Antegrade and Retrograde Continuous Warm Blood Cardioplegia: A ^{31}P Magnetic Resonance Spectroscopic Study

by

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A thesis

submitted to the Faculty of Graduate Studies

in Partial Fulfillment of the Requirements for the Degree of

Master of Science

Department of Physiology, University of Manitoba

Winnipeg, Manitoba

and

Institute for Biodiagnostics

National Research Council Canada

and

St. Michael's Hospital, University of Toronto

ISBN 0-612-13192-0

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ANTEGRADE AND RETROGRADE CONTINUOUS WARM BLOOD CARDIOPLEGIA: A ^{31}P MAGNETIC RESONANCE SPECTROSCOPIC STUDY

BY

EDWARD FRANK HOFFENBERG

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

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Abstract

Retrograde Normothermic Blood Cardioplegia (RNBC) was studied to aid in optimizing the protective strategy used for open heart surgery. Isolated beating pig hearts were perfused antegradely (aortic root pressure = 85-95 mmHg) for 30 minutes. Arrest was induced with high K^+ blood cardioplegia delivered either antegradely (Group I, n=8), or retrogradely (Group II, n=8, coronary sinus pressure=35-55 mmHg), arrest was maintained for 60 minutes followed by 30 minutes of recovery. Intracellular pH, phosphocreatine (PCr), inorganic phosphate (Pi), and ATP were monitored continuously and non-invasively with ^{31}P Magnetic Resonance Spectroscopy (MRS) throughout the experiment and functional parameters (rate pressure product (RPP), and ± dP/dt) were assessed concurrently. Antegrade cardioplegia maintained high energy metabolites, pH, and myocardial function. RNBC resulted in an increase of Pi (197% ± 15% of control) and a decrease in PCr (51% ± 6% of control). Recovery of myocardial function was consistently lower in the retrograde group. The MRS data indicate ischemia occurred within 2 minutes of initiating retrograde perfusion. This study suggests that in the normal pig heart retrograde perfusion causes a transition into ischemic metabolism.

Acknowledgments

This project was completed at the Institute for Biodiagnostics at the National Research Council Canada in collaboration with St. Michael's Hospital, University of Toronto.

I would like to express my sincere gratitude to my supervisors, Drs. Roxanne Deslauriers and Tomás Salerno for their guidance, patience, and encouragement throughout my graduate studies.

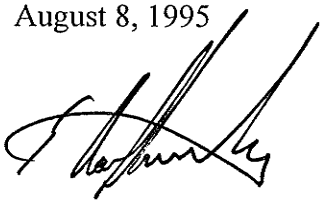
Many thanks are also extended to Drs. Alexander Aronov and Jian Ye for their assistance and perseverance during the many hours of surgery and experimental work. Technical assistance was provided by Lori Gregorash and Rachelle Perchaluk and statistical consultation was provided by Randy Summers.

Statement

The work reported in this thesis was undertaken in the Institute for Biodiagnostics of the National Research Council Canada in conjunction with the Department of Physiology of the University of Manitoba, and St. Michael's Hospital in Toronto. Supervision of this work was provided by Drs. Roxanne Deslauriers and Tomas Salerno.

I declare that the work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text; and that this material has not previously been submitted, either in part or in full, for a degree at this or any other university.

August 8, 1995



Edward Frank Hoffenberg

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1 General Introduction

Cardiac surgery has evolved from an age of venerated circumvention; a time when the heart was considered untouchable, "the fountaine of the vitall spirits" [65], to an age where remodeling, artificially assisting, or entirely replacing the heart has become common place. The increased complexity of surgical procedures performed today demand precision, time, and excellent visualization. This has traditionally been accomplished by arresting the heart, providing a stable and blood free surgical field. While the heart is arrested during the bypass procedure, the extracorporeal blood circulation provided by an artificial pump, oxygenator, and filter maintains the patient's metabolic stability. The procedure is performed in the following order; initiation of bypass, cardiac arrest, required surgical procedure, recovery of normal sinus rhythm, and finally *weaning* the patient off bypass. There are approximately 450 000 patients undergoing open heart surgery each year in North America, the majority of them requiring elective cardiac arrest. During cardiac arrest the normal coronary circulation is arrested and the challenge lies in providing the optimum degree of protection to the heart during this period. This challenge has led to the development of a number of protective strategies, all involving different forms of cardioplegia or cardioplegic delivery (see *Cardioplegia* page 1-7). It has been established that the peri- and post-operative recovery is highly dependent on the extent of *myocardial protection* provided during the arrest

period [42]. The extensive use of cardioplegia each year combined with the impact that the cardioplegic strategy has on patient outcome, makes cardioplegic research very pertinent. This study will investigate one form of cardioplegia known as *retrograde normothermic blood cardioplegia (RNBC)*.

1.1 *Statement of the Problem*

In order to adequately protect the heart during elective arrest when continuous normothermic blood cardioplegia is the strategy of choice, it is imperative to provide a sufficient supply of nutrient perfusion, supplying oxygen and removing metabolic waste products. Normally this is provided by antegrade perfusion by means of the proximal aorta, however for surgical procedures involving the aortic valve or for bypass procedures in patients with severe coronary occlusion, antegrade perfusion is most likely insufficient [13,19,40,58]. The other alternative is to perfuse *retrogradely*, into the coronary sinus and through the venous system (see *Coronary Venous System*, page 1-4) to reach tissue which would normally be perfused by the occluded coronary artery; a technique that was conceived almost one hundred years ago by Pratt [69]. Since the 1950's when retrograde perfusion was first applied clinically there has been controversy over its ability to adequately protect the heart during arrest. There has been a great deal of research probing the efficacy of RNBC, and a number of these studies have supported its clinical use. They demonstrated it provides equal, if not superior, protection for the myocardium relative to antegrade normothermic blood cardioplegia (ANBC) during some procedures

[2,10,40,58,78]. There is, however, reluctance on the part of surgeons to embrace RNBC as a reliable cardioplegic strategy. Concern stems from studies which have shown that with significant collateral vascularization in the venous bed of the myocardium, during retrograde perfusion a large percentage (65 - 75%) of the perfusate is shunted through the Thebesian veins as non-nutritive flow [80]. This, combined with the typically lower flow rates used in retrograde perfusion, means a potentially hypoperfused and poorly protected myocardium. The purpose of this study was to use ^{31}P magnetic resonance spectroscopy (MRS) to observe the continuous metabolic events in the isolated porcine heart during RNBC in order to evaluate its protective ability. There have been numerous clinical and animal studies on retrograde perfusion where a number of functional parameters have been examined, however never before has retrograde normothermic blood cardioplegia been looked at with the revealing eye of magnetic resonance spectroscopy (MRS).

1.2 *The Coronary Vessels*

In order to discuss RNBC adequately, it is first necessary to review the basic anatomy of the coronary vasculature. The coronary venous system is of particular relevance to RNBC.

i. Coronary Venous System

Venous drainage of the heart is ensured by a myriad of interconnecting networks of veins, with subepicardial routes communicating with all layers of the myocardium. This sponge-

like arrangement provides excellent drainage for the coronary circulation. The venous system can be divided into two groups; what has been traditionally known as the greater and lesser systems. *The greater system* consists of a subepicardial system of venous drainage which directly involves the coronary sinus, and the anterior cardiac vein system which drains directly in the right atrium. *The lesser system* consists of deeper venous system which drains directly into the cardiac chambers.

The coronary sinus is located in the right atrium, between the orifice of the inferior vena cava and the leaflets of the atrial-ventricular valve. The coronary sinus provides drainage primarily for the left side of the heart and is fed from the great, middle, and small cardiac veins as well as the left ventricular posterior veins, and the left atrial oblique vein of Marshall.

The anterior right ventricular wall is drained either by the anterior cardiac veins which empty directly into the right atrium, or by branches of the small cardiac vein which empties into the coronary sinus.

The lesser system consists of the arterioluminal, arteriosinusoidal, and Thebesian veins. The Thebesian veins provide drainage from capillary bed to cardiac chambers predominately in the right ventricle. Arterioluminal vessels provide bi-directional flow from capillary bed to coronary arteries. Arteriosinusoidal vessels are irregular and originate from coronary vessels and empty into either the capillary bed or directly into the ventricles.

ii. Coronary Arterial System

The heart's blood supply is provided with its own vasculature originating from the right and left coronary arteries and terminating mostly in the coronary sinus of the right atrium. The ostia of the coronary arteries are located at the base of the aortic root, superior to the leaflets of the aortic valve. The left coronary artery originates at the left posterior aortic sinus as the coronary osteum, and travels medially to divide into two branches, the left anterior descending (LAD) and the circumflex branches (Circ). The LAD travels inferiorly towards the apex through the anterior interventricular groove, and further branches off supplying the majority of the interventricular septum. The Circ. branch travels posteriorly around the left side of the heart within the left atrioventricular sulcus to terminate at the posterior interventricular sulcus. The right coronary artery originates at the right coronary sinus and travels along the right atrioventricular groove curving posteriorly and turns caudally to follow the posterior atrioventricular groove. There is extensive branching of the right coronary artery irrigating the right side of the heart. These branches include the conus artery, sinus and AV node arteries, right ventricular branches, right atrial branches, an acute marginal branch, posterior descending artery, and some terminal branches to the left atrium and ventricle.

1.3 *Cardioplegia*

The goal of cardioplegia is to provide an optimal protective environment for the myocardium allowing for a prolonged safe operative period. Cardioplegia achieves this by ensuring that the supply of oxygen and substrates is always sufficient to meet the metabolic demand of the heart. The supply is determined by the carrying capacity of the perfusate and the adequacy of its distribution. The demand of the myocardium is reduced significantly by imposing diastolic arrest (*see page 1-13*). Cardioplegia is any solution which electively halts all mechanical activity and provides nutrients when perfused through the heart. The membrane potential of the cardiomyocyte is normally approximately -90 mV. According to the Nernst equation $E_K = -60 \log_{10} \frac{[K^+]_i}{[K^+]_o}$, an increase in the extracellular concentration of K^+ causes the reversal potential and the membrane potential to decrease and the myocardial membrane becomes depolarized. With increasing depolarization, the generation and propagation of action potentials is prevented, thereby arresting the heart. The most common form of cardioplegia is the use of a solution containing KCl at a concentration of between 15 and 30 mM which depolarizes the membrane to approximately -58 and -40 mV, respectively. A successful cardioplegic strategy impedes the two proposed culprits in myocardial injury during surgery, ischemia and reperfusion injury (see pages 1-14 and 1-18, respectively).

i. History

To describe the evolution of cardioplegia one must go back to the beginnings of bypass surgery. Greater demands of more intricate procedures requiring a stable and clear surgical field brought about the evolution of bypass surgery. During this revolutionary type of surgery the heart is voluntarily arrested, blood is routed extracorporeally, artificially oxygenated then returned to the body. Initially an arrested heart meant an ischemic one and protection against ischemic injury was achieved by reducing the metabolic demand of the heart. This was accomplished through hypothermic diastolic arrest, using ice slush to cool the epicardium. This technique of heart preservation was brought into clinical use by Bigelow, Lindsay, and Greenwood in 1950 [5]. The idea that the heart could be arrested chemically was introduced by Melrose in 1955. Melrose helped bring about what is now the most common form of cardioplegia [53]. The use of crystalloid cardioplegic solution for induction and maintenance of arrest meant that not only could the metabolic demand of the heart be decreased (typically a reduction in oxygen consumption from 9 ml/min/100g to 0.6 ml/min/100g at 11°C) [9], there was now a means of metabolic waste removal and delivery of nutrients during arrest. Despite the development of chemical arrest in 1955, it was not until 1967 that the importance of myocardial management was fully recognized. Najafi and associates suggested that post-operative deaths and low cardiac output syndrome immediately following surgery were likely related to the lack of myocardial management during the surgery [28]. This realization brought about the development of new forms of cardioplegic solution. In

1972 Bretschneider and associates developed a new cardioplegic solution which was used clinically for elective cardiac arrest, and was named Bretschneider's solution [8]. The use of hypothermic blood cardioplegia, instead of crystalloid solution, was introduced by Buckberg and associates in 1978 [17]. This led to the next stage in the evolution of myocardial protection; a rise in the cardioplegic temperature towards normothermia (37°C in humans). Normothermic blood cardioplegia began in 1986 with Buckberg's recognition of its resuscitative capabilities as a *hot shot* in reperfusion [72]. The idea of continuous normothermic blood cardioplegia was introduced at the Myocardial Protective Symposium in Oxford in 1990 [75] and was tested clinically at St. Michael's Hospital, University of Toronto that same year. All the techniques discussed thus far have used antegrade delivery of cardioplegia. Surprisingly, the notion of perfusing retrogradely was first introduced in 1898 by Pratt [69] but was not used clinically until 1957 by Gott and associates, who used the technique in patients undergoing aortic valve procedures [20]. After more than 40 years of development and experience, retrograde perfusion has now evolved to a stage where RNBC may be initiated with a relatively uncomplicated and low risk, trans-atrial cannulation, which has been clinically shown to provide protection for the myocardium during arrest [10,16,40,54,58,77].

ii. Controversy

The developments in myocardial protection have generated a quagmire of controversy over which method provides optimal protection for the myocardium during arrest. The debate continues up until today over which methods are superior, blood versus crystalloid

and normothermic versus hypothermic. Although the literature in this area resembles a contest between the different techniques, I will discuss them with the notion that they should all be considered as a medley of techniques from which the surgeon may choose the optimal and most suitable method of protecting the heart.

a. Blood vs. Crystalloid

Crystalloid cardioplegia consists of an iso-osmotic salt solution with varying concentrations of electrolytes. An example is given in Table 1-1 and Table 1-2, showing the composition of standard *St. Thomas* and *modified Krebs Henseleit* solution, respectively. Crystalloid solution was originally developed with the advent of chemical arrest in the 1950's (*see page 1-8*) and has the advantages of being available in unlimited quantities, relatively inexpensive, offers a low risk of infection from blood borne disease, eliminates the need to conserve volume during surgery, does not have to be returned to the body after bypass, and is not affected by the roller pump during prolonged perfusion times.

Content	**(mM)**	**Content**	**(mM)**
NaCl	110.0	CaCl	1.2
MgCl	16	$NaHCO_3$	25
KCl	16	pH	7.4

Table 1-1: St. Thomas solution.

Content	(mM)	Content	(mM)
NaCl	118.0	CaCl	1.75
$MgSO_4$	1.2	$NaHCO_3$	25
KCl	3.5	KH_2PO_4	1.2
Glucose	11.0	BSA	0.5%
Dextran	2.0%	pH	7.4
EDTA	0.5		

Table 1-2: Modified Krebs Henseleit solution. Abbreviations BSA and EDTA are bovine serum albumen and ethylenediaminetetraaceticacid, respectively

The major disadvantage of crystalloid solution is that it is non-physiological. Although it has similar electrolyte concentrations as blood, there are no human proteins or factors such as complements and immunoglobulins. In the absence of red blood cells it is necessary to use a high oxygen content (PO_2 > 600 mmHg) in order to provide sufficient oxygen distribution. This extreme saturation may be toxic to endothelial cells. The toxicity of an ultra high PO_2 and the abnormally low concentration of proteins is thought to be responsible for the myocardial extracellular edema observed with prolonged perfusion of crystalloid solutions.

Blood based cardioplegia has the advantage of containing red blood cells which aid in the delivery of oxygen and contain abundant oxygen-derived free radical scavengers [37]. In addition blood has a buffering capacity provided by histidine and imidazole groups of blood proteins [42].

b. Normothermic vs. Hypothermic

As discussed previously cardioplegia had its debut with hypothermic temperatures of between 10 - 15 °C. Since its introduction in 1950, hypothermic cardioplegia has been popular as a means of myocardial protection. Its popularity is due, in part, to a dramatic reduction of the heart's metabolic demand, requiring less dependence on continuous flow. This provides the surgeon with the option of using brief ischemic periods for better visualization and a measure of safety in case of complications. Unfortunately there are a number of disadvantages to cooling the heart. Cooling has deleterious effects on enzymatic function reducing glucose utilization, intracellular hydrogen regulation, and (ironically) ATP generation and tissue oxygen uptake [38,48,57]. The ability of hemoglobin to deliver oxygen is severely inhibited by hypothermia because of the left-ward shift in the hemoglobin-oxygen dissociation curve. At a temperature of 12°C dissociation of oxygen from hemoglobin does not contribute to oxygen delivery [23]. Other reported disadvantages of hypothermia are a reduction in membrane stability [52], a decrease in calcium sequestration [74], and a reduction of platelet function [83]. The lower temperature has been shown to cause an increase in intracellular edema [45], increase in blood viscosity sometimes leading to *sludging*, and increase in extracellular glucose concentration sometimes leading to severe hyperglycemia [23,32]. Hypothermic cardioplegia is very often used intermittently allowing for drift in myocardial

temperature. Christakis gives a good summary of some problems associated with hypothermic cardioplegia.

> ...during the majority of the cross-clamping period myocardial temperatures are higher than 10 - 11 °C and closer to 18 - 20 °C. We are, therefore, faced with warming hearts with increasing metabolic rates that receive boluses of cold blood whose oxygen cannot be easily used because of the left-ward shift in the oxygen-haemoglobin dissociation curve. Furthermore the heart suffers from the negative effects of hypothermia [77].

Another observed disadvantage of cooling the heart is the metabolic imbalance created by unequal slowing of different energy dependent reactions. For instance the energy dependent sodium-potassium pump is inhibited preferentially with decreasing temperature as opposed to other energy dependent pumps [23].

The argument for using hypothermic arrest is to minimize the metabolic demand of the heart, however there is little difference between the metabolic demand of the arrested myocardium at 12°C and 37°C. The oxygen demand of a beating, unstressed heart is approximately 10 ml/100g/min. Normothermic cardioplegia reduces the demand to approximately 1 ml/100g/min. Reducing the temperature to 12°C from 37°C only reduces the oxygen demand a further 0.8 ml/100g/min [9]. Normothermic cardioplegia provides a period of warm aerobic arrest, during which it has been shown to resuscitate the heart and avoids all the disadvantages described above which have been associated with hypothermia. One of the disadvantages of normothermic cardioplegia is that any area of the myocardium which is inadequately perfused is subject to normothermic ischemia and possible irreversible injury. Situations where the myocardium may be

inadequately perfused could occur during antegrade delivery in patients with severe coronary disease, where retrograde delivery may not adequately perfuse the right ventricle, or during surgery where flow must be interrupted for any reason. Studies have shown that these disadvantages of normothermic cardioplegia are manageable. One reason to stop flow during surgery is to improve visualization, however Salerno and associates have shown that adequate visualization may be achieved by using a jettison of air or saline [76]. Our group has shown that normothermic blood cardioplegia may be turned off if needed for a period of up to 10 minutes without causing irreversible damage. This may be repeated 6 times, with 5 minutes of reperfusion between ischemic episodes [85].

1.4 *Ischemia*

In order to optimize cardioplegic delivery, one must fully understand the two factors involved in myocardial injury that cardioplegia strives to overcome: *ischemia* and *reperfusion injury*. Although these two pathologies are very closely related they will be discussed separately. Ischemia is derived from the Greek words *ischo* meaning to "hold back " and *haima* "blood", literally meaning not enough blood. Perhaps a more suitable definition of ischemia is given by Hearse, "[Ischemia is] a condition in which coronary blood flow is inadequate to permit the maintenance of a steady-state metabolism" [89]. Ischemic injury does not only occur because of inadequate substrate delivery, but

includes inadequate washout of wastes such as lactate, protons, and CO_2. Myocardial ischemic injury can result from a number of pathologies which restrict blood flow through the coronary circulation, such as coronary occlusion or spasm. The complications ensuing ischemic injury are manifest in signs such as loss of contractile force, depletion of high energy phosphates, an increase of inorganic phosphate, lactate, intracellular acidosis, and an increase in creatine kinase (MB) activity [39]. More severe and prolonged ischemia is likely to lead to a myocardial infarction (MI) [42].

i. Mechanisms

Cardiomyocytes change from aerobic to anaerobic metabolism when the rate of O_2 delivery becomes less than is required to meet the metabolic rate of the myocardium. A consequence of anaerobic metabolism is the decline of mitochondrial adenosine triphosphate (ATP) production. As a result there is a gradual decrease in ATP, however the decline is buffered by replacement of ATP from the creatine kinase catalyzed reaction breaking down creatine phosphate (CP):

$$CP + ADP + H^+ \Leftrightarrow \text{creatine} + ATP, \qquad ATP \Rightarrow ADP + Pi.$$

Therefore a decrease in the ratio CP/Pi is a more sensitive indicator of anaerobic metabolism than changes in ATP concentration. This buffering effect will maintain energy supplies for a few minutes until the supply of PCr is depleated. The reason ATP continues to decline even with buffering is multifactorial. The major causes can be

attributed to an increased flux of Ca^{2+} at the sarcoplasmic reticulum, inhibition of transport of ATP from mitochondria by acyl CoA, glycogen breakdown and resynthesis, and triglyceride breakdown [63]. The decrease in high energy phosphates such as ATP and CP, and the increase in Pi, removes inhibition from and stimulates phosphofructokinase (PFK), hexokinase, and phosphorylase *b*; augmenting glycolysis.

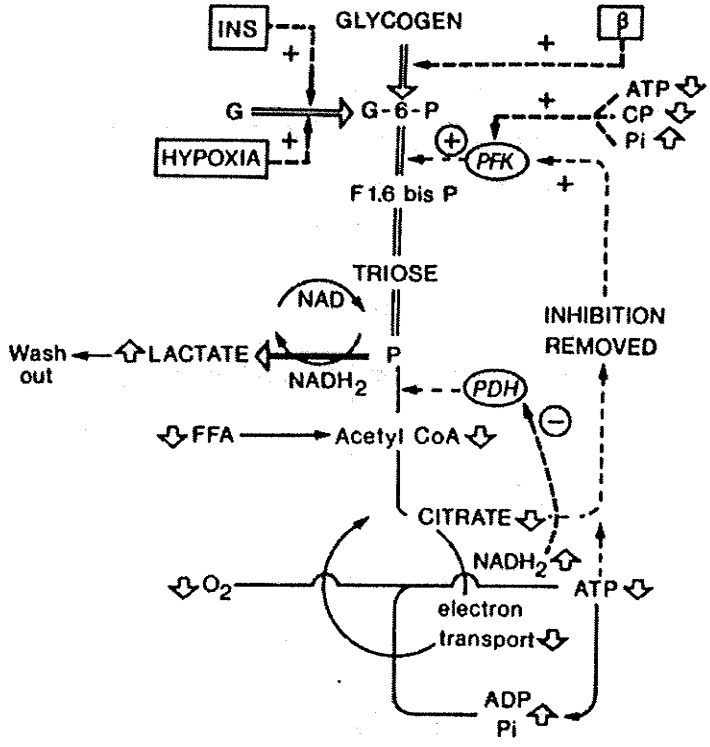


Figure 1-1: Effects of mild ischemia on glycolytic flux.

During ischemia fatty acid is unable to be oxidized leading to an accumulation of free fatty acids, acyl CoA, and acyl carnitine [63]. The accumulation of these metabolites inhibits some membrane function such as mitochondrial translocase, the sodium/potassium pump, and phospholipid cycles [63]. Accumulation of the breakdown products of phospholipids form micelles and are thought to be highly membrane active,

perhaps responsible for early ischemic arrhythmias [63]. The decrease in fatty acid oxidation indicates a decrease in citrate which removes the normal inhibition on PFK and further augments glycolysis. All of these factors cause a net increase in glycolytic flux in mild ischemia (Figure 1-1).

Major sources for protons are the breakdown of ATP and lactate from glycolysis. If anaerobic glycolysis is complicated with low or no flow ischemia there is an accumulation of protons and tissue pH is lowered. Low tissue pH is a strong inhibitor of glycolysis through its action on PFK, overpowering any stimulation of glycolytic flux seen in mild ischemia. In addition, the accumulation of NADH strongly inhibits glycolysis. Mild and severe ischemia can be differentiated by their effects on the glycolytic rate: mild ischemia accelerates glycolysis and severe ischemia inhibits glycolysis [60].

The decrease in contractility accompanying ischemia can be explained by protons competing with Ca^{2+} for binding sites on contractile proteins (i.e. troponin), impairing actin-myosin interaction. A decrease in intracellular pH also closes Ca^{2+} channels, inhibits Ca^{2+} channel activity, and reduces sensitivity of the sarcoplasmic reticulum to Ca^{2+}, all of which results in a decrease in contractility. CO_2 is released from bicarbonate during acidosis and may also be involved in decreasing contractility. The accumulation of Pi reduces the Ca^{2+} sensitivity of contractile proteins, providing another explanation for the observed decrease in contractility seen during ischemia [60].

1.5 *Reperfusion Injury*

When ischemia has caused reversible biochemical changes in the myocardium, the most effective method of recovery is reperfusion. There is, however, a paradoxical risk involved in reperfusing ischemic tissue, which has been observed to cause additional injury other than from the ischemia itself. This phenomenon is known as *reperfusion injury*. This is a clinically relevant phenomenon which is indicated by a depression of myocardial function, arrhythmias, stunning, and/or microvascular damage after revascularization or use of thrombolytic agents such as streptokinase in patients suffering from ischemic heart disease [61].

i. Mechanisms

Calcium overload has been implicated as a possible cause of reperfusion injury. The theory is based upon the observation that after a period of severe ischemia where there was irreversible damage to the myocardium, an excessive accumulation of intracellular calcium (Ca_i^{2+}) occurred during reperfusion [82]. In addition, it has been proposed that oxygen derived free radicals (ODFR) may be responsible for development of reperfusion injury [64]. I will briefly discuss the current theories explaining both calcium overload and ODFR and how they interrelate.

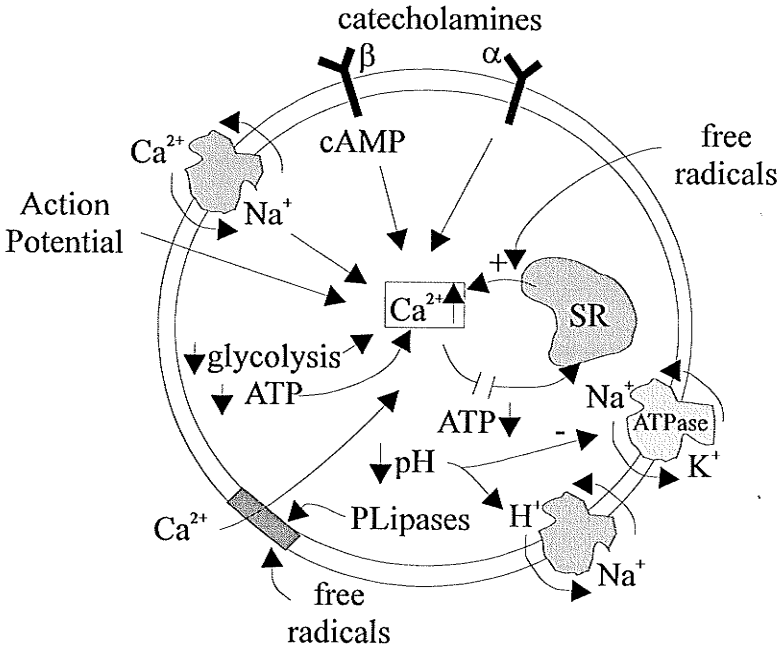


Figure 1-2: Proposed mechanisms for calcium overload during reperfusion.

a. Calcium Overload

Rapid increases in Ca_i^{2+} during reperfusion have led to the belief that intracellular calcium overload may contribute to myocardial injury during reperfusion. The mechanisms of injury are not well understood. One proposal for the influx of Ca^{2+} is that during ischemia damage is inflicted upon the sarcolemma and its Ca^{2+} channels, allowing the internal passage of Ca^{2+}. During reperfusion there is a large influx of Ca^{2+} intracellularly leading to excess accumulation of Ca^{2+} in the mitochondria and a subsequent decline in ATP production. This is one possible explanation for the observed reperfusion related cell necrosis [61]. During reperfusion, with the restoration of ATP stores, there will be uptake of Ca^{2+} into the sarcoplasmic reticulum (SR) and an

augmented release of Ca^{2+} during the next systole. This *overcycling* of Ca^{2+} is thought to be involved in reperfusion induced arrhythmias because the effect may be lessened by ryanodine or caffeine which inhibits Ca^{2+} sequestration in the SR [84]. Another proposed mechanism for Ca^{2+} influx involves the Na^+/Ca^{2+} and Na^+/H^+ exchanges. During ischemia there is a rise in Na_i^+ because of gradual leakage through fast Na^+ channels, which remain active during ischemia [82]. In addition, the increase in the intracellular proton concentration as pHi decreases during ischemia (see *Lactate and pH* page 1-29), causes a further rise in Na_i^+ by driving the Na^+/H^+ exchanger. The net increase in Na_i^+ drives the Na^+/Ca^{2+} exchange in the reverse direction, causing an increase in Ca_i^{2+}. The Na^+/Ca^{2+} exchanger is sensitive to pH so that during ischemia when the extracellular pH drops significantly, the Na^+/Ca^{2+} exchanger will be inhibited [68]. However during reperfusion when pH is returned to normal, inhibition of the Na^+/Ca^{2+} exchanger is removed and there is a rapid reactivation of the exchanger causing a subsequent inward flow of Ca^{2+}. The Na^+/K^+ ATPase pump plays an important role in Na^+ homeostasis. During ischemia the loss of ATP and the accumlation of Pi and H^+ inhibit the normal function of the energy dependent Na^+/K^+ ATPase pump, causing a further increase in Na^+_i [31]. Upon reperfusion, the restoration of ATP and the washout of H^+ and Pi restore the pump's function. In addition, the pump's activity is augmented by the increased Na^+_i. It is thought that the Na^+/K^+ ATPase pump is responsible for most of the decline in Na^+_i observed during reperfusion [82].

b. Oxygen Derived Free Radicals (ODFR)

ODFR are formed during ischemia and particularly during reperfusion [7]. The primary sources for ODFR related to the ischemic myocardium, are postulated to be from xanthine oxidase involved in purine degradation, the activation of neutrophils, activation of the arachidonate cascade, autoxidation of catecholamines, and damage of the mitochondrial respiratory chain [7]. During reperfusion they are thought to be responsible for compounding the effects of Ca^{2+} overloading, discussed above. Reintroduction of oxygen during reperfusion can form ODFR through the reaction:

$O_2 + e^- \rightarrow \bullet O_2^-$ (superoxide radical)

$\bullet O_2^- + e^- + 2H^+ \rightarrow H_2O_2$ (hydrogen peroxide)

$\bullet O_2^- + H_2O_2 + H^+ \rightarrow O_2 + H_2O_2 + \bullet OH$ (hydroxyl radical)

$\bullet OH + e^- + H^+ \rightarrow H_2O$

ODFR help increase Ca_i^{2+} during reperfusion through two proposed mechanisms. Hydroxyl radicals, formed in the reaction described above, react with lipids and initiate another reaction known as *lipid peroxidation.* Lipid peroxidation alters trans-membrane transport mechanisms and increases Ca^{2+} permiability [46]. ODFR are also believed to oxidize certain residues of membrane-bound proteins which are involved in ion transport. For example the Na^+/Ca^{2+} exchange has been shown to be activated through oxidation of the methionine residue by ODFR [70]. There is still a great deal of uncertainty about the exact mechanisms by which ODFR affect Ca_i^{2+} concentration. Attempts to reduce the

effect of lipid peroxidation by enhancing the activity of the free radical scavanger, *glutathione reductase*, have failed to demonstrate any observed protection from reperfusion injury [82]. In addition, lipid peroxidation only occurs during the late events of reperfusion and therefore does not explain the early rise in Ca_i^{2+} seen during the early stage of reperfusion. Other possibilities include stimulation of α or β adrenergic receptors by catecholamines released during reperfusion.

1.6 *Evaluating Cardiac Condition*

As stated above the goal of cardioplegia is to provide optimum myocardial protection for optimal post-operative recovery. The recovery of the patient is dependent on the post-operative ventricular function, or how effectively the heart moves blood through the body. This is measured in terms of cardiac output (CO) which is equivalent to the heart rate (HR) multiplied by the stroke volume (SV), CO = HR x SV. The SV is dependent on the loading conditions and on the contractile function. Although it is known that the normal cycling of calcium which occurs during contraction and relaxation is dependent on the HEP, there is no clear correlation between the contractile function of the heart and the intracellular concentrations of HEP. However it has been demonstrated that if the level of ATP drops below 80% of normal, there is a dramatic reduction in functional recovery of the ischemic heart. A number of conclusions about cardiac condition can be made based on the levels of these essential metabolites. Therefore in this study, the focus

for evaluating cardiac condition was the contractile function and metabolic status of the myocardium.

i. Contractile Function

Contractility of the heart describes the dynamics of ventricular contraction and can be defined as, the rate of contraction and ability to reach peak tension or pressure independent of other factors such as heart rate, preload, and afterload [62]. Contractility may be equated with *inotropic state* and may be increased with inotropic agents such as digitalis or adrenaline. With experimental animals it is possible to measure contractility using cardiac function curves, however it is more difficult to make clinical assessments of contractility. One method which is thought to be an appropriate measure of cardiac contractility is *dP/dt* [25].

a. dP/dt

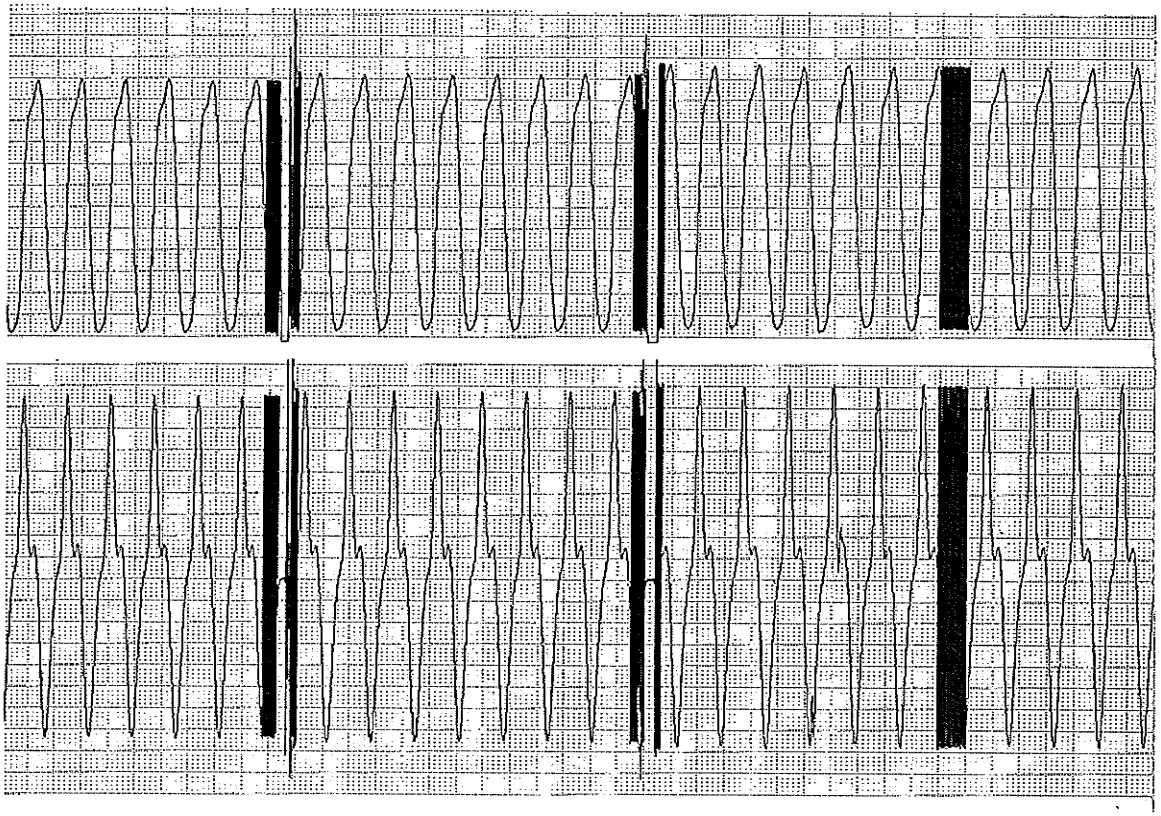


Figure 1-3: Tracing of left ventricular pressure curve (top) and dP/dt (bottom).

dP/dt is the rate of change in ventricular pressure with respect to time. dP/dt is normally generated by a computer which differentiates the standard pressure curve from the ventricular contractions. Figure 1-3 shows a sample tracing of the left ventricular pressure curve and dP/dt. Maximum dP/dt corresponds to the maximum rate of increase in pressure, which correlates to the maximum contraction strength of the ventricle [26]. Minimum dP/dt corresponds to the maximum rate of relaxation of the ventricle, which is of particular interest because it is related to the function of the SR and therefore the metabolic status of the myocardium. Although information about the contractile state of

the myocardium may be inferred from dP/dt, there is a problem with using this parameter as an absolute quantification. dP/dt is affected by factors such as preload and afterload, and is therefore not an independent variable. In this study preload and afterload are kept constant, maintaining dP/dt as an independent variable, and is of value in assessing contractility.

b. Myocardial Relaxation

When thinking of cardiac function one naturally considers contraction of paramount importance, but of equal importance is cardiac relaxation. The left ventricle must eject its volume of blood into a high pressure arterial system, then fill under sufficiently low pressure to avoid atrial or pulmonary hypertension. The dependence of pulmonary vascular function on ventricular relaxation is illustrated by a number of cardiopathologies where relaxation is restricted, such as pericarditis, constrictive pericarditis, and restrictive and hypertrophic cardiomyopathies. All of these conditions decrease minimum dP/dt, increase filling pressure, and progressively lead to pulmonary hypertension and pulmonary edema. Myocardial relaxation is the process by which the myocardium returns to its initial or resting length and tension. Relaxation begins after the second phase of ejection in the cardiac cycle and ends with the onset of systole. The process of relaxation is highly energy dependent. A decrease in availability of intracellular ATP will lead to a decrease in its availability for ATPase induced cross-bridge detachment and active sequestration of Ca^{2+} in the SR, leading to the depression of - dP/dt [4].

c. RPP

The developed pressure (DP) is an indicator of contractility because it measures the strength of the contraction from end diastole to peak systole. One difficulty with using the DP as a functional parameter is that it varies with heart rate. One solution is to pace the heart and standardize the HR for all experiments, however it is more technically challenging to pace the heart while in the bore of the MR instrument without decreasing the signal to noise ratio (*see* MR Equipment *page 1-39*). An alternative is to use the rate pressure product (RPP) which takes the HR into account; RPP = HR x DP. Many studies have utilized RPP as a funtional parameter for ventricular contractility [3].

ii. Vascular function

At the level of the capillary a single endothelial cell layer and a basement membrane is all that separates the contents of the vessel from the interstitium. The endothelium plays an essential role as a filter, containing pores which allow the free passage of plasma, ions, small solutes, glucose, lactate, and amino acids, but prevents the free passage of macromolecules such as proteins and globulins. Barricading the passage of these macromolecules creates an *oncotic pressure*, a force which prevents most plasma from filtering out into the interstitium. Therefore the barrier provided by the endothelium to macromolecules in the blood is one of the forces which prevents interstitial edema by creating an oncotic pressure of sufficient magnitude to allow only a small amount of

filtration into the interstitium. The forces involved in capillary exchange are summarized in the Starling equation:

The net force on capillary exchange = $(P_c + \pi_i) - (P_i + \pi_c)$,

where P_c and P_i are the capillary and interstitial hydrostatic pressures, respectively and π_c and π_i are the capillary and interstitial oncotic pressures, respectively.

The coronary microvasculature is capable of autoregulation of its blood supply. This autoregulation of blood flow is mediated through a number of neural, metabolic, and endothelial derived factors. Neural mediation acts predominantly through sympathetic activation. Therefore a neurostimulant such as adrenaline, which increases contractility and heart rate, also increases coronary blood flow through the activation of β-receptors and subsequent vasodilation. The metabolic status of the myocardium has a direct effect on coronary blood flow, primarily mediated through the vasodilator adenosine. As the supply of ATP is reduced there is an increase in ADP and Pi. This is thought to activate the 5' nucleotidase mediated breakdown of AMP into adenosine. Adenosine then acts by stimulation of AMP formation, and by hyperpolarizating the smooth muscle and preventing the activation of voltage dependent Ca^{2+} channels. Endothelium-derived relaxation factor (EDRF) is released in response to platelet aggregation and subsequent release of ADP and serotonin [61]. EDRF is actually nitric oxide and acts to dilate coronary vessels by stimulating smooth muscle cGMP dependent reuptake of Ca^{2+} into the sarcoplasmic reticulum.

In addition to myocyte injury, ischemia can cause significant microvascular damage. The production of ODFR during ischemia (*see Oxygen Derived Free Radicals page 1-21*) is thought to be the primary cause of microvascular damage [71]. The damage caused by ODFR during ischemia makes the endothelium more permeable to macromolecules, thereby reducing the oncotic pressure, and allowing a greater amount of filtration into the interstium. If filtration is extensive enough to cause interstitial edema, the swelling tissue will compress the local microvasculature, preventing normal distribution of the perfusate. This is one explanation for the "no-reflow" phenomenon observed during reperfusion [71].

In addition to damage from ischemia, the endothelium may be damaged by the cardioplegia itself. There is a growing concern of the deleterious effects of hyperkalemic cardioplegia. It has been proposed to cause reversible disturbances in cardiomyocyte calcium handling and irreversible alterations of myofibril function [43]. Studies have shown that hyperkalemic cardioplegia may also cause endothelial cell damage [27,49]. One proposed mechanism involves an increase in intracellular calcium due to the electrogenic Na^{+}/Ca^{2+} exchanger being drivien in response to membrane depolarization.

iii. Metabolic Status

The concept of supply vs. demand and the consequences of an inbalance between them was discussed in *Ischemia page 1-14*. The changes in HEP during ischemia and their causes was also discussed. In this study NMR is used to monitor HEP to determine the

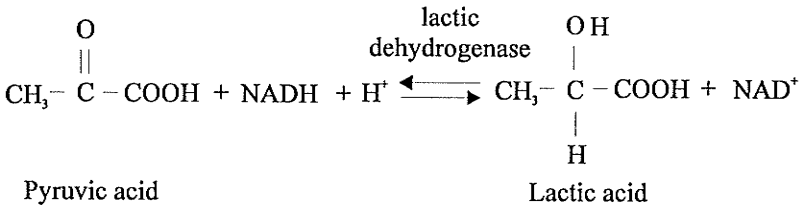
metabolic status of the myocardium, however there are other parameters which may be considered.

a. Lactate and pH

Intracellular pH is a good indicator of the metabolic status of the myocardium. The pH can be altered by the two end products of glycolysis, pyruvic acid and hydrogen atoms, shown here in the net equation for glycolysis;

Glucose + 2ADP + 2 PO_4 $\Leftrightarrow$ 2 Pyruvic acid + 2ATP + 4H.

The hydrogen atoms combine with NAD^+ to form NADH and H^+. This combines with pyruvic acid in the lactic dehydrogenase catalyzed reaction to form lactic acid and NAD^+.



During anaerobic conditions there is a build up of pyruvic acid and H^+, and a subsequent increase in the formation of lactic acid. Since lactate easily leaves the cell to be washed away by extracellular fluid, it acts as a sink for protons, delaying the dissolution of glycolysis (see *Ischemia* page 1-14). It is possible to monitor the level of lactate continuously with ^1H NMR spectroscopy [79]. In addition to levels of lactate, NMR is also able to monitor pHi continuously (see *Intracellular pH* page *1-38*). A gradual decline in pHi occurs when the buffering capacity of lactic acid is insufficient.

1.7 *Magnetic Resonance Spectroscopy*

i. Background

Since the Nobel prize winning discovery of nuclear magnetic resonance (NMR) by Purcell and Bloch in 1952, it has rapidly evolved to become one of the most important analytical tools of the 20th century. The best known application of NMR is imaging (MRI), which can be used to non-invasively scan tissue *in vivo* and produce sharp three dimensional images without the use of ionizing radiation.

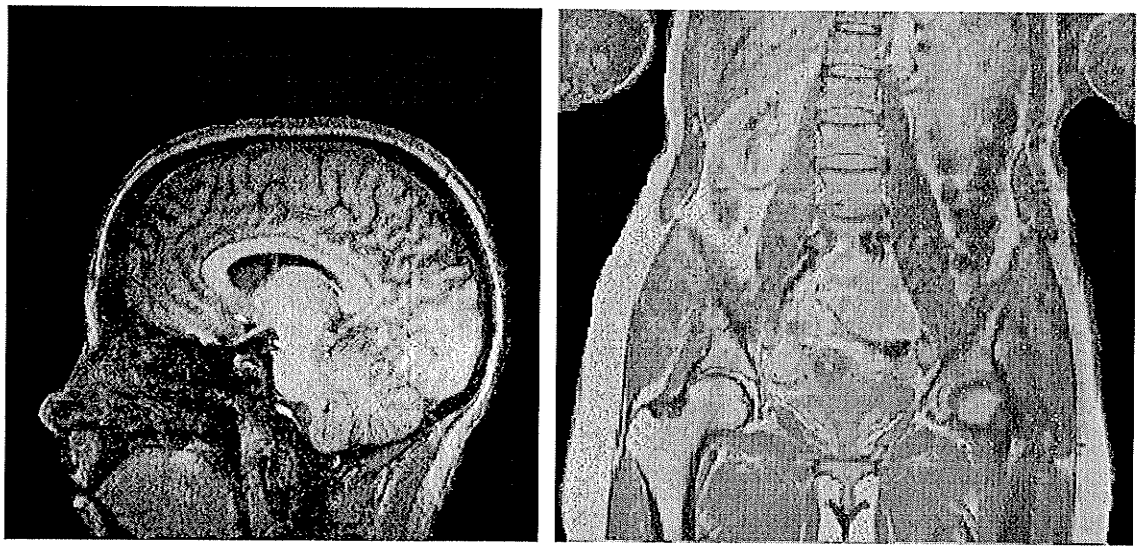


Figure 1-4: Left) sagital section of skull, right) transverse section of thorax and pelvis.

Another application of NMR is magnetic resonance spectroscopy (MRS), which has traditionally served the chemist or physicist in determining structures of solids, chemical compositions, and molecular structures, but is now emerging as a diagnostic tool. ^{13}C-MRS has been used successfully to follow brain amino acid metabolism in humans [24]

and to study glycogenolysis and gluconeogenesis during fasting in the liver [73]. ^{1}H MRS may be used to resolve a number of compounds *in vivo*, such as the levels of inositols, choline-containing compounds, N-acetyl aspartate (NAA), and glycine from brain tissue [14]. In addition, ^{1}H MRS may be used to observe changes in levels of creatine, glycine, lactate, lipids and many other metabolites. ^{31}P MRS has been used to study utilization and recovery of high energy metabolites in human skeletal muscle [6], to provide prognostic information from neonatal brain tissue for disorders such as asphyxia, focal seizures, and stroke [29], and to study differences in patients' liver metabolites with cirrhosis and hepatitis [56]. However of all the applications of ^{31}P MRS, its use for evaluation of the heart is among the most difficult. This is due to difficulty in obtaining the spatial resolution necessary to define relevant volumes of interest (VOI) and to the undesirable (from an NMR point of view) motion of the beating heart. Despite these difficulties ^{31}P MRS has become invaluable to cardiac research because it offers unique opportunities to examine the heart while it is subjected to a variety of physiological conditions. ^{31}P MRS is a powerful tool because it can monitor not only cardiac function, but also the metabolic status.

Quantification of high energy phosphates (HEP) in tissue has classically been performed with the technique known as *freeze clamping*. This technique involves removal of tissue samples with a biopsy needle which are rapidly frozen (in less than a second) in liquid nitrogen. High Performance Liquid Chromatography (HPLC) is then used to quantify the levels of HEP, such as PCr, and ATP, as well as Pi. This has proven an accurate and

reliable method of analysis, but it is both highly invasive and cannot practically be performed continuously.

The energy requirements of the heart necessary for the mechanical function are met directly by ATP and PCr. As long as the supply of blood and oxygen are meeting the demands of the myocardium Pi, PCr, and ATP will remain unchanged. However as soon as flow is interrupted, or oxidative phosphorylation is not meeting the energy demands of the myocardium, there is a drop in PCr and a concurrent sharp rise in Pi within seconds [79]. The PCr/Pi ratio provides information about the metabolic state of the myocardium and a sensitive test for ischemia (see *Ischemia* page 1-14).

Before an evaluation of ^{31}P MRS and a discussion of different NMR techniques and their limitations is conducted, a brief overview of the underlying principles behind NMR will be provided.

ii. Resonance

Nuclear magnetic resonance (NMR) is a technique based on the principle that certain nuclei have an odd number of protons giving them what is known as *net nuclear spin.* Some examples are ^{1}H, ^{2}H, ^{15}N, ^{17}O, ^{39}K, ^{31}P, ^{13}C, and ^{23}Na. Since the nucleus is charged its spin causes a small magnetic field producing a *magnetic moment.* It is this magnetic moment which causes the nucleus to behave as a small magnet. If these nuclei are placed in a strong magnetic field, referred to as B_0, the magnetic moments of the nuclei will align themselves parallel or antiparallel to the axis of the field, along the z axis (Figure 1-5). It is important to clarify that by alignment of the nuclei it is meant that the

larger population of spins are aligned in parallel with the field. The difference in populations is proportional to the strength of the B_0 field and results in a net macroscopic magnetization. The process of alignment with B_0 is known as the *longitudinal or spin-lattice relaxation* and has a characteristic time constant called T_1.

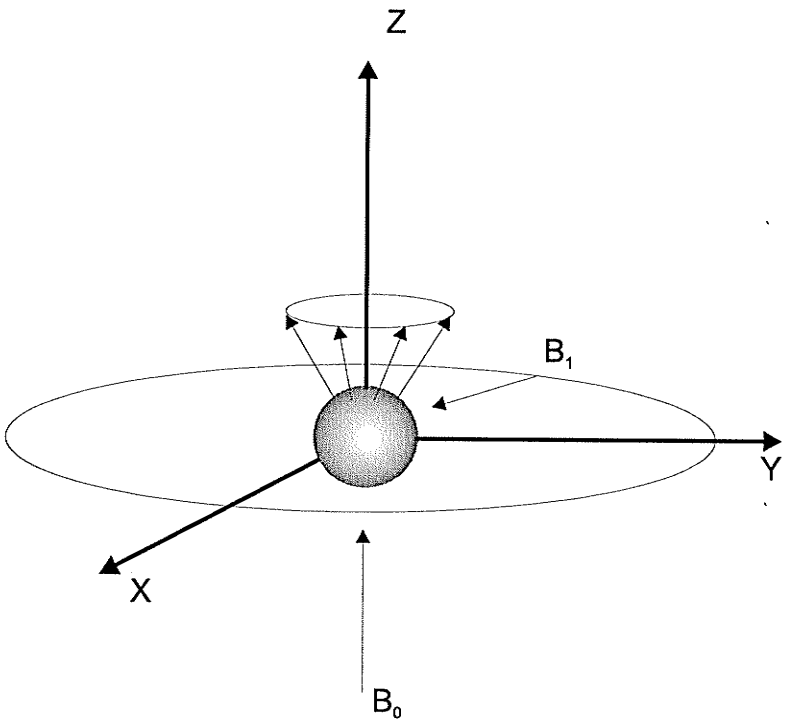


Figure 1-5: Nucleus precessing about the z axis with B_0 parallel to z.

The nucleus spins about its axis, however if it is tipped away from B_0 then it will precess about the z axis (Figure 1-5) with a natural angular frequency, known as the Larmor frequency (ω_0). ω_0 is equal to γB_0, where γ = proportionality constant, known as the magnetogyric ratio of the nucleus [18]. A typical magnetic field strength for use with humans is between 0.1 tesla (T) and 2.0T. The field strength typically used for a ^{31}P

MRS experiment involving animals is very large; between 1.5 and 11.4T. In order to compare the strength of this kind of magnetic field, keep in mind that the magnetic field strength of the earth is approximately 5×10^{-5}T.

The magnetic field that is experienced by a particular nucleus in a strong magnetic field is known as the local magnetic field B_L and is dependent on its chemical environment. For example small magnetic fields may be caused by neighboring electrons or other charged species altering B_L. Therefore two identical nuclei with different chemical environments will experience different B_L, and with the Larmor equation ($\omega_0 = \gamma \mathbf{B_0}$ **(1-s)**, s = contribution of a small secondary field caused by surrounding electrons) can be shown to have different positions (known as *chemical shift*) in the NMR spectrum, caused by their chemical environments. The chemical shift is usually expressed in the dimensionless units of parts per million (ppm).

It is possible to apply another magnetic field which is perpendicular to and rotates about the z axis with equal frequency to the Larmor frequency. This is known as a rotating frame of reference and the field is defined as B_1 (see Figure 1-5). This rotating field B_1 may be generated by a small coil with a voltage across it oscillating with the Larmor frequency. Applying B_1 causes the nucleus to tip towards the x-y plane, by an angle θ, where $\theta = \gamma \mathbf{B_1 tp}$ and tp is the time the field is applied. A pulse of B_1 that has a duration $\gamma \mathbf{B_1 tp} = \pi/2$ is known as a 90° pulse [18]. A 90° pulse causes the nucleus' magnetic moment to tip onto the x-y plane. Now the net magnetization precesses in the x-y plane, about B_0, at ω_0, and causes an electromotive force (e.m.f.) to be induced in the coil of the

probe which surrounds the sample. A coil is, in essence, a loop of wire which encompasses the sample with its axis perpendicular to the axis of B_0. Typically coils consist of numerous wrappings of wire which may incorporate a number of variable capacitors for tuning and optimizing the field homogeneity. The e.m.f. oscillates at ω_0 and according to Faraday's law of magnetic induction, it's magnitude depends on the amount of net magnetization, which is proportional to the concentration of nuclei in the sample. As soon as precessing in the x-y plane begins there is a natural decay representing the transverse (or spin-spin) relaxation, caused by spin-spin interaction. The time for this decay to reach 63% is the *transverse (spin-spin) relaxation time constant* (T_2).

iii. The NMR spectrum

The e.m.f. detected by the coil is amplified and digitized in the spectrometer. This signal is called a free induction decay (FID, see Figure 1-6). The FID represents the initial e.m.f. induced by the magnetization precessing in the x-y plane with a decay *approximately* equal to T_2. In order to separate the different components of the FID a mathematical operation known as a *Fourier transformation* is performed. A Fourier transformation converts the time domain signal (FID) to a frequency domain signal (spectrum). This conversion produces a spectrum with peaks at distinct frequencies (Figure 1-7).

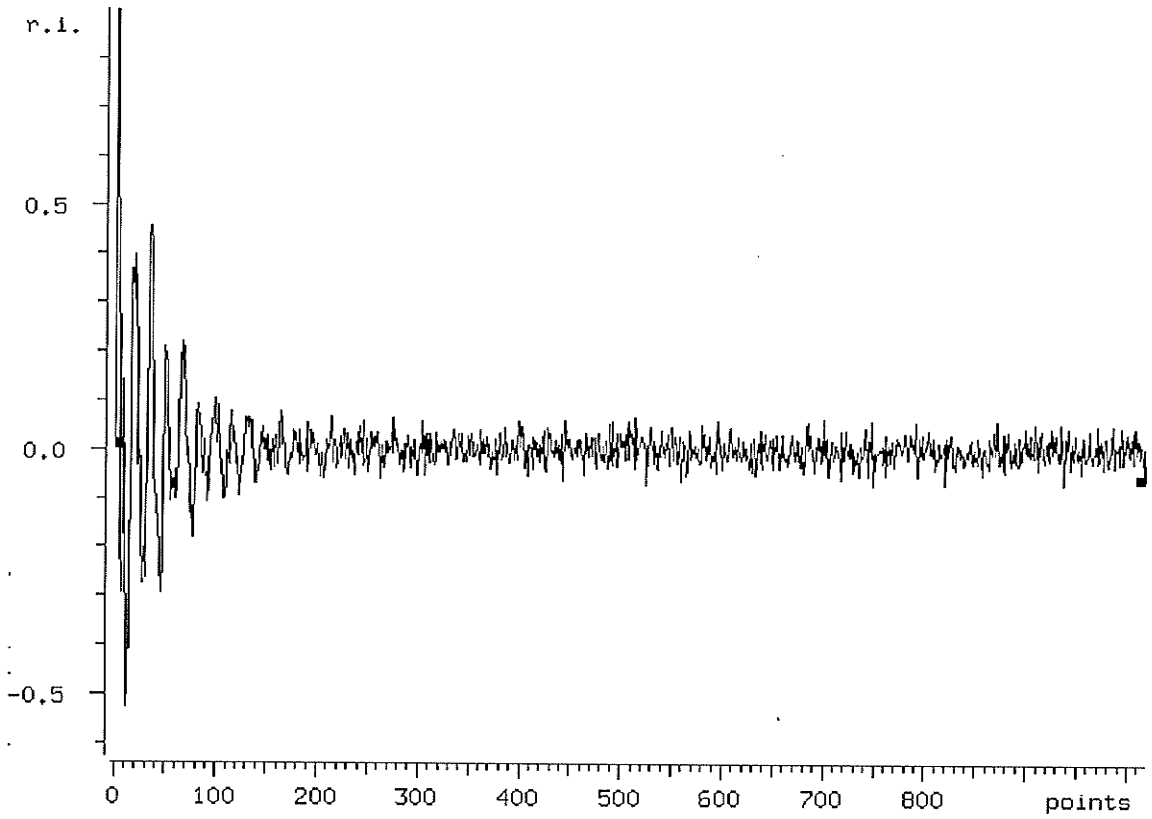


Figure 1-6: ^{31}P Free induction decay signal of a beating heart at 7T.

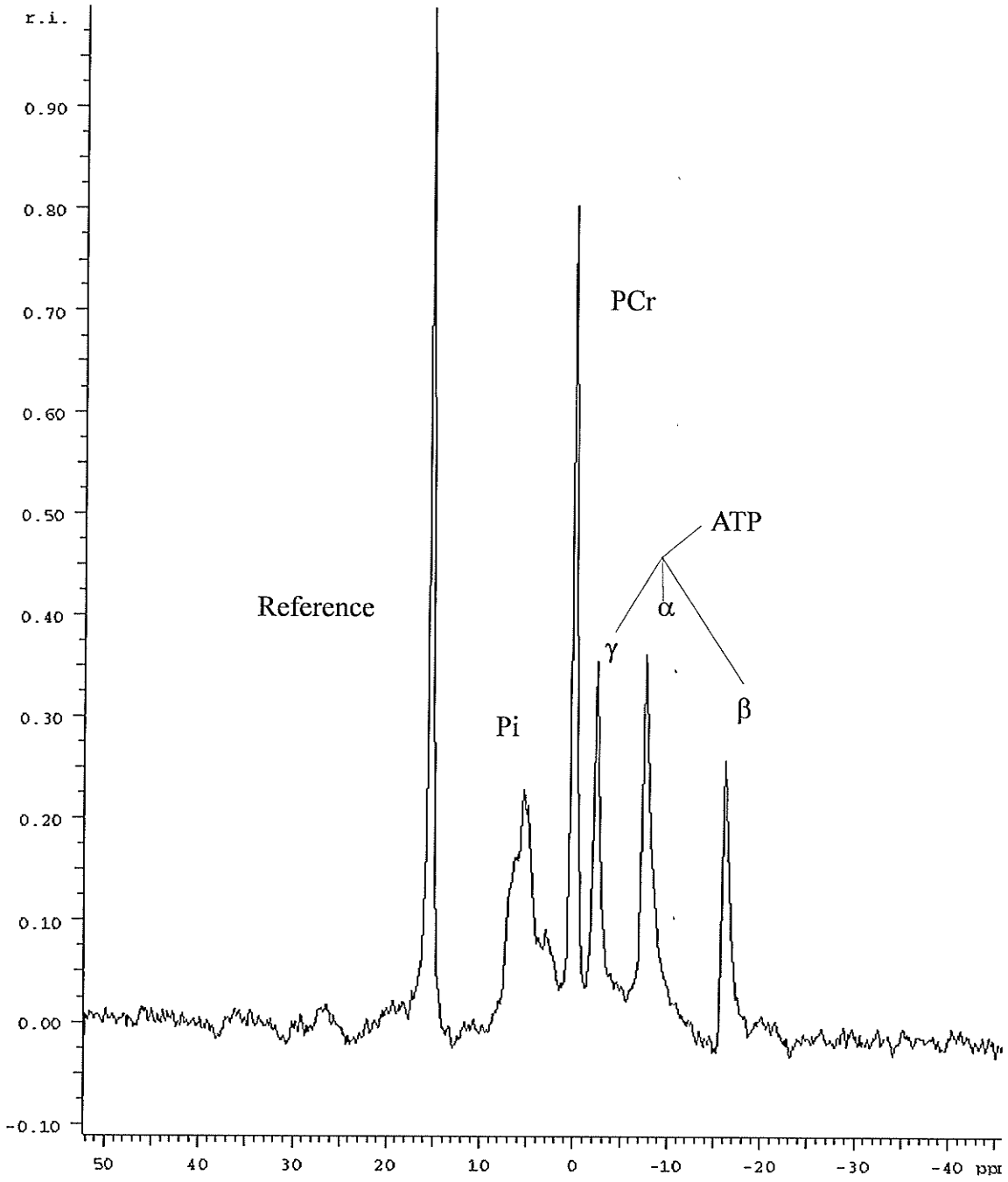


Figure 1-7: ^{31}P spectrum after a Fourier transformation of Figure 1-6.

Figure 1-7 shows an example of a phosphorus spectrum from an isolated beating heart with six peaks of distinct chemical shifts. Working from left to right there is a reference

peak which is phenylphosphonic acid (PPA), then Pi, then PCr, followed by the three phosphates of ATP. All three ATP phosphates have unique chemical environments resulting in the clear separation in their chemical shifts. There is significant overlapping of resonances from closely related compounds in the phosphorus spectrum. For instance the Pi peak has overlapping resonances from the second phosphate of 2, 3 diacylphosphoglycerate (2, 3 DPG) contained in blood, and a broad sugar phosphate resonance. γ and α-ATP peaks also contain two resonances of ADP. In addition the NAD resonance appears as a shoulder on the α-ATP resonance. Therefore the peak labeled γ-ATP is comprised of γ-ATP and β-ADP, and the α-ATP peak consists of α-ATP, α-ADP, and NAD. The β-ATP peak is considered to be mostly ATP with less than 5% contribution from other nucleotide triphosphates and for this reason is used to quantify ATP [34]. It is possible to quantitate the levels of all the high energy phosphates by measuring the areas of the peaks obtained in the spectrum (see *Analysing Spectra,* page 1-42).

In addition to being able to quantify the concentrations of HEP it is also possible to extract information about other ionic species from the chemical shift of certain peaks. For instance the chemical shifts of the ATP groups are sensitive to the intracellular concentration of magnesium, and thus magnesium may be monitored continuously and non-invasively using ^{31}P MRS [36]. Intracellular pH (pHi) may be determined from the chemical shift of the Pi peak.

iv. Intracellular pH

In living systems inorganic phosphate exists predominantly as either HPO_4^{2-} or $H_2PO_4^-$. In solution these two forms give rise to one resonance with a frequency dependent on the pHi [18]. It is possible to form a standard titration curve with which the chemical shift of Pi may be compared and the pHi calculated. Therefore using ^{31}P MRS one may monitor pHi continuously and non-invasively. There are, however, limitations to calculating pHi in this manner which result from overlapping resonances in the Pi peak (see *Analysing Spectra*, page 1-42). In addition there is a source of error due to the non-linearity of the relationship between pHi and the chemical shift. This may be overcome by dividing the intensity axis of the resonance by the derivative of the Henderson-Hasselbalch equation [22]. Another source of error is that the standard titration solution only estimates intracellular composition but may not accurately reflect the Mn^{2+} concentration, the salt concentration, or the viscosity. There is a method of accurately calculating both pHi and Mn^{2+} concentration from the chemical shifts of ATP resonances [88].

v. Quantification

The goal of MRS is to be able to measure concentrations of metabolites continuously and non-invasively, however, in order to do so one must first be able to quantify the spectra. In theory, one need only measure the area under the peaks to determine the contribution from a particular resonance, which is related to the concentration of a particular metabolite. In practice, however, there are a number of difficulties which must be

overcome to analyze the data. There are a number of factors which are potential sources of error in interpretation of the spectra, such as errors arising from the MR equipment, the biological system, and the final spectral analysis.

vi. MR Equipment

Inhomogeneity of RF fields has been mentioned above, however the effect on the data has not been discussed. Conclusions about the sample concentrations from peak intensities makes the assumption that there is homogeneity of flip angles throughout the sample. If this is not the case, i.e. if 10% of the spins are at less than 90° angles, then the signal will be weaker and there will be error in quantifying the concentration of the sample. The extent of this error is dependent on a number of factors, one of which is the degree of inhomogeneity in the coil. An additional problem may be found as a result of the inhomogeneity of the B_0 field caused by changes of the irregularly shaped sample, such as a beating heart. Therefore it is not only the MR equipment which can cause error, but also the sample itself.

vii. Biological System

The use of MRS by a chemist for analysis of *in vitro* compounds and determining molecular structure of solutions, is relatively uncomplicated compared to the complexity presented by biological systems. In a biological system there is a myriad of interactions between compounds of completely different electronic environments, providing different chemical environments. This is responsible for such observable phenomena as alterations

in T_1 and T_2, nuclear coupling such as spin-spin coupling (J-coupling), and distortions in the phase of spectral lines and of the baseline. A biological system contains a relatively high concentration of ions and is therefore able to conduct electrical current. The generation of an electrical current within the sample is responsible for creating noise and diminishing the SNR. It is also responsible for the phenomenon known as *eddy currents*. An RF pulse causes the magnetic moment to tip onto the x-y plane producing net magnetization and an induced e.m.f. in the coil, however in a biological system there is an additional *back e.m.f.* induced in the sample. The e.m.f. induced currents are conducted through the sample, the extent of which depends on the amplitude and frequency of the RF pulse, and on the conductivity of the sample [18]. Eddy currents dissipate power, which may be equated to resistance; affecting another parameter known as *coil loading*. Essentially coil loading refers to the receiver efficiency, which involves the inherent resistance of both the coil and the sample; also coined the *quality factor* or Q factor [12]. The Q factor is related to the dielectric constant (conductivity) of the sample. The difficulty in using biological systems, such as a whole heart, is that the dielectric constant changes throughout the experiment with changes in volume; such as edematous swelling seen during ischemia. A change in the dielectric constant alters Q and therefore can alter the observed height, phase, and width of the peaks in the spectrum, as well as a loss of signal. This source of error is difficult to account for when acquiring MR spectra, however it may be minimized by using specialized coils (tuned coupling coil for matching), and special electronics (low impedance preamplifiers) which partially

remove the dependencies of receiver efficiency on sample volume, therefore significantly reducing the problem of changes in coil loading [30].

viii. Analysing Spectra

In order for MRS to be useful as an analytical tool, a reliable and efficient method for analysis of the spectra must be available. There are a number of complications which must be overcome before the data can be meaningfully interpreted. A standard method for quantifying FID's is a Fourier transformation and then quantification of the areas under the peaks. The traditional method of manually measuring the area under the peaks is extremely labour intensive and is not practical for large quantities of data. Computer analysis is possible, but at present involves first fitting the spectra with Lorentzian approximations through function fitting algorithms. Although this method offers a high degree of automation and provides a means of accounting for contributions from overlapping resonances, it brings with it the error involved in approximating a fit [15]. The software used to fit the spectra generally require a great deal of coaxing and definition which is intrinsically a subjective process. There are a number of overlapping resonances which constitute the ^{31}P MR spectrum of living tissue (see *The NMR spectrum* page 1-35). Overlapping resonances present a number of difficulties in interpretation because of the their uncertain relative contribution to the observed peak. The calculation of pHi is a good example of this complication. Typically the calculation of pHi uses the chemical shift of the Pi peak from ^{31}P spectra. There are, however, a number of overlapping resonances at the same position as Pi which obscure the exact location of the

Pi resonance. This problem is acute during the normal metabolic state when the Pi peak is low. Ischemia reduces the problem because of the dramatic elevation of Pi and diminished relative contribution from the other resonances. There are a number of techniques for overcoming this dilemma. Firstly, estimating the contribution from the other contaminating resonances is possible by modifying the biological system, such as lowering the concentration of blood to diminish the contribution from the second phosphate of 2, 3 DPG, imposing ischemia on the heart to identify the location of the other resonances, or comparing to spectra of a sample of whole blood. Once the contribution of the other resonances have been estimated, they may be eliminated by subtraction using processing software such as *Allfit* (in-house software developed by Informatics, IBD, NRC used for peak fitting through algorithm functions).

ix. Summary

Studies using experimental animals have demonstrated ^{31}P MRS to be an invaluable analytical tool for studying metabolic changes of the heart. It is ideally suited, and has been extensively used for the study of a number of clinically related pathologies involving cardiac energetics. Some examples are ischemia [90], reperfusion injury [59], myocardial stunning [41], cardiomyopathies [50], myocardial protection (Tian G, et al., 1994 J Thorac Cardiovasc Surg; *in press*), transplant preservation [44], and the kinetics of creatine kinase reactions [21]. Its sensitivity to ischemic metabolism has led to its use in studies of cardioplegia such as this one. The limitations and sources of error with ^{31}P MRS are being addressed with technological advances, such as superior forms of data

analysis with fitting software such as *Allfit*, which set out to minimize interpretation error in the data and increase the efficiency of data processing by providing a significant amount of automation. At its present state of development ^{31}P MRS has not only been shown capable of monitoring HEP and pHi from a large volume of tissue such as the right and left ventricles, but also by using localization techniques can monitor a small volume of interest such as the apical septum of the heart *in vivo* [86]. In addition it is able to monitor levels of HEP in discrete layers of the myocardium, such as the subepicardial and subendocardial layers of the ventricular wall [55]. ^{31}P MRS is potentially an extremely powerful analytical tool which provides insight into the metabolic process of the heart. It is able to provide information about the key energy metabolites essential to cardiac function and contribute to our overall understanding of effects of external conditions on myocardial metabolic status.

2 Materials and Methods

i. The Perfusion System

The perfusion system (see Figure 2-1) was designed with the following goals: 1) The whole isolated beating heart preparation had to be inserted in the MR instrument while providing continuous perfusion. In order to accomplish this, the tubing had to be of sufficient length to reach in the NMR instrument while keeping the perfusion pump at a safe distance from the strong magnetic field. 2) The system had to be remotely switched from a normokalemic to a hyperkalemic system, and from antegrade to retrograde flow. This was accomplished by using a two reservoir system connected to the arterial line through a common Y connector. Entirely remotely (without disturbing the position of the heart within the NMR instrument) antegrade flow was switched to retrograde cardioplegic delivery by opening stopcock B and closing stopcock A, closing the hydro-occluder to prevent bypassing of the coronary sinus, filling the retroplegic cannula balloon, and opening the aortic vent to allow venous return (See Figure 2-2). 3) In order to keep the blood in the system autologous and eliminate the need for a donor pig, a minimal prime was necessary. This was achieved with the use of components designed

for use with human neonates and minimal internal diameter tubing. The blood was oxygenated using a Capiox

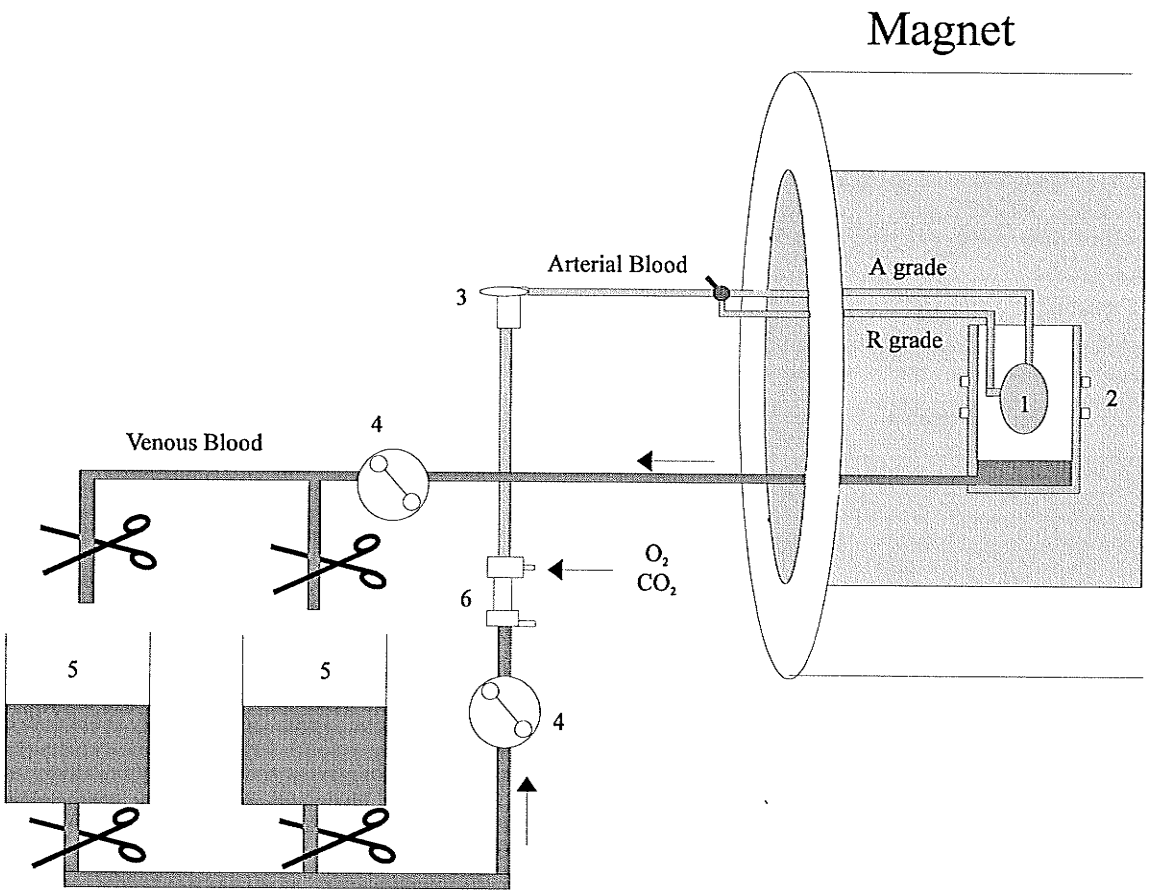


Figure 2-1: Perfusion system showing the heart (1), the coil (2), the arterial filter (3), roller pumps (4), 2 reservoirs (5), and neonatal oxygenator (6).

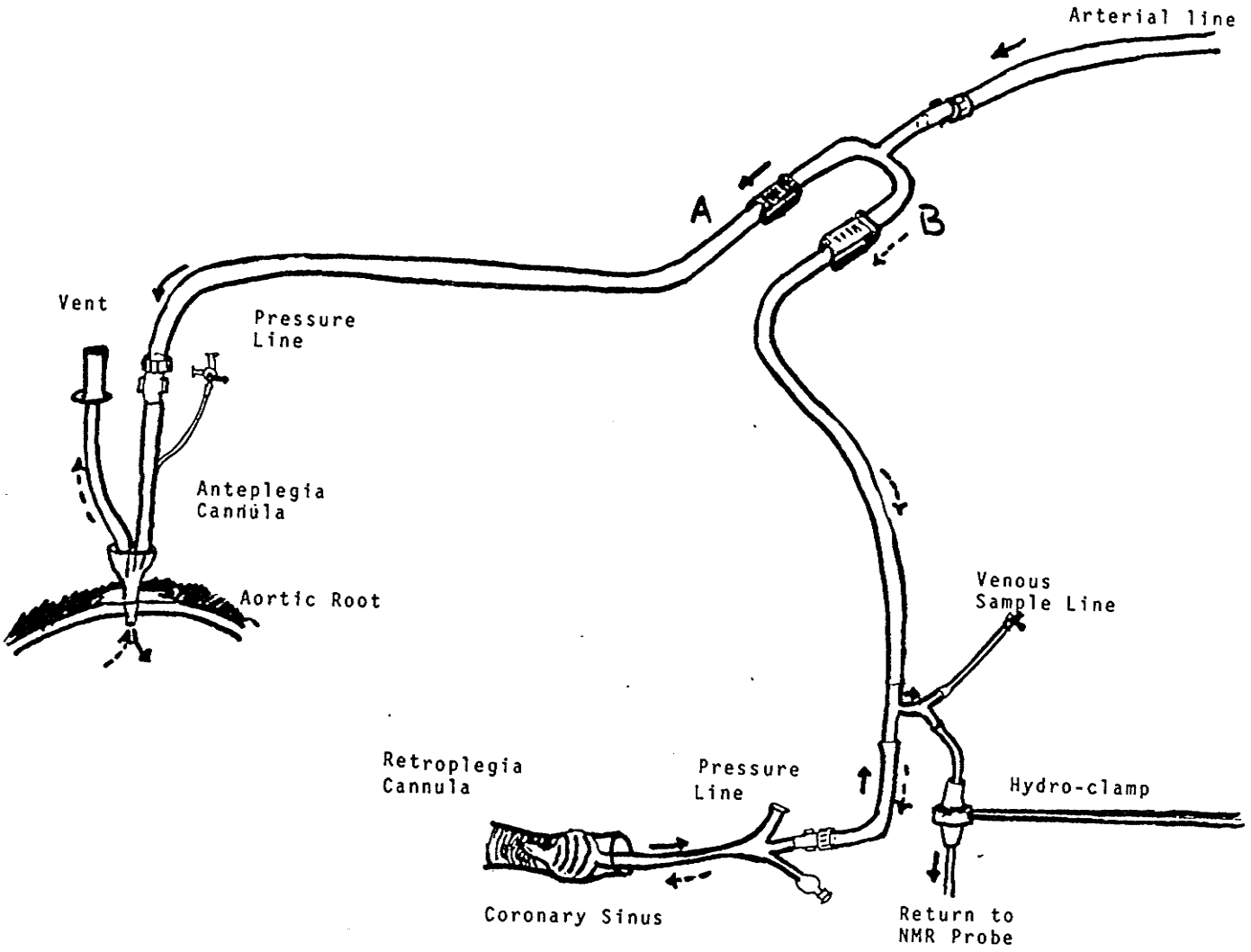


Figure 2-2: Arterial infusion line specially designed for use in the NMR instrument.

neonatal, hollow fiber oxygenator (Stat Health Corporation, Calgary, Canada). The oxygenator was connected to a gas mixer, which was set at approximately 95/5 O_2/CO_2. The blood was filtered of any particulate matter with a Dideco 20 μm pore neonatal arterial filter (Sorin Biomedica Canada, Richmond Hill, Ontario). Normothermia (39°C in the pig) was maintained with a heat exchanger in the venous reservoir and the oxygenator. The perfusion system was minimally primed with 1 L of Lactated Ringer's solution (Na^+ 130 mM, K^+ 4 mM, Ca^{2+} 1.5 mM, Cl^- 109 mM, Lactate 28 mM) and mixed with 350 ml of whole blood collected during surgery for a hematocrit of between 12 and 15%.

ii. Surgical procedure

Sixteen pigs (30 - 50 kg) of either sex were sedated with an intramuscular injection of ketamine hydrochloride (20 mg/kg), xylaxine (2.2 mg/kg), and atropine (0.03 mg/kg). Topical xylocaine was sprayed on the larynx to prevent spasmotic tightening during insertion of the endothracheal tube, which was used for ventillation and maintenance of anaesthesia. Anaesthesia was maintained with Isoflurane (1 - 1.5% at 2 L/min) and inspired and expired values were monitored using an Ohmeda 5250 respiratory gas monitor (Ohmeda, Madison, MI, USA). A small midline incision was made just superior to the thyroid cartilage and the internal carotid and jugular were dissected. The carotid artery was cannulated for blood pressure measurement and exsanguination of approximately 350 ml of blood over the whole surgery. The blood was used for minimal prime of the perfusion system (see *The Perfusion System* above). Lactated Ringer's

solution was used for volume replacement and was infused in the external jugular vein. After a midline sternotomy the brachiocephalic artery was isolated. Intravenous heparin was administered (660 units/kg) and the aorta was cannulated with an RMI 9 Fr, 3 lumen, anteplegic cannula (Canadian Cardiovascular, Mississauga, Ontario, Canada). The anteplegic cannulaton was performed by first placing 2 concentric purse string sutures on the proximal aorta. The cannula, which contains its own stylette, was stabbed into the aorta at the center axis of the purse string sutures. The sutures were then used to fasten the cannula to the aorta. Three lumens simultaneously provided a pressure line, perfusion line, and a vent for removing air. During retrograde perfusion the vent provided a means of venous sampling. Umbilical tape was placed around the brachiocephalic artery and around the aortic arch between the brachiochephalic and left subclavian arteries. Perfusion down the aortic root was initiated keeping the perfusion pressure in the aorta between 75 and 85 mmHg, requiring a flow rate of 250 to 550 ml/min. The heart and lungs were isolated en-bloc taking care not to cut the esophagus. The weight of the heart was distributed equally through suspension from the aorta. This was done to ensure that aortic distortion would not cause aortic valve incompetence. The hemiazygous vein was closed with a purse string suture and the coronary sinus was cannulated by a transatrial insertion of a dual lumen 13 Fr retroplegic cannula (Sarns, 3M, London, Ontario, Canada), providing concurrent perfusion and pressure monitoring. The position of the cannula was determined by palpation of the coronary sinus and by visual examination so as to be deep enough to prevent extensive leaking into the right atrium, but not too deep

where blockage of proximal venous tributaries might occur. The position was secured by fastening the cannula to the atrial wall with a loop suture. A stabilization period began with antegrade flow and blood content was adjusted whenever necessary to ensure potassium and calcium concentrations of approximately 4 mM and 1 mM, respectively. Hematocrit was maintained at 15% - 20%. PCO_2 and PO_2 were maintained at 35 - 40 mmHg and above 200 mmHg, respectively. PO_2 was held above 200 mmHg to ensure sufficiently high oxygen saturation of the blood (>99%).

iii. Preparation for Physiological Monitoring

The mitral valve leaflets were freed by carefully cutting the cordae tendenae. A phenylphosphonic acid (PPA) charged, compliant balloon (unstressed volume >50 cc) was inserted in the left ventricle via the left atrium for assessment of the diastolic and systolic function before and after the arrest period. The balloon was held in place with a purse-string suture enclosing the mitral valve leaflets. PPA was used as a chemical shift and peak area standard for the ^{31}P MRS (see *The NMR spectrum* page 1-35). The aortic root, coronary sinus, and left ventricular (balloon) pressure lines were connected to independent pressure transducers (Cobe Canada Ltd., Scarborough, Ontario, Canada) and cleared of all in-line air. The transducers were calibrated and zeroed and the entire preparation was placed in the NMR probe. The coronary blood flow was 1 - 1.5 ml/g/min to maintain aortic root pressure of 75 - 85 mmHg. The whole heart weight (HW) was estimated from the previously measured body weight (BW) using the

empirical formula; HW (g) = (BW (kg) x 4.118) + 5.4 (Tian, G, unpublished). Placement of the retroplegic cannula was reassured by monitoring the coronary sinus pressure, which was maintained at 45-55 mmHg, requiring a flow rate of 100-150 ml/min.

iv. Functional Measurements

Pressure transducers were connected to a multichannel Gould TA 5000 polygraph recorder with digital output (Gould, USA). End diastolic pressure was set at 5-10 mmHg by filling the ventricular balloon with a known quantity of PPA. Cardiac function was assessed using the first derivative of left ventricular pressure, giving rates of myocardial contraction (+ dP/dt) and relaxation (-dP/dt). Heart rate and developed pressure were incorporated into the functional parameter rate-pressure product (RPP).

v. Protocol for NMR Study

After tuning the NMR probe and optimizing the magnetic field, a series of 2 minute ^{31}P spectra were acquired on the beating heart perfused with normokalemic blood at 39°C (30 min). The normothermic blood cardioplegia (blood mixed with Lactated Ringer's solution for a hematocrit of 15-20% and adjusted to obtain a final KCl concentration of 18 mM) was initiated antegradely then maintained (group I), or switched to retrograde flow (group II), for one hour during which a series of ^{31}P spectra was acquired. After the arrest period of one hour, recovery of normal sinus rhythm was achieved by switching to the normokalemic venous reservoir and allowing the hyperkalemic solution to wash out.

Once a stable sinus rhythm was achieved a final series of NMR spectra was acquired (30 min) for a total acquisition time of 2 hours. The spectra were acquired using a 7T, 40 cm bore magnet. At 7T, ^{31}P resonanted at 121.5 MHz. Sixty scans with a delay of 2 seconds and a sweep width of 12 000 Hz were used for one file of 2K. The pulse length was between 60° and 80°.

vi. Processing the NMR Data

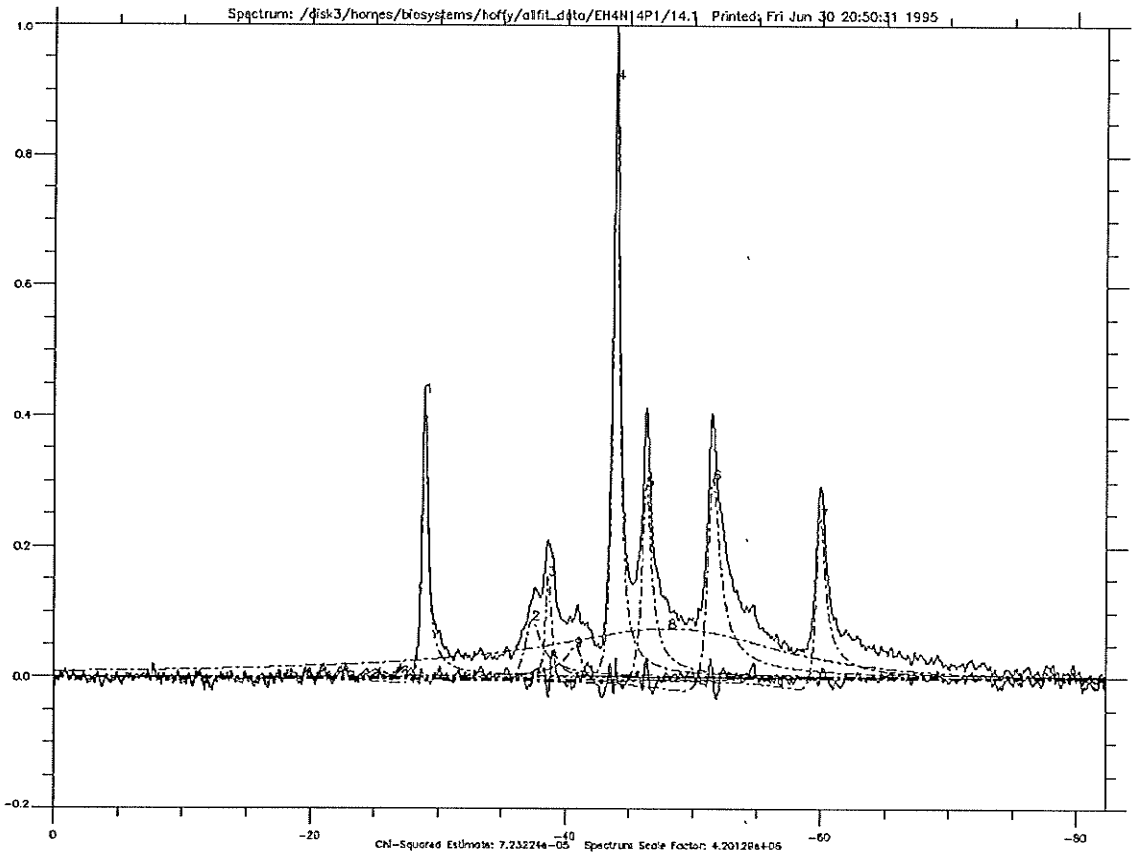


Figure 2-3: A spectrum approximated with the use of Lorenztian peaks.

The raw FID's were transferred to another software package called UXNMR- P (Bruker, Germany). UXNMR-P was used to process the data with a line broadening of 20 Hz and

auto phase correction. No baseline correction was performed. The spectra were then converted to ASCII files to be read by the in-house software, Allfit. Another spectra processing software package called Xprep was used to find the centroids of the data sets, which could be used for placement of the Lorentzian peaks for fitting of the spectra. Nine peaks were placed for an optimal fit of the spectra (see Figure 2-3) . Once the peaks were placed, they could be saved into a peak table and entered into a journal file for automatic fitting of all the spectra in one data set. The journal file is a set of instructions with a number of constraints for Allfit to be able to opitmally fit the spectra. Figure 2-4 shows an sample journal file which was developed to optimally fit a wide range of spectra from these experiments. The first 9 lines give the placement instructions of the 9 peaks which Allfit will use to fit the spectra.

```
addpeak heights=0.991422 widths=70.3125 positions=-37.3395 phases=0.00000
addpeak heights=0.226587 widths=150.000 positions=-45.9401 phases=0.00000
addpeak heights=0.398015 widths=407.812 positions=-47.3736 phases=0.00000
addpeak heights=0.889224 widths=98.4375 positions=-52.6295 phases=0.00000
addpeak heights=0.391422 widths=182.812 positions=-55.3052 phases=0.00000
addpeak heights=0.444169 widths=126.562 positions=-60.2744 phases=0.00000
addpeak heights=0.272741 widths=126.562 positions=-68.9706 phases=0.00000
addpeak heights=0.0749385 widths=942.187 positions=-53.9673 phases=0.00000
addpeak heights=0.0551582 widths=150.000 positions=-50.6227 phases=0.00000

constrain peaks=(1,2,3,4,5,6,7,9)  phases=(0,0,0,0,0,0,0,0)
phasefreedom=(20,20,20,20,20,20,20,20)
constrain  peaks=(1,2,3,4,5,6,7,9)  positions=(-37.33,-45.94,-47.37,-52.62,-55.30,-60.27,-68.97,-
50.62) posfreedom=(0.6,0.3,0.6,0.6,0.6,0.6,0.6,0.6)
constrain peaks=(8) widths=2600 widthfreedom=2400
constrain peaks=(2) widths=75 widthfreedom=75
constrain peaks=(9) widths=75 widthfreedom=75

%LinPhaseCorr off
%Marquardt_Method
curvefit rp
%SMSimplex_Method
curvefit sp
%SMSimplex_Method
curvefit sp
%SMSimplex_Method
curvefit sp
%SMSimplex_Method
curvefit sp
```

Figure 2-4: Example of a journal file used by Allfit.

The constraints described in Figure 2-4 involve constraining peaks 1-7, and 9 to a phase from -20° to +20° and a defined position with 0.6 or 0.3 degrees of freedom in position. The 8th peak was used as a baseline correction. This purpose was assured by giving no position or phase constraints for this peak. A width constraint was used to reduce the probability of the peak being used by Allfit to fit one of the 6 peaks of interest. The 8th peak was permitted to have a width between 200 and 5000 Hz. The Pi peak was fit with 3 Lorenztian peaks in order to account for the overlapping resonances in that region of the

spectrum (see page 1-35). Knowing that peaks 2 and 9 were used for the region of the Pi peak that should not change throughout the experiment they were constrained in width between 0 and 150 Hz. Therefore the resulting changes seen in peak 3 may be assumed to be primarily due to changing Pi concentration.

vii. Data Analysis

The heights of MR spectral peaks were normalized with respect to the reference peak and were used to determine the relative changes in Pi, PCr, and ATP (the resonance of the ß-peak of ATP). The values were then further processed using Microsoft Excel, version 5.0 (Microsoft Corporation, USA), appended to functional data and exported to the software package, Statistica 4.5 (StatSoft, Tulsa, Oklahoma, USA) for statistical analysis. Data are presented as the mean ± standard error of the mean. The data were tested for significant differences using both the Student unpaired t test for independent samples and the non-parametric Mann-Whitney U test. Data were considered significant at p values less than 0.05.

viii. Animal Care

All animals received humane care in compliance with the "Guide to the Care and Use of Experimental Animals" published by the Canadian Council on Animal Care [11].

3 Results

3.1 *Effect of ANBC and RNBC on Myocardial High Energy Metabolites*

The relative changes in Pi, PCr, and ATP in both groups during arrest are shown in Figure 3-1. Antegrade cardioplegia caused no significant change in high energy metabolites such as PCr, and ATP, or in Pi. Retrograde cardioplegia caused immediate (occurring within 2 minutes, see Figure 3-4) changes in Pi and PCr, 197% ± 15% and 51% ± 6% of control, respectively. RNBC resulted in a gradual drop in ATP to 72% ± 3% of control. When compared to ANBC all changes in high energy metabolites during RNBC were statistically significant ($p<0.05$). The extent of recovery in levels of high energy metabolites are shown in Figure 3-2. The alterations in Pi and PCr which occurred during RNBC, were reversed (within 2 minutes, see Figure 3-2) after restoration of function, with no significant difference compared to the ANBC group ($p<0.05$). ATP, however, did not completely recover in the RNBC group and remained at 79% ± 2.4% of its initial control. The difference in recovery of ATP between the two groups (ANBC and RNBC) was significant ($p<0.05$). Figure 3-2 gives an example of the changes seen in

high energy metabolites throughout the experiment in the retrograde group. It is clear from this diagram that the levels of HEP were relatively stable until at 22 minutes into the experiment when RNBC was initiated. Within 2 minutes of initiation of RNBC, PCr dropped precipitously until it reached a plateau which lasted throughout the arrest period. There was a concurrent, dramatic rise of Pi which also reached a plateau and was maintained throughout the arrested period. Both PCr and Pi returned to approximately 100% of initial levels within minutes of switching to antegrade perfusion. In the antegrade group the levels of HEP were maintained throughout the experiment (see Figure 3-3).

Group	Arrest % Pi	Recov % Pi	Arrest % PCr	Recov % PCr	Arrest % ATP	Recov % ATP
Antegrade	105.07	126.90	104.78	102.49	93.66	100.97
SE	6.16	8.42	5.18	9.50	4.43	8.46
Retrograde	197.74	102.71	51.29	98.29	72.59	79.22
SE	14.92	16.51	6.03	5.05	3.22	2.44
	*		*		*	*

Table 3-1: Summary of changes in HEP from control during arrest and recovery. An asterisk indicates a significant difference ($p<0.05$) between the two groups.

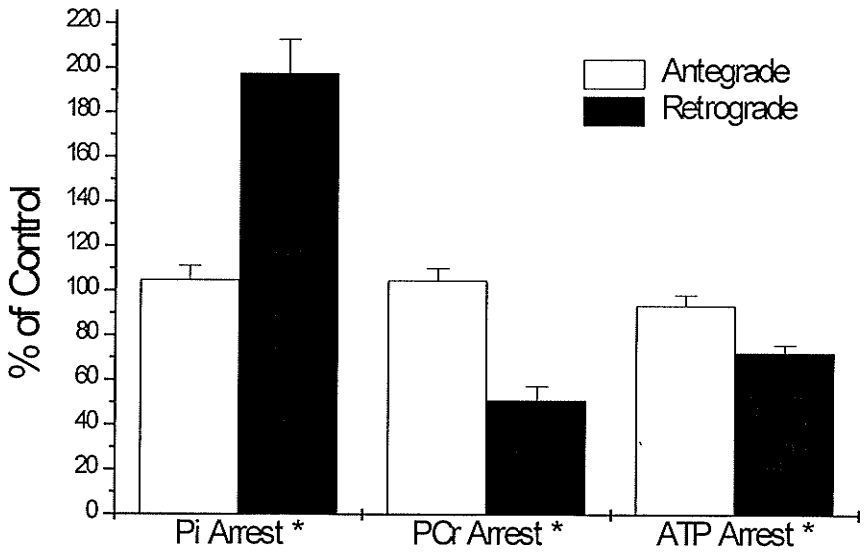


Figure 3-1: Effect of ANBC and RNBC on high energy metabolites during arrest. An asterisk indicates a significant difference ($p<0.05$) between the two groups.

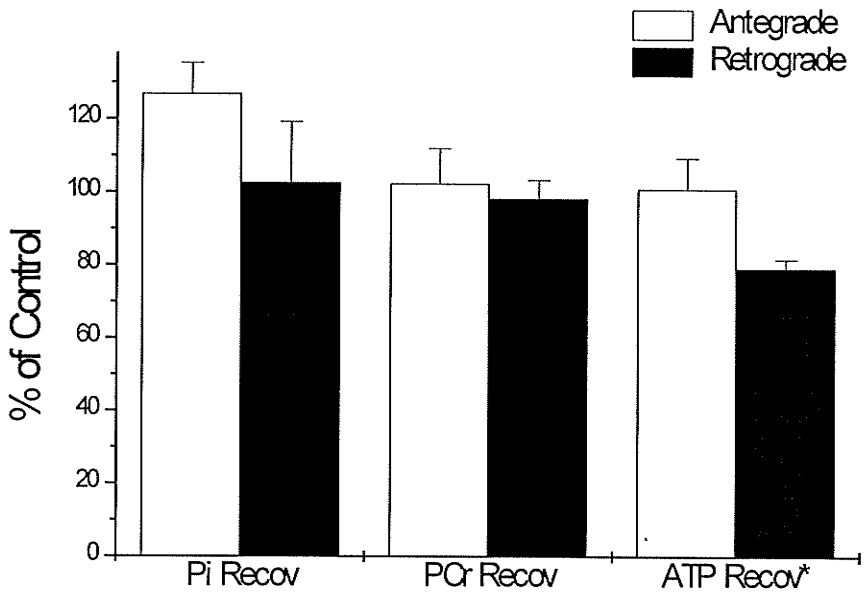


Figure 3-2: A comparison of the recovery of Pi, PCr, ATP after either RNBC or ANBC. An asterisk indicates a significant difference ($p<0.05$) between the two groups.

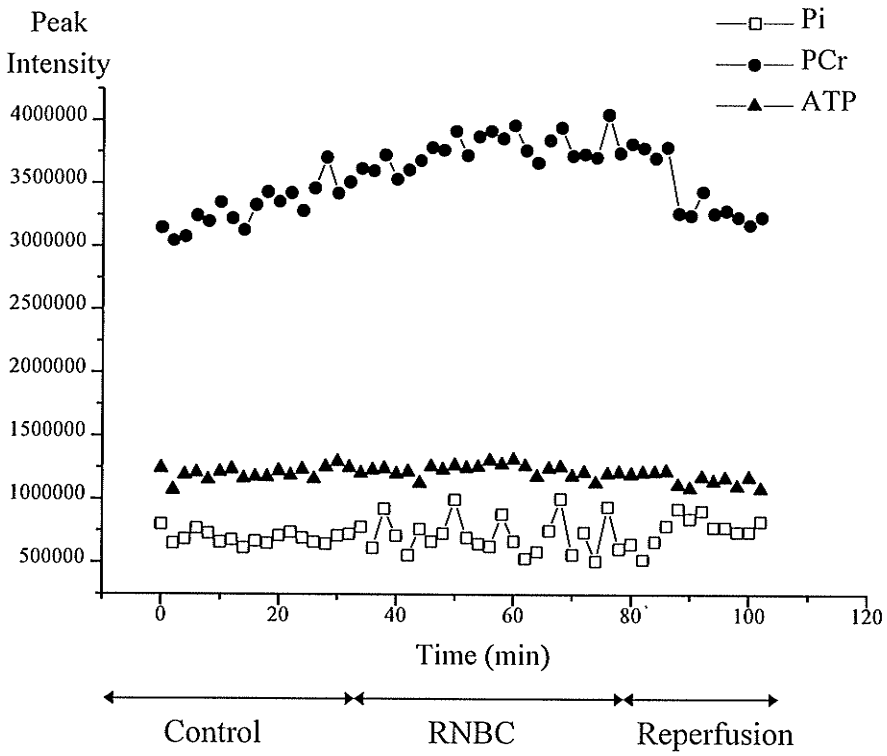


Figure 3-3: Effect of ANBC on Pi, PCr, and ATP versus time.

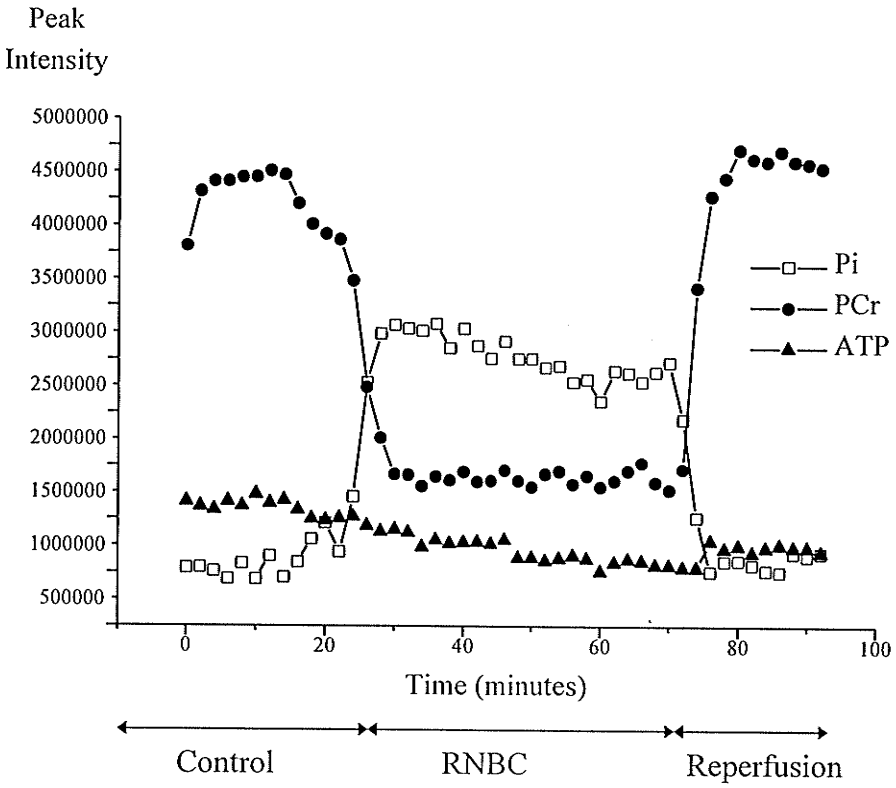


Figure 3-4: Effect of RNBC on Pi, PCr, and ATP versus time.

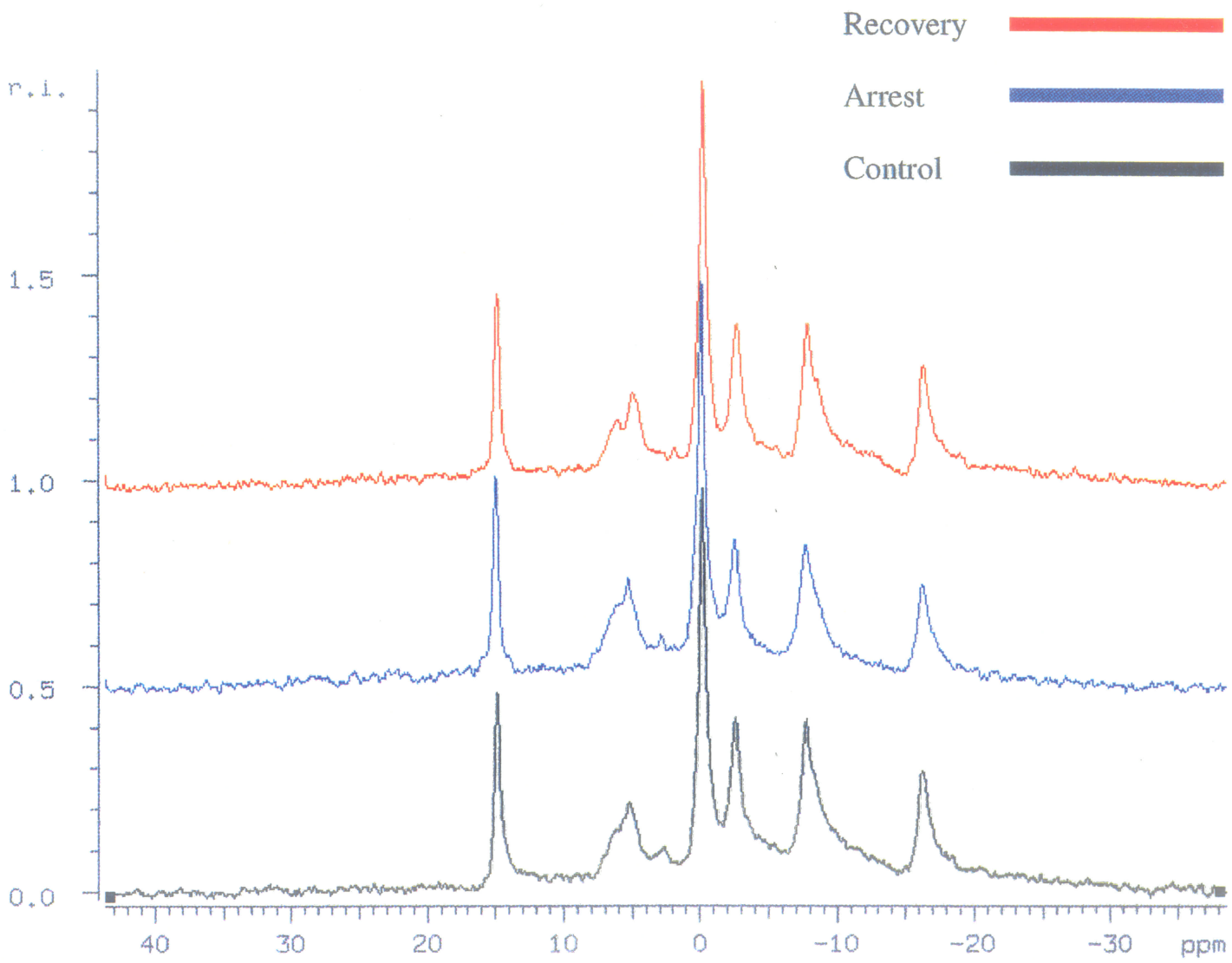


Figure 3-5: Example of changes seen in spectra during control, antegrade cardioplegia, and recovery

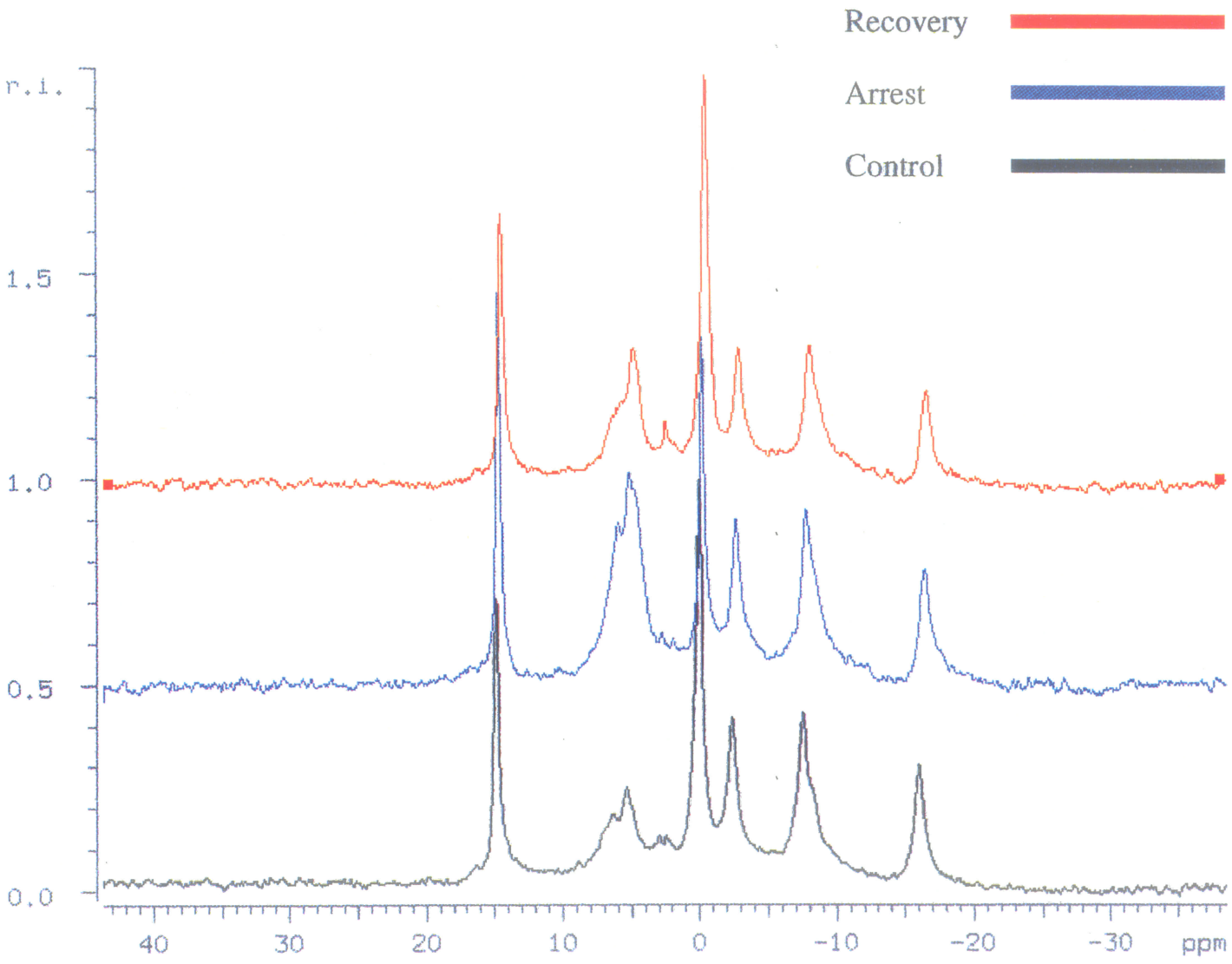


Figure 3-6: Example of changes seen during control, retrograde cardioplegia, and recovery.

Figures 3-5 and 3-6 show an examples of spectra during the control beating, arrest, and recovery periods. Note the stable Pi and PCr peaks in the antegrade group. In the retrograde group there is a visible increase in the Pi peak and a decline in the PCr peak.

3.2 *Intracellular pH*

A summary of the changes seen in pHi for both groups is given in Figure 3-7 and Table 3-2. Antegrade cardioplegia maintained normal pHi values, however retrograde cardioplegia caused a gradual decline in pH of almost 0.4 units to 6.8 ± 0.2 over a period of 40 minutes (see Figure 3-7). When normal sinus ryhthm was restored after one hour of RNBC by switching to the normokalemic reservoir, pHi returned to normal and was not statistically significant compared to the ANBC group ($p > 0.05$).

Group	Control pH	Arrest pH	Recovery pH
Antegrade	7.32	7.43	7.19
SE	0.08	0.08	0.06
Retrograde	7.14	6.84	7.26
SE	0.18	* 0.17	0.24

Table 3-3: Changes in pHi during arrest and recovery. An asterisk indicates a significant difference ($p<0.05$) between the two groups.

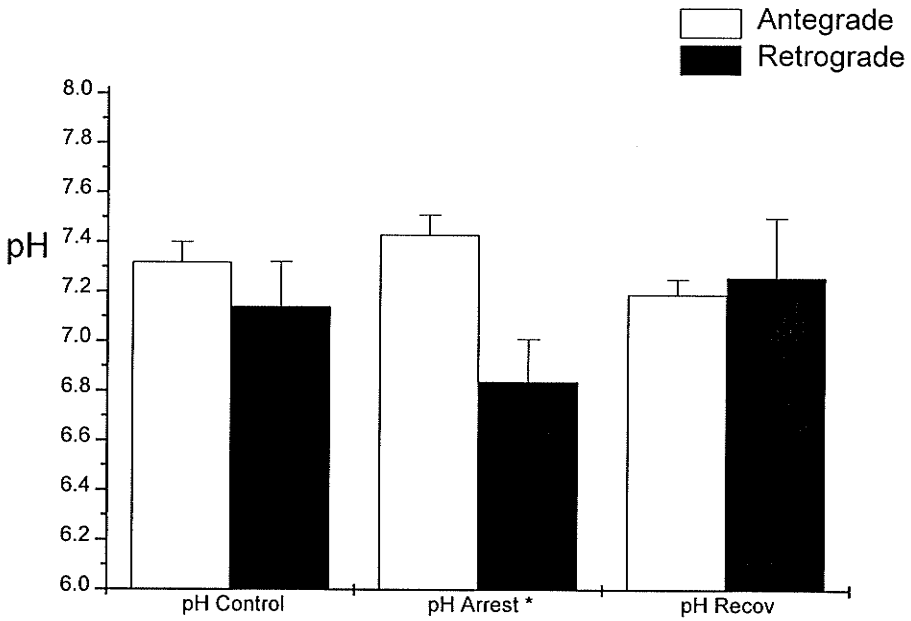


Figure 3-7: Changes in pHi seen in the ANBC and RNBC groups. An asterisk indicates a significant difference ($p<0.05$) between the two groups.

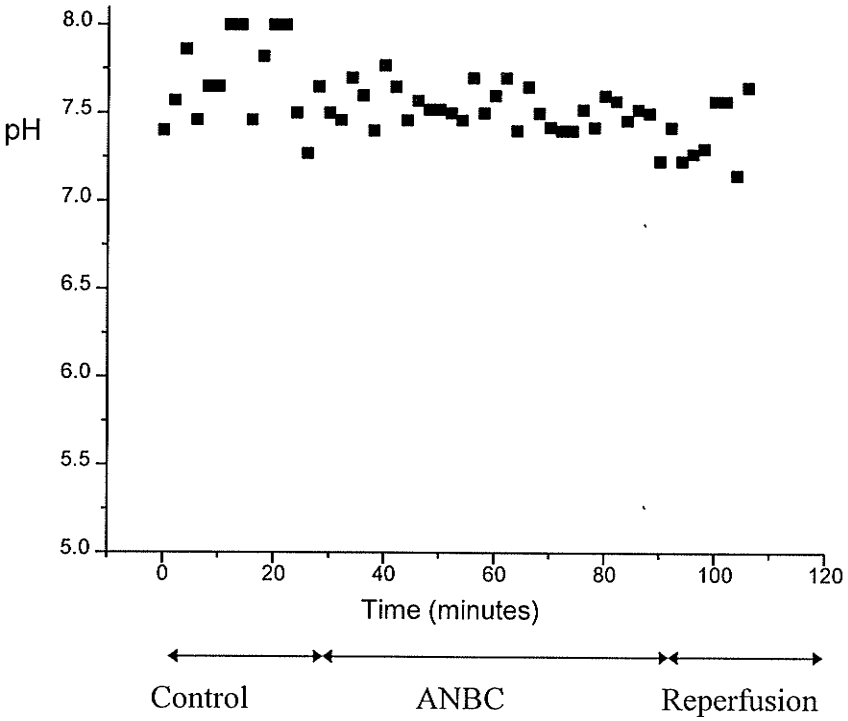


Figure 3-8: Changes seen in pHi versus time with ANBC.

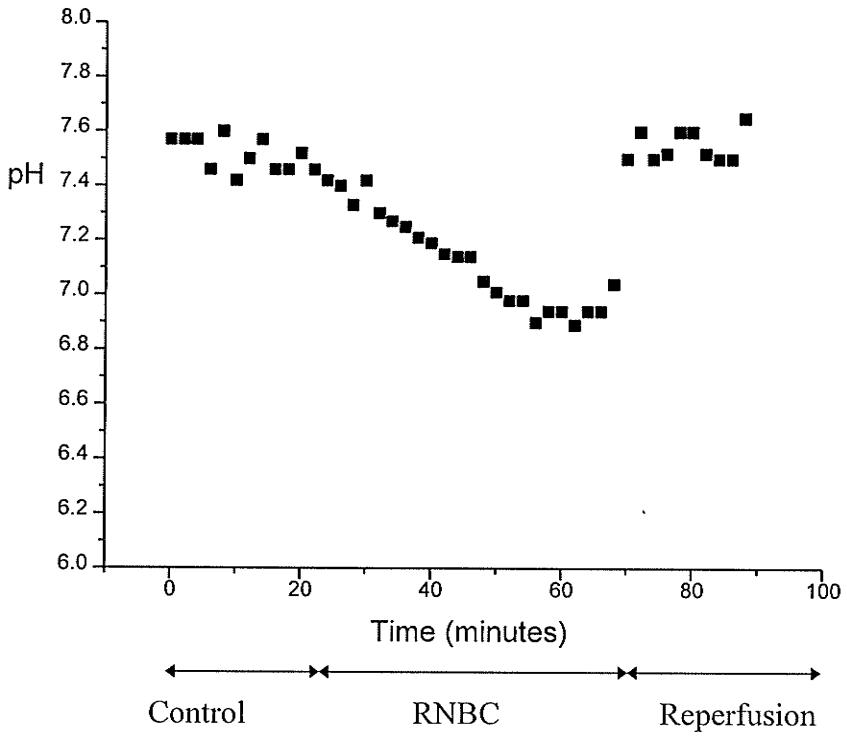


Figure 3-9: Changes seen in pHi versus time with RNBC

3.3 *Myocardial Contractile Function*

Figure 3-8 and Table 3-3 show a comparison of the recovery in +dP/dt, -dP/dt, and RPP between ANBC and RNBC groups. All values are expressed as a percentage of the control, recorded at the end of 30 minutes of function prior to initiation of cardioplegia. The mean recovery of +dP/dt and -dP/dt for the antegrade group was 68% ± 9% and 65% ± 7%, respectively. In the retrograde group the recovery of +dP/dt and -dP/dt was 61% ± 7% and 57% ± 7%, respectively. RPP was 52% ± 8% in the RNBC group and 64% ± 7% in the ANBC group. Although retrograde cardioplegia caused a consistently lower

recovery of myocardial function, the differences between the two groups were not statistically significant ($p>0.05$).

Group	Recov % +dP/dT	Recov % -dP/dT	Recov % RPP
Antegrade	67.90	64.76	63.88
SE	8.49	6.94	8.23
Retrograde	60.50	57.32	52.58
SE	6.82	6.61	6.84

Table 3-4: Recovery of left ventricular function.

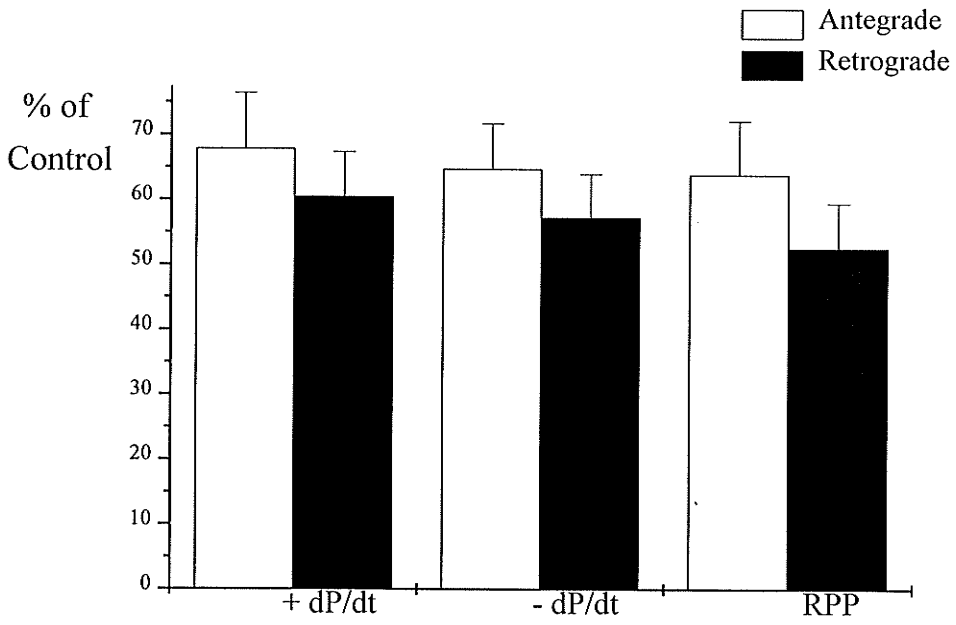


Figure 3-10: Recovery of left ventricular function after ANBC or RNBC.

4 Discussion

4.1 *The Model*

i. Using NMR to Study Cardioplegia

There has been a great deal of research probing the efficacy of RNBC to protect the myocardium during arrest. A number of these studies have supported the clinical use of RNBC, demonstrating it provides equal, if not superior, protection for the myocardium during some procedures relative to ANBC [2,10,40,58,78]. Recently work has evaluated the changes in myocardial metabolism, including pHi and ATP in retrograde warm blood cardioplegia [81]. However there has not been any evaluation of myocardial metabolism during RNBC using the continuous and non-invasive probe of cellular metabolism, ^{31}P MRS.

^{31}P MRS can provide continuous measurement of Pi, PCr, ATP, and pHi. The protocol chosen for acquiring data in this study allowed for a time resolution of 2 minutes throughout the experiment. This time resolution was chosen as optimal with both a good signal to noise ratio, and an adequate time for observing changes in metabolism. The ^{31}P

MRS model allowed initiation of RNBC while continuously monitoring myocardial metabolic status.

ii. The Isolated Heart Model

The model for this study was developed to allow isolation of a perfused beating porcine heart without any incidence of ischemia or hypothermia. The absence of ischemic or hypothermic insult during isolation prevents any undesired secondary effects, such as preconditioning or stunning of the myocardium. The isolated heart was chosen over the *in situ* model to remove unwanted secondary neural and humoral influences on myocardial metabolism and function. In so doing, observed changes could be attributed to the effects of a particular cardioplegic method on myocardial metabolism and function, instead of secondary corporeal effects. In addition, the isolated heart preparation is more easily placed in the bore of the 7T magnet than the whole body preparation used for *in vivo* studies.

iii. The Experimental Animal

The porcine heart model was chosen over other models because it is more similar to that of the human heart in terms of biochemistry, size, and susceptibility to fibrillation than that of the more commonly used rat or dog. The porcine heart is also similar to the human heart in its anatomy of the coronary system, which is crucial to this study because of the issue of heterogeneic perfusion distribution during RNBC (see below).

4.2 *Interpretation of Results*

i. Effect of ANBC and RNBC on High Energy Phosphates

Inorganic phosphate and high energy metabolites such as PCr and ATP were preserved in the myocardium during antegrade cardioplegia. However homeostasis failed to be preserved when retrograde cardioplegia was used and within minutes there was nearly a 2 fold increase in Pi, and PCr decreased by half. This is a strong indication of transition to ischemic metabolism [60]. Under anaerobic conditions when oxydative phosphorylation shuts down, ATP is primarily generated with the breakdown of PCr through the creatine kinase catalyzed reaction:

$PCr + ADP + H^+ \Leftrightarrow$ creatine + ATP, $\quad ATP \Rightarrow ADP + Pi.$

Accordingly the decline of mitochondrial ATP production means that Pi will accumulate and PCr will decrease. This makes the PCr/Pi ratio a very sensitive indicator of ischemic metabolism. The buffering effect of ATP production with the breakdown of PCr explains why the changes seen in ATP, in response to the onset of ischemia, are gradual compared with Pi and PCr which alter dramatically within minutes. The observed rapid transition from aerobic to anaerobic metabolism after initiation of retrograde cardioplegia is indicative of inadequate supply relative to demand typical of hypoperfusion ischemia. The oxygen demand of a beating, unstressed heart is normally approximately 10 ml/100g/min. Normothermic cardioplegia reduces the demand to approximately 1 ml/100g/min [9]. In our study the typical flow for RNBC was between 80 and 150

ml/min with an arterial PO_2 of approximately 500 mM. The hematocrit was maintained between 15 and 20% with a hemoglobin concentration of approximately 5-7 g/dl. Total arterial oxygen delivery capacity is equivalent to the total blood oxygen content multiplied by the flow rate. Arterial content may be calculated by using the following formula:

Hb (g/dl) x SO_2 /100x 1.39 ml/g + 2.35/760 x PO_2,

where 1.39 ml/g is the binding co-efficient for milliliters of O_2 to grams of hemaglobin (Hb) and 2.35 is the carrying co-efficient for milliliters of O_2 per 100 ml of blood per atmosphere of pressure. Therefore assuming the following conditions:

coronary flow rate = 150 ml/min
hematocrit = 20%
hemaglobin concentration = 7 g/dl
PO_2 = 500 mM
arterial O_2 saturation (SO_2) = 100%
The arterial O_2 content (ACO_2) would be;

(7 g/dl x 1 x 1.39 ml of O_2 /g of Hb + 2.35 ml of O_2 /760 Torr x 500mM of O_2) = 11.3 ml O_2 /dl.

The O_2 delivery capacity is the ACO_2 multiplied by the flow rate,

0.113 ml O_2 /ml x 150 ml/min = 16.9 ml/min.

Therefore for a heart weighing 300g this O_2 delivery would be 5.6 ml/100g/min. However if 65-75% of that flow is non-nutritive, i.e. it is shunted through the Thebesian vessels [80], then only approximately 1.4 ml/100g/min of O_2 are delivered. The oxygen demand of the arrested heart is 1ml/100g/min therefoe this calculation suggests a sufficient nutrient flow to meeting the metabolic demands of the arrested myocardium.

However this calculation makes the basic assumption that there is homogeneous flow distribution, which other studies have shown not to be the case during retrograde flow [1,47]. This suggests that there are areas of the myocardium which may be inadequately perfused, despite the total oxygen delivery of 1.4 ml/100g/min.

Antegrade cardioplegia is able to maintain aerobic metabolism as indicated by the lack of significant change in pHi. Retrograde cardioplegia, however, causes a significant decline in pHi ($p < 0.05$) to a value of 6.84 ± 0.17. pHi recovers upon antegrade reperfusion with ultimately no significant differences between the two groups ($p > 0.05$). The decrease in pHi during retrograde cardioplegia indicates a moderate intracellular accumulation of protons and is further evidence that RNBC does not maintain aerobic metabolism. There seems to be sufficient coronary flow during RNBC to washout metabolic wastes thereby limiting the accumulation of protons and preventing the onset of severe ischemia. These observations are consistant with work by Stahl, et al. where it was found that initiation of RNBC caused a decrease in pHi (< 6.8 after 2 hours), a concurrent rise in myocardial lactate concentration, and a significant decrease in recovery of left ventricular function [81].

The rates of isovolumic contraction and relaxation are given by the first derivative of the left ventricular pressure curve (±dP/dt). In this model the preload is constant therefore ± dP/dt are dependent on myocardial contractility and reflect cardiac function [51]. Although the recovery of all functional parameters in the retrograde group was consistently lower than the antegrade recovery, there was no significant difference

between the groups ($p > 0.05$). This may explain why some clinical studies have not seen any significant difference in function between the two cardioplegic methods [58].

One hour of arrest was chosen because it mimics the typical cross clamp period during bypass surgery, however it is possible that no significant differences in function between the two groups was observed because the time of RNBC was not long enough. At the end of one hour arrest in the retrograde group, ATP was reduced to approximately 80% of its initial amount. Studies have shown that there is a threshold of 80% ATP, below which the extent of functional recovery diminishes significantly [87]. It is possible that if the period of RNBC were longer, ATP stores would be further diminished and a significant difference in functional recovery would have been observed between the two groups.

ii. Limitations of the Model

All the limitations previously discussed (see *Magnetic Resonance Spectroscopy* page 1-30) in acquiring and processing NMR data are inherent in a project of this nature. The potential error caused by overlapping resonances at the same frequency of Pi has been minimized by using Allfit to estimate the other peaks. Included in this potential error is the lack of accuracy which remains in calculating pHi values during normal metabolism. However the goal of this study was to determine the relative changes of HEP and pHi during RNBC and this was accomplished by demonstrating a visible change in these parameters compared to ANBC.

The coil used for the acquisition of MRS is large and encompasses the majority of both the right and left ventricles. Therefore information about the metabolic status of the

myocardium from this study is limited to an average of both ventricles. Therefore little information is available concerning the flow distribution during either cardioplegic technique. Although this model is limited because it does not provide localized information, the results obtained from this study provide a useful indication of the overall metabolic status of the myocardium during either retrograde or antegrade perfusion and correlate those effects to function. The metabolic and functional data given here, combined with previous flow distribution studies, allow us to draw a number of conclusions about the efficacy of RNBC in normal porcine hearts. Cardioplegic delivery could not be confirmed other than by monitoring the coronary sinus pressure and observating the venous return from the aortic root during RNBC. This may be improved in the future by the combination of this model with the use of radiolabeled microspheres which would confirm the delivery of cardioplegia to the end organ target.

The limitation in using a porcine heart involves species specificity. Other than the primate heart, the pig heart is thought to be the closest model to that of the human. There are however limitations in extrapolating data obtained with this model for application to humans. For instance it has been suggested that the porcine heart has a more extensively developed venous system, including shunts such as the Thebesian veins. In addition, there are other minor differences in anatomy between the porcine and human heart. One such difference is the existence of an anomylous vein called the hemiazygous which, in pigs only, anastamoses with the coronary sinus. Without closing this anomylous vein any attempt at perfusing into the coronary sinus would be fruitless.

The isolated heart model has the advantage of removing external influences such as neural and hormonal responses, however the isolation is relatively invasive and maintenance on bypass without any hormonal protection can result in cell damage and, in combination with the lower hematocrit used for blood cardioplegia, can result in extracellular edema. In addition, perfusion for one hour with hyperkalemic cardioplegia may cause reversible disturbances in cardiomyocyte calcium handling and irreversible alterations of myofibril function causing the relatively low functional recovery observed in both groups [43]. Use of a normal, healthy heart provides a less clinically relevant model than one which has been ischemically injured or one on which acute coronary occlusion has been imposed. Although the results of this study have shown that RNBC causes a transition to ischemic metabolism, it is possible that in the more clinically relevant, coronary obstucted model, RNBC could provide superior protection to the myocardial tissue supplied by the occluded artery compared to ANBC. This advantage of RNBC in the occluded LAD model has been demonstrated by a number of studies [66,67]. One hypothesis for this observed advantage of RNBC in superiorly protecting myocardial tissue distal to an occluded LAD is the observed preferential perfusion of the more susceptible sub-endocardial tissue distal to an occluded coronary artery [67]. This issue is currently being addressed with the development of a model in which the LAD branch of the coronary artery is occluded and localized ^{31}P NMR spectroscopy is used to evaluate and compare the metabolic state of the myocardium distal to the occlusion. In addition the localized spectroscopy may be used transmurally, with the use of a surface

gradient coil, to observe distinct layers of the ventricular wall [35]. This approach should resolve whether RNBC is advantageous in protecting the susceptible regions of both ventricles in cases involving coronary occlusion.

The possible failings of RNBC to adequately perfuse certain areas of the myocardium have been addressed by Ihnken et al. where it was shown that RNBC and ANBC could be safely combined to provide simultaneous perfusion of the coronary sinus and the proximal aorta [33]. This was a comprehensive study which included both an experimental animal model and a clinical trial. Porcine hearts were used for with 1 hour of aortic clamping, during which simultaneous retrograde and antegrade cardiolegia was delivered at 200 ml/min. Coronary sinus pressure was maintained at less than 30 mmHg, there was no right or left ventricular edema, lactate production, or lipid peroxidation, and there was good recovery of postbypass left ventricular end-systolic elastance and preload recruitable stroke work index (101% ± 3% and 109% ± 90%, respectively) [33]. The clinical segment of the study included 155 high risk patients with an average clamping time of 94 minutes, 18 patients required post-operative circulatory assistance, 16 of whom were in cardiogenic shock pre-operatively. The incidences of post-operative myocardial infarction was 2% and the mortality rate was 4% [33]. This study demonstrated that simultaneous aortic root and coronary sinus perfusion is a viable cardioplegic strategy which takes advantage of the benefits of both antegrade and retrograde delivery.

4.3 *Conclusion*

Retrograde flow requires lower flow rates (0.3 - 0.5 ml/min/g) to avoid damaging the coronary sinus. In addition a large percentage of the perfusate flowing through the coronary sinus is diverted through collateral veins and is therefore non-nutritive. This study has shown that in the normal isolated porcine heart, RNBC causes a transition to ischemic metabolism within minutes of initiation; indicated by a rise in Pi, decrease in PCr, pHi, and gradual decline of ATP. The RNBC reduced pHi to 6.8 after one hour therefore ischemia was only mild, and with the exception of ATP, there was no significant difference in recovery between the two groups ($p > 0.05$). Although clinical data suggests that RNBC provides adequate protection of the myocardium, in this model RNBC did not provide sufficient nutritive flow to maintain aerobic metabolism. It is possible that the deleterious effects of RNBC are not observable other than with NMR or with an intracellular pH probe [81], however these effects are still relevant because they indicate a compromised myocardium which demands caution should flow need to be interrupted for any reason during surgery. It is also possible that given the limitations of this model (see *limitations of the model* above) that RNBC is more efficacious in human than in porcine hearts. The results from this study suggest that the continuing research in this area utilizing localized spectroscopy and more clinically relevant models are warranted, and should provide information furthering our evaluation of RNBC as a means of cardioplegia.

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