collateral response to coronary occlusion was similar in both dogs and baboons in terms of their resultant haemodynamic and metabolic changes. Flameng *et al.* (1986) showed that, following left anterior descending coronary artery occlusion in baboons and dogs, subendocardial collateral flow was higher than subepicardial collateral flow in baboons (and therefore similar to that of humans) while in dogs the subendocardial collateral flow was less than the subepicardial collateral flow. Opie *et al.* (1983) have however pointed out that, although the baboon is phylogenetically close to man in terms of its cardiac receptors, immunology and coronary artery anatomy, the degree of the

collateral circulation remaining after coronary occlusion is often less than that demonstrated following ischaemic heart disease in humans.

Existing epicardial collaterals in the canine model consist of endothelium and several smooth muscle layers (similar to arterioles of 20 to 60 μm diameter), whilst those of the pig are closer to capillaries or pre-capillary arterioles (Schaper *et al.* 1988). Transformed or newly-developed collaterals of all class and size (up to 1mm diameter) of arterial and capillary blood vessels have been noted following coronary artery occlusion in the human, porcine and canine models. The anatomy of newly-developing collaterals has been closely investigated in the canine model by Schaper *et al.* (1988); the collaterals, which were found to develop in a narrow sub-epicardial zone adjacent to the ischaemic region, first appear thin-walled and vein-like, but then develop sub-intimal muscular proliferation and later show both medial and adventitial blood vessel wall layers.

2.2.4 THE FUNCTIONAL SIGNIFICANCE OF CORONARY COLLATERALS.

An important factor of ultimate infarct size is the remaining blood flow to ischaemic tissue following a restriction of blood flow. It is reasonable therefore to expect a reduction in infarct size in the presence of good collateral blood flow, following coronary occlusion. The degree of collateral blood flow existing 5 minutes after ligation of a major coronary artery (expressed as a percentage of non-ischaemic blood flow) has been assessed in various species using radioactive microspheres, and shown

to be; dog 16%, cat 12%, rat 6%, baboon 2% (n = 6), rabbit 2% and pig 0.6% (Maxwell *et al.* 1987). Lubbe *et al.* (1974) has however noted in the baboon that, as early as 10 minutes after left anterior descending coronary artery ligation, there was a considerable blood supply (5% to 15%) to the border zone of the resulting infarct, and that this blood flow stemmed from the unligated adjacent coronary artery. He concluded that in the baboon the blood flow to the infarct edge is dependent on the existence of readily available collateral circulation channels.

Crozatier *et al.* (1978) noted that baboons had a far smaller index of collateral flow (using both retrograde flow and histology techniques) than dogs, but that both animals exhibited a gradual increase in the collateral blood flow over one month, following coronary occlusion. Rivas *et al.* (1976), Bishop *et al.* (1976) and Judgutt *et al.* (1979) have shown significant correlations between collateral blood flow and infarct size in dogs, while Cohen and Yipintsoi (1979) have demonstrated a protective effect of the developed collaterals on myocardial function (in this case cardiac failure, as judged by left atrial pressure measurements), when the left circumflex was occluded during treadmill running in canines. Recently Yanagisawa-Miwa *et al.* (1992) demonstrated that the intracoronary injection of basic fibroblast growth factor improved cardiac systolic performance and reduced infarct size in a canine experimental myocardial infarct model. There is little available evidence for such correlations or improvements of cardiac function following acute coronary artery occlusion in baboons.

Since limitation of infarct size in the baboon model has been shown to be dependent on early reperfusion (Vatner *et al.* 1987), the sparse early collateralization after coronary occlusion in baboons may explain the failure of many pharmacological interventions to reduce infarct size in these primates (Geary GG *et al.* 1982, Geary *et al.* 1983; Flameng *et al.* 1986; Vatner *et al.* 1987). Thus any intervention which may improve collateralisation in baboons might be expected to improve ventricular function, reduce infarct size or to have a synergistic action on the positive effects of other intervention agents during the primate myocardial ischaemia or infarction.

Human ischaemic heart disease subjects with well developed collaterals show less necrosis, less depressed heart function and longer survival times following myocardial infarction (Williams *et al.* 1976): It is generally accepted that; in humans, coronary collaterals are developed in the presence of ischaemia and myocardial infarction; that this process of collateralisation is complete within two to four weeks following significant coronary occlusion; and that these coronary collaterals help to preserve the functional integrity of the myocardium in those hearts with coronary obstructive coronary disease (Cohen, 1985; Fujita *et al.* 1988).

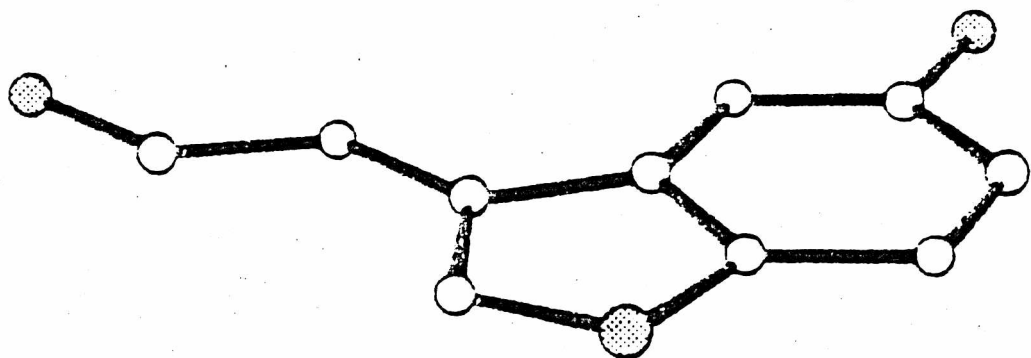
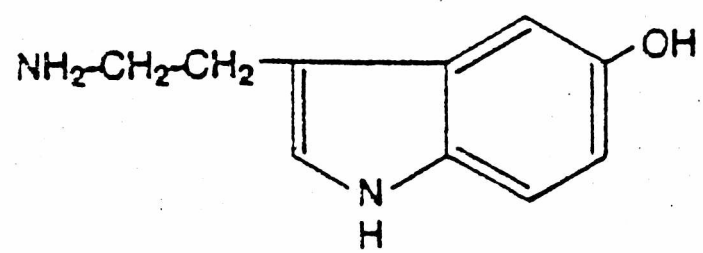
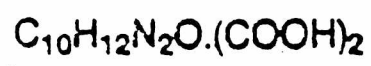
2.3 SEROTONIN AND THE CARDIOVASCULAR SYSTEM.

2.3.1 INTRODUCTION: THE SEROTONIN MOLECULE.

Serotonin (Five-Hydroxytryptamine, 5-HT) was first recognised in 1949 as a vasoconstricting agent. Serotonin is a monoamine, vasoactive peptide (Figure 2.1; page 16) synthesised in the amine precursor uptake and decarboxylation enterochromaffin cells (APUD cells) of the gastro-intestinal tract. Important quantities of this monoamine are found in the plasma, platelets and serotonergic neurons of the central nervous system and gastro-intestinal wall (Houston and Vanhoutte, 1986).

Serotonin crosses the blood-brain barrier poorly; the serotonin located within the central nervous system is synthesised locally. Central serotonergic pathways are involved in the regulation of blood pressure (Kuhn *et al.* 1980). Free plasma serotonin is actively taken up and stored in platelets, and destroyed by the liver and vascular endothelial cells (by monoamine oxidase). The rapid removal of serotonin by endothelial cells makes serum assays of this hormone difficult. This fact makes it more likely that the cardiovascular effects of serotonin predominate at discrete sites where platelet aggregation occurs, rather than by contributing towards the overall homeostasis of the cardiovascular system.

Figure 2.1: The molecular structure of Serotonin.



Serotonin

2.3.2 SEROTONIN RECEPTOR THEORY AND SEROTONIN ANTAGONISM.

2.3.2.1 SEROTONIN RECEPTOR THEORY.

The nomenclature of 5-HT peripheral receptors has recently been re-classified using several new available serotonin antagonists. The antagonists have also allowed a more specific descriptive profile of serotonin binding sites (Janssen, 1983; Bradley *et al.* 1986; Leff and Martin, 1988; Chester *et al.* 1990):

Functional peripheral serotonin receptors are divided into three classes; 5-HT₁-like receptors, 5-HT₂ receptors and 5-HT₃ receptors (Bradley *et al.* 1986). The nomenclature and pharmacology of 5-HT₂ and 5-HT₃ receptors is well described through the use of selective antagonists. These two receptor types correspond to the older nomenclature of "D" and "M" receptors. A number of serotonin's vascular actions are distinct from the effects of 5-HT₂ and 5-HT₃ receptors, but because selective antagonists for these different receptors are not yet available, they are currently classified as 5-HT₁-like receptors.

5-HT₁-like receptors have been located on dog endothelial cells where they mediate the release of Nitric Oxide, with subsequent smooth muscle relaxation, by stimulating a rise in intracellular cyclic GMP (Cohen *et al.* 1983). A second 5-HT₁-like receptor has also been located on the smooth muscle myocyte, and here it's relaxation response is mediated by a rise in cyclic AMP. A ligand known as GR 43175, which is selective for

5-HT₁-like receptors, has been found to mediate contraction of the dog saphenous vein as well as contraction of human isolated coronary arteries, but to a much lesser degree than that of the normal maximum artery serotonin response (Angus, 1989). 5-HT₁-like receptors have also been located on cardiac pacemaker cells and central and peripheral neurones of different species including humans (Gothert and Schlicker, 1987).

Highly selective serotonin antagonists have led to both the identification and localisation of the specific 5-HT₂ receptor. The diverse list of the responses to 5-HT₂ receptor stimulation includes vasocontraction, platelet aggregation and neuronal depolarisation; the receptor is located on vascular smooth muscle myocytes, platelet membranes and noradrenergic nerve endings, as well as in the human cortex (Schutte *et al.* 1983). Since my study was specifically concerned with antagonism of 5-HT₂ receptors, the other known actions of 5-HT₂ receptors are summarised in Table 2.1; page 21.

Gothert *et al.* (1987) have located 5-HT₃ receptors on peripheral and central neurones of different species including humans, while Kaumann (1985) has named certain porcine sino-atrial and left atrial receptors as 5-HT₃. The introduction of more specific antagonists for 5-HT₄ receptors has led to the recent acceptance of their existence (Bockaert *et al.* 1992), and their localisation at specific sites in different species: They are found on neurones of the mouse, guinea pig and rat; the guinea-pig ileum plus ascending colon and the rat oesophagus, and the human right atrium and ventricle (Kaumann, 1985).

The wide intra- and inter-species variation of the density, types and localisation of 5-HT receptors has led to a hypothesis of the possible cellular regulation of 5-HT receptors (Leff and Martin, 1988). Serotonin receptors have not been extensively studied in primates and **have not been studied at all in baboons to date.**

2.3.2.2 SEROTONIN ANTAGONISM.

Commonly used agonists at 5-HT₁ and 5-HT₂ receptors are serotonin, spiroperidol and lysergic acid. The classical serotonin antagonists are methysergide, metergoline, mianserin, cyproheptadine and spiperone. Methysergide is a mixed receptor agonist-antagonist. More recently, a compound R41468 known as ketanserin was found to bind selectively to 5-HT₂ receptors (Janssen, 1985). The structure of ketanserin and certain other selective 5-HT₂ antagonists is shown in figure 2.2; page 23. The side chains of all these compounds end in common typical heterocyclic structures. Ketanserin is the most selective of these compounds, being almost devoid of dopaminergic activity.

Ketanserin is a potent, highly selective and competitive 5-HT₂ serotonergic receptor antagonist. The compound is a piperidine derivative substituted in position 4: (3-(2-(4-(4-fluorobenzoyl)-1-piperinyl)ethyl-2,4(iH,3H)-quinazolinedione: The molecular weight = 395, but with tartrate, the injectable form, the molecular weight = 545). Ketanserin does not bind to 5-HT₁ receptor sites at all, but has measurable affinity for H₁-histaminergic, α_1 - and α_2 -adrenergic and D₂-dopaminergic (low affinity) binding sites. Ketanserin is devoid of agonistic properties (unlike methysergide) and is a potent and

selective antagonist of the effects of serotonin on the vasculature smooth muscle and on platelets. Ketanserin also acts as an anti-hypertensive agent in some species including humans (Van Oene, 1990), either by serotonergic blockade or by its weak α_1 -blocking properties (Andren, 1983; Janssen, 1983).

LY 53857 (Eli Lilly Co.) is a highly selective and potent central and peripheral competitive 5-HT₂ receptor antagonist, and has recently been used by several researchers as a 5-HT₂ blocker (Fuller and Snoddy, 1979; Cohen *et al.* 1983; Eidt *et al.* 1989; Simpson *et al.* 1991). This agent has an approximately 300 000 fold lower affinity for α -adrenergic receptors than for 5-HT receptors. Although this affinity is an advantage over ketanserin because no anti-hypertensive effects are evident, LY 53857 is a less potent 5-HT₂ antagonist, has a relatively short plasma half-life of 30 minutes and is not as yet suitable for oral administration (Cohen *et al.* 1983).

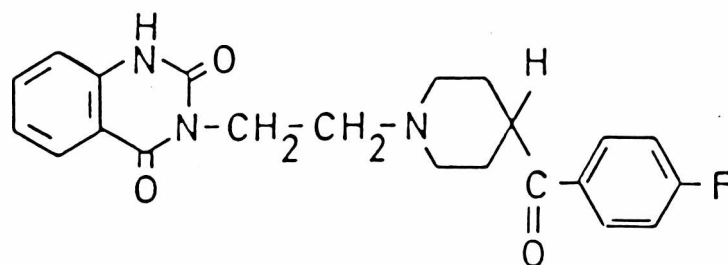
TABLE 2.1: 5-HT₂ RECEPTOR RESPONSES TO SEROTONIN IN VARIOUS SPECIES.

(Adapted from Bradley *et al.* 1986)

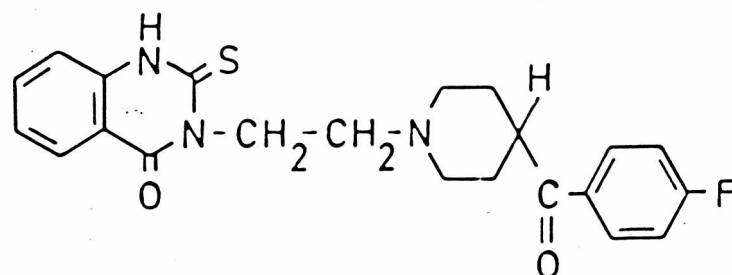
Responses to 5-HT	Species/location	Specific antagonism by ketanserin or methysergide
<i>In vitro</i>		
Contraction of vascular smooth muscle	Rabbit aorta	Apperley, Humphrey and Levy (1976); Feniuk <i>et al.</i> (1985a)
	Rat tail artery	Bradley, Humphrey and Williams (1985);
	Dog gastrosplenic v.	Van Nueten <i>et al.</i> (1981); Van Nueten <i>et al.</i> (1981)
Contraction of extra-vascular smooth muscle	Guinea-pig trachea	Van Nueten, Leysen, Vanhoutte and Janssen (1982)
	Rat uterus	Ichida <i>et al.</i> (1983)
	Guinea-pig ileum (atropine treated)	Engel <i>et al.</i> (1985)
Platelet aggregation	Cat	De Clerck, Xhonneux, Leysen and Janssen (1984)
	Rabbit and man	No data
<i>In vivo</i>		
Vasopressor responses	Pithed/anaesthetized rat	Van Nueten <i>et al.</i> (1981); Feniuk, Humphrey and Perren (1982); Fozard (1982); Saxena and Lawang (1985);
	Conscious rat	Dalton <i>et al.</i> (1985)
Bronchoconstriction	Anaesthetized cat	Reiche and Frey (1983); Connor <i>et al.</i> (1986)
	Anaesthetized guinea-pig	Saxena and Lawang (1985)
5-Hydroxytryptophan-induced heat twitch	Conscious rat/mouse	Malick, Dosen and Barnett (1977)
Oedema	Rat paw	Ortmann, Bischoff, Radeke Buech and Delini-Stula (1982)
Urinary bladder contraction (second phase)	Anaesthetized cat	Saxena, Heiligers, Mylecharane and Tio (1985a)

Figure 2.2: Structural formulae of certain serotonin 5-HT₂ receptor antagonists (Janssen, 1989).

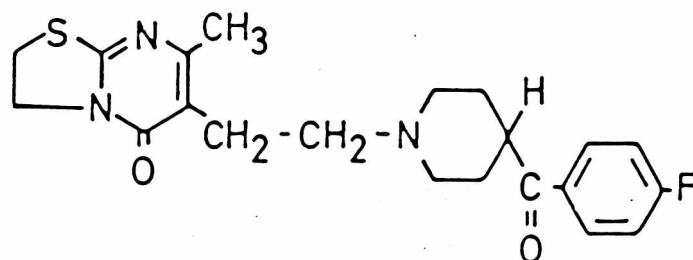
Ketanserine
(R 41468)



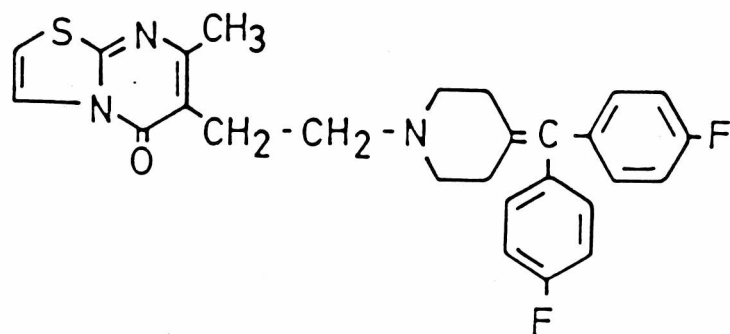
Altanserine
(R 53200)



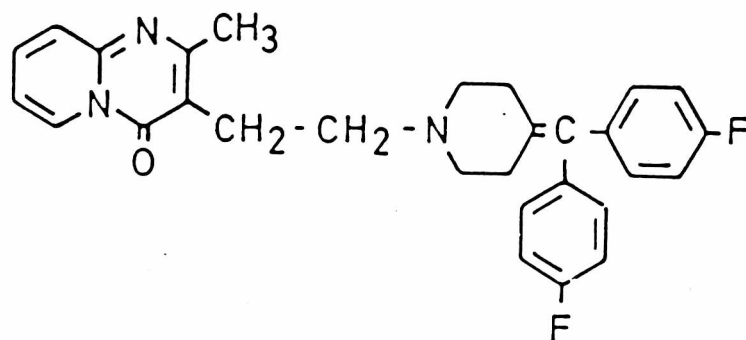
Setoperone
(R 52245)



Ritanserine
(R 55667)



R 56413



2.3.3 SEROTONIN AND THE VASCULATURE.

2.3.3.1 INTRODUCTION.

Some of the **diverse** cardiovascular effects of serotonin include:-

(Houston, 1986; Houston and Vanhoutte, 1986; Reneman and Bollinger, 1986).

- Displacement of noradrenaline from adrenergic nerve endings (at high concentrations of serotonin), with a subsequent positive chronotropic and inotropic (β_1) myocardial effect.
- Inhibition (at a pre-junctional site) of vascular sympathetic nerves.
- Alteration of blood rheology (thereby adversely affecting the blood's flow properties); a decrease of in vitro filterability, increased vascular permeability and a decreased red cell pliability (Dormandy, 1986).
- Activation of platelets and the potentiation of other platelet aggregating agents and vasoactive agents: Serotonin-induced platelet activation stimulates the release of Thromboxane- A_2 (and its metabolites), as well as stimulating further platelet serotonin release via a platelet 5-HT₂ receptor. The effect of this is an escalation in plasma serotonin concentration, the resulting concentration of which is sufficient to act on endothelial cells, myocytes and adrenergic nerve terminals. Since platelet aggregation and the release of clotting cascade activating factors is a function of thrombus formation, serotonin also plays an important role in haemostasis.

- Regulation of intravascular blood flow and blood pressure: Serotonin has direct and indirect **vasodilator and vasoconstrictor effects**: (see ensuing chapters).

2.3.3.2 SEROTONIN AND THE PERIPHERAL VASCULATURE.

Serotonin exerts a consistent vascular contractile response in a variety of isolated peripheral vascular preparations. In contrast to this, the *in vivo* response to serotonin is one of both large artery segment constriction and vasodilation of the muscle microvasculature, with a net increase in blood flow (Figure 2.3; page 28).

Blackshear *et al.* (1985), using angiography and blood flow measurements of canine femoral arteries *in vivo*, demonstrated that intra-arterial serotonin reduced the canine large artery cross-sectional area by 50% or more, whilst concomitant arteriolar dilatation led to a net increase in regional blood flow. They further went on to show that this serotonin-induced large artery contraction was inhibited by methysergide and ketanserin, thereby implicating the 5-HT₂ receptor as that responsible for the vasoconstrictor effects of serotonin. The use of selective 5-HT₂ blockade also failed to block serotonin-induced increments in blood flow in their experiments. Thus 5-HT₂ antagonists may antagonise large vessel constriction caused by serotonin, while still permitting serotonin's vasodilatory responses to occur.

The above findings led researchers to look at the effects of serotonin and serotonin antagonism on the peripheral circulation and on collateral formation in arterial disease such as occlusion (Figure 2.3; page 28). Orlandi *et al.* (1984) found that, following superficial femoral arterial ligation in dogs, a 5-HT₂ receptor-dependent mechanism mediated decreases in the perfusion of the regions served by collaterals (contrary to the in vivo unligated normal artery response to serotonin). Heistad (1984) demonstrated that atherosclerotic monkeys were supersensitive to the vasoconstrictor effects of serotonin when compared with controls: In diseased limbs the large artery constrictor effect of serotonin became dominant, resulting in a fall of the hindlimb blood flow. Imhoff (1961) was the first to show that when ligation of the infra-renal aorta was combined with thrombus formation in cats, the local collateral circulation blood flow was severely reduced. Schaub *et al.* (1976, 1977) confirmed these findings on the feline hindlimb collateral circulation, further showing that serotonin depresses collateral blood flow, and that this collateral inhibitory effect could be partially reversed by pre-treatment with cinanserin (a serotonin antagonist). Nevelsteen *et al.* (1984) then produced an acute obstruction of the cat aorta and found that daily i.p. pre-treatment with ketanserin led to a significant improvement in the blood flow and a decrease in the collateral vascular resistance of the ischaemic area at risk, thereby further implicating the role of 5-HT₂ receptors in the inhibition of collateral circulations.

In summary, the development of collateral arterial blood vessels appears to be sensitive to inhibition by serotonin.

Figure 2.3: The normal and the pathological in vivo peripheral vascular responses to an intra-arterial serotonin infusion.

Normal
Response to 5-HT:

Pathological
Response to 5-HT:

Artery:
vasoconstriction

Arterioles:
vasodilatation

Lesion:
supersensitive,
vasoconstriction

Collaterals:
supersensitive,
vasoconstriction

Blood Flow:
Increased

Blood Flow:
Decreased

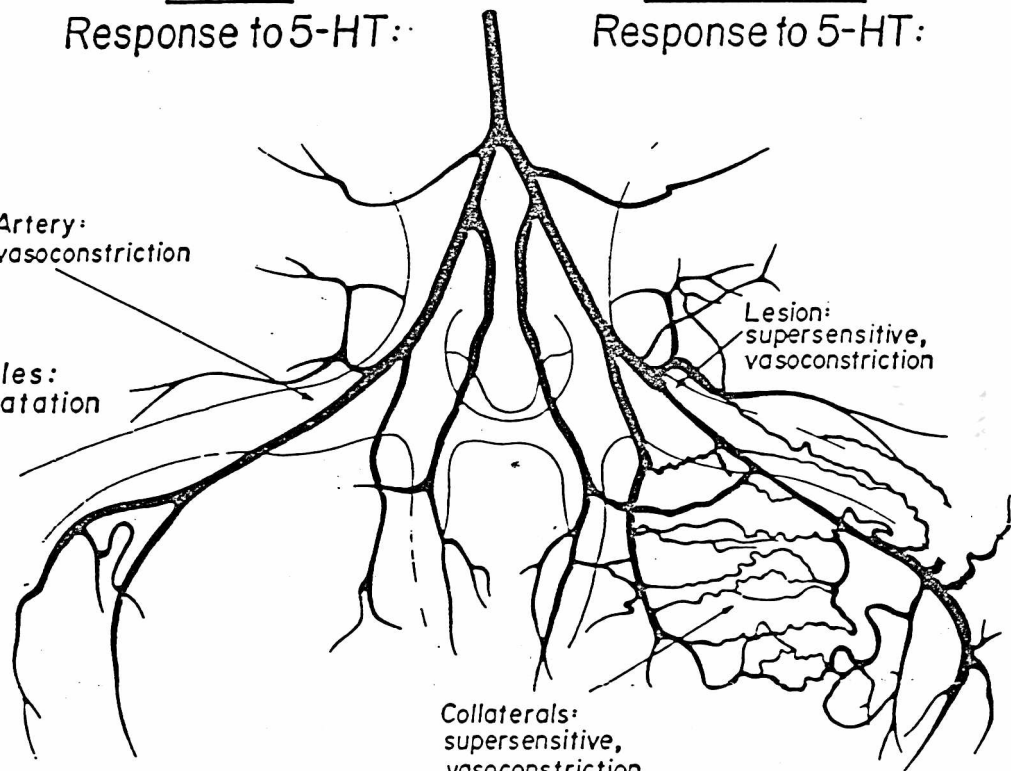
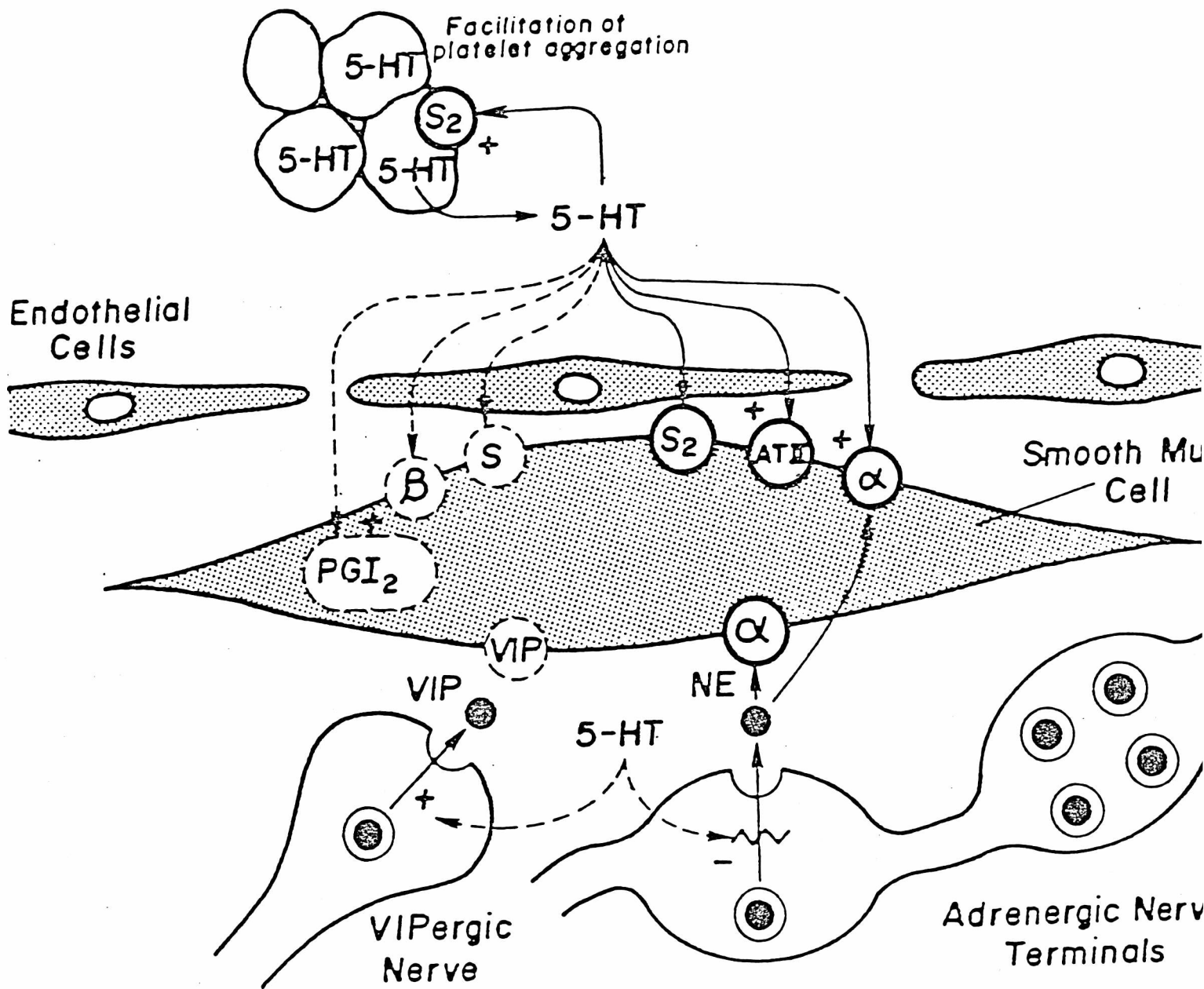


Figure 2.4: Serotonin's vascular modes of action; see text for explanation. (Vanhoutte, 1987)



2.3.3.3. THE MECHANISM OF SEROTONIN'S VASCULAR ACTION.

(Figure 2.4; page 30)

The association of arterial thrombus formation with the inhibition of collateral circulation development is possibly linked by the release of serotonin derived from activated platelets, when they undergo aggregation during the formation of thrombi. The presence of platelet cell membrane-associated 5-HT₂ receptors is well documented (Arnout *et al.* 1985; De Clerck and Xhonneux, 1985; De Cree *et al.* 1985; Buhler *et al.* 1987). The action of serotonin at these receptors is to enhance platelet aggregation and to mediate further release of serotonin; ketanserin has been shown to inhibit these effects.

The vasoconstrictor influence of serotonin is primarily via its direct action on 5-HT₂ receptors located on vascular smooth muscle myocytes. In addition, serotonin causes displacement of noradrenaline from adrenergic nerve terminals and potentiates the action of other vasoconstrictors such as histamine, noradrenaline, angiotensin II, thromboxane-A₂ and prostaglandin-F₂α (Figure 2.4; page 30).

Serotonin-mediated vasodilation occurs by the release of an endothelial derived relaxing factor (EDRF, ? nitric oxide) from endothelial cells which incorporate 5-HT₁ receptors (Cocks and Angus, 1983; Cohen *et al.* 1983), as well as by a direct action on smooth muscle cells incorporating an as yet unidentified 5-HT receptor (Housten and Vanhoutte, 1986; Janssen, 1983). Other possible mechanisms of serotonin's

vasodilatory effect include; direct pre-junctional adrenergic nerve inhibition, β -adrenergic receptor stimulation, and the activation of inhibitory autonomic nerves with the release of vaso-active intestinal polypeptide (Reneman and Bollinger, 1986; Vanhoutte, 1987).

2.3.3.4 SEROTONIN AND THE CORONARY CIRCULATION.

(i) CORONARY ARTERY RESPONSES TO SEROTONIN.

The effects of serotonin on the baboon coronary circulation have not been extensively investigated, however numerous studies have been performed on other species' coronary circulations, in particular that of the dog. As early as 1982 Brazenor and Angus, using an in vitro model, demonstrated that ketanserin could inhibit serotonin-induced canine coronary circumflex constrictor activity. Since that time the role of serotonin in the coronary circulation has become topical and serotonin was shown to be a potent in vivo coronary artery constrictor in anaesthetized dogs (Perez *et al.* 1983). Chu and Cobb (1987) then described the effect of serotonin on the proximal circumflex arteries of awake dogs as one of a biphasic motor response, characterised by initial vasodilation (mediated through the endothelium) followed by delayed vasoconstriction.

The typical response to experimental canine coronary stenosis is the development of episodic spontaneous decreases in coronary blood flow, interrupted by restorations of

blood flow, in the portion of the artery proximal to that site of stenosis. This phenomenon has been termed cyclic flow variation (CFV) and is thought to be related to platelet aggregation at the sites of stenosis. Serotonin is capable of initiating such flow variations and ketanserin has been noted to abolish the existing CFV's in canines (Bush *et al.* 1984; Apprill *et al.* 1985; Ashton *et al.* 1986; Bush, 1987; Ashton *et al.* 1989). In one study of canine experimental thrombolysis, following thrombotic coronary occlusion and the production of CFV's, spontaneous arterial re-occlusion was found to be promoted by the presence of serotonin and delayed by the presence of Thromboxane-A₂ and 5-HT₂ receptor antagonists (Golino *et al.* 1988). The vasospasm found following coronary artery balloon angioplasty has also been illustrated to be reduced by the 5-HT₂ receptor antagonist LY 53857 in rabbits (Sigal *et al.* 1991).

It has been shown that the concentration of serotonin at sites of canine experimental coronary occlusion is elevated (Ashton *et al.* 1986). Benedict *et al.* (1986) correlated coronary sinus plasma serotonin concentrations with in vivo platelet aggregation, during canine coronary thrombus formation. It has been noted subsequently that serotonin is released into the coronary circulation in increased amounts in patients with coronary artery disease (Van den Berg, 1989).

Most coronary-circulation serotonin-related studies have concentrated on coronary diameter changes rather than on coronary blood flow or regional myocardial blood flow changes: Myocardial blood flow (using a microsphere technique) has been noted to increase in the presence of ketanserin after canine coronary artery stenosis and this was

related to a decline in the cyclic flow reductions of blood flow in the ketanserin group (Bush *et al.* 1984). In addition, using the Langendorff technique and manual coronary flow measurements, Luscher *et al.* (1986) exhibited serotonin-induced reductions of coronary flow in the isolated heart of the spontaneously hypertensive rat.

Ruocco *et al.* (1988), using the radioactive microsphere technique, established that serotonin had no effect on the regional myocardial blood flow of pigs unless there was a severe coronary stenosis; under the latter condition infused exogenous serotonin caused a modest impairment of endocardial blood flow. In primates, coronary artery cross-sectional area and blood flow were measured using angiography and doppler techniques by Golino *et al.* (1991); they concluded that serotonin has a vasodilating effect in humans with normal coronary arteries but a vasoconstrictor effect, which was alleviated in part by ketanserin, in patients with ischaemic heart disease.

(ii) SEROTONIN'S INTERACTION WITH THE VASCULAR ENDOTHELIUM.

One might expect that the normal serotonergic-mediated vasodilator response would be blunted in states of coronary artery disease such as occlusion or atherosclerosis, because in such pathology the endothelium lining the arteries and arterioles would be damaged. Consequently, most serotonin-related coronary studies have concentrated on the coronary circulation in the presence of arterial disease of various species:

Vascular endothelial damage has the effect of:

- 1) Promoting platelet aggregation and thrombus formation (and consequently the release of increased amounts of platelet-stored serotonin).
- 2) Decreasing serotonin breakdown by endothelial monoamine oxidase.
- 3) Enhancing the vascular myocyte 5-HT₂ receptor exposure to circulating serotonin and accordingly encouraging vasoconstriction.

The expected response of coronary endothelial damage on serotonin's vasoconstrictor responses has been investigated. Cocks and Angus (1983) found that both serotonin and noradrenaline were more powerful vasoconstrictors in the absence of endothelium in pig and dog isolated coronary arteries, following mechanical removal of the endothelium. Accordingly, Lamping *et al.* (1985) and Chu and Cobb (1987), using in vivo models of anaesthetized and awake dogs respectively, established that mechanical disruption of the coronary artery endothelium potentiated the constrictor response to intracoronary infusion of serotonin. These findings were expanded by Shimokawa *et al.* (1987) who illustrated that the ability of porcine coronary arteries with a regenerated endothelium (following mechanical denudation) to evoke endothelium dependent relaxation, in response to serotonin in vitro, was reduced; the morphology of the regenerated endothelial cells was different from that of the native non-denuded cells. Moreover, Nyborg and Mikkelsen (1988) looked at the effects of aging on rat coronary resistance arteries and noted that with increasing age the coronary vascular constrictor response to infused serotonin was elevated.

(iii) CONCLUSION.

In summary, some of the possible advantages of blocking the effects of serotonin at coronary 5-HT₂ receptors in the presence of coronary artery occlusive disease may be;

- decreased platelet aggregation and thrombus formation;
- decreased platelet activation and reduced additional platelet serotonin release;
- decreased serotonin-mediated vascular myocyte contraction;
- decreased serotonin-mediated inhibition of the developing coronary collateral circulation.

2.4 METHODS OF REGIONAL AND COLLATERAL **MYOCARDIAL BLOOD FLOW MEASUREMENT.**

2.4.1 TECHNIQUES OF COLLATERAL CIRCULATION MEASUREMENT.

Numerous direct and indirect measurements of the collateral circulation have been employed by different researchers over the past two decades. Studies of the collateral circulation require the measurement of both coronary pressures and blood flows, and their changes over time. No single used method is ideal. Schaper *et al.* (1965, 1988) and Cohen (1985) have experimented with and reviewed the usefulness of several of these methods:

Direct visual techniques of assessing collaterals encompass the exploitation of both histology and arteriography (coronary angiography). Both of these techniques involve the counting and the grading according to size of visualised collateral anastomoses. The histology methods are time-consuming and give no assessment of either temporal fluctuation or functionality of the collaterals. The angiographic assessments often do not offer sufficient definition for quantification of collaterals, since many collaterals are microscopic in size and are located subendocardially. In addition, it is well known that a significant pressure gradient across a collateral network is a prerequisite for the angiographic visualisation of coronary collaterals (Fujita *et al.* 1988). The use of traditional coronary cineangiography also has inherent problems relating to; the discordance of anatomy and functionality of the vasculature; limitations of the X-ray

imaging chain (such as the maximum obtainable speed of successive exposures); operator experience, and the biological characteristics of the animal under surveillance (Verani, 1983; Theron *et al.* 1991).

A system of cannulating the coronary artery distal to the site of occlusion, and measuring the rate of blood flow through this cannula, gives an assessment of "retrograde flow". This technique has been used as an index of collateral flow, however retrograde flow has often been shown to be either greater than or less than actual collateral blood flow. The discrepancy noted is due to factors such as ongoing venous drainage due to retrograde venous blood flow, or differences in the resistance to blood flow of the cannula used versus that of the original coronary artery.

"Peripheral coronary pressure" is another indirect collateral index that refers to intracoronary pressure distal to a site of coronary occlusion. The principle behind this method, first used by Schaper *et al.* (1965), is that an increased collateral blood flow results in a greater filling of the vascular bed at risk, and hence a higher residual distal arterial pressure (in the absence of anterograde flow). The measurement is unreliable since peripheral coronary pressure is also related to changes in left ventricular pressure, ventricular wall tension and aortic blood pressure.

Another two methods which have been employed as indices of the collateral circulation are the myocardial clearance of inert gases (the time taken for washout of injected gamma-isotopes), and the myocardial uptake of isotope potassium analogues (blood

flow is reflected by the supply of the tracer to the myocardium and hence myocardial uptake of this tracer). Rapid uptake of inert gases by pericardial fat results in tailing off of the washout curve, which can affect blood flow determinations, whilst the potassium analogue is lost from ischaemic and normal myocardium at different rates, interfering with blood flow determinations as a function of time.

2.4.2 THE RADIOACTIVE MICROSPHERE TECHNIQUE.

2.4.2.1 HISTORICAL BACKGROUND.

Because of the technical and theoretical problems which arise when using the above-mentioned assessments, the technique of utilising radioactive microspheres to measure blood flow has gained much popularity and acceptance in the field of circulation research. Collateral flow can be measured reliably and reproducibly when using this tracer technique if the right precautions are taken. As a result, radioactive microspheres are generally thought to be the best form of measurement of the collateral circulation.

In 1967 Rudolph and Heymann first described a successful technique of measuring the distribution of blood flow using radionuclide labelled 50 μm diameter particles in goats. Following their experiments radio-labelled microspheres have become a gold standard for regional blood flow measurements in experimental animals. The microspheres are nowadays composed of an inert, insoluble carbonized plastic, and range in size from 1 to 100 μm in diameter. Nen-Trac spheres (Nen^R) are labelled with one of 7 possible

gamma nuclides, and two beta emitter nuclides, which nuclides are incorporated into the microsphere plastic. The density of the plastic microspheres is manufactured to be close to that of red blood cells.

The major disadvantages of the microsphere technique are the hazards of the exposure of researchers to the radio-isotope and the expense of the radio-isotopes. The advantages of this technique over that of others is that the spheres give a measurable indication of the amount of blood flowing to the myocardium. As a result regional blood flows of various myocardial areas can be determined, furnishing a quantitative assessment of myocardial blood flow, transmural blood flow ratios, and a functional assessment of vascular collateral formation (Schaper, 1978).

2.4.2.2 TECHNICAL POINTS PERTAINING TO THE USE OF MICROSPHERES.

The physical characteristics of microspheres which may affect blood flow determination include the size and shape of the spheres, the isotope's specific activity, radionuclide label purity and leaching of the nuclide from the microspheres. Leaching refers to the physical loss of radioactive nuclide from the plastic microspheres per unit time.

Microspheres are usually suspended in a solution of either saline or Dextran. Tween-80 (polyoxyethylene sorbiton mono-oleate, a detergent) is added to the suspension to prevent sphere aggregation. Tween-80 in concentrations of greater than 0.05 % may

affect haemodynamics and the concentration most commonly used is therefore 0.01 % (Warren and Ledingham, 1974). Most commercially available microsphere preparations meet nuclide leach criteria of less than 1 % leaching in saline over four days, and less than 2 % leaching in plasma over 2 days in vitro. The in vivo use of spheres for greater than twenty four hours may result in different sphere isotope leach characteristics.

Following intravascular injection the microspheres are trapped in arterioles or capillaries. In order to establish accurate flow measurements, the spheres must be well mixed within the blood stream immediately after injection; uniform distribution of the microspheres throughout the cross section of the vessel is important and should approximate the normal red blood cell distribution (Wagner et al 1969). Two factors which affect this uniformity of microsphere distribution are the site of the spheres' injection and the number of microspheres injected. The best possible site for injection is that which is most proximal to an organ under consideration since this allows the greatest time for sphere mixing to occur in the bloodstream prior to entering the organ's vascular bed. Hence, if accessible, the left atrium should be preferentially used, particularly in studies involving the coronary circulation.

After the experiment a comparison of the number of microspheres per gram of tissue in the left and right cerebral hemispheres or kidneys gives an indication of the evenness of sphere distribution within the blood stream. Alternatively, bifemoral blood samples can be withdrawn simultaneously and their radioactive counts compared. It is shown

that the number of microspheres per sample of tissue being counted should number around 400 in order to have a distribution variability of within 10 % of the mean distribution (Buckberg *et al.* 1971).

The number and size of microspheres per injection, and the frequency of different injections may also have an effect on haemodynamics. In general, depending on the animal species used, the repeated intra-arterial injection of 300 000 to 500 000 15 μ m diameter spheres causes no significant acute or chronic haemodynamic alterations. The number of spheres applied per injection must obviously be reduced when using spheres of larger diameters, since the degree of obstruction of the microcirculation is related to the diameter of vessels obstructed. In smaller animals fewer microspheres are used even if small diameter microspheres have been selected.

The physical size of the microspheres affects both the myocardial entrapment of spheres and the site of entrapment. Large diameter spheres of 50 μ m have been noted to preferentially precipitate out in the subendocardial layers of the dog heart (Kaihara *et al.* 1969). In addition, the diameters of capillaries and arteriovenous anastomoses may vary between different organs and between animal species, thus affecting the site of sphere entrapment as well as the number of spheres trapped. An alteration of physiological conditions such as temperature, or the use of anaesthetic agents may affect microsphere entrapment. The in vivo time-related loss of microspheres previously trapped in tissue has also been documented (Crystal *et al.* 1979). The degree of sphere entrapment can be assessed by measuring the microsphere activity of that organ's total

venous drainage, or by counting the activity of organs which receive that venous drainage.

In general, 15 μm diameter microspheres satisfy the conditions of having a vascular distribution similar to that of red blood cells, the absence of significant non-entrapment and a lack of haemodynamic effects.

2.4.2.3 TECHNICAL POINTS PERTAINING TO THE ADMINISTRATION OF MICROSPHERES.

Prior to left atrial injection the spheres must be agitated by vortexing and ultrasonication in order to reduce sphere clumping and aggregation. The absence of significant aggregation is confirmed microscopically. Attention to sterility is important in animal survival experiments and the ultrasonication proves to be useful as a bactericidal means. Spheres are drawn up into a teflon-coated or glass syringe and injected via the left atrial cannula over 10 or 20 seconds, after which the cannula is flushed with 10 ml of saline over 10 or 20 seconds. The solution of spheres for injection should be warmed to body temperature to reduce the incidence of thermal-related ventricular ectopy. In the author's experience, the ultrasonication of the microsphere vial alone usually results in an adequate rise of the injectate's temperature.

If calculations are made based on the total number of microspheres injected (such as for cardiac output determination), caution must be taken to ensure that there is no loss of microspheres during the injection procedure and the left atrial cannulas should also be counted for residual radioactivity. In addition, the time difference between the counting of the spheres injected and the counting of the reference blood samples and tissue blood samples should be accounted for using the known radioactive decay constants of the different isotopes as follows;

CALCULATION OF RADIOACTIVE NUCLIDE DECAY:

let c_1 = the sample's counts per minute (cpm) at time t_0 .

let $c_{1/2}$ = the sample's cpm at time t_1 .

let $t_1 - t_0 = x$ = the known half-life (T) of the isotope, in days.

let y = the number of cpm which have decayed in time x .

then,

$y = \ln(c_1) - \ln(c_{1/2})$, where $\ln$ = natural logarithm.

and

$$dy/dx = (\ln(c_1) - \ln(c_{1/2})) / (t_1 - t_0) = \ln 2 / T = 0.693 / T$$

Therefore,

For any given time difference (t), the original cpm (O) of a given sample's currently measured cpm (M) is calculated from:

$$O = e^{\ln(M) + (0.693/T).t}$$

2.4.2.4 CALCULATION OF BLOOD FLOW.

(i) METHOD OF TISSUE SAMPLE COUNTING.

It is important to procure similar geometry of the prepared samples because geometry (sample height, volume, density and composition) affects the radioactive counts obtained (Heymann *et al.* 1977). Glass and plastic containers attenuate the counts differently, so the counting vials must all be of the same type. The myocardium is cut up to facilitate packing of tissue into the vials and the tissue must occupy the same height in each vial. After the sample weights have been determined, the tissue can be counted in a wet or carbonized form. In the author's experiment, the technique of dissolving the myocardium in nitric acid (after packing the tissue into the counting vials) was used: The result of tissue degradation by nitric acid causes the release of spheres from tissue, and the spheres settle out at the bottom of the counting vial.

Samples are counted in a gamma-scintillation counter or multi-channel pulse-height analyzer using window-spectrometry, according to the types of isotopes used. The energy range which is selected for the counting of each isotope is also an important determinant of the final counts obtained: Different isotopes tend to have different energy spectrum peaks and this fact is exploited to obtain energy range windows with a high specificity for each isotope. Ideally, the energy spectrum that is selected for each isotope should extend from the centre of the pre-photoelectric-peak trough to as close to the background count as possible on the high energy side of the peak. The advantages of choosing a wide window for each isotope (higher counting efficiencies) are offset by the greater overlap (or lesser separation) of the different isotopes' energy spectra.

Samples are counted over the entire energy spectrum as well as in their respective window ranges simultaneously. All samples are counted on the same day, but a 2 or 3 day difference is, depending on the isotope used, usually insignificant. The duration of sample counting-time is dependent on the various activities of the isotopes used: The greater the activity of an isotope the better is the counting efficiency of the gamma-counter, and the less is the time that is needed to be spent on counting the tissue sample; in the author's experiment, a 10 minute count, repeated once (and the mean of the two counts taken) was used for each tissue sample.

(ii) ANALYSIS OF COUNTS.

Calculation of regional blood flow can be performed in two ways: Cardiac output can be measured by an independent means, in which case the individual organ flow derived from the proportion of microspheres found in that organ is related to either the total number of spheres injected or to the total number of spheres present in the whole animal (the latter method is obviously not practical in large animal studies).

The second technique requires the measurement of flow to one organ by an independent means; flow to any organ can then be calculated from the formula:-

$$Q = (Z \times M)/N$$

Where Q = Unknown organ flow in ml.min⁻¹

Z = Known organ flow in ml.min⁻¹

M = Number of microspheres in organ with unknown flow

N = Number of microspheres in organ with known flow

Currently, the most commonly employed method to obtain "Z" and "N" is that of knowing a surrogate organ's flow. This is acquired by exploiting the "reference sample technique" which involves the withdrawal of blood from an artery at a constant rate

using a (Harvard) withdrawal pump. The blood withdrawal is started 10 seconds prior to the injection of the spheres and is continued for usually 1 - 2 minutes. (In the author's experiment the blood was withdrawn from the aorta over an average period of 2 minutes).

After the blood withdrawal the syringe is weighed and the volume of blood that is withdrawn, and hence the reference blood flow rate, is calculated by using the formula:-

$$Z = ((W_{B2} - W_{B1})/D_B)(60/S)$$

Where;

**Z = The rate of the reference blood sample withdrawal, in
ml.min⁻¹**

**W_{B1} = The measured weight of the syringe prior to blood
withdrawal, in grams.**

**W_{B2} = The measured weight of the syringe after the blood
withdrawal, in grams.**

D_B = The known density of blood.

S = The time of withdrawal, in seconds.

The blood is then placed into counting vials with care being taken to flush the glass withdrawal syringe using distilled water to ensure that no spheres are left adhering to the syringe sides. A flow rate ("Z") of 8 - 12 ml.min⁻¹ is usually sufficient to ensure that the spheres do not adhere to the suction tubing during the withdrawal procedure. In large animals, more than one reference sample may be withdrawn simultaneously for purposes of comparing the spheres' mixing uniformity and to improve on the precision of the technique.

(iii) SPECTRAL DISTRIBUTION STRIPPING.

When using more than one isotope to determine regional myocardial blood flows the background count and the count due to other isotopes' energy emission must be

u ^ s T 1 0 1 4 b 3 s

percentage of counts found in the windows of the other isotopes which were used is noted. This is performed by counting either a pure nuclide sample or by making use of the counted reference withdrawal blood sample (Table 2.2, page 50);

Energy Range: (KeV)	0-450 (^{113}Sn)	460-690 (^{85}Sr)	700-2000 (^{46}Sc)
Percentage count of total energy spectrum occupying each window:	H		
	F	E	
	C	B	A

Table 2.2

For the nuclide with the highest energy range peak (Sc):

Let K1 = measured counts per minute (cpm) of the Sc tissue sample in the chosen Sc window.

Let L1 = measured cpm of the Sc reference blood sample, in the chosen Sc window.

and,

Let total measured tissue sample Sc activity over the entire energy spectrum = M1.

Let total measured reference blood sample Sc activity over the entire energy spectrum = N1.

Then,

$$M1 = K1/A \text{ (A is derived from Table 2.2 and expressed as a decimal).}$$

and

$$N1 = L1/A$$

Therefore,

$$Q1 = (Z1.M1.100)/(N1.W1)$$

Where

Q1 = Unknown tissue sample flow of Sc nuclide, in ml/min.100g

Z1 = Known reference sample flow rate, in ml.min⁻¹

W1 = Mass of tissue sample with unknown flow, in grams.

Similarly, for the nuclide with the second highest primary energy range peak (Sr):

$$M2 = (K2/E) - (B.M1)/E$$

$$N2 = L2/E$$

$$Q2 = (Z2.M2.100)/(N2.W2)$$

Similarly, for the nuclide with the lowest primary energy range peak (Sn):

$$M3 = (K3/H) - ((F.M2 + C.M1)/H)$$

$$N3 = L3/H$$

$$Q3 = (Z3.M3.100)/(N3.W3)$$

Thus the absolute counts of each isotope in their respective windows is determined, and the tissue sample flows are calculated using the above equations. Note that this calculation assumes that each isotope has little forward overlap into the energy ranges of the other isotopes which occupy higher energy spectra.

The use of computer software programs makes these calculations very much less time consuming, however such commercially available software is often expensive, and a simple Basic-Language program written by the author (compatible with DOS Q-Basic, GW-Basic and IBM Basic-A) suffices for the above-described calculations (Table 2.3; page 55).

(iv) VALIDITY OF THE RADIOACTIVE MICROSPHERE TECHNIQUE.

The distribution of microspheres within the bloodstream and organs correlates well with that found using dye-indicator and radioactive potassium-uptake techniques. Cardiac output measurements show good correlation with dye indicator techniques however the

author's experience is that, unless extremely meticulous measures are taken to ensure accurate activity-counts of the injected spheres and of the spheres remaining behind in the syringes and cannulas, large errors may be encountered in the estimation of cardiac output. Organ blood flows, derived using the microsphere technique, also correlate well with those obtained from electromagnetic flow probe measurements or by the direct collection of venous effluent (Sasaki and Wagner, 1971; Heymann *et al.* 1977). An attempt was made by Maxwell *et al.* 1987 to demonstrate that a component of coronary collateral flow could not be detected with microspheres; thus the regional distribution of blood flow using the microsphere method was compared with several other isotope-uptake techniques but the results showed that there was no biologically significant microsphere-insensitive component of collateral blood flow in dogs.

Table 2.3: Authors example of a simple computer program for the radioactive microsphere spectral distribution stripping technique.

```

list
20 REM MICROSPHERE CALCULATION
40 PRINT"PROGRAM MICROHART:MAX 3 MICROSPHERES"
45 PRINT"This program may only be copied with written permission of Dr Hartford."
50 PRINT
55 PRINT "Check printer is on line!!"
56 PRINT
60 PRINT"INPUT THE FOLLOWING GENERAL DATA"
80 PRINT
100 INPUT"ENTER l1;cpm blood Sc";L1
110 PRINT"l1=",L1
120 INPUT"ENTER l2;cpm blood Sr";L2
130 PRINT"l2=",L2
140 INPUT"ENTER l3;cpm blood Tn";L3
150 PRINT"l3=",L3
160 INPUT"ENTER z1;blood flow in ml/min Sc";Z1
170 PRINT"z1=",Z1
180 INPUT"ENTER z2;blood flow in ml/min Sr";Z2
190 PRINT"z2=",Z2
200 INPUT"ENTER z3;blood flow in ml/min Tn";Z3
220 INPUT"ENTER a;% energy of Sc occupying Sc range,as a decimal";A
230 PRINT"a=",A
240 INPUT"ENTER b;% of Sc occupying Sr range";B
250 PRINT"b=",B
260 INPUT"ENTER c;% of Sc occupying Tn range";C
270 PRINT"c=",C
280 INPUT"ENTER e;% energy of Str occupying Str range";E
290 PRINT"e=",E
300 INPUT"ENTER f;% of Str occupying Tn range";F
310 PRINT"f=",F
320 INPUT"ENTER h;% energy of Tn occupying Tn range";H
330 PRINT"h=",H
335 PRINT
340 PRINT"PREPARE TISSUE SAMPLE DATA"
350 PRINT
360 PRINT
370 INPUT"ENTER R;THE SAMPLE CODE/NO.";R$
380 PRINT"R=",R
390 INPUT"ENTER the mass of tissue in grams-w";W
400 PRINT"w=",W
410 INPUT"cpm tissue Sc,k1=";K1
420 PRINT"k1=",K1
430 INPUT"cpm tissue Str,k2=";K2
440 PRINT"k2=",K2
450 INPUT"cpm tissue Tn,k3=";K3
460 PRINT"k3=",K3
470 PRINT
480 PRINT
490 PRINT
500 PRINT"NOW CALCULATING"
510 M1=K1/A
520 PRINT"m1=",M1
530 N1=L1/A
540 PRINT"n1=",N1
550 Q1=(Z1*M1*100)/(N1*W)
560 M2=(K2/E)-((B*M1)/E)
570 PRINT"m2=",M2
580 N2=L2/E
590 PRINT"n2=",N2
600 Q2=(Z2*M2*100)/(N2*W)
610 M3=(K3/H)-(((F*M2)+(C*M1))/H)
620 PRINT"m3=",M3
625 N3=L3/H
630 PRINT"n3=",N3
640 Q3=(Z3*M3*100)/(N3*W)

```

```

650 PRINT
660 PRINT
670 PRINT"FOR SAMPLE NO.";R$
680 PRINT
690 PRINT"Sc TISSUE FLOW in ml/min,q1=";Q1
700 PRINT"Str TISSUE FLOW in ml/min,q2=";Q2
701 PRINT"Tn TISSUE FLOW in ml/min, q3=";Q3
718 PRINT
719 PRINT"check printer is on-line"
720 LPRINT"For tissue sample no.";R$
725 LPRINT " " " " ;"flow ml/min"
730 LPRINT "Tn/q3" USING "#####.f" ;Q3
740 LPRINT "Sc/q1" USING "#####.f" ;Q1
750 LPRINT "Sr/q2" USING "#####.f" ;Q2
760 PRINT
762 GOTO 370
770 PRINT
780 INPUT"TO RUN ANOTHER SAMPLE ON THIS SERIES DEPRESS KEY 1,otherwise press any
NUMBER";S
790 IF S=1,GOTO 340,ELSE GOTO 810
810 STOP

```

```

5 REM "BACKGROUND DEDUCTIONS"
10 INPUT"mean blank 210-450?/",A1
20 INPUT"mean blanks450-690/",A2
30 INPUT"mean blanks700-2000/",A3
40 INPUT"mean blanks open A/",A4
50 INPUT"mean blanks open B/",A5
60 INPUT"sample no.?/",Z
70 INPUT"cpm1 210/",B1
80 INPUT"cpm1 460/",C1
90 INPUT"cpm1 open1a/",D1
100 INPUT"2cpm 210/",B2
110 INPUT"2cpm 460/",C2
120 INPUT"cpm open2a/",D2
121 INPUT"3cpm 210\ ",B3
122 INPUT"3cpm 460\ ",C3
123 INPUT"open3a\ ",D3
130 INPUT"cpm1 700/",E1
140 INPUT"cpm open1b/",F1
150 INPUT"2cpm 700/",E2
160 INPUT"cpm open2b/",F2
161 INPUT"3cpm 700\ ",E3
162 INPUT"open3b\ ",F3
170 B4=((B1+B2+B3)/3)-A1
180 C4=((C1+C2+C3)/3)-A2
190 E4=((E1+E2+E3)/3)-A3
200 D4=((D1+D2+D3)/3)-A4
210 F4=((F1+F2+F3)/3)-A5
220 G4=(D4+F4)/2
230 PRINT"mean210=",B4
240 PRINT"mean460=",C4
250 PRINT"mean700=",E4
260 PRINT"mean open=",G4
270 LPRINT
280 LPRINT Z="";CINT(B4),CINT(C4),CINT(E4),CINT(G4)
290 GOTO 60
300 INPUT"press 1 to cont",W
310 IF W=1,GOTO 60,ELSE320
320 STOP
Ok

```


2.5 EXPERIMENTAL PRODUCTION OF MYOCARDIAL INFARCTION.

2.5.1 OBSTRUCTION OF CORONARY ARTERIES.

The most logical method of inducing a myocardial infarct in experimental animals is to interrupt the myocardial oxygen supply. This interference of coronary blood flow can be produced in several ways:

- A)** In the open or closed chest models: Options are; the closed chest model alone, the closed chest model following an open chest, or the open chest model alone. The major advantage of the open chest model is the technical ease of animal instrumentation. The main disadvantage of the open chest model is the many haemodynamic disturbances which may result, thereby influencing the interpretation of measured variables. Such haemodynamic disturbances may arise from;
- (i) A reduced cardiac venous return and hence a reduced right and left sided cardiac output. The venous return is reduced by the absence of negative intra-pleural and intrathoracic pressures (secondary to mandatory open-chest artificial ventilation) and hence a decreased gradient of blood flow between the peripheral veins and the right atrium.

- (ii) The necessary supine position of the animal (the normal erect positional effects of gravity on venous return are altered)
- (iii) The surgical manipulation involved leads to neurohumoral responses such as the release of inotropic and chronotropic catecholamines (particularly when performing the sternotomy or thoracotomy).
- (iv) The opening of the pericardial sac (a change in the viscous properties of the fluid surrounding the heart).
- (v) Thermal disturbances, resulting from exposure of the thoracic cavity to the atmosphere.
- (vi) Regional myocardial blood flow disturbances; thoracotomy is known to affect the endocardial:epicardial blood flow ratio in dogs (Bishop *et al.* 1984).

Disadvantages of the closed chest model are;

- (i) The difficulty with which interruption of coronary blood flow is achieved.
- (ii) The necessary peripheral introduction of the vascular catheters, and their associated haemodynamic effects, to record haemodynamic variables.

B) Acute versus chronic interruption of coronary blood flow: Acute ligation of a coronary artery usually ensures myocardial infarction, where a chronic interruption may give varying degrees of stenosis in the experimental subjects. The major advantages of the chronic arterial occlusion method are;

- (i) The duration of gradual occlusion resembles more closely that of human

ischaemic heart disease and hence the experimentally produced pathophysiology is likely to be more authentic.

- (ii) There is a lower incidence of ventricular fibrillation and other acute ischaemia related cardiac arrhythmias.

2.5.2 CORONARY ARTERY LIGATION.

The simplest and most widely employed technique of coronary blood flow interruption is by ligation of the coronary arteries. This procedure is usually performed with the chest open, although a system of placing the ligature around the coronary artery and extending the loop through the chest wall in a snare has been used in a closed chest model to allow progressive partial arterial occlusion. In most cases ligation is performed with silk suture as a one stage procedure but it is feasible to perform a two-stage procedure, in which the first ligation is only partial, to reduce the incidence of ventricular fibrillation post-occlusion. It is also necessary in certain animal models to ligate more than one arterial site to produce an infarct because of the existence of a good coronary collateral circulation. In the dog, both the left anterior descending (LAD) and the circumflex arteries have been ligated for this reason (Schaper, 1978).

In the baboon acute single vessel ligation produces a well circumscribed infarct, and this technique has often therefore been utilised for myocardial infarct studies in these primates (Lubbe *et al.* 1974; Barker *et al.* 1980; Geary *et al.* 1981; Flameng *et al.* 1986; Flameng *et al.* 1990). Vessel ligation in the baboon requires careful dissection

of the coronary artery and the threading of the silk suture beneath the artery prior to ligation. Usually the left anterior descending coronary artery (Lubbe *et al.* 1974), or one of its large diagonal branches (Geary *et al.* 1981; Ruf *et al.* 1986), is chosen as the site of ligation .

Different researchers have tied the baboon LAD at various sites;- the junction of the distal one third and proximal two thirds of its length, the mid-LAD and just distal to the first diagonal branch have all been used. In the author's experience, ligation of the distal LAD proves to be technically difficult, particularly in smaller female baboons, and may not produce a large enough infarct: On the other hand, ligation of the LAD at a site excessively proximal was associated with a very high incidence of ventricular fibrillation. Care is taken not to ensnare the adjacent coronary vein in the arterial ligation since this may lead to haemodynamic interferences and atypical infarct pathology. Acute coronary artery ligation in the baboon results in a well circumscribed infarct, with a clearly defined edge (Opie *et al.* 1983).

2.5.3 ADDITIONAL METHODS OF PRODUCING CORONARY OCCLUSION.

Certain other methods which have been successfully employed (but not in baboons as yet) permit closed chest or gradual restriction of coronary arteries (Tillmanns *et al.* 1975). Examples are: Chronically implanted balloon cuffs placed around the artery and linked to a micro-flow infusion pump; releasable clips; screw forceps and screw clamps for gradual occlusion; intracoronary balloon catheters introduced under fluoroscopic

guidance; techniques which involve the production of coronary thrombosis and embolisation (see below), through the use of injected large plastic spheres or metals and springs. Experimentally produced atherosclerosis has also been attempted in dogs, rats, rabbits and baboons, utilising high cholesterol diets.

Ameroid constrictors can be used to produce gradual coronary occlusion. These devices consist of a hygroscopic material (a casein derivative) encased in a rigid silver or stainless steel capsule. Once placed around the coronary artery they absorb water and constrict the arterial lumen over a gradual period of time (which time period is adjustable).

Hydraulic vessel occluders are reported to have been used to study myocardial infarction in awake baboons (Croizatier *et al.* 1978; Vatner *et al.* 1987).

2.5.4 METHODS OF EXPERIMENTAL ARTERIAL THROMBOSIS.

To simulate the conditions of coronary artery disease found in humans the production of a thrombus within an animal coronary artery is imperative. The presence of associated thrombi with human myocardial infarction following coronary artery disease is very frequent (80 - 90 %), and the superimposition of an acute thrombus on a pre-existing coronary vascular plaque (causing a critical coronary stenosis) is thought to be the precipitating event for human myocardial infarction. As previously mentioned (section 2.3.3), thrombi contain activated platelets which release vaso-active substances

(serotonin). These agents can, in turn, influence coronary blood flow and the development of coronary collateral circulations.

Techniques to accomplish thrombogenesis in coronary arteries have ranged from the introduction of thrombogenic foreign bodies such as metal wires (aluminium, magnesium, copper and lead) to the placement of electrical and thermal intraluminal currents across the vessel walls (Tillmans *et al.* 1975).

As early as 1961, Imhoff recognised the importance of vascular thrombi in the pathogenesis of sequelae to vascular occlusion. He pioneered the technique of producing an in vivo thrombus in cat aortas by introducing thrombin into an arterial sac, created by two aortic ligations; the second ligation was tied at a site approximately 1 cm proximal to the first. Schaub *et al.* (1976) were the first researchers to follow up on the work of Imhoff by testing the effects of producing an aortic thrombus in cats on the hindlimb collateral circulation. The intravascular thrombus created in their experiments was induced using thromboplastin in place of thrombin, but the ligation and arterial sac creation techniques used were the same as those of Imhoff. Schaub subsequently made further use of this arterial sac technique to look at the effects of serotonin and thrombus formation on the feline collateral circulation (Schaub *et al.* 1977). More recently it has been shown that low-dose thrombin activates platelets and causes the release of serotonin from in vitro human platelets (Marche *et al.* 1985).

The method of intravascular thrombus formation using platelet aggregation agents has

been extended to the coronary circulation (Flameng *et al.* 1985): Flameng *et al.* produced an occlusive thrombus by thrombin-induced coagulation in the LAD (left anterior descending) artery of open-chest baboons, in a study of myocardial infarction; after dissection of the LAD a 1 cm length of artery was ligated proximally and distally, and approximately 10 u of thrombin was injected into the sac so created. The LAD segment was also repeatedly crushed with a small forceps. This resulted in a localised thrombus occluding the LAD. The technique was later used again for infarct-size studies in baboons. (Flameng *et al.* 1990).

2.6 METHODS OF DETERMINING INFARCT SIZE.

The demonstration and quantification of an infarct's size can be performed indirectly (techniques usually used in living animals) or directly (post-mortem techniques). Indirect techniques include electrocardiographic ST-segment maps, measurements of plasma cardiac enzymes such as creatine kinase (De Scheerder *et al.* 1987), radionuclide-uptake ventriculography and echocardiography. The combination of serum creatine kinase-MB isoenzyme (CK-MB) measurements and ST-segment mapping has also often been used as an index of infarct size (Rogers *et al.* 1982; Burke *et al.* 1983; Rogers *et al.* 1983).

Direct infarct size measurements are more definitive, being based on macroscopic evaluation or microscopic histology: At the macroscopic level, the interface between

viable and infarcted myocardium is frequently not sharply defined, particularly if the infarct is of recent origin (2 - 3 days). Therefore, the dyes para-nitro blue tetrazolium (pNBT) and triphenyl tetrazolium chloride (TTC), which are coloured blue or red respectively when reduced by viable cardiac tissue's dehydrogenases (such as NADH), are used to define the infarct's boundaries more clearly. TTC, which is less expensive, can be applied directly to post-mortem slices after the ventricles are sectioned in 1 - 2 cm intervals from apex to base. TTC can also be perfused post-mortem or in vivo into the myocardium via the coronary arteries (Reimer and Jennings, 1984).

When macroscopically viewed, myocardial infarct size can be quantified following ventricular sectioning by planimetry of the ventricle slices and the subsequent calculation of area, volume or mass. Although the TTC technique has been used in baboons (Flameng *et al.* 1986), myocardial infarction in the baboon, unlike that of the dog, tends to show a clearly defined infarct edge (Lubbe *et al.* 1974; Crozatier *et al.* 1978) and therefore the above staining techniques are unnecessary to delineate the baboon's infarct border.

When assessing the impact of therapeutic interventions on myocardial infarct size, it is useful to determine the perfusion bed of an occluded coronary artery, since this defines the myocardial area at risk for infarction. If experiments are performed to determine the effects of drugs on infarct size, the ratio of infarct size to area at risk is more useful than the quantification of infarct size alone. The microscopic techniques employed to delineate the perfusion bed of a coronary artery have been used in baboons

(Geary *et al.* 1981; Geary *et al.* 1982; Geary *et al.* 1983; Flameng *et al.* 1985; Flameng *et al.* 1986). One of these methods involves the placement of cannulas in the LAD proximal and distal to the site of occlusion, in the proximal left circumflex artery and in the proximal right coronary artery. Following cannula placement the arteries are injected simultaneously, and at equal flow rates, with coloured silicone microvascular dyes. Serial cross-sections of the ventricles are then viewed microscopically allowing the anatomical areas of perfusion and infarction to be determined by planimetry. The disadvantage of the microscopic technique of infarct-size measurement is that it precludes the simultaneous use of radio-active tracer microspheres to determine regional myocardial blood flows.

CHAPTER 3: METHODS.

3.1 EXPERIMENTAL MODEL.

A total of twenty seven baboons underwent surgery, of which twelve were excluded from the final analysis due to mortality or morbidity. Permission to perform the experiments was granted by the University of the Witwatersrand Animal Ethics Screening Committee (AEC No.'s 89/176/5 and 90/15/5). Fifteen female baboons (*Papio ursinus*) weighing 19.3 ± 1.2 kg were anaesthetized and subjected to coronary artery ligation. Nine of the animals received ketanserin tartrate (Janssen Pharmaceutica) 1 mg/kg i.m. and six received the vehicle i.m. (injectable water) 24 hours pre-operatively and daily at 0800 hours thereafter. Haemodynamic variables were recorded and radioactive microspheres were injected in the open chest model before and after coronary artery ligation. All the instrumentation was then removed and the animals were allowed to recover with buprenorphine i.m. (a synthetic opiate-receptor agonist analgesic) 0.03 mg/kg.day. The haemodynamic variables were recorded again at one week post-infarct, after anaesthesia and re-instrumentation (Figure 3.1; page 72).

3.2 PROCEDURE.

The animals were immobilised using ketamine 10 mg/kg intramuscularly. A 20-gauge Jelco cannula, linked to an infusion set with a distal Y-injection site, was introduced into a dorsal vein of the left upper-limb forearm. A slow infusion of 0.9% Na⁺Cl⁻ in

water (Normal Saline, Sabax) was administered using a Sabax Buretrol Paediatric micro-dropper set. Each experimental subject was repeatedly cleansed with Hibitane-in-water solution and the chest shaved of any hair, before being transported to a sterile operating theatre where the animal was moved to a horizontal operating table.

A rectal thermocouple was inserted and an electrical heating pad used to maintain core body temperature at 37° Celsius. The subject's anaesthesia was then induced by means of an anaesthetic mask applied over the nasal and oral cavities (a mask was manufactured by the author to fit the baboons facial anatomy, thereby creating an acceptable seal). The induction agents used were a combination of the immobilising dose of ketamine and an inhalational volatile agent, halothane. Once Stage III of surgical anaesthesia was obtained (as judged by pupillary diameter, pupillary reflexes, corneal reflexes, lacrimation and muscle tone), the baboon was pre-oxygenated with 100% inspired oxygen. The anaesthetic was delivered through a Boyle's apparatus using a Magill anaesthetic circuit which incorporated a one-way expiratory valve near the baboon's mask. The oxygen flow rate was regulated with a calibrated rotameter (Coxeter King, England) and the concentration of halothane varied by means of a pre-calibrated Fluotec Mark II vaporiser (Cyprane Ltd. Keighley, England).

Following induction the animal was endotracheally intubated after tracheal suction, under laryngoscopic guidance. Depolarising and non-depolarising nicotinic-receptor neuro-muscular junction blockers for muscle relaxation were not used in this experiment. Maintenance of anaesthesia was effected with halothane at 0.5% to 1.5%

inspired concentrations, and the baboon was ventilated on medical air and oxygen with an open circuit dual-phase respirator (Harvard and Bodine Electric Co., U.S.A.). The gas flow rates, tidal volume and ventilation rate were adjusted to support physiological acid-base conditions, according to frequent arterial blood gas measurements (Corning Analyzer; Medfield, Massachusetts). Vital life-support systems were connected to electrical outlets with back-up generators in the event of a power failure. In view of the high incidence of ventricular fibrillation following myocardial ischaemia in captivated baboons (Opie *et al.* 1983), a lignocaine infusion was administered i.v. at 10 $\mu\text{g/kg.min}$ as a prophylactic anti-arrythmic agent for the duration of the surgical procedures. This prophylactic procedure has been previously used during baboon experimental myocardial infarction (Geary *et al.* 1983).

3.3 INSTRUMENTATION.

Electrocardiography standard vertical leads I, II and III were connected to the animal by subcutaneous needles, to detect and monitor arrhythmias and ischaemia. Observing theatre sterility precautions, a midline sternotomy was performed and the heart exposed by suspension in a pericardial cradle. In five of the animals a 5-French catheter was introduced into the aorta via the right common carotid artery, which was ligated (Figure 3.2; page 74). Due to embolic phenomena (anti-coagulation was inappropriate for this experiment) an 18-gauge teflon-coated cannula was introduced directly into the ascending aortic arch in the remaining animals, with sham right common carotid arterial ligation. Six of the ketanserin group and four of the control group received the latter

procedure. A 20-gauge cannula was introduced into the left atrium in all the baboons.

High-frequency conduction, saline-filled and heparin-free catheter lines were connected to magnetic-coiled dome pressure transducers (Statham-Gould P50; Oxnard, California), for the measurement of mean aortic (MAP) and mean left atrial (LAP) pressures and heart rate (HR). The transducers were located at mid-heart level. Aortic flow was measured using an electromagnetic probe (Narcomatic series-500; Houston, Texas) placed around the ascending aorta. The flow probe sizes were chosen, according to the size of the aorta, to fit tightly around the external aortic wall circumference. An electromagnetic flux-transparent jelly was applied to the probes to increase transmission of the electromagnetic field. Haemodynamic measurements were recorded in digital and analogue form on a multichannel recorder (Hellige Servomed; Freiburg, Germany) after the halothane concentration was adjusted to obtain the least possible cardiovascular depressive effect yet still allowing for adequate anaesthesia.

The left anterior descending coronary artery was dissected and ligated at approximately its midpoint, below the first diagonal branch. Care was taken to ensure that the adjacent coronary vein was not included in the ligation. The artery was then clamped 5 millimetres proximal to the ligation and 20u bovine thrombin (Thrombostat, Parke-Davis) in 0.9% saline injected into the arterial sac created to produce a thrombus (Figure 3.3; page 76). The clamp was removed after 20 min (technique modified from previously described procedures: Schaub *et al.* 1976; Schaub *et al.* 1977). The ability of the bovine thrombin to coagulate baboon whole blood was confirmed in vitro prior

to the experimental procedure.

During the surgical procedure, blood loss, as judged by the number of blood-saturated surgical swabs and the volume of blood in the suction bottle, was replaced with intravenous fluids. Blood loss was minimised by the use of an electro-cautery and electro-cutting apparatus (Birtcher 755, California, U.S.A.). Following myocardial infarction and the second set of haemodynamic measurements the catheters were removed, the pericardium re-opposed and the chest closed in layers using Vicryl suture material. Sternal closure was secured with synthetic Vetafil. A triple antibiotic regime consisting of ampicillin (broad spectrum penicillin), gentamycin (an aminoglycoside for gram negative bacteria cover) and Flagyl (metronidazole; anaerobic bacteria cover) was used i.v. intra-operatively and i.m. post-operatively to minimise thoracic cavity infection.

After these initial procedures the anaesthetic was terminated and the animal was returned to the cage once the recommencement of spontaneous respiration had been ensured. Seven days after myocardial infarction the animal was again subjected to anaesthesia, sternotomy and instrumentation as described above. A third set of haemodynamic measurements was made and the baboon was euthenased by using an excess i.v. bolus injection of barbiturate (to effect central nervous system depression) and excess potassium chloride (to arrest the heart in flaccidity).

3.4 REGIONAL MYOCARDIAL BLOOD FLOW.

Fifteen μm diameter Nen-Trac ^{113}Sn , ^{85}Sr and ^{46}Sc radioactive microspheres suspended in 0.9 % saline with 0.01 % Tween 80 added were injected over 10 s into the left atrium immediately following the haemodynamic recordings, after agitation and sonication, according to previously described techniques (Buckberg *et al.* 1971; Heymann *et al.* 1977; Plus see Literature Review section 2.4.2). The microspheres were flushed into the atrium with saline and a simultaneous reference blood sample was withdrawn from the indwelling aortic cannula at $10 \text{ ml} \cdot \text{min}^{-1}$ for 2 minutes.

Post-mortem the heart was immediately excised and washed in saline. The ventricles were trimmed of all fat, viewed and sectioned from apex to base at 1 cm intervals (Figure 3.4; page 78). The full-thickness ventricle wall containing the infarct, which was clearly defined, was excised with a 1-2 mm edge of macroscopically non-infarcted myocardium to include the border zone. These infarct-ischemic segments, along with the remaining normal myocardial tissue segments, were weighed and dissolved in nitric acid for gamma-ray scintillation spectrometry (Packard 500C; Illinois, USA). Total infarct-ischemic myocardium and normal myocardium blood flows were calculated using a spectral distribution stripping technique (Heymann *et al.* 1977; Plus see Literature Review section 2.4.2). After the removal of tissues to be counted, the baboon was returned to the Central Animal Service for concreting and safe disposal. All the researchers involved were registered radiation workers, and subject to radiation dosimetry monitoring.

Figure 3.1: Sequential steps of the study design. HM = haemodynamic measurements; RM = radioactive microsphere injection.

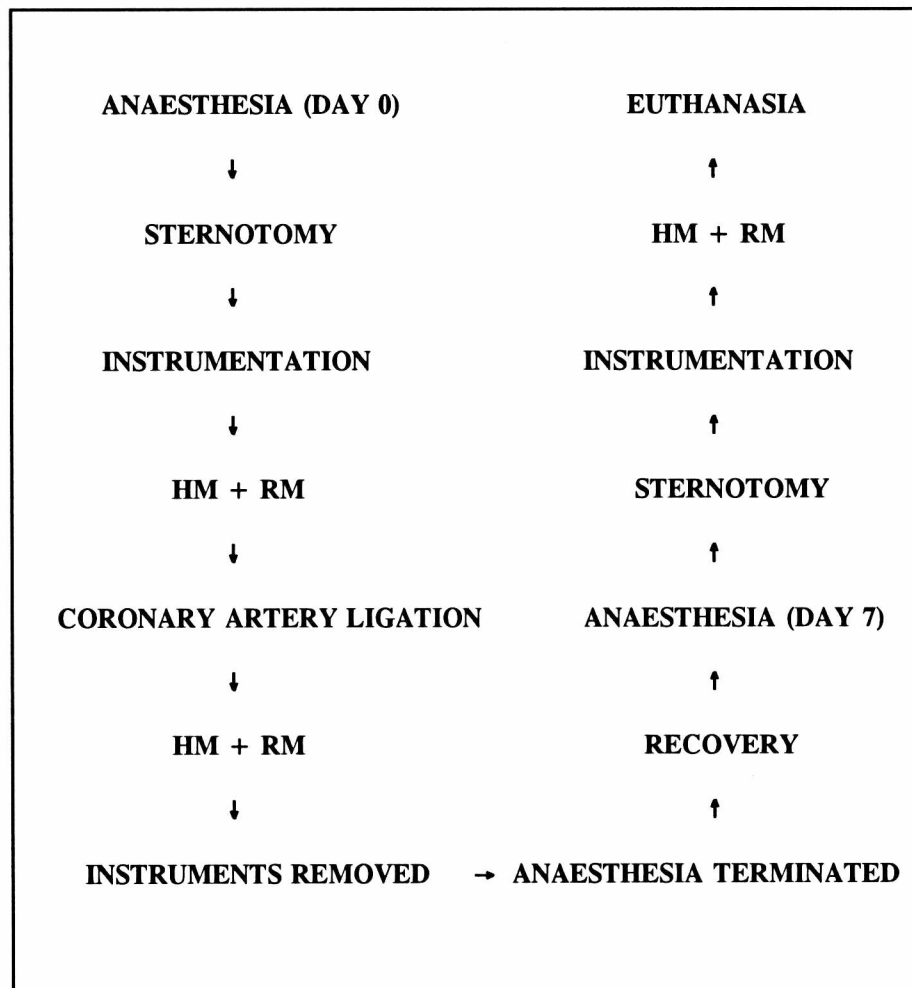
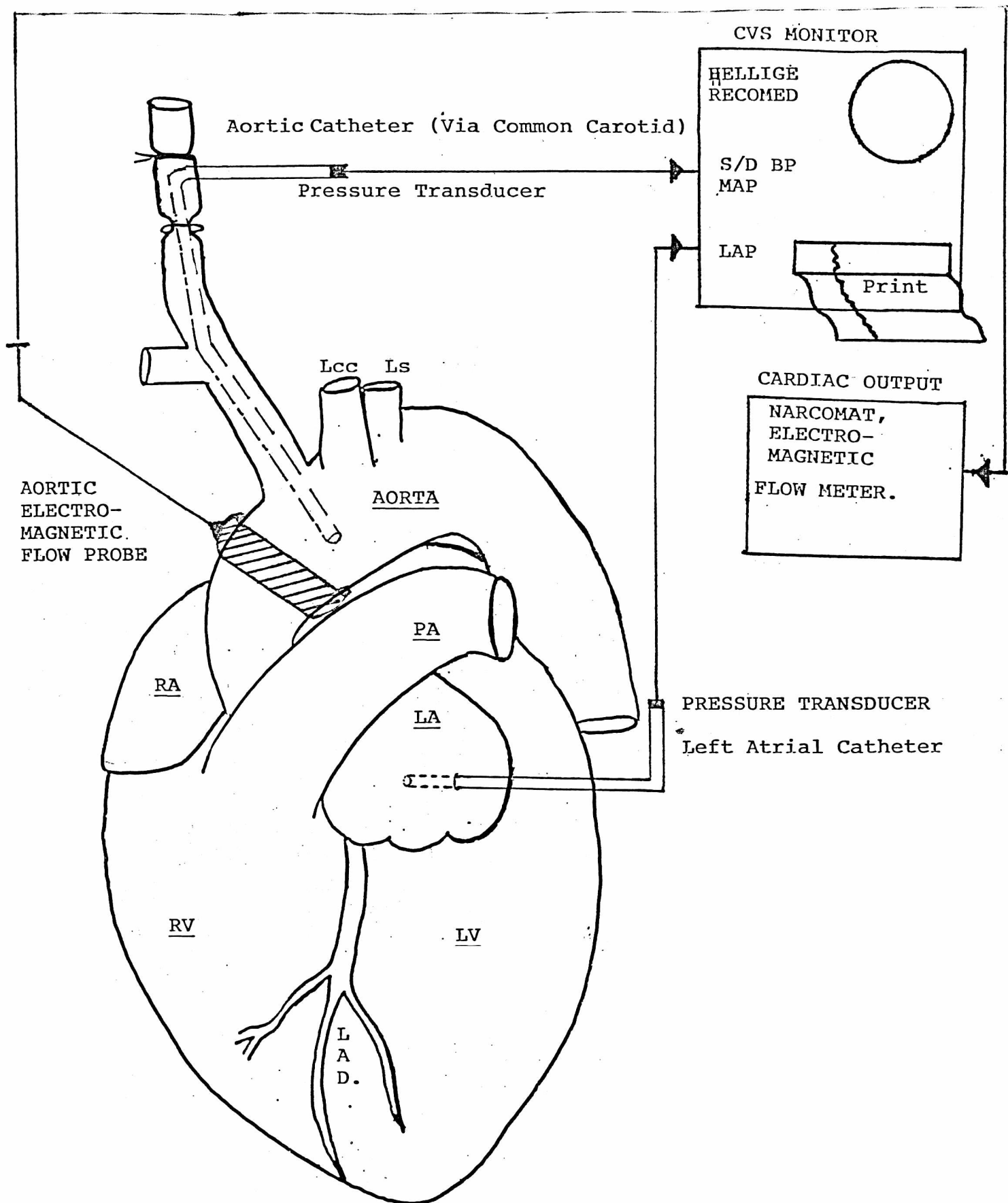


Figure 3.2: Diagram depicting the placement of the aortic and left atrial cannulas and the electromagnetic flow probe.

The aortic cannula was later introduced directly through the aortic wall into the aortic arch.



OPEN CHEST MODEL.

Figure 3.3: Schematic illustration of coronary artery ligation and thrombus induction.

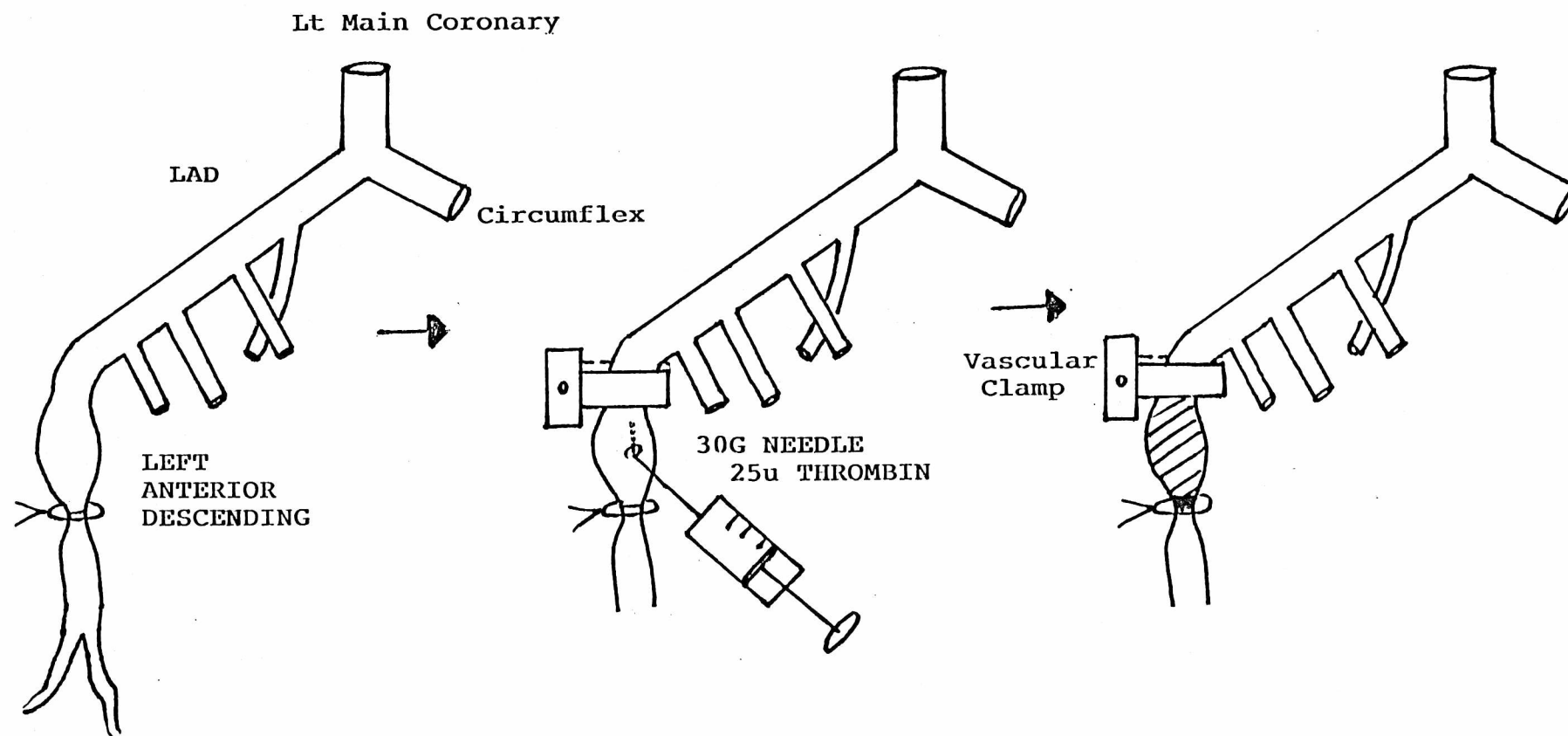
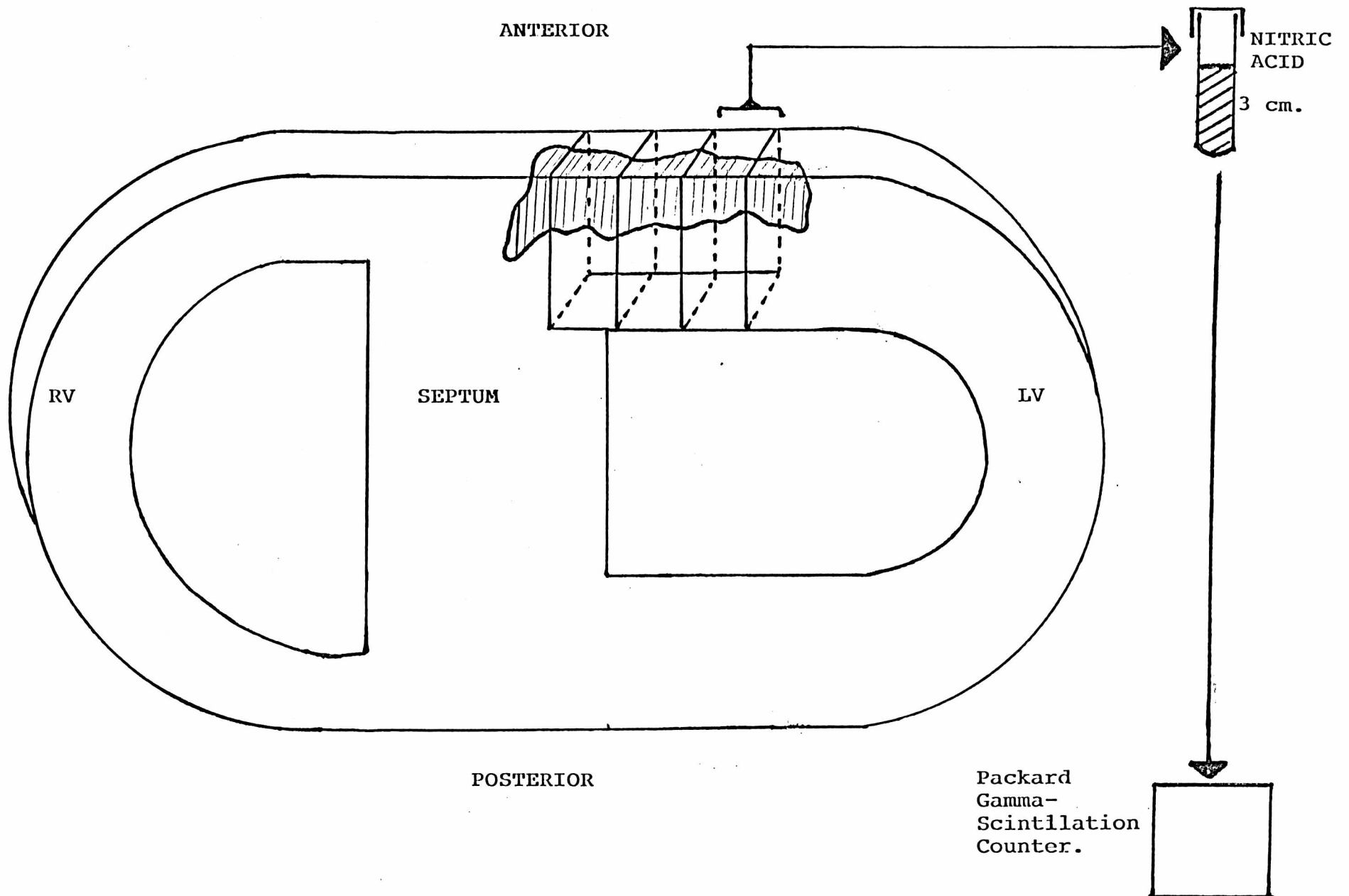


Figure 3.4: Schematic illustration of myocardial sectioning for tissue radioactivity analysis. The shaded areas represent the infarct zone, around which a 1 - 2 mm edge of normal tissue was included in each sample.



3.5 CALIBRATION AND VALIDATION.

Prior to blood pressure measurements the recording system was calibrated as follows: An internal electronic linearity millivoltage check was first performed on the digital screen and the analogue recorder displays. The pressure transducers were then zeroed relative to atmosphere and a manual calibration performed against either a mercury manometer (Baumanometer) for the aortic pressure channel, or a pure-water manometer for the left atrial pressure transducer. The water manometer was manufactured by the author to increase the accuracy of pressure calibration over the range 0 - 15 mm Hg: a pressure of 1 mm Hg is equal to 13.61 mm H₂O. The electromagnetic flow probe was zeroed against motionless fluid and thereafter an internal electronic calibration performed, before application of the probe to the ascending aorta.

Just prior to the injection of the microspheres a drop of the vortexed and sonicated sphere suspension was placed on a slide and examined with low-power light microscopy to ensure the absence of significant microsphere aggregation. The syringes and tubing, through which the microspheres had been flushed into the atrium, were also divided into smaller pieces after the surgery and their radio-activity checked to ensure that no significant amount of microspheres remained behind in these items. In addition, the left and right kidney radioactivity was compared to verify adequate mixing of the microspheres within the bloodstream.

Coronary thrombus formation was confirmed histologically: Post-mortem the LAD coronary artery was removed and fixed in 10% formaldehyde. The specimens were then impregnated in a paraffin wax and sectioned manually with a microtome at 0.5 μm intervals. The specimens were slide-mounted and stained with Hematoxylin and Eosin. The slides were examined microscopically for the presence of intracoronary thrombus formation, lamination and organisation. The presence of the macroscopically obvious myocardial infarction was also verified histologically.

3.6 CALCULATIONS.

The method of regional blood flow calculation is discussed in the Literature Review (section 2.4.2.4.). Pulse pressure (PP) is obtained from the difference of systolic pressure (SP) and diastolic blood pressure (DP). The mean arterial pressure (MAP) may be obtained from the formula;

$$\text{MAP} = 1/3(\text{PP}) + \text{DP} = 1/3(\text{SP}-\text{DP}) + \text{DP} = 1/3\text{SP} + 2/3\text{DP}.$$

This formula assumes that diastole occupies 2/3 of the cardiac cycle time, which is untrue for states of tachycardia. However, an electronically derived mean is independent of heart rate and therefore in this study the MAP is taken as the electronic mean of the aortic pressure cycle. Heart rate (HR) is taken as the number of systoles per unit time. Cardiac output is taken as the electronic mean over 10s to allow for variations of venous return with respiratory movements (although this variation is not marked in the

open-chest animal on positive pressure ventilation). Stroke volume (SV) is calculated from the division of CO by HR. Systemic vascular resistance is derived from the principle inherent in Ohm's law;

$$I = V/R$$

Where I = The current, in amperes.

V = The potential difference, in volts.

R = The resistance of the conductor to flow, in Ohms.

This principle can be applied to any point of the biological cardiovascular system:

$$F = \Delta P/R$$

Where F = Flow of blood.

ΔP = The gradient of blood pressure in the section of the closed circuit.

R = The resistance of the blood vessels to flow.

Therefore,

$$CO = (MAP - RAP)/SVR$$

Where CO = Cardiac output in $\ell \cdot \text{min}^{-1}$

MAP = Mean arterial pressure in mm Hg.

RAP = Right atrial pressure in mm Hg (assumed to be zero in the absence of right ventricular failure).

SVR = The systemic vascular resistance in mm Hg.min/ ℓ

3.7 STATISTICS.

All data are given as means $\pm$ SEM. The Kruskal-Wallis method and Wilcoxon matched pairs signed-ranks test for intragroup and the Mann-Whitney test for intergroup comparisons were used (Zar, 1974). A two-tailed probability of < 0.05 was taken as statistically significant.

CHAPTER 4: RESULTS.

4.1 BLOOD CHEMISTRY.

The absorption of ketanserin following i.m. injection in baboons was confirmed by high pressure liquid chromatographic assay of serum levels (HPLC method, Janssen Pharmaceutica, Belgium). The serum levels were 1562 ± 395 ng/ml at 10 min after administration in 7 animals and 69 ± 9 ng/ml at 2 h post-administration in 3 animals.

Table 4.1 (page 84) shows pre-experimental, in-captivity electrolyte concentrations and haematology indices of the animals used for experimentation. The values were similar between the control ($n = 6$) and ketanserin ($n = 9$) groups and are in agreement with previously described values for *Papio ursinus* baboons (Steyn *et al.* 1975).

4.2 HISTOLOGY.

Coronary thrombus formation and myocardial infarction were successfully produced using the methods described. Figure 4.1 (page 86) illustrates the typical laminated thrombi which results from coronary artery ligation and the injection of thrombin. The thrombi showed organisation and, in some cases, early recanalisation. Figure 4.2 (page 88) illustrates sections of the left ventricular organising infarcts one week after coronary artery ligation.

TABLE 4.1**BLOOD CHEMISTRY VALUES OF PAPIO URSINUS BABOONS IN CAPTIVITY.**

BLOOD TEST (value $\pm$ SD)	CONTROL	KETANSERIN	COMBINED
Sodium (mmol/ ℓ)	144.7 $\pm$ 1.4	144.3 $\pm$ 2.4	144.5 $\pm$ 2.0
Potassium (mmol/ ℓ)	3.9 $\pm$ 0.3	4.0 $\pm$ 0.8	3.9 $\pm$ 0.7
Haemoglobin (g/d ℓ)	13.9 $\pm$ 1.2	14.9 $\pm$ 1.3	14.5 $\pm$ 1.3
Red cell count (x 10 ¹² / ℓ)	5.7 $\pm$ 0.5	5.9 $\pm$ 0.6	5.8 $\pm$ 0.6
Haematocrit (ratio)	0.431 $\pm$ 0.032	0.460 $\pm$ 0.035	0.446 $\pm$ 0.036
Mean cell volume (f ℓ)	75.8 $\pm$ 3.5	78.2 $\pm$ 3.7	77.1 $\pm$ 3.7
Mean cell haemoglobin (pg)	24.5 $\pm$ 1.2	25.4 $\pm$ 1.0	25.0 $\pm$ 1.1
Mean cell haemoglobin concentration (g/d ℓ)	32.3 $\pm$ 1.3	32.5 $\pm$ 1.2	32.4 $\pm$ 1.2

FIGURE 4.1:

Sections of the baboons' coronary arteries proximal to the site of ligation, viewed and photographed under the light microscope.

The top plate shows a cross section of the LAD artery, stained with Hematoxylin and Eosin, one week following its ligation. A large thrombus is situated within the lumen and is adherent to the intimal surface of the blood vessel wall at the inferior aspect of the lumen (bottom end of the photograph). The thrombus contains fibrin layers (pale areas at top right of the lumen) enmeshing platelets. Trapped in the fibrin-platelet mass are scattered red blood cells as well as white blood cells, most of which are eosinophils. There is invasion by fibroblasts and capillaries on the inferior intimal surface, indicative of early organisation and recanalisation of the thrombus. The lumen is surrounded by a well defined arterial muscular media.

The middle and bottom plates demonstrate a different LAD artery at low and high power fields, one week following ligation of the artery. The arterial lumen is entirely obliterated by thrombus. Specific Giemsa staining techniques highlight the platelet and fibrin layers indicative of an ante-mortem thrombus.

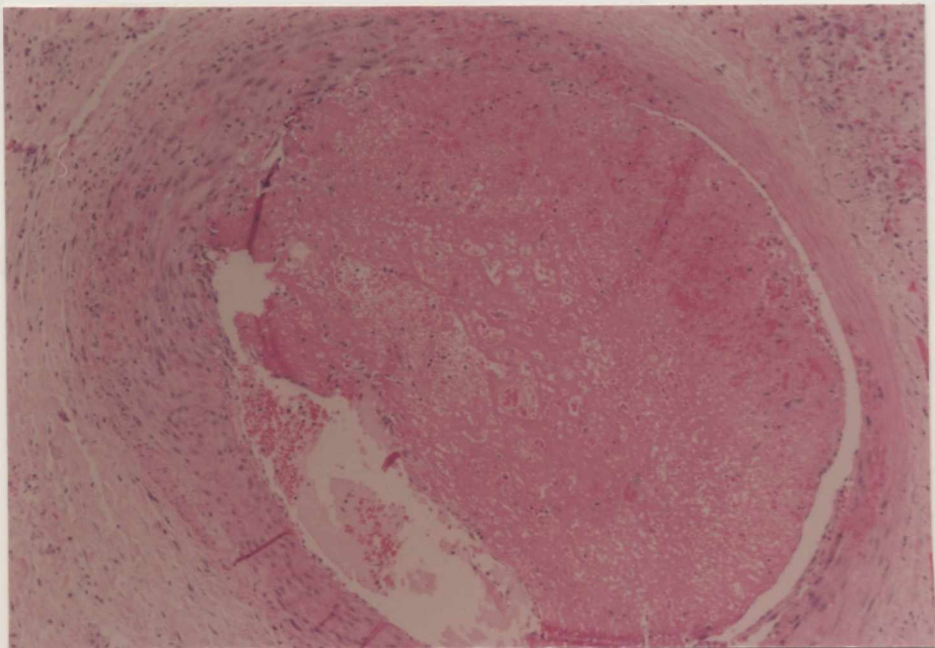
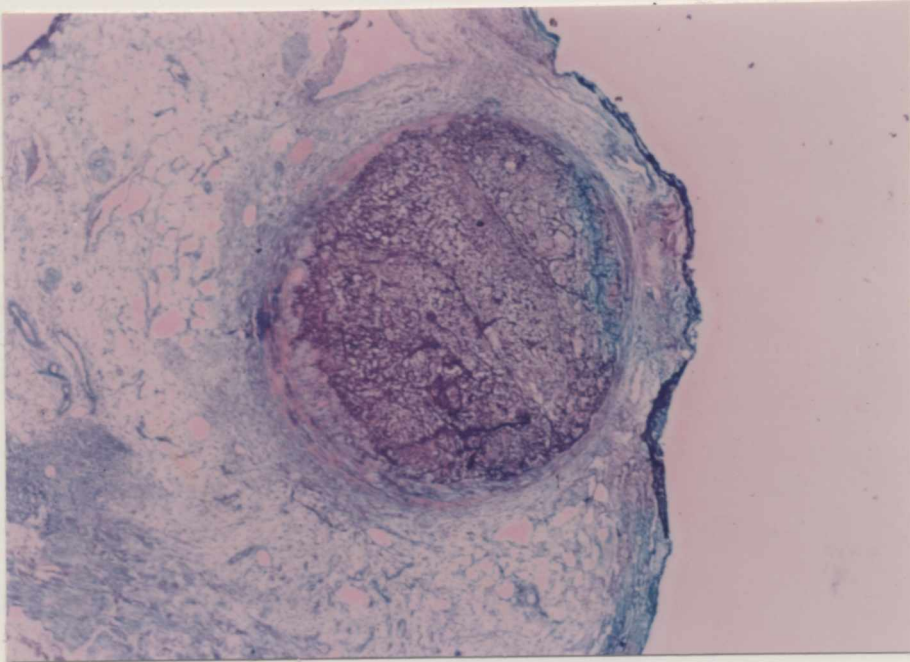
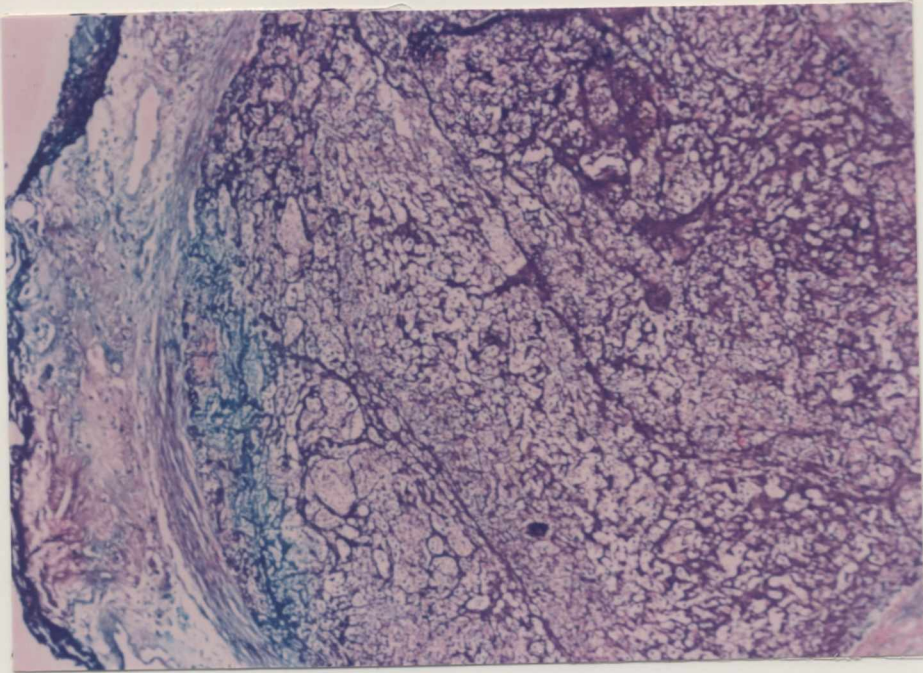


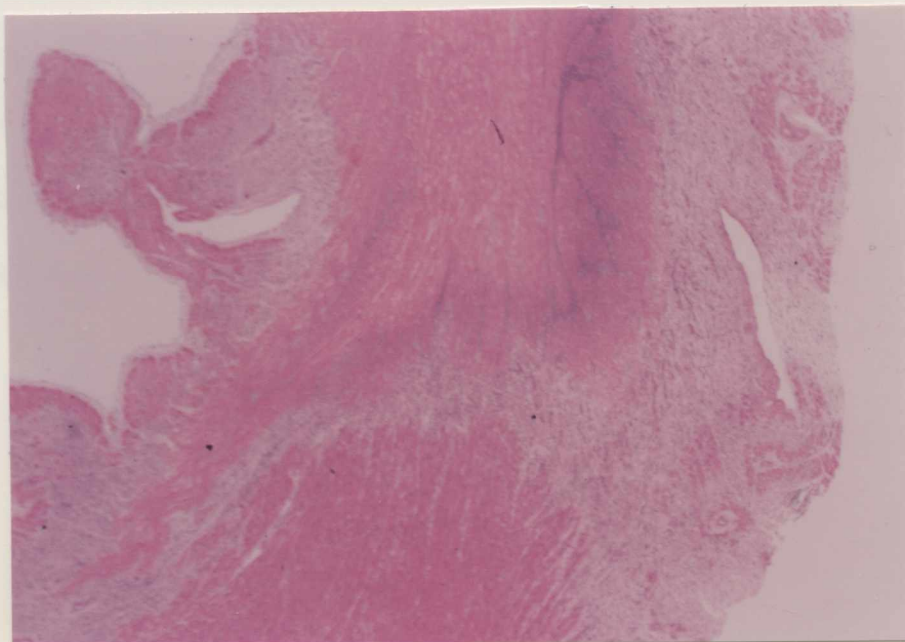
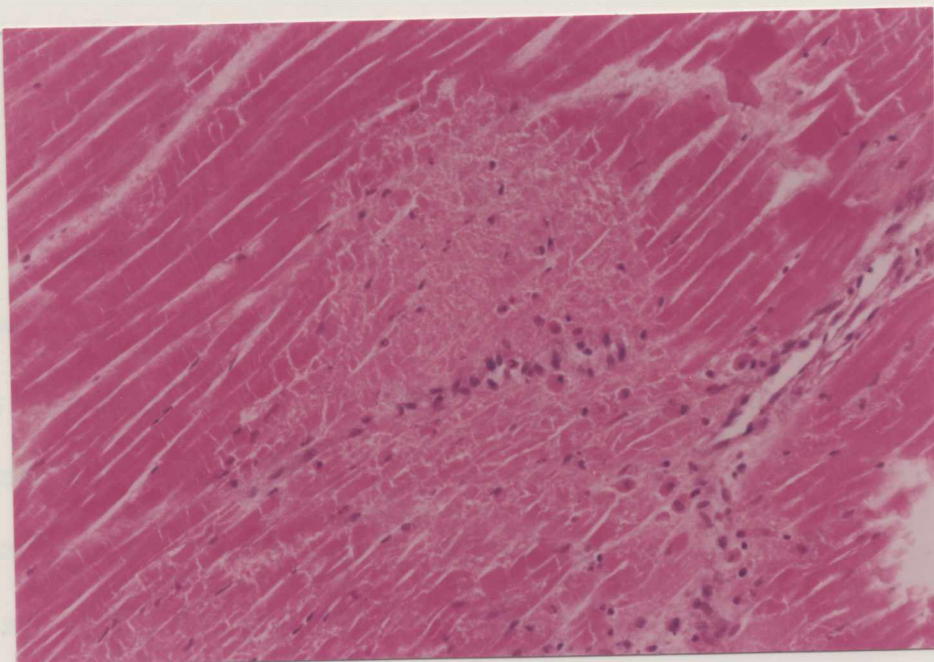
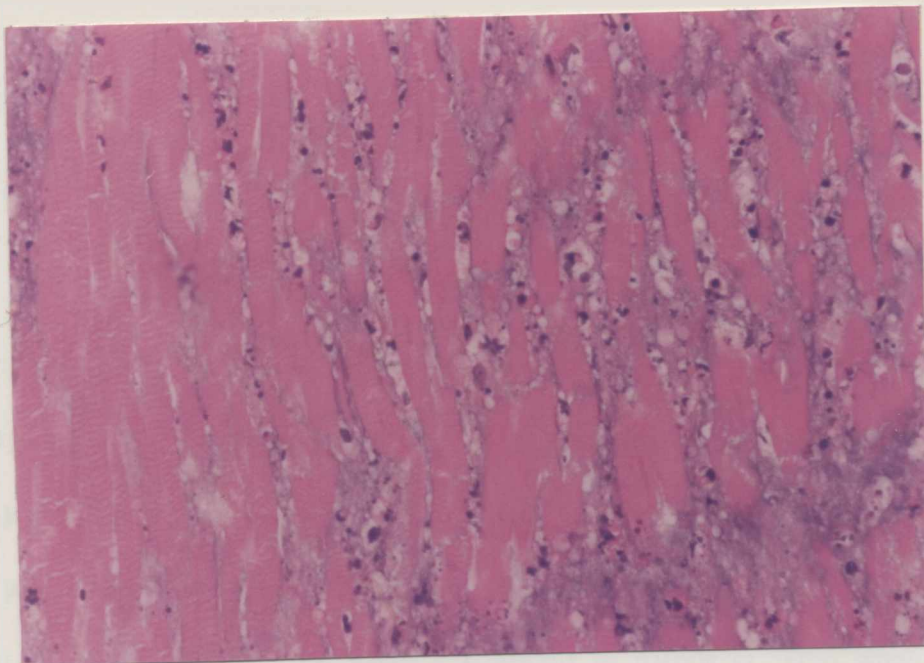
FIGURE 4.2

Sections of the baboons' myocardium demonstrating ventricular myocardial infarction.

The top plate shows a hematoxylin and eosin stained section of the myocardium under low power light microscopy. The deeply eosinophilic areas are the infarcted cardiac muscle. The edges of the infarct are invaded with inflammatory cells forming granulation tissue.

The middle plate shows a hematoxylin and eosin section of the left ventricular myocardium at one week. The infarcted dead muscle (lower right) stains deeply and lacks nuclei. The ischaemic and living muscle fibres stain more palely and are nucleated.

The lower plate shows a high power section of the infarcted myocardium. The muscle fibres are still striated, but deeply eosinophilic and without nuclei. Between the cardiac muscle fibres are polymorphs, macrophages and fibroblasts, which later digest the dead muscle and form scar tissue.



4.3 HAEMODYNAMICS.

There was no difference in the incidence of ventricular fibrillation between the two groups; three ketanserin and two control animals were low-amperage direct-current cardioverted for ventricular fibrillation following coronary artery ligation.

Table 4.2 (page 90) and Table 4.3 (page 91) present the haemodynamics recorded before, after (mean 45 ± 9 min after coronary occlusion) and 7 days after coronary artery ligation in the ketanserin and control groups. MAP and aortic flow were similar within and between both groups, and did not therefore influence the regional myocardial blood flow measurement interpretations. Systemic vascular resistance in the ketanserin group was not significantly different from the control group. The LAP increments noted post-occlusion were not significant in both groups. The HR increase at 7 days was significant within the ketanserin group only.

An example of the coronary angiography, performed in the early part of the experiments only, is shown in Figure 4.3 (page 93). The angiographic method of collateral assay was discontinued due to high morbidity rates associated with the prolonged anaesthesia required to include this technique, and because of the poor visualisation of collaterals using this procedure (see Literature Review, section 2.4.1.)

TABLE 4.2: HAEMODYNAMICS AFTER KETANSERIN ADMINISTRATION AND CORONARY OCCLUSION.

		SP	DP	MAP	PP	LAP
Treatment	Time	(mm Hg)	(mm Hg)	(mm Hg)	(mm Hg)	(mm Hg)
Ketanserin	Pre-occlusion	92 ± 5.0	71 ± 3.5	82 ± 3.8	26 ± 2.5	5.6 ± 0.9
	45 min post-occlusion	88 ± 5.4	69 ± 3.8	79 ± 4.2	23 ± 1.8	7.1 ± 1.0
	7 days post-occlusion	71 ± 4.8	62 ± 4.2	75 ± 4.5	26 ± 3.0	9.3 ± 1.1
Placebo	Pre-occlusion	97 ± 1.9	73 ± 1.7	85 ± 1.7	24 ± 0.9	5.2 ± 0.9
	45 min post-occlusion	87 ± 7.1	65 ± 5.9	77 ± 6.1	22 ± 1.8	6.0 ± 1.3
	7 days post-occlusion	90 ± 5.0	63 ± 2.7	75 ± 3.8	27 ± 3.4	6.7 ± 1.0

SP = Systolic pressure; DP = Diastolic pressure; MAP = mean aortic pressure; PP = pulse pressure;

LAP = mean left atrial pressure.

TABLE 4.3: HAEMODYNAMICS AFTER KETANSERIN ADMINISTRATION AND CORONARY OCCLUSION.

		HR	CO	SV	SVR
Treatment	Time	(beats/min)	(ℓ /min)	(ml)	(mm Hg.min/l)
Ketanserin	Pre-occlusion	102 $\pm$ 5.1	1.12 $\pm$ 0.11	11.3 $\pm$ 1.27	78.4 $\pm$ 5.8
	45 min post-occlusion	105 $\pm$ 2.6	0.99 $\pm$ 0.09	9.52 $\pm$ 0.97	84.0 $\pm$ 6.6
	7 days post-occlusion	118 $\pm$ 4.1 ^a	1.19 $\pm$ 0.11	10.3 $\pm$ 1.25	66.8 $\pm$ 8.1
Placebo	Pre-occlusion	101 $\pm$ 6.8	1.02 $\pm$ 0.05	10.3 $\pm$ 0.97	84.5 $\pm$ 4.3
	45 min post-occlusion	106 $\pm$ 7.1	1.11 $\pm$ 0.12	10.7 $\pm$ 1.41	70.4 $\pm$ 3.9
	7 days post-occlusion	116 $\pm$ 4.9	0.95 $\pm$ 0.06	8.3 $\pm$ 0.72	79.3 $\pm$ 3.9

HR = heart rate; CO = mean aortic blood flow; SV = mean left ventricular stroke volume;

SVR = systemic vascular resistance. ^ap < 0.05, significantly different from

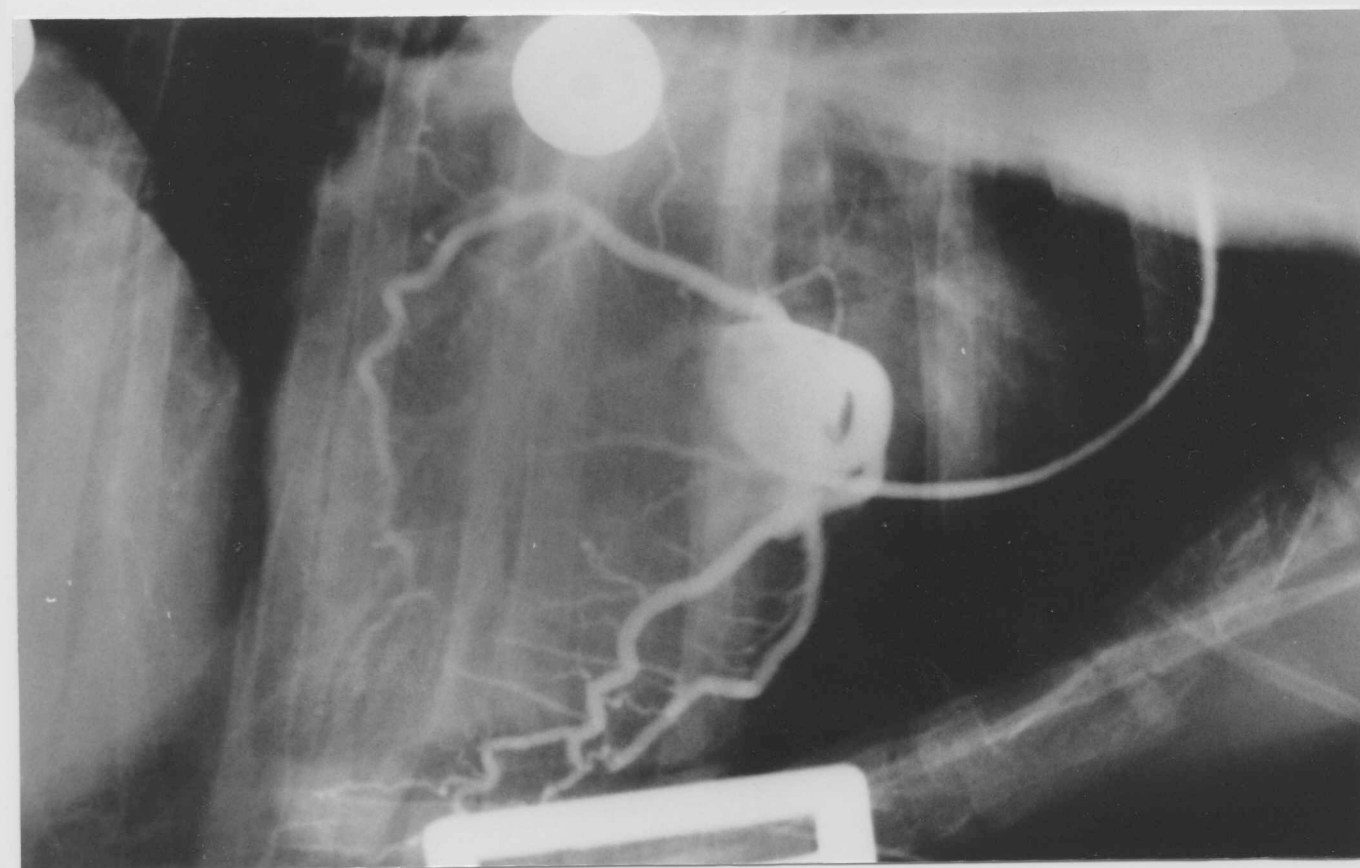
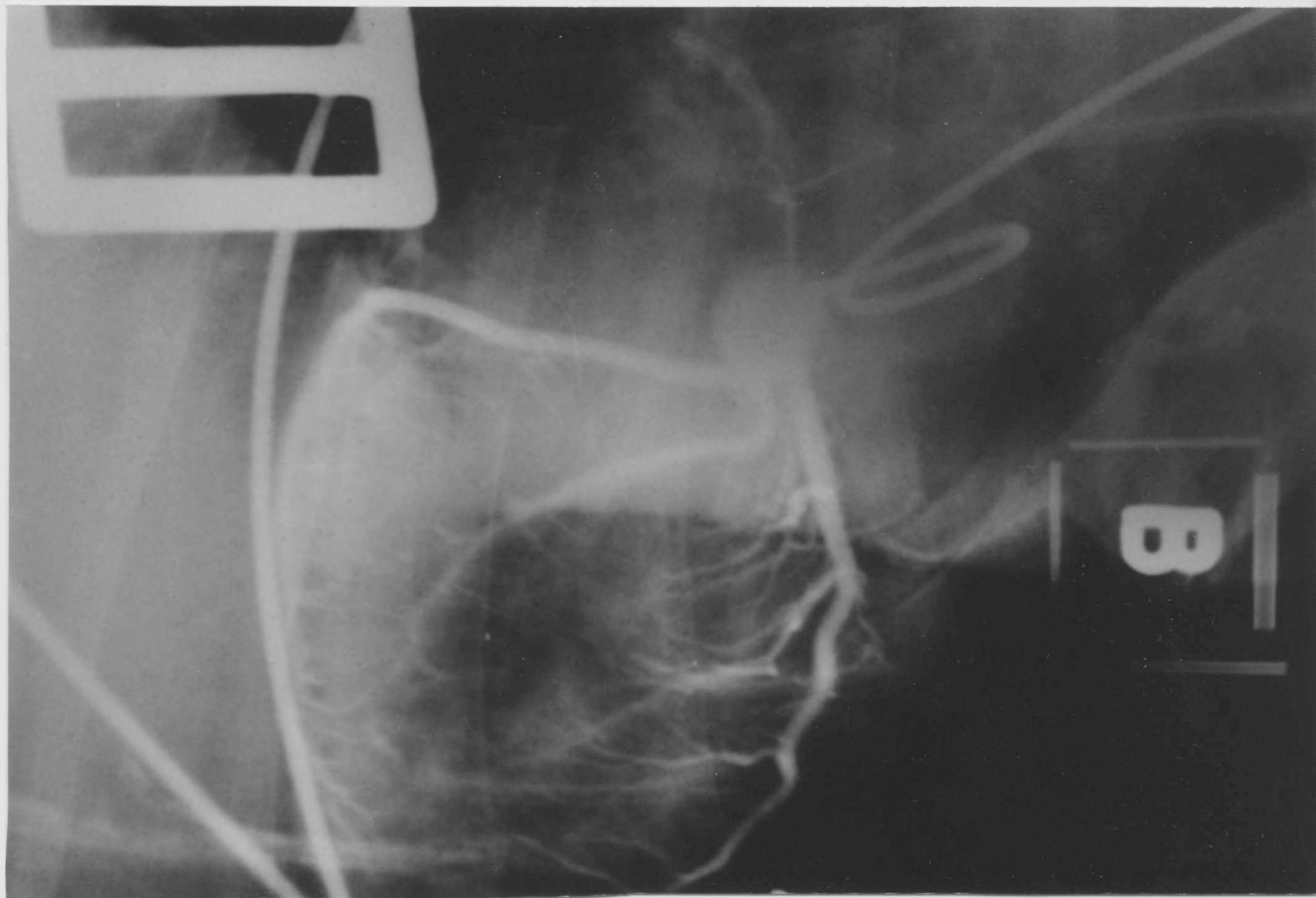
ketanserin pre-occlusion and 45 min post-occlusion.

FIGURE 4.3 :**Coronary angiography of the baboon heart prior to coronary artery ligation.**

The photographs show antero-posterior and lateral views of the heart after the injection of radio-opaque Urograffin® dye. The definition obtained is not sufficient for the quantification of smaller collaterals, which may be microscopic in size (See Literature Review section 2.4.1 paragraph 2).

FIGURE 4.3 :**Coronary angiography of the baboon heart prior to coronary artery ligation.**

The photographs show antero-posterior and lateral views of the heart after the injection of radio-opaque Urograffin[®] dye. The definition obtained is not sufficient for the quantification of smaller collaterals, which may be microscopic in size (See Literature Review section 2.4.1 paragraph 2).



4.4 REGIONAL BLOOD FLOWS.

Infarct size, calculated from the infarcted ventricular tissue:total ventricular weight ratio, was $15.3 \pm 2.8\%$ and $16.1 \pm 0.8\%$ in the ketanserin and control groups respectively (Figure 4.4, page 96). The mean heart ventricle:body weight ratio at one week was $0.3 \pm 0.01\%$ in both groups. The mean weight of both groups' myocardial tissue samples (which were counted for radioactivity) was 1.29 ± 0.02 grams/sample (interquartile range 0.8 - 1.7 grams).

Table 4.4 (page 97) presents the ventricular blood flows to the normal and the infarct-ischemic areas before, 45 min after and 7 days after coronary artery occlusion. Pre-occlusion regional blood flows were the same in both groups. The ratio of the blood flow in the infarct-ischemic left ventricular myocardium to that of the normal ventricular myocardium shows an expected decrease post-coronary artery occlusion on day 0, when compared with the pre-occlusion value (Figure 4.5, page 99). This decrease is significant ($p < 0.05$) and similar in both groups. At 7 days post-occlusion both groups show a significant ($p < 0.05$) but equal increase in this ratio compared with day 0 post-occlusion, suggesting a similar improvement of regional blood flow to the infarct-ischemic area at 7 days in both groups.

Figure 4.4

Infarct size resultant upon mid-length left anterior descending coronary artery ligation in the *Papio ursinus* baboon. Similar sized infarctions were produced in both the control and ketanserin groups.

Size of Ventricular Infarct.

(calculated from ventricle weights)

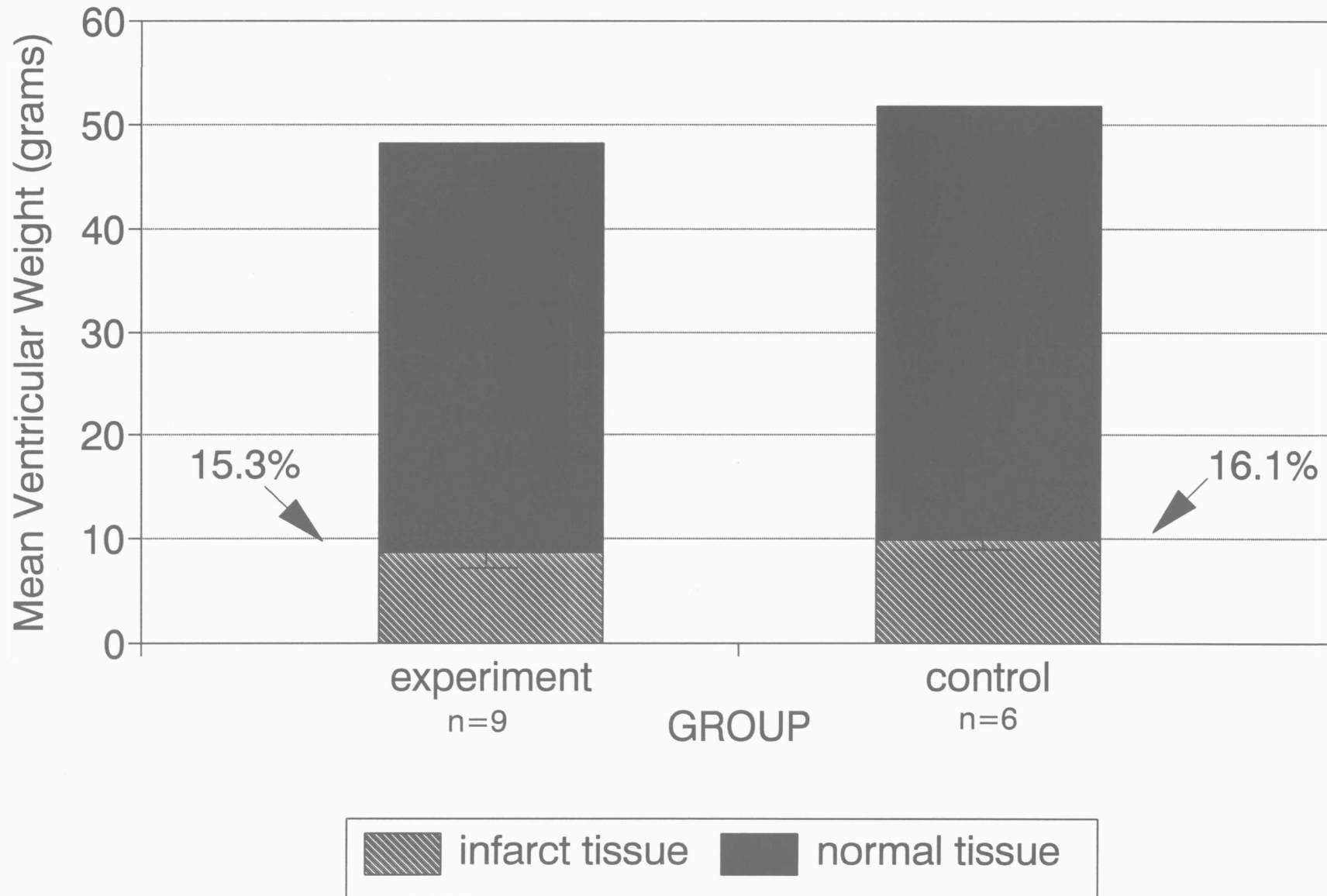


TABLE 4.4. Effect of ketanserin and coronary occlusion on regional myocardial blood flow

Treatment	Time	Regional blood flow ml/min.100g	
		Infarct-ischemic ventricle	Normal ventricle
Ketanserin	A. Pre-occlusion	51.9 ± 3.8	62.1 ± 7.3
	B. 45 min post-occlusion	28.4 ± 2.8 ^a	72.1 ± 9.1 ^c
	C. 7 days post-occlusion	63.8 ± 6.1	102.9 ± 10.8 ^{b,c}
Placebo	D. Pre-occlusion	50.0 ± 6.6	60.7 ± 9.9
	E. 45 min post-occlusion	19.8 ± 3.6 ^a	59.2 ± 7.7 ^d
	F. 7 days post-occlusion	53.7 ± 6.9	97.0 ± 12.4 ^{b,d}

^ap < 0.05 for infarct-ischemic ventricle; B vs. A and C, E vs. D and F.

^bp < 0.05 for normal ventricle; C vs. A and B, F vs. D and E.

^cp < 0.01 for; normal ventricle B vs. infarct-ischemic B; normal ventricle C vs. infarct-ischemic C.

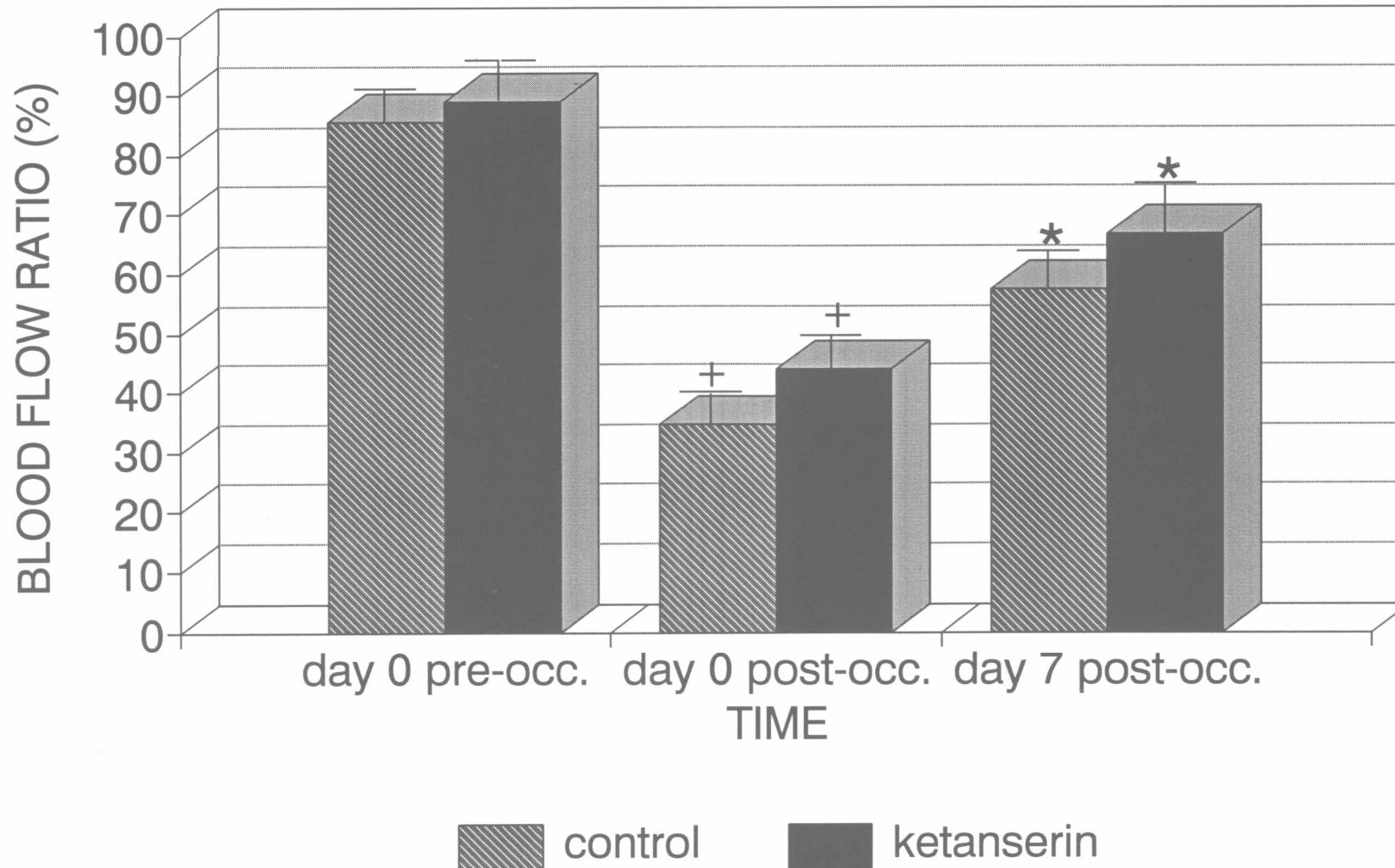
^dp < 0.01 for; normal ventricle E vs. infarct-ischemic E; normal ventricle F vs. infarct-ischemic F.

FIGURE 4.5

The effect of ketanserin on the ratio of infarct-ischaemic ventricle:normal ventricle blood flow, before and after coronary occlusion. Occ. = occlusion. For both groups; ⁺p < 0.05 significantly lower than day 0 pre-occlusion; *p < 0.05 significantly greater than day 0 post-occlusion and significantly less than day 0 pre-occlusion.

Regional Blood Flows

(ratio of infarct to normal zone flow)



CHAPTER 5: DISCUSSION.

5.1 HAEMODYNAMICS.

The LAP increments post-occlusion were not significant in both groups. This latter finding agrees with a previously noted absence of significant chronic increments in left ventricular end diastolic pressure following anterior descending coronary artery occlusion, in baboons (Crozatier *et al.* 1978). The HR increase at 7 days, which was significant within the ketanserin group only, may be a cumulative response to ketanserin's effect of increasing circulating catecholamines (Grobecke *et al.* 1985).

Hypokalemia has been advocated as a predisposing factor for cardiac arrhythmias in captivated baboons (Opie *et al.* 1983). The baboon serum potassium levels measured in captivity, prior to experimentation, in my study were within the normal range for most primates (Steyn *et al.* 1975). Hypokalemia was therefore unlikely to have been an associated causative factor for ventricular fibrillation in this experiment. The use of halothane anaesthesia is however well recognised to increase the sensitivity of the myocardium to circulating catecholamines, thereby exacerbating the arrhythmic potential of the heart under ischaemic conditions.

5.2 KETANSERIN AND REGIONAL BLOOD FLOW.

The results show that 5-HT₂ receptor antagonism in baboons has no effect on regional myocardial blood flow in the infarct-ischæmic areas or in the normal myocardial areas immediately and one week after thrombotic left anterior descending coronary artery occlusion. Perfusion of the ischaemic-infarct myocardial areas does however increase relative to the non-infarcted regions at one week, in both groups (Figure 4.5; page 99). This increment of regional blood flow can be attributed either to functional collateral formation over this period, or to selective coronary vasodilation of the inflamed ischaemic myocardial areas at one week. Collateral formation is, however, the more likely mechanism involved here, since inflammatory-related vasodilation in the ischaemic areas would already have formed at the time of the second radioactive microsphere measurements (i.e. 45 min after coronary ligation). In addition, early collateral blood supply to the myocardium at risk, in the absence of inflammation, has previously been implicated in baboons following coronary occlusion (Lubbe *et al.* 1974). Furthermore, the myocardial collateral circulation following coronary artery ligation in baboons has also previously been shown to increase over one month (Croizatier *et al.* 1978).

Thus ketanserin does not appear to affect the functional extent of the collateral circulation which exists at the time of occlusion, nor its functional development over one week. In addition, prior to coronary artery occlusion in both groups, the pre-infarct left ventricular apex had a similar blood flow to the remainder of the ventricles,

suggesting that ketanserin does not alter the typical distribution of myocardial blood flow in the normal baboon heart.

5.3 KETANSERIN AND INFARCT SIZE.

In keeping with this study's findings that no difference in the infarct-ischaemic zone blood flows or most haemodynamic variables was found between the two groups and, with the premise that the site of coronary occlusion and the myocardial region at risk were similar in both groups, infarct size was also not affected by ketanserin. This result agrees with a recent observation that LY53857, a selective 5-HT₂ antagonist, did not alter infarct size in dogs following 90 min of coronary artery occlusion (Simpson *et al.* 1991).

In my study the female baboon total ventricle weight:body weight ratio (3.3 ± 0.3 g/kg, $n = 15$) is lower than the male baboon heart weight:body weight ratios found by Weisse *et al.* 1976 (5.2 ± 0.3 g/kg, $n = 16$) and Maxwell *et al.* 1987 (8.6 g/kg, $n = 6$). Atrial weight was not included in the ratio used for my study, which partially explains the difference. Apart from the study animal sex differences, Weisse *et al.*'s study fails to mention the species of baboon used, while the baboon species used in the Maxwell *et al.* study was *Papio anubis* and not the *Papio ursinus* used in my study.

5.4 MICROSPHERE LOSS.

The measured increase in the regional myocardial blood flows of both the infarct-
ischaemic and the normal myocardium at one week, in both groups, is contributed to
by a number of factors: Firstly, the time-related loss of microspheres from myocardium
has been described in various experimental animals (Marshall *et al.* 1976; Judgutt *et*
al. 1979; Lekuen and Andersein, 1980; Consigny *et al.* 1982). This loss would
contribute to the higher radioactive microsphere tissue counts of the isotope used at day
7 compared with the two isotopes used at day 0, since the animals were euthenased
immediately following the day 7 isotope injection. The loss of 15 μ m microspheres from
ischaemic myocardium was 26% over 10 hours in one canine study (Lekuen and
Andersein, 1980), whilst the quantity of chronic microsphere loss from the normal and
ischaemic canine myocardium was found to be similar from both these areas (Consigny
et al. 1982): In addition, the use of halothane anaesthesia increased the total myocardial
microsphere loss in this latter study.

The microsphere loss phenomenon has not been extensively investigated in baboons.
Our data, in agreement with a previous baboon study (Lavallee and Vatner, 1984),
show no significant difference between the pre-occlusion infarct-ischaemic zone and the
normal zone myocardial blood flows. This fact does not however exclude chronic
myocardial microsphere loss, but verifies that, in baboons, any chronic microsphere
loss which occurs from the infarct-ischaemic zone is similar to that of the normal zone.

The presence of tissue oedema or haemorrhage in the infarct-ischaemic zone has been implicated as an interfering factor in the interpretation of microsphere-derived regional blood flows following myocardial infarction (Reimer and Jennings, 1979). Since, in this study, the pre-occlusion ischaemic-infarct zone blood flows were not significantly different to the normal zone pre-occlusion blood flows (which finding would not be expected if post-occlusion oedema or haemorrhage had subsequently increased the weight of the ischaemic-infarct zone, thereby artificially reducing the calculated blood flows), this influence could not have interfered with my interpretation of the myocardial collateral blood flow data or infarct size.

A second factor which may have contributed to the increased myocardial blood flows, in both the normal and the infarct-ischaemic myocardium at one week, is the trend towards an increased heart rate in both groups at this time. This increment in heart rate may have produced autoregulatory coronary vasodilation with the greater oxygen demand imposed on the myocardium. Since I have used the ratio of infarct-ischaemic:normal myocardium blood flow to demonstrate that ketanserin has no effect on collateral formation, the above-mentioned factors would not have interfered with this interpretation of my data.

5.5 SEROTONIN ANTAGONISM.

The dose of ketanserin administered in this study is known to produce 5-HT₂ receptor blockade in several animal models, and is comparable with the 1.25 mg/kg.day used by Nevelsteen *et al.* 1984. The 2 h plasma concentration of ketanserin measured in the baboons is similar to the concentration required to block serotonin induced dog coronary artery constriction (Brazenor and Angus, 1982) and to the concentration found following a 10 mg i.m. or i.v. bolus injection in humans (Heykants *et al.* 1986). The study data were not significantly affected by post-junctional α_1 -adrenoreceptor blockade since there was no change in the blood pressure measurements at any time with the dose of 1 mg/kg ketanserin daily.

Intra-arterial infused serotonin is vasoactive in coronary arteries, mediating vasoconstriction, vasodilation and cyclic flow variations in different species (Lamping *et al.* 1985; Ashton *et al.* 1986; Bush, 1987; Chu and Cobb, 1987). In my study, in order to approach the pathophysiological conditions of myocardial disease more closely, no exogenous serotonin was infused, the use of heparin was avoided and thrombin was injected locally to mediate platelet aggregation. These procedures are unlike most other serotonin investigations which have been performed to date, since serotonin was usually injected as an exogenous agent in these studies.

Although 5-HT₂ receptor antagonism has often been shown to reverse serotonin-induced coronary artery constriction, the exact role of serotonin in ischaemic heart disease is

still controversial, and my findings corroborate those of many other researchers: It has been noted that in swine epicardial and transmural regional blood flows were not affected by serotonin infusion following coronary artery stenosis (Ruocco *et al.* 1988). Platelet-activating factor has been shown to restore cyclic blood flow variations (CFV's) in stenosed canine coronary arteries despite pre-treatment with ketanserin (Apprill *et al.* 1985); ketanserin did not significantly attenuate the delayed vasoconstrictor response to infused serotonin in awake dog proximal coronary arteries (Chu and Cobb, 1987) and ketanserin, given by itself, did not prevent the re-occlusion of thrombolysed canine coronary artery thrombosis, where the combination of both ketanserin and a thromboxane A₂/prostaglandin H₂-receptor antagonist did produce a deterrent effect (Golino *et al.* 1988). In addition, the non-selective serotonin antagonist methysergide, but not ketanserin, was found to abolish serotonin-induced reductions in blood flow following canine dynamic coronary artery stenosis (Ichikawa *et al.* 1989). Our primate data also support the findings that ergonovine-induced myocardial ischemia (Freedman *et al.* 1984) and Prinzmetal's angina (De Caterina *et al.* 1984) are not improved by the administration of ketanserin in humans.

5.6 CONCLUSION.

I have shown that 5-HT₂ receptor antagonism does not potentiate coronary vasodilation, regulate ischaemic zone blood flow or modify functional collateral formation in anaesthetized baboons following acute thrombotic coronary artery occlusion, and that ketanserin has neither a protective nor an adverse effect on the resulting myocardial infarct. Since the possible routine use of serotonin antagonists for the management of human ischaemic heart disease is a current issue, my findings necessitate further investigation into the exact roles of serotonin, the identification of serotonergic receptors and the potential use of combinations of 5-HT₂ receptor antagonists with thromboxane-A₂ inhibitors or with 5-HT₁-like receptor antagonists, in these non-human primates.

6. REFERENCES.

Andren E, Svensson A, Dahlof B, Eggerstein R, Hansson L (1983)

Ketanserin in hypertension. *Acta Medica Scandinavica*, 214:125-130.

Angus JA (1989)

5-HT receptors in the coronary circulation. *Trends in Pharmacological Sciences*, 10:89-90.

Apprill P, Schmitz JM, Campbell WB, Tilton MD, Ashton J, Raheja S, Buja LM, Willerson JT (1985)

Cyclic blood flow variations induced by platelet-activating factor in stenosed canine coronary arteries despite inhibition of thromboxane synthetase, serotonin receptors, and α -adrenergic receptors. *Circulation*, 72:397-405.

Arnout J, Van Russelt M, Deckmyn H, Vermeylen J, Fiocchi R, Lijnen P, Amery A (1985)

Platelet hypersensitivity to serotonin after prolonged ketanserin intake. *Journal of Cardiovascular Pharmacology*, 7: 520-522.

Ashton JH, Benedict CR, Fitzgerald C, Raheja S, Taylor A, Campbell WB, Buja LM, Willerson JT (1986)

Serotonin as a mediator of cyclic flow variations in stenosed canine coronary arteries. *Circulation*, 73:572-577.

Ashton JH, Golino P, McNatt JM, Buja LM, Willerson JT (1989)

Serotonin S_2 and thromboxane A_2 -Prostaglandin H_2 receptor blockade provides protection against epinephrine-induced cyclic flow variations in severely narrowed canine coronary arteries. *Journal of the American College of Cardiology*, 13:755-763.

Barker B, Rosario MD, Grant V, McNamara JJ, Suehiro G (1980)

Infarct distribution in sub-human primates after coronary artery occlusion. *Cardiovascular research*, 14:671-674.

Benedict CR, Matthew B, Rex KA, Cartwright J, Sordahl LA (1986)

Correlation of plasma serotonin changes with platelet aggregation in an in vivo dog model of spontaneous occlusive coronary thrombus formation. *Circulation Research*, 58:59-67.

Bishop SP (1984)

How well can we measure coronary flow risk zones and infarct size? In: *Therapeutic approaches to myocardial infarct size limitation*. Hearse DJ and Yellon DM (Eds), New York, Raven Press, 139-162.

Bishop SP, White FC, Bloor CM (1976)

Regional myocardial blood flow during acute myocardial infarction in the conscious dog. *Circulation Research*, 38:429-438.

Blackshear J, Orlandi C, Garnic JD, Hollenberg NK (1985)

Differential large and small vessel responses to serotonin in the dog hindlimb in vivo: Role of the 5-HT₂ receptor. *Journal of Cardiovascular Pharmacology*, 7:42-49.

Bockaert J, Fozard JR, Dumuis A, Clarke DE (1992)

The 5-HT₄ receptor: A place in the sun. *Trends in Pharmacological Sciences*, 13:141-145.

Bradley PB, Engel G, Feniuk W, Fozard JR, Humphrey PPA, Middlemiss DM, Mylecharane EJ, Richardson BP, Saxena PR (1986)

Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacology*, 25:563-576.

Brazenor RM, Angus JA (1982)

Actions of serotonin antagonists on dog coronary artery. *European Journal of Pharmacology*, 81:569-576.

Buckberg GD, Luck JC, Payne DB, Hoffmann HJE, Archie JP, Fixler DE (1971)

Some sources of error in measuring regional blood flow with radioactive microspheres. *Journal of Applied Physiology*, 4:598-604.

Buhler FR, Amstein R, Fetkouska N (1987)

Serotonin and pre-activated platelets in essential hypertension. *Journal of Cardiovascular Pharmacology*, 10:S32-S34.

Burke SA, Lefer AM, Smith GM, Smith BS (1983)

Prevention of extension of ischaemic damage following acute myocardial ischaemia by Dazoxiben. *British Journal of Clinical Pharmacology*, 15:975-1015.

Bush LR, Campbell WB, Kern K, Tilton GD, Apprill P, Ashton J, Schmitz J, Buja M, Willerson JT (1984)

The effect of α_2 -adrenergic and serotonergic receptor antagonists on cyclic blood flow alterations in stenosed canine coronary arteries. *Circulation Research*, 55:642-652.

Bush LR (1987)

Effects of serotonin antagonists, cyproheptadine, ketanserin and mianserin, on cyclic blood flow reductions in stenosed canine coronary arteries. *Journal of Pharmacology and Experimental Therapeutics*, 240:674-682.

Camping KG, Marcus ML, Dole WP (1985)

Removal of the endothelium potentiates canine large coronary artery constrictor responses to 5-HT in vivo. *Circulation Research*, 57:46-54.

Chester AH, Martin GR, Bodelsson M, Arneklo B, Tadjkarimi S, Tornebrandt K, Yacoub MU (1990)

5-Hydroxytryptamine receptor profile in healthy and diseased human epicardial coronary arteries. *Cardiovascular Research*, 24:932-939.

Chu A, Cobb FR (1987)

Vasoactive effects of serotonin on proximal coronary arteries in awake dogs. *Circulation Research*, 61(Suppl II):II81-II83.

Cocks TM, Angus JA (1983)

Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. *Nature*, 305:627-629.

Cohen MV, Yipintsoi T (1979)

Myocardial performance and collateral flow after transient coronary occlusion in exercising dogs. *American Journal of Physiology*, 237:H520-H527.

Cohen ML, Fuller RW, Kurz KD (1983)

LY53857, a selective and potent serotonergic (5HT₂) receptor antagonist, does not lower blood pressure in the spontaneously hypertensive rat. *The Journal of Pharmacology and Experimental Therapeutics*, 227:327-332.

Cohen RA, Sheperd JT, Vanhoutte PM (1983)

5-Hydroxytryptamine can mediate endothelium-dependent relaxation of coronary arteries. *American Journal of Physiology*, 245:H1077-H1080.

Cohen MV (1985)

Functional significance of coronary collaterals in experimental animals. In: *Coronary Collaterals; Clinical and Experimental Observations*. New York: Futura Publishing Co., 343-372.

Consigny PM, Verrier FD, Payne BD, Edelist G, Jester J, Baer RW, Vlahkes GJ, Hoffman JIE (1982)

Acute and chronic microsphere loss from canine left ventricular myocardium. *American Journal of Physiology*, 242:H392-404.

Crozatier B, Ross J, Franklin D, Bloor CM, White FC, Tomoike H, McKown DP (1978)

Myocardial infarction in the baboon: regional function and the collateral circulation. *American Journal of Physiology*, 235:H413-H421.

Crystal GJ, Boatwright RB, Downey HF, Bashoup FA (1979)

Shunting of microspheres across the canine coronary circulation. *American Journal of Physiology*, August:H7-H12.

De Caterina R, Carpeggiani C, L'Abbate A (1984)

A double blind, placebo-controlled study of ketanserin in patients with Prinzmetal's angina. Evidence against a role for serotonin in the genesis of coronary vasospasm. *Circulation*, 69:889-894.

De Clerck F, Xhonneux B (1985)

Continuous inhibition of platelet S_2 serotonergic receptors during chronic administration of ketanserin in humans. *Journal of Cardiovascular Pharmacology*, 7:523-525.

De Cree J, Leempoels J, Demoen B, Roels V, Verhaegen H (1985)

Effects of ketanserin on the hyperactivity of platelets to 5-hydroxytryptamine in patients with cardiovascular diseases. *Journal of Cardiovascular Pharmacology*, 7:526-528.

De Scheerder I, Buyzere M, Delanghe J (1987)

Early detection of acute myocardial infarction. *Medical Focus*, 2:36-39.

Dormandy JA (1986)

Haemorheological aspects of serotonin. *Progress in Applied Microcirculation*, 10:62-75.

Egerton K, Schmitz J, Benedict CR, Malloy CR, Willerson JT, Dehmer GJ (1989)

Transcardiac serotonin concentration is increased in selected patients with limiting angina and complex coronary lesion morphology. *Circulation*, 79: 116-123.

Eidt JF, Ashton J, Golino P, McNatt J, Buja M, Willerson JT (1989)

Treadmill exercise promotes cyclic alterations in coronary blood flow in dogs with coronary artery stenosis and endothelial injury. *Journal of Clinical Investigation*, 84:517-527.

Flameng W, Vanhaecke J, Stump DC, Van de Werf F, Holmes W, Guenzler WA, Flohe L, Collen D (1985)

Coronary thrombolysis by intravenous infusion of recombinant single chain urokinase-type plasminogen activator or recombinant urokinase in baboons: effect on regional blood flow, infarct size and haemostasis. *Journal of the American College of Cardiology*, 8:118-124.

Flameng W, Van der Werf F, Vanhaecke J, Verstraete M, Collen D (1985)

Coronary thrombolysis and infarct size reduction after intravenous infusion of recombinant tissue type plasminogen activator in non-human primates. *Journal of Clinical Investigation*, 75:84-90.

Flameng W, Vanhaecke J, Vandeplasse G (1986)

Studies on experimental myocardial infarction. Dogs or Baboons? *Cardiovascular research*, 20:241-247.

Flameng W, LeSaffre E, Vanhaecke J (1990)

Determinants of infarct size in non-human primates. *Basic Research in Cardiology*, 85:392-403.

Francis GS, Cohn JN (1984)

Angina Pectoris and chronic congestive heart failure: Pathophysiology and therapy. In: *Cardiovascular, Pharmacology*, Antonaccio MJ (ed.) New York: Raven Press, 296-328.

Freedman SB, Chierchia S, Rodriguez-Plaza L, Bugiardini R, Smith G, Maseri A (1984)

Ergonovine-induced myocardial ischaemia: no role for serotonergic receptors? *Circulation*, 70:178-183.

Fuller RW, Snoddy HD (1979)

The effect of metergoline and other serotonin receptor antagonists on serum corticosterone in rats. *Endocrinology*, 105:923-928.

Fujita M, Sasayama S, Ejiri M, Asanoi H, Nakajima H, Miwa K (1988)

Coronary Collateral Development after acute myocardial infarction. *Clinical Cardiology*, 11:525-528.

Geary GG, Smith GT, McNamara JJ (1981)

Defining the anatomic perfusion bed of an occluded coronary artery and the region at risk to infarction. A comparative study in the baboon, pig and dog. *The American Journal of Cardiology*, 47:1240-1247.

Geary GG, Smith GT, Suehiro GT, McNamara JJ (1982)

Failure of a nifedipine therapy to reduce myocardial infarct size in the baboon. *American Journal of Cardiology*, 49:328-331.

Geary GG, Fenton L, Cheng G, Smith GT, Siu B, McNamara JJ (1983)

Failure of pre-treatment with propranolol to reduce the zone of myocardial infarction after 2 hours of coronary occlusion in the primate heart. *American Journal of Cardiology*, 52:615-620.

Golino P, Ashton JH, Glas-Greenwalt P, McNatt J, Buja LM, Willerson JT (1988)
Mediation of reocclusion by thromboxane A₂ and serotonin after thrombolysis with tissue-type plasminogen activator in a canine preparation of coronary thrombosis. *Circulation*, 77:678-684.

Golino P, Piscione F, Willerson JT, Capelli-Bigazzi M, Focaccio A, Villari B, Indolfi C, Russolillo E, Condorelli M (1991)
Divergent effects of serotonin on coronary-artery dimensions and blood flow in patients with coronary atherosclerosis and control patients. *New England Journal of Medicine*, 324:641-648.

Gothert M, Schlicker E (1989)
Classification of serotonin receptors. *Journal of Cardiovascular Pharmacology*, 10 (Supplement 3):53-57.

Grobecker H, Gessler I, Delius W, Dominiak P, Kees F (1985)
Effect of ketanserin on hemodynamics, plasma-catecholamine concentrations and serotonin uptake by platelets in volunteers and patients with congestive heart failure. *Journal of Cardiovascular Pharmacology*, 7 (Supplement 7):S102-S104.

Heistad DD (1984)
Augmented responses to vasoconstrictor stimuli in hypercholesterolemic and atherosclerotic monkeys. *Circulation Research*, 54:711-718.

Henderson AH, Marcus ML (1984)
The problem of infarct size limitation. In: *Therapeutic approaches to myocardial infarct size limitation*. Hearse DJ, Yellon DM (eds.), New York: Raven Press, 1-17;91-107.

Heykants J, Van Peer A, Woestenborghs R, Gould S, Mills J (1986).

Pharmacokinetics of ketanserin and its metabolite ketanserin-ol in man after intravenous, intramuscular and oral administration. *European Journal of Pharmacology*, 31:343-350.

Heymann MA, Payne BD, Hoffman JIE, Rudolph AM (1977)

Blood flow measurements with radionuclide-labelled particles. *Progress in Cardiovascular Disease*, 20:55-79.

Heyndrickz GR, Amano J, Patrick TA, Manders WT, Rogers GG, Rosendorff C, Vatner SF (1985)

Effects of coronary artery reperfusion on regional myocardial blood flow and function in conscious baboons. *Circulation*, 71:1029-1037.

Hollenberg NK (1987)

Collateral arterial tree and responses to serotonin. *Journal of Cardiovascular Pharmacology*, 10(Supplement 3):S35-S38.

Houston DS, Vanhoutte PM (1986)

Serotonin and the vascular system. Role in health and disease, and implications for therapy. *Drugs*, 31:149-163.

Ichikawa Y, Yokoyama M, Akita H, Fukuzaki H (1989)

Constriction of large coronary artery contributes to serotonin-induced myocardial ischaemia in the dog with pliable coronary stenosis. *Journal of the American College of Cardiology*, 14:449-459.

Imhoff RK (1961)

Production of aortic occlusion resembling acute aortic embolism syndrome in cats. *Nature*, 192:979-980.

Janssen PAJ (1983)

5-HT₂ receptor blockade to study serotonin induced pathology. *Trends in Pharmacological Sciences*, May:198-206.

Janssen PAJ (1983)

The mode of action of ketanserin. *Trends in Pharmacological Sciences*, October:412-413.

Janssen JA (1985)

Pharmacology of potent and selective S₂-serotonergic antagonists. *Journal of Cardiovascular Pharmacology*, 7(Suppl 7):S2-S11.

Judgutt BI, Becker LL, Hutchins GM (1979)

Early changes in collateral blood flow during myocardial infarction in conscious dogs. *American Journal of Physiology*, 237:H371-H380.

Judgutt BI, Hutchins GM, Bulkley BH, Becker LC (1979)

The loss of radioactive microspheres from canine necrotic myocardium. *Cardiac Research*, 45:746-756.

Kaihara S, Rutherford RB, Schwentker EP, Wagner HM (1969)

Distribution of cardiac output in experimental haemorrhagic shock in dogs. *Journal of Applied Physiology*, 27:218-221.

Katz AM (1983)

The ischaemic heart. In: *Physiology of the Heart*. New York: Raven Press, 175-197; 419-434.

Kaumann AJ (1985)

Two classes of 5-HT receptors that are neither 5-HT₁ nor 5-HT₂. *Journal of Cardiovascular Pharmacology*, 7 (Supplement 7):577-578.

King RB, Bassingthwaite JRS, Rowell LB (1985)

Stability of heterogeneity of myocardial blood flow in normal awake baboons. *Circulation Research*, 57:285-295.

Kuhn DM, Wolf WA, Lovenberg W (1980)

Review of the role of the central serotonergic neuronal system in blood pressure regulation. *Hypertension*, 2:243-255.

Lamping KG, Marcus ML, Dole WP (1985)

Removal of the endothelium potentiates canine large coronary artery constrictor responses to 5-HT in vivo.

Circulation Research, 57:46-54.

Lavallee M, Vatner SF (1984)

Regional myocardial blood flow and necrosis in primates following coronary occlusion. *American Journal of Physiology*, 246:4635-4639.

Leff P, Martin GR (1988)

Differences in agonist dissociation constant estimates for 5-HT and 5-HT₂ receptors: a problem of acute desensitisation? *British Journal of Pharmacology*, 95:569-577.

Lekuen J, Andersein KS (1980)

Migration of 15 micron microspheres from infarcted myocardium. *Cardiovascular Research*, 14:280-287.

Lubbe WF, Peisach M, Pretorius R, Bruyneel KJJ, Opie LH (1974)

Distribution of myocardial blood flow before and after coronary artery ligation in the baboon. Relation to early ventricular fibrillation. *Cardiovascular Research*, 8:478-487.

Luscher TF, Rubanyi GM, Aarhus LL, Edoute Y, Vanhoutte P (1986)

Serotonin reduced coronary flow in the isolated heart of the spontaneously hypertensive rat. *Journal of Hypertension*, 4(Suppl 5):S148-S150.

Marche P, Rendu F, Macclouf J, Girard A, Levy-Toledano S (1985)

Serotonin release and phosphoinositide breakdown in thrombin-induced activation of human platelets. *Journal of Cardiovascular Pharmacology*, 7(Supplement 7):S18-S19.

Maroko PR, Kjekshus JK, Sobel BE, Watanabe T, Covell JW, Ross J, Braunwald E (1971)

Factors influencing infarct size following experimental coronary artery occlusion. *Circulation*, 43:67-82.

Maroko PR, Braunwald E (1975)

Effects of metabolic and pharmacological interventions on myocardial infarct site following coronary occlusion. *Acta Medica Scandinavica*, 587:125-136.

Marshall WG, Boakman GB, Dickerson G, Perlin A, Todd EP, Utley JR (1976)

Shunting, release and distribution of nine and fifteen micron spheres in myocardium. *Surgery*, 79:631-637.

Maxwell MP, Hearse DJ, Yellon DM (1987)

Species variation in the coronary collateral circulation during regional myocardial ischaemia: a critical determinant of the rate of evolution and extent of myocardial infarction. *Cardiovascular Research*, 21:737-746.

Maxwell MP, Hearse DJ, Yellon DM (1987¹)

Is there a component of coronary collateral flow which cannot be detected by radio-labelled microspheres? *Cardiovascular Research*, 21:747-754.

Montesano R, Pepper M, Orci L (1990)

Angiogenesis in vitro: morphogenetic and invasive properties of endothelial cells. *News in Physiological Sciences*, 5:75-79.

Nevelsteen A, De Clerck F, Loots W, De Gryse A (1984)

Restoration of post-thrombotic peripheral collateral circulation in the cat by ketanserin, a selective 5-HT₂-receptor antagonist. *Archives Internationales de Pharmacodynamie et de Therapie* (reprint), 270:268-278.

Nyborg NCB, Mikkelsen EO (1988)

Serotonin response increases with age in rat coronary resistance arteries. *Cardiovascular Research*, 22:131-137.

Opie LH, Bruyneel KJ, Lubbe WF (1983)

What has the baboon to offer as a model of experimental ischaemia? *European Heart Journal*, 4 (Supplement C):55-60.

Orlandi C (1986)

Augmented arterial sensitivity to serotonin in vascular disease; implications for tissue perfusion. *Progress in Applied Microcirculation*, 10:76-82.

Orlandi C, Blackshear JL, Hollenberg NK (1984)

Increased sensitivity to serotonin of hindlimb arterial collateral vessels. *Circulation*, 70(Suppl II):231.

Perez JE, Saffitz JE, Gutierrez FA, Henry PD (1983)

Coronary artery spasm in intact dogs induced by potassium and serotonin. *Circulation Research*, 52:423-431.

Reimer KA, Jennings RB (1979)

The changing anatomic reference base of evolving myocardial infarction. *Circulation*, 60:866-876.

Reimer KA, Jennings RB (1984)

Can we really quantitate myocardial cell injury? In: *Therapeutic approaches to myocardial infarct size limitations*. Hearse DJ, Yellon DM (Eds). Raven Press, New York, 163-185.

Reneman RS, Bollinger A (1986)

Vascular and microvascular effects of serotonin. *Progress in Applied Microcirculation*, 10:83-86.

Rivas F, Cobb FR, Bache RJ, Greenfield, JC (1976)

Relationship between blood flow to ischaemic regions and extent of myocardial infarction. *Circulation Research*, 38:439-447.

Rogers GG, Rosendorff C, Shimell CJ, Coull A (1982)

Effects of Tiapamil on hemodynamics and myocardial salvage during myocardial infarction in the baboon. *Cardiology*, 69 (Supplement 1):58-67.

Rogers GG, Rosendorff C, Coull A, Warner SJC, Jarvis AC (1983)

Sotalol and infarct size after coronary ligation in the baboon. *Journal of Cardiovascular Pharmacology*, 5:28-34.

Rudolph AM, Heymann MA (1967)

The circulation of the fetus in utero: methods for studying distribution of blood flow, cardiac output and organ blood flow. *Circulation Research*, 21:163-184.

Ruf W, Suehiro GT, Suehiro A, McNamara JJ (1986)

Regional myocardial blood flow in experimental myocardial infarction after pre-treatment with aspirin. *Journal of the American College of Cardiology*, 7:1057-1062.

Ruocco NA, Most AS, Sasken H, Steiner M, Gewirtz H (1988)

Influence of serotonin on myocardial blood flow in the presence and absence of a coronary arterial stenosis: observations in domestic swine. *Proceedings of the Society for Experimental Biology and Medicine*, 197:416-424.

Sasaki Y, Wagner HM (1971)

Measurement of the distribution of cardiac output in unanesthetized rats. *Journal of Applied Physiology*, 6:879-894.

Schaper WKA, Xhonneux R, Jagenau AHM (1965)

Stimulation of the coronary collateral circulation by lidoflazine. *Archives of Experimental Pathology and Pharmacology*, 252:1-8.

Schaper W (1978)

Experimental coronary artery occlusion. *Basic Research in Cardiology*, 73: 584-594.

Schaper W, Gorge G, Winkler B, Schoper J (1988)

The collateral circulation of the heart. *Progress in Cardiovascular Diseases*, 31:57-77.

Schaub RG, Meyers KM, Sande RD, Hamilton G (1976)

Inhibition of feline collateral vessel development following experimental thrombotic occlusion. *Circulation Research*, 39:736-743.

Schaub RG, Meyers KM, Sande RD (1977)

Serotonin as a factor in the depression of collateral blood flow following experimental arterial thrombosis. *Journal of Laboratory and Clinical Medicine*, 90:645-653.

Schutte A, Maloteaux JM, Laduron PM (1983)

Characteristics and regional distribution of serotonin- S_2 receptors in human brain. *Brain Research*, 276:231-235.

Shimokawa H, Lawrence LA, Vanhoutte PM (1987)

Porcine coronary arteries with regenerated endothelium have a reduced endothelium-dependent responsiveness to aggregating platelets and serotonin. *Circulation Research*, 61:256-270.

Sigal SL, Gellman J, Sarembock JJ, LaVeau PJ, Chen QS, Cabin HS, Ezekowitz MD (1991)

Effects of serotonin-receptor blockade on angioplasty-induced vasospasm in an atherosclerotic rabbit model.

Arteriosclerosis and Thrombosis, 11:770-783.

Simpson PJ, Schelm JA, Jakubowski JA, Smallwood JK (1991)

The role of serotonin 5HT₂ receptor blockade in myocardial reperfusion injury: effects of LY53857 in a canine model of myocardial infarction. *The Journal of Pharmacology and Experimental Therapeutics*, 258:979-985.

Steyn DG, Hamilton-Bruce RJ, Zuurmond TJ, Pharo R (1975)

Standard serum chemical and haematological values in the chacma baboon. *Journal of the South African Veterinary Association*, 46(2):191-196.

Theron HD, Lambert CR, Conti CR (1991)

Quantification of coronary artery stenosis. *Cardiovascular Journal of Southern Africa*, 2:18-24.

Tillmanns H, Keda S, Bing RJ (1975)

Production of myocardial infarcts in animal experiments: In: *Handbook of Experimental Pharmacology*. Schmier J and Eichler O (eds.), Springer-Verlag, New York, 16:117-130.

Van de Werf T (1980)

Review of the Physiology of the Cardiovascular System. In: *Cardiovascular Pathophysiology*. London, Walton Street: Oxford University Press, 1-50.

Van den Berg EK, Schmitz JM, Benedict CR, Malloy CR, Willerson JT, Dehmer GJ (1989)

Transcardiac serotonin concentration is increased in selected patients with limiting angina and complex coronary lesion morphology. *Circulation*, 79:116-123.

Vanhoutte PM (1986)

Serotonin, adrenergic nerves, endothelial cells and smooth muscle. *Progress in Applied Microcirculation*, 10:1-11.

Vanhoutte PM (1987)

Cardiovascular effects of serotonin. *Journal of Cardiovascular Pharmacology*, 10 (Supplement 3):S8-S11.

Van Oene JC (1990)

Oral dosing with ketanserin to control high blood pressure in the elderly. *Cardiovascular Drugs and Therapy*, 4:111-114.

Vatner SF, Patrick TA, Knight DR, Manders WT, Fallon JT (1987)

Effects of calcium channel blockers on responses of blood flow, functional arrhythmias and extent of infarction following reperfusion in conscious baboons. *Circulation Research*, 62:105-115.

Verani MS (1983)

The functional significance of coronary collateral vessels. *Catheterization Cardiovascular Diagnosis*, 9:333-337.

Wagner HN, Rhodes BA, Sasaki Y, Ryan JP (1969)

Studies of the circulation with radio-active microspheres. *Investigative Radiology*, 4:374-396.

Warren DJ, Ledingham JGG (1974)

Measurement of cardiac output distribution using microspheres; some practical and theoretical considerations. *Cardiovascular Research*, 8:570-581.

Weisse AB, Kearney K, Narang R, Regan T (1976)

Comparison of the coronary collateral circulation in dogs and baboons after coronary occlusion. *American Heart Journal*, 92:193-200.

Williams DO, Amsterdam EA, Miller RR (1976)

Functional significance of coronary collateral vessels in patients with acute myocardial infarction: Relationship to pump performance, cardiogenic shock and survival. *American Journal of Cardiology*, 37:345-351.

Yanagisawa-Miwa A, Uchida Y, Nakamura F, Tomaru T, Kido H, Kamijo T, Sugimoto T, Kaji K, Utsuyama M, Kurashima C, Ito H (1992)

Salvage of infarcted myocardium by angiogenic action of basic fibroblast growth factor. *Science*, 257:1401-1403.

Zar JH (1974)

Non-parametric statistics. In: *Biostatistical Analysis*. Engelwood Cliff, New Jersey: Prentice Hall Inc; 109-114, 124-126, 139-142.

7 APPENDIX.

- 7.1 Acceptance of paper for publication.
- 7.2 Animal ethics clearance certificates.
- 7.3 Clearance for use of radioactive isotopes.

Journal of Cardiovascular Pharmacology

Jan Koch-Weser, M.D.
Director and Head of Clinical Research
F. HOFFMANN-LA ROCHE LTD
CH-4002 Basel, Switzerland
Telephone: (61) 588 51 29
Telex: 962 292 965 542 air ch
Fax: (61) 691 93 91 / 691 96 00

Editors-in-Chief

Paul M. Vanhoutte, M.D., Ph.D.
Professor of Medicine
Director, Center for Experimental Therapeutics
Baylor College of Medicine
One Baylor Plaza, R. 826E
Houston, Texas 77030 U.S.A.
Telephone: (713) 798-6452
Telefax: (713) 799-2469

CITATION INDEX = 2.2

August 14, 1992

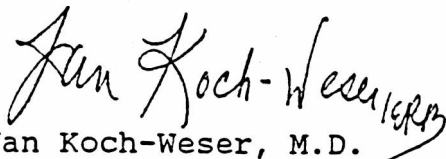
Dr. Craig G. Hartford
Department of Physiology
University of the Witwatersrand Medical School
7 York Road
Parktown, Johannesburg, 2193
South Africa

Dear Doctor Hartford:

Thank you for the revision of your manuscript (No. 771)
"The Influence of Ketanserin on Regional Myocardial Blood
Flow Following Myocardial Infarction in Baboons".

The article has been sent to the publisher and should
appear in about three months.

Sincerely,


Jan Koch-Weser, M.D.
Editor-in-Chief

JKW:erb

(Dictated but not Read)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

ANIMAL ETHICS SCREENING COMMITTEE

MODIFICATIONS AND EXTENTIONS TO EXPERIMENTS

- a) Name: C. HARTFORD
- b) Department: MEDICAL PHYSIOLOGY
- c) Experiment to be modified/extended: AEC No.

00	15	5
----	----	---
- d) Project title: KETANSERIN EFFECT ON CORONARY COLLATERALS

- e) Number and species of animals originally approved:
- | | |
|----|---------------|
| 20 | Papio ursinus |
| | |
| | |

- f) Number of animals used to date:
- | | |
|---|--|
| 3 | |
| | |
| | |

- g) Specific modification/extension requested
See attached page

- h) Motivation for modification/extension
Current femoral catheterisation is contributing to excessive arterial thrombosis.

Date: 22/03/1990 Signature: *[Signature]*

RECOMMENDATIONS

Approved: The modification as set out

Signature: *G. Lunn* (Chairman, AESC)
Date: 28.3.90

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

ANIMAL ETHICS SCREENING COMMITTEE

CLEARANCE CERTIFICATE NO:

90	15	5
----	----	---

APPLICANT: Dr C Hartford

DEPARTMENT: Physiology, Medical School

PROJECT TITLE: The influence of serotonin antagonism....

SPECIES	NUMBER	DATE OF EXPIRY
Baboons	10 additional	December 1992

The use of these animals is subject to AESC Guidelines for the use and care of animals, to the procedures specified in the application form, and to:

Noted Progress Report dated 3/12/90.

Signed *G. Lunn*
(Chairman: Animal Ethics Screening Committee)

Date 12/12/90

AP7.2

APPLICATION FOR CLEARANCE OF USE OF RADIOACTIVE ISOTOPES/
IONISING RADIATIONS IN AN ANIMAL EXPERIMENT

(Including neutral nuclear beams but excluding X-ray radiographic procedures).

1. Which ionising radiations are to be used?

Radioactive Materials

Machine Produced

Type	Activity (μ Ci or Bq)	Type	Dose (Gy)
15um Radio-labelled microspheres		X-ray	20 mins x 4 sessions (total of 80 mins per animal)
46 Scandium 500 μ Ci			
85 Strontium 500 μ Ci			
141 Cerium 500 μ Ci			
113m In 500 μ Ci			

2. For radioactive nuclides indicate:

(i) Where dispensing and administration will be done

Room No. 6M13

Building Medical School, York Road, Parktown

(ii) The quantities of radioactive materials to be given to each animal

.... 25 μ Ci of each isotope

.....

(iii) Where the animals will be housed, sampled and killed

.. Housed in Central Animal Unit .. Sample 6M13 .. Killed 6M13 ..

.. Congregated by Central Animal Unit

(iv) Where the examination and measurement of carcase material, organs body fluids etc. will be carried out

.. 6M13: Central Scintillation Counter Service

.....

(v) With which instruments the measurements will be made

Auto gamma Scintillation Spectrometer

Applicant's Signature *[Signature]* Date 7.12.84 Tel No. 647.7343 / 2256

FOR ADMINISTRATIVE USE ONLY

APPROVED



NOT APPROVED



Instruction for mode of disposal of carcase material/organs/body fluids:

1. Incineration without any restrictions.



2. Storage under refrigeration for ___ days before incineration.



3. Incapsulate in lime for disposal to ACE by Health Physics Service.



Comments and Recommendations:

Signature

[Signature]

Date

7/12/84

Director of Health Physics Service
or Deputising Officer

NOTE

Queries regarding the completion of this form should be directed to Professor R J Keddy at the Schonland Research Centre Tel. Ext. 3163.

APD 7.3