Retrograde Cardioplegia: Effects of Coronary Venous Perfusion on Myocardial Energy Metabolism, Contractile Function and Myocardial Perfusion in Neonatal Hearts.

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A thesis submitted to the faculty of graduate studies in partial fulfillment of the requirements of the degree

Master of Science (MSc)

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RETROGRADE CARDIOPLEGIA: EFFECTS OF VENOUS PERFUSION ON MYOCARDIAL ENERGY METABOLISM, CONTRACTILE FUNCTION AND MYOCARDIAL PERFUSION IN NEONATAL HEARTS

BY

GODWIN I. ORIAKU

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

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Abstract

Objective: This study tested the hypothesis that a heart maintained in arrest by retrograde cardioplegia (RC) can recover metabolic and contractile function to a level comparable to that of antegrade cardioplegia (AC).

Methods: Isolated Langendorff piglet hearts were subjected a protocol consisting of 30 minutes of control perfusion, 45 minutes of arrest, and 30 minutes of reperfusion. During the control and reperfusion periods, the hearts were perfused using Krebs-Henseleit (K-H) solution. Cardioplegic arrest was induced with two minutes of AC. Arrest was maintained for 43 minutes with continuous RC in group 1. The hearts in group 2 were subjected to 45 minutes of continuous AC. Myocardial energy metabolism and contractile function in the two groups were compared by assessing energy metabolites using ^{31}P MR spectroscopy and determining myocardial O_2 consumption (MVO_2) and measuring intraventricular pressure.

Results: No significant difference was observed in the levels of ATP and PCr in the two groups, suggesting that RC did sustain oxidative phosphorylation in the immature heart under normothermic conditions. Both groups of hearts also showed similar recovery of left ventricular developed pressure, LVDP (87.5 ±0.25 versus 84.5 ±0.25). The left ventricular end-diastolic pressure, LVEDP was 25.0 mmHg ± 0.31 in retrograde group and 20.5mmHg ±0.12 in the antegrade group ($P < 0.05$). Myocardial O_2 consumption (MVO_2: ml/minute/100g) for the retrograde group was also higher (11.69±0.2) during reperfusion compared to the antegrade group (10.66 ±0.75 $P < 0.05$). MR images showed that myocardial perfusion during RC was heterogeneous. This study suggest that in spite of the underperfusion of the right

ventricular myocardium during RC, the hearts sustained in diastolic arrest using this method of cardioplegic delivery did recover contractile and metabolic function to levels above 75% during reperfusion.

1.0 General Introduction

1.1. Statement of the problem.

Precision surgery requires a bloodless field and quiet heart. This is achieved by clamping the inflow and outflow tracts of the heart and diastolic arrest. This procedure decreases the O_2 and energy supply to the heart and may lead to ischemia. During the arrest period when the heart is not beating the heart cannot pump blood to itself and to the rest of the body. It is known that if nutrients can be channelled into the heart, survival of the arrested heart will be enhanced. When an oxygenated nutrient solution is channelled into the heart through its vessels to minimise ischemia during surgery, it is called cardioplegia. There are two ways of achieving cardioplegia: through the veins of the heart (this is called retrograde cardioplegia) or through the arteries of the heart (this is called antegrade cardioplegia). Other methods that are also used include: hypothermia (cooling the heart) and prolonged circulatory arrest (complete cut-off of blood flow in the body).

In neonates noncardioplegic schemes such as prolonged circulatory arrest and topical hypothermia are often used to minimize ischemic stress and provide a bloodless field. Avalaible evidence shows that, in addition to not providing adequate protection, this method may contribute to reperfusion injury and post-reperfusion complications (Fisk et al, 1993, Rosefeldt 1998; Rebekka, 1990). This is necessitating efforts to seek other ways of protecting the heart of

the newborn. The cardioplegic and noncardioplegic techniques with potential for application in neonates are presented in Figure 1.1.

The protocols used for neonatal heart operations are influenced by factors such as the type of anatomical and physiological corrections to be made and the state of the myocardium before the operation. Patients with congenital heart defects may have a pressure and/or volume-overloaded cardiac chamber, which makes the heart more susceptible to ischemia than the normal myocardium. To achieve excellent results, optimal preservation of the myocardium is absolutely necessary (Wittnich et al, 1991). Moreover, most complex corrective repairs require long "safe times" (duration of time during which the blood supply of the heart can be cut off without the risk of significant ischemic injury). Progressive hypothermia, to degrees used during infant heart operations (10-20°C) and prolonged circulatory arrest may not guarantee the optimal time for surgery. Some workers have reported fatal air embolism when temperatures greater than 10°C were used to induce deep hypothermic cooling (Fisk et al, 1971). Buckberg et al (1993), reported compression of the endocardial vasculature leading to unintentional ischemia, while Rosenfeldt (1988) and Kaijser (1985), reported epicardial frostbite and phrenic nerve paralysis, an epicardial endocardial temperature gradient leading to inadequate protection of the deeeper layers of the heart and edema due to depression of the Na^+-K^+ pump respectively. Based on the available evidence, I believe that the coronary sinus could provide a reliable route for maintenance of diastolic arrest and delivery of cardioplegic perfusates.

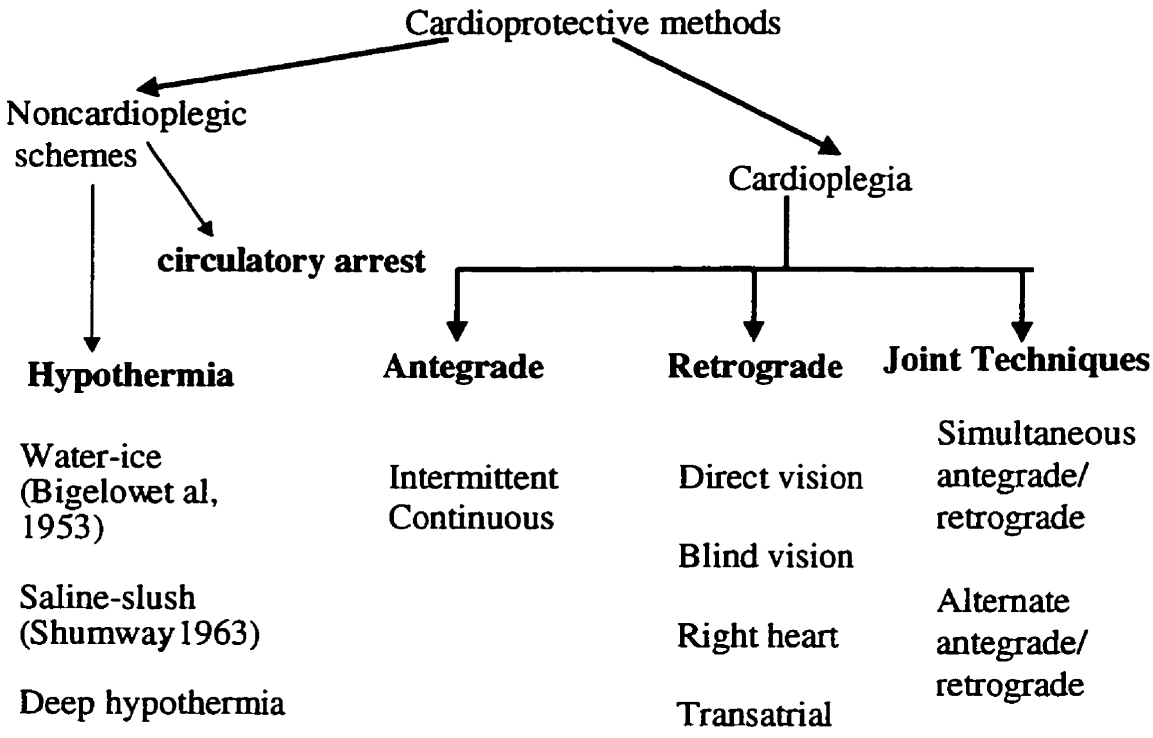

Figure 1.1. Schematic diagram illustrating some major cardioprotective methods with potential applications in neonates.

1.2. Working hypotheses.

Information regarding the efficacy of retrograde cardioplegia, RC emanates from experimental work on adult models. These reports show that RC is unable to meet the metabolic needs of a beating heart, however, in the setting in which the bioenergetic demands of the myocardium are reduced by cardioplegic arrest, these reports have established the effectiveness of RC. Using an isolated Langendorff crystalloid-perfused neonatal piglet heart model, we tested the following hypotheses:

1. A heart maintained in a state of arrest by coronary sinus cardioplegia, CSC can be restored to full contractile function by reperfusion with a nomorkalemic K-H solution delivered antegradely.
2. The ability of CSC to preserve myocardial ATP and PCr levels in the neonatal heart is less than that derivable using conventional aortic root cardioplegia (ARC). However, if the heart is maintained in a state of arrest, the amount of perfusate delivered to the neonatal heart by CSC is adequate to sustain myocardial metabolism and contractile function.
3. The flow of cardioplegia delivered via the coronary sinus may be slow due to high impedance and pooling in the extensive networks of veins leading to decreased coronary flow rate during arrest. This stagnation of perfusate in the networks of veins of the heart may be associated with edema, loss of compliance and increase in left ventricular developed pressure (LVEDP).

4. The functional and metabolic events observed during reperfusion in a neonatal heart maintained in arrest by RC do not differ significantly from those of a neonatal heart arrested using antegrade cardioplegia (AC).

1.3 Goals of Study

The objective of every cardiac operation must be a technically perfect anatomic result and avoidance or limitation of intraoperative damage in pursuit of this goal. This goal may be realized using a good cardioprotective strategy. Surgery in neonates involves maneouvres to repair major intracardiac defects and malformations affecting the great vessels and coronary arteries which makes the use of the antegrade route for delivery of cardioplegia technically difficult. The goal of this study was to develop an alternative method for channeling cardioplegic perfusates to the neonatal myocardium in situations where the antegrade route cannot be used efficiently and includes:

1. To study CSC with emphasis on neonatal hearts, the efficacy of CSC and to explore the technique as a viable option for neonatal myocardial protection in cardiac surgery.
2. To determine the ability of CSC to sustain ATP and PCr levels in the arrested neonatal myocardium and compare the results with those obtained using conventional aortic root cardioplegia (ARC).
3. To ascertain the ability of CSC to maintain the neonatal heart in a complete state of quiescence throughout the duration of cardiac surgery.

4. To determine, relative to conventional ARC, the possibility of restoring normal contractile function in a neonatal heart maintained in arrest using CSC by reperfusing with normokalemic Krebs-Henseleit (K-H) solution delivered via the aortic root.
5. To assess the ability of coronary sinus perfusion to provide sufficient coronary flow to sustain myocardial energy metabolism.
6. To determine the extent of loss of function and compliance relative to antegrade cardioplegia, when the retrograde route is used to perfuse the heart.
7. To determine relative to the antegrade method, the distribution of cardioplegia delivered through the coronary sinus.

2.0. Review of Literature

Myocardial preservation in neonates dates back to the 50's when Senning (1952) used the technique of anoxic arrest and fibrillation in an effort to preserve the heart during surgery. He clamped the inflow and outflow tracts of the heart to eliminate the coronary blood supply. The heart stopped beating when its store of energy substrates was depleted. Anoxic arrest and fibrillation provided a safe ischemic time of only 15 minutes, too short for most neonatal repair operations and was reported to be associated with serious complications and arrhythmia. Some workers have also reported compression of the subendocardial vasculature leading to unintended ischemia (Buckberg, 1993). Bigelow and associates in 1953 popularized the water-ice topical hypothermia technique. The method provided some of the earliest evidence that cooling the heart of newborns to low temperatures could provide protection against ischemia. In this technique, the patient is covered with crushed ice or ice blankets to induce a state of hypothermia. Its clinical efficacy was tested in 1953, when Dr. John Lewis successfully used it to close an atria septal defect (ASD) under direct vision. The popularity of this technique waned with the discovery of an epicardium-endocardium temperature gradient, particularly in hypertrophied hearts, which drastically limits its ability to adequately protect the endocardial layer of the heart. Efforts to provide more homogeneous cooling to the heart led Shumway and associates to replace water with saline-slush (Shumway et al, 1963). With this method, it was possible to achieve cooling to very low temperatures. However, its major disadvantage arose from the

closeness of the melting temperature of saline-slush (-0.5°C) and the freezing temperature of heart tissue (-0.55°C). This predisposes the patient to epicardial frostbite and phrenic nerve paralysis resulting in very serious arrhythmia and respiratory problems.

When the presenting pathophysiology permits, topical hypothermia may be combined with or be replaced by prolonged circulatory arrest and intermittent antegrade perfusion in most centers. Circulatory arrest, unlike Senning's method, was induced by draining most of the patient's blood into a reservoir and clamping the great vessels and venae cavae, with a view to having it reinfused at the conclusion of the operation. Intermittent AC does not prevent ischemia and anaerobic metabolism. The disadvantage of prolonged circulatory arrest is that it imposes an ischemic duration far beyond the acceptable safe ischemic time and in most cases has to be combined with hypothermia to minimize the ischemic stress (Oschner JL et al, 1976). Hypothermia per se, has deleterious effects on membrane stability, myocardial metabolism, calcium homeostasis and cellular O_2 uptake. There have also been several reports of postsurgical neurological complications in patients whose hearts were protected during surgery using this prolonged circulatory arrest. (Brunberg et al, 1974; Deverall, 1981; de Leval, 1983; Barratt-Boyes, 1981).

Techniques based on antegrade cardioplegia, which have been in use in adults for more than a decade, are yet to be universally accepted in neonates. Some centers use low flow bypass combined with hypothermia (Barrat-Boyes et al, 1971), there is concern as to whether the low perfusion pressure and flow

rates used during the bypass can provide adequate nutritive flow. Intermittent delivery via the aortic root is also used but is highly dependent on age and the presenting pathophysiology because of the increased susceptibility of the coronary arteries to the occurrence of congenital anomalies. This technique has also been reported to be associated with longer global ischemic and circulatory arrest times (Report of Congenital Heart Surgeon Society, 1989).

The work of Pratt, 1898 and Batson et al, 1930; provided some documentation that reversal of flow in the coronary sinus and its tributaries could confer some nutritive benefit on the myocardium. Pratt was able to maintain myocardial contraction for 60 minutes using perfusate delivered via the coronary sinus. However, the fact that the coronary sinus is not affected by atherosclorosis (Chiu, 1975) triggered further investigations of the coronary sinus as alternative route for delivery of nutritive flow to the myocardium. Coronary sinus perfusion promises to minimize global ischemia and eliminate the need for long periods of circulatory arrest. This will guarantee longer "safe times" required for most corrective repairs. The coronary venous system has very low incidence of malformations and anatomical variations. Its use for delivery of cardioplegia may eliminate the present dependence on presenting pathophysiology associated with antegrade perfusion, guarantee a bloodless field and minimize clutter of the field by catheters and cannulae.

2.1. Structure and Function of the Coronary Venus System

The coronary venous system is an important drainage channel of venous blood of the myocardium. It consists of a dense vascular meshwork that is interconnected through numerous pathways or tributaries. These pathways remain unaffected by coronary vascular diseases and congenital malformations (Mohl W, 1994). This is one of the reasons for the present effort to the coronary venous system as a mode of cardioplegic delivery especially in situations where it is difficult to use the antegrade approach.

In general, the venous drainage channel of the heart consists of the following: the coronary sinus, the anterior cardiac vein and the thebesian veins. The anterior cardiac veins and the thebesian veins drain less than 20% of the coronary venous blood. The lymph vascular pathways account for 7%. The anterior cardiac vein drains into the right atrium while the thebesian veins channel directly into all the chambers of the heart. The coronary sinus drains blood mainly from the left ventricular wall, the anterior part of the interventricular septum, both atria and most parts of the right ventricle. Approximately 75% of the coronary venous circulation is drained by the coronary sinus (Chih et al, 1994), which originates as a continuation of the great cardiac vein. The origin of the coronary sinus is partially covered by the left atrium. Less frequently, it may originate by the union of the great cardiac vein with the oblique vein of the left atrium or by the union of the great cardiac vein with the left marginal vein. The coronary sinus runs in the posterior interventricular sulcus (coronary sulcus) receiving the following tributaries: the

left marginal vein, the posterior vein of the left ventricle and the middle cardiac vein, which joins it close to its opening into the right atrium. This description is based on work performed on pigs, calves, dogs and human cadavers (Chih et al, 1994; Gundry et al, 1990; Hammond et al, 1971). However some species variations have been reported (Gates et al, 1993; Pakalska et al, 1980).

The ostium of the coronary sinus most frequently bears a thick valve of varying dimensions with a free, concave border. This valve has been termed valve of the coronary sinus or thebesius and has been found to be present in up to 75% of adults and 85% of children and newborns. Its length and width varies from.

1.7 x 1.3 cm to 0.5 x 0.7 cm in adults

1.4 x 1.0 cm to 0.6 x 0.4 cm in adolescents

0.9 x 1.0 cm to 0.2 x 0.3 cm in children

0.3 x 0.4 cm to 0.2 x 0.2 cm in newborns (Piffer et al, 1990).

Valves are also present in the vascular lumen of the coronary sinus. Experimental data shows these valves to be incompetent in adults. In pediatric hearts, 50% of the valves are competent and play a significant role in determining the impedance and distribution of retrograde perfusion (Chih et al, 1994; Mohl W, 1994).

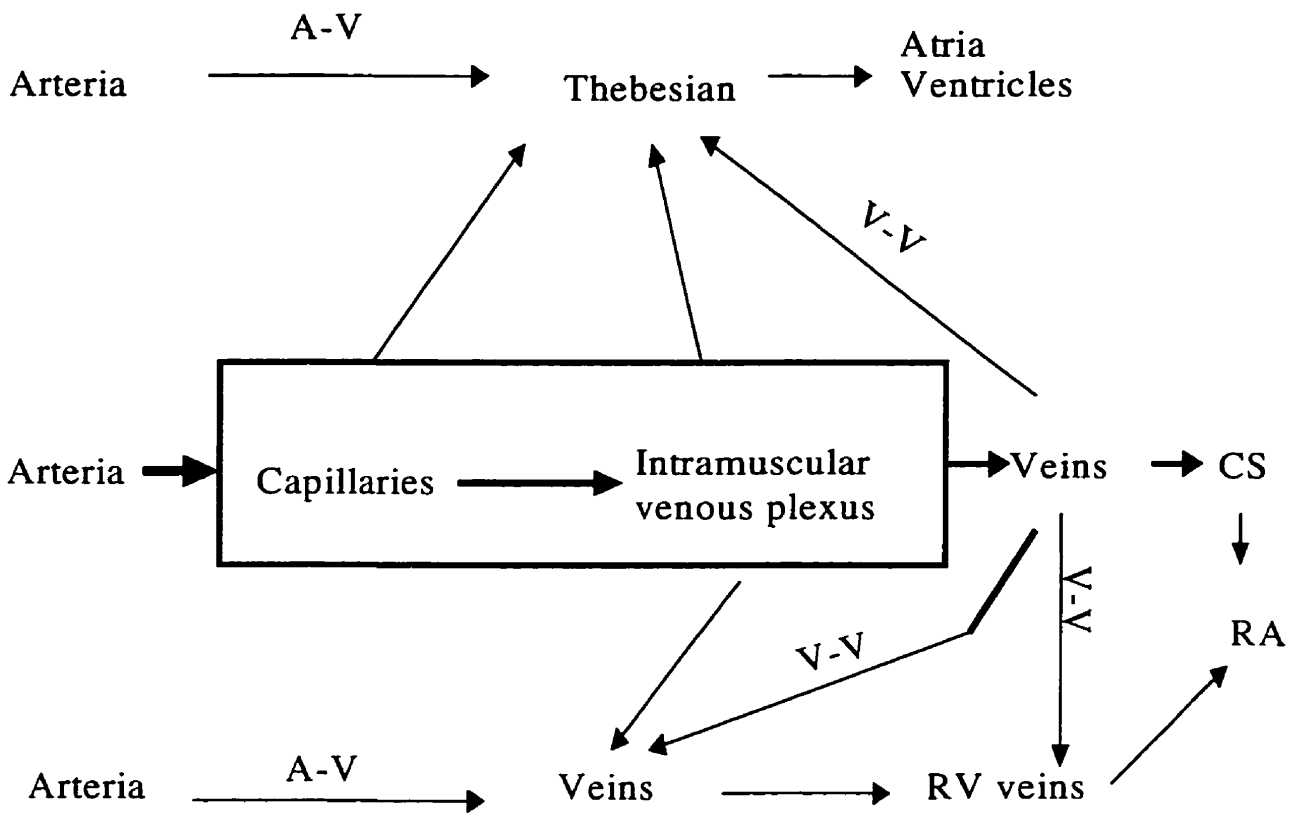

Fig. 2.1. Schematic diagram illustrating the pattern of arterial supply and venous drainage of the normal myocardium.

2.2. The pathway of cardioplegia delivered retrogradely.

The pathway taken by cardioplegia delivered retrogradely has been a subject of controversy and intense investigations. In 1977, Hochberg using radioactive microspheres, quatified the contributions of the cardiac veins to drainage of inflow through the left anterior descending coronary artery, LAD (Hochberg, 1977). His work showed that anterior cardiac veins drains 12%, coronary sinus 48%, thebesian veins 34% and arteriosinusoidal vessels, 5%. Many other workers have reported values between 60% and 70% as the contribution of the coronary sinus to drainage of cardiac venous blood (Hammond et al, 1967; Gates et al, 1996). Hammond and Austen reported that blood draining into the right ventricle had the same level of desaturation as that in the coronary sinus, showing that this blood may have exited via the arteriovenous (A-V) and venovenous (V-V) vessels without traversing the capillary bed. On the contrary, the blood in the left ventricle was only partially desaturated, indicating that some of this blood may have traversed the capillary bed.

Three possible pathways have been proposed for cardoplegia that is delivered retrogradely (Gates et al, 1996). These are:

1. The classical pathway
2. The alternate pathway and
3. The second window pathway.

The classical pathway.

This is the route of retrograde cardioplegia when the coronary sinus is closed to prevent backward leakage of perfusate and the aortic root vented continuously such that only delivery through the retroplegia cannula enters the coronary sinus. This is presented schematically in the diagram below. About 65 to 70% of the cardioplegia will exit via the coronary arteries in this setting while the rest is shunted via the thebesian venous system into the ventricles.

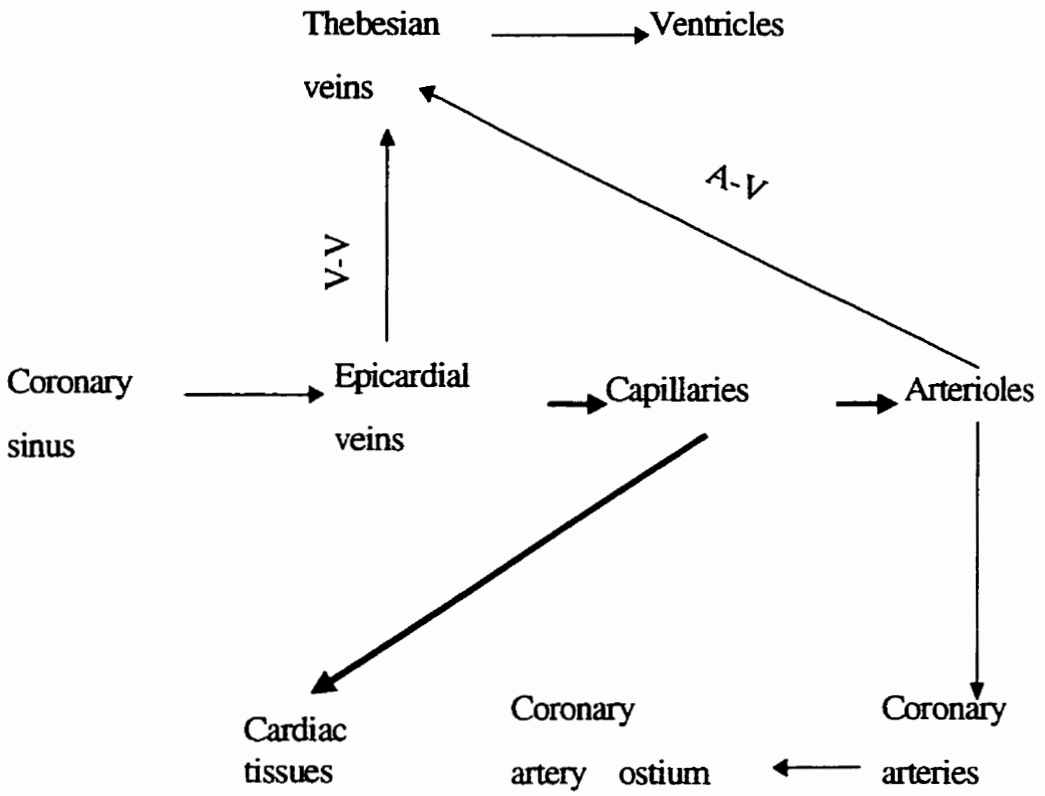

Figure 2.2. Schematic diagram illustrating the classical pathway of cardioplegia delivered retrogradely.

2. The alternate pathway.

This is the route followed by the perfusate when the aortic root or coronary artery ostium is occluded and not vented. This is illustrated below.

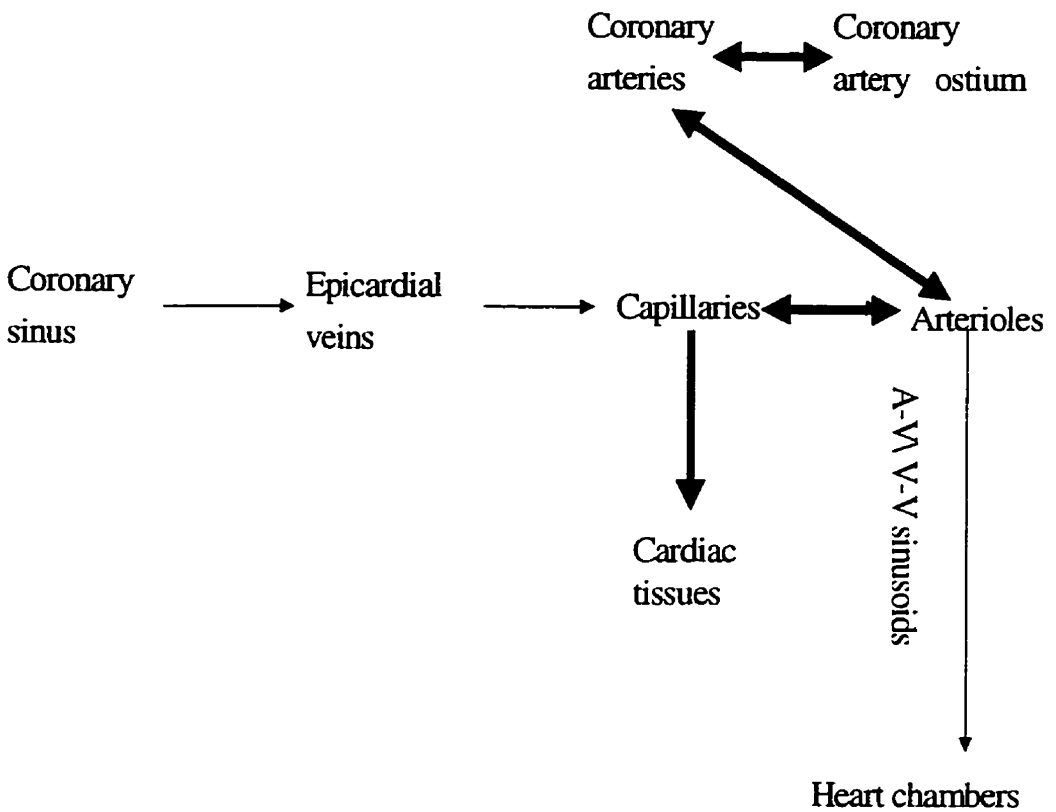

Figure 2.3. Schematic diagram illustrating the alternate pathway of cardioplegia delivered retrogradely.

2. The second window pathway.

This pathway has been hypothesized to be recruited in two situations

I. When antegrade and retrograde delivery are performed simultaneously

II. If the aortic root is ligated and not vented during retrograde perfusion.

The second window pathway is presented schematically in figure 2.4.

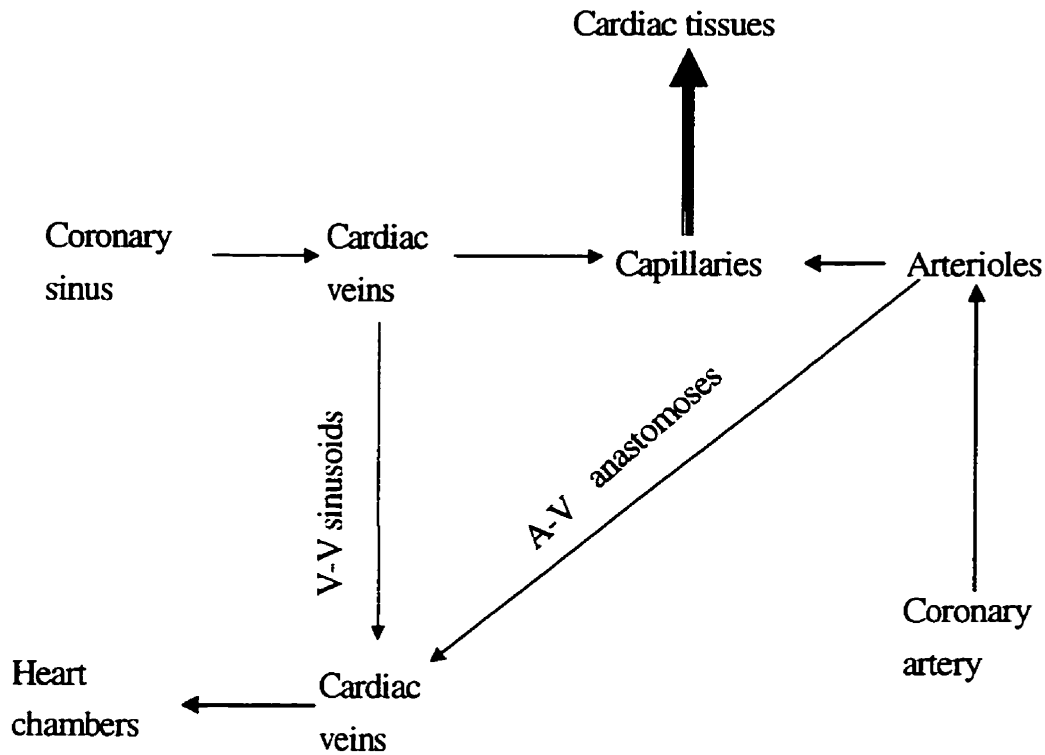

Figure 2.4. Schematic diagram illustrating the second window pathway of cardioplegia delivered retrogradely.

The second window pathway has been hypothesized to be accountable for the added benefits that are derived when simultaneous antegrade/retrograde cardioplegia is used (Gates et al, 1996).

2.3. Rationale.

Congenital heart lesions and malformations of the great vessels are among the most common birth defects. Indeed, children with congenital heart disease now constitute the largest single patient population in many pediatric inpatient services. Of 30,000 infants born annually with congenital heart disease, more than 40% will require cardiac surgery early in life. Although morbidity and mortality of corrective cardiac surgery in infancy has declined, it is still more than 3 times that associated with adult cardiac surgery. This has been attributed to the inability of present cardioprotective techniques to meet the needs of infant cardiac surgery. This fact is leading the exploration for alternative methods of myocardial protection in neonates (Wells et al, 1983).

During cardiac surgery visualization is improved by providing a bloodless field. This is achieved by clamping the inflow and outflow tracts of the heart, and induction of diastolic arrest. These procedures cut off the blood supply to the heart and impose a period of global ischaemia on the myocardium, thus necessitating a method for protecting the heart during this period. Presently, most centers rely mainly on such protective methods as hypothermia with or without circulatory arrest, low flow bypass with hypothermia and intermittent antegrade perfusion. There is increasing concern about the use of circulatory

arrest in neonatal cardiac surgery. Recent reports emanating from clinical monitoring of newborns who were protected with circulatory arrest during cardiac surgery to correct congenital defects have revealed a state of neuropsychological disability and reduced intellectual competence (Jonas RA, 1993, Ye et al, 1997).

Intermittent antegrade cardioplegia is also used in some centres for myocardial preservation when the presenting pathophysiology permits. However, there are still some problems with its use in neonates. These include:

I. Difficulty in cannulation of the diminutive aorta and the coronary ostia especially in malformations involving the aortic root and coronary circulation.

II. Its dependence on the presenting pathophysiology.

III. The increased risk of coronary ostial stenosis from direct cannulation.

IV. The presence of aortic root cannulae clutters the small operative field.

V. The requirement to maintain continuous delivery of oxygenated cardioplegic solution during neonatal heart operations becomes more difficult to achieve with intermittent antegrade perfusion.

In adults, the limitations associated with delivery of cardioplegia through the aortic root can be overcome by the use of direct vein graft (a piece of vein obtained from a vein in the extremeties used in replacing occluded or stenosed coronary vessels or the aorta) and performing proximal grafting before aortic ligation. In neonates, these maneuvers are technically difficult and limited by the small size of the heart and the vessels.

Myocardial energy requirements are determined principally by cardiac electromechanical work and secondarily by temperature. It has been determined that diastolic arrest decreases the O_2 consumption of the heart (MVO_2) by up to 90% by eliminating cardiac electromechanical work. This is equivalent to a decrease from 8-10ml of O_2/minute/100g required by the normal working heart to 1ml/minute/100g required by the arrested myocardium. Deep hypothermia combined with circulatory arrest decreases the MVO_2 to about 0.3ml/minute/100g of heart tissue (Buckberg, 1993), which is not significantly different from that associated with diastolic arrest. Furthermore, it has been determined that retrograde cardioplegia can deliver a total nutritive flow (i.e % of perfusate reaching the capillary bed) of about 40% of that required by a working heart (Tian et al, 1996). This translates to about 3-4ml/minute/100g of heart tissue, which far surpasses the O_2 consumption rate of 1ml/minute/100g of arrested heart tissue. Experimental results from work on adult models suggests that if the heart is arrested, the nutritive flow (the amount of perfusate reaching the capillary bed) derivable from coronary sinus perfusion will be sufficient to sustain myocardial energy metabolism (Buckberg, 1993; Fabiani, 1984; Tian et al, 1997).

2.4. Anatomical Basis for Retrograde Cardioplegic Protection in Neonates.

The coronary circulation exhibits some unusual characteristics. It does not completely follow the accepted circulatory pattern which is present in smooth or

skeletal muscle; i.e. blood moving from artery to arteriole to capillary, venule and then to vein. In cardiac muscle, this orderly pattern is intercepted by sinusoids which constitute irregular, interconnecting, endothelial-lined channels. They can connect veins to veins (veno-venous channels), arteries to veins (arterio-venous channels), arteries to ventricular lumen (arterio-luminal channels) and veins to ventricular lumen (veno-luminal channels). The frequency of occurrence of each of these types of channels remains to be ascertained (Piffer et al, 1990; Hammond et al, 1971; Pakalaska et al, 1980). The sinusoids wander through the myocardium and constitute an arterial or venous blood pool from which the myocardium could be nourished. They are capable of transporting blood to the myocardium under conditions of reduced coronary pressure and when coronary pressure and ventricular systolic pressure are equal. Whether these sinusoids transport blood to the myocardium and simultaneously provide drainage at normal as well as altered pressures is not known. The low pressure situation, the complete absence of autoregulatory mechanisms and systolic squeeze (reduced coronary resistance) present in a heart stilled by diastolic arrest provides a favorable physiological climate for transport of blood or cardioplegic perfusates through these channels and exchange across the endothelium. In a heart whose energetic requirements have been reduced by hypothermia and/or arrest, transport through the sinusoids may be sufficient to sustain the myocardial energy function (Solorzano et al, 1978; Menasche et al, 1982).

It is difficult to explain the unusual characteristics of the cardiac muscle vasculature. It is however supported by some embryological evidence (Hammond et al, 1967). It is believed that the myocardial vasculature in arriving at its mature state evolved through an early vertebrate phase that had no coronary arteries. These early vertebrate hearts were supplied by blood from the ventricular chamber that washed into and out of the myocardial sinusoids and in doing so, bathed the muscle bundles in oxygenated blood (Jensen, 1966). As animals ascended the phylogenetic ladder however, heart muscle became more and more compact, compressing the sinusoids to the point where blood could no longer enter and leave freely. According to this view, coronary arteries developed as the need for an extramyocardial blood supply rose (Grant et al, 1926). Avalaible evidence show that the sinusoids, even in the mature human, still retain this embryological characteristic making it possible for them to transport nourishment to the myocardium when they are filled with blood or crystalloid solution retrogradely or antegradely (Batson et al, 1930).

2.5. Clinical Basis for Retrograde Cardioplegic Protection in Neonates

Noncardioplegic methods such as prolonged circulatory arrest and hypothermia have remained the cornerstone of neonatal heart operations. Available reports show that these methods do not provide a safe ischemic time that meets the increasing demands of current neonatal heart repairs. Intermittent antegrade infusion of cardioplegic solution is also used. However, the short

intermittent periods of non-perfusion associated with each aortic clamping could translate into significant ischemic stress. Retrograde cardioplegia has been shown to overcome some of the inadequacies of antegrade perfusion which include inadequate perfusion in aortic incompetence and beyond stenosed coronary arteries in adults. It has also been recommended as the technique of choice for delivering cardioplegia during arterial switch operations, which are currently popular for treatment of d-transposition of the great arteries (Vaage, 1993). This anomaly is associated with multiple ventricular defects and abnormal origins of the coronary arteries, which may make antegrade delivery of cardioplegia technically difficult (Mohl, 1994; Ludinghausen, 1987).

With the increased complexity of operations presently possible through cardiopulmonary bypass and the fact that most of the operations in neonates involve the aortic root, coronary vasculature and other great vessels, coupled with the very small operative field in neonates, the efficiency of antegrade coronary perfusion has become limited. This is true particularly in aortocoronary bypass surgery. The use of antegrade perfusion in this procedure will lead to excessive bleeding and blood in the operative field. Another drawback of delivery of cardioplegic solution through the coronary arteries is the presence of the perfusion cannula in the small operating field of the neonates (Chiu, 1984).

Retrograde coronary sinus perfusion (RCSP) affords the modern cardiac surgeon an easier and more efficient technique for protecting the heart especially in situations where the use of the antegrade mode of delivery has been found undesirable. It can also eliminate the prolonged ischemic period associated with

circulatory arrest and intermittent antegrade perfusion and the observed epicardium-endocardium temperature gradient and possible damage to the phrenic nerve or frostbite of the epicardium that can occur when topical hypothermia alone is used. RCSP reduces the technical difficulties of neonatal open heart operations by ensuring continuous delivery of cold cardioplegic solution in a heart whose muscles are relaxed and natural motion stilled by diastolic arrest. It may allow efficient repair of major congenital malformations associated with the left ventricular outflow tract and aortic reconstruction without the need for long periods of circulatory arrest.

2.6. Controversies Associated with RCSP

The concept that reversal of flow in the coronary venous system could confer nutritive benefit on the myocardium is controversial. This is due to a variety of factors perceived to affect its efficacy. The factors include: the anatomy of the coronary venous system, the time it takes to induce arrest, the possibility of edema and tissue swelling, the loss of cardioplegic solution through direct drainage into the heart chambers, the integrity of the neonatal vascular network, inadequate nutritive flow to the right ventricular myocardium and inefficient washout of reactive oxygen free radicals and other metabolites.

There is a high level of non-uniformity in the reports of various workers. This may be due to the use of different animal models, different experimental protocols and different physiological, anatomical and biochemical parameters. Menasche et al (1982) evaluated the efficacy of retrograde and antegrade modes

of cardioplegic delivery using myocardial functional parameters such as cardiac output and ventricular stroke work index. Each cardioplegic delivery method was applied to a group of 12 patients. No statistically significant difference was reported between the two groups, leading to the conclusion that retrograde coronary sinus perfusion is a simple, safe and effective means of cardioplegic protection. Using radioactive microspheres in the *in vivo* dog heart, Partington et al (1989), showed that nutritive flow during retrograde and antegrade cardioplegia was 65% and 87%, respectively. However, using a similar technique and animal model, Solorzano and associates (1928) found that nutritional flow of retrograde cardioplegia was about 20% of that arising from antegrade perfusion. Similar experiments by Tian and associates show inadequate perfusion of the right ventricular myocardium and a near normal nutritive flow to the other chambers of the heart (Tian et al, 1996). Many other workers have reported different values of nutritional flow for cardioplegia delivered retrogradely. These include: 93% (Caldarone et al, 1994); 52% (Cohen et al, 1988); 35% (Ardehali et al, 1995); and 20-30% (Menasche et al, 1991; 1982). It is possible that variations in species of animals used, experimental protocols and techniques may be responsible for the reported differences in nutritional flow. On the other hand, it should be noted that nutritional flow values obtained using radionuclide microspheres or colored microspheres are highly dependent on the size of the microspheres. The large variations in numerical results indicate that the quantitative aspects of nutritive flow of retrograde cardioplegia are not well established. Foglia et al (1979) reported that

the use of low perfusion pressure and a hyperosmolar perfusate can be effective in preventing tissue swelling and edema.

Based on the impedance distribution in the coronary vascular system it is expected that nutritive flow during retrograde perfusion may not have a fixed value but may depend on perfusion pressure, which raises a question regarding the optimal perfusion pressure for retrograde cardioplegic perfusion (Tian et al, 1996). Currently, the perfusion pressure used clinically in adults for retrograde cardioplegia varies from 30 to 65 mmHg (Menasche et al, 1991). This large difference in perfusion pressure may be responsible, at least partially, for the conflicting results.

The extensive venous networks developed throughout the myocardium are expected to make RCSP an efficient means of providing core cooling and delivery of cardioplegic perfusates in an arrested heart. Shunting of blood through the arteriosinusoidal and thebesian systems, which tend to limit the nutritive value of retrogradely delivered perfusates, can be drastically reduced by the use of optimal perfusion pressure, hyperosmolar perfusate and substituting crystalloid with blood.

3.0. Materials and Methods

3.1. Animals.

Hearts were obtained from domestic piglets aged 5 to 11 days and weighing 3.0-4.5 Kg. The hearts were divided into two groups. The hearts in group 1 were maintained in arrest by coronary sinus perfusion while those in group 2 were maintained in arrest by aortic root perfusion. All animals received humane care in strict compliance with the regulations of the Canadian Council on Animal Care and approved by the Animal Care Committee at the Institute of Biodiagnostics.

By necessity, research designed for application in humans must be carried out on animal models that share many similarities with humans. The neonatal piglet heart resembles the neonatal human heart in terms of coronary venous anatomy, size, electrical activity, susceptibility to fibrillation and metabolism. It allows the use of equipment similar to that used in human cardiac surgery and simulates very closely the conditions obtainable in the theatre during cardiac surgery and cardiopulmonary bypass. The seven day old piglet is large enough to allow the study of hemodynamic, biochemical and other cardiovascular functions as they relate to neonatal humans. It can withstand extended periods of cardiopulmonary bypass. The isolated piglet heart rather than the *in vivo* heart was chosen to remove unwanted secondary neural and humoral influences on myocardial metabolism, function and homeostasis. In this way observed changes could be attributed to the effects of a particular

method of cardioplegic protection on myocardial metabolism instead of secondary corporeal effects.

3.2. Premedication and anaesthetic treatment.

The essence of good preanaesthetic treatment is based on the fact that it calms the animal and helps ensure increased efficiency of the anaesthetic treatment. In this study premedication consisted of intramuscular injection of midazolam (0.3 mg/Kg body weight), ketamine (20mg/Kg body weight) and atropine sulphate (0.025 mg/Kg body weight). General anaesthesia was induced by inhalational administration of 3-4% isoflurane. The animal was then intubated, and mechanical ventilation commenced using medical air with an inspired oxygen fraction of 40% at a rate of 20 to 25 cycles/minute and an inspiratory pressure of 20 mm Hg. This was mixed with 1.5 to 2.0% isoflurane for maintenance anaesthesia throughout the duration of surgery. The respiratory rate and volume were monitored continuously during the surgery and adjusted as necessary to keep the arterial blood PO_2 and PCO_2 within the normal physiological levels.

3.3. Surgery and isolation of piglet heart.

Under appropriate anaesthesia, the brachiocephalic artery was cannulated at the level of the common carotid artery for arterial pressure monitoring, blood sampling and infusion of cardioplegic solution to arrest the heart. A sternotomy was performed and the brachiocephalic and subclavian arteries dissected. The

pericardium was exposed longitudinally along the midline and the incision extended to the right and left in cruciform fashion. Umbilical tapes were threaded around the origins ascending aorta and the pulmonary trunk in readiness for ligation of these vessels prior to excision of the heart. A cannula was inserted centrally in the brachiocephalic via the carotid artery. Following anticoagulation with an intravascular injection of heparin (1000 IU) into the superior vena cava, the brachiocephalic artery, subclavian artery, superior and inferior venae cavae were ligated in succession using the umbilical tapes already in position. The descending aorta was clamped and heparinized cold cardioplegic solution at the designated temperature (approximately 4° C) infused (20 mL/kg body weight) into the aortic root via the brachiocephalic arterial cannula. The right and left atria were cut to allow drainage of the cardioplegic solution and to prevent the warm blood in the lungs from returning to the heart. The heart was then rapidly excised by transection of the great vessels, vena cavae, and the pulmonary hilae and immersed in a cold cardioplegic solution containing heparin (4° C).

3.4. Instrumentation of the isolated heart.

Instrumentation of the isolated heart involved insertion of a cannula into the aortic root by way of the brachiocephalic artery, which was connected to the Langendorff perfusion apparatus and used for antegrade delivery of cardioplegic perfusates (antegrade group). An incision was made in the right atrium and through it, a retroplegia cannula (Research Medical Inc., Salt Lake city, UT) was passed through the coronary ostium into the coronary sinus (retrograde group).

This was used for retrograde delivery of cardioplegia. A fluid filled latex balloon was passed into the left ventricle via a small incision in the left atrium. This was connected to the pressure transducer/multichannel recorder and used to measure the left ventricular pressure and control preload during control perfusion and reperfusion. A ligature was placed around left atrio-ventricular orifice to secure the balloon position within the left ventricle. The balloon was connected to a pressure transducer (Model P23 XL Spectramed Inc. Oxnard CA) by a short length of polyethylene tubing. Accumulation of solution in the ventricles from thebesian flow and coronary sinus flow was prevented by the use of a small length of polyethylene tubing pierced through the apex of the left ventricle and the free wall of right ventricle. A small glass ball filled with phenyl phosphonic acid (PPA) was inserted into the right ventricle as a reference for the ^{31}P nuclear magnetic resonance signal intensities.

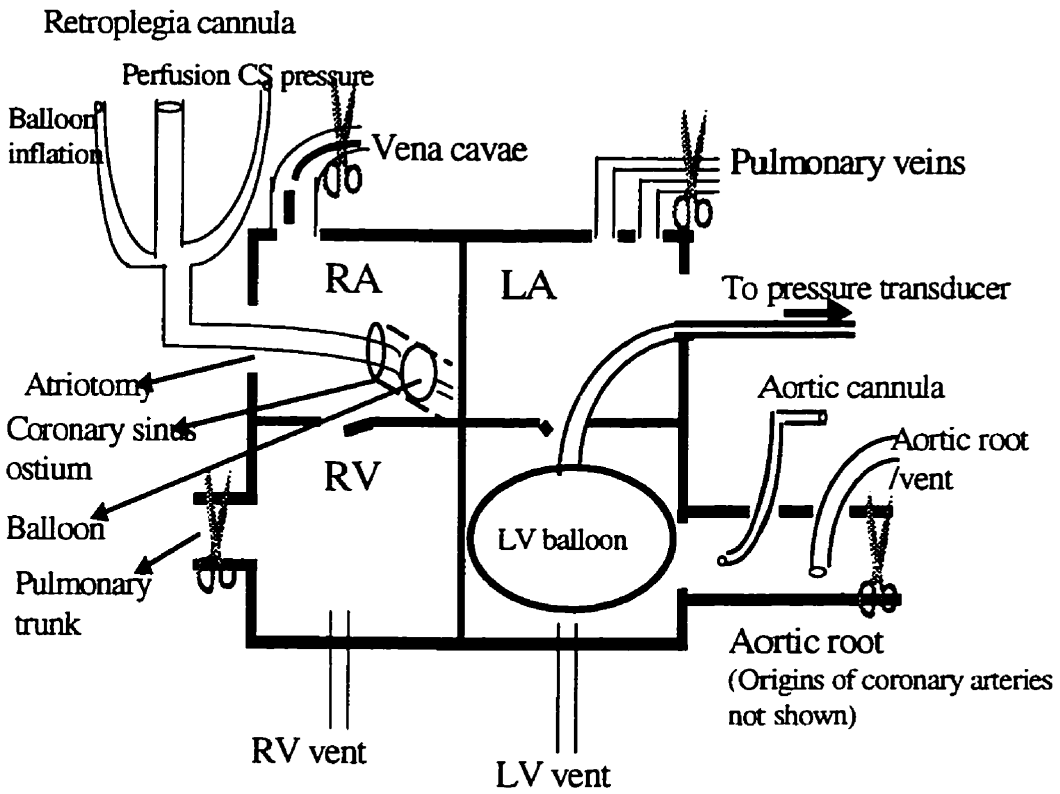

Figure 3.1. Schematic diagram showing instrumentation of the piglet heart preparation used in the study. CS: coronary sinus, RA and LA: right and left atrial chambers; RV and LV: right and left ventricles.

3.5. Perfusion system and experimental set-up.

Because the heart must be cannulated outside the spectrometer magnet and subsequently moved into it, a Langendorff perfusion apparatus that provided optimal spatial flexibility was designed. It consists of two thermostatically regulated reservoir/oxygenator systems, A and B (Capiox 308, Terumo Corp., Tokyo, Japan) and a roller pump (Cobe Laboratories, Inc., Lakewood, Colo.) located inside the laboratory. These systems were used to supply oxygenated K-H solutions (hyperkalemic and nomorkalemic) to another set of two thermostatically regulated glass reservoirs (C and D) by means of a silicon and tygon tubing system connecting the two. Thermoregulation was achieved with the aid of two heat exchanger systems (RM 6 Lauda super, Lauda DR. R Wobser GMBH and CO), one circulates temperature regulated water to the reservoir/oxygenator systems and the other to the thermoregulatory overhead glass reservoirs and the shiley heater/air bubble trap system (Shiley BCD, Shiley Inc, Irvine, Ca). The outlet arm of the heat exchanger was connected to inlet arm of the shiley/air bubble trap system with the aid of plastic tubing made of nonkinkable material. The shiley contains multiple arms that permit attachment to the aortic and retrograde cardioplegic cannulae and helps warm up the perfusate to the desired temperature while permitting continuous debubbling of the perfusate. The shiley is debubbled and primed before hook up to the aortic and/or coronary sinus cannulae.

The two glass reservoirs were suspended close to the isolated heart/sample chamber in the center of the spectrometer magnet (1.4 meters for

the hyperkalemic reservoir and 1.8 meters for the normokakemic reservoirs respectively). Gas exchange was accomplished with a mixture of 95% O_2 \5% CO_2 passed through the perfusate with the aid of the reservoir-oxygenator system. Perfusate temperature was maintained at 37°C by a heat exchanger. O_2 tension was uniformly maintained at between 500 and 550mmHg; pH and CO_2 tension was maintained within the normal range. The perfusion system used in the study is presented schematically in Figures 3.2 and 3.3.

In the antegrade group, infusion of perfusate was via a cannula inserted into the aortic root by way of the brachiocephalic trunk. Another cannula was passed into the subclavian artery and used for monitoring aortic pressure, decompression of retrograde return and for venting (removal of air or fluid). In adults, aortic vein grafts are also used. Grafts can also be connected to the aortic root before or during aortic clamping to be used for perfusate infusion during surgery.

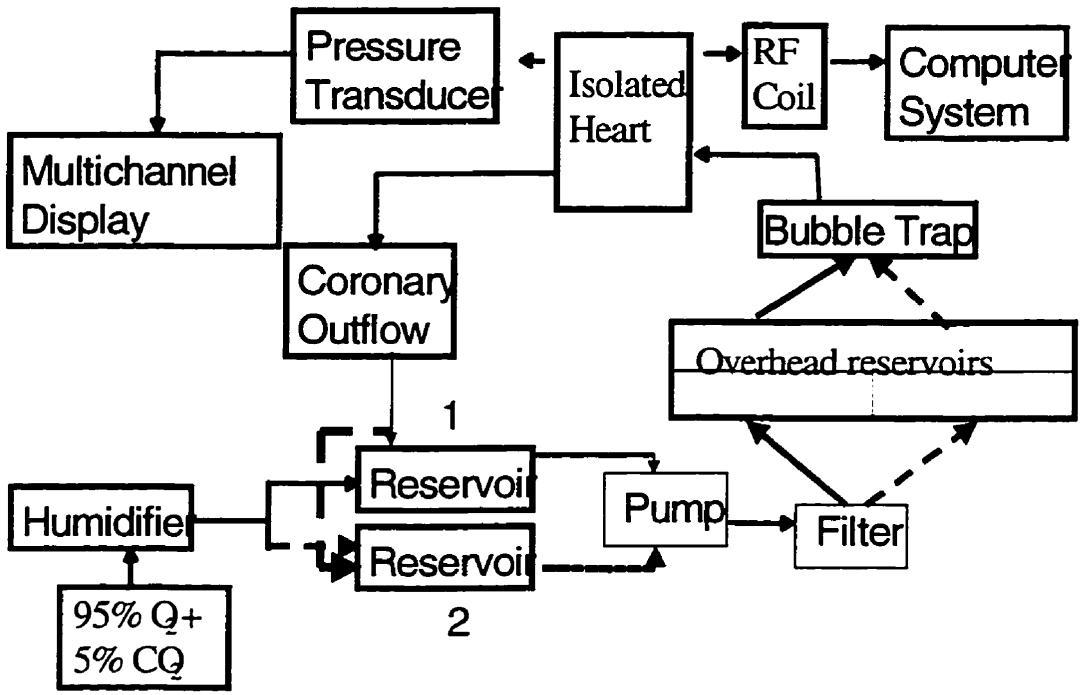

Fig. 3.2. Diagrammatic illustration of the experimental design and set-up.

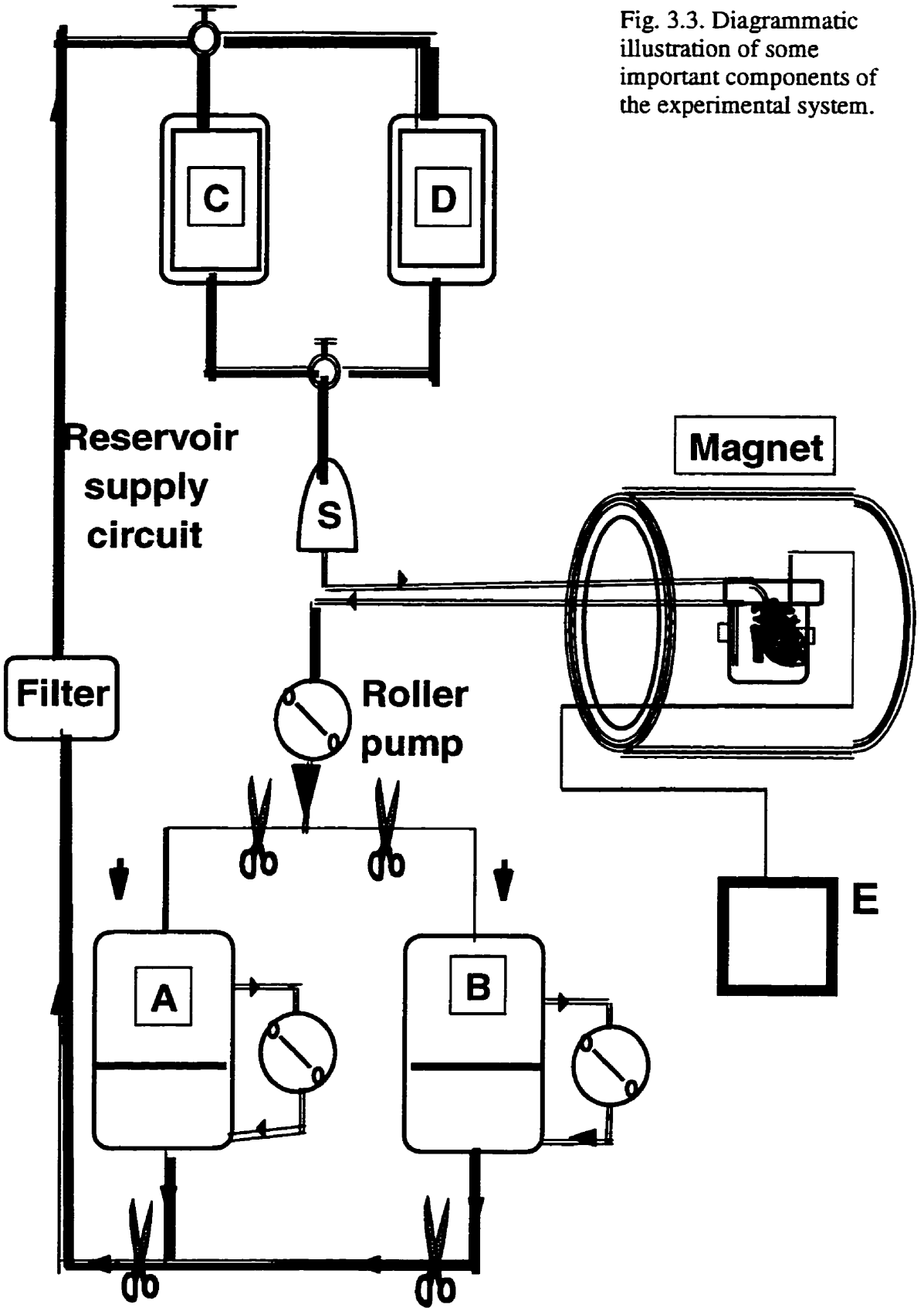

Fig. 3.3. Diagrammatic illustration of some important components of the experimental system.

Key to Figure 3.3.

A and B, reservoir-oxygenator system

C and D, suspended thermoregulated reservoirs

E, Pressure transducer/multichannel display system

S, Shiley heater/air bubble trap

Heat exchanger systems (not shown)

Overhead thermoregulated reservoir suction lines (not shown)

Table 3.1: Composition of the solutions used in the study of retrograde/antegrade cardioplegia.

Components	A:Cardioplegic Arrest (mM/L)	B:Hyperkalemic K-H (mM/ L)	C: Normokalemic K-H (mM/L)
NaCl	100	100	118
KCl	26	16	3.5
MgSO4	-	-	1.2
MgCl	16	16	-
EDTA	0.5	0.5	0.5
Glucose	11	11	11
$NaHCO_3$	25	25	25
$CaCl_2$	1.75	1.75	1.75
$KHPO_4$	-	-	0.9
BSA (%)	0.5	0.5	0.5

3.6. Solutions used.

Three types of cardioplegic solutions were used. The composition are presented in the table 1. The goals of effective cardioprotection are to achieve elctromechanical quiescence, maintain a bloodless field, deliver optimal nutritive flow and O_2 to the heart, and ensure full recovery of contractile and energetic function during reperfusion. To test the efficacy of retrograde cardioplegia, three types of solutions differing only in the K^+ and SO_4^{2-} concentrations were designed and used to achieve the following aims:

1. Rapidy arrest the heart prior to removal from the thoracic well (solution A). This minimizes injury and in combination with the bathing media, minimizes ischemia during the short interval between isolation and hook-up to Langendorff perfusion apparatus.

2. Maintain the heart in an isovolumic contraction mode during control perfusion and reperfusion (solution C). This permits effective monitoring of systolic, diastolic and contractile functions during these periods.

3. Allow transition from isovolumic contraction to diastolic arrest (solution B). During this period, hearts in group one were perfused through the coronary sinus (retrograde cardioplegia) while those in group two were perfused through the aortic root (antegrade cardioplegia).

The general principle of formulation of good cardioplegic solution is that it should mimic either the extracellular fluid or blood. Another criteria is the purpose to be achieved. For example, for rapid alteration of the biochemical (intracellular and extracellular) milieu of the myocyte leading to rapid arrest, the

K^+ in the Krebs-henseleit solution was increased to 26.0mM/L (solution A in Table 1). For maintenance of arrest, this was decreased to 16.0mM/L (solution B). To induce a rapid transition to the isovolumic contractile state, the concentration of K^+ in the perfusate was decreased to between 3.5 and 4.0mM/L; (solution C, approximately equal to that of blood). The other components of the three sets of solutions used in the experiments are approximately equal to those in blood. Thus the three solutions differ from blood only by virtue of their K^+ concentration and the absence of some plasma proteins and cells.

3.7. Experimental time course and methods

This study compared the efficacy of retrograde cardioplegia (RC) and antegrade cardiolegia (AC) in protecting the neonatal heart. Isolated piglet hearts 5-11 days old and weighing 3.0 to 4.5 Kg were divided into two groups (n = 8 per group). The experimental protocol consisted of 30 minutes of control perfusion, followed by 45 minutes of cardioplegic arrest, and 30 minutes of reperfusion. During control perfusion and reperfusion, the hearts were perfused using nomorkalemic Krebs-Henseleit (K-H) solution. In group 1 hearts, cardioplegic arrest was rapidly induced using an initial 2-minute period AC and maintained with continuous RC for the remaining 43 minutes. The hearts in group 2 were subjected to continuous AC throughout the experimental time course. Myocardial energy metabolism and contractile function in the two groups were compared by assessing energy metabolites using ^{31}P MR spectroscopy and

determining myocardial O_2 consumption (MVO_2) and measuring intraventricular pressure.

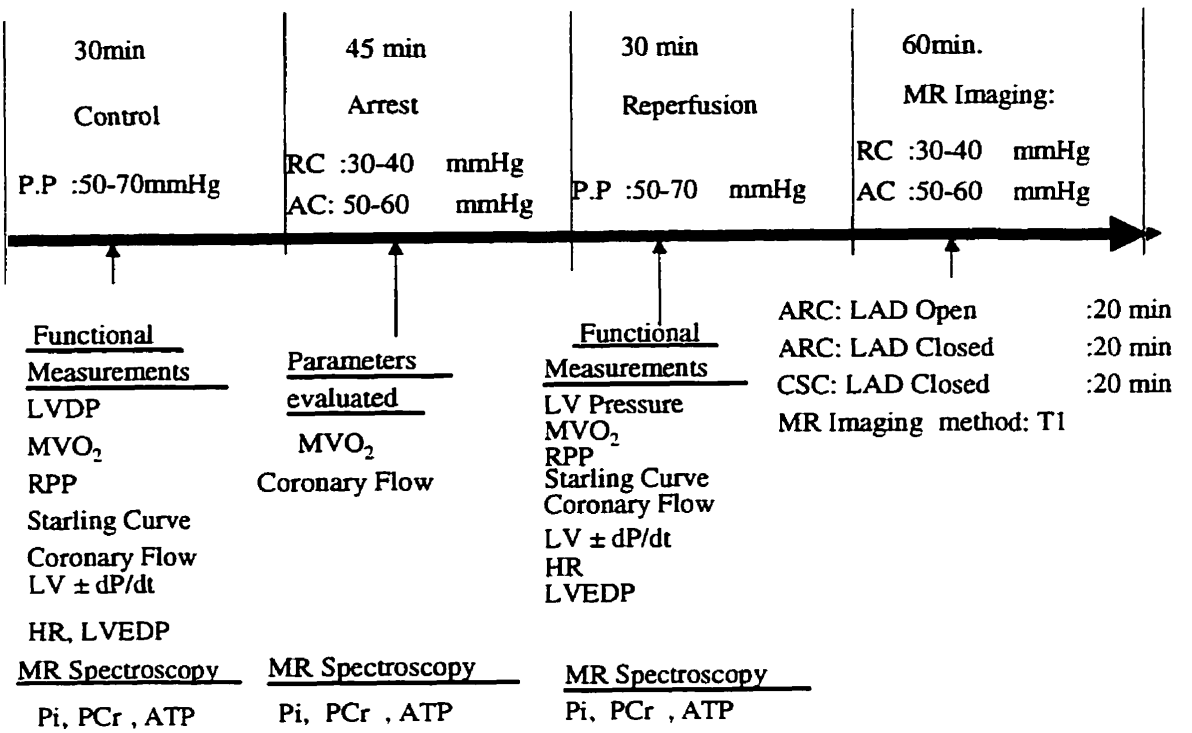

Figure 3.4. Schematic diagram illustrating the experimental time course and methods used in the study. LV; left ventricle, LVEDP; left ventricular end-diastolic pressure, MVO_2; myocardial oxygen consumption, dP/dt; rate of change of left ventricular pressure, Pi; inorganic phosphate, PCr; phosphocreatine, ARC; aortic root cardioplegia, CSC; coronary sinus cardioplegia.

4.0. Measurements

4.1. Assessment of Contractility and Myocardial function.

The term contractility is used to describe the dynamics of the cardiac contractile process and refers to the rate of contraction of the myocardium and its ability to reach maximal tension or pressure. In our isolated, isovolumically contracting heart preparation the assessment of myocardial contractility and function was done on the basis of systolic function, diastolic function and myocardial compliance. A small incision was made in the left atrium and through it a latex balloon (of volume 2.0-3.0mL) fixed at the end of a polyethylene tubing was carefully maneouvered into the left ventricular chamber. A ligature was placed around the left atrioventricular orifice to secure the balloon position within the left ventricle. The polyethylene tubing from the balloon was connected to a pressure transducer (Model P23 XL, Spectramed Inc., Oxnard CA), which in turn was connected to a multichannel pressure recorder (EEG and Polygraph data recording system, Grass Model 79E, Grass Instrument CO, Mass.). With the aid of the pressure transducer-multichannel recorder system, changes in the intraventricular balloon volume associated with the cardiac contractile process are electronically differentiated and displayed as cardiac contraction curves on a monitor or chart recorder/display system.

4.2. Cardiac Contraction Curve (CCC).

The cardiac contraction curve is a graphical time course display of the following functional curves:

I. The systolic pressure curve,

II. The velocity of increase or decrease of left ventricular developed pressure (± dP/dt curve),

111. The aortic and/or coronary sinus pressure.

4.3. Systolic Pressure Curve.

This is the curve that shows maximal or peak pressure associated with the cardiac contractile process. It is generated during systole. In our isolated heart preparation, the contraction of the left ventricle impinges on the intraventricular balloon, producing a squeezing effect and generating mechanical signals. This is picked up by the pressure transducers. In the pressure transducer, the mechanical information is converted into electronic information that is channelled into the multichannel recorder/display system.

Systolic pressure in the past has been used by various workers as an index for assessment of systolic function of the myocardium (i.e the ability of the heart to generate enough peak pressure to direct the flow of blood in the circulation). It is however, affected by the diastolic volume, diastolic pressure and compliance of the myocardium. This necessitated the design of another parameter (left ventricular developed pressure, LVDP) that takes into account the effects of these factors on the systolic pressure.

4.3. The Left Ventricular Developed Pressure (LVDP).

The LVDP refers to the difference between left ventricular systolic pressure and left ventricular diastolic pressure. It is a modification of systolic pressure that takes into account the effect of factors such as end-diastolic volume and compliance on the effective systolic pressure. In the intact organism, it is also used to assess the ability of the myocardium to eject the venous return into the pulmonary and systemic circulation. A decrease of LVDP indicates reduced force for systolic ejection. Under such conditions venous return will accumulate in the heart and result in an increased pre-load, which will lead to heart failure. The LVDP is highly dependent on heart rate. Changes in heart rate will lead to changes in LVDP. One way to minimize this dependence is to continuously pace the heart during an experiment. In an NMR setting, this is technically difficult since the heart is in the center of the spectrometer magnet and may not be easily accessible. Furthermore, pacing can lead to interference with the signal-to-noise ratio and other experimental conditions. In this study continuous pacing of the heart was achieved with the aid of magnetically shielded cables that connected the ventricles to the pacing machine. Another parameter the rate-pressure product, RPP which is the product of the LVDP and heart rate have been designed to account for the changes in LVDP that accompanies changes in heart rate.

In this study the assessment of myocardial contractility and systolic function during control perfusion and reperfusion was achieved by measuring

the LVDP, heart rate (HR), +dp/dt and calculating the RPP during isovolumic contractions of the myocardium.

4.4. Diastolic Function.

Diastolic function refers to the sum of events that accompany the return of the myocardium to its presystolic state (diastolic relaxation). The volume of the ventricular chambers in this state is called the diastolic volume. It is the maximum volume of the ventricular chambers during the cardiac cycle. The pressure associated with the diastolic volume is called diastolic pressure and is the minimum pressure generated by the ventricles during the cardiac cycle. Diastolic pressure is usually measured at the end of the diastolic phase of the cardiac cycle and is also often referred to as the end-diastolic pressure, EDP. If the site of measurement is the left ventricle, it is called left ventricular end-diastolic pressure, LVEDP. It is the pressure in the left ventricle at the end of diastole and is generated by failure of the systolic pressure to return to the presystolic pressure at the end of diastole.

The heart muscle relaxes after developing tension because the sarcoplasmic reticulum actively removes Ca^{2+} from the sarcomeres. This process requires energy, thus LVEDP could also be used as an indirect mechanism for assessing the metabolic competence of the myocytes. However, it is not an independent parameter since its value may be affected by factors such as increased heart rate and edema.

Some workers have proposed that engorgement of the extensive cardiac venous networks following coronary perfusion pressure increases can lead to increased LVEDP (Salisbury et al, 1961). Under our experimental conditions, engorgement of the venous bed was minimized by continuously venting the aortic root and the ventricular chambers. With the high impedance associated with retrograde cardioplegia, perfusate can accumulate in the venous networks of the myocardium leading to decreased EDV and increased LVEDP. This will lead to an increase in heart rate and decreased velocity of relaxation (–dP/dt).

In this study, assessment of diastolic function of the myocardium during control perfusion and reperfusion was achieved by measuring LVEDP at a constant balloon volume of 5.0 mL, plotting LVEDP-volume curve and assessment of the –dP/dt. This balloon volume was chosen to ensure reproducible LVEDP since at lower balloon volumes, the balloon may not have totally filled the LV cavity. LVEDP at constant balloon volume can also provide an indirect assessment of the ventricular wall stiffness (Mirsky, 1984). The LVEDP-volume curve is used to assess myocardial mechanical tissue properties (ventricular wall stiffness). Changes in myocardial tissue properties are reflected by displacements of the LVEDP-volume curves. An increase of ventricular wall stiffness may arise from myocardial infarction, edema, venous engorgement and injury and results in a leftwards and upwards displacement of the LVEDP-volume curve (Baumgart et al, 1993).

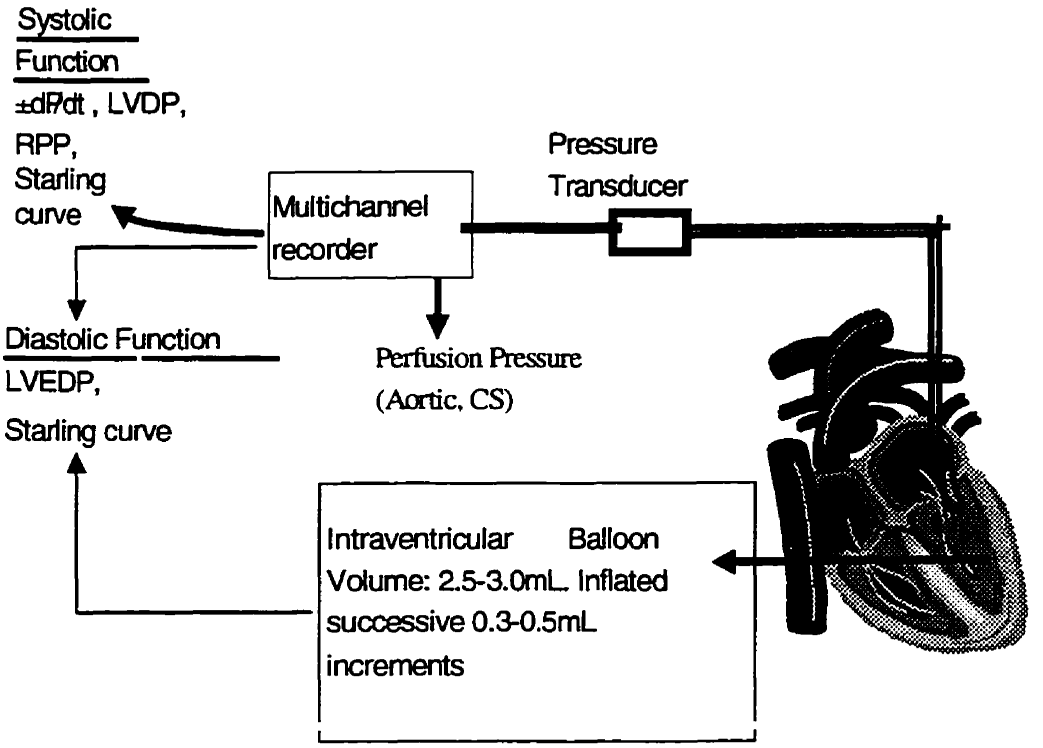

Figure 4.1. Schematic diagram illustrating some of the equipment used in acquiring cardiac function parameters and for measuring aortic and coronary sinus pressures.

4.5. Myocardial Compliance/Chamber Stiffness.

Systemic function depends on end-diastolic volume (more precisely end-diastolic sarcomere length as described by the Frank-Starling relation). Systolic function is governed by parameters such as systolic pressure, LVDP, heart rate, and dP/dt. End-diastolic volume is determined by compliance, an intrinsic property of the myocardium that determines its distensility in the presence of increasing intrachamber pressure. Myocardial compliance is defined as the ratio of change in volume (dV) to change in pressure (dP). The inverse dP/dV (ratio of change in pressure to changes in volume) represents the chamber stiffness. The ability of increasing ventricular chamber distension to cause increased generation of tension and pressure provides a good assessment of myocardial performance and could be used to monitor recovery of the heart during reperfusion.

In our isolated, Langendorff crystalloid perfused heart preparation, increasing distension of the ventricular chamber (increased ventricular chamber volume) was produced with the aid of a fluid filled latex ballon (of volume 2.0-3.0 mL) inserted into the left ventricle via a small atriotomy on the lateral wall of the left atrium. The balloon position was maintained by a ligature placed around the left atrioventricular orifice. The balloon was connected via polyethylene tubing to a pressure transducer/multichannel recorder and display system. Changes in volume of the intraventricular balloon were produced using a saline filled syringe by injecting increasing volumes of saline (0.3-0.5 mL)

into the intraventricular balloon. Filling was continued until a LVEDP of 25.0 mmHg was reached. The pressure associated with each balloon volume was electronically differentiated and displayed on a chart recorder by the pressure transducer/multichannel recorder system as cardiac function curves. From the cardiac function curve, the LVDP associated with each balloon volume is calculated by subtracting the LVEDP from the peak systolic pressure. The value of LVDP generated at each increase of intraventricular balloon volume was used to plot a pressure-volume curve with changes of LVDP on the Y-axis and volume changes on the X-axis. The slope of the curve represents the chamber stiffness of the myocardium. An increase in chamber stiffness is reflected by an upward and leftward displacement of the LVDP-volume curve.

Increasing balloon volume stretches the myocardium and results in increases in the systolic function parameters (LVDP, heart rate, dP/dt). This relationship was first proposed by Starling in 1918. He conducted some rigorous experiments on isolated myocardium and observed that an increase in diastolic volume leads to an increase in systolic performance. This phenomenological observation has come to be known as the Frank-Starling law of the heart which states that the energy of cardiac muscle contraction however measured, is proportional to the initial length of the cardiac muscle fibre (up to the optimal length, Lmax (starling, 1918). In the intact heart, the initial length of the cardiac muscle fibre is proportional to the end-diastolic volume. The relation between ventricular stoke volume (the volume ejected into the circulation by the

ventricles in one systolic contraction or cardiac output), which is proportional to LVDP, is called the Frank-Starling curve (Ganong, 1995).

Although the mechanism by which stretching the myocardium induces an inotropic response of the heart is still under investigation, available evidence suggests that it is mediated by activation of various membrane based Ca^{2+} fluxes. These include activation of sarcolemmal Ca^{2+} conductance (Allen et al, 1974, 1988), activation of length-dependent intracellular Ca^{2+} release (Allen and Kentish, 1985), decrease excitatory threshold of the myocardium due to opening of stretch-activated K^+ channels (Tung and Zou, 1995), and increased synthesis and release of various intracellular signal transduction molecules such as cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP) and inositol triphosphate (IP_3) leading to increased sarcolemmal Ca^{2+} influx and sarcoplasmic reticulum Ca^{2+} release (Singh, 1982; Haneda et al, 1989, Boer et al, 1994; Miki et al, 1994). Some workers have reported increased atrial natriuretic peptide, ANP (Lang et al, 1985; Bilder et al, 1986; Page et al, 1991) while others have reported increased gene transcription and protein synthesis leading to increased availability of signal transduction molecules.

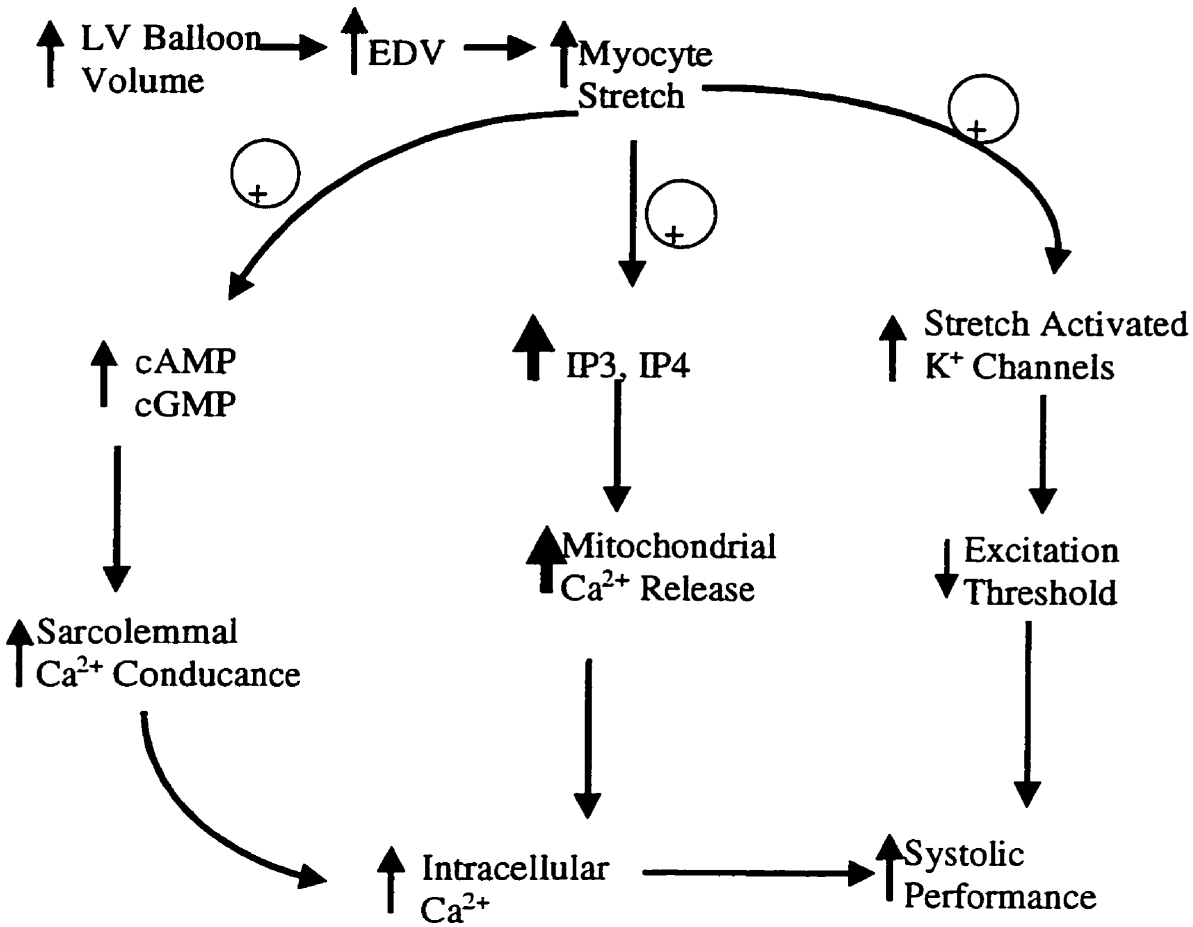

Figure 4.2. Mechanisms leading to increased systolic performance arising from increased end-diastolic volume.

4.6. Rate of increase/decrease of LVDP, ±dP/dt.

A healthy myocardium should not only be able to generate adequate systolic pressure to sustain the flow of blood in the circulation but should attain this pressure at the optimal speed. It should also be able to return to its presystolic state in readiness for the next systole at an optimal speed. The speed at which the myocardium attains peak systolic pressure is determined by its elastic properties, which in turn depend on chamber and myocardial stiffness. Factors such as edema, engorgement of the venous bed by perfusates, injury and inadequate venting that decrease myocardial compliance can significantly affect the speed of increase/decrease of peak systolic pressure ±dP/dt. The first derivative of systolic pressure dP/dt (defined as the velocity of change of ventricular pressure or rate of change of systolic pressure with respect to time) is used as an index for direct assessment of the speed at which the peak systolic pressure is attained and indirect assessment of chamber and myocardial stiffness. ±dP/dt is generated by the pressure transducer/multichannel recorder system which is equipped with a computer system that electronically differentiates the standard pressure curve from ventricular contractions. It consists of two important components that include;

I. Rate of increase of LVDP (+dP/dt):

Positive dP/dt refers to maximum velocity of increase of systolic pressure and correlates with the maximum contraction strength of the ventricles. Isovolumic contraction is primarily an active process brought about by the ATP and Ca^{2+}

mediated crossbridge cycling of actins and myosins of the sarcomere. The value of +dP/dt can constitute an indirect assessment of the performance of the factors that affect transmembrane Ca^{2+} fluxes and intracellular Ca^{2+} level including fluctuations in intracellular ATP levels.

II. Rate of decrease of LVDP (-dP/dt):

Negative dP/dt refers to the minimum velocity of decrease of systolic pressure and correlates with the maximum relaxation strength of the ventricles. Myocardial relaxation is energy dependent. The value of –dP/dt can be used as an indirect assessment of the metabolic viability of cardiac myocytes and the metabolic function of the myocardium. The relaxation of the myocardium is principally governed by active sarcoplasmic sequestration of intracellular Ca^{2+}. Negative dP/dt is thus related to function of the sarcoplasmic reticulum and can constitute an indirect assessment of the factors that control intracellular Ca^{2+} levels.

4.7. Perfusion Pressure.

Perfusion pressure refers to hydrostatic pressure that delivers perfusates to the myocardium. The most popular method for generating adequate perfusion pressure to sustain the flow of cardioplegic perfusates has been to use a variety of pumps. The method has produced very successful results in adult models but requires intricate and careful calibration procedures to minimize errors. In neonates errors arising from improper calibration of centripetal and roller pumps may have a significant effect on post surgical cardiac performance.

In this study, the perfusion pressure was generated by perfusing the myocardium from two thermoregulated glass reservoirs (C and D, see figure 3.3) suspended very close to the isolated heart/sample chamber from heights of 1.4 meters (for hyperkalemic perfusion) and 1.8 meters (for nomorkalemic perfusion) respectively. Water from the heat exchanger (temperature: 36-37°C) was circulated around the inner layer of the glass reservoirs to maintain adequate thermoregulation. By a trial and error process, the heights corresponding to various pressures were determined. These were carefully marked on the stand carrying the reservoirs to facilitate the process of adjusting the heights to select any desired pressure. A higher perfusion was used during nomorkalemic perfusion to overcome the effect of systolic squeeze and autoregulatory processes of the myocardium.

In the retrograde group, measurement of perfusion pressure was achieved by connecting the pressure line of the retroplegia cannula to the pressure transducer and multichannel recorder/display system by means of a polyethylene tubing and stopcock. In the antegrade group, measurement of perfusion pressure was done by connecting the aortic vent line through a two-way stopcock to the pressure transducer and multichannel recorder/display system. The stopcock allowed this route to be used for both decongestion of the aortic root and measurement of aortic pressure. The multichannel recorder differentiates the pressure changes that accompanies increased volume delivery to the coronary sinus and aortic root and displays the associated pressure along with the cardiac function curves on the chart recorder/display system.

4.8. Coronary Flow rate (CFR).

The total amount of solution returning in the suction line from the sample chamber was considered to represent total coronary flow and was measured by timed collection of the solution returning in the suction line into a graduated cylinder. This measured CFR closely approximates that read from a flowmeter in the retrograde and antegrade perfusion lines. The values were expressed as mL/minute.

4.9. Myocardial O_2 Consumption (MVO_2)

The main advantage of retrograde cardioplegia over the noncardioplegic methods is that in addition to ensuring a quiet, bloodless operative field for a prolonged time, it provides continuous delivery of substrates and O_2 thereby preserving biochemical and mechanical function of the myocardium postoperatively. The ability of the myocardium to provide metabolic activity in excess of demand is dependent not only on the temperature and composition of perfusate administered during the aortic cross-clamp interval but also on the distribution, duration and volume of perfusion. Retrograde cardioplegia, by maintaining myocardial energy and substrate supply that is in excess of electromechanical and biochemical demand during diastolic arrest, optimizes the relationship between energy supply and consumption thereby sustaining oxidative phosphorylation. This prevents large decreases in ATP and PCr levels and anaerobic metabolism.

In this study, myocardial O_2 consumption (MVO_2) was calculated as the product of coronary flow rate (CFR) and the arterio-venous O_2 difference, corrected for the weight of the heart. The collection of the arterial and venous samples was done with the intraventricular balloon deflated to minimize myocardial tension. Measurements of coronary flow and MVO_2 were done twice prior to and after ventricular function measurements in the isovolumically contracting heart and twice during diastolic arrest. For the retrograde series, arterial samples were collected from the coronary sinus (CS) line very close to the heart without exposure to air and venous samples from the aortic vent line. It has been reported that only about 30% of retrograde flow exits through this route (Gates et al, 1996). The aortic vent line was used in this study because the perfusate exiting through this route during retrograde cardioplegia may have traversed the capillary bed, the site of tissue respiration. For the antegrade group, arterial samples were collected from the aortic root cannula very close to the heart while the venous sample were obtained from the CS perfusion line. Approximately 50mL of solution was discarded from the line before collection of the MVO_2 sample. O_2 saturation, PO_2; was obtained using a blood gas analyzer (Stat profile 9, Nova Medical, Mass) calibrated for analysis of non-blood samples. MVO_2 was derived using the following equations:

$$MVO_2\ (mL/min/100g) = \frac{PO_2\ (arterial - venous)}{760} \times 2.35 \times \frac{CFR}{weight\ of\ heart}$$

2.35 is the coefficient of O_2 solubility (O_2mL/100mL/atm) at 37°C (Tian et al, 1994).

This equation is based on the principle proposed by Adolph Fick (1870), which states that cardiac output (represented by the CFR corrected to heart size) can be determined by measuring the O_2 of the body (represented by the isolated heart) and dividing by the difference in arterial O_2 tension (PO_2) and PO_2 of mixed venous blood (Berne et al, 1993).

5.0. NMR measurements.

5.1. Design of the sample chamber and probe.

The sample chamber and probe constitute an important component of the NMR apparatus and anomalies in the design and construction of the sample chamber and probe will lead to distortion of the NMR data. High field magnets such as the 7.0 Tesla magnet used in this study are homogeneous to only about ± 100ppm. During NMR experiments, a variety of procedures are used to enhance the field homogeneity and optimize the signal-to-noise ratio (S/N ratio). In this study, the steps taken included:

1. Shimming
2. Enhancement of the filling factor
3. Quality of the environment of the NMR spectrometer magnet.

1. Shimming:

This is an important process used to improve the homogeneity of the external magnetic field B_0 around the sample. In our isolated Langendorff crystalloid perfused piglet heart experiments, shimming was performed on the sodium signal of the sample.

2. Enhancement of filling factor:

The size of the neonatal pig heart was taken into consideration in the design and construction of the sample chamber and probe. With the dimensions of our system the heart occupies 70-80% of the sample chamber volume.

Precautions were also taken to minimize the presence of conductors around the sample and sample chamber. These included:

i. Minimizing the size of the perfusion tubes taking buffer into and out of the sample chamber

ii. Preventing formation of bubbles within the sample

iii. Continuously removing the effluent of the heart from the sample chamber during the experiment

iv. Arranging for maximum symmetry around the sample by positioning the sample chamber in the center of the magnet with the heart in a vertical position. This also ensured that the axis of the probe was perpendicular to the B_0 field of the magnet.

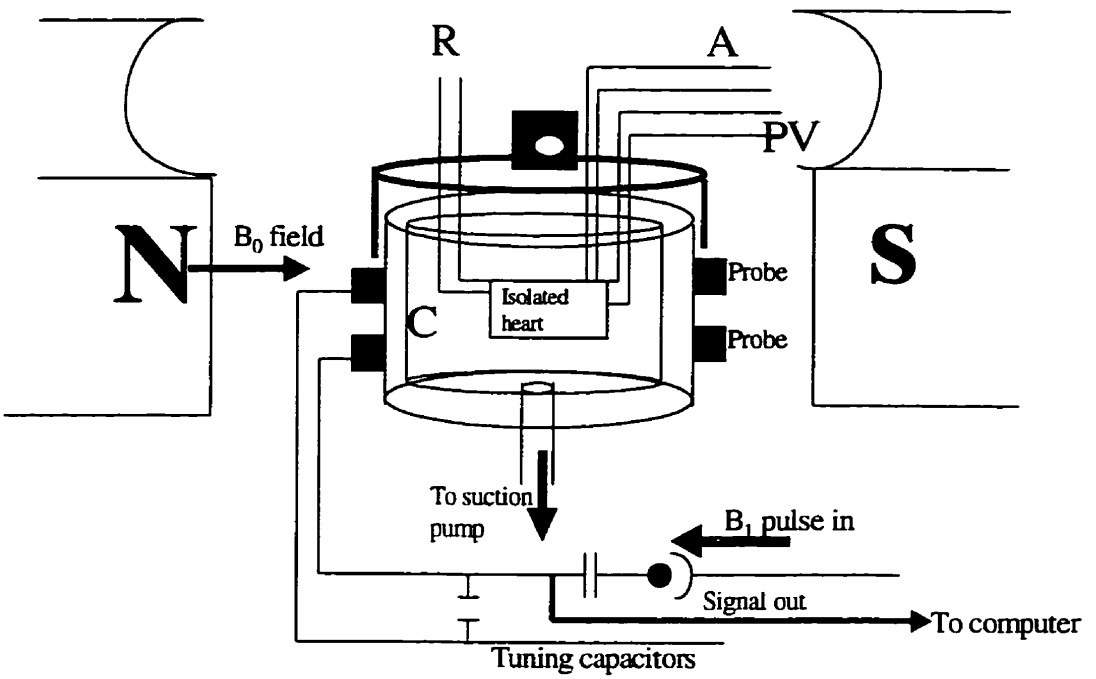

Figure 5.1. Schematic representation of the NMR experiment. R, retroplegia cannula; A, aortic root cannula; C, double walled sample chamber; PV, aortic pressure/vent line; N and S represents the north and south poles of the 7.0 Tesla spectrometer magnet; left ventricular pressure line not shown.

The sample chamber consisted of a double-walled transparent plastic cylinder 30 cm in height, an internal diameter of 13 cm and an external diameter of 15 cm. The base contained a central outlet tube. This was connected to a suction pump with the aid of tygon or silicon tubing to permit continuous suctioning of the fluid effluent from the heart. Surrounding the sample chamber were two loops of coil separated by a distance of 6.0 cm, which constituted the probe used to transmit radiofrequency (rf) pulses to the sample and receive the associated resonance signal.

5.2. Nuclear magnetic resonance (NMR) Spectroscopy.

Magnetic resonance spectroscopy is an important technique for assessing the metabolic function of animal tissues in vivo and ex vivo. It is based on the principle that moving charges generate a magnetic field around them. The interaction of this magnetism associated with charged particles in motion gives rise to a net magnetization. Living tissues such as the isolated heart preparation consist of various charged nuclei in motion which are surrounded by orbital electrons. These nuclei are oriented randomly and are associated with magnetic property called magnetic moment or spin. For imaging and spectroscopy purposes only the net magnetization is important. In their random orientation, no net magnetization is generated. Only particles possessing odd number nuclei and hence an unpaired spin such as ^{1}H, ^{31}P, ^{13}C can generate net magnetization and are of interest in NMR imaging and spectroscopy. Particles with even number

nuclei such as ^{12}C, ^{16}O do not generate net magnetization. NMR measurements are usually accomplished by applying a radiofrequency pulse (a function usually accomplished by the probe or coil) to a sample positioned symmetrically in the center of the magnet. This tilts the net magnetization Mz away from its equilibrium plane. The return of the net magnetization to the equilibrium position following discontinuation of the B_1 pulse generates rf signal, this can be Fourier transformed to give the NMR spectrum.

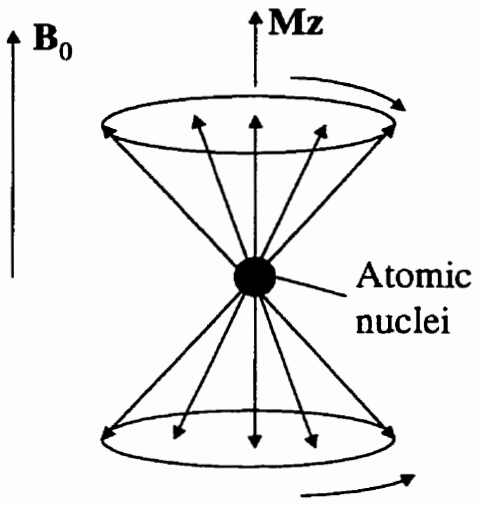

Figure 5.2. Schematic diagram illustrating the possible spins of the nuclei in the isolated heart preparation on exposure to the high strength magnetic field of the 7.0 Tesla spectrometer magnet. Mz, net magnetization on the z plane.

When an isolated Langendorff crystalloid perfused heart preparation is moved to the center of a high field magnet, the randomly oriented spins of its nuclei become aligned, leading to the generation of a net magnetic field due to interaction with the B_0 field of the magnet. In their aligned position the spins of the charged nuclei can assume two possible orientations: parallel and antiparallel. However, precession in the parallel orientation contributes most to the net magnetization. In the presence of the B_0 field of the magnet, these nuclei spin at specific frequencies which are dependent on the strength of the external magnetic field with which their own fields are interacting. This is called the resonance or Larmor frequency. It is directly proportional to the magnetic field strength of the magnet and is related to it by the following equations.

Larmor frequency ω_0 = gyromagnetic ratio γ x magnetic field strength B_0.

Gyromagnetic ratio γ is the proportionality constant. It is different for each nucleus.

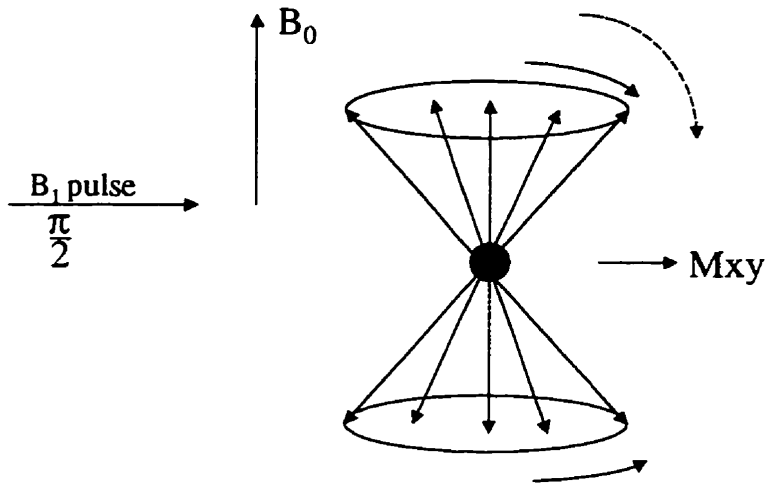

Figure 5.3. Schematic diagram illustrating the effect of the B_1 pulse on the net magnetization. Mxy, net magnetization on the xy plane.

5.3. Free induction decay.

The net magnetization associated with the charged nuclei in the isolated heart preparation can be tilted away from the equilibrium position under the influence of a B_1 magnetic field. This is achieved with a radiofrequency (rf) pulse delivered by the loop of coil or probe wound around the outer layer of the transparent sample chamber. The rf pulse is capable of penetrating the wall of the chamber and isolated heart tissue to supply the energy required to tilt the net magnetization away from the equilibrium position. When the rf pulse is

discontinued, the net magnetization returns to the position of equilibrium. The energy difference is released as radiofrquency (rf) signal. This constitutes the free induction decay (FID). The FID is received by the probe and is channeled into a computer system. This is the data that is processed and analyzed in both NMR imaging and NMR spectroscopy.

5.4. Relaxation parameters.

The term relaxation parameters refers to the time dependent constants that are used to describe the return of the net magnetization vector tilted away from its position of equilibrium by the rf pulse back to its position of equilibrium or more appropriately the exponential decay of the net magnetization M_{xy} with time when the applied pulse B_1 is discontinued. The following relaxation parameters have been described.

Longitudinal relaxation parameter, T1.

T1 is defined to be the time that describes the recovery of the net magnetization M_z after a perturbation (B_1 pulse) delivered by the probe is discontinued. It is generated by the return of the nuclei to the parallel orientation in complete alignment with the external magnetic field vector B_0 when the rf pulse B_1 is discontinued. In this study rf pulse B_1 at 90° (delivered by the probe) was used to initiate the perturbation of the net magnetization. In general T1 values are dependent on the strength of the field, and are generally longer at higher field strengths.

Transverse relaxation time, T2.

T2 is the time constant that is used to describe the loss of alignment of the net magnetization vector (in the x-y plane) associated with the spin of the individual nuclei when the rf pulse B_1 is discontinued.

Transverse relaxation time, T2*.

This type of transverse relaxation time is generated by the following factors

i. Loss of alignment or phase of the net magnetization vector of the individual nuclei from the external magnetic field vector B_0 following discontinuation of the rf pulse B_1.

ii. Contributions arising from inhomogeneity of the external magnetic field. This may be due to imperfections in the probe and magnet manufacturing processes.

iii. Changes in magnetic field susceptibility in different parts of the same tissue for example, air/tissue interfaces have a different magnetic susceptibility from tissue/tissue interfaces.

The sum of these factors is called the T2* transverse relaxation time constant. Unlike T1, the T2 and T2* values are not dependent on the strength of the external field magnetic vector B_0.

5.5. The procedure of NMR measurements.

Assessment of the efficacy of cardioprotective techniques requires that certain functional and metabolic parameters be monitored continuously and noninvasively. Laboratory techniques such as high performance liquid chromatography (HPLC) and fluorometry can provide accurate and reliable quantitative assessment of various metabolic parameters such as adenosine triphosphate (ATP) and phosphocreatine (PCr) levels. However, these techniques are invasive and require tissue specimens for preservation by freezing in liquid nitrogen. These techniques are impractical when there is need to follow the time course metabolic events. Although, NMR spectroscopy may not readily provide accurate quantitative assessment of metabolic parameters, it allows continuous and systematic qualitative monitoring of the time course of metabolic events.

In this study, assessment of the time course of metabolic event which occurs in the myocardium during control perfusion, arrest and reperfusion was achieved by acquiring ^{31}P NMR spectra. These were recorded at 121 MHz in a high field spectrometer (7.0 Tesla Bruker Biospec spectrometer, Bruker, Karlsruhe, Germany) with 40cm bore diameter. Magnetic field homogeneity in the region of the sample was optimized by shimming on the sodium signal of the sample. The heart was suspended inside the sample chamber/probe system and shimmed to optimize the field homogeneity. The probe was then switched to the frequency for acquisition of ^{31}P spectra (121.47mHz). This was followed by the

acquisition of free induction decay (FID) using 4K data points and 40 to 90° radiofrequency pulses. Each spectrum was acquired over 2 to 5 minutes sample period. The accumulated FID was then exponentially multiplied, resulting in 20-30Hz line broadening. This helped to further enhance the signal to noise ratio. The following phosphorus metabolites i.e inorganic phosphate, Pi; phospocreatine PCr and adenosine triphosphate, ATP were monitored continuously during control perfusion, arrest and reperfusion to determine the ability of retrograde and antegrade cardioplegia to preserve metabolic function of the myocardium. All spectral data were expressed as a percentage of the initial intensity.

5.6. Data analysis.

Following exponential multiplication of the time function and line broadening, the FID was transferred to a software package (Win NMR, Bruker, 1997). With the aid of the software, the magnetic resonance spectra were Fourier transformed, phased and baseline corrected. After baseline correction, the spectra were fitted with lorentzian curves and then quantified using the integrals of the curve. All data were presented as mean ±standard error of the means. Statistical analyses were performed with the use of Microsoft Excel 97 (version 5.0) and Statistica 5.0 (Statsoft, Tulsa, Okla) software. The student t-test and one-way anova were used to compare data between groups. Data comparison across time within the two groups was achieved using the paired student t-test. Results of the data were expressed as % of the initial value.

6.0. Results

6.1. General physiological measurements

Assessment of the consequences of perfusing the neonatal myocardium retrogradely was achieved by monitoring changes of various physiological indices during control perfusion, arrest and reperfusion. These parameters includes; left ventricular developed pressure, LVDP; rate-pressure product, RPP; velocity of increase/decrease of LVDP, ±dP/dt; heart rate, HR; left ventricular end-diastolic pressure, LVEDP; myocardial O_2 consumption, MVO_2 and coronary flow rate, CFR. These values expressed as mean ± standard error of mean are shown in tables 6.1 (retrograde group), 6.2 (antegrade group) and as percentage of control values (table 6.3). Hearts in both groups demonstrated similar recovery of left ventricular developed pressure, LVDP; heart rate, HR; rate-pressure product, RPP and the velocity of increase/decrease of LVDP, ±dP/dt.

Table 2: Recovery of Physiologic Indices in Hearts Perfused by Retrograde Cardioplegia (RC)

Retrograde Group

Parameters	Control: minutes (AC)	Arrest: minutes (RC)	Reperfusion: 30 (AC)
LVDP (mmHg)	90.0 ± 0.26		84.5 ±0.25
Rate-Pressure Product	10326.6±2.66		1073.7.58±2.6
MVO_2 (mL/minute/100g	11.69 ±0.20	2.30 ±0.09	12.97 ± 0.22
+dP/dt	672.77 ±0.72		581±0.66
-dP/dt	599.99 ± 0.68		469.99 ± 0.60
HeartRate (beats/minute)	114.74 ± 0.29		131.05 ±0.31
LVEDP (mmHg)	13.5 ±0.10		25.0 ±0.12
CFR (ml/minute)	128.39 ± 0.17	25.15 ± 0.078	133.31 ± 0.18

Data are means ± SE. LVDP, left ventricular developed pressure; MVO_2, myocardial O_2 consumption; +dP/dt; velocity of increase of LVDP; -dP/dt, velocity of decrease of LVDP; LVEDP, left ventricular end-diastolic pressure; CFR, coronary flow rate.

Table 6.2: Recovery of Physiologic Indices in Hearts Perfused by Antegrade Cardioplegia (AC)

Antegrade Group

Parameters	Control: 30 Minutes (AC)	Arrest: 45 Minutes(AC)	Reperfusion:30 Minutes (AC)
LVDP (mmHg)	90.05±0.25		87.5±0.25
Rate-Pressure Product	9077.03 ±2.40		7768.40±2.44
MVO_2(ml/min./ 100g)	10.66 ± 0.20	5.52 ±0.14	11.64 ± 0.21
+dP/dt	746.25 ± 1.70		663.33 ± 1.60
-dP/dt	680.83 ±1.60		510.83 ± 1.41
Heart Rate beats/minute)	100.8 ± 0.6		107 ±0.64
LVEDP (mmHg)	13.5 ±0.10		20.5 ±0.31
CFR(ml/minute)	117.05 ± 0.30	91.33 ±0.26	127.0 ± 0.31

Data are mean ±SE: LVDP, left ventricular developed pressure: MVO_2, myocardial O_2 consumption; +dP/dt, velocity of increase of LVDP; -dP/dt: velocity of decrease of LVDP, LVEDP: Left ventricular end-diastolic pressure, CFR: coronary flow rate.

Table 6.3. Recovery of Physiological Indices in Hearts Arrested by Retrograde Cardioplegia (group 1) and Antegrade Cardioplegia (group 2).

Groups	LVPD	HR	RPP	CFR	MVO_2	+dP/dt	-dP/dt	LVEDP
1	95 ± 2	114 ± 2	98 ± 2	88 ± 2	110 ± 2	86 ± 2	78 ± 1	151 ± 2
2	99 ± 2	107 ± 2	103 ± 2	108 ± 2	109 ± 2	88 ± 2	75 ± 1	119 ± 2

Group1, retrograde cardioplegia (n = 6); group 2, antegrade cardioplegia (n = 6); MVO_2, myocardial O_2 consumption; HR, heart rate; RPP, rate-pressure product; LVDP, left ventricular developed pressure; ±dp/dt, peak positive and negative rate of increase/decrease of left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; CFR, coronary flow rate. All values are mean of % of control values ± standard error of mean.

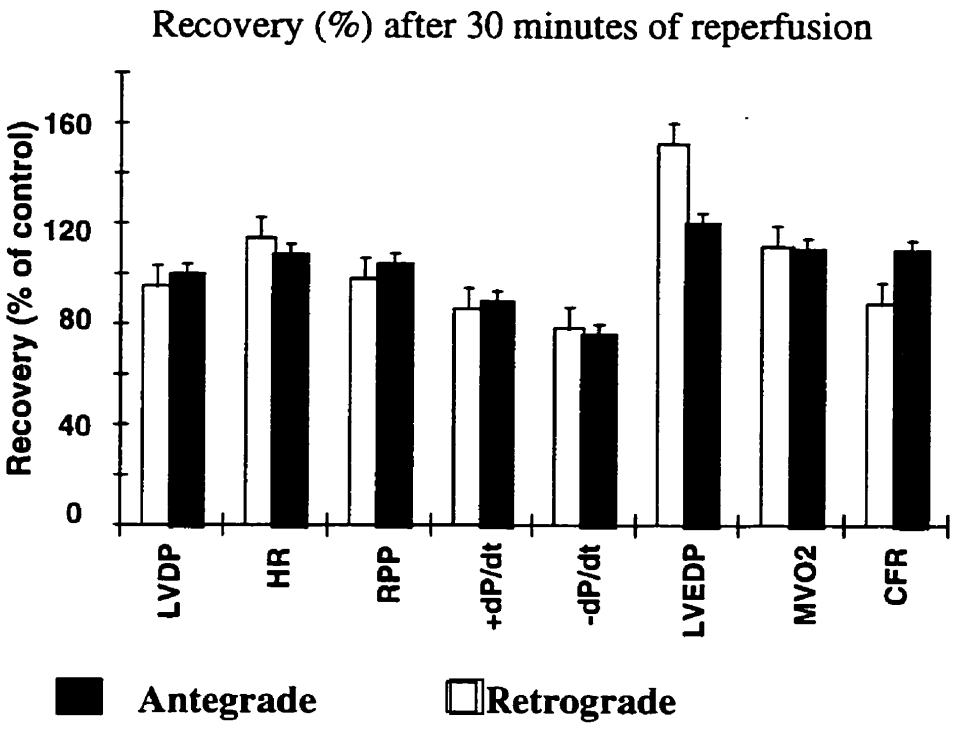

Figure 6.1 Recovery of physiologic indices during reperfusion of hearts arrested by retrograde and antegrade cardioplegia. Values are mean ±SEM. Retrograde group: n = 6, antegrade group: n = 6

6.2. Systolic and diastolic function.

The recovery of LVDP and LVEDP (mean ±SEM) is shown in Figure 6.2. In the retrograde group, the LVDP decreased from 90±0.26 mmHg during control perfusion to 84.5±0.25 mmHg ($p>0.05$) during reperfusion and from 90.05±0.25 mmHg in the antegrade group ($p>0.05$). A time course presentation of the changes of LVDP (Figure 6.3) and heart rate (figure 6.4) during 30 minutes of control perfusion, 45 minutes of arrest followed by 30 minutes of reperfusion did not show any significant difference between the two groups.

The LVEDP measured at a constant intraventricular balloon volume of 5.0 ml (± standard error of the mean) are shown in Figure 6.2. In the retrograde group the LVEDP increased from 13.5±0.10 to 25.0 ±0.31 mmHg and from 13.5 ±0.10 to 20.5 ±0.12 mmHg in antegrade group, $P<0.05$. These data show that both methods of cardioplegic protection are associated with loss of compliance. The loss of compliance in the RC group was greater than that associated with the use of AC. Hearts in both groups demonstrated similar recovery of LVDP during reperfusion.

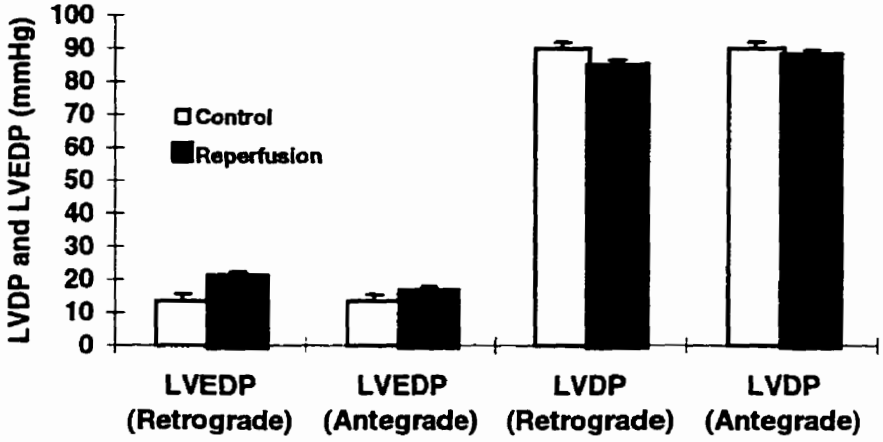

Figure 6.2. Comparison of recovery of LVDP and LVEDP in hearts arrested using retrograde and antegrade cardioplegia during reperfusion. (Data plotted are the mean ± standard error of the mean).

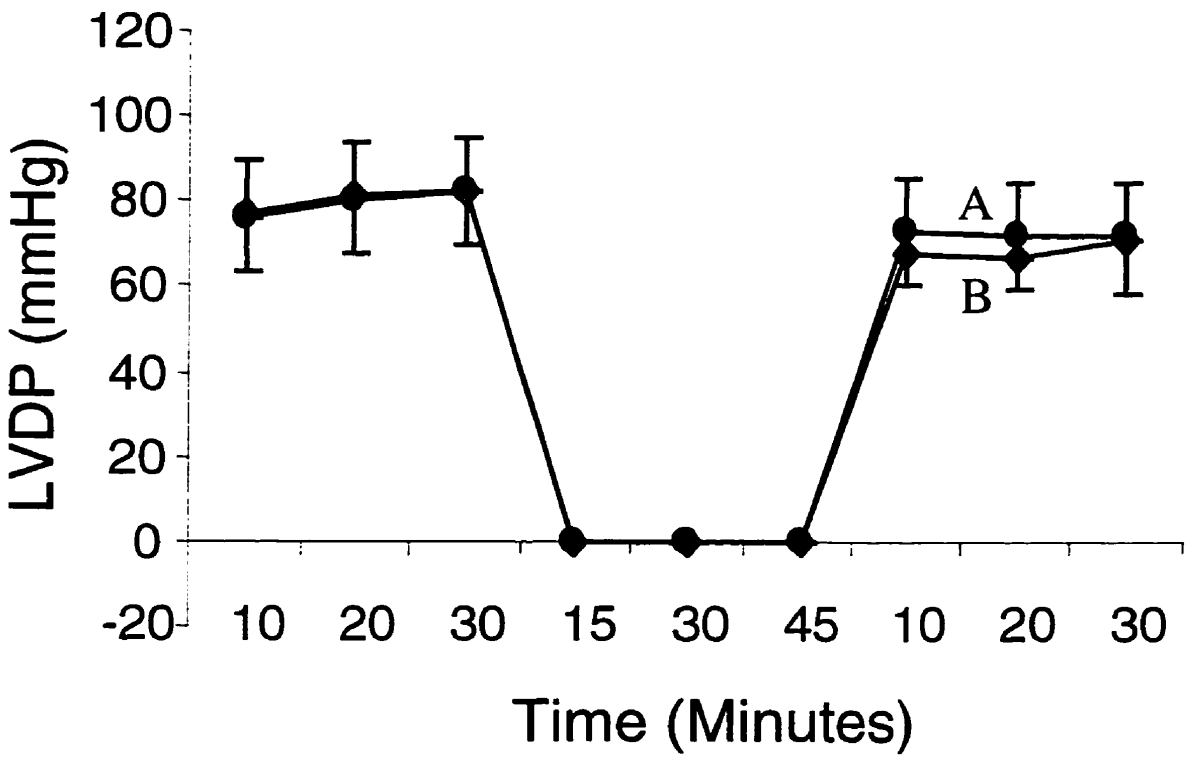

Figure 6.3. Changes of LVDP during 30 minutes of control perfusion, 45 minutes of arrest and 30 minutes of reperfusion in the retrograde (B) and antegrade groups (A).

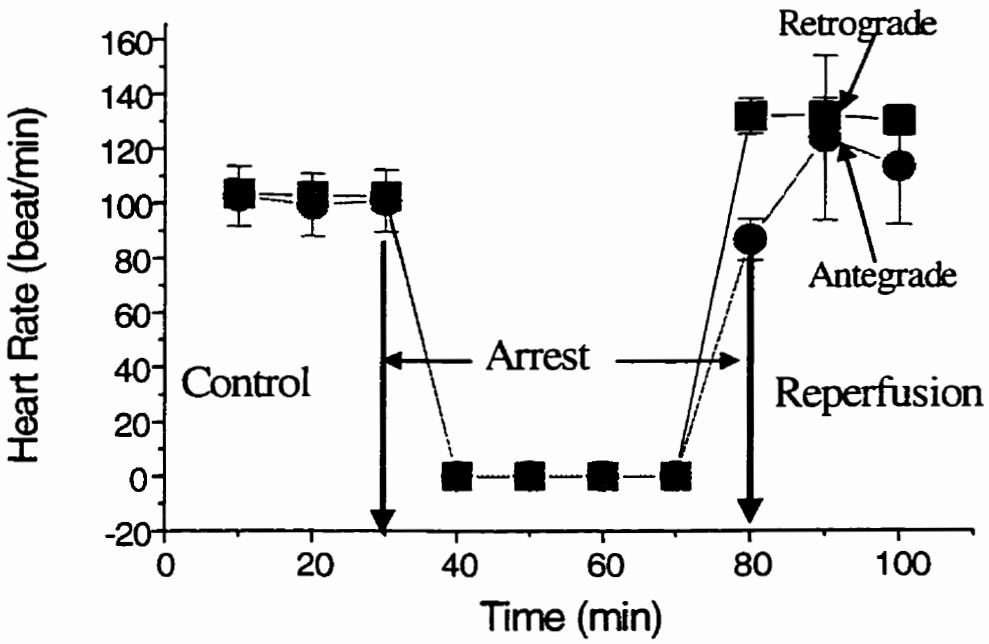

Figure 6.4. Changes in heart rate with time during 30 minutes of control perfusion, 45 minutes of arrest and 30 minutes of reperfusion in hearts arrested using retrograde cardioplegia and hearts arrested using antegrade cardioplegia.

6.3. Myocardial compliance/chamber stiffness.

Figure 6.5 and 6.6 shows the velocity of contraction and relaxation in the hearts arrested using retrograde cardioplegia and in the hearts arrested using antegrade cardioplegia. During reperfusion, the hearts arrested using antegrade cardioplegia showed similar contractile velocity, +dP/dt (Figure 6.5) and relaxation velocity -dP/dt (Figure 6.6) to that observed during the initial control perfusion period. There was a slight depression of contractile velocity from the initial values observed during the control perfusion period in the retrograde group but this was not significant ($p>0.05$). There was also greater depression of relaxation velocity, -dP/dt in the retrograde group compared to antegrade group during reperfusion ($p>0.05$). However, this also was not significant.

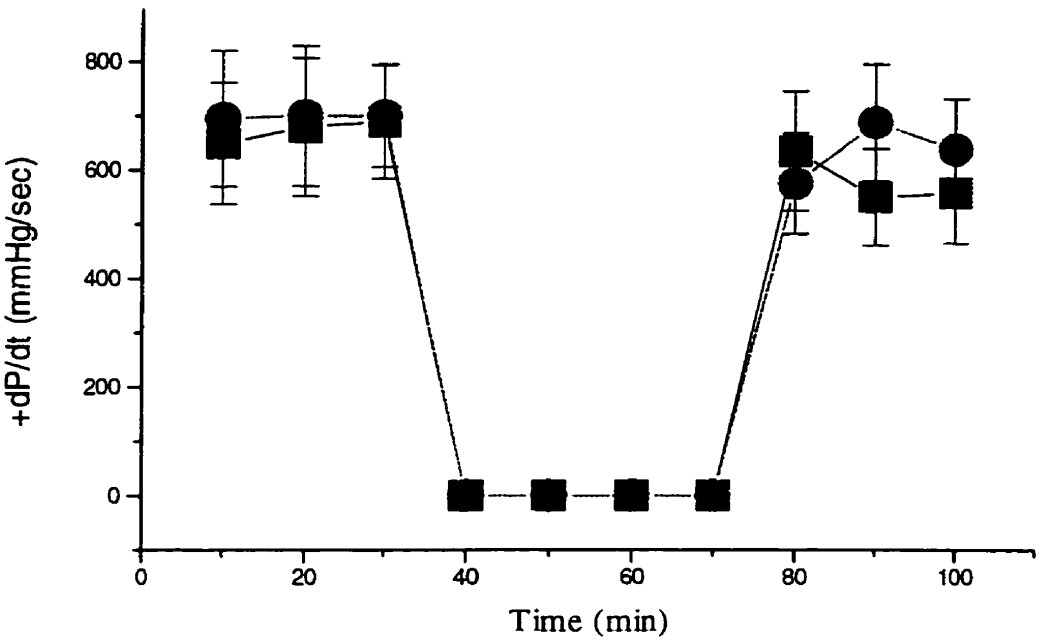

Figure 6.5. Velocity of increase of LVDP, +dP/dt in hearts arrested using retrograde cardioplegia (squares) and hearts arrested using antegrade cardioplegia (circles) during 30 minutes of control perfusion, 45 minutes of arrest and 30 minutes of reperfusion.

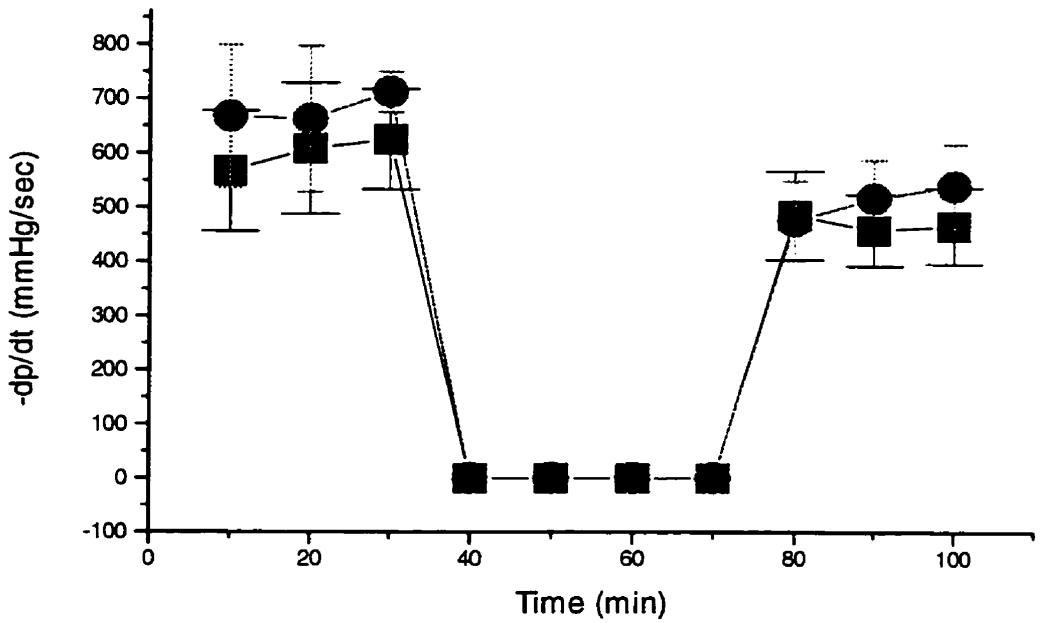

Figure 6.6. Velocity of decrease of LVDP, –dP/dt in hearts arrested using retrograde cardioplegia (squares) and hearts arrested using antegrade cardioplegia (circles) during 30 minutes of control perfusion, 45 minutes of arrest followed by 30 minutes of reperfusion.

Figure 6.7 shows the effect of increasing the end-diastolic volume, EDV, on the LVDP (Starling relationship) during reperfusion. There was a decrease in sensitivity of the hearts arrested using RC which increased at greater EDV. During reperfusion, the sensitivity in the antegrade group was similar to that observed during control perfusion. The effect of increasing EDV on the LVEDP during reperfusion is presented in Figure 6.8. At EDV $\leq$ 4.5 mL, hearts arrested using AC returned to the presystolic EDV. At EDV > 4.5 mL the LVEDP-volume curve for the antegrade group was displaced upwards from the volume-axis, indicating a loss of compliance and an increase in ventricular wall stiffness. In the hearts arrested using RC, the presystolic LVEDP was not restored during reperfusion. The LVEDP-volume curve for this group was displaced upwards and to the left, indicating an increase in myocardial wall stiffness and loss of compliance.

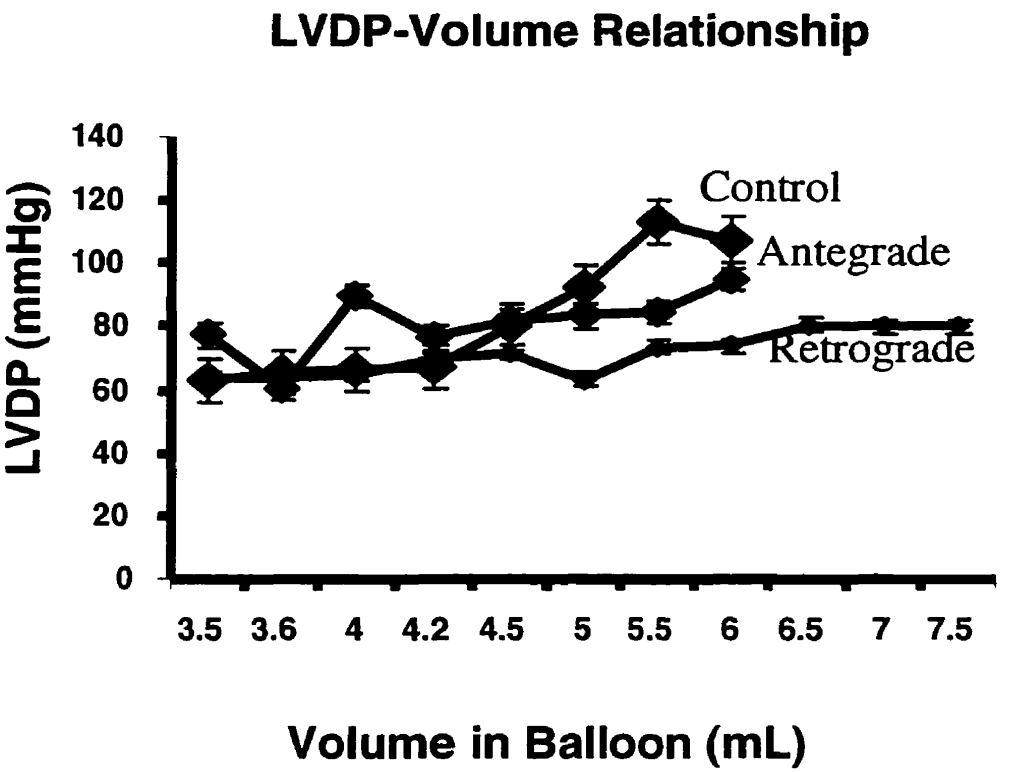

Figure 6.7. Effect of increasing end-diastolic volume on LVDP (systolic pressure minus end-diastolic pressure). The isovolumically contracting Langendorff crystalloid perfused isolated heart was subjected increasing end-diastolic volume with the aid of a latex balloon placed in the left ventricle.

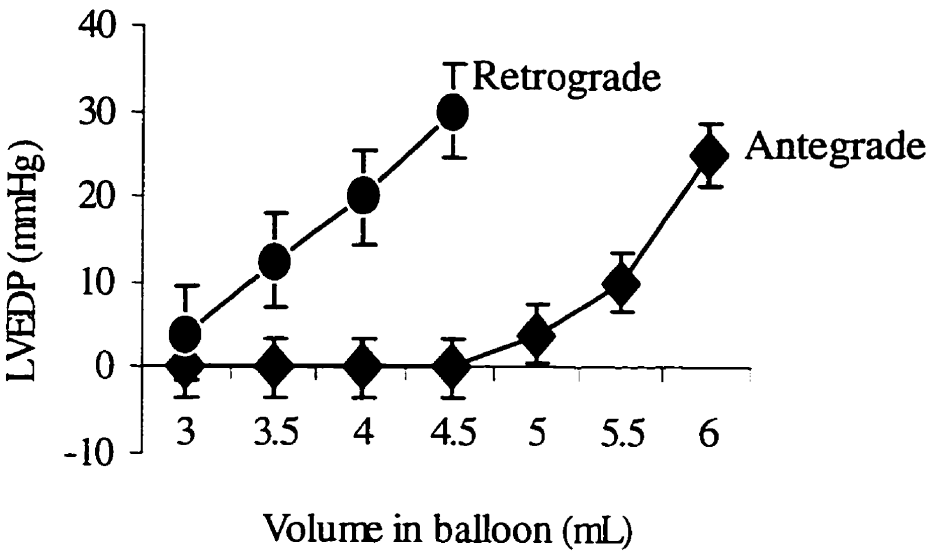

Figure 6.8. Comparison of the effect of increasing end-diastolic volume on the end-diastolic pressure of the left ventricle during reperfusion in hearts arrested using retrograde cardioplegia (45 minutes) and hearts arrested using antegrade cardioplegia (45 minutes).

6.4. Myocardial O_2 Consumption (MVO_2)

The MVO_2 (measured at a left ventricular balloon volume of 2.0-3.0mL) for the retrograde group during reperfusion was higher than in the antegrade group (Figures 6.9 and 6.10). This is in spite of the higher coronary flow rate provided by AC (Table 6.3 and Figure 6.1).

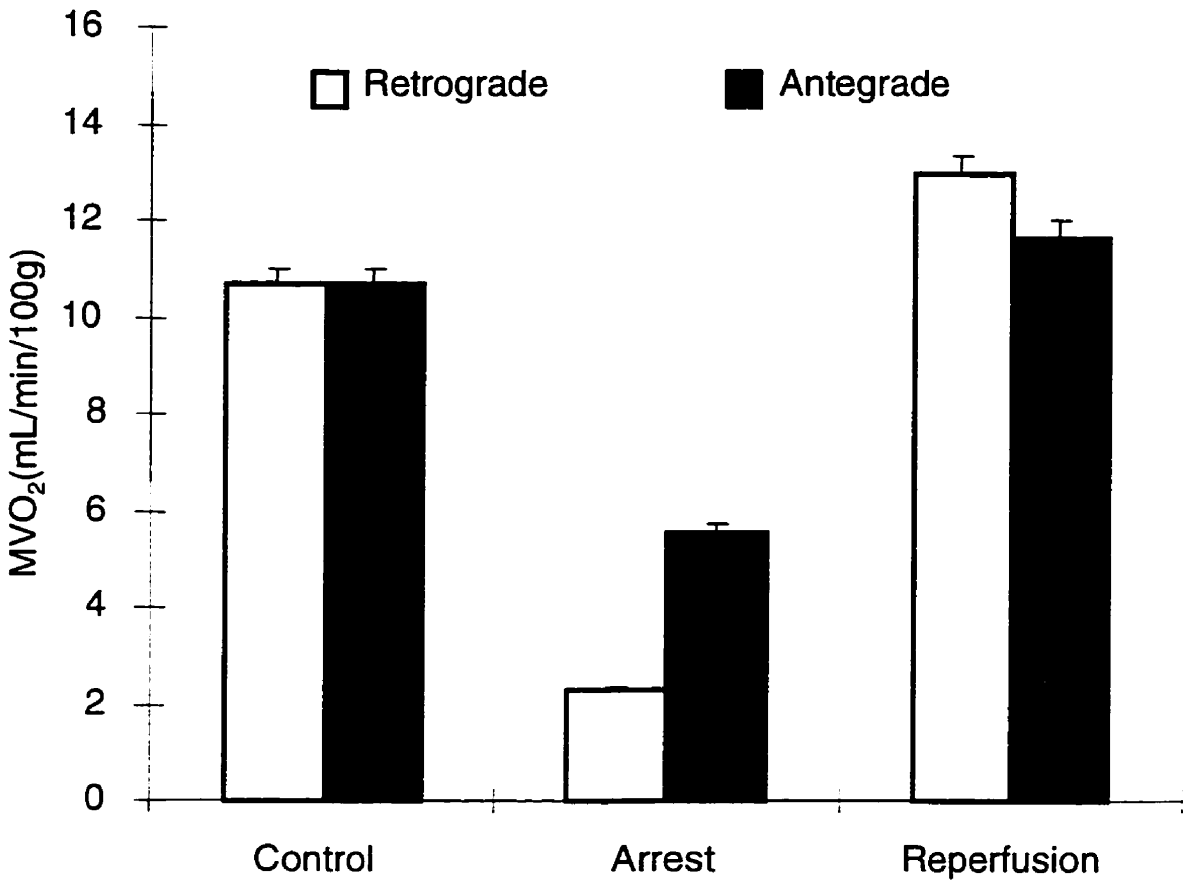

Figure 6.9. MVO_2 at a balloon volume of 2.0-3.0mL (± standard error of mean) during control perfusion, arrest and reperfusion.

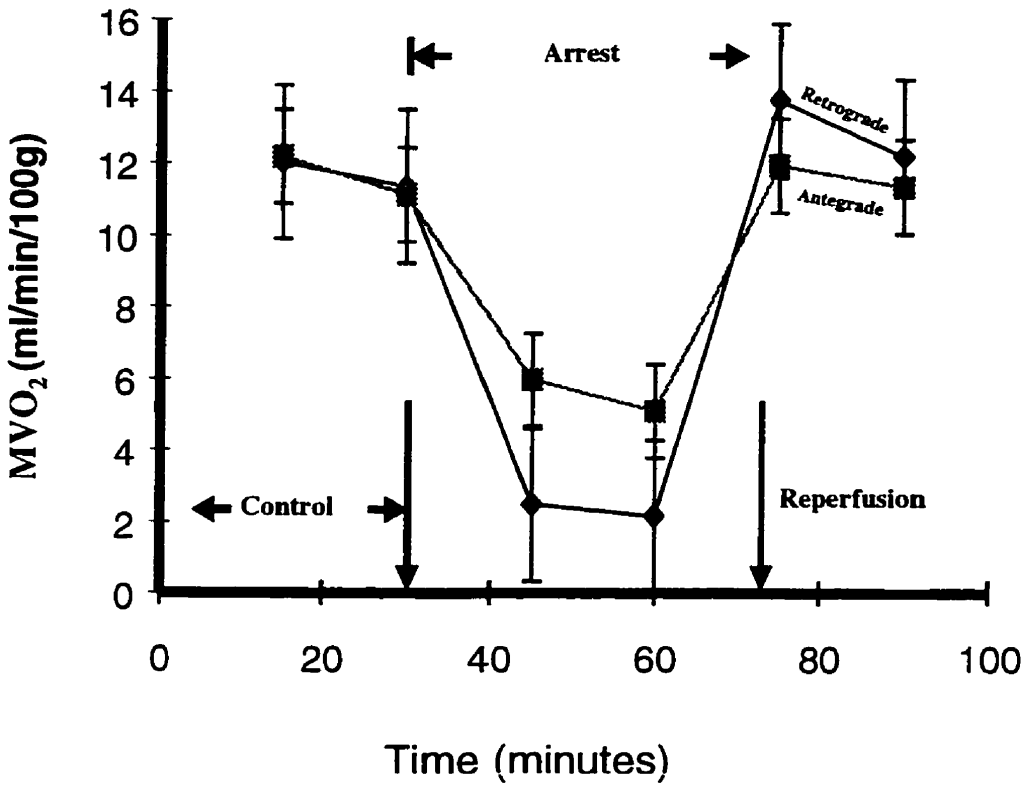

Figure 6.10. Comparison of changes of MVO_2 at a left ventricular balloon volume of 2.0-3.0mL during the experimental time course.

6.5. ^{31}P Nuclear magnetic resonance (NMR) spectroscopy

Figures 6.11 and 6.12 show the changes of ATP and phosphocreatine (PCr) during 30 minutes of control perfusion, 45 minutes of arrest and 30 minutes of reperfusion. The bar chart represent average for four piglets ± standard error of mean. Hearts arrested using antegrade cardioplegia showed a slight decrease in the levels of ATP and PCr during 45 minutes of arrest which recovered rapidly on reperfusion. In the retrograde group, there was a greater fall in the levels of ATP and PCr during arrest and slow recovery to control values during reperfusion ($P< 0.05$).

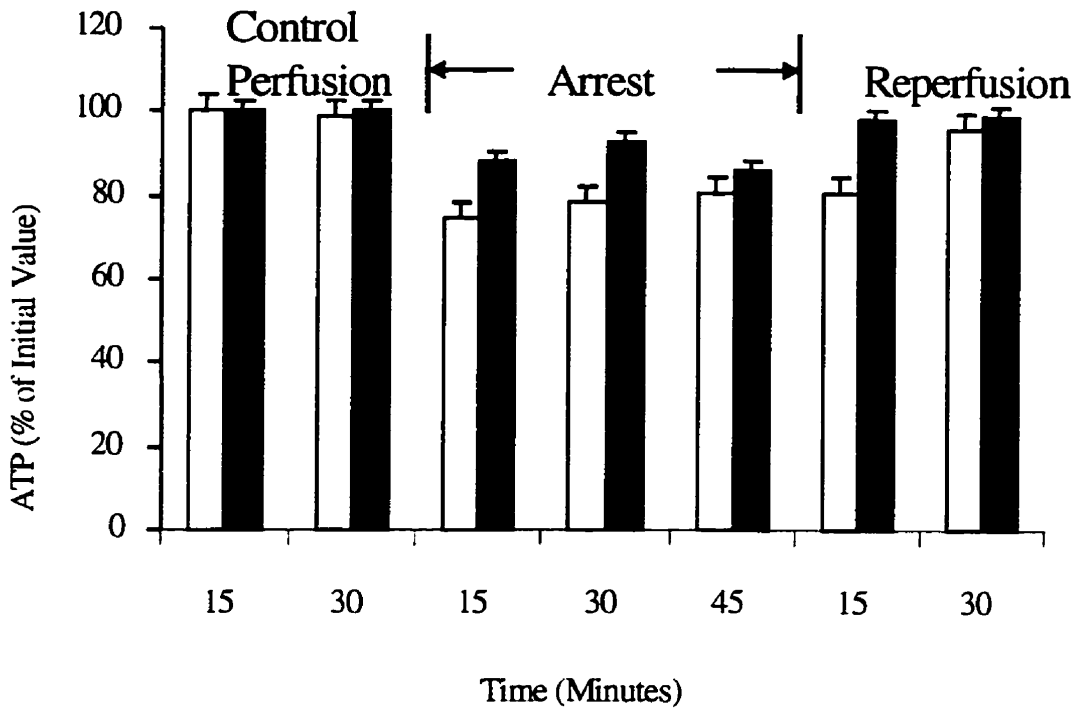

Fig. 6.11. Changes in ATP levels during 30 minutes of control perfusion, 45 minutes of arrest and 30 minutes of reperfusion in hearts arrested using retrograde cardioplegia and in hearts arrested using antegrade cardioplegia. The bars represent averages of % of control values of 4 hearts ± standard error of mean.

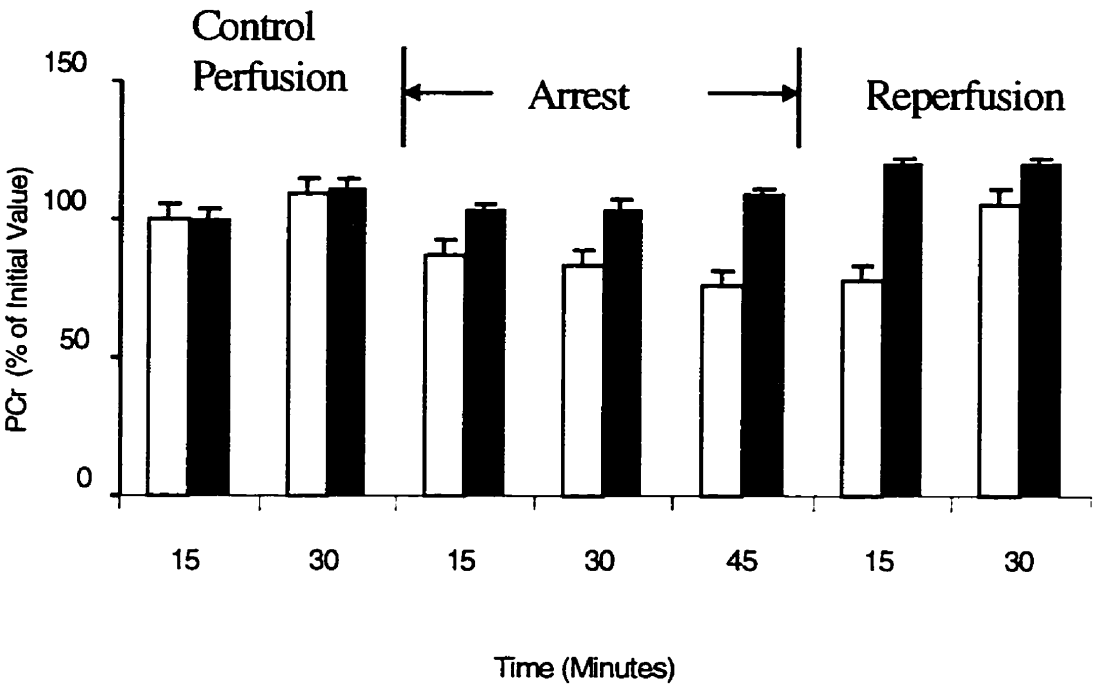

Figure 6.12. Changes of phosphocreatine levels during 30 minutes of control perfusion, 45 minutes of arrest and 30 minutes of reperfusion in hearts arrested using retrograde cardioplegia and hearts arrested using antegrade cardioplegia. The bars represent averages of % of control values of 4 hearts ± standard error of mean.

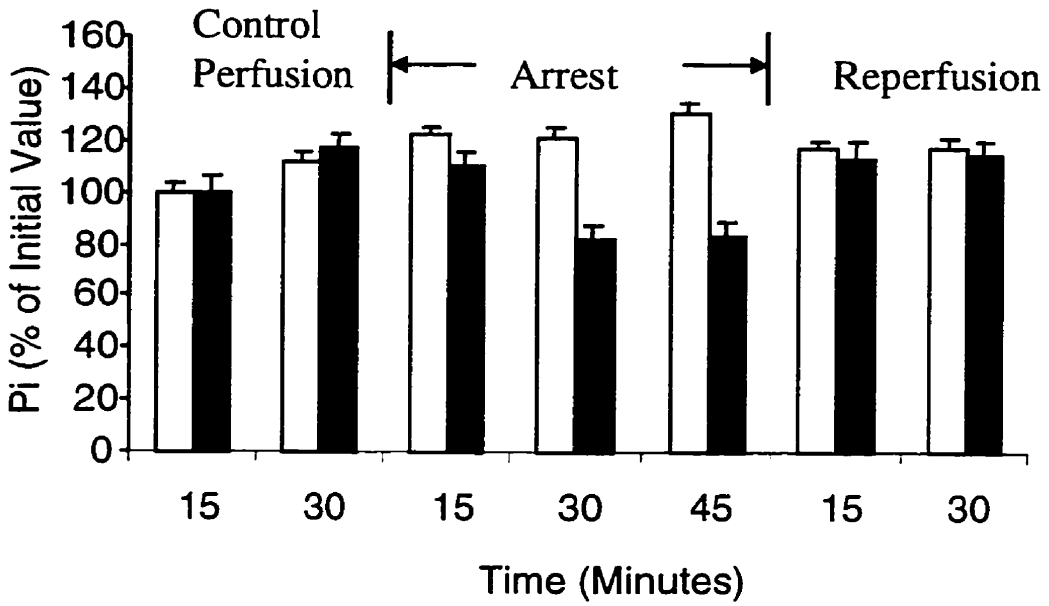

Figure 6.13. Changes of inorganic phosphate levels during 30 minutes of control perfusion, 45 minutes of arrest and 30 minutes of reperfusion in hearts arrested using retrograde cardioplegia and hearts arrested using antegrade cardioplegia. The bars represent averages of % of control values of 4 hearts ± standard error of mean.

6.6. Distribution of retrograde cardioplegia

At the end of 30 minutes of reperfusion, hearts from both groups that showed recovery of function greater than 85% of controls were rearrested. During this period the hearts in both groups were subjected to magnetic resonance imaging using the contrast agent Gadolinium-DPTA polylysine. For the first 20 minutes, the hearts were perfused antegradely with the left anterior descending coronary artery (LAD) open. The LAD was then occluded at its origin with the aid of a manually inflated balloon and perfused retrogradely with a K-H solution containing Gadolinium-DPTA for another 20 minutes and then antegradely again for 20 minutes. T1-weighted images acquired during this period (Figure 6.13) showed the myocardium in the LAD region was not perfused during antegrade perfusion however, retrograde cardioplegia provided solution to the boundary of the area supplied by LAD adjacent to the non-LAD supplied regions. The right ventricular myocardium received more perfusion during antegrade than during retrograde perfusion, indicating RC provided less capillary flow to the right ventricular myocardium. The distribution of antegrade cardioplegia was homogeneous in both the right and the left ventricles.

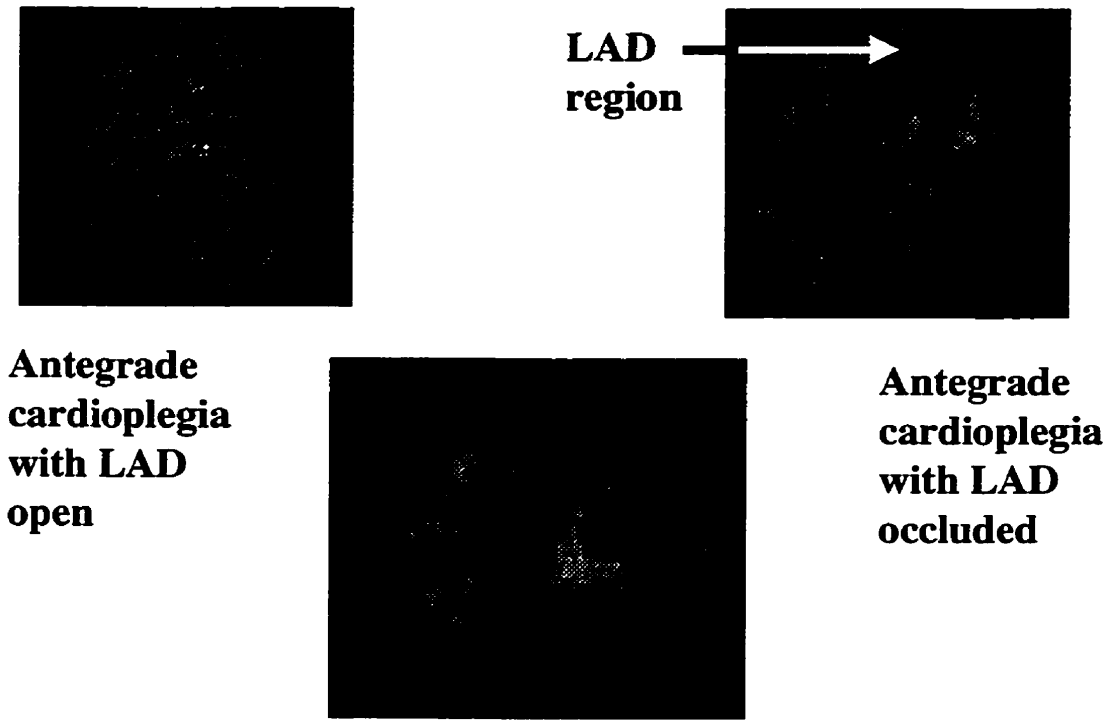

Figure 6.14. T1-weighted images showing the distribution of cardioplegia delivered retrogradely and cardioplegia delivered antegradely. The left anterior descending coronary artery (LAD) was occluded to demonstrate the ability of both methods to deliver solution to parts of the myocardium supplied by an occluded LAD.

7.0. Discussion.

7.1. The principle of cardioplegic arrest.

Cardioplegia means artificial diastolic arrest and involves the infusion of various pharmacological and physiological preparations through the major arteries (antegrade cardioplegia) and veins of the heart (retrograde cardioplegia) to reach the myocardial tissues with a view to arrest its contractile activity, provide necessary oxygenation and metabolic substrates during the interval when the blood supply of the heart is stopped to provide a clear field of view and permit necessary corrective repairs on the heart.

In this study, arrest of the myocardium was achieved by perfusing the heart via the aortic root or via the coronary sinus with a physiological solution containing a high concentration of K^+ (26mM/L for rapid arrest prior to removal of the heart from the pericardial/thoracic cavity and 16mM/L for maintenance of arrest during continuous antegrade or retrograde cardioplegia). The net effect of this was an increase of extracellular K^+ concentration.

An increase in the concentration of extracellular K^+ of the myocardium causes diastolic arrest by deactivating the fast Na^+ and slow Ca^{2+} and Na^+ channels of the myocardial sarcolemmal membrane. It has been determined that cardioplegic arrest based on the use of perfusate containing high K^+ concentration occurs at a K^+ concentration of about 30 mM (Lochner et al, 1968). This is however dependent on the temperature of the cardiac tissues and the ionic milieu of the cardiac extracellular space. Thus, diastolic arrest using a solution containing a high concentration of K^+ is usually combined with measures such as reducing the concentration of Ca^{2+} in the extracellular space, increasing Mg^{2+} concentration and lowering of the cardiac temperature.

The resting membrane potential is primarily the result of the activity of the K^+ leak channels located on the membrane of the cardiac myocyte which is more permeable to K^+ than to Na^+ and other ions. This allows more K^+ than Na^+ to leak out down its concentration gradient, leaving the intracellular space more negative. The potential difference arising from this can be predicted from the Nernst equation and is given below.

$$E_k = -60\log_{10} \frac{[K^+] \text{ inside the cell}}{[K^+] \text{ outside the sell}}$$

Increasing the extracellular of K^+ decreases the concentration gradient driving the efflux of K^+ from the intracellular space. The net effect of this is an increase of excitation threshold of the cardiac myocyte. Thus fewer action potentials are generated for the induction of the cross-bridge cycling of the cardiac contractile process.

7.2. Retrograde cardioplegia: Overview.

The concept of protecting the heart by an artificial circulation of blood or physiological solution was first suggested by Legalloise in 1812 when he postulated that any organ in or outside the body could be kept alive by external perfusion. This has since been confirmed experimentally and its clinical application bolstered by the development of the heart-lung machine in 1951 and the membrane oxygenator in 1944. These advances culminated in the adoption of antegrade cardioplegia as a mode of myocardial protection in adults. The difficulty of perfusing the myocardium beyond stenosed coronary arteries lead to several experimental work exploring the possibility of using the coronary sinus as alternative route for delivery of cardioplegic perfusates to the myocardium. This was however, many years after Pratt perfused devascularised feline hearts using the coronary sinus route in 1898. He reported maintaining

cardiac contraction for up to 10 minutes using perfusate delivered via the coronary sinus (Pratt, 1898). Since then, several experimental and clinical studies have confirmed this (Blancho and colleagues, 1956; Lillehei et al, 1956; Vaage J, 1993; Menasche et al, 1982 and Tian et al, 1996).

In neonates, the evolution of myocardial protection has been relatively slow with most centers still relying on the noncardioplegic methods such as prolonged circulatory arrest and hypothermia which have remained the cornerstone of neonatal heart protection. Available reports shows that these methods does not provide adequate duration of safe ischemic time to meet the increasing demands of todays neonatal heart operations. Intermittent antegrade perfusion is used in some centres and has been reported to be effective in maintaining the heart in arrest but the cumulative effect of the intermittent ischemia arising from intermittent clamping of the aorta, have been confirmed to translate into severe ischemic stress (Report of Congenital Heart Surgeon Society, 1989). The need to guarantee long safe ischemic times to meet the increasing demands of neonatal heart operations is spurring efforts aimed at exploring the coronary sinus as an alternative route for delivery of cardioplegic perfusates.

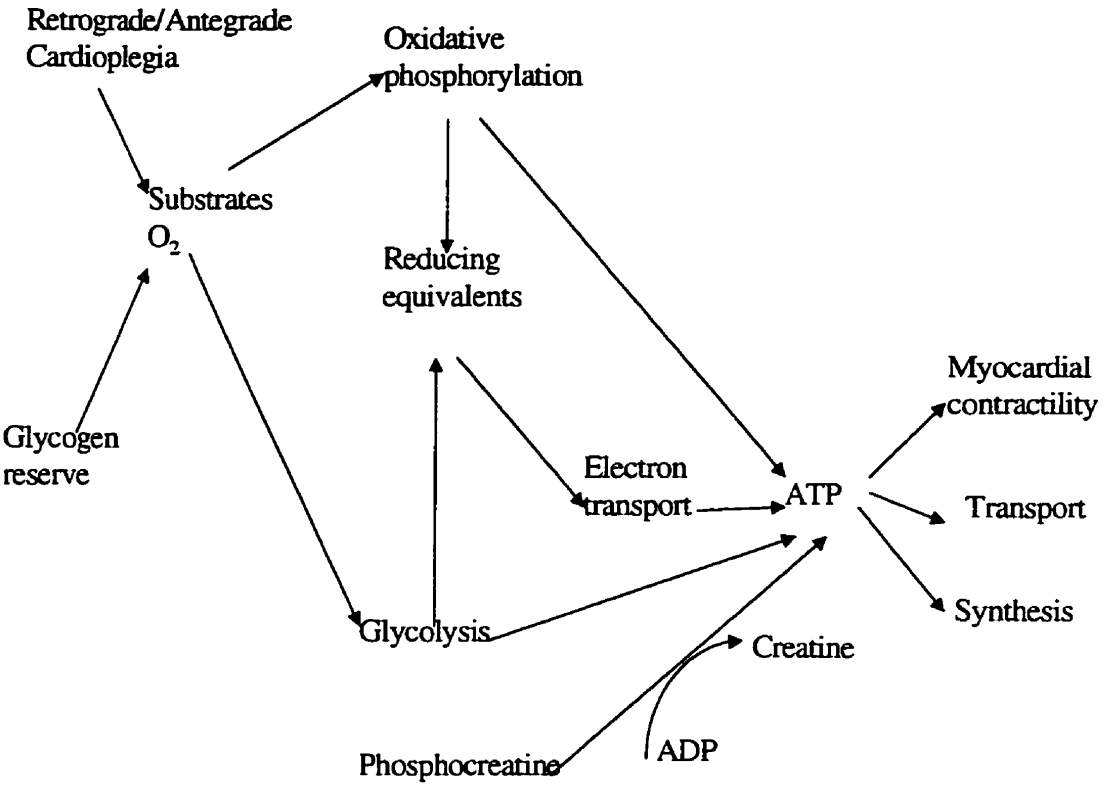

Figure 7.1. Schematic diagram illustrating some of the metabolic events of an isolated heart protected using antegrade and/or retrograde cardioplegia.

7.3. Retrograde cardioplegia techniques.

Direct vision retrograde cardioplegia. (Also called open technique).

This method was first reported by Menasche and associates in 1982 and has also been investigated by other workers (Guirandon et al, 1986 and Vaage, 1993). It involves the isolation of the right side of the heart and clamping of the inflow and outflow tracts of the heart to create a bloodless field. A right atriotomy is made to visualize and cannulate the coronary ostium directly. This technique allows precise control of the positioning of the coronary sinus cannula. The disadvantages includes: the requirement for right heart isolation and double caval cannulation, the need to place pursestring sutures to secure the cannula and maintain the intracoronary sinus pressure and the requirement for a right atriotomy.

Blind vision retrograde perfusion (also called facile method).

The principal disadvantage of the open technique is the large size of the right atriotomy, which predisposes to increased risk of atrioventricular node damage. Bicaval and aortic clamping imposes ischemic stress on the myocardium. The facile or blind method was developed to minimize these problems. In this technique, the right side of the heart is isolated, pursestring suture is made to form the boundaries of a small right atriotomy, through which a cannula can be carefully maneuvered in the coronary sinus ostium. This is the most popular retrograde cannulation method and has been recommended by various workers (Alder et al, 1992; Aron et al, 1992; Blancho et al, 1956; Buckberg et al, 1989; Chitwood et al, 1992 and Gundry et al, 1990). It is however, associated with the following disadvantages:

- Difficulty in precise control of the positioning of the coronary sinus cannula

- The operative field is filled with blood
- There is requirement for right heart isolation.

Transatrial method.

This method was advocated by Buckberg and associates as one way of further minimizing the size of atriotomy associated with the open and facile methods. The cannula normally includes a malleable or semi rigid stylet and self or manually inflating balloons. The stylet is used to create a puncture on the right side of the right atrium to allow the passage of the retroplegia cannula into the atrial chamber and finally maneuvered into the ostium of the coronary sinus. The position of the cannula is secured by inflating the balloon. The advantages of this method lies on its none requirement of bicaval ligation and placement of pursestring sutures.

Right heart retrograde perfusion.

The major complications associated with coronary sinus cardioplegia is the increased risk of injury to the coronary sinus, inadequate perfusion of the right ventriclular myocardium due to and shunting of cardioplegic perfusates through the thebesian venous system. Right heart retrograde cardioplegia was advocated by Fabiani and associates in 1984 as a way of overcoming these problems. The two caval veins and aortic root are clamped and a transatrial puncture created to allow the passage of a cannula. The cannula is positioned to lie either in the right atria or right ventricle. This method provides adequate protection to the right ventricular myocardium and other parts of the heart but has the disadvantage of involving the use of large volumes of solution which predisposes to increased risk of hyperkalemia. It is also associated with the

distention of the right ventricle. Available evidence shows that this distention is not associated with ventricular dysfunction (Fabiani et al, 1984).

7.4. The model

Isolated Langendorff perfused heart preparations have been used extensively in studies designed to elucidate the biochemical, functional and structural alterations that occur in the myocardium in response to anatomical, physiological, pathological and pharmacological challenges such as those that arise from when artificially formulated solutions are substituted for blood during surgical repairs. This is because they allow precise regulation of experimental conditions such as pH, temperature, O_2 and CO_2 tensions, perfusate composition, heart rate, coronary flow rate, perfusion pressures and other factors that influence cardiac function. The neural and hormonal influences that are present in the intact heart are eliminated when the heart is studied in the isolated mode.

To assess the consequences of perfusing the arrested neonatal myocardium through the coronary sinus, we developed an isolated Langendorff crystalloid perfused piglet heart model (a heart preparation in which oxygenated Krebs-Henseleit (K-H) solution at 37°C is delivered to the aortic root by means of a cannula in the brachiocephalic trunk with the aortic arch ligated (antegrade group) or by means of a cannula passed through the coronary sinus ostium into the coronary sinus (retrograde group). The preparation is physiologically stable and can be used within the bore of a high field (7.0 Tesla), 40 cm horizontal bore magnet. Its advantages include:

1. The experimental set-up allows alternate perfusion of the isolated heart with either normokalemic K-H or hyperkalemic K-H solutions.

2. The system allows remote switching from one reservoir to the other as well as collection of samples for monitoring the electrolyte level and oxygen saturation of the cardioplegic solutions.
3. The system allows the isolated heart preparation to be connected to pressure monitoring equipment.
4. The system can keep the heart beating or relaxed for long periods. Because the pump system is only used to maintain the solution levels in the thermoregulated reservoirs, errors and problems with the perfusion circuit do not affect the isolated heart directly.
5. The system can easily be adapted for studies of both cellular and subcellular mechanisms of the heart.
6. The system can be easily adapted for clinical use, since the water jacketed reservoirs can easily be replaced by a set of blood bags suspended from an appropriate height to allow perfusion of the fragile neonatal heart with the aid of gravity. This may eliminate the requirement for perfusion pumps.
7. The system allows precise control of the experimental conditions such as pH, temperature, O_2 and CO_2 tensions.

The isolated Langendorff crystalloid perfused heart model used in this study was developed to allow experimental studies aimed at assessing the efficacy of various cardioprotective methods in neonates. The hearts were perfused at perfusion pressures of about 70 mmHg during antegrade perfusion (control perfusion and reperfusion) and 40 and 60 mmHg during diastolic arrest using continuous retrograde and antegrade cardioplegia respectively. These pressures were achieved by perfusing the hearts from two thermoregulated reservoirs suspended very close to the isolated heart in the magnet at heights of 1.4 m (for hyperkalemic

perfusion) and 1.8 m (for nomorkalemic perfusion). The model was stable for more than 12 hours and permitted repeated switching from normokakemic perfusate (for studies on isovolumic beating heart during control perfusion and reperfusion) and hyperkalemic perfusate (for studies on arrested myocardium during diastolic arrest and NMR imaging). Diastolic arrest eliminates physical factors like ventricular squeeze and autoregulation that protects the myocardium against surges in perfusion pressure. This increases the susceptibility of the coronary vascular system to damages that could arise when perfusion is suddenly initiated from a pump system. This may be worsened by operator errors and errors due to inaccurate pump calibration. Perfusion from a suspended reservoir system eliminates these errors and may help to minimize damages that may arise from sudden exposure of the vasculature to sudden surge of pressure such as could occur during diastolic arrest of the myocardium. Our isolated heart model also allowed continuous sampling of arterial and venous effluents to determine oxygen tension while allowing functional and metabolic assessments.

7.5. Limitations of the Study

Over the course of the experiments and in the process of analyzing the data a number of limitations of the work came to light. The major points to emerge were:

I. Limitations based on the species of animal used in the study. Although the piglet heart is judged to be closest to that of humans, there may be limitations in extrapolating data obtained from such an experimental study to humans. For example, the pig heart has been reported to have a more extensive venous system including shunts such as the thebesian veins (Hoffenberg et al, 1995). The pig heart also differs from the human heart in having an anomalous vein called the hemiazygos vein that opens directly into the coronary sinus.

This vein must be ligated prior to commencement of retrograde perfusion if an optimal level of coronary sinus pressure is to be sustained during the perfusion process.

II. Monitoring of the efficacy of cardioplegic delivery during the experiments was achieved by monitoring the coronary sinus and aortic root pressures continuously. A high pressure indicated that the cannula was inserted far too deep into the vessel while a low pressure may have indicated puncture of the vessel, inadequate balloon occlusion or withdrawal of the balloon from the ostium of the coronary sinus. This procedure did not monitor the position of the balloon in the coronary sinus. Insertion of the cannula too the deeply in the coronary sinus can cause occlusion of some tributaries such as the great cardiac vein, which joins the coronary sinus very close to its ostium.

III. Processing of NMR data normally involves efforts to separate overlapping resonances to allow accurate quantification of various parameters. This is however limited by the type of software used. In this study processing of the NMR data was achieved using the Win NMR software (Win NMR, Bruker, 1997). This software have been reported to be an excellent tool for display and analysis of multidimensional data such as those obtained in NMR imaging and spectroscopy. This is however dependent on the operators skill, patience and carefulness. Inability to adequately separate overlapping resonances as could arise due to operator error can contributes to errors in quantification of the values of Pi, PCr and ATP.

7.6. The Future/Clinical implications.

The heart is an organ with a very high aerobic capacity and comparatively little capacity for anaerobic metabolism. Thus no effort should be spared in designing techniques that prevent

the drift to an anaerobic state. The present study demonstrated over 80% recovery of metabolic and contractile function during reperfusion in hearts maintained in arrest for 43 minutes using perfusate delivered via the coronary sinus. This indicates sustained arrest using retrograde cardioplegia does lead significant depression of cellular activities relying on cellular oxidative metabolism. Our imaging data shows large segments of the right ventricular myocardium remain unperfused or underperfused during retrograde cardioplegia. This is because the right ventricular myocardium is predominantly drained by the thebesian and anterior cardiac veins. This event did not impact very adversely on recovery of contractile and metabolic function during reperfusion. In the future, efforts should be made to determine how the myocardium of various chambers of the heart respond when cardioplegia is delivered retrogradely.

This study demonstrated that the coronary flow of retrograde cardioplegia is much slower than that of antegrade cardioplegia. Previous workers have reported that this is due to pooling of perfusate in the extensive venous networks of the myocardium leading to engorgement of the vascular networks and an increase in LVEDP (Baumgart et al, 1993). The data presented in this study show that antegrade and retrograde cardioplegia are associated with a rise in diastolic pressure during reperfusion but is inconclusive regarding the source of the rise in diastolic pressure. I hypothesize that the increase of diastolic pressure observed with the use of antegrade cardioplegia may have come from the use of K^+ cardioplegia while that observed with the use of retrograde cardioplegia came predominantly from pooling of perfusate in the extensive venous networks of the myocardium as well as from K^+ cardioplegia. In the future, efforts should be made to uncouple the contribution arising from the use of K^+ cardioplegia from those of other factors such as free radicals, reperfusion injury and the technique of cardioplegic delivery and

should aim at determining the extent of injury associated with the use of antegrade and retrograde cardioplegia during diastolic arrest.

The theoretical and experimental information reviewed in this study show that although some areas of the right ventricular myocardium remain unperfused or are underperfused during retrograde cardioplegia, the hearts did recover metabolic and contractile function during reperfusion to levels close to 80% of that observed during control perfusion. Further investigations are necessary to determine the effect of perfusion heterogeneity at the tissue, cellular and subcellular levels of the myocardium.

7.7. Summary of findings

Preamble

In the last 40 years neonatal heart protection has passed through eras of anoxic arrest and fibrillation (Senning, 1952), hypothermia (Oschner JL et al, 1976; Bigelow et al, 1953; Shumway et al, 1963), prolonged circulatory arrest (Jonas RA, 1993, Brunberg et al, 1974; Deverall, 1981; de Leval, 1983; Barratt-Boyes, 1981) and intermittent antegrade perfusion (Report of Congenital Heart Surgeon Society, 1989). Similarly, the surgical operations possible in neonates have improved from simple closure of ASDs to complex repairs such as arterial switches. Retrograde perfusion via the coronary sinus have been recommended by various authors as an option for addressing the increasing demand of these complex neonatal repairs (Kofsky et al, 1991; Vaage, 1993). This is based on the following reasons.

1. The need to guarantee longer "safe times" for corrective repairs.
2. The dependence of the antegrade technique on the presenting pathophysiology.

3. The very low susceptibility of the coronary venous system to malformations, anatomical variations and occlusive diseases.
4. The need to minimize clutter of the operation field by catheters and cannulae.
5. The requirement to minimize long ischemic and circulatory arrest times.

Using our isolated Langendorff crystalloid perfused neonatal pig heart model we monitored the changes in contractile and metabolic function in hearts arrested using retrograde and antegrade cardioplegia. A summary of the analyses is presented below.

Coronary flow of retrograde cardioplegia.

During the arrest period with constant perfusion pressure of 30-40 mmHg for retrograde group and 50-60 mmHg for the antegrade group, the coronary flow rate (CFR) was lower in the retrograde group (25.15±0.01 ml/minute) relative to the antegrade group (91.33±0.2 ml/minute). However, on reperfusion at a perfusion pressure of 70 mmHg, no significant difference was observed in the CFR in the two groups (retrograde group: 133.31±0.2 ml/minute; antegrade: 127.0±0.3 ml/minute). The coronary flow of perfusate delivered via the coronary sinus was much slower than that delivered via the aortic root. Previous investigations have demonstrated that this is due to pooling of perfusate in the extensive venous networks of the myocardium during retrograde cardioplegia (Baumgart et al, 1993). Coronary artery flow occurs during diastole while venous flow occurs during systole and is aided by atrial and ventricular squeeze. During arrest these important factors are eliminated, thus most of the perfusate delivered via the coronary sinus stagnates in the venous networks of the myocardium. This leads to engorgement of the vascular networks and an increase in LVEDP. This observation is supported by the

significantly lower CFR observed in the retrograde group. In the past, recovery of CFR has been used as an index for assessment of recovery of autoregulatory potential of the myocardium (Bruno et al, 1997). During reperfusion the CFR recovered to levels close to that observed during control perfusion. The preservation of autoregulatory potential in the hearts arrested using retrograde cardioplegia shows that retrograde cardioplegia did not lead to irreversible injury.

Myocardial compliance/chamber stiffness.

This was assessed during the experimental time period by continuously monitoring the LVEDP at a constant intraventricular balloon volume of 5.0 mL, monitoring the response of the myocardium to increasing EDV and using the measured values to plot LVEDP-volume curve. The LVEDP were adjusted by changing the left ventricular balloon volume with a calibrated syringe. The LVEDP measured at a constant balloon volume has in the past have been used as an index for assessment of ventricular wall stiffness (Mirsky I, 1984). The LVEDP measured at a constant balloon volume of 5.0 mL during reperfusion was higher in the retrograde group (25.0±0.12 mmHg) than in the antegrade group (20.5±0.31 mmHg).

The LVEDP-volume curve at ventricular end-diastolic volumes, EDV of 2.0-3.0 ml was displaced upwards and to the left in the retrograde group, showing that the hearts never recovered their presystolic volume. For the antegrade group the curve was only displaced upwards but not to the left at volumes greater than 4.5 ml, indicating that the hearts recovered their presystolic volumes even when stretched to very large EDV. The LVEDP-volume curve is used to assess myocardial mechanical tissue properties

(ventricular wall stiffness). Changes in myocardial tissue properties are reflected by displacements of the LVEDP-volume curves (Baumgart et al, 1993). An increase in ventricular wall stiffness may arise from venous engorgement and results in a leftwards and upward displacement of the LVEDP-volume curve. The low CFR in the retrograde group during arrest may have been due to pooling of perfusate in the venous networks of the myocardium leading to an increase of myocardial stiffness. Some workers have proposed that engorgement of the extensive cardiac venous networks following increases in coronary perfusion pressure can lead to increased LVEDP (Salisbury et al, 1961). Under our experimental conditions, engorgement of the venous bed was minimized by continuously venting the aortic root and the ventricular chambers. With the high impedance associated with retrograde cardioplegia, perfusate can accumulate in the venous networks of the myocardium leading to increased LVEDP. This will lead to an increase in heart rate and decreased velocity of contraction and relaxation (±dP/dt).

Myocardial contractility/function.

There was no significant difference in the recovery of LVDP, ±dP/dt, O_2 consumption, MVO_2 (measured as % of the control) during reperfusion between the hearts arrested using retrograde cardioplegia and those arrested using antegrade cardioplegia Figure 4. The % of increase of heart rate was significantly higher in the retrograde group (114±2) than in the antegrade group (107±2). The higher beats/minute in the retrograde group may be associated with an inability of the hearts in this group to recover their presystolic EDV before commencing another systole.

The total energy expenditure of the myocardium arises from demands to sustain structural integrity such as the activity of ion channels and pumps and demands for contractile and metabolic function. Thus parameters such as LVDP, ±dP/dt and RPP that address these functions could be used to assess the ability of a cardioprotective technique to deliver enough perfusate to meet the demands of these functions. Under our experimental conditions the hearts perfused through the coronary sinus recovered LVDP, ±dP/dt and RPP to similar levels as those perfused via the aortic root, indicating adequate preservation of structural, metabolic and contractile integrity of the cells.

Myocardial metabolic function.

In this study assessment of myocardial metabolic function was achieved by continuously monitoring the changes in the PO_2 in the aortic and coronary sinus delivery lines, determining MVO_2 and using ^{31}P MR spectroscopy to monitor changes of myocardial ATP, PCr and Pi. The MVO_2 recovered to a level of 110% ±2.0 in the retrograde group and 109% ±2.0 in the antegrade group. MVO_2 is principally a function of the contractile system. Thus it could be decreased drastically by maneouvres that eliminate the contractile activity of the myocardium. Under our experimental conditions, retrograde cardioplegia was able to sustain the myocardium in complete electromechanical quiescence thereby decreasing the energy demand and expenditure. Under this condition, the perfusate delivered retrogradely was able to preserve myocardial metabolic and electrophysiologic integrity in readiness for resumption of contractile function during reperfusion.

MVO_2 serves as an excellent index of total energy expenditure of the heart. It thus constitutes an index for assessment of the metabolic integrity of the cells and the ability of the tissues to use the O_2 delivered to drive the oxidative reactions that lead to ATP generation. The hearts perfused using retrograde cardioplegia showed a slight increase of MVO_2 during reperfusion relative to the antegrade group, this is inspite of the higher CFR observed in the antegrade group during reperfusion. In the past a rise of MVO_2 has been correlated with the presence of subtle injury and may therefore represent increased energy demand by the cell to reestablish depressed ion pump activity and perform necessary cellular repair (Kojima et al, 1992). The optimal recovery of LVDP, ±dP/dt and RPP during reperfusion observed in the hearts protected using retrograde cardioplegia under our experimental conditions did not correlate with the reports of Kojima and his group.

The high energy phosphates such as phosphocreatine, PCr and adenosine triphosphate, ATP are called on during diastolic arrest to meet the demands arising from efforts by the cell to sustain its structural, metabolic and electrophysiologic integrity in readiness for resumption of contractile function when optimal conditions are restored during reperfusion. ^{31}P MR spectra obtained during control perfusion, arrest and reperfusion showed AC provided better recovery of ATP and PCr during reperfusion. These data show that retrograde perfusion was able to sustain the ATP and phosphocreatine, PCr levels above 80% (for ATP) and 65% (for PCr) of the control values and those derived from antegrade perfusion. Evidence from work on adult models shows that this may be sufficient to meet the energy requirements of a myocardium in diastolic arrest (Buckberg et al, 1993; Tian et al, 1996).

Myocardial Perfusion.

It is obvious from an anatomical point of view that because of the high density of meshwork of the coronary venous system, retrograde infusion of oxygenated cardioplegic perfusates will result in adequate and homogeneous perfusion to an extent comparable to that achieved when perfusates are delivered through the aortic root. However, only 70% of the coronary circulation empty through the coronary sinus, with the remaining 30% draining via the thebesian and anterior cardiac veins. The consequence of this is that large areas of the right ventricular free wall and interventricular septum remain unperfused and underperfused during retrograde cardioplegia. This theoritical observation correlates closely with the results obtained from T1-weighted magnetic resonance images (Figure 8) Representative T1-weighted images acquired during retrograde and antegrade perfusion show that the distribution of perfusate during RC is heterogeneous and homogeneous during AC, indicating that some parts of the myocardium received more perfusate than others during retrograde cardioplegia. The myocardium in the region supplied by the occluded LAD was not perfused during antegrade perfusion however, retrograde cardioplegia provided solution to the boundary of the area supplied by the LAD adjacent to the non-LAD supplied regions. The right ventricular myocardium was perfused adequately during antegrade cardioplegia but remain relatively underperfused during retrograde perfusion.

In summary this study showed the following: (1) RC when used to sustain the neonatal myocardium in arrest preserves contractile function during reperfusion. (2) The PCr level during diastolic arrest dropped to about 65% of its initial level in hearts arrested

by RC and recovered rapidly during reperfusion, to a level greater than 90% of control values. The ATP during arrest remained at a level of 75-80% of control in the retrograde group and recovered to a level greater than 95% of control value during reperfusion indicating that RC, like AC, preserved oxidative phosphorylation. The distribution of perfusate delivered retrogradely was heterogeneous, with the right ventricular myocardium receiving less flow.

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IMAGE EVALUATION
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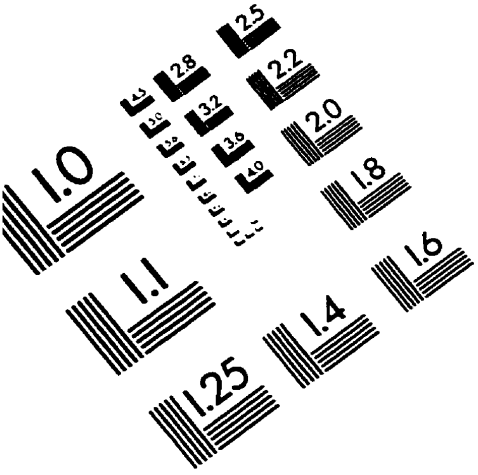

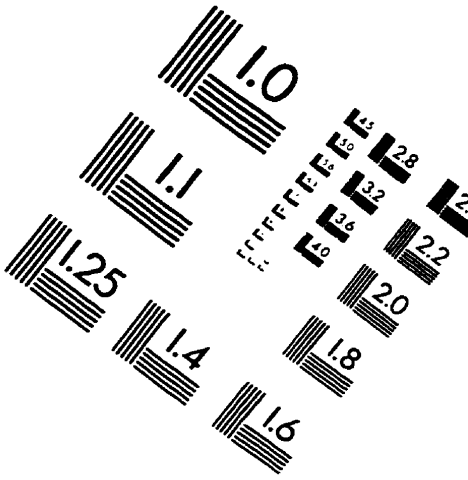

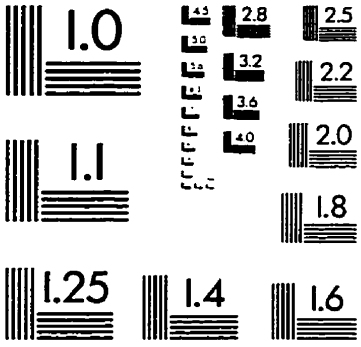

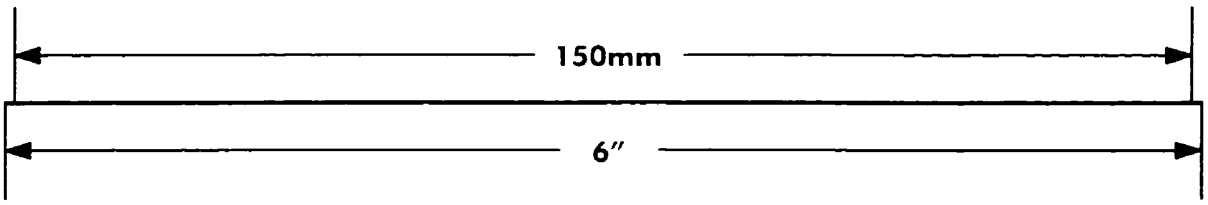

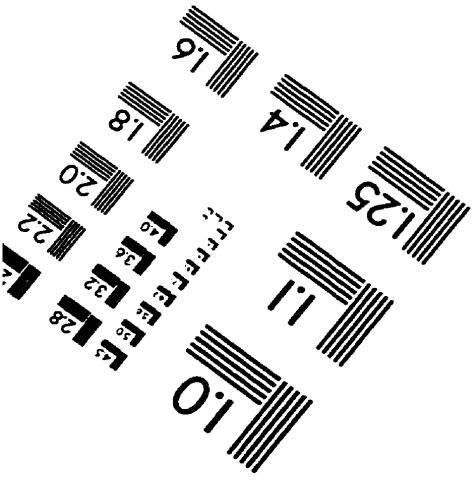

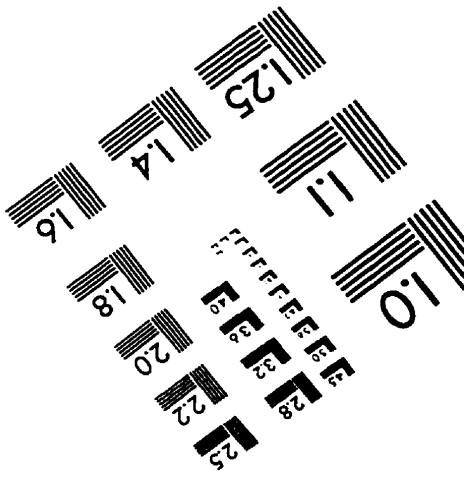

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