SUBSTRATE ENHANCEMENT UTILIZING RIBOSE PRETREATMENT IN NORMAL AND PATHOLOGIC STATES

by

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A thesis submitted in conformity with requirements for the degree of Master of Science Graduate Department of Physiology University of Toronto

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These studies investigated the effects of advanced intravenous (IV) and intramuscular (IM) pretreatment with D-ribose on energy stores in myocardium, skeletal muscle (SKM), and liver (LG), and myocardial tolerance to global ischemia.

Ribose significantly ($p<0.05$) elevated myocardial glycogen when administered IV but not IM, but did not alter SKM or LG. Myocardial tolerance to global ischemia (TIC) was significantly improved by both routes, associated with a reduced net consumption of myocardial energy stores during early ischemia. When administered in the presence of the inhalation anesthetic halothane, TIC was shortened ($p<0.05$) by ribose pretreatment, associated with an increased net consumption of myocardial energy stores. No differences in SKM were observed, while IV ribose appeared to prevent a significant depression in LG. In the presence of hypertension/myocardial hypertrophy, IV ribose pretreatment significantly ($p<0.05$) improved *in vivo* left ventricular performance, but did not alter energy stores or TIC.
ACKNOWLEDGEMENTS

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Finally, I must thank the members of my family for their love and support. To my stepfather, George Harris, my brother Dave, his wife Anne, and their children, Christopher, Kathryn, and Nicholas, and my sister Beth; I know you all think that I'm going to be a student forever, but see, there is an end in sight! I must also mention my mother, who encouraged me throughout my education, but who did not live to see the completion of this thesis. I hope she would be pleased with the results.

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<td>adenosine</td>
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<tr>
<td>ADP</td>
<td>diphosphate</td>
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<tr>
<td>AMP</td>
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<td>AMP-S</td>
<td>monophosphate</td>
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<tr>
<td>AnER</td>
<td>adenylsuccinate</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<td>APRT</td>
<td>adenine phosphoribosyl-transferase</td>
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<tr>
<td>ATP</td>
<td>adenosine tri-phosphate</td>
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<tr>
<td>bpm</td>
<td>beats per minute</td>
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<tr>
<td>CABG</td>
<td>coronary artery bypass surgery</td>
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<td>CAD</td>
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<td>cyclic AMP</td>
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<td>CO</td>
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<td>CPB</td>
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<td>electron transport chain</td>
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<td>fructose-1,6-bisphosphate</td>
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<td>PCA</td>
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<td>Pi</td>
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<td>PPI</td>
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<tr>
<td>PRPP</td>
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<td>QH₂</td>
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<td>RV</td>
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<td>SHR</td>
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<td>SKM</td>
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<tr>
<td>UA</td>
<td>uric acid</td>
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4. *In vivo* LV metabolites from Sprague-Dawley rats expose to IV ribose for either 3 to 8 hours or 12 hours
CHAPTER ONE

INTRODUCTION
SECTION I: CLINICAL RELEVANCE

Heart disease remains the most common cause of death in North America, accounting for approximately 57,000 deaths in Canada in 1994 or 30% of total mortality (Statistics Canada 1995, Gorodeski 1994). Of these deaths, the large majority are due to ischemic heart disease. Systemic hypertension and left ventricular hypertrophy have been shown to be important risk factors for the development of heart disease in both men and women (McKee et al. 1971, Lerner and Kannel 1986, Ettinger 1990, Davidson 1991, Gorodeski 1994, Kitler 1994). Epidemiological studies have shown that risk factors for heart disease are cumulative; thus, the hypertensive patient with cardiomegaly is at even greater risk for increased morbidity and mortality (Kaplinsky 1994).

Treatment has progressed significantly in the past decades, with the development of both pharmacological and surgical interventions designed to alleviate the acute effects of heart disease. Invasive procedures, including percutaneous transluminal coronary angioplasty (PTCA) and coronary artery bypass grafting (CABG), have proved effective in improving perfusion, yet in themselves these procedures subject the myocardium to transient periods of ischemia. That hypertrophied hearts tolerate ischemia less well than normal hearts has been demonstrated in both human and animal studies (Cooley et al. 1972, Katz and Tada 1972, Sink et al. 1981, Peyton et al. 1982, Snoeckx et al. 1989, Allard et al. 1994). Depressed levels of high-energy phosphates in the preischemic hypertrophied myocardium is thought to be at least partly responsible for this increased susceptibility (Sink et al. 1981). A variety of techniques have been developed to reduce the damage associated with myocardial ischemia, including hypothermia, cardioplegia, and various biochemical interventions.
intended to promote postischemic recovery of function and metabolism. To date, the majority of these biochemical interventions have focused on postischemic administration, with little attention paid to the effort to improve ischemic tolerance prior to the ischemic stress.

Aside from contributing to the development of myocardial hypertrophy, chronic systemic hypertension has adverse effects on other organs. Hypertensive patients have been found to be insulin resistant and hyperglycemic compared with normotensives (Reaven 1988, Hall et al. 1994). Furthermore, significant alterations in skeletal muscle characteristics have been found to occur with hypertension. Chronic hypertension is associated with a shift toward more anaerobically active fiber types, characterized by increased activity of glycolytic enzymes (Bassett 1994). Thus, hypertension is associated with dramatic shifts in carbohydrate metabolism. The liver is the primary storage site for carbohydrates, allowing it to regulate plasma glucose concentration, while skeletal muscle comprises the majority of body mass and thus utilizes large amounts of substrate. Since the substrate metabolized by the heart is primarily determined by its plasma concentration, the studies detailed in this thesis include examination of myocardium, skeletal muscle, and liver.
SECTION II: MYOCARDIAL HYPERTROPHY

As with any living tissue, the heart must have the capacity to adapt to changing conditions in order to maintain adequate tissue perfusion. Thus, increasing workloads bring about an adaptive response allowing the heart to improve its performance to compensate for the augmented demand. If the exposure to the increased workload is prolonged, the adaptation takes the form of organ enlargement, such that the larger heart is better able to function in the face of increased demand. Two possible mechanisms are available for this to occur; increasing cell numbers via multiplication (hyperplasia) or increasing cell size (hypertrophy), although in the adult, the heart uses only the latter means to enlarge. Cardiomyocytes are thought to lose the ability to divide shortly after birth (Oparil 1985); thus, cellular hypertrophy is the only available mechanism for the heart to respond to increased workload. There are two categories of hypertrophy that can occur: physiological and pathological. Physiological hypertrophy occurs response to increased demand from regular aerobic exercise. Hearts that undergo physiological hypertrophy are able to meet increased demand with no loss of contractile function or biochemical detriment, since blood supply is maintained at appropriate levels, in contrast to the pathological heart. This category of response will not be further discussed in this thesis.

Pathological myocardial hypertrophy can be stimulated by two different types of work overload: pressure overload and volume overload. Each stimulus generates a characteristic form of hypertrophy; concentric hypertrophy arises from pressure overload, while eccentric hypertrophy arises from volume overload. In order to understand the different responses elicited by these stimuli, it is first necessary to understand the nature of
the mechanical forces that exist in the myocardium. These are described in the next section.

The heart's response to increased workload has been divided into three stages: 1) an initial stage of developing hypertrophy, 2) a compensatory stage of stable hypertrophy, and 3) heart failure, in which the load exceeds the heart's capacity to function (Crozzatier and Hittinger 1988, Opie 1991). The response to increased demand on the myocardium is the development of hypertrophy, in which structural remodelling occurs that relieves the stress of the increased demand, leading to compensatory hypertrophy. This stage is associated with normal or hyperfunction of the heart. However, prolonged exposure to increasing workload eventually leads to the final stage, which, depending on its severity, results in signs and symptoms of heart failure, concomitant with cell necrosis. Clinically, this manifests as left heart failure and pulmonary congestion, or right heart failure and peripheral edema.

**Myocardial Mechanics:**

The primary purpose of the heart is to maintain adequate perfusion of all tissues in the body dependent on their individual needs. This is accomplished by pumping blood at a variable rate that can be expressed as cardiac output (CO). CO, measured in litres of blood per minute, is determined by both the heart rate (HR, beats per minute) and the stroke volume (SV, ml), the volume of blood ejected by the heart per beat. This relationship is expressed by the following equation:

\[
CO = HR \times SV
\]

Contraction of the myocardium, leading to ventricular ejection, is due to the forceful movement of contractile proteins, specifically actin and myosin, within the contractile unit of the cell, the sarcomere. The controlled movement of these proteins, whereby tension is
developed, shortens the sarcomere and exerts force. The coordinated contraction of cells within the ventricle decreases the ventricular chamber volume, thus elevating intraventricular pressure that eventually overcomes extraventricular pressure, resulting in the ejection of blood. Thus, the forces exerted by the myocardium can be measured, and the factors contributing to ventricular remodeling explained, in terms of these forces.

Contraction increases **wall stress**, which is the tension across a unit area in the ventricle. Interestingly, even at rest a degree of ventricular wall stress exists. Wall stress is dependent on the shape and dimensions of the chamber, and the pressure within, and is expressed by Laplace's Law:

\[
Wall\ Stress\ = \frac{P \cdot r}{h} \ (\text{dynes/cm}^2)
\]

*Laplace's Law*

where:
- \(P\) = intraventricular pressure (dynes/cm\(^2\)),
- \(r\) = chamber radius (cm), and
- \(h\) = wall thickness (cm)

Although this equation, which assumes a thin-walled spherical ventricle, is overly simplistic for the elliptical ventricle, it does illustrate the significance of wall thickness in the determination of wall stress (Yin 1981, Oparil 1985, Opie 1991, Katz 1992). It is this feature that is exploited in the pressure-overloaded ventricle's attempt to normalize wall stress. As intraventricular pressure rises in the overloaded ventricle, the ventricular walls thicken so that wall stress is maintained at normal levels.

In the next section, both types of pathologic hypertrophy (eccentric and concentric) and their respective stimuli, specifically volume and pressure overload, will be described. Attention will be focused on pressure overload, since systemic hypertension has been
identified as a key determinant in the development of left ventricular hypertrophy and, if uncontrolled, eventual heart failure (Vasan and Levy 1996). After discussing the global changes in the heart's response to these stimuli, a detailed characterization of the ultrastructural and biochemical changes occurring with myocardial hypertrophy will be given.

**Volume Overload**

Ventricular overload due to increased intraventricular volume can occur clinically in patients with aortic regurgitation, in which valvular insufficiency allows the back flow of blood into the left ventricle, or arteriovenous fistula, in which arterial blood is shunted to the right ventricle (Spotnitz and Sonnenblick 1973). Both situations increase ventricular preload; that is, the degree of stretch present in the myocardium prior to contraction. This increases the end diastolic wall stress, which forces the heart to compensate producing eccentric hypertrophy.

Eccentric hypertrophy (Figure 1) is characterized by an increased chamber size (radius) and an increased ventricular mass, without any change in wall thickness (Spotnitz and Sonnenblick 1973). Chamber enlargement occurs due to the replication of myocyte sarcomeres in series, resulting in elongation of muscular fibres (Oparil 1985, Opie 1991). However, as described in Laplace's Law, the chamber enlargement actually increases ventricular wall stress, since chamber radius is greatly enhanced while wall thickness increases only moderately. Therefore, the ratio of chamber radius to wall thickness \((r/h)\) increases in eccentric hypertrophy.
Pressure Overload

Patients with chronic systemic hypertension, aortic stenosis, or pulmonary disease experience an increased resistance to ventricular ejection (afterload) due to the increased pressure load against which the heart must work. The resultant pressure overload predisposes these patients to development of concentric hypertrophy in an effort to compensate for the increased systolic wall stress (Figure 2).
Concentric hypertrophy is characterized by significantly augmented wall thickness (h') at the expense of chamber size, which decreases r/h below that of the normal heart (Spotnitz and Sonnenblick 1973, Oparil 1985, Opie 1991). The combined effects serve to minimize wall stress by spreading the increased stress over a greater number of contractile elements. Calculations have shown that a 50% increase in wall thickness results in a 67% increase in ventricular mass (Spotnitz and Sonnenblick 1973). In contrast to eccentric hypertrophy, sarcomere replication occurs in parallel in concentric hypertrophy (Oparil 1985, Opie 1991).

**Cellular Morphology and Ultrastructural Changes**

Ultrastructural changes to cardiac muscle in response to pressure overload are striking. However, tissue remodelling involves not only cardiomyocytes but non-myocyte tissue as well. Alterations in the composition of interstitial mass and perfusion patterns have profound effects on the global performance of the heart, and must be considered along with myocardial changes to understand the impact of hypertrophy.

The gross anatomical indicators of myocardial hypertrophy, increased ventricular mass and wall thickness, can be explained in terms of histological changes that accompany alterations in protein synthesis. In rats subjected to chronic renovascular hypertension, left ventricular papillary muscles were shown to significantly increase in diameter and cross sectional area, but not in length (Capasso et al. 1986), in accordance with the parallel replication of sarcomeres occurring in concentric hypertrophy. Similarly, studies in Spontaneously Hypertensive Rats (SHR), a genetic model of chronic systemic hypertension, show that left ventricular wall thickness, myocyte cross sectional area, and fibre diameter are
significantly increased compared with normotensive controls (Lund et al. 1979, Engelmann et al. 1987).

Accompanying changes in muscle mass, alterations occur in the degree of vascularization in the hypertrophied heart. As the myocardium hypertrophies in response to increased workload, the vascular bed increases to a smaller degree, so that the myocardium is relatively underperfused compared with the normal heart. In addition, increasing systolic wall stress is associated with increased tissue oxygen consumption (Strauer 1983, Strauer 1984). This tends to create regions of relative hypoxia within the myocardium, particularly in the subendocardial layers where wall stress is greatest. The reasons for this poor perfusion are related to the significant increase in myocyte diameter that occurs without a similar increase in capillary density. Myocardial capillary density, expressed as the number of capillaries/mm² of myocardium, is 37% lower and myocyte cross-sectional area (μm²) is 30% greater in 6 month old SHR than that of age-matched Wistar-Kyoto (WKY) rats, their normotensive controls (Engelmann et al. 1987). This is associated with a significant and progressive increase in average diffusion distance in the SHR due to the increase in myocyte size. Similar reductions in capillary density were seen in the myocardium of cats hypertrophied via aortic banding (Breisch et al. 1984) and in patients with LV hypertrophy (Kaplinsky 1994). Basal coronary flow (ml/minute/g ventricle) is similarly reduced by 35% in the SHR compared with the WKY, associated with a significantly reduced arterial lumen diameter in the SHR (Anderson et al. 1989). These studies suggest that the hypertrophied myocardium is relatively underperfused compared with the normal myocardium.

Pressure overload hypertrophy is accompanied by an increase in the number of
mitochondria, such that the mitochondria to myofibril ratio remains constant during the compensatory phase (Breisch et al. 1984, Oparil 1985, TenEick et al. 1989). This ratio becomes significantly reduced with advanced hypertrophy and failure (Breisch et al. 1984). Further alterations in the biochemical machinery of the hypertrophied myocardium are discussed in Section III.

To understand the functional consequences of hypertrophy, it is first necessary to briefly discuss the contractile machinery of the myocyte. Any changes in this machinery can then be related to performance changes in the whole organ. As with any striated muscle, the contractile proteins myosin and actin are structured into sarcomeres, which comprise the contractile unit of the cell. The globular head of myosin, which directly interacts with actin to cause sarcomere shortening, consists of myosin heavy chains (MHC) and myosin light chains (MLC). The enzymatic properties of the myosin head account for the hydrolysis of ATP that powers the contractile process. Two different MHC isoforms exist, denoted α and β. Thus, the myosin molecule can be composed of αα or ββ homodimers, or an αβ heterodimer (Mercadier et al. 1983). Electrophoretic separation and monoclonal antibody identification in the normal human ventricle shows that over 80% of total myosin is composed of the ββ homodimer, designated as the V3 isoform, while the remainder is mostly αα (V1) myosin, with very little existing as αβ (V2) (Mercadier et al. 1983; Gorza et al. 1984). Adult rats and mice express primarily V1 myosin, while rabbits, pigs, dogs, and cattle express V3 only (Lompre et al. 1981, Mercadier et al. 1981, Capasso et al. 1986). A gradual increase in the percentage of V3 isoform occurs with increased workloads. Strong correlations have been demonstrated between the percentage of V3 myosin and the degree of
ventricular hypertrophy (r= 0.70) (Mercadier et al. 1981) and between mean arterial blood pressure and percent left ventricular β-MHC composition (r = 0.87) (Haddad et al. 1995).

This increase in $V_3$ expression is observed whether the hypertrophy is induced by aortic or renal artery constriction, valvular incompetence, or by genetic hypertension in the SHR strain (Gorza et al. 1981, Mercadier et al. 1981, Capasso et al. 1986, Bugiasky et al. 1990, Haddad et al. 1995). Subendocardial layers show significantly greater percentage $V_3$ in response to aortic constriction than subepicardial layers (Bugiasky et al. 1990). Similar shifts in ventricular myosin isoform composition are not found in hypertrophied human hearts.

The reasons for this isoenzyme shift with the development of ventricular hypertrophy in rats have been studied regarding mechanical and biochemical properties of the proteins. $V_1$ myosin has a high ATPase activity and is associated with a rapid velocity of shortening, while $V_3$ has a low ATPase activity and a slower shortening velocity (Opie 1991, Katz 1992). Maximum shortening velocity has been demonstrated to be proportional to ATPase activity (Harris et al. 1994). Studies using pressure overloaded rabbit ventricles illustrated that myocardial hypertrophy is associated with an increased efficiency of force production compared to normal muscle (Alpert and Mulieri 1982). Recently, investigations into the forces generated in cross-bridge interactions between a single, fluorescently labelled actin filament and $V_1$ and $V_3$ myosin showed that the ATPase activity and shortening velocity of $V_1$ were between two and three times that of $V_3$, yet generated only one half the force per cross-bridge of $V_3$ (VanBuren et al. 1995). These differences were attributed to the amino acid sequence of the $\alpha$ and $\beta$ chains. This may explain the increased efficiency of force generation in the hypertrophied myocardium without loss of mechanical ability in the
compensated phase of development.

In addition to cardiomyocyte changes, significant alterations in the extracellular matrix occur with the development of hypertrophy. Interstitial collagen content has been shown to increase significantly in response to aortic banding (Doering et al. 1988, Jalil et al. 1988). Similar results were seen at autopsy in patients with hypertrophy with both compensated and decompensated function (Pearlman et al. 1982). Factor and Robinson (1988) hypothesized that the normal organization of this matrix, in which fibrous interconnections exist between individual myocytes and between muscle bundles, "... may account for the forceful coordinated contraction and recoil of the entire ventricle." The increase in collagen content initially enhances the ability of the overloaded heart to generate force, but eventually impairs the diastolic properties of the heart due to its increased stiffness (Jalil et al. 1989). Dramatic increases in left ventricular fibrosis in 52 and 90 week old SHR rats is concomitant with a reduced pumping ability (Pfeffer et al. 1979). Thus, observed alterations in this fibrous matrix also affect global function.
SECTION III: MYOCARDIAL METABOLISM

Overview: The maintenance of the normal mechanical activity of the heart requires the continuous expenditure of large amounts of energy in the form of adenosine triphosphate (ATP). Degradation of ATP to adenosine diphosphate (ADP) via ATPase activity releases chemical energy that can be used for mechanical work. Myocardial contraction accounts for approximately 65% of energy utilization in the heart, the remainder being required for cellular homeostasis (Opie 1991). ATP use is estimated to be approximately 1 μmol/g dry tissue per beat in studies of the isolated working rat heart (Hearse 1979), and increases with increasing work load, from about 0.66 μmol/sec/g tissue at low load to as much as 2.35 μmol/sec/g tissue at high work loads in the rat (Jacobus 1985). However, tissue ATP content is only about 5 μmol/g tissue (Katz 1992). Thus, the heart exerts fine control over metabolic pathways so that ATP utilization is matched by ATP production. This control is possible due to the variety of substrates and pathways used to generate ATP, allowing the heart to adapt to changes in energy demand and fuel availability.

There are two mechanisms for ATP production in the heart: aerobic and anaerobic metabolism. Aerobic metabolism encompasses a number of metabolic pathways, specifically glycolysis, β-oxidation, the tricarboxylic acid (TCA) cycle, and the electron transport chain (ETC) (Opie 1991, Katz 1992). The principal fuel utilized by the normal adult heart for aerobic energy production is lipid, in the form of free fatty acids (FFA), which yield large amounts of ATP when they are oxidized. Carbohydrates can also be metabolized aerobically, allowing the heart to utilize blood glucose, pyruvate, and lactate. Availability is the primary
determinant of which substrate is utilized. Thus, when blood FFA levels are high, lipids become the primary energy source. Carbohydrates become important following heavy exercise which raises blood lactate and after a carbohydrate load (Opie 1991). When oxygen availability falls, as occurs when the heart experiences ischemia such that delivery of oxygen is insufficient to maintain aerobic metabolism, anaerobic metabolism becomes the only means for ATP synthesis. Anaerobic glycolysis is the only pathway operating during this period, forcing the heart to rely on carbohydrates. Both aerobic and anaerobic metabolic pathways will be discussed in detail in the following sections.

**Energy Stores:** Cardiac myocytes have limited stores of compounds that can be used to generate ATP. Creatine phosphate (CP), and myocardial glycogen (MG) can serve as immediate and more prolonged sources of ATP, respectively, without the need for extracellular substrate delivery. However, these sources are insufficient to meet energy demand for more than a short period if external substrates are unavailable. The mechanisms for ATP production from each of these sources are detailed below.

(i) **Creatine phosphate** levels in the heart have been reported to be about 50% higher than ATP levels in both normal and hypertrophied hearts (Gudbjarnason *et al.* 1970, Snoeckx *et al.* 1989). Despite this, CP does not serve as the direct energy source for myocardial contraction, but rather is used to rapidly regenerate ATP via creatine kinase (CK).

\[
CP + ADP \xrightarrow{CK} ATP + creatine + energy
\]

Gudbjarnason *et al.* (1970) showed that myocardial CP levels could be reduced by as much
as 40% in the dog before a significant reduction in ATP occurred. The importance of the relationship between CP and ATP in the heart is further illustrated by the observation that contractile activity ceases when CP is 75% depleted by ischemia but ATP content is still 80% of normal (Gudbjarnason et al. 1970). Myocardial CP content has been shown to be significantly reduced in SHR at 6 months of age compared with age-matched WKY rats (Shimamoto et al. 1982). This may contribute to the increased susceptibility to ischemia of hypertrophied hearts.

Cellular CK exists in two forms, cytosolic (CKc) and mitochondrial (CKm). These two enzymes act in concert to transfer high-energy phosphates from the mitochondrial matrix to the cytosol. CP is generated via the CK reaction only when ATP is abundant, as in the mitochondrial matrix. ATP synthesized in the mitochondrial matrix via oxidative phosphorylation is transferred to the cytosol by the action of CKm, which transfers the high energy phosphate from ATP to creatine, generating CP (Opie 1991, Katz 1992). CP then serves as a phosphate donor to form ATP and creatine within the cytosol via CKc. In this

![Diagram of the creatine phosphate shuttle](image-url)

**Figure 3.** The creatine phosphate shuttle. Modified from Kammermeier 1987, Katz 1992.
manner, ATP is constantly supplied to areas of high metabolic activity which produce large amounts of ADP, with creatine acting as the messenger of the phosphate group. This "shuttle" mechanism is shown in Figure 3.

(ii) **Myocardial glycogen (MG)** can function as an endogenous substrate reserve that can provide a substantial proportion of the energy requirements of the heart when external substrates are unavailable. MG content is only about 5% of that of the liver (Wittnich et al. 1982, Wittnich et al. 1986), and is unevenly distributed in the heart, such that higher concentrations are seen in the subendocardial layers compared with the subepicardium, corresponding to the higher workloads experienced in the subendocardium (Naveri et al. 1987). MG levels in SHR have been shown to be similar to (Shimamoto et al. 1982, Snoeckx et al. 1989) or significantly lower (Raizada et al. 1993) than those of age-matched rats with normal hearts.

Glycogen exists as a large, branched polymer of glucose residues linked by α-1,4-glycosidic bonds and α-1,6-glycosidic bonds at branch points, which occur about every ten residues (Stryer 1988).

![Figure 4. Structure of the glycogen polymer, showing bonds between glucose residues. Modified from Stryer 1988.](image-url)
By degrading into its constituent glucosyl units, glycogen can be used to produce ATP via glycolysis, as detailed in the next section. The process of glycogen synthesis (glycogenesis) occurs by a different mechanism than that of its degradation. Both processes are highly regulated via the reciprocal interconversion of enzymes between active and inactive forms, dependent on their state of phosphorylation and dephosphorylation. The phosphorylation state of these enzymes is controlled by the action of hormones, such as epinephrine and insulin (Ramachandran et al. 1983). $^{13}$C-NMR studies of the mechanisms of glycogen synthesis and degradation showed that these processes occur in a controlled order, such that the first glucosyl units formed are the last to be mobilized (Brainard et al. 1989). These processes are described in detail next.

**Glycogen Synthesis**

Uridine diphosphate glucose (UDP-glucose) is used as the glucose donor for glycogen synthesis. This activated form of glucose is synthesized from glucose-1-phosphate (G-1-P) and uridine triphosphate (UTP) via UDP-glucose pyrophosphorylase (Stryer 1988).

Synthesis of glycogen is catalyzed by glycogen synthase, which can exist in an active dephosphorylated form (glycogen synthase a) and an inactive phosphorylated form (glycogen synthase b). This enzyme has been shown to be rate limiting for glycogen synthesis in hearts from normal, diabetic, and fasted rats (Laughlin et al. 1990). Active glycogen synthase catalyzes the formation of $\alpha$-1,4-glycosidic bonds between UDP-glucose and the nonreducing terminal of the glycogen polymer, generating UDP and an elongated glycogen molecule (Stryer 1988). A separate branching enzyme is required to form the $\alpha$-1,6-glycosidic bonds. Conversion of the active form of glycogen synthase to the inactive form is regulated by a
cascade reaction, initiated by adrenergic stimulation via epinephrine (Stryer 1988, Katz 1992) (Figure 5). Binding of epinephrine to membrane β-receptors activates adenylate kinase, which catalyzes the formation of cAMP from ATP. In turn, cAMP activates a cAMP-dependent protein kinase, which directly phosphorylates glycogen synthase, generating the inactive β form. Binding of epinephrine to α-adrenergic receptors increases intracellular Ca\(^{++}\) concentration, which activates a Ca\(^{++}\)-dependent protein kinase, also leading to the production of inactive glycogen synthase, although a different site on glycogen synthase is phosphorylated (Ramachandran 1983). Reconversion of glycogen synthase to the active form is governed by synthase phosphatase, which dephosphorylates inactive glycogen synthase β (Katz 1992).

Glycogen Breakdown

Degradation of the glycogen polymer is controlled by glycogen phosphorylase, the activity of which is also dependent on its phosphorylation state. In contrast to glycogen synthase, the phosphorylation of glycogen phosphorylase results in the active form, phosphorylase α, catalyzed by phosphorylase kinase (Stryer 1988, Katz 1992). Phosphorylase kinase can be phosphorylated into its active form by both Ca\(^{++}\) and cAMP dependent protein kinases, and is thus regulated by adrenergic agonists. Phosphorylase kinase phosphatase inactivates phosphorylase kinase. Dephosphorylation of glycogen phosphorylase produces the inactive β form, catalyzed by phosphorylase phosphatase. Johnson and Hammer (1993) found no significant difference in phosphorylase activity between 8 month old SHR and WKY rats.

The reciprocal phosphorylation and dephosphorylation of glycogen synthase and
Figure 5. Reciprocal control of glycogen synthesis and degradation by catecholamines. Modified from Stryer 1988, Katz 1992.
glycogen phosphorylase under the influence of cyclic AMP (cAMP) and Ca\textsuperscript{++} links glycogen synthesis and degradation, allowing the heart to respond to varying conditions. Thus, glycogen synthase is active when phosphorylase is inactive, and vice versa. This enables glycogen to be synthesized when metabolic requirements do not necessitate the utilization of stored carbohydrates. For example, net glycogen synthesis occurs following a meal when plasma glucose is elevated, since plasma insulin levels rise leading to increased glucose uptake, while net glycogen degradation occurs to meet the additional metabolic need when energy demand increases. The pathways of energy production from carbohydrates will be described later.

Active glycogen phosphorylase catalyzes the breakdown of glycogen to G-1-P by sequential removal of glucosyl residues at \(\alpha-1,4\)-glycosidic bonds (Stryer 1988). Phosphorylase is incapable of cleaving branches in the glycogen polymer; thus, a debranching enzyme hydrolyzes the \(\alpha-1,6\)-glycosidic bonds. The G-1-P generated from glycogen breakdown is then able to enter the glycolytic pathway for ATP production.

**Pathways of ATP synthesis:**

Production of chemical energy in the form of ATP occurs via two main pathways in the heart: glycolysis and oxidative phosphorylation. Although the latter route produces substantially more ATP than glycolysis, both processes serve important roles for the proper functioning of the myocardium. Unlike oxidative phosphorylation which has an absolute requirement for the presence of oxygen, glycolysis can also operate anaerobically. Thus, glycolysis is the only source of ATP during periods of myocardial hypoxia and ischemia.
The capacity of the heart to utilize both major pathways and a variety of substrates to generate ATP, allows it to adapt metabolically to constantly changing conditions without impairment of normal mechanical function. The manipulation of these pathways using metabolic intermediates to "short cut" the production of myocardial energy stores in both normal and pathologic states is the focus of this thesis.

**Glycolysis:** Glycolysis (Figure 6) is a highly regulated sequence of reactions that utilizes glucose, derived from exogenous sources or from MG, to generate ATP. In the aerobic process, pyruvate is also generated that can enter oxidative pathways and be further metabolized, while lactate is the final product of anaerobic metabolism. Generation of either pyruvate or lactate from glucose occurs only in the cytosol. Further processing of pyruvate via oxidative mechanisms requires that pyruvate be transported into the mitochondria and converted to acetyl coenzyme A (CoA) via pyruvate dehydrogenase (PDH) (Stryer 1988). The complete aerobic metabolism of glucose, through glycolysis and oxidative phosphorylation, yields a net 32 ATP, while anaerobic glycolysis yields only 2 ATP (Stryer 1988, Tropp 1997). Myocardial ATP content has been shown to be 16% lower in 6 month old SHR compared with age-matched WKY rats, although this did not achieve statistical significance (Shimamoto *et al.* 1982). However, a reduced myocardial energy state in the SHR at this age is supported by significantly higher concentrations of lactate, ADP, and AMP, associated with significant LV hypertrophy (Shimamoto *et al.* 1982). This may be related to an increased reliance on glycolysis.

The glycolytic pathway can be divided into two phases: reactions involving hexose
Figure 6. Biochemical pathway of glycolysis, showing major regulatory enzymes. Modified from Stryer 1988.
sugars, and those involving trioses. The first step in glycolysis is the production of glucose-6-phosphate (G-6-P) from glucose by hexokinase (HK), using ATP as a phosphate donor, and from G-1-P from glycogen breakdown via phosphoglucomutase (Katz 1992; Stryer 1988; Opie 1991). Subsequent reactions produce fructose-1,6-bisphosphate (F-1,6-BP) with the expenditure of a second ATP as a phosphate donor (Katz 1992; Stryer 1988; Opie 1991). Triose sugar reactions lead to the formation of two moles of pyruvate, 4 moles of ATP, and 2 moles of reduced nicotinamide adenine dinucleotide (NADH) per mole of glucose. NADH plays an important role as an electron donor in the electron transport chain, described below.

Control of the glycolytic pathway occurs at three points, specifically, the reactions catalyzed by the enzymes HK, phosphofructokinase (PFK), and pyruvate kinase (PK) (Stryer 1988).

HK activity is primarily regulated by intracellular levels of G-6-P, such that high levels are inhibitory, halting glycolytic flux (Stryer 1988, Katz 1992). The inhibitory effect of G-6-P on HK is modulated by intracellular phosphate compounds, including high-energy phosphates. Thus, high concentrations of ATP enhance the inhibitory action of G-6-P, while products of ATP degradation (ADP, AMP, Pi) have the opposite effect (Katz 1992). This control allows the entry of substrate into the glycolytic pathway to be matched by flow through the reaction sequence, coupling energy status with production of ATP.

PFK is the major regulatory site in glycolysis. Activity of PFK is under strict allosteric control, by both metabolic end products and non-metabolites. ATP has a strong inhibitory effect on PFK at high concentrations, whereas ADP, AMP, and Pi have the opposite effect (Stryer 1988, Katz 1992). Both citrate, an intermediate of the TCA cycle, and H⁺, which may accumulate during hypoxia and ischemia, exert strong inhibitory effects on the
activity of PFK (Stryer 1988, Opie 1991, Katz 1992). These compounds halt glycolytic flux when it is unnecessary or potentially harmful. PFK is also regulated by the compound fructose-2,6-bisphosphate (F-2,6-BP), which exerts strong positive feedforward control of PFK and can overcome the inhibition by ATP (Rousseau and Hue 1993). F-2,6-BP is formed from F-6-P by phosphofructokinase 2 (PFK2), and is degraded to F-6-P by fructose bisphosphatase 2 (FBPase2). Interestingly, both enzymes are present in a single protein, known as a tandem enzyme (Stryer 1988). F-6-P increases the kinase activity and decreases the phosphatase activity of the enzyme, leading to increased formation of F-2,6-BP, thus stimulating glycolysis (Rousseau and Hue 1993). As well, phosphorylation of the enzyme by protein kinase A in response to cAMP activates the PFK2 activity and inactivates FBPase2 in muscle, but has the opposite effect in liver; dephosphorylation reverses these effects (Stryer 1988, Rousseau and Hue 1993). The FBPase2 activity of the enzyme is much lower in the heart than it is in the liver, so that adrenergic stimulation increases the glycolytic activity of the heart (Rousseau and Hue 1993).

PK represents the final control point in glycolysis, regulating the outflow of pyruvate from the pathway. As with the other regulatory enzymes, high levels of ATP allosterically inhibit PK activity (Stryer 1988). Outflow through PK is matched with glycolytic flux by F-1,6-BP, which activates PK (Stryer 1988). Recent studies demonstrated that the LV of 6 month old SHR has 28% higher PK and 79% greater lactate dehydrogenase (LDH) activities, indicative of higher glycolytic activity in the SHR (Atlante et al. 1995). This is supported by studies of isolated perfused hearts from aortic banded and sham operated rats, where higher glycolytic rates (Allard et al. 1994) and significantly greater lactate production during ischemia (Anderson et al. 1990) were observed.
Although it does not represent a significant site in the regulation of glycolysis in the well-oxygenated heart, glyceraldehyde-3-phosphate dehydrogenase (G-3-P DH) becomes important under anaerobic conditions (Katz 1992). The forward direction of this reaction involves the production of NADH, which accumulates in the anaerobic myocardium due to cessation of oxidative metabolism. High concentrations of NADH inhibit G-3-P DH, halting glycolytic flux and energy production (Katz 1992). The conversion of pyruvate to lactate via LDH regenerates NAD\(^+\) from NADH. This relieves the inhibition of G-3-P DH, thereby sustaining the production of ATP through glycolysis.

\[
\text{LDH} \\
\text{pyruvate} + \text{NADH} \rightleftharpoons \text{lactate} + \text{NAD}^+
\]

**Lipid Metabolism:** Although the heart can utilize a variety of substrates for ATP production, lipids provide the highest energy yield and, consequently, are the major fuel source for the adult heart under aerobic conditions. For example, the complete oxidation of palmitate, a 16-carbon fatty acid, yields 129 ATP. Lipid oxidation involves a complex series of reactions, including fatty acid activation, \(\beta\)-oxidation, the tricarboxylic acid cycle, and oxidative phosphorylation. Each process will be described in turn.

Metabolism of fatty acids occurs in the mitochondrial matrix, necessitating their transfer across the outer mitochondrial membrane. This process, known as activation, occurs on the surface of the outer mitochondrial membrane, and involves the generation of long chain acyl CoA molecules from CoA and the fatty acid, with the hydrolysis of one ATP (Stryer 1988). Acyl CoA is then modified by carnitine acyltransferase I, located on the
cytosolic side of the inner mitochondrial membrane, into acyl carnitine. Acyl carnitine is then transported across the mitochondrial membrane by a translocase enzyme (Stryer 1988). Carnitine acyltransferase II, located on the matrix side of the inner membrane, then regenerates acyl CoA for oxidation and carnitine which returns to the cytosol.

Tissue carnitine levels have been shown to be altered in pathologic conditions, including pressure-overload myocardial hypertrophy (Reibel et al. 1987). Significantly reduced total myocardial carnitine contents have been observed in rats with left ventricular hypertrophy induced by aortic banding (Reibel et al. 1987) and in cardiomyopathic hamsters (Kobayashi et al. 1992). In contrast, total carnitine is significantly elevated in the hearts of 4-month old SHR rats compared with normotensive WKY, associated with increased serum carnitine levels in the SHR (Foster et al. 1985). The levels of free myocardial carnitine are also significantly reduced in left ventricular papillary muscles of patients with congestive heart failure, although total myocardial carnitine levels (free carnitine + acyl carnitine) are not significantly different from patients without heart failure because the levels of long-chain acyl carnitine are elevated (Kobayashi et al. 1992). Therefore, myocardial carnitine metabolism may be unaltered in patients with heart failure.

\[\text{\textit{\beta-oxidation:}}\] Acyl CoA next undergoes \(\beta\)-oxidation (Figure 7), during which the long chain lipid is degraded in a four-step process into two carbon units (acetyl CoA). As acyl CoA is degraded, two oxidation reactions occur, with flavin adenine dinucleotide (FAD) and NAD\(^+\) functioning as electron acceptors, generating one FADH\(_2\) and one NADH, respectively, for each turn of the oxidation spiral (Stryer 1988). These reduced coenzymes are later used to generate ATP in subsequent oxidation reactions known as the electron transport chain (ETC).
Figure 7. β-oxidation.  
Acetyl CoA is then metabolized in the TCA cycle with the further production of NADH and FADH$_2$. Pyruvate from aerobic glycolysis is also used to generate acetyl CoA via PDH, linking glycolysis with the TCA cycle. The rate of β-oxidation is controlled by the availability of fatty acyl CoA in the mitochondrial matrix, which is governed by the activities of carnitine acyltransferase I and II, which function to transfer acyl CoA from the cytosol to the mitochondria (Stryer 1988, Katz 1992). The utilization of acetyl CoA in the TCA cycle and the oxidation of NADH and FADH$_2$ in the ETC regulate β-oxidation as well (Naveri et al. 1987, Stryer 1988, Opie 1991). Johnson and Hammer (1993) showed that the activity of hydroxyacyl dehydrogenase was significantly reduced (27%) in the LV of SHR compared with age-matched WKY, although the depression was not considered to be sufficient to affect fatty acid utilization.

**Tricarboxylic Acid (TCA) Cycle:** The TCA cycle (Figure 8) is a complex sequence of reactions beginning with the production of a 6-carbon compound, citrate, from acetyl CoA and oxaloacetate, and the subsequent regeneration of oxaloacetate. One complete cycle generates 3 NADH, 1 FADH$_2$, and 1 GTP, eventually producing a total of 12 ATP per acetyl CoA (Stryer 1988, Opie 1991, Katz 1992). The TCA cycle is strictly regulated at a number of sites, beginning with the production of acetyl CoA from pyruvate by PDH. PDH activity is inhibited by both acetyl CoA and the products of the cycle, ATP and NADH. Aside from regulating the formation of acetyl CoA from pyruvate, control of the cycle is also achieved through regulation of the activities of citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase (Stryer 1988), such that the cellular ATP requirement is matched with ATP production. ATP inhibits both citrate synthase and isocitrate.
Figure 8. The Tricarboxylic Acid Cycle
Modified from Stryer 1988.

Net Reaction:
Acetyl-CoA + 2H₂O + 3NAD⁺ + FAD + ADP + Pi → 2CO₂ + CoA + 3NADH + FADH₂ + ATP
dehydrogenase, while ADP stimulates isocitrate dehydrogenase (Katz 1992; Stryer 1988).

The production of succinyl CoA via α-ketoglutarate dehydrogenase is regulated by feedback inhibition via the products of the reaction, succinyl CoA and NADH (Stryer 1988). In addition, the redox state of the mitochondrion, expressed by the NAD⁺/NADH ratio, exerts control over the cycle. Increased NAD⁺ levels, indicative of a low energy state, augment flow through the cycle by stimulating the activities of isocitrate dehydrogenase and α-ketoglutarate dehydrogenase (Stryer 1988, Opie 1991, Katz 1992). This allows ATP demand to be matched by ATP production, since augmented ATP hydrolysis to ADP under high workloads increases the oxidation of NADH to NAD⁺.

Recent studies showed that the activity of isocitrate dehydrogenase was significantly lower in 16 and 24-week old SHR compared with age-matched WKY (Torii and Ito 1990, Tokoro et al. 1995). Thus, aerobic capacity of the hypertrophied heart is depressed compared with normal hearts.

The Electron Transport Chain (ETC): The final and most productive stage in ATP generation is the electron transport chain (Figure 9), in which oxidative phosphorylation produces large amounts of ATP by converting the electron-transfer potential of NADH and FADH₂ to the phosphate-transfer potential of ATP (Stryer 1988, Katz 1992). Three enzyme complexes located on the inner mitochondrial membrane, NADH-Q reductase, cytochrome C reductase, and cytochrome C oxidase, successively transport electrons through a series of reduction-oxidation reactions, with oxygen as the final electron acceptor (Stryer 1988). This transfer is used to generate a proton gradient across the inner mitochondrial membrane, which powers the machinery of ATP synthesis.
**Figure 9.** The electron transport chain.
In addition to the NADH and FADH$_2$ generated in the TCA cycle, NADH from glycolysis also serves as an electron donor for the ETC. The inner mitochondrial membrane, however, is impermeable to charged molecules; thus, transporters are present that allow cytosolic ADP into the mitochondrial matrix and ATP out of the matrix into the intermembrane space (Tropp 1997). In contrast, cytosolic NADH is shuttled into the mitochondrial matrix via the malate-aspartate shuttle (Figure 10) (Katz 1992). The oxidation of cytosolic NADH to NAD$^+$ is coupled to the reduction of oxaloacetate to malate, which crosses the mitochondrial membrane via a carrier molecule. Malate is then reoxidized to oxaloacetate, in the process regenerating NADH. Because the inner mitochondrial membrane lacks a carrier for oxaloacetate, a series of transamination reactions occurs to restore cellular compartments to their original state. Mitochondrial oxaloacetate is converted to aspartate, for which a membrane carrier exists, via transamination with glutamate (Katz 1992). Oxaloacetate is reformed in the cytosol via transamination of aspartate with $\alpha$-ketoglutarate, which is carried to the cytosol via the same membrane carrier used by malate, also producing glutamate. Glutamate then enters the mitochondrion via the same carrier which shuttles aspartate out of the matrix.

NADH-Q reductase, the first enzyme complex in the ETC, transfers electrons from NADH to coenzyme Q (ubiquinol), forming reduced ubiquinol (QH$_2$) (Stryer 1988, Katz 1992). QH$_2$ also serves as the point of entry for FADH$_2$ into the ETC. NADH-Q reductase is the first site at which protons are pumped from the matrix to the intermembrane space, creating an electric potential, or proton-motive force (Stryer 1988). From QH$_2$, electrons are transferred via cytochrome reductase, the second proton pump, to cytochrome c, and then to O$_2$ via cytochrome oxidase, the final proton pump. Overall, the net reaction occurring in the
Figure 10. The malate-aspartate shuttle. Modified from Katz 1992.
ETC is:

\[
\text{NADH} + \frac{1}{2} \text{O}_2 + \text{H}^+ \rightarrow \text{H}_2\text{O} + \text{NAD}^+
\]

The energy generated by each site of proton transfer drives the synthesis of ATP from ADP; thus, one mole of NADH entering the ETC produces 2.5 moles of ATP, while one mole of FADH\(_2\) generates 1.5 moles of ATP (Tropp 1997). The proton-motive force is used by ATP synthase, located on the inner mitochondrial membrane, to generate ATP from ADP in two steps. Flow of H\(^+\) down its gradient through ATP synthase provides the energy required to generate a high-energy phosphate bond between ADP and Pi (Stryer 1988, Katz 1992).

Lanying et al. (1995) showed that the activity of cytochrome c oxidase was 17% lower in SHR compared with WKY, reflective of a depressed flux through the ETC. As well, basal mitochondrial ATP synthase activity is 32% greater in SHR than in WKY, but is unable to increase above this basal activity in response to increased energy demand in SHR (Das and Harris 1990). This may contribute to the progression to heart failure in the SHR.

The Pentose Phosphate Pathway

The pentose phosphate pathway (PPP, Figure 11) serves as a source of reducing equivalents, in the form of reduced nicotinamide adenine dinucleotide phosphate (NADPH), for biosynthesis of fatty acids, and to generate pentose phosphates for purine nucleotide synthesis (Eggleston and Krebs 1974, Stryer 1988, Zimmer 1992). The pathway has links with glycolysis via the transketolase and transaldolase reactions, in which pentose sugars are converted to glyceraldehyde-3-phosphate and fructose-6-phosphate.

Glucose-6-phosphate from glycogenolysis and glycolysis enters the PPP and is
Figure 11. The pentose phosphate pathway.
Modified from Stryer 1988.
converted to ribose-5-phosphate (R-5-P) in a series of reactions, with the production of 2 NADPH (Stryer 1988; Zimmer 1992). The activity of the first and rate limiting enzyme in the pathway, glucose-6-phosphate dehydrogenase (G-6-P DH), in the heart is only about 25% that of the enzyme in the liver, in which this pathway is crucial for fatty acid synthesis (Eggleston and Krebs 1974; Zimmer et al. 1990). In humans and a variety of animal species, myocardial G-6-P DH activity has been found to be significantly lower than that of 6-phosphogluconate dehydrogenase, the second enzyme in the PPP (Zimmer et al. 1984).

Induction of cardiac hypertrophy by aortic constriction in normal rats was associated with an increase in the activity of G-6-P DH and the PRPP pool compared with control (Zimmer et al. 1980c, Zimmer 1996). This suggests that hypertrophied hearts can incorporate ribose into the metabolic pathways more efficiently than normal hearts, potentially increasing the production of energy stores.

The PPP is regulated both in the short-term and in the long-term at the G-6-P DH reaction. NADPH exerts a strong inhibitory influence at physiological concentrations, such that the enzyme is expected to be 100% inhibited under normal conditions (Eggleston and Krebs 1974). Thus, control of the pathway is exerted by de-inhibition of this reaction, which has been shown to occur in the presence of AMP and oxidized glutathione (GSSG), although only GSSG was effective at physiological concentrations (Eggleston and Krebs 1974, Zimmer 1992). GSSG plays an important role in removing harmful reactive oxygen radicals. GSSG stimulation of the PPP may therefore become significant under ischemic conditions, when oxygen radicals are generated from degradation of adenine nucleotides (see Section VI). Long-term control of the PPP is governed by adrenergic stimulation, which also alters the activity of G-6-P DH. Administration of the sympathomimetic isoproterenol to rats has
been shown to increase G-6-P DH activity in a dose and time dependent manner, and that the apparent mechanism of this effect was activation of the synthesis of the enzyme (Zimmer et al. 1990). This long-term control via catecholamines would be an effective means of increasing the availability of PRPP for adenine nucleotide production when myocardial ATP levels are reduced from adrenergic stimulation.

The preceding discussion describes the production of ATP from substrate fuels, which provide the majority of ATP utilized by the heart. However, two other pathways of ATP generation are available, *de novo* synthesis and adenine nucleotide salvage, which will be detailed in the next section.

**De Novo Adenine Nucleotide Biosynthesis:**

Under normal conditions adenine nucleotide biosynthesis contributes only a minor amount of the total adenine nucleotide pool used by the heart for normal function, due to the extremely slow nature of the *de novo* synthetic pathway. However, under certain conditions, specifically in the postischemic myocardium, *de novo* synthesis becomes an important source of adenine nucleotides.

*De novo* synthesis (Figure 12) is the process by which inosine monophosphate (IMP) is generated in a step-wise manner on a phosphoribosyl backbone. PRPP is generated from R-5-P, from the PPP, at the cost of one ATP. This reaction is controlled via negative feedback inhibition of ribose phosphate pyrophosphokinase by purines and nucleotides. PRPP is involved in the first committed step of *de novo* synthesis, in which 5-phosphoribosyl-1-amine is generated using the NH$_2$ chain from glutamine via adenine phosphoribosyltransferase (APRT) (Stryer 1988). In subsequent reactions, the five
Figure 12. De novo adenine nucleotide biosynthesis.
Modified from Stryer 1988.
membered purine ring is synthesized with contributions from glycine, \(N^{10}\)-formyl-tetrahydrofolate, and glutamine, with the hydrolysis of three more ATP (Stryer 1988). In the next series of reactions, atoms 6, 1, and 2 of the purine ring are added to 5-aminoimidazole ribonucleotide to form the six-membered ring. These constituents are derived from \(\text{CO}_2\), aspartate, and \(N^{10}\)-formyltetrahydrofolate, respectively, with the hydrolysis of another ATP. The purine base of IMP is known as hypoxanthine (Hxn). Overall, \textit{de novo} synthesis of IMP consumes a total of 6 ATP, including the hydrolysis of pyrophosphate in the formation of 5-phosphoribosylamine (Stryer 1988, Opie 1991). IMP is used as a precursor in the production of AMP, from which the higher adenine nucleotides are generated. In this process, the carbonyl oxygen atom at C-6 is replaced by an amino group donated by aspartate, using guanosine triphosphate (GTP) as the energy source (Stryer 1988).

\[
\text{IMP} + \text{aspartate} + \text{GTP} \rightarrow \text{adenylosuccinate} \rightarrow \text{AMP}
\]

ATP is generated from AMP in the salvage reactions, described below.

Biosynthesis of purine nucleotides is regulated at several points via feedback inhibition by a number of compounds. The production of PRPP from R-5-P, and 5-phosphoribosyl amine from PRPP, are inhibited by high levels of IMP and AMP, such that cellular PRPP levels are tightly controlled (Stryer 1988). AMP similarly inhibits the formation of adenylosuccinate (AMP-S) from IMP.

\textbf{Adenine Nucleotide Salvage:}

Salvage of adenine nucleotides (Figure 13) involves a variety of reactions in which preformed purine bases, Hxn and xanthine (Xan), and the nucleosides adenosine (Ado) and inosine (Ino), are rapidly returned to the cellular nucleotide pool. The mechanisms of these
Figure 13. Adenine nucleotide salvage pathways. Modified from Manfredi and Holmes 1985.
reactions constitute the salvage pathways. In combination with the de novo synthesis of adenine nucleotides, the loss of purine nucleotides during normal metabolic activity is balanced by their regeneration (Manfredi and Holmes 1985). In order to better understand the balance between adenine nucleotide salvage and loss, it is necessary to understand the process of nucleotide degradation, from which salvage reactants are generated. This process is described next.

**Purine Nucleotide Degradation**

Hydrolysis of ATP via ATPase yields free energy for contractile function, ion pumps, and other biochemical processes. Degradation of ATP yields ADP, inorganic phosphate, H⁺, and energy. However, in the cell, ATP is chelated to Mg⁡⁺⁺, such that the overall degradation of ATP to ADP is actually:

\[
\text{MgATP}^{2-} + \text{H}_2\text{O} \rightarrow \text{MgADP}^- + \text{Pi}^{2-} + \text{H}^+ + \text{energy}
\]

ADP exists only transiently in the cell, as it will either be used to (1) reform ATP via CK, (2) combine with a second ADP to yield ATP and AMP via myokinase (see below), or (3) enter the mitochondria for use in oxidative metabolism (Opie 1991).

**ADP + ADP → ATP + AMP**

The Myokinase Reaction

AMP is the final of the non-diffusible products of ATP degradation, referring to the fact that ATP, ADP, and AMP are unable to cross the sarcolemma freely. AMP is degraded to Ino via two intermediates: (1) AMP to Ado and inorganic phosphate via AMP-specific 5'-nucleotidase; or (2) AMP to IMP and NH₄⁺ via AMP deaminase. Ado is then converted to Ino via adenosine deaminase, while a distinct, IMP-specific 5'-nucleotidase catalyzes the formation of Ino from IMP. Recent studies suggest that the principle route of AMP
degradation is via the action of AMP-specific 5'-nucleotidase leading to Ado (Bak and Ingwall 1994). Both H+ and inorganic phosphate inhibit the activity of 5'-nucleotidase, while AMP deaminase is inhibited by GTP. Inosine is degraded in turn via nucleoside phosphorylase to Hxn, xanthine oxidase to Xan plus NADH, and finally to urate plus NADH (Manfredi and Holmes 1985, Opie 1991). Torii and Ito (1990) showed that myocardial 5'-nucleotidase activity in 16-week old SHR was 18% lower than that of age-matched WKY.

Purine Salvage

The rate of purine salvage is at least 10-fold greater than that of de novo synthesis (Manfredi and Holmes 1985), and thus serves as the major route by which adenine nucleotide pools are maintained in the normal myocardium. The process of purine salvage involves the conversion of a purine nucleoside to the corresponding nucleotide catalyzed via a salvage enzyme (Manfredi and Holmes 1985). In the myocardium, the action of phosphoribosyl-transferases leads to the production of nucleotides from purine bases, either Hxn or adenine, and PRPP. Hxn is salvaged via hypoxanthine phosphoribosyltransferase (HPRT) in the following reversible reaction:

\[ Hxn + PRPP \rightleftharpoons IMP + PPi \]

The forward reaction, IMP production, is normally favoured. HPRT has an absolute requirement for PRPP to serve as the R-5-P donor, while IMP and inorganic pyrophosphate (PPi) exert inhibitory control (Manfredi and Holmes 1985). Adenine is salvaged by adenine phosphoribosyl-transferase (APRT) to AMP in a similar fashion:

\[ \text{adenine} + PRPP \rightleftharpoons AMP + PPi \]

As with HPRT, the equilibrium of this reaction favours the forward reaction. PRPP is the
only suitable R-5-P donor, while AMP and PPI inhibit the salvage of adenine, and the presence of Mg$^{2+}$ is absolutely required for enzyme activity (Manfredi and Holmes 1985). Thus, the salvage of Hxn and adenine is dependent on intracellular PRPP content.

Adenosine is salvaged via adenosine kinase, with the hydrolysis of one ATP. Adenosine kinase also has an absolute requirement for Mg$^{2+}$ for normal activity, presumably indicating that Mg-ATP is the necessary substrate in this reaction. Free Mg, unchelated ATP, ADP, and AMP inhibit adenosine kinase (Manfredi and Holmes 1985).
The preceding section detailed the various biochemical pathways utilized by the heart to provide sufficient energy to maintain homeostasis and normal function. Ultimately, the majority of ATP (65%) is used to provide energy for myocardial contraction. As described in Section II, ventricular hypertrophy occurs to maintain function in the face of increased workload; when workload exceeds the heart's ability to adapt, heart failure ensues. Thus, myocardial function is intimately tied to both metabolism and hypertrophy. A brief discussion of myocardial function will be presented next.

Two types of myocardial function can be distinguished; systolic and diastolic. Systolic function involves contraction of the ventricles, whereby force (pressure) is generated so that blood is ejected from the heart. Diastolic function involves the relaxation of the ventricles between contractions, which allows the chambers to fill with blood. Both types of function will be described separately.

**Systolic Function:**

There are three principal determinants of systolic function: ventricular preload, ventricular afterload, and myocardial contractility (Dalla-Volta et al. 1988, Greenberg 1989, Katz 1992). Preload is the degree of stretch of the myocardium arising from the volume of blood within the ventricle prior to ejection. The mechanism of this effect is defined by the Frank-Starling relationship (Katz 1992). With greater stretch of the myocardium, the degree of overlap of the myofibrils increases so that more force is generated with each contractile event. At a specific point, overlap reaches an optimal level, so that
force begins to decline if stretch is further increased. On a global scale, increasing end-diastolic volume leads to increased pressure generated during systole, which increases stroke volume and cardiac output. Ventricular afterload is the force encountered during contraction that resists myofibril shortening (Greenberg 1989). Thus, an increase in afterload will decrease the degree of shortening, reducing stroke volume and cardiac output. Systemic vascular resistance is the primary determinant of ventricular afterload, although blood viscosity and wall stress also contribute to afterload (Dalla-Volta et al. 1988). Thus, as systemic blood pressure increases in hypertensive individuals, such as the SHR, afterload increases dramatically. Studies in patients with both pressure and volume overload-induced hypertrophy show that ventricular function, assessed using the ejection fraction, is inversely correlated with systolic wall stress (Strauer 1984). Myocardial contractility is the potential of the muscle to do work, determined by the interaction of the myofibrils, that is independent of the effects of preload and afterload (Dalla-Volta 1988, Katz 1992). Thus, any factor that alters the formation of cross-bridges will affect contractility, and will subsequently alter cardiac output. For example, catecholamine stimulation increases intracellular calcium concentration which increases the formation of actomyosin cross-bridges and subsequently improves myocardial contractility (Perreault et al. 1993).

Ventricular hypertrophy in response to pressure-overload maintains systolic function within normal ranges until the changes become maladaptive (heart failure). Thus, studies have shown that, at 6-months of age, SHR and WKY exhibit similar indices of systolic function, including ejection fraction, stroke volume, and cardiac output (Pfeffer et al. 1976, Pfeffer et al. 1979).
Diastolic Function:

Proper filling of the ventricle during diastole ensures that normal systolic function can occur. The degree of ventricular filling is dependent, in part, on the ability of the ventricle to relax after contraction. Relaxation is determined by the removal of calcium from the cytosol at the cellular level (Swynghedauw et al. 1992), and, on a global scale, by the stiffness of the ventricle, which is dependent on the collagen content of the myocardium (Jalil et al. 1989). Left ventricular hypertrophy is associated with diastolic dysfunction (Douglas et al. 1989, Sugishita et al. 1994), even when systolic function is normal.

Calcium uptake by the sarcoplasmic reticulum (SR) is impaired in the hypertrophied rat myocardium, due to a reduction in the number of SR calcium pumps (Swynghedauw et al. 1992). As well, left ventricular collagen composition is significantly increased in rats subjected to aortic banding, leading to an increase in myocardial stiffness (Jalil et al. 1989). Honda et al. (1993) showed that the myocardial concentration of collagen in 5-month old SHR was significantly less than that of age-matched WKY, and attributed this to a proportionally greater increase in myocyte mass in the SHR. By 8-months of age, the SHR had significantly higher myocardial collagen concentrations than WKY. Dramatic increases in myocardial fibrosis occur after the age of 6-months in the SHR (Pfeffer et al. 1979, Bing et al. 1995). Together, the impaired SR calcium uptake and increased fibrosis account for the observed depression in diastolic function in hypertrophied hearts.

Because the outcome of changes in either preload, afterload, or contractility affects systolic function, a change in cardiac output cannot be attributed to a single factor unless the other two are held constant. Ventricular preload can be measured by determining end-diastolic volume or, indirectly, end-diastolic pressure. Ventricular afterload is easily
measured using systemic pressure. A variety of procedures are available to measure myocardial contractility. Several common indices of function are discussed next.

The rate of rise of LV systolic pressure, \( +\frac{dP}{dt_{\text{max}}}(\text{mm Hg/sec}) \) was among the first indices of myocardial contractility. This index is depressed in the failing heart (Katz 1992). Apart from the contractility of the myocardium, \( +\frac{dP}{dt_{\text{max}}} \) is also dependent on preload, afterload, and heart rate (Mason 1969). Increased preload augments \( +\frac{dP}{dt_{\text{max}}} \) via the Frank-Starling relationship. Elevations in afterload, as determined using systemic arterial pressure, delays the opening of the aortic valve, prolonging the period of isovolumic contraction, which increases \( +\frac{dP}{dt_{\text{max}}} \) (Mason 1969). As well, an increased heart rate, indicative of an enhanced inotropic state, also increases \( +\frac{dP}{dt_{\text{max}}} \). Myocardial relaxation can be measured using the rate of fall of LV pressure, \( -\frac{dP}{dt_{\text{max}}}(\text{mm Hg/sec}) \). In an attempt to obtain a load-independent index of myocardial contractility, the quantity \( \frac{dP}{dt/P} \), where \( P \) is LV pressure, has been calculated (Mirsky et al. 1972). The principle hemodynamic determinant of \( -\frac{dP}{dt_{\text{max}}} \) is peak LV systolic pressure or wall stress (Weisfeldt et al. 1974). These indices were utilized to assess in vivo myocardial function in this thesis.
SECTION V: LIVER and SKELETAL MUSCLE

Liver

The liver serves as the largest storage site of glycogen in the body, and is responsible for buffering fluctuations in plasma glucose. Glycogen synthesis in the liver is controlled by glycogen synthase, which is stimulated by insulin following a meal (Ganong 1993). However, the liver is also responsive to glucagon, secreted when plasma glucose concentrations fall, and catecholamines, both of which stimulate glycogen breakdown (Guyton 1981, Stryer 1988). Unlike muscle, the liver contains glucose-6-phosphatase, which converts the G-6-P generated from glycogen into glucose, enabling plasma glucose levels to rise.

Differences in systemic and liver metabolism have been observed between SHR and WKY. In response to a 12 hour fast, SHR had significantly higher plasma glucose and glucagon compared with WKY (Hörl et al. 1988). Studies have shown the SHR to be either insulin resistant (Rao 1993, Swislocki and Tsuzuki 1993) or have normal or slightly elevated sensitivity (Frontoni et al. 1992). In response to metabolic stress induced by hemorrhage, the liver of SHR showed significant alterations in adenine nucleotide concentrations, with reductions in ATP and elevations in ADP and AMP, compared with WKY (Shiino et al. 1996). This was attributed to strain differences in hepatic microcirculation. The activity of liver G-6-P DH was found to be significantly reduced in 10-week old SHR compared with age-matched WKY (Iritani et al. 1977). How this would affect the liver's response to ribose is unknown.
Skeletal Muscle

Systemic hypertension is also associated with alterations in skeletal muscle in both humans and animal models. Recent studies showed that skeletal muscle CP concentration was 30% lower in hypertensive patients than in controls (Ronquist et al. 1995). Contractile function is depressed in 6-month old SHR, and is associated with alterations in intracellular sodium and calcium (Carlsen et al. 1996). In fasted SHR, skeletal muscle glycogen concentration was 36% higher than in WKY (Hörl et al. 1988), with a 41% higher glycogen synthase activity and 57% faster rates of glycogen synthesis (Farrace et al. 1995). This corresponds with the reported shift towards more glycolytically active skeletal muscle fibers in hypertensive individuals (Bassett 1994).

The administration of compounds intended to improve myocardial tolerance to ischemia, which forms the focus of the studies reported in this thesis, may result in dramatic changes in tissue glycogen in liver and skeletal muscle. Therefore, both liver glycogen (LG) and skeletal muscle glycogen (SKM) were measured.
SECTION VI: MYOCARDIAL ISCHEMIA

The ability of the heart to maintain normal work is dependent upon the production of large amounts of ATP via oxidative phosphorylation which, in turn, is dependent on the continuous supply of substrates and oxygen to the cardiomyocytes. As shown in Section III, energy reserves are limited in the myocardium, particularly in hearts with pathologic hypertrophy, and are insufficient to sustain normal activity for more than a brief period. Therefore, any interruption in tissue perfusion, a condition known as ischemia, rapidly leads to metabolic and functional deterioration. It is essential that a distinction be made between the ischemic myocardium and the hypoxic myocardium. In hypoxic tissue, oxygen availability is reduced such that oxidative phosphorylation ceases, without any alteration in tissue perfusion. Ischemia has the additional effect of allowing harmful metabolic intermediates to accumulate in the tissue, potentially increasing damage (Naveri et al. 1987). Throughout this thesis, use of the term ischemia will refer to the condition of inadequate tissue perfusion.

Clinically, a number of conditions can produce, or are produced by, myocardial ischemia. Angina pectoris is caused by transient episodes of ischemia resulting from the partial occlusion of coronary arteries or vasospasm, such that an increased metabolic demand cannot be matched by an increased blood flow (Opie 1991, Katz 1992). Surgical and medical procedures intended to improve organ perfusion paradoxically produce transient ischemia. For example, blood flow to the heart is interrupted both during PTCA with the insertion of balloon catheters into the coronary arteries, and with aortic cross clamping during open-heart surgery. Prolonged ischemia can lead to cell necrosis, generating areas of infarction which
are akinetic and irreversibly damaged. The size of the infarcted area has been shown to be directly related to the duration of the ischemic episode (Jennings et al. 1969). Recent studies of age-matched SHR and WKY rats showed that when infarction was induced by coronary artery occlusion, hypertrophied myocardium showed a smaller degree of infarct expansion after 7 days (Morita et al. 1996). Myocardial wall stress is a primary determinant of infarct expansion; the reduced expansion in the hypertrophied group was attributed to reduced wall stress in the SHR despite hypertension.

To understand the consequences of myocardial ischemia, it is necessary to examine the metabolic alterations occurring as a result of cessation of blood flow, and to relate these to the functional changes.

Numerous studies have investigated the metabolic changes occurring as a result of myocardial ischemia. Figure 14 shows the pattern of changes in myocardial high energy phosphates, as a percentage of preischemic values, in the rat heart in response to acute global ischemia. Once blood flow ceases, myocardial oxygen concentrations are insufficient to maintain aerobic metabolism, removing oxidative phosphorylation as the primary source of ATP. As shown, ATP content declines despite the rapid degradation of CP in an effort to maintain ATP levels. The reduced ATP and G-6-P concentrations release the inhibition of PFK, promoting glycolysis, and accelerate glycogenolysis by promoting the conversion of phosphorylase b to phosphorylase a (Sobel 1974, Levitsky and Feinberg 1975, Opie 1991, Katz 1992). The increased glycolytic flux is soon halted due to the accumulation of H^+, which inhibits PFK. Degradation of adenine nucleotides continues past the AMP stage, releasing adenosine and their diffusible products into the extracellular space. These compounds are then lost upon reperfusion of the ischemic tissue, reducing the available pool
of compounds that can be used to reform adenine nucleotides.

![Graph of % Preischemic Value vs Ischemic Time (minutes)]

**Figure 14.** Changes in myocardial high energy phosphates in response to ischemia. Modified from Neely *et al.* 1973, Hearse *et al.* 1977, Kocolassides *et al.* 1996.

Loss of contractility is characteristic of myocardial ischemia. Isolated, buffer perfused rat heart studies demonstrated that the onset of global ischemia was followed by a rapid reduction in left ventricular systolic pressure, cardiac output, and coronary flow (Neely *et al.* 1973). Similar decrements in functional indices with ischemia have been noted by others (Gudbjarnason *et al.* 1970, Levitsky and Feinberg 1975, Vial *et al.* 1982, Humphrey *et al.* 1985).

**Ischemic Contracture**

Severe and prolonged ischemia can lead to irreversible cellular injury, which manifests morphologically as swollen cells, loss of mitochondrial cristae, nuclear swelling
with chromatin aggregation, disruption of the sarcolemma, and disorganization of the myofibrils (Jennings and Ganote 1974, Miura et al. 1989). In 1972, clinical observations of ischemic contracture in cardiac surgery patients were first reported (Cooley et al. 1972).

This phenomenon, in which the heart is locked in systole, arises due to the formation of rigor complexes in the sarcomere. Cross-bridge formation and movement is dependent, in part, on the availability of ATP. Cleavage of ATP to ADP and Pi by myosin ATPase provides energy for cross-bridge motion, leaving ADP bound to myosin (Opie 1991, Katz 1992).

Dissociation of the actomyosin complex also relies on ATP to dislodge the bound ADP, resetting the contractile apparatus (Katz 1992). Thus, it has been theorized that ATP depletion leads to the formation of rigor complexes, in which the dissociation of the contractile proteins is prevented, globally evident as ischemic contracture (Katz and Tada 1972). In a landmark report, Hearse et al. (1977) showed that the onset of contracture occurred when myocardial ATP had declined by approximately 50%, and that this could be delayed by interventions that increased energy supply. Recently, ³¹P-NMR studies of isolated perfused rat hearts showed that the onset of contracture occurs when anaerobic glycolysis ceases (Kingsley et al. 1991). Although the onset of contracture did not correspond to a specific concentration of myocyte ATP in this report, values were indeed approximately 50% of preischemic values.

Numerous studies have shown that hypertrophied hearts are more susceptible to ischemic injury than normal hearts. In the original description of ischemic contracture ("stone heart"), electrocardiographic evidence of left ventricular hypertrophy was seen in over half of the 13 patients identified (Cooley et al. 1972). Studies in both SHR and banded rats, and patients with aortic stenosis demonstrate that the onset of contracture and
postischemic recovery of function is significantly reduced compared with normal hearts (Sink et al. 1981, Peyton et al. 1982, Fenchel et al. 1986, Snoeckx et al. 1989, Anderson et al. 1990, Snoeckx et al. 1993). This has been attributed to depressed energy stores (Sink et al. 1981), poorer recovery of tissue perfusion (Snoeckx et al. 1989), and increased glycolytic activity resulting in increased accumulation of harmful byproducts (Anderson et al. 1990) in hypertrophied hearts compared with normal hearts.
SECTION VII: SUBSTRATE ENHANCEMENT

A variety of compounds have been utilized to enhance myocardial energy reserves in an attempt to protect the heart against ischemic injury. There are two basic means by which myocardial energy reserves can be enhanced: 1) reduction of energy utilization; and 2) elevation of energy production.

The principal means by which energy utilization can be reduced is through the use of hypothermia, in which the heart itself is cooled, as in donor hearts destined for transplantation, or the entire patient is cooled, as in CPB procedures. In both of these procedures, the heart's mechanical activity is halted with cardioplegia to further preserve energy stores. The use of cold cardioplegia therefore provides improved protection, by rapidly halting the energy consuming mechanical activity of the heart, and slowing other metabolic activities, including the action of ion pumps. A variety of cardioplegic solutions and preservation protocols have been devised in an effort to maximize preservation (Havel et al. 1991, Wheeldon et al. 1992, Ferrera et al. 1994, Fremes et al. 1995). However, these procedures are limited to surgical cases in which hearts are subjected to extreme episodes of ischemia, for example, harvested organs which undergo long periods of global ischemia. Thus, the use of cardioplegia and cold preservation is effective only acutely, and cannot be used well in advance of the ischemic episode to provide myocardial protection.

Calcium channel blockade has been employed to provide ischemic protection. Aside from decreasing Ca++ influx and reducing energy utilization, these agents have vasodilatory effects that decrease afterload and energy demand on the heart. Perfusion of isolated rat hearts with calcium channel antagonists prior to the ischemic episode significantly improved myocardial ATP and CP concentrations and recovery of function upon reperfusion, likely by

Aside from slowing the utilization of ATP using hypothermia or chemicals to reduce work, ischemic protection of the myocardium may be accomplished by enhancing myocardial energy stores. Since rapid postischemic recovery of myocardial ATP concentration is primarily dependent on the availability of AMP that can be phosphorylated to ADP and then to ATP, mechanisms to enhance this procedure attempt either to prevent AMP degradation or enhance its phosphorylation (Pasque and Wechsler 1984). Studies investigating methods to elevate energy production in the heart have incorporated a large number of compounds with the potential to enhance ATP (Pasque and Wechsler 1984). Among these are intermediates along glycolytic and TCA cycle metabolic pathways, and precursor molecules to these intermediates. Synthesis of ATP can also be enhanced by supplying the heart with important precursors in both the salvage and de novo pathways. A brief description of some of these compounds (ATP, glucose, adenosine) will be given next, although this is not intended to be a comprehensive discussion of all possible mechanisms to improve myocardial ATP. This brief discussion will be followed by an extensive review of the use of the pentose sugar ribose to improve myocardial energy reserves. Ribose was chosen since it has been shown to significantly increase the rate of de novo synthesis and improve postischemic recovery of the myocardial ATP pool, and to retain its effectiveness in the presence of drugs commonly employed in the treatment of systemic hypertension and angina (Zimmer 1996). However, studies into the use of ribose to alter metabolism and function in hypertrophied myocardium are lacking.

**Glucose**

During myocardial ischemia or hypoxia, anaerobic glycolysis serves as the primary
source of ATP. Thus, enhancement of myocardial glycogen stores should improve tolerance to metabolic stress such as ischemia. Several studies have shown this to be the case.

Elevation of MG by glucose administration has been shown to preserve function during anoxia in isolated rat hearts (Scheuer and Stezoski 1970). Administration of glucose to patients undergoing CABG surgery significantly elevated MG concentration and reduced the incidence of perioperative complications (Iyengar et al. 1976, Lolley et al. 1979). Perfusion with glucose in isolated rabbit hearts improved tolerance to ischemia, as shown by better recovery of function upon reperfusion, better preservation of ATP and CP, and decreased ultrastructural damage (Apstein et al. 1983). Addition of glucose to cardioplegic solutions enhanced ATP stores and improved functional recovery after long-term cold storage (Fremes et al. 1994). Elevations in MG were observed following glucose infusion in dogs with myocardial hypertrophy (Wittnich et al. 1986). In contrast to these reports, studies using aortic banding to induce LV hypertrophy in rats showed that postischemic functional recovery was improved when tissue glycogen content was reduced prior to no-flow ischemia (Allard et al. 1994). Recently, it was shown that high myocardial glycogen content delayed the onset of ischemic contracture due to brief low-flow ischemia, but reduced postischemic functional recovery from prolonged ischemia (Cross et al. 1996). These negative effects of increased MG were attributed to prolonged glycolysis in the high glycogen hearts, leading to greater accumulation of the end-products of glycolysis, lowering tissue pH. Na⁺/H⁺ exchange activity increased in an attempt to counter the increased H⁺ concentration, increasing intracellular Na⁺, which ultimately increases Ca²⁺ influx by reversal of the Na⁺/Ca²⁺ exchanger.

During ischemia, myocardial AMP is rapidly degraded via 5'-nucleotidase to
diffusible metabolites, primarily Ado but also Ino (Smolenski et al. 1992), that readily cross the sarcolemma, subsequently limiting AMP availability to the cell upon reperfusion. Therefore, prevention of AMP degradation or incorporation of salvage pathway intermediates in the reperfusate should elevate AMP content and ATP repletion.

*Adenosine Triphosphate (ATP)*

Attempts to improve myocardial energy stores via administration of ATP have met with limited success. Despite the fact that ATP is required in the postischemic myocardium, Reibel and Rovetto (1978) showed that perfusion of isolated rat hearts with 0.5 mM ATP prior to 30 minutes global ischemia followed by 30 minutes reperfusion did not significantly affect tissue adenine nucleotide or CP concentrations. Furthermore, perfusion with ATP did not alter postischemic functional recovery compared with control hearts. Radiolabelling studies suggest that extracellular ATP crosses the sarcolemma in a modified form, presumably as adenosine, followed by rephosphorylation to ATP (Hoffmann and Okita 1965). Due to its negative chronotropic effect on pacemaker cells and negative dromotropic effect on AV node conduction (Pelleg et al. 1990, Rankin et al. 1990), exogenous ATP is currently utilized in the treatment of paroxysmal supraventricular tachycardia (Belhassen and Viskin 1993). Thus, administration of ATP itself seems to have no role in enhancing myocardial energy reserves. However, administration of ATP together with MgCl₂ increased cardiac output and decreased myocardial oxygen demand during low flow ischemia (Chaudry 1990), suggesting that Mg-ATP has potential therapeutic benefits. As with ATP alone, Mg-ATP has potent vasodilator effects that may preclude its use. Extracellular ATP has also been shown to increase the cytosolic Ca²⁺ concentration of the myocardium by binding to sarcolemmal purine receptors, activating phospholipase C which mobilizes intracellular Ca²⁺
stores (Dubyak and El-Moatassim 1993). This may exacerbate injury of the ischemic myocardium due to Ca^{++} overload.

**Adenosine**

(i) *Inhibition of Ado transport:* Preventing the loss of Ado during ischemia should allow the heart to replete its pool of adenine nucleotides and speed recovery of ATP and normal function. Both dipyridamole and R75231, inhibitors of nucleoside transport, have been shown to inhibit nucleoside transport in the ischemic myocardium (Pasque and Wechsler 1984, Galiñanes et al. 1993). Although postischemic tissue adenosine content was elevated 13-fold when rabbit hearts were pretreated with R75231 prior to ischemia/reperfusion compared with controls, no significant improvement in the recovery of contractile function or adenine nucleotides was seen (Galiñanes et al. 1993). It was suggested that this may be evidence for compartmentalization of adenosine that prevents it being used to replenish adenine nucleotide content.

(ii) *Adenosine administration:* Rapid recovery of tissue AMP following myocardial ischemia/reperfusion should occur if the heart is supplied with the necessary compounds needed for the salvage pathways. Administration of exogenous adenosine to the ischemic/reperfused heart increases the recovery of AMP and ATP (Pasque and Wechsler 1984). The potent vasodilatory action of adenosine increases coronary blood flow, thereby enhancing tissue oxygen delivery; however, this may also contribute to reperfusion injury of the postischemic myocardium. As well, the negative chronotropic effect of adenosine (Pelleg et al. 1990) may reduce metabolic demand postischemically at the expense of function. Similar to ATP, adenosine can also bind to sarcolemmal purine receptors and activate intracellular cascades, leading to negative inotropic effects that could reduce ventricular
function (Tucker and Linden 1993). Thus, adenosine may not be appropriate for use in the postischemic heart. This compound will not be further discussed.

Ribose

The pentose sugar ribose (Figure 15) has been investigated for its use as an agent to reduce ischemic injury to the myocardium and to promote postischemic recovery. Ribose is ubiquitous in nature, comprising a portion of the nucleotide bases of DNA and RNA. In addition, ribose forms a part of the ATP molecule (Figure 16). An examination of the pentose phosphate and de novo pathways shows that ribose plays a crucial role in the synthesis of nucleotides (refer to Section III). It is this feature that led to studies into the potential use of ribose as a means of elevating myocardial energy stores.

De novo synthesis of ATP is dependent on an adequate supply of PRPP supplied via the pentose phosphate pathway. PRPP production is regulated at the conversion of G-6-P to 6-phosphogluconolactone by G-6-P DH, the rate limiting enzyme of the pentose phosphate pathway. The activity of G-6-P DH in the heart is very low compared with other organs (Glock and McLean 1954); thus, myocardial PRPP concentration is very low in the heart. More recent studies show that the rate of flux through the PPP in the rat heart is only 5% that of the liver (Pfeifer et al. 1986). Elevating myocardial PRPP should provide a mechanism by which ATP stores can be elevated. Ribose bypasses the rate limiting G-6-P DH catalyzed
step of the PPP after direct phosphorylation to R-5-P, and subsequently elevates PRPP as reported in the landmark study of Zimmer and Gerlach (1978). This increase in myocardial PRPP may potentially promote adenine nucleotide salvage.

Numerous studies have investigated the effect of ribose administration on myocardial adenine nucleotide content in non-hypertrophied hearts. Bolus intravenous injection of 100 mg/kg ribose resulted in a 4-fold acceleration in the rate of myocardial \textit{de novo} synthesis in rats (Zimmer and Gerlach 1978). In dogs, continuous IV administration of ribose following aortic cross-clamping returned myocardial ATP to 69% of preischemic concentrations after 4 hours, and to 85% after 24 hours, versus no significant repletion by 24 hours in saline-treated animals (St.Cyr \textit{et al.} 1989). These benefits were also seen in total adenine nucleotide and CP levels. Similar results were reported in rat hearts subjected to 15 minutes of coronary artery ligation, followed by continuous IV ribose, where myocardial ATP returned to preischemic concentrations within 12 hours, versus 72 hours in control rats (Zimmer and Ibel 1984). The depletion of myocardial ATP in rats due to administration of the synthetic catecholamine isoproterenol was prevented by continuous (24 hours) IV infusion of 200 mg/kg/hr ribose, due to a significant elevation in the PRPP pool (Zimmer \textit{et al.} 1980b; Zimmer and Ibel 1983). Assuming that ribose exerts its effects via the pentose phosphate pathway, analysis of the quantity of ribose administered in these experiments was sufficient to account for the improvements in metabolite levels. In patients with documented coronary artery disease (CAD) of at least one main vessel, long-term oral ribose administration significantly improved exercise tolerance and delayed the onset of angina compared with those receiving placebo (Pliml \textit{et al.} 1992). Although tissue metabolite concentrations were not measured in these subjects, the large body of evidence from animal studies suggests that
these patients benefited from an elevation in myocardial energy stores due to ribose.

Administration of oral ribose to patients with myoadenylate deaminase deficiency, in which skeletal muscles fatigue rapidly upon exertion due to the loss of adenine nucleotides, has been shown to reduce postexertional pain (Zöllner et al. 1986, Wagner et al. 1991). Furthermore, intramuscular injection of ribose elevated liver glycogen concentration by 45% within 60 minutes and 143% within 120 minutes in fasted mice (Naitô 1944). The effects of ribose administration on liver and skeletal muscle glycogen in rats with systemic hypertension is unknown.

Ribose administration has also been shown to have an impact on myocardial function. Continuous IV infusion of high-dose ribose (450 mg/kg/hr) for 12 hours in rats treated with isoproterenol further enhanced left ventricular contractility, assessed using the rate of development of pressure (+dP/dt\text{\text{max}}), compared with those receiving isoproterenol alone (Zimmer 1982). This functional improvement was not due to a change in heart rate. However, this study showed that ribose alone produced no inotropic or chronotropic effects. Postischemic recovery of LV function in isolated rat hearts was significantly improved by perfusion with ribose during the pre- and postischemic periods (Pasque et al. 1982). Ribose treated hearts also had higher tissue ATP concentration compared with control hearts (Pasque et al. 1982).

Clearly, ribose plays a significant role in myocardial metabolism, which can be harnessed to protect the heart during periods of metabolic stress. For patients scheduled for procedures such as CABG and PTCA, administration of ribose prior to the intervention could significantly reduce the risk of injury to the heart. However, studies into the efficacy of ribose pretreatment and the duration of its beneficial effects are lacking. The impact of the
route of administration of ribose has never been investigated. Furthermore, in the high risk hypertrophied heart, where the benefits of ATP elevation could be even more important, studies into the effects of ribose administration are lacking. This thesis details investigations into these areas.
CHAPTER TWO

HYPOTHESES
To date, investigations into the cardioprotective effects of ribose have utilized long-term, continuous administration to bring about significant metabolic effects. Little is known about the metabolic effects of bolus ribose administration on the heart and other tissues, or the efficacy of different routes of administration. As well, no studies into the potential use of ribose in a model of chronic systemic hypertension with concomitant myocardial hypertrophy have been conducted. To address these issues, the following hypotheses were investigated.

1. Advance bolus administration of D-ribose will elevate myocardial energy stores and improve tolerance to a subsequent episode of global ischemia. D-ribose will also elevate glycogen stores in skeletal muscle and liver.

2. Administration of D-ribose via intravenous and intramuscular routes will have similar effects.

3. The presence of inhalation anesthetic during advance bolus administration of D-ribose will not alter its efficacy.

4. In the presence of chronic hypertension and myocardial hypertrophy, advance bolus administration of D-ribose will elevate myocardial energy stores and improve myocardial function, and will improve myocardial tolerance to global ischemia. Skeletal muscle and liver glycogen stores will also be elevated.
CHAPTER THREE

MATERIALS and METHODS
Animal Care

All experiments were conducted using male rats, either the Sprague-Dawley strain for studies in normal hearts, or Spontaneously Hypertensive Rats (SHR) for studies of myocardial hypertrophy. All animals were supplied by the Charles River Breeding Laboratories as pure strain animals. Upon arrival at the animal facility, the rats were transferred to plastic cages, and were housed in pairs with free access to food and water. All animals were maintained under identical conditions with a 12 hour light-dark cycle, and were treated in accordance with the guidelines of the Canadian Council on Animal Care. The rats were allowed at least one week to acclimatize to the new facilities before experiments were performed.

Two general studies were performed. The initial study investigated the metabolic benefits of ribose administration on Sprague-Dawley rats, with respect to the impact of the route of administration and inhalation anesthetics. The second study investigated the effects of ribose administration on metabolism and myocardial function in SHR. A description of the specific protocols for both studies is given next.

Study Protocol

Twelve hours after the specific pretreatment protocol (see Page 72), the rats were returned to the laboratory for the acute study of metabolism and myocardial function.

Acute Protocol

Rats were anesthetized with halothane to an appropriate plane, as determined by the abolition of all pain reflexes. Anesthesia was maintained with halothane and an oxygen source. A transthoracic intracardiac blood sample was taken from the left ventricle using a
22 gauge needle, and immediately analyzed for blood glucose levels using a glucose oxidase/peroxidase reaction sequence via the "Accu-Chek" chemstrip method (Boehringer Mannheim Ltd., Laval, Que.), and for blood gases (PaO₂ and PaCO₂) and acid-base status (pH, HCO₃⁻, base excess) using an ABL30 Acid-Base Analyzer (Radiometer, Copenhagen, Denmark). A midline sternotomy and laparotomy was performed, the heart was exposed, and in vivo left (LV) and right (RV) ventricular, and liver biopsies were taken using the freeze-clamp technique (Belanger et al. 1992), and immersed in liquid nitrogen. A skeletal muscle (SKM) biopsy of the semimembranosus was also taken using the same procedure. This muscle exhibits high glycolytic activity, and contains both fast and slow twitch fibers. The heart was immediately excised and the time noted as the onset of global ischemia.

Myocardial tolerance to ischemia was assessed by determining the time to the development of ischemic contracture (TIC), as noted below.

**Ischemic Tolerance:** A compliant latex balloon connected to a fluid-filled pressure transducer was inserted into the LV through the first biopsy site, sutured in place, and connected to a pressure monitor (Model 413, Tektronix, Beaverton, Oregon). The balloon was inflated with saline to achieve a baseline pressure of 10 mm Hg. Throughout the assessment, the heart was submerged in a 37°C substrate-free Krebs-Henseleit solution, composed of the following compounds (mM): NaCl 120.0, KCl 5.6, CaCl₂ 1.2, MgSO₄ 1.3, NaH₂PO₄ 1.17, NaHCO₃ 25.0, pH 7.4. Balloon pressure was continuously monitored to determine the time of the onset (TICO, minutes), defined as a 2 mm Hg rise in intraventricular pressure, and peak (TICP, minutes), defined as pressure plateau, of ischemic contracture (Hearse et al. 1977). A second RV biopsy ("early ischemia") was taken after 3 minutes of global ischemia. All biopsies were freeze-dried for 24 hours (Lyph-Lock 6 Litre Benchtop
Model 77520, Labconco, Kansas City, MS) and stored at -80°C until analysis.

**Biochemical Analyses:** Tissues were processed as detailed in Appendix I. Myocardial samples were analyzed for individual adenine nucleotides (ATP, ADP, AMP), total adenine nucleotides \((TAN = ATP + ADP + AMP)\), creatine phosphate (CP), lactate, and glycogen concentrations. SKM and liver samples were analyzed for glycogen only. Metabolite values are expressed as \(\mu\)moles/g dry wt. Adenine nucleotide concentrations were determined by high performance liquid chromatography (HPLC) using the method of Smolenski et al. (1990). Tissue CP, lactate, and glycogen concentrations were determined using an enzymatic fluorometric technique (Passoneau and Lowry 1993).

**Myocardial Function Studies:** Functional assessment was performed on 6 month old SHR after IV pretreatment according to the awake protocol as described later. The rats were anesthetized with 100 mg/kg intraperitoneal thiobutabarbital (INACTIN, Research Biochemicals International, Natick, MD). The neck, chest, and right inner thigh were shaved after the rat was quiescent. Once the appropriate plane of anesthesia was obtained, determined by the abolition of all pain reflexes, the trachea was surgically exposed and a 16 gauge blunt needle was inserted and secured with suture material to maintain an open airway. Rats were ventilated with room air using a rodent ventilator (Kent model RSP1002, Kent Scientific Corp., Litchfield, CT). Total time from the initial incision to intubation was approximately 60 seconds. The tracheostomy incision was then extended laterally to expose the right carotid artery, which was cannulated with a length of PE50 tubing and sutured in place. The right femoral artery was similarly exposed and cannulated with PE50 tubing. One hundred IU heparin (Hepalean) was administered via the arterial cannula to prevent coagulation, after which a blood sample was obtained for blood gas analysis. Ventilation was
adjusted as required to ensure normal blood gas and acid-base parameters. Once an adequate arterial pressure trace was obtained, the carotid artery cannula was advanced through the aortic valve into the left ventricle to record intraventricular pressures. Blood pressures and heart rate were continuously monitored using the Biopac Systems model MP100 and AcqKnowledge software (Biopac Systems Inc., Goleta, CA) at a sampling rate of 250 samples/second. Functional recordings were made by saving a 10 second window of data every 5 minutes. The LV pressure trace at each recording interval was duplicated and

![Figure 17. Sample function trace from male Sprague-Dawley rat.](image-url)

differentiated with respect to time to yield the rate of pressure development (dP/dt, mm Hg/sec). A sample tracing from a Sprague-Dawley rat, including LV and femoral artery pressures and LVdP/dt, is shown in Figure 17.

Values for LV systolic (LVSP) and diastolic (LVDP) pressures, and the maximal rates of pressure rise (+dP/dt_max, contractility) and fall (-dP/dt_max, relaxation) and heart rate were then obtained. LV contractile function was assessed using LV developed pressure (DP = LVSP - LVDP, mm Hg), "normalized" contractility (dP/dt/P = +dP/dt_max / LVSP, sec⁻¹),
and the pressure-rate product (DP x HR, mm Hg/min) (Clarke et al. 1987, Katz 1992). Two consecutive, reproducible traces yielding similar values for +dP/dt_max were taken as the LV function from which all indices were calculated.

**Pretreatment Protocol**

To determine the duration of exposure to bolus ribose administration that produced the optimal metabolic benefits, studies of the long-term effects of ribose administration on tissue metabolism were conducted on Sprague-Dawley rats. Details of the effect of the duration of exposure to ribose are given in Appendix II.

**Ribose Administration - Route of Administration**

Sprague Dawley rats were randomly allocated to either control or experimental groups and either IM (saline n=17, ribose n=17) or IV (saline n=10, ribose n=9) routes of administration. On the day of pretreatment, the rats were marked for identification and weighed to the nearest gram. The ribose solution was prepared by dissolving 25.0 g D-ribose (Eastman Kodak Co., Rochester, NY) in approximately 20 ml sterile 0.9% saline. This was filtered using 0.45 µm syringe filters (Gelman Sciences, Ann Arbor, MI) and brought up to a total volume of 500 ml with 0.9% saline. This solution was maintained at 12°C prior to use. Rats were brought from the animal facility to the laboratory and allowed to settle for at least 30 minutes prior to administration of solutions. Rats in the experimental group were injected with 100 mg/kg of a 50 mg/ml solution of D-ribose. The rats were placed on a clean towel, and securely wrapped to prevent movement. IV injections were given in the lateral caudal (tail) veins using sterile 30 gauge needles and 1 cc syringe. IM injections were given in
alternate posterior thigh muscles using a sterile 30 gauge needle and 1 cc syringe. Twelve hours following the first injection, all animals received an identical second injection while anesthetized. The rats were returned to the animal facility after each administration.

**Ribose Administration - Role of Inhalation Anesthetic on Route of Administration**

Sprague-Dawley rats were randomly allocated to control and experimental groups. Rats in the experimental group received 100 mg/kg ribose (IV n=3, IM n=6), while control rats (n=5) received equivolume normal (0.9%) saline. Animals were prepared as before. Ribose solution was prepared as described previously. Prior to injection, the rats were placed in a sealed chamber saturated with the inhalation anesthetic halothane (Halothane B.P., MTC Pharmaceuticals, Cambridge, Ont.). Once asleep, injections were given as described previously. Each animal was allowed to recover fully from the anesthetic before being returned to the animal facility.

**Ribose Administration - Impact of Pathology (Hypertension and Myocardial Hypertrophy)**

Six month old SHR were randomly allocated to control and experimental groups. All pretreatment injections were given IV, with the experimental group receiving 100 mg/kg ribose (n=12), and controls receiving equivolume normal (0.9%) saline (n=11). Ribose solution was prepared as before. Twelve hours following the second pretreatment injection, the rats were prepared for the function study as described previously. After recording between 5 and 10 intraventricular traces, the heart was exposed via midline sternotomy, and *in vivo* LV, RV, SKM, and liver biopsies were taken as previously described. The heart was then assessed for ischemic tolerance using the TIC model. An additional RV biopsy was
taken after 6 minutes of global ischemia.

**Statistical Analysis**

All data are expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using the Statistical Analysis System (SAS) Program (Release 6.10, SAS Institute, Cary, NC). Differences in measured parameters in the awake study groups across treatments (saline vs. ribose) and routes of administration (IM vs. IV) were assessed using two-way analysis of variance (ANOVA). Differences in ischemic tolerance (TICo, TICp) and tissue metabolites among treatment groups in the anesthetized study were assessed using one-way ANOVA with Scheffe's test post hoc. The functional responses of saline vs. ribose treated normal and hypertrophied hearts were assessed using Student's t-test. Statistical significance was accepted at p≤0.05.
CHAPTER FOUR

RESULTS
I: Effect of Route of Administration

To date, all studies into the cardioprotective effects of ribose have utilized intravenous administration, while other, clinically relevant routes of administration have been neglected. Thus, IV versus IM routes of administration were compared. Based on preliminary studies to determine the optimal time course of exposure to ribose that provided the best protection (Appendix II, Table 4), animals were studied 12-hours following the second pretreatment injection. A dose of 100 mg/kg ribose was utilized in all studies.

Table 1 shows in vivo LV metabolites from both pretreatment groups (ribose, saline) for both routes of administration (IV, IM). In the IM group, CP was significantly lower (p<0.05) in the ribose group compared with saline. This was not seen in the IV group. Neither IV nor IM ribose produced any significant elevation in MG or AnER compared with saline controls.

Table 1. In vivo left ventricular ATP, total adenine nucleotides (TAN), CP, myocardial glycogen (MG), and AnER concentrations for Sprague-Dawley rats pretreated with either ribose or saline via intravenous (IV) or intramuscular (IM) routes. Values are μmoles/g dry wt. (mean±SEM).

<table>
<thead>
<tr>
<th></th>
<th>IV</th>
<th></th>
<th>IM</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>saline</td>
<td>ribose</td>
<td>saline</td>
<td>ribose</td>
</tr>
<tr>
<td>ATP</td>
<td>16.4±1.6</td>
<td>17.0±0.8</td>
<td>17.6±0.6</td>
<td>19.4±1.3</td>
</tr>
<tr>
<td>TAN</td>
<td>24.9±1.9</td>
<td>25.7±1.3</td>
<td>24.1±1.4</td>
<td>28.4±2.1</td>
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<tr>
<td>CP</td>
<td>7.8±2.4</td>
<td>11.0±3.2</td>
<td>17.3±1.8</td>
<td>11.4±1.8*</td>
</tr>
<tr>
<td>MG</td>
<td>121.2±25.1</td>
<td>139.5±31.6</td>
<td>129.9±26.9</td>
<td>135.5±26.5</td>
</tr>
<tr>
<td>AnER</td>
<td>145.4±25.7</td>
<td>167.5±34.1</td>
<td>164.8±27.0</td>
<td>166.3±25.1</td>
</tr>
</tbody>
</table>

(TAN = ATP + ADP + AMP; AnER = ATP + CP + MG), * p≤0.05 vs. saline
Table 2 shows in vivo RV metabolites from both ribose and saline pretreatment groups and the effect of different routes of administration (IV, IM). Compared with saline controls, IV ribose significantly (p<0.05) elevated MG by 41% and AnER by 34%. IM ribose pretreatment did not produce any elevation in these metabolites. CP was elevated by 48% in the IV ribose group, although this did not attain statistical significance, while IM ribose, as in the LV, resulted in significantly (p<0.05) lower CP than saline control.

Table 2. In vivo right ventricular ATP, total adenine nucleotides (TAN), CP, myocardial glycogen (MG), and AnER concentrations for Sprague-Dawley rats pretreated with either ribose or saline via intravenous (IV) or intramuscular (IM) routes. Values are μmoles/g dry wt. (mean±SEM).

<table>
<thead>
<tr>
<th></th>
<th>IV</th>
<th></th>
<th>IM</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>saline</td>
<td>ribose</td>
<td>saline</td>
<td>ribose</td>
</tr>
<tr>
<td>ATP</td>
<td>16.4±1.1</td>
<td>16.8±1.1</td>
<td>19.5±1.1</td>
<td>17.0±1.0</td>
</tr>
<tr>
<td>TAN</td>
<td>27.2±1.6</td>
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<td>24.9±1.0</td>
<td>25.8±0.9</td>
</tr>
<tr>
<td>CP</td>
<td>9.9±3.0</td>
<td>14.7±3.3</td>
<td>21.3±2.3</td>
<td>13.3±2.6*</td>
</tr>
<tr>
<td>MG</td>
<td>61.2±6.9</td>
<td>86.0±4.6*</td>
<td>72.1±4.9</td>
<td>76.0±5.5</td>
</tr>
<tr>
<td>AnER</td>
<td>87.6±8.5</td>
<td>117.5±7.7*</td>
<td>112.9±6.9</td>
<td>106.2±7.4*</td>
</tr>
</tbody>
</table>

(TAN = ATP + ADP + AMP; AnER = ATP + CP + MG). *p<0.05 vs. saline

Figures 18 and 19 show the rates of consumption of right ventricular ATP, TAN, CP, AnER, and MG, and the rate of production of lactate, during the first 3 minutes of global myocardial ischemia from IV and IM pretreated rats. Although these differences did not achieve statistical significance, both IV and IM ribose pretreated hearts had 56% lower rates of ATP consumption compared with saline. Rates of CP consumption were not statistically different in either the IV or IM ribose groups, although the 56% lower rate in the IM ribose group showed a trend towards significance (p=0.07). Compared with saline controls, the net
Figure 18. Rates of consumption (+) or production (-) of RV metabolites over 3 minutes global ischemia from Sprague Dawley rats pretreated with either IV saline or IV ribose. Values are mean±SEM. *p<0.05 vs saline.
Figure 19. Rates of consumption (+) or production (-) of RV metabolites over 3 minutes global ischemia from Sprague-Dawley rats pretreated with either IM saline or IM ribose. Values are mean±SEM. ‡p=0.07 vs. saline
consumption of TAN was significantly (p<0.05) lower in the IV ribose pretreatment group, while no significant difference was seen with IM ribose pretreatment. No significant differences in the net rates of MG or AnER consumption were seen with either IV or IM ribose pretreatment.

These metabolic profiles resulted in significant improvements in tolerance to global myocardial ischemia in both groups of ribose pretreated hearts. Figures 20 and 21 show values for TICo and TICp for IM and IV pretreated rats, respectively. IV ribose pretreatment significantly (p<0.05) prolonged both TICo and TICp compared with saline pretreatment, by 25% and 22%, respectively. Administration of IM ribose delayed the onset of ischemic contracture by 15% (p<0.05) and the time to peak contracture by 10% compared with saline pretreatment. Despite these differences in the percent improvement versus saline control, there were no significant differences in either TICo or TICp between the different routes of administration.

Values for in vivo liver and skeletal muscle glycogen are shown in Table 3. There were no significant differences in either metabolite between saline and ribose groups, or between IM and IV pretreatment routes.

<table>
<thead>
<tr>
<th></th>
<th>IV saline</th>
<th>IV ribose</th>
<th>IM saline</th>
<th>IM ribose</th>
</tr>
</thead>
<tbody>
<tr>
<td>LG</td>
<td>919.9±90.0</td>
<td>860.9±86.2</td>
<td>778.6±36.8</td>
<td>845.3±55.6</td>
</tr>
<tr>
<td>SKM</td>
<td>158.9±6.4</td>
<td>157.8±6.2</td>
<td>146.8±9.5</td>
<td>159.3±7.8</td>
</tr>
</tbody>
</table>

Table 3. In vivo liver (LG) and skeletal muscle (SKM) glycogen for Sprague-Dawley rats pretreated with either ribose or saline via intravenous (IV) or intramuscular (IM) routes. Values are μmoles/g dry wt. (mean±SEM).
Figure 20. Time to ischemic contracture onset (TICO) and peak (TICp) from Sprague-Dawley rats pretreated with either IV saline or ribose. Values are mean±SEM. * p≤0.05 vs. saline.
Figure 2. Time to ischemic contracture onset (TICO) and peak (TICp) from Sprague-Dawley rats pretreated with either IM saline or ribose. Values are mean±SEM. * p≤0.05 vs. saline.
II: Administration of Ribose in the Presence of Inhalation Anesthesia

Male Sprague-Dawley rats were subjected to pretreatment with either saline or ribose in the presence of halothane to assess the impact of inhalation anesthesia on the metabolic effects of substrate enhancement. Table 4 shows in vivo LV metabolites from both ribose and saline pretreatment groups and routes of administration (IV, IM). There were no significant differences among the groups, although IM ribose pretreated rats showed a statistical trend \((p=0.09)\) toward higher CP concentrations than either IV ribose or saline controls.

**Table 4.** In vivo left ventricular ATP, total adenine nucleotides (TAN), CP, myocardial glycogen (MG), and AnER concentrations for Sprague-Dawley rats pretreated with either ribose or saline via intravenous (IV) or intramuscular (IM) routes in the presence of halothane anesthesia. Values are µmoles/g dry wt. (mean±SEM).

<table>
<thead>
<tr>
<th></th>
<th>IV/IM saline</th>
<th>IV ribose</th>
<th>IM ribose</th>
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<tbody>
<tr>
<td>ATP</td>
<td>11.4±1.3</td>
<td>14.0±0.8</td>
<td>14.1±0.7</td>
</tr>
<tr>
<td>TAN</td>
<td>23.8±3.1</td>
<td>26.1±0.6</td>
<td>25.6±0.5</td>
</tr>
<tr>
<td>CP</td>
<td>0.6±0.3</td>
<td>1.9±1.0</td>
<td>3.5±1.1*</td>
</tr>
<tr>
<td>MG</td>
<td>98.1±30.2</td>
<td>43.7±10.2</td>
<td>37.6±12.6</td>
</tr>
<tr>
<td>AnER</td>
<td>110.1±29.3</td>
<td>59.6±9.7</td>
<td>55.1±13.9</td>
</tr>
</tbody>
</table>

\((\text{TAN} = \text{ATP} + \text{ADP} + \text{AMP}; \ \text{AnER} = \text{ATP} + \text{CP} + \text{MG})\), \# \(p=0.09\) vs. others

Table 5 shows in vivo RV metabolites from both pretreatment groups (ribose, saline) and routes of administration (IV, IM). There were no significant differences in any metabolite among the different groups.
Table 5. *In vivo* right ventricular ATP, total adenine nucleotides (TAN), CP, myocardial glycogen (MG), and AnER concentrations for Sprague-Dawley rats pretreated with either ribose or saline via intravenous (IV) or intramuscular (IM) routes in the presence of halothane anesthesia. Values are μmoles/g dry wt. (mean±SEM).

<table>
<thead>
<tr>
<th></th>
<th>IV/IM saline</th>
<th>IV ribose</th>
<th>IM ribose</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>14.4±0.5</td>
<td>16.5±0.2</td>
<td>13.0±1.0</td>
</tr>
<tr>
<td>TAN</td>
<td>25.0±0.6</td>
<td>28.2±0.6</td>
<td>24.6±0.9</td>
</tr>
<tr>
<td>CP</td>
<td>1.9±0.3</td>
<td>1.4±0.2</td>
<td>1.9±0.9</td>
</tr>
<tr>
<td>MG</td>
<td>41.7±8.6</td>
<td>50.1±5.0</td>
<td>44.3±10.5</td>
</tr>
<tr>
<td>AnER</td>
<td>58.0±8.8</td>
<td>67.9±5.0</td>
<td>59.1±11.6</td>
</tr>
</tbody>
</table>

(TAN = ATP + ADP + AMP; AnER = ATP + CP + MG)

Figure 22 shows the rates of consumption of right ventricular ATP, TAN, CP, AnER, and MG, and the rate of production of lactate, during the first 3 minutes of global myocardial ischemia from saline and ribose pretreated rats. Although there were no significant differences among the groups, the ribose pretreated hearts actually showed 40% greater net rates of consumption of CP and 4 to 6 fold greater net rates of consumption of total adenine nucleotides compared with saline controls.

When compared with the previous study, the increased net rates of consumption in ribose pretreated hearts appeared to have a negative impact on myocardial tolerance to global ischemia. Figure 23 shows values for TICo and TICp for IM and IV pretreated rats. Rather than improving tolerance to ischemia, administration of ribose IV did not significantly delay either the onset or peak of ischemic contracture compared with saline pretreatment. TICo was significantly (p≤0.05) shorter in the IM ribose group compared with both saline and IV ribose groups, by 61% and 50%, respectively. TICp was also shorter in the IM ribose group (17.5±4.7 minutes) compared with either saline (22.9±1.2 minutes) or IV ribose (21.2±6.5...
Figure 22. Rates of consumption (+) and production (-) of RV metabolites over 3 minutes global ischemia from Sprague Dawley rats pretreated with saline, IV ribose, or IM ribose in the presence of halothane. Values are mean±SEM.
Figure 23. Time to ischemic contracture onset (TICO) and peak (TICp) from Sprague-Dawley rats pretreated with saline, IV ribose, or IM ribose in the presence of halothane. Values are mean±SEM. * p<0.05 vs. others
minutes) pretreated rats (NS).

Values for \textit{in vivo} liver and skeletal muscle glycogen are shown in Table 6. Liver glycogen was significantly ($p \leq 0.05$) elevated in the IV ribose pretreated rats compared with either saline or IM ribose pretreated groups. There were no significant differences in skeletal muscle glycogen among saline and ribose pretreatment groups.

\textbf{Table 6.} \textit{In vivo} liver (LG) and skeletal muscle (SKM) glycogen for Sprague-Dawley rats pretreated with either ribose or saline via intravenous (IV) or intramuscular (IM) routes in the presence of halothane anesthesia. Values are $\mu$moles/g dry wt. (mean±SEM).

<table>
<thead>
<tr>
<th></th>
<th>IV/IM saline</th>
<th>IV ribose</th>
<th>IM ribose</th>
</tr>
</thead>
<tbody>
<tr>
<td>LG</td>
<td>261.5±70.5</td>
<td>592.5±119.1*</td>
<td>237.8±44.3</td>
</tr>
<tr>
<td>SKM</td>
<td>150.1±11.9</td>
<td>181.9±13.8</td>
<td>170.2±6.4</td>
</tr>
</tbody>
</table>

*$p \leq 0.05$ vs others.

\textbf{III: Comparison of Saline Pretreated Groups}

Metabolic profiles and TIC data from rats pretreated with saline in the presence of halothane, and via IV and IM routes of administration, were compared to determine whether inhalation anesthesia alone accounted for the effects seen in part II.

Figures 24 and 25 show \textit{in vivo} metabolites from the left and right ventricles, respectively. Significant differences were seen among the three groups, with rats exposed to halothane during pretreatment having lower metabolite concentrations than those not exposed to anesthesia during pretreatment. Despite these \textit{in vivo} metabolic differences, there were no
Figure 24. *In vivo* left ventricular metabolites from Sprague-Dawley rats pretreated with saline either in the presence or absence of halothane. Values are mean±SEM. * p≤0.05 vs. others.
Figure 25. In vivo right ventricular metabolites from Sprague-Dawley rats pretreated with saline either in the presence or absence of halothane. Values are mean±SEM. * p<0.05 vs. others, † p<0.05 vs. IM.
Figure 26. Time to ischemic contracture onset (TCo) and peak (T Cp) from Sprague-Dawley rats pretreated with saline either in the presence or absence of halothane. Values are mean±SEM.
significant differences in either TICo or TICp among the three groups (Figure 26).

IV: Ribose Administration in a Pathologic State

Chronic systemic hypertension and myocardial hypertrophy are associated with dramatic alterations in metabolism. Therefore, the metabolic effects of ribose administration were studied using Spontaneously Hypertensive Rats to model essential human hypertension and left ventricular hypertrophy. Based on the previous results, only the intravenous route of administration was employed, utilizing the same injection schedule that produced improvements in Sprague-Dawley rats. All animals were mechanically ventilated to maintain normal blood gas and acid base status. Ischemic RV biopsies were taken after 6 minutes of global ischemia rather than 3 minutes as in the previous studies, to investigate the effects of a greater metabolic stress on the consumption of energy stores. There was no significant difference in systemic blood pressures (Table 9) or heart to body weight ratios (mg/g) between ribose (3.8±0.1) and saline (3.8±0.1) pretreated rats. Both of these parameters confirm the presence of systemic hypertension and myocardial hypertrophy.

Table 7 shows in vivo LV and RV metabolites from both ribose and saline pretreatment groups for 6 month old SHR. There were no significant differences between groups.
Table 7. *In vivo* left and right ventricular ATP, total adenine nucleotides (TAN), CP, myocardial glycogen (MG), and AnER concentrations for SHR rats pretreated IV with either saline or ribose. Values are μmoles/g dry wt. (mean±SEM).

<table>
<thead>
<tr>
<th></th>
<th>LV</th>
<th>RV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>saline</td>
<td>ribose</td>
</tr>
<tr>
<td>ATP</td>
<td>16.6±0.5</td>
<td>17.0±0.5</td>
</tr>
<tr>
<td>TAN</td>
<td>25.5±0.7</td>
<td>25.4±0.5</td>
</tr>
<tr>
<td>CP</td>
<td>13.6±1.4</td>
<td>13.0±1.3</td>
</tr>
<tr>
<td>MG</td>
<td>153.6±10.1</td>
<td>141.0±5.2</td>
</tr>
<tr>
<td>AnER</td>
<td>180.9±10.2</td>
<td>171.0±5.9</td>
</tr>
</tbody>
</table>

(TAN = ATP + ADP + AMP; AnER = ATP + CP + MG)

Figure 27 shows the rates of consumption of right ventricular ATP, TAN, CP, AnER, and MG, and the rate of production of lactate, during the first 6 minutes of global myocardial ischemia from ribose and saline pretreated SHR. There were no significant differences between saline and ribose pretreatment groups in the net rates of consumption of any metabolite. However, production of lactate was 33% greater in ribose pretreated SHR than in saline controls (p<0.05), associated with a 75% greater rate of MG consumption (NS).

These metabolic profiles did not result in significant improvements in myocardial tolerance to global ischemia in ribose pretreated hearts. Figure 28 shows values for TICo and TICp for IV pretreated SHR rats.

Values for *in vivo* liver and skeletal muscle glycogen are shown in Table 8. There were no significant differences in either metabolite between saline and ribose groups.
Figure 27. Rates of consumption (+) and production (-) of RV metabolites over 6 minutes global ischemia from SHR pretreated IV with saline or ribose. Values are mean±SEM. * p≤0.05 vs. saline.
Figure 28. Time to ischemic contracture onset (TICo) and peak (TICp) from SHR rats pretreated IV with either saline or ribose. Values are mean±SEM.
Table 8. *In vivo* liver (LG) and skeletal muscle (SKM) glycogen from SHR pretreated IV with either saline or ribose. Values are μmoles/g dry wt. (mean±SEM).

<table>
<thead>
<tr>
<th></th>
<th>saline</th>
<th>ribose</th>
</tr>
</thead>
<tbody>
<tr>
<td>LG</td>
<td>553.9±137.0</td>
<td>554.6±90.5</td>
</tr>
<tr>
<td>SKM</td>
<td>128.4±14.2</td>
<td>145.4±11.4</td>
</tr>
</tbody>
</table>

V. Effects of Ribose Administration on Myocardial Function in the SHR

Intraventricular pressures were used to derive various functional indices in SHR pretreated with either IV ribose or saline. The data presented below are from those animals in which appropriate hypertensive pressures were obtained. There were no significant differences in *in vivo* heart rates, arterial pressures, or LV pressures between pretreatment groups (Table 9).

Table 9. *In vivo* heart rate (HR), arterial systolic (SBP) and diastolic (DBP) pressures, and left ventricular systolic (LVSP) and diastolic (LVDP) pressures from SHR pretreated IV with either saline or ribose. Values are mean±SEM.

<table>
<thead>
<tr>
<th></th>
<th>HR (bpm)</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>LVSP (mm Hg)</th>
<th>LVDP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>saline</td>
<td>305.6±8.7</td>
<td>203.8±12.4</td>
<td>125.4±9.4</td>
<td>196.1±7.8</td>
<td>-3.1±5.7</td>
</tr>
<tr>
<td>ribose</td>
<td>347.0±18.9</td>
<td>234.2±9.9</td>
<td>137.5±6.2</td>
<td>216.5±8.1</td>
<td>-5.6±4.0</td>
</tr>
</tbody>
</table>

Figures 29 and 30 show values for indices of ventricular performance between saline and ribose pretreatment groups. Despite no differences in *in vivo* hemodynamics between groups, measures of LV contractile performance in the SHR were significantly elevated by ribose
pretreatment. Compared with saline controls, LV +dP/dt_{max} was elevated by 25% (p≤0.05), while normalized contractility (+dP/dt_{max}/LVSP) was 13% higher in the ribose pretreated group (p≤0.05). No significant differences were seen in -dP/dt_{max} or pressure-rate product between the pretreatment groups, although both were increased by ribose pretreatment, by 44% and 25%, respectively.
Figure 29. *In vivo* LV contractile index (+dP/dt<sub>max</sub>) and relaxation index (-dP/dt<sub>max</sub>) from saline and ribose pretreated SHR rats. Values are mean±SEM. * p≤0.05 vs. saline.
Figure 30. *In vivo* normalized LV contractile index (\(+\frac{dP}{dt_{\text{max}}} / \text{LVSP}\)) and LV pressure-rate product from saline and ribose pretreated SHR rats. Values are mean±SEM. * p≤0.05 vs. saline.
CHAPTER FIVE

DISCUSSION
Choice of Model

The ultimate goal of any medical research is to generate data that may eventually be useful in addressing clinical issues. However, ethical considerations constrain research to the use of animal models before studies on human subjects can begin. This raises important considerations about the choice of model with which to conduct experiments. Clearly, the more closely related the animal species is to humans, the better is the model. As well, to examine the physiology of a disease state, an appropriate model must be chosen that mimics as closely as possible the disease state in humans.

Rats were chosen for the studies described in this thesis for two reasons. First, the vast majority of studies into the cardioprotective effects of ribose have utilized rats as the experimental model. Therefore, it is logical to investigate new administration protocols for ribose using a model in which certain of its metabolic benefits have been documented. Second, Zimmer and Gerlach (1978) demonstrated that administration of ribose significantly elevated the levels of PRPP and accelerated \textit{de novo} adenine nucleotide synthesis in the rat heart, and speculated that this occurred via the pentose phosphate pathway. Later studies showed that the rate limiting enzyme of this pathway, G-6-P DH, has similar activities in hearts from a variety of species, including rat, rabbit, guinea pig, and human (Zimmer et al. 1984). Thus, the capacity for ribose to modify the energy stores of the myocardium in rats could have application in humans.

Bolus ribose administration was chosen in an effort to mimic possible clinical scenarios in which protection against a future ischemic episode is desired, but where chronically indwelling intravenous lines may not be available. Two injections, 12-hours apart, were chosen to reproduce the time span of studies utilizing 24-hour continuous
delivery of ribose, which was previously shown to prevent the isoproterenol-induced loss of myocardial adenine nucleotides in rats (Zimmer et al. 1980b). In the present studies, however, ribose was delivered as a bolus. The ribose dosage (100 mg/kg) was based on previous studies showing that de novo adenine nucleotide biosynthesis was accelerated 3-fold in rat hearts receiving this dose by bolus IV ribose administration (Zimmer and Gerlach 1978). Previous studies in rats (Zimmer 1983), dogs (Mauser et al. 1985), and pigs (Bulley 1993) showed that the ribose-induced enhancement of myocardial energy stores following metabolic stress required up to 24-hours before their benefits were seen. This reflects the slow rate of de novo adenine nucleotide biosynthesis in the heart, which, even with acceleration due to ribose, still requires time to result in significant improvements in tissue metabolite concentrations. However, despite the time required for continuous IV ribose infusion to exert its effects, it did significantly shorten the time required to replenish myocardial adenine nucleotide levels following a brief episode of myocardial ischemia (Zimmer and Ibel 1984). A time response study was conducted (Appendix II) which showed that the maximum metabolic benefit of bolus ribose administration occurred 12-hours following the second injection. This time was therefore selected for the studies discussed in this thesis.

Because IV injection introduces compounds directly into the circulation, the dosage used is generally lower than it is for the same compound given by IM injection. This is also due, in part, to the slower and less direct absorption of an IM injection, which may result in lower concentrations of the compound entering the circulation but maintaining elevated levels for longer periods of time compared with the same dose delivered IV. Continuous IV infusion of a compound results in a rapid rise in plasma concentration, that is maintained at
the desired level for the duration of the infusion. In contrast, bolus IV administration results in a sudden and dramatic rise in plasma concentration which then declines over time (Burgen and Mitchell 1977). Thus, IV bolus ribose administration would have produced high initial concentrations of plasma ribose that subsequently decline as the ribose is metabolized, which would have been more gradual with IM. Because no information is available to convert IV doses of ribose that would produce similar circulating concentrations in an IM dose, 100 mg/kg was used for both IV and IM routes of administration to allow direct comparisons of route efficacy to be made. Future studies could be conducted to determine the optimal dose required to achieve similar plasma levels and optimal protection by both IV and IM routes of administration.

Effects of Bolus Ribose Administration - IV vs. IM

Despite elevated CP, MG, and AnER in the RV, bolus IV administration of ribose 24- and 12-hours prior to an ischemic event did not alter myocardial adenine nucleotide levels in normal hearts. Previous studies in pigs showed that a single bolus injection of ribose also elevated CP and MG, but contrasting these results in rats, also elevated myocardial ATP and TAN (Bulley, 1993). These differences may be due to the faster metabolic rate of rats compared with larger species (Schmidt-Nielsen 1967), which may increase the rate of adenine nucleotide turnover but not actually alter baseline ATP and TAN levels. Aside from ATP, the lower adenine nucleotides, ADP and AMP, may also be elevated by ribose due to enhancement of de novo adenine nucleotide biosynthesis or salvage pathways. Since ATP can be salvaged from both of these nucleotides, an elevation in either ADP or AMP could improve ATP stores. Since these three compounds can contribute to the store of high energy
phosphates, TAN levels were calculated to assess the overall impact of ribose on these compounds. However, as with in vivo ATP, no difference was seen following bolus ribose pretreatment in the total adenine nucleotide pool. Since ATP, CP, and MG are all utilized during global ischemia, AnER actually represents the total available energy store that can be utilized by the heart. Due to the relative concentrations of these components in the myocardium, AnER is heavily dependent on the MG level, which was also significantly elevated by ribose.

In the time response study, no significant elevations in myocardial energy stores were seen when rats were exposed to ribose for shorter durations than 12 hours before the acute study. Therefore, although bolus ribose administration may accelerate de novo adenine nucleotide synthesis acutely (Zimmer and Gerlach, 1978), this does not appear to result in elevated adenine nucleotide concentrations in rats in the present study. Despite this, the elevations in MG in response to ribose administration suggest ribose does have a sparing effect on other metabolic pathways in the heart. Whether exposures to ribose longer than 12 hours would have further metabolic benefits is unknown.

One mechanism for these enhancements of MG in rats may involve the pentose phosphate pathway. Ribose can be converted to fructose-6-phosphate, which serves as a point of entry into glycolysis or glycogen synthesis, via the transketolase and transaldolase reactions of the pentose shunt. At physiological ATP concentrations of 5 mM (Katz 1992), PFK would be significantly inhibited, slowing glycolysis and promoting the conversion of fructose-6-phosphate to glucose-6-phosphate, leading to glucose-1-phosphate and, ultimately, glycogen synthesis and the elevated MG levels observed. Mahoney et al. (1989) showed that the rate of utilization of ribose via the pentose phosphate pathway and glycolysis in the
isolated rat heart is too slow to support metabolism in the absence of other substrates. This slow rate of incorporation of ribose into the pentose phosphate pathway would slow any elevation in the production of energy stores, possibly explaining why the optimal interval for metabolic enhancement post-ribose administration was at least 12 hours.

Despite the fact that CP is not directly involved in the pentose phosphate pathway, providing ribose to the heart appeared to have an impact on LV and RV CP concentrations. Although the results did not achieve statistical significance, IV ribose administration did elevate CP in both the LV and the RV by over 40%. In contrast, CP was significantly lower in both the LV and the RV in the IM ribose pretreated hearts. Normally, the ratio of CP to ATP in the myocardium is about 1.5 to 1 (Gudbjarnason et al. 1970); thus, excess CP is available to rapidly generate ATP as required to maintain normal function and homeostasis. Providing ribose to the heart may reduce the conversion of CP to creatine in the cytosol. This could occur if ribose accelerates adenine nucleotide synthesis by bypassing the rate limiting G-6-P DH step (Zimmer 1992), which would raise cytosolic ATP levels. This could spare the transfer of phosphate from cytosolic CP to ADP, resulting in elevated myocardial CP levels as seen with IV ribose administration. Although elevated myocardial ATP levels were not observed with ribose administration in the present studies, ATP may have been elevated in different cellular compartments that did not result in overall tissue elevations. Because of the slower entry of ribose administered IM into the circulation, low levels of ribose may have been maintained that could have prevented this sparing of CP. Because adenine nucleotide synthesis is an energy consuming process, the longer exposure of IM pretreated hearts to ribose could have resulted in an increased conversion of CP to creatine in an effort to maintain tissue ATP levels. Previous studies utilizing continuous IV infusion of
ribose to the energy depleted heart showed an improved recovery of adenine nucleotide concentrations (Zimmer and Ibel 1984), but did not measure myocardial CP. Thus, although in the present studies ribose administration did not directly result in elevated myocardial ATP, its incorporation into the pentose shunt is the likely explanation for the elevation in other energy stores, such as CP and MG. Studies into the effects of ribose on the activities of enzymes controlling tissue glycogen levels could also clarify these metabolic effects.

Despite the elevations in in vivo myocardial CP and MG that were observed in IV but not IM ribose pretreated rats, both ribose pretreated groups showed significant improvement in tolerance to global ischemia as measured using TIC, although the magnitude of this was diminished in the IM route of administration. With the observed metabolic benefits using IV ribose administration, both TICo and TICp were prolonged by about 25%, while IM ribose elicited only a 10-15% prolongation in these parameters. Thus, despite the fact that IM ribose administration did not improve in vivo myocardial metabolites, it did provide some benefit against global ischemia. The best effect was produced by IV ribose, which elevated myocardial energy stores and provided about twice the improvement in TIC.

Thus, the present studies showed that significant prolongation of time to ischemic contracture occurred with both IV and IM ribose administration. Ischemic contracture has long been used as a marker of ischemic injury. In the earliest discussion of the mechanisms of this phenomenon, Katz and Tada (1972) attributed contracture to the depletion of myocardial ATP due to ischemia, which prevents the active relaxation of contractile proteins. The relationship between the depletion of myocardial energy stores and contracture was confirmed by Hearse et al. (1977), Sink et al. (1980), and Ventura-Clapier and Veksler (1994). Furthermore, Kingsley et al. (1991) showed that rather than being determined by
total cellular ATP content, contracture is, in fact, related to the cessation of ATP production via anaerobic glycolysis. While elevations in *in vivo* metabolites might explain the prolonged TIC in the IV ribose pretreated group, similar elevations were not observed with IM ribose. Therefore, the net consumption of myocardial metabolites during early ischemia was studied to determine whether this could account for the improvement in ischemic tolerance. Since both ribose pretreated groups showed slower net rates of ATP and CP consumption compared with their saline controls, the depletion of energy stores leading to contracture would take longer to occur in both groups, thereby prolonging TIC compared with controls. It must be appreciated that the net rate of consumption of metabolites represents a balance between the overall consumption versus the production of the metabolite. During ischemia, the production of CP and ATP would be profoundly compromised; thus, reduced consumption of these metabolites is the likely explanation for the lower net rates of consumption in the ribose groups.

Although there were no significant differences in the net rate of consumption of most metabolites between ribose and saline groups, the combined effects of lower rates of high energy phosphate consumption and increased consumption of MG with IM ribose pretreatment may explain the different efficacies of IV versus IM pretreatment. During early ischemia, compared with their saline controls, both IV and IM ribose pretreated groups showed about a 50% slower net consumption of ATP, which should result in improved tolerance to global ischemia in both. As well, a significantly lower net rate of TAN consumption was seen in the IV ribose group that was not seen in the IM ribose group, suggesting that the IV group maintained its pool of adenine nucleotides as possible energy stores, at least during early ischemia, possibly explaining why TIC was delayed to a greater
extent in the IV group. Although not achieving statistical significance, differences were seen in the net consumption of other metabolites between IV and IM groups. IV ribose showed a 21% slower net consumption of CP and 19% faster net consumption of MG compared with IV saline. IM ribose showed a 56% slower rate of CP consumption, but a 66% faster rate of MG consumption than their saline control. Thus, compared with IV ribose, IM ribose spared CP but had a much greater reliance on the consumption of glycogen during ischemia. This increased glycogen consumption may have caused an earlier cessation of glycolysis due to faster metabolite accumulation, initiating contracture earlier than in the IV ribose group. Both IV and IM ribose pretreatment groups showed similar net rates of consumption of AnER as their saline controls; however, due to its emphasis on MG, the slower net rates of consumption of ATP and CP are masked in AnER. The differences in the net consumption of MG between IV and IM ribose groups does not appear to be due to differences in in vivo MG levels, as there was no elevation with IM ribose compared with a 40% elevation in the IV ribose group, yet the IM group had greater net MG consumption. The apparently enhanced reliance of the IM group on MG during ischemia would increase the glycolytic production of ATP, possibly sparing CP as was observed, but would also increase the production of lactate and H⁺, reducing intracellular pH. If intracellular H⁺ accumulated to sufficiently high levels, anaerobic glycolysis would stop, initiating contracture. This sparing of CP by elevated MG consumption in the IM ribose group may account for the difference in the consumption of CP between IV and IM pretreatment groups. The reduced efficacy of IM ribose pretreatment likely reflects a balance between the beneficial effects of reduced net ATP and CP consumption and increased net consumption of MG and increased H⁺ production.
Ribose pretreated hearts probably showed decreased net rates of consumption of high energy phosphates due to either slowed consumption or enhanced production. Because ribose participates in a number of metabolic pathways, including *de novo* adenine nucleotide biosynthesis, salvage pathways, and the pentose phosphate pathway, it is feasible that intermediates in these pathways were elevated by ribose pretreatment. Thus, the ribose pretreated groups may have possessed substrates, such as PRPP, glucose, and ribose-5-phosphate, required to generate ATP during the ischemic period. Utilizing these intermediates to generate ATP via salvage and glycolytic pathways during ischemia would enhance production, and manifest as a slowing of the net consumption of high energy phosphates, as observed. It is more likely that the absolute rate of consumption was reduced, however, since production of high-energy phosphates in the absence of oxygen is severely compromised. Further metabolic assays are required to determine the mechanism of these effects. For example, the rate of glycolytic flux could be determined using radiolabelled substrates.

Alternatively, ribose may have acted through pathways not directly involved in energy metabolism. In five separate bolus injections at 100 mg/kg ribose to 200 g rats, the total amount of ribose delivered (approximately 700 µmoles) was sufficient to prevent the 5 µmole drop in myocardial adenine nucleotides due to isoproterenol (Zimmer and Gerlach 1978). Similarly, continuous IV ribose was also sufficient to account for an elevation in myocardial ATP (Zimmer and Ibel 1983). These calculations assume that the total quantity of ribose is available to the heart. However, in the present studies, two bolus injections of 100 mg/kg ribose to 300-350 g rats would have resulted in a maximum of approximately 450 µmoles ribose, producing an initial plasma ribose concentration of approximately 12 mM
ribose, much greater than the 1.7 mM concentrations used to enhance postischemic metabolic and functional recovery of isolated perfused rat hearts (Pasque et al. 1982, Clay et al. 1988). It seems unlikely that the total amount of ribose was available to the heart over the course of 24 hours, since the heart comprises only a small percent of total body weight. Thus, the quantity of ribose administered may not account for the metabolic benefits of ribose pretreatment observed in the present studies. Pasque et al. (1982) calculated that their perfusion protocol delivered 5.5 mg ribose per gram of heart each minute of perfusion. Despite the higher plasma concentration of ribose produced by bolus IV ribose pretreatment in the present study, over the 12-hour interval between injections, the maximal ribose delivery per gram of heart per minute was possibly less than 1% of Pasque's study. IM ribose administration would result in even less ribose entering the circulation, further lowering the amount of ribose reaching the heart. These levels of ribose may be too low to produce the long term benefits in the heart's tolerance to ischemia seen in the present studies. This suggests that ribose exerts its effects via mechanisms not involving the pentose phosphate pathway. Ribose may act via another mechanism, possibly by interacting with cell surface receptors and initiating intracellular cascades leading to metabolic improvements. For example, a family of receptors known as lectins, which contain carbohydrate recognition domains for sugars, such as mannose and galactose, are involved in signal transduction in a variety of cell types (Gabius 1997). Whether ribose binding lectins exist is currently unknown. Glycoproteins, which may serve as lectins, also have a role in signal transduction. For example, glycoprotein hormones, such as follicle stimulating hormone, activate G-proteins leading to the production of cAMP (Sairam 1989). If ribose binding proteins do exist, then the administration of bolus ribose could initiate intracellular cascades leading to
adenine nucleotide or glycogen synthesis. Further studies are required to determine whether ribose acts in this manner.

Ribose may have acted indirectly through purine receptors on the cell surface. ATP can be released from secretory granules into the plasma by a number of cell types, including mast cells, leukocytes, and platelets, in response to mechanical and chemical stimulation (Dubyak and El-Moatassim 1993). ATP can then initiate intracellular cascades via purine receptors, leading to the release of intracellular Ca^{++} (Dubyak and El-Moatassim 1993). Thus, it may be that ribose exerts its effects by promoting the release of ATP into the extracellular fluid, leading to the activation of purine receptors. However, ATP has a half-life of only a few seconds in the blood, due to the presence of cell surface nucleotidases (Motte et al. 1995). Thus, it is unclear how pretreatment with ribose could have resulted in any sustained activation of purine receptors via ATP, since any elevation in extracellular ATP would have been rapidly eliminated. Further studies are required to determine whether ribose exerts its effects via cell surface purine receptors.

Contrary to the beneficial effects of ribose pretreatment on glycogen levels in the heart, there was no elevation in glycogen with ribose pretreatment in either skeletal muscle or liver. Bolus IV ribose administration has been shown to elevate the rate of *de novo* adenine nucleotide biosynthesis in skeletal muscle from undetectable levels to levels that were 73% of the rate seen in the heart (Zimmer and Gerlach, 1978). More recently, Tullson and Terjung (1991) showed that perfusion of rat hindlimb with ribose accelerated *de novo* adenine nucleotide biosynthesis 3 to 4 fold compared with control muscle. Thus, skeletal muscle is able to utilize ribose similar to cardiac muscle. Therefore, IM administration of ribose could result in the direct incorporation of ribose into the skeletal muscle, reducing the
amount of ribose entering the circulation. If this were the case, IM ribose administration might have been expected to increase skeletal muscle glycogen levels, yet this was not seen. However, if ribose was utilized by the skeletal muscle via pathways not leading to glycogen synthesis, such as adenine nucleotide biosynthesis or salvage, which were not measured, this could still diminish its effect on skeletal muscle glycogen. Further biochemical analyses would be required to verify this hypothesis.

The pentose phosphate pathway is active in both skeletal muscle and liver. However, the activity of G-6-P DH in skeletal muscle is only about 17% that of the heart (Glock and McLean, 1954). Due to the very low activity of the pentose phosphate pathway in skeletal muscle, ribose may be unable to induce a significant increase in flux through the pathway, preventing any elevation in skeletal muscle glycogen. The activity of G-6-P DH in the liver, however, is double that of the heart (Glock and McLean, 1954), and the PRPP pool is much higher in the liver than in skeletal muscle (Zimmer and Gerlach, 1978). This reflects the importance of NADPH production for fatty acid synthesis in the liver, and suggests that the pentose shunt could be exploited in the liver by ribose administration. For example, in rats fasted for 24-hours, bolus administration of ribose was shown to elevate liver glycogen to a much greater extent compared with other pentose sugars, such as xylose and arabinose, which do not participate in the pentose phosphate pathway (Naito, 1944). However, the present studies showed that bolus ribose administration does not increase liver glycogen above nonfasted levels. This apparent discrepancy in results could be due to the presence of fasting in Naito's study. Liver glycogen in rats has been shown to be significantly depleted by fasts of similar duration (Wittnich et al., 1986). Thus, under fasting conditions, it appears that ribose can contribute to the resynthesis of liver glycogen stores, resulting in higher LG with
ribose administration in the face of fasting. Also, Naitō (1944) showed that liver glycogen synthesis in fasted rats was accelerated by ribose for up to 3 hours after administration; longer durations were not investigated. It is also possible that 12 hours following ribose administration may be too long to detect any effect on liver glycogen, due to the high level of activity of the pentose phosphate pathway in the liver, and the role of liver glycogen in maintaining plasma glucose. Alternatively, as discussed previously for the heart, the dose of ribose delivered to the body results in only very small quantities being delivered to various organs. Although the liver receives the majority of the cardiac output at rest (Ganong 1993), based on a plasma ribose concentration of 12 mM, this would still deliver only μg quantities of ribose to the liver per minute. This small amount may be insufficient to produce any significant impact on total liver glycogen stores. As well, it cannot be ruled out that ribose had an impact on other metabolites involved in either the pentose phosphate pathway or adenine nucleotide metabolism in either skeletal muscle or liver. Further biochemical analyses are required to answer these questions.

Despite these issues, it is clear that pretreatment using bolus ribose administration is an effective cardioprotective agent when given IV, while IM administration is not as effective. Neither route of administration significantly altered either skeletal muscle or liver glycogen levels.

It was interesting to note that in vivo myocardial glycogen concentrations in the LV were more than double those seen in the RV from both saline and ribose pretreated groups. As well, the variability in MG values were larger in the LV than in the RV. Other metabolites did not show this difference. This may reflect an adaptation of the LV to the higher, fluctuating workloads imposed by the systemic circulation, compared with the lower
and more stable workloads associated with the pulmonary circulation. Since both pretreatment groups showed this MG difference, ribose does not appear to impact on this response in the LV.

**Effects of Bolus Ribose Administration in the Presence of Halothane**

Clinical requirements may make it necessary to administer ribose in the presence of inhalation anesthetics. Therefore, studies were conducted to confirm that the presence of halothane during bolus administration of ribose would not alter its metabolic benefits to the heart nor its capacity to improve myocardial tolerance to global ischemia. Halothane was chosen since it is commonly available in animal research and it is still employed clinically (Kaus and Rockoff 1994, Fee and Thompson 1997).

Surprisingly, in the presence of the inhalation anesthetic halothane, *in vivo* myocardial metabolites no longer showed any improvement with either IV or IM ribose administration. As well, the presence of halothane during ribose administration reduced myocardial tolerance to global ischemia, shortening TICo by 21% in the IV ribose group and as much as 61% in the IM ribose group, while TICp was affected much less. This is the first report of ribose having a detrimental effect on the heart. One possible explanation for these detrimental effects on ischemic tolerance is the presence of halothane itself. *In vivo* myocardial metabolites were significantly depressed in the rats repeatedly exposed to halothane compared with rats in the first study. However, when TIC was compared among saline controls both in the presence and the absence of halothane, no significant difference was seen across these groups. Thus, it appears that it is the combination of halothane and ribose together that results in a detrimental effect on myocardial tolerance to global ischemia.
To clarify how halothane could have caused this detrimental effect with ribose, the net consumption of myocardial metabolites during early ischemia was examined. In the presence of halothane, IV and IM ribose pretreated hearts actually showed a faster net rate of ATP consumption compared with saline pretreated controls. Similarly, the net rate of CP consumption was also accelerated by ribose in the presence of halothane. This is in direct contrast with the effects of ribose in the absence of halothane, where a sparing of both ATP and CP during early ischemia was observed. Halothane has been reported to have direct mitochondrial effects. For example, studies in isolated mitochondria from rat liver show that halothane impairs oxidative phosphorylation, possibly by interfering in conformational changes necessary to the catalytic activity of mitochondrial ATP synthase (Rottenberg 1983, Branca et al. 1989, Vincenti et al. 1989). Although it is unknown whether similar effects of halothane on mitochondria occur in the myocardium, Blaise et al. (1991) showed that halothane significantly decreased myocardial ATP and CP levels in isolated rat hearts by 30% compared with controls. Furthermore, halothane has also been shown to significantly decrease CP levels, increase glycolytic flux and reduce glycogen synthesis in rat diaphragm (Rosenberg et al. 1977). The rate of glycolysis in isolated hepatocytes was significantly increased by halothane compared with control cultures (Olson et al. 1990), consistent with a reduction in oxidative phosphorylation. It is unknown whether similar effects are seen in the heart. However, in the present study, the presence of halothane during saline pretreatment significantly decreased the levels of ATP and CP in both the LV and the RV, suggesting that oxidative metabolism was impaired by halothane. An increased flux through the pentose phosphate pathway due to ribose administration may have accelerated the rate of de novo adenine nucleotide biosynthesis, which requires ATP (Stryer 1988). Combined with an
impairment in oxidative phosphorylation due to the presence of halothane, this additional consumption of ATP stimulated by ribose may account for the increased net consumption of high energy phosphates in the ribose pretreated groups, as shown by the increased net rates of consumption of ATP, CP, and TAN.

While both IV and IM ribose pretreated groups showed similar elevations in the net consumption of ATP and CP during early ischemia, dramatic differences were seen in their net consumption of glycogen. IV ribose in the presence of halothane resulted in an almost 7-fold faster rate of glycogen consumption during ischemia. This increased consumption of glycogen may be due to the enhanced glycolytic flux and impairment of glycogen synthesis, together with an increased ATP consumption due to the entry of ribose into the pentose phosphate pathway and an acceleration of de novo adenine nucleotide biosynthesis, as described above. This would increase the heart's reliance on glycogen for energy during ischemia compared with the saline pretreated hearts. In contrast, hearts pretreated with ribose IM appeared unable to consume glycogen, which may account for the significant shortening of TIC that was observed. This is potentially due to either the inhibition of glycogen phosphorylase, which would prevent the degradation of glycogen, or by a decreased entry of glucosyl units derived from glycogen into the glycolytic pathway, or by inhibition of glycolysis. Since there were no significant differences among the pretreatment groups in the production of lactate, it is likely that the mobilization of glycogen itself accounts for the observed inability to consume glycogen. An inability to utilize glycogen during ischemia, combined with a faster net consumption of high-energy phosphates, would rapidly deplete myocardial energy stores and hasten ischemic contracture. These differences in MG consumption are supported by differences in the net consumption of AnER, with IV ribose
again showing faster rates than saline, while IM ribose appears unable to consume AnER, due to the inability to consume MG. Further studies are required to determine the mechanism of this inability to utilize glycogen.

Halothane is reported to require up to 24-hours to be 80% eliminated via the lungs (Marshall and Wollman, 1985). Although the rate of elimination is probably much faster in the rat, the multiple short exposures to halothane may have maintained a level of the anesthetic in the body below clinically significant levels, yet enough to exert long-term effects on basal metabolism, such as impairing oxidative phosphorylation and enhancing glycolysis. Compared with the results of the first study, the hearts from animals pretreated with halothane showed signs of metabolic stress, and it is possible that the metabolic effects of halothane itself may account for the depressed myocardial energy levels seen in saline controls and both groups of ribose pretreated rats. Whether this would occur with all anesthetics or only inhalation anesthetics, is unknown.

Similar to the previous study, bolus ribose administration by either route of administration did not alter skeletal muscle glycogen levels even when administered in the presence of halothane. In contrast to the first study, in the presence of halothane, IV ribose pretreatment did result in higher liver glycogen. This was not observed with IM ribose pretreatment. However, when compared with values obtained for liver glycogen in animals not exposed to halothane during pretreatment, it appears that IV ribose actually prevented an almost 75% depletion of liver glycogen seen with halothane, rather than elevating basal levels. This depletion could indicate some degree of stress to the liver. Halothane has been shown to be hepatotoxic in some cases, due to its biotransformation into degradation products including trifluoroacetic acid via the liver (Gut et al. 1993). Furthermore, these
degradation products appear to be involved in activation of the immune system in some cases, which may lead to liver damage. It is unknown whether this could deplete liver glycogen. As previously stated, halothane significantly increases the rate of glycolysis in hepatocytes (Olson et al. 1990). If halothane does contribute to the loss of liver glycogen, IV ribose, but not IM, appears to protect against this effect.

Thus, these studies demonstrate that bolus ribose administration in the presence of the inhalation anesthetic halothane does not protect the heart against ischemic challenge, and that it is actually detrimental with IM administration. No effect was seen on skeletal muscle glycogen with either route of administration. However, glycogen levels in the liver were elevated by IV ribose administration, apparently by preventing the depletion of liver glycogen occurring in saline controls and with IM ribose.

**Effects of Ribose in the Presence of Chronic Hypertension and Myocardial Hypertrophy**

The spontaneously hypertensive rat was developed through selective mating of Wistar rats showing elevated systemic blood pressures (Okamoto and Aoki 1963), and are used as a model of essential human hypertension. In the present studies, 6 month old SHRs were used to determine the effects of ribose administration in a pathologic model. At 10 weeks of age, the SHR shows significantly elevated blood pressure and left ventricular hypertrophy compared with WKY rats, the "normotensive" control for the SHR (Schoemaker et al. 1994). Arterial blood pressures and heart to body weight ratios of the SHRs used in this thesis are comparable to those previously reported for age-matched SHRs (Shimamoto et al. 1982), and confirm the presence of significant hypertension and myocardial hypertrophy. These values
are substantially higher than those seen in our normotensive Sprague-Dawley rats, which have arterial pressures and heart weight to body weight ratios that are 35% lower than values in age-matched SHRs. The dosing regimen selected for this study was based on the finding that the best cardioprotective effects were seen with IV bolus administration in the absence of halothane; this protocol was used for all SHR studies.

Surprisingly, contrary to the benefits seen with IV ribose administration in normal hearts, no significant benefits were seen on *in vivo* myocardial metabolites in either the LV or the RV in the presence of hypertension and hypertrophy. The lack of an improvement in *in vivo* myocardial metabolites with IV ribose administration may indicate that the dose or pretreatment protocol was insufficient in this pathologic state, or that hypertrophied hearts are unable to respond to ribose. Both the doses used and the durations of exposure to ribose were based on studies using Sprague-Dawley rats. Further studies are necessary to determine whether a different dose or duration of exposure to ribose would be beneficial in the SHR.

Differences in myocardial metabolism between normal and hypertrophied hearts could cause the SHR heart to respond very differently to ribose than normal hearts. For example, the increased reliance of the SHR heart on glycolytic energy metabolism (Atlante et al. 1995) may have shunted the ribose through glycolysis rather than into glycogen synthesis, preventing any elevation in MG levels. This emphasis on glycolytic metabolism may also explain the lack of *in vivo* benefits on ATP and CP with ribose pretreatment. Interestingly, the activities of myocardial G-6-P DH from Sprague-Dawley rats (Zimmer et al. 1980) and SHR (Heckmann and Zimmer 1992) are similar, suggesting that the myocardial pentose phosphate pathway is not more active in the SHR.

Despite the lack of *in vivo* metabolic benefits, significant improvements in ventricular
performance in SHR were observed in response to ribose pretreatment. The ribose pretreated SHRs did exhibit a 25% elevation in \( +\frac{dP}{dt_{\text{max}}}. \) Even when normalized for differences in afterload by calculating \( +\frac{dP}{dt_{\text{max}}}/LVSP \), contractility was still elevated by 13% in the ribose pretreated SHRs. As well, LV pressure-rate product, an indicator of the work performed during a set time interval, was elevated by 26% in the ribose pretreated hearts. The maximum rate of relaxation, \( -\frac{dP}{dt_{\text{max}}} \), was also improved by 44% by IV ribose pretreatment, although this difference did not achieve statistical significance. These improvements in ventricular performance in the absence of any improvement in hemodynamics suggest that ribose has a positive inotropic effect in the hypertrophied myocardium. It appears that ribose has a greater effect on the relaxation properties of the ventricle, since the relaxation index showed a greater magnitude of improvement than the contractility indices, despite no significant difference compared with saline pretreatment. The improvement in ventricular relaxation could improve filling during diastole, which, in turn, could improve systolic function. Since active relaxation is dependent on the myocardial ATP concentration (Swynghedauw et al. 1992), an increased ATP turnover due to ribose pretreatment may have enhanced the dissociation of contractile proteins.

Previous studies showed that ribose prevented the functional deficit associated with aortic constriction (Zimmer 1983), enhanced the positive inotropic effects of adrenergic stimulation (Zimmer 1982, Zimmer and Ibel 1983), and improved postischemic recovery of function (Pasque et al. 1982). However, these studies involved the use of ribose in a stressed, metabolically challenged heart, and also showed improvements in myocardial energy stores, neither of which was the case in the studies discussed in this thesis. In the present study, despite no apparent effects on \textit{in vivo} myocardial metabolites, ventricular performance was
significantly improved by IV ribose pretreatment in the SHR, as shown by elevations in ventricular contractility and relaxation. Thus, for similar energy stores, ribose pretreated hearts performed at a significantly higher level, suggesting that ribose increases the efficiency of energy consumption. Alternatively, an increased production of adenine nucleotides due to ribose administration may enable the heart to function at a higher level of activity without experiencing a metabolic deficit. Further studies, particularly those examining the turnover of adenine nucleotides, are required to clarify these effects. Ribose may act via catecholamine stimulation, which would improve both contractility and relaxation indices. An increased inotropic state may also have been caused by an increased Ca\textsuperscript{++} sensitivity associated with ribose administration. Since activation of purine receptors elevates the concentration of intracellular Ca\textsuperscript{++} (Dubyak and El-Moatassim 1993), ribose may have increased ventricular performance via an interaction with cell surface receptors. Further studies are required to determine whether ribose binds to purine receptors in the heart. Ventricular relaxation is an active, energy consuming process, involving uptake of Ca\textsuperscript{++} into the sarcoplasmic reticulum (Katz 1992). Enhancing this uptake, via increased activity of SR Ca\textsuperscript{++} pumps, would improve relaxation. Whether ribose directly affects the SR, or acts indirectly through catecholamines to improve relaxation, is unknown and requires further investigation.

Ventricular ±dP/dt\textsubscript{max} in SHRs are much higher than values from \textit{in vivo} Sprague-Dawley rat hearts (Dowell 1991). This is an adaptation to the increased workload to which the SHR hearts are subjected. It has been shown that when ribose was continuously infused IV for 24 hours in rats with normal hearts, it did not alter either arterial pressures or LV performance (Lortet and Zimmer 1989). This suggests that the hypertrophied myocardium
may be more functionally responsive to ribose than the normal heart. In response to reductions of high-energy phosphates by ischemia/reperfusion, isolated hearts from SHR showed a greater loss of ventricular performance than age-matched normal hearts (Fenchel et al. 1988). Therefore, the SHR heart appears to be more sensitive to changes in energy stores or turnover, which may be exploited by ribose to improve in vivo performance.

Contractility is also affected by changes in preload and afterload. For example, increases in preload and afterload can lead to an elevation in $+\frac{dP}{dt_{\text{max}}}$ (Mason 1969). An elevation in heart rate is also associated with increased $+\frac{dP}{dt_{\text{max}}}$, indicative of an elevated inotropic state. Although it did not achieve statistical significance, ribose pretreated SHR hearts did show a 15% increase in heart rate, which could have affected ventricular performance. As an indicator of preload, no difference in left ventricular diastolic pressure was seen between saline and ribose pretreated SHRs, eliminating this as a potential source for the elevation in $+\frac{dP}{dt_{\text{max}}}$ in the ribose group, assuming that there was no difference in ventricular compliance between groups. Afterload, the force resisting shortening of the contractile elements of the myocardium, is related to the arterial diastolic pressure, which must be overcome by the left ventricle before ejection occurs. The ribose pretreated SHRs did exhibit a modest 10% elevation in diastolic pressure compared with the saline pretreated group, which may also have contributed to the functional increase. However, the modest 10%-15% differences in hemodynamics do not account for the significant 25% improvement in contractile function, further supporting the idea of ribose exerting a positive inotropic effect. Relaxation is determined by the compliance of the ventricle, which is dependent on the rate of dissociation of contractile proteins and the fibrous matrix of the ventricle. Since both saline and ribose pretreated SHRs were age-matched, no significant difference in the
fibrous matrix would exist. Thus, the improvement in $-dP/dt_{\text{max}}$ must be related to an enhancement in the dissociation of contractile proteins, possibly via increased SR Ca$^{++}$ uptake due to catecholamine stimulation.

Also in contrast to normal hearts, IV ribose pretreatment did not improve tolerance to global ischemia of hypertrophied hearts. Surprisingly, the TIC results were not dramatically different from those seen in normal hearts. In contrast, Snoeckx et al. (1993) showed that SHR showed a reduced recovery of mechanical function and adenine nucleotide content following global ischemia/reperfusion compared with age-matched normotensives (WKY). Those studies showed that the recovery of the hypertrophied heart from ischemia is depressed compared with normal hearts. However, TIC is a measure of the heart's response to ischemia. Sink et al. (1981) showed that, in Sprague-Dawley rats subjected to aortic banding to induce myocardial hypertrophy, TICo was significantly shorter than in normal hearts, but that TICp was significantly longer. This discrepancy was not explained. It must be remembered that TICo reflects the onset of cellular injury in response to ischemia, while TICp indicates peak injury. One further issue that must be considered when comparing the responses of normal and hypertrophied hearts to ribose pretreatment is that Sprague-Dawley and SHR are very different strains of rats; thus, any comparisons between the two must be done with caution.

Differences in the net consumption of myocardial metabolites during ischemia between pretreatment groups provide some evidence that ribose did have an effect in the SHR. In contrast to the first study, the second ventricular biopsy was taken after six minutes of global ischemia rather than three minutes. This was done to determine whether a protective effect of ribose would be seen after a greater metabolic stress than was used in the
first study. This extended biopsy time was based on previous studies utilizing bolus ribose administration in pigs that showed that ribose pretreatment prevented an approximately 50% decline in myocardial ATP content during the first 15 minutes of global ischemia (Bulley 1993). In the first studies done in normal hearts, the decline in myocardial ATP after three minutes of ischemia was only 22%, while six minutes of ischemia resulted in a 58% decline in SHR controls. Thus, it was felt that 6-minutes would be the optimal time to observe a beneficial effect of ribose on myocardial metabolism in hypertrophied hearts. Despite these differences in the duration of ischemia, by calculating the net rate of consumption over the ischemic interval (μmoles/g dry wt./min), some general comparisons can be made. For these comparisons to be valid, however, the consumption of these metabolites must be linear over time. Recent studies utilizing NMR to measure myocardial metabolites in rats showed that the decline in ATP was reasonably linear for the first 10 minutes (Kolocassides et al. 1996). Thus, calculating the net rate of consumption does permit some comparisons to be made.

Contrary to the 56% lower net rate of consumption of ATP seen in normal hearts, hypertrophied hearts showed no difference in the net consumption of ATP, nor was there any difference in the consumption of TAN. Yet, both hypertrophied and normal hearts showed similar reductions in the net rate of consumption of CP (21%). However, unlike normal hearts, hypertrophied hearts showed a faster net rate of consumption of glycogen during early ischemia and a significant increase in the production of lactate. This indicates an increased glycolytic flux in the ribose pretreated SHRs. Overall, these differences in the net rates of ATP, CP, and MG consumption between saline and ribose pretreated hearts did not result in any difference in the consumption of AnER. Incorporation of ribose into the pentose phosphate pathway, associated with an increased activity of G-6-P DH in the hypertrophied
heart, could potentially increase the production of glycolytic intermediates such as fructose-6-phosphate. As previously described, increased glycolytic flux would also increase the production of harmful metabolites, particularly H\(^+\), decreasing intracellular pH leading to the inhibition of PFK and cessation of glycolysis. The increase in the net consumption of MG in hypertrophied hearts secondary to ribose pretreatment does not appear to have been sufficient to have caused an earlier cessation of glycolysis, since there were no differences in either TICo or TICp between pretreatment groups.

As seen in the previous study, ribose pretreatment did not significantly alter either skeletal muscle or liver glycogen levels in the SHR. The activity of glycogen synthase and the rate of glycogen synthesis in skeletal muscle from SHRs have been shown to be significantly higher than in normotensive controls (WKY) (Farrace et al. 1995). This suggests that an adequate dose of ribose should have had a dramatic impact on skeletal muscle glycogen, which was not seen. This further supports the belief that bolus injections of ribose may not provide sufficient substrate to significantly influence metabolism in skeletal muscle. This may also account for the lack of effect seen on liver glycogen. The low activity of liver G-6-P DH in SHR versus normotensive rats (Iritani et al. 1977) may have reduced or prevented the incorporation of ribose into the pentose phosphate pathway and, via glycolytic intermediates, glycogen synthesis, resulting in no difference in liver glycogen stores. Alternatively, ribose could be exerting its effects in the heart via cell surface receptors. Tissue differences in purine receptors have been observed. For example, extracellular ATP causes vasodilation and acts as a positive inotrope in the heart, while glycogenolysis is stimulated in the liver (Dubya and El-Moatassim 1993).

These studies demonstrate that bolus administration of ribose does not alter \textit{in vivo}
myocardial metabolites, but did improve ventricular performance. Ribose pretreatment did not improve myocardial tolerance to global ischemia, as measured using TIC, possible due to an early cessation of anaerobic glycolysis. This treatment regimen also had no effect on skeletal muscle or liver glycogen levels.

Regardless of which pretreatment was used in hypertrophied hearts, it was interesting to note that, as seen previously in normal hearts, myocardial glycogen was dramatically higher in the left ventricle than the right ventricle. This may reflect the metabolic adaptation of the left ventricle to a significantly higher workload, the elevated systemic pressures of the SHR, or a decrease in myocardial perfusion. For example, in rats in which myocardial hypertrophy was induced by aortic banding, the rate of glycolysis in hypertrophied hearts was greater than that of normal hearts, while rates of oxidative metabolism were lower (Allard et al. 1994). This is also supported by studies showing that SHRs have higher activities of glycolytic enzymes and lower activities of mitochondrial enzymes than WKY controls (Atlante et al. 1995, Lanying et al. 1995, Torii and Ito 1990). Therefore, the presence of myocardial hypertrophy is associated with an increase in the utilization of glycolytic metabolism for ATP generation. The left ventricle of the SHR, subjected to a more severe hypertrophic stimulus, may therefore express metabolic characteristics reflecting myocardial hypertrophy, while the right ventricle does so to a lesser degree. This may predispose the left ventricle to increased storage of glycogen for glycolysis compared with the right ventricle. At 6-months of age, the SHR shows dramatically elevated systemic blood pressures and clear evidence of concentric left ventricular hypertrophy, while the right ventricle does not exhibit morphological signs of hypertrophy (Pfeffer et al. 1979, Engelmann et al. 1987). At this age, there is no evidence of functional deficit in the SHR (Pfeffer et al. 1979, Mirsky et al. 1983,
Conrad et al. 1991). Thus, the SHRs used in this thesis were in a state of functionally compensated hypertrophy. SHRs beyond the age of 18-months show significant depression of myocardial function compared with age-matched WKYs (Mirsky et al. 1983, Conrad et al. 1991). Six-month old SHRs were selected for the present studies to determine the metabolic and functional effects of ribose pretreatment without the confounding influence of functional depression. Future studies could investigate the impact of heart failure on the response to ribose. Clearly, the adaptations to chronic pressure-overload and myocardial hypertrophy must be considered if we are to optimize the pretreatment benefits of ribose in pathologic hearts.
CHAPTER SIX

CONCLUSIONS
The following conclusions can be drawn for the studies detailed in this thesis.

1. Advance bolus administration of D-ribose elevated myocardial energy stores and significantly improved myocardial tolerance to global ischemia as assessed using TIC. However, no elevation in skeletal muscle or liver glycogen stores occurred.

2. The metabolic benefit of intravenous administration of D-ribose in the myocardium was not observed with intramuscular administration. Similarly, while both IV and IM administration prolonged TIC compared with saline controls, intramuscular administration delayed TIC only half as well as intravenous administration.

3. The presence of the inhalation anesthetic halothane during bolus administration of D-ribose prevented the improvement in ischemic tolerance with IV administration, and actually proved to be detrimental with IM administration. No alteration in skeletal muscle glycogen was observed; however, IV ribose administration resulted in higher liver glycogen levels compared with the other groups.

4. In the presence of chronic hypertension and myocardial hypertrophy, advance bolus IV administration of D-ribose did not alter \textit{in vivo} myocardial energy stores, but appeared to significantly improve ventricular function. D-ribose did not appear to improve myocardial tolerance to ischemia, nor did it elevate skeletal muscle or liver glycogen.
CHAPTER SEVEN

LIMITATIONS

and

FUTURE DIRECTIONS
The findings of this thesis must be interpreted within the boundaries of the experimental protocols that were used. The studies have several limitations that will be discussed briefly, and suggestions for possible solutions that could lead to future studies will be presented.

1. Animal Model

The studies described in this thesis made use of two very different strains of rat. The SHR is an inbred strain of hypertensive rat that develops severe LV hypertrophy, while the Sprague-Dawley is a normotensive strain sharing little genetic lineage with the SHR. Therefore, some of the differences between the normal and hypertensive groups may be related to strain, and not the presence or absence of pathology. However, differences between treatments (saline vs. ribose) within a group are valid. The purpose of the SHR study was to compare the response of ribose pretreated rats under conditions of hypertension and myocardial hypertrophy. Future studies using WKY rats, the normotensive control for SHR, could be performed to permit direct comparisons of normal and pathologic hearts. However, investigations of the WKY strain showed that significant biological variability exists between colonies obtained from different suppliers that was not found in SHR (Kurtz and Morris 1987). It was concluded that the WKY do not constitute an adequately pure inbred strain of rat. As well, the WKY rat can present as moderately hypertensive with some degree of myocardial hypertrophy (Christe and Rodgers 1995). Thus, no ideal normal control exists for the SHR.

2. Metabolic Measures

The metabolic benefits of ribose on the \textit{in vivo} myocardium were subtle. However,
the tissue concentration of PRPP, reported in previous studies to be increased by ribose, was not measured, so it cannot be confirmed that the metabolic effects occurred due to an elevation in this pool. It is also unknown to what extent ribose was converted to intermediates in other metabolic pathways, which could account for the modest increases in high-energy phosphate and glycogen stores that were observed.

To what extent advanced pretreatment with ribose would aid metabolic recovery from an ischemic insult is not known, since the biopsies taken in these studies were either in vivo or purely ischemic, with no reperfusion. Since previous studies clearly show that recovery from ischemia in normal hearts is enhanced with continuous ribose infusion, ischemia/reperfusion studies need to be conducted to further explore the use of ribose pretreatment as a means assisting the pathologic heart in recovering from an ischemic episode.

The majority of studies on the metabolic effects of ribose employed continuous, long-term intravenous administration of the compound to effect significant metabolic changes. The studies detailed in this thesis employed bolus administration of ribose that resulted in modest metabolic changes in myocardium, skeletal muscle, and liver. Thus, differences between previous studies and this thesis may be due to the method of delivery. Dose-response studies in pathologic hearts have not been conducted to determine which is the most suitable ribose concentration that provides the best protection in these hearts. Future studies could investigate this to determine the optimal dose of ribose.

3. **In vivo Myocardial Function**

The studies of the effects of ribose pretreatment on in vivo myocardial function utilized isovolumic indices, specifically ±dP/dt\textsubscript{max}, that are based on the development of
intraventricular pressure. These measures are limited by the preload, afterload, and heart rate dependency of the rate of pressure development. The rat hearts were not paced at a uniform rate, nor was end-diastolic volume (preload) controlled. Therefore, any functional alterations in response to ribose pretreatment must be interpreted with caution, since changes in the functional indices may not represent alterations in the contractile characteristics of the myocardium per se. However, no significant differences in heart rate between saline and ribose pretreatment groups was found, so any differences in functional indices reflect changes in the heart's performance. Although load-independent functional studies can be performed using an isolated, perfused rat heart model, this technique excludes neural and humoral input (i.e., the whole body response). One possible method to preserve the whole body response yet obtain indices of myocardial function is to measure the velocity of ventricular segmental shortening via ultrasonics. This procedure would allow for the direct measurement of any change in the contractile performance of the myocardium in response to ribose pretreatment, although it is still heart rate and load dependent. Studies using an isolated, paced heart model would allow load independent functional assessments of the potential therapeutic benefit of ribose administration as a cardioprotective agent.

As well as the load-dependent nature of the functional indices, the metabolic data indicate that ribose pretreatment has the greatest effect on the heart during an ischemic episode; thus, any functional effect of ribose may only be observed during or following a metabolic stress. However, function in each animal was monitored while the heart was normally perfused. Measuring in vivo function and then applying a metabolic stress, such as acute hypoxia or regional ischemia, and recording the recovery of function after a return to
control conditions could help to identify any functional benefits from ribose pretreatment during the recovery period.

4. **Mechanism of Ribose Effects**

Presently, the metabolic fate of ribose administered as a pretreatment is unknown. Future studies could ascertain the mechanism by which ribose exerts its effects through the various metabolic pathways, using radiolabelled (¹⁴C) ribose. This would enable the fraction of ribose present in the PRPP pool, tissue glycogen, and glycolytic intermediates to be detected. These analyses would reveal the contribution of ribose pretreatment to high-energy phosphate pools, and would indicate any difference in the uptake of ribose due to the route of administration. Any tissue-specific differences in the response to ribose could also be elucidated using this procedure.

The mechanisms by which mammalian cells uptake ribose is unknown. Identification of possible sarcolemmal transporters for ribose should be undertaken to further clarify the mechanism of action of ribose pretreatment.
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APPENDICES
APPENDIX I: BIOCHEMICAL METHODOLOGIES

Tissue metabolites were quantified using two distinct biochemical methods; fluorescence spectrophotometry (fluorometry), and high performance liquid chromatography (HPLC). Details of each procedure will be considered separately.

Tissue Metabolite Extraction

All tissue samples were freeze-dried prior to biochemical analyses. Fibrous connective and vascular tissues were carefully removed from all samples before extraction, and 2-5 mg of cleaned tissue was used. Extraction of metabolites was accomplished using perchloric acid (PCA) for the analysis of adenine nucleotides (ATP, ADP, AMP), CP, and lactate. Tissue extraction for glycogen content was accomplished using hydrochloric acid (HCl).

Perchloric Acid (HClO₄) Extraction: Cleaned tissue was extracted in 600 μl of 0.5 M PCA for 10 minutes at 0°C with constant agitation to ensure adequate penetration of the acid into all layers of tissue. Samples were then centrifuged at 15000g at 0°C for 10 minutes (Eppendorf model 5402, Brinkmann Instruments Inc., Rexdale, Ont.) to remove the protein precipitate, which may include enzymes, that remained intact in the acid. After, 540 μl of supernatant was removed and rapidly frozen at -80°C. To neutralize the extract, 135 μl of 2.3 M KHCO₃ was added to the frozen extract to prevent foaming due to the release of CO₂, and this was then centrifuged a second time. The supernatant was removed and maintained at -80°C until analysis.

HCl Extraction: Due to the high molecular weight of the glycogen polymer, PCA extraction
was not used since a significant proportion of the glycogen can remain unextracted in the precipitate. To ensure adequate extraction, a strong acid is used. In addition, because the tissues may contain endogenous glucose that would interfere with the glycogen determination, the extraction was performed at a high temperature to destroy any extant glucose. Cleaned tissue was extracted with 2.0 N HCl (650 μl for muscle, 1000 μl for liver) at 100°C for 120 minutes, with mixing after the first 60 minutes. The acid was allowed to cool to room temperature, and neutralized with equivolume 2.0 N NaOH. The liquid extract was separated and maintained at -80°C until analysis.

**Fluorescence Spectrophotometry ("Fluorometry")**

Tissue was analyzed for ATP, CP, lactate, and glycogen via a fluorometric procedure using a Hitachi model F-2000 fluorescence spectrophotometer (Hitachi Ltd., Tokyo, Japan). Figure 1 is a schematic representation of the principles of fluorometry. Light from the xenon

![Fluorescence spectrophotometer diagram](https://example.com/fluorescence_diagram.png)

**Figure 1.** A fluorescence spectrophotometer. Modified from Freifelder 1982.
(Xe) lamp is narrowed to a specific wavelength by the excitation monochromator before entering the sample. This wavelength is absorbed by the compounds of interest in the sample, causing them to gain energy. As the excited molecules lose energy, they emit light (fluoresce) at longer wavelengths than that of the incident light. This emitted light passes through a second monochromator and into a detector.

The fluorometric quantification of tissue metabolites is based on the fact that NADH and NADPH absorb strongly at ultraviolet wavelengths and fluoresce in the visible range. Enzymatic analysis utilizes this property by generating NADH and NADPH in the sample extract. Thus, the fluorometer is set to an excitation wavelength of 365 nm and an emission wavelength of 455 nm for all compounds analyzed using this procedure.

*ATP/CP analysis:* The enzymatic determination of ATP and CP is based on the following biochemical steps.

1. $CP + ADP \rightarrow creatine + ATP$

2. $ATP + glucose \rightarrow ADP + G\_6\_P$

3. $G\_6\_P + NADP^+ \rightarrow 6\_P\_gluconolactone + NADPH$
Each sample was analyzed twice, while three water blanks and three ATP standards (200 μM) were also prepared. The analysis was performed as follows. A 20 μl aliquot of water blank, ATP standard, or sample was added to acrylic cuvettes, followed by 2.0 ml of dilute reagent containing 50 mM TRIS buffer (Sigma-Aldrich, # T-1378, pH 8.1), 1 mM MgCl₂ (Sigma-Aldrich, # M-9272), 0.5 mM dithiothreitol (Sigma-Aldrich, # D-5545), 100 μM glucose (Sigma-Aldrich, # G-5767), and 50 μM NADP (Boehringer Mannheim, # 128058), and 0.02 U/ml G-6-P DH (EC 1.1.1.49, Boehringer Mannheim, # 127035). An initial fluorescence measurement (R₁) was taken to establish a baseline. Dilute hexokinase (HK, EC 2.7.1.1) was prepared by adding 25 μL HK (Sigma-Aldrich, # H-5750, 280 U/ml) to 1.0 ml dilute reagent. A 50 μl aliquot of this dilute enzyme was added to each cuvette, and they were incubated in the dark for 30 minutes. A second measurement (R₂) was taken.

Next, a dilute solution of creatine kinase (CK, EC 2.7.3.2) was prepared by adding 10 μl of 10% bovine serum albumin (Sigma-Aldrich, # A-2153), 2 mg ADP (Boehringer Mannheim, # 236675), and 2 mg CK (Sigma-Aldrich, # C-7886, 240 U/mg) to 1.0 ml dilute reagent. A 40 μl aliquot of dilute CK was added to each cuvette, and they were incubated in the dark a second time for 60 minutes. A final measurement (R₃) was taken. The concentrations of ATP and CP were calculated as follows.

\[
ATP = \frac{\Delta \text{sample} - \Delta \text{blank}}{\Delta \text{standard} - \Delta \text{blank}} \times \frac{[\text{standard}]}{(\text{tissue wt.} \times DF)}
\]

where: \( \Delta = R₂ - R₁ \); [standard] = 200 μM; DF = 1.333.

\[
CP = \frac{\Delta \text{sample} - \Delta \text{blank}}{\Delta \text{standard} - \Delta \text{blank}} \times \frac{[\text{standard}]}{(\text{tissue wt.} \times DF)}
\]
where: $\Delta = R_3 - R_2$; [standard] = 200 $\mu$M; DF = 1.333.

Mean values of the blanks, standards, and samples were used in the calculations.

**Lactate analysis:** Quantification of lactate is based on the following biochemical steps.

\[
\text{LDH} \\
1. \text{lactate} + \text{NAD}^+ \rightarrow \text{pyruvate} + \text{NADH} \\
2. \text{pyruvate} + \text{hydrazine} \rightarrow \text{hydrazone}
\]

Each sample was analyzed twice, while three water blanks and three lactate standards (500 $\mu$M) were also prepared. The analysis was performed as follows. A 20 $\mu$l aliquot of water blank, lactate standard, or sample was added to acrylic cuvettes, followed by 2.0 ml of dilute reagent (pH 10.0) containing 100 mM hydrazine (Sigma-Aldrich, # H-7294), 100 mM glycine (Sigma-Aldrich, # G-7403), and 0.5 mM NAD (Boehringer Mannheim, # 127973). An initial fluorescence measurement (R1) was taken to establish a baseline. Dilute lactate dehydrogenase (LDH, EC 1.1.1.27) was prepared by adding 250 $\mu$L LDH (Boehringer Mannheim, # 106984, 1250 U/ml) to 1.0 ml dilute reagent. A 50 $\mu$l aliquot of this enzyme was added to each cuvette, and each was incubated in the dark for 60 minutes. A second measurement (R2) was taken. The lactate concentration (µmoles/g dry wt) was calculated using the following equation:

\[
\frac{\Delta \text{sample} - \Delta \text{blank}}{\Delta \text{standard} - \Delta \text{blank}} \times \frac{\text{[standard]}}{(\text{tissue wt.} \times \text{DF})}
\]
where: $\Delta = R2 - R1$; [standard] = 500 µM; DF = 1.333.

Mean values of the blanks, standards, and samples were used in the calculations.

**Glycogen analysis:** Quantification of glycogen is based on the following biochemical steps.

1. **ATP + glucose $\rightarrow$ ADP + G-6-P**

2. **G-6-P + NADP$^+$ $\rightarrow$ 6-P-gluconolactone + NADPH**

Each sample was analyzed twice, while three water blanks and three glucose standards (myocardium, 500 µM; liver, 2 mM) were also prepared. The analysis was performed as follows. A 20 µl aliquot of water blank, glucose standard, or sample was added to acrylic cuvettes, followed by 2.0 ml of dilute reagent containing 50 mM TRIS buffer (Sigma-Aldrich, # T-1378, pH 8.1), 1 mM MgCl$_2$ (Sigma-Aldrich, # M-9272), 0.5 mM dithiothreitol (Sigma-Aldrich, # D-5545), 300 µM ATP (Boehringer Mannheim, # 519987), and 50 µM NADP (Boehringer Mannheim, # 128058), and 0.02 U/ml G-6-P DH (EC 1.1.1.49, Boehringer Mannheim, # 127035). An initial fluorescence measurement (R1) was taken to establish a baseline. Dilute hexokinase (HK) was prepared by adding 25 µL HK (Sigma-Aldrich, # H-5750, 280 U/ml) to 1.0 ml dilute reagent. A 50 µl aliquot of this dilute enzyme was added to each cuvette, and each was incubated in the dark for 60 minutes. A second measurement (R2) was taken. The glycogen concentration (µmoles/g dry wt) was calculated using the following equation:
\[ \frac{\Delta \text{sample} - \Delta \text{blank}}{\Delta \text{standard} - \Delta \text{blank}} \times [\text{standard}] \times \frac{1}{(\text{tissue wt.} \times DF)} \]

where: \( \Delta = R2-R1 \); [standard] = 500 \( \mu \)M (myocardium and skeletal muscle) or 2mM (liver); DF = 1.3 (myocardium) or 2.0 (liver). Mean values of the blanks, standards, and samples were used in the calculations.

**High Performance Liquid Chromatography (HPLC)**

Chromatography is a technique by which mixtures of compounds are separated into individual components, allowing them to be quantified. Separation is achieved by passing a solvent containing the mixture ("mobile phase") across a solid surface ("stationary phase") to which the various compounds can reversibly bind. Different compounds will have different binding strengths to the solid; thus, each compound will be held in place for different durations ("retention time"). The various retention times are used to identify the unknown compounds by comparing them with known standards. *Reversed phase chromatography*, utilized to separate adenine nucleotides, is a specific application of HPLC in which the mobile phase is more polar than the stationary phase. Thus, non-polar or weakly polar compounds can be eluted from the stationary phase by gradually increasing the polarity of the mobile phase. In reversed phase HPLC, the solid surface consists of a column of octadecylsilane bonded to a silica gel, while the mobile phase is a phosphate buffer:methanol:acetonitrile mixture.

Tissues for HPLC analysis were extracted using PCA as detailed above. Sample and solvent delivery was accomplished using a 128 Programmable Solvent Module, and 507e Autosampler (Beckman Instruments Canada Inc., Mississauga, Ont.). Compound separation
was achieved using a Supelcosil LC-18-T column (15.0 cm. long x 4.6 mm I.D. # 5-8970) and a Supelcosil LC-18-T guard column (2.0 cm. x 4.6 mm I.D., Supelco Inc., Bellefonte, PA, # 5-9621). Peak detection was performed using a Beckman 168 Diode Array Detector Module (Beckman Instruments Canada Inc., Mississauga, Ont.) at ultraviolet wavelengths (254 nm). Retention times and peak areas were calculated using System Gold *Nouveau* software (Beckman Instruments Canada Inc., Mississauga, Ont.). The mobile phase was a phosphate buffer, prepared as follows. *Buffer A* consisted of 150 mM KH$_2$PO$_4$ and 150 mM KCl in deionized HPLC-grade water. Buffer pH was adjusted to 6.0 with 12.0 N KOH. *Buffer B* consisted of 80% Buffer A, 15% methanol, and 5% acetonitrile. All buffers were filtered through a 0.45 μm filter before use. A solvent gradient was used to separate tissue metabolites, in which the percentage of Buffers A and B were varied, from 100% A:0%B to a maximum of 60%A:40%B. A 25 μl injection volume of both standards and samples were run at 1.0 ml/min at 2400 PSI. ATP, ADP, and AMP standards were prepared at known concentrations to enable quantification of the sample adenine nucleotides. A representative standard chromatogram with appropriate retention times is shown in Figure 3.
Figure 3. Standard chromatogram.
APPENDIX II: RIBOSE - DURATION STUDY

The studies discussed in this thesis employed a single protocol for the administration of ribose, two injections 12 hours apart, followed by the acute experiment 12 hours after the second injection. However, the time course of the metabolic effects of IV ribose administration is unknown. The following study was conducted to determine when the maximal metabolic protection is seen following the second pretreatment injection. Sprague-Dawley rats were administered D-ribose (100 mg/kg) IV at 12 hour intervals according to the awake study protocol. The acute study was then conducted 3, 4, 6, 7, or 8 hours following the second injection. Table 1 shows in vivo LV and RV metabolites according to the duration of exposure to ribose. No significant differences were found in any of the LV metabolites across the different durations of exposure. Table 2 shows LV metabolite concentrations after 12-minutes of global ischemia from these same groups of rats. No significant effects of the duration of exposure were apparent in the ischemic LV metabolites.

Figure 1 shows the percent decrease in LV concentrations of ATP, TAN, CP, MG, and AnER from in vivo to 12 minutes of global ischemia. There were no significant differences in any parameter across the different durations. Similarly, no differences were found in the rate of utilization (μmoles/g dry wt./minute) of these compounds during global ischemia (Figure 2).
Table 1. *In vivo* myocardial metabolites from Sprague-Dawley rats sacrificed after various intervals post IV ribose administration. Values are mean±SEM (μmoles/g dry wt.).

<table>
<thead>
<tr>
<th></th>
<th>3-hours (n=5)</th>
<th>4-hours (n=1)</th>
<th>6-hours (n=3)</th>
<th>7-hours (n=2)</th>
<th>8-hour s (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LV</td>
<td>RV</td>
<td>LV</td>
<td>RV</td>
<td>LV</td>
</tr>
<tr>
<td>ATP</td>
<td>18.8±1.2</td>
<td>19.9±1.3</td>
<td>18.9</td>
<td>19.5</td>
<td>18.1±1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18.4±1.5</td>
<td>17.8±2.0</td>
<td>19.5±2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13.1±6.8</td>
<td>17.8±2.0</td>
<td>20.3±1.1</td>
</tr>
<tr>
<td>TAN</td>
<td>25.5±1.1</td>
<td>25.8±1.4</td>
<td>29.4</td>
<td>27.0</td>
<td>23.7±1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>23.9±1.4</td>
<td>24.2±1.2</td>
<td>26.9±0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26.2±2.2</td>
<td>25.5±1.5</td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>19.5±3.2</td>
<td>21.4±3.0</td>
<td>13.5</td>
<td>17.8</td>
<td>17.5±3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>19.3±3.9</td>
<td>13.7±12.1</td>
<td>17.9±4.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8.8±7.3</td>
<td>17.9±4.8</td>
<td>22.4±4.3</td>
</tr>
<tr>
<td>MG</td>
<td>140.2±16.3</td>
<td>147.2±17.6</td>
<td>135.2</td>
<td>106.0</td>
<td>127.3±28.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>113.7±11.6</td>
<td>97.5±10.6</td>
<td>128.6±1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>125.0±14.8</td>
<td>119.1±36.5</td>
<td></td>
</tr>
<tr>
<td>AnER</td>
<td>178.5±16.8</td>
<td>188.5±13.8</td>
<td>167.6</td>
<td>143.3</td>
<td>162.9±20.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>151.4±7.2</td>
<td>142.4±5.3</td>
<td>155.2±8.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>162.4±8.6</td>
<td>161.8±33.5</td>
<td></td>
</tr>
</tbody>
</table>

TAN = ATP + ADP + AMP; AnER = ATP + CP + MG
Table 2. Left ventricular metabolites after 12 minutes global ischemia from Sprague-Dawley rats exposed to IV ribose for various durations. Values are mean±SEM (µmoles/g dry wt.).

<table>
<thead>
<tr>
<th></th>
<th>3-hours (n=5)</th>
<th>4-hours (n=1)</th>
<th>6-hours (n=3)</th>
<th>7-hours (n=2)</th>
<th>8-hours (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>7.7±0.8</td>
<td>5.0</td>
<td>6.1±1.5</td>
<td>7.2±0.7</td>
<td>6.7±1.9</td>
</tr>
<tr>
<td>TAN</td>
<td>19.6±2.0</td>
<td>16.4</td>
<td>16.8±2.4</td>
<td>17.7±1.5</td>
<td>16.3±2.8</td>
</tr>
<tr>
<td>CP</td>
<td>4.4±1.6</td>
<td>1.6</td>
<td>1.6±0.2</td>
<td>2.9±0.0</td>
<td>1.9±0.8</td>
</tr>
<tr>
<td>MG</td>
<td>64.4±28.1</td>
<td>51.6</td>
<td>70.2±28.0</td>
<td>27.1±6.2</td>
<td>65.7±37.3</td>
</tr>
<tr>
<td>AnER</td>
<td>76.5±52.3</td>
<td>58.2</td>
<td>78.8±26.6</td>
<td>37.2±6.9</td>
<td>74.3±34.8</td>
</tr>
</tbody>
</table>

TAN = ATP + ADP + AMP; AnER = ATP + CP + MG

Because metabolite concentrations did not show significant differences across the various durations of exposure to ribose, the data from the five groups were combined to form a single group of rats. Table 3 shows the *in vivo* and ischemic metabolite concentrations for this group of rats.

Table 3. *In vivo* LV and RV and ischemic LV metabolites from grouped data. Values are mean±SEM (µmoles/g dry wt.).

<table>
<thead>
<tr>
<th></th>
<th>LV <em>in vivo</em> (n=14)</th>
<th>RV <em>in vivo</em> (n=14)</th>
<th>LV 12 min. ischemia (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>18.0±1.1</td>
<td>19.4±0.2</td>
<td>6.9±0.6</td>
</tr>
<tr>
<td>TAN</td>
<td>25.3±0.7</td>
<td>25.6±0.6</td>
<td>17.8±1.0</td>
</tr>
<tr>
<td>CP</td>
<td>17.5±2.0</td>
<td>19.1±2.0</td>
<td>3.1±0.6</td>
</tr>
<tr>
<td>MG</td>
<td>127.7±8.9</td>
<td>128.4±10.0</td>
<td>59.7±13.2</td>
</tr>
<tr>
<td>AnER</td>
<td>163.2±9.0</td>
<td>166.8±9.1</td>
<td>69.6±12.5</td>
</tr>
</tbody>
</table>

TAN = ATP + ADP + AMP; AnER = ATP + CP + MG

Because it was speculated that ribose requires time to provide metabolic benefits, another group was studied 12-hours following the second ribose injection. The *in vivo* values from the 3-8 hour duration groups were compared with those obtained from Sprague-Dawley rats studied 12-hours following IV ribose administration (Table 4).
Figure 1. Percent decrease in LV metabolites from *in vivo* to 12 minutes ischemia in Sprague-Dawley rats exposed to ribose for various durations. Values are mean±SEM (μmoles/g dry wt.).
Figure 2. Net rate of consumption of LV metabolites during 12 minutes ischemia in Sprague-Dawley rats exposed to ribose for various durations. Values are mean±SEM (μmoles/g dry wt./minute).
Myocardial glycogen concentration was significantly (p≤0.05) higher in the 12-hour group than that of the rats in the grouped data. This resulted in significantly (p≤0.05) higher AnER in the 12-hour group, since there were no differences in either ATP or CP concentrations between groups. Based on these results, 12 hours post-injection was chosen as the optimal interval after which to conduct the acute study for this thesis.

**Table 4.** In vivo LV metabolites from Sprague-Dawley rats exposed to IV ribose for either 3 to 8 hours (Grouped data) or 12 hours. Values are mean±SEM (μmoles/g dry wt.).

<table>
<thead>
<tr>
<th></th>
<th>Grouped Data (n=14)</th>
<th>12-hour Data (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>18.0±1.1</td>
<td>17.26±1.80</td>
</tr>
<tr>
<td>TAN</td>
<td>25.3±0.7</td>
<td>23.34±1.03</td>
</tr>
<tr>
<td>CP</td>
<td>17.5±2.0</td>
<td>14.83±6.75</td>
</tr>
<tr>
<td>MG</td>
<td>127.7±8.9</td>
<td>201.88±38.72*</td>
</tr>
<tr>
<td>AnER</td>
<td>163.2±9.0</td>
<td>233.98±41.77*</td>
</tr>
</tbody>
</table>

TAN = ATP + ADP + AMP; AnER = ATP + CP + MG

* p≤0.05 vs Grouped data.