ROLE OF OXIDATIVE STRESS IN HEART FAILURE SUBSEQUENT TO MYOCARDIAL INFARCTION

Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfilment of the requirements for the Degree of:

DOCTOR OF PHILOSOPHY

BY

MICHAEL F. HILL

Department of Physiology
Faculty of Medicine
1998

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

ROLE OF OXIDATIVE STRESS IN HEART FAILURE SUBSEQUENT TO MYOCARDIAL INFARCTION

BY

MICHAEL F. HILL

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

of

DOCTOR OF PHILOSOPHY

ACKNOWLEDGMENTS

I would like to express my deep gratitude and appreciation to my supervisor, Dr. P.K. Singal, for his guidance, leadership, encouragement, friendship and wealth of support during the course of my studies. His tremendous expertise in academic and personal matters served as a role model for me and in addition, also served as a constant source of inspiration.

I would also like to thank Drs. N.S. Dhalla, I. Dixon and V. Panagia for their assistance and willingness to provide support and helpful suggestions throughout my Ph.D. training.

My sincere thanks to my lab colleagues Dr. Thomas, Neelam, Timao, Julieta, Vince and Dinender for their help and friendship. Special thanks to Mrs. Diane Stowe for her assistance in the typing of this thesis as well as for her friendship during my Ph.D. training.

I would like to acknowledge the Manitoba Health Research Council for their fellowship support during my Ph.D. training.

Finally, I would like to express my gratitude to my parents, who always provided me with encouragement and support, as well as my two sisters who were always there for me.

CONTENTS

LIST OF TABLES

Page No.

LIST OF FIGURES

LIST OF ABBREVIATIONS

CHF	-	Congestive Heart Failure
GSH	-	Reduced Glutathione
GSHPx	-	Glutathione Peroxidase
GSSG	-	Oxidized Glutathione
LVEDP	-	Left Ventricular End-Diastolic Pressure
LVSP	-	Left Ventricular Systolic Pressure
MI	-	Myocardial Infarction
RVEDP	-	Right Ventricular End-Diastolic Pressure
RVSP	-	Right Ventricular Systolic Pressure
TBARS	-	Thiobarbituric Acid Reactive Substances

ABSTRACT

Congestive heart failure (CHF) following myocardial infarction (MI) continues to be an important clinical problem. Although the etiology of heart failure is multifactorial, one mechanism that appears to play a key role is an increase in oxidative stress. We have earlier demonstrated that mild, moderate and severe stages of heart failure subsequent to MI are accompanied by an antioxidant deficit and elevated oxidative stress in the myocardium. The objective of the present research was to characterize regional changes in antioxidants and oxidative stress in the right and viable left ventricles separately in relation to the hemodynamic function in each of the respective ventricles. Another goal of this research was to establish whether a relative deficit in the antioxidant reserve is the cause or simply an effect of the CHF due to MI. The hypothesis tested was that an increase in oxidative stress aided by a deficit in the antioxidant reserve plays a role in the pathogenesis of heart failure subsequent to MI and that chronic antioxidant therapy involving vitamin E may modulate the development of heart failure.

Left ventricular MI was produced in male Sprague-Dawley rats weighing 150 ± 10 g by occlusion of the left coronary artery. Animals were monitored daily for their general behaviour and any clinical signs of heart failure. At the end of 1, 4, 8 and 16 week post-MI periods, left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), right ventricular systolic pressure (RVSP) and right ventricular end-diastolic pressure (RVEDP) were recorded. Viable left and right ventricular tissues were studied separately with respect to antioxidant enzyme activities [catalase (CAT) and

glutathione peroxidase (GSHPx)], glutathione (reduced and oxidized), lipid peroxidation and myocardial content of vitamin E.

In 1 week sham-operated controls, LVEDP was 2.4 ± 0.26 and LVSP was 125 ± 1.0 mmHg. Baseline value for RVEDP was 1.1 ± 0.6 and RVSP was 31.8 ± 0.7 mmHg. Catalase activity in the left ventricle was 31.1 ● 0.61 and in the right ventricle was 31.2 ± 2.4 U/mg protein. Glutathione peroxidase in the left ventricle was 82.1 ± 2.3 and in the right ventricle was 83.7 ± 3.3 nmoles/mg protein. GSH/GSSG ratio in the left ventricle was 13.4 while this ratio in the right ventricle was 14.0. Baseline value for myocardial TBARS in the left ventricle was 66.6 ± 2.8 and in the right ventricle it was 64.5 ± 2.3 nmol/gm wet weight. Thus, except for the systolic pressure being higher in the LV, there were no differences between the left and the right ventricle with respect to any of the parameters studied. All of these values recorded in 1 week sham controls were not changed at 4, 8 and 16 weeks of post-surgical durations. However, in the MI animals, a characteristic pattern of changes was noticed with respect to most of these parameters.

At one week post-MI, rats demonstrated sustained LV function as indicated by the maintenance of LVEDP and LVSP. RVEDP was also maintained while RVSP was increased, indicating hyperfunctionality of the RV. Catalase and GSHPx activities in the viable LV were unchanged while in the RV, a significant increase in their activities was observed. No change in lipid peroxidation was observed in either the viable LV or RV. GSH/GSSG ratio was depressed in the viable LV while a marginal increase in this ratio was seen in the RV. Clinical signs of heart failure involving lung and liver congestion were not evident in these animals.

At 4 weeks post-MI, development of LV failure was manifested in a decreased LVSP and increased LVEDP. RV function was maintained as seen by sustained RVSP and RVEDP. Clinical signs of heart failure were not evident, indicating mild depression of LV function. Catalase and GSHPx activities were reduced in the viable LV and were not changed in the RV. An increase in lipid peroxidation was seen in the viable LV and no change was observed in the RV. GSH levels were decreased and GSSG levels increased in the viable LV, bringing down the ratio of GSH/GSSG, while no changes were seen in the RV.

At 8 weeks post-MI, LVSP was further depressed and LVEDP further increased. As well, RVEDP was elevated while RVSP was maintained. The occurrence of lung congestion in these animals indicated a worsening of cardiac function. Catalase and GSHPx activities were further reduced in the viable LV while no change in their activities was seen in the RV. A further increase in lipid peroxidation was seen in the viable LV while no change was observed in the RV. GSH/GSSG ratio was further depressed in the viable LV and at this stage, a decrease in this ratio was also seen in the RV.

At 16 weeks post-MI, a most pronounced elevation in LVEDP (31.4 ± 1.5 mmHg) and depression in LVSP (89.2 ● 1.4 mmHg) was seen. In addition, RVEDP was increased (10.8 ± 0.8 mmHg) and RVSP decreased (19.9 ± 0.5 mmHg) , indicating failure of the right ventricle. Occurrence of both lung and liver congestion in these animals indicated severe heart failure. Catalase and GSHPx activities in the viable LV were most severely reduced at this time point of post-MI with values of 9.3 ± 0.26 U/mg protein and 41.7 ± 1.3 nmoles/mg protein, respectively. At this stage, these values in the RV (catalase, 18.4 ± 0.62

U/mg protein and GSHPx, 61.4 ● 1.8 nmoles/mg protein) were also decreased but the magnitude of this reduction was less as compared to the viable LV. Lipid peroxidation was maximally elevated in the viable LV (126.8 ± 1.5 nmol/gm wet weight) and an increase was also seen in the RV (95.0 ± 1.1 nmol/gm wet weight). GSH/GSSG ratio was severely reduced in the viable LV (0.86) and also in the RV (3.2).

These characteristic hemodynamic and biochemical changes demonstrated that ventricular failure subsequent to MI is associated with an antioxidant deficit and increased oxidative stress first in the LV, followed by the RV. These oxidative stress changes also correlated with the hemodynamic function in each of the ventricles. In order to establish a "cause and effect" relationship for oxidative stress, beneficial effects of dietary supplementation with vitamin E (a strong biological antioxidant) were analyzed.

For vitamin E treatment, animals in the control and infarcted group were divided into two subgroups prior to infarct induction. One subgroup in each category received vitamin E while the other did not. Vitamin E treatment was administered by dietary supplementation consisting of 1545 mg tocopherol acetate/kg in the basal diet. Treatment of these animals was initiated 2 weeks prior to induction of MI and continued until 16 weeks post-MI. Untreated animals were maintained on a regular rat chow that contained 40 mg/kg vitamin E. In the 16 week post-MI vitamin E treatment group, vitamin E therapy completely normalized RV and significantly improved LV hemodynamic function. Complete normalization of LV function was not achieved. Absence of both lung and liver congestion indicated that the residual cardiac dysfunction was not severe enough to exert any influence on the tissues upstream. Catalase and GSHPx activities were increased in the viable LV,

though these levels remained lower than that of controls. In the RV, catalase and GSHPx activities were normalized to the point that they were not different from respective controls.

Myocardial vitamin E concentration in the LV of 16 week sham controls was 65.4 ± 2.3 and in the RV it was 53.5 ± 3.2 μg/g wet weight. Dietary supplementation with vitamin E increased myocardial vitamin E content in both the LV (105.9 ● 7.5 μg/g wet weight) and RV (81.4 ± 6.8 μg/g wet weight). Myocardial vitamin E content was significantly reduced in the viable LV of untreated 16 week post-MI animals (44.5 ± 6.1 μg/g wet weight) while no change was observed in the RV. In the vitamin E supplemented MI group, the content was 86.5 ± 2.3 μg/g wet weight in the viable LV and 92.8 ± 11.3 μg/g wet weight in the RV. Lipid peroxidation was completely normalized in the RV, while in the viable LV, a reduction was seen. GSH and GSSG levels were completely normalized in both the viable LV and RV as was the GSH/GSSG ratio in each of the ventricles. Thus, improved cardiac function in each of the respective ventricles with vitamin E treatment was associated with an enhanced antioxidant status, improved redox ratio and reduced lipid peroxidation.

In conclusion, it is suggested that decreased antioxidants and increased oxidative stress may have a causative role in the pathogenesis of heart failure subsequent to myocardial infarction. Clinical potential and applicability of these findings in surviving patients with myocardial infarction needs to be addressed.

I. INTRODUCTION

Congestive heart failure (CHF) following myocardial infarction (MI) is a common clinical syndrome with a poor long-term prognosis. About 15-25% of surviving patients with MI ultimately go on to develop overt heart failure in the subsequent 5 years (Kannel et al., 1979; Francis et al., 1993). The syndrome of heart failure is dominated by hemodynamic abnormalities, impaired exercise capacity and fluid retention (Cohn, 1993). Loss of contracting myocardium due to MI results in a chronic increase in the work load of the remaining viable myocardium. If this load is allowed to continue for a prolonged period, cardiac pumping function is compromised and heart failure supervenes.

Clinical as well as basic research efforts have focussed on understanding the pathophysiology of heart failure resulting from a variety of etiologies. Several different mechanisms have been postulated to explain the pathogenesis of heart failure and its associated clinical manifestations, including defects in the production and utilization of high-energy phosphates (Bing, 1983), abnormalities in excitation-contraction coupling and calcium movements (Bing, 1983; Gwathmey et al., 1987), downregulation of β-adrenergic receptors (Bristow et al., 1982; Vatner et al., 1985), alterations in ventricular geometry and architecture (Gaudron et al., 1993a; Pfeffer and Braunwald, 1990; McKay et al., 1986), free radicals and lipid peroxidation (Belch et al., 1991; Dhalla and Singal, 1994; Siveski-Iliskovic et al., 1994) and apoptosis (Narula et al.,1996; Yao et al., 1996). However, as no single mechanism fully explains the development of the depressed cardiac function, it is likely that the cause is multifactorial. In this regard, increased free-radical mediated lipid peroxidation

and oxidative stress appears to play an important role in the pathogenesis of cardiac dysfunction and failure (Kaul et al., 1993; McMurray et al., 1990; Sobotka et al., 1993; Dhalla et al., 1996; Khaper and Singal, 1997; Hill and Singal, 1997).

Although a relative deficit in the myocardial antioxidant reserve and concomitant increase in oxidative stress have been reported in the surviving myocardium during heart failure subsequent to MI (Hill and Singal, 1996; Khaper and Singal, 1997), regional changes in antioxidants and oxidative stress remain to be examined. In the present study, the question of regionally specific changes was approached by comparing the antioxidant enzyme activities and oxidative stress in the viable left and right ventricles separately in relation to the hemodynamic function. Thus, using an already established animal model of heart failure (Hill and Singal, 1996), myocardial levels of catalase and glutathione peroxidase, vitamin E, lipid peroxidation, reduced and oxidized glutathione and hemodynamics were analyzed in each of the ventricles at 1, 4, 8 and 16 weeks post-MI.

Another question that remains to be resolved is whether increased free radical-mediated lipid peroxidation and oxidative stress was the "cause" or an "effect" of heart failure. We approached this problem by instituting a chronic antioxidant treatment therapy involving dietary vitamin E supplementation and examining its effects on myocardial antioxidants and oxidative stress in relation to hemodynamic function in each of the ventricles.

The findings described in this study advance our understanding of the pathogenesis of heart failure and in addition, provides further evidence of a novel therapeutic

strategy for preventing/mitigating its progression. The results from this study suggest that surviving patients with MI may accrue benefit from vitamin E supplementation.

II. LITERATURE REVIEW

A. GENERAL BACKGROUND

Heart failure constitutes the most rapidly rising population with cardiovascular disease globally and is presently amongst the commonest of all cardiovascular syndromes in North America. This can be attributed, in part, to the fact that heart failure is a common clinical endpoint of many cardiac conditions. Because of improved emergency help as well as wider use of cardiopulmonary resuscitation, the number of patients surviving from heart attacks has increased. However, this group of myocardial-infarct patients are also prone to eventual heart failure. Since the prevalence of heart failure is age-dependent and the number of people over the age of 65 is steadily growing, its incidence will continue to rise as our population ages. Heart failure continues to be the most frequent diagnostic-related group diagnosis (Geltman, 1993) and the 5-year mortality from the time of diagnosis is ≈ 50% (Armstrong and Moe, 1994). Therefore, heart failure remains a daunting therapeutic challenge in view of continued mortality. As a result, efforts focussing on improving our understanding of the pathophysiology of this syndrome have intensified to develop new therapeutic strategies aimed not only towards treatment of the established condition, but also at preventing or retarding its progression.

B. MECHANISMS OF HEART FAILURE

Both acute and chronic forms of heart failure involve mechanical dysfunction during systolic and/or diastolic phases of the cardiac cycle (Morgan et al., 1990) and is suggested to result from a variety of subcellular abnormalities (Dhalla et al., 1982). Diverse metabolic

as well as morphological alterations involving abnormalities in myocardial energy and calcium metabolism, neurohormonal changes, alterations in contractile proteins and gene expression as well as ventricular remodeling have been observed in the failing myocardium. More recently, a surge in experimental and clinical studies have begun to document and characterize the phenomenon of oxidative stress as a novel and important mechanism in the development of heart failure. A detailed account of the proposed pathophysiological mechanisms that may underlie the development and progression of heart failure is provided below.

B.1. Abnormalities in Myocardial Energy Metabolism

Under normal conditions, myocardial energy metabolism is aerobic. A substantial quantity of ATP, needed for contraction as well as relaxation of the myocardium, is supplied by fatty acids and carbohydrate oxidation in the mitochondria. This high level of oxidative capacity in the heart is also reflected morphologically as mitochondria occupy approximately 35% of cardiac cell volume (Page et al., 1974). During the 1960's and early 1970's, considerable argument revolved around the question of abnormal energy production during cardiac failure (Chidsey et al., 1966; Schwartz and Lee, 1962). The gradation of mitochondrial oxidative phosphorylation with the severity of myocardial failure was first reported in cardiomyopathic hamsters (Lindenmayer et al., 1970) and in rabbits with aortic coarctation (Sardahl et al., 1973). In both of these animal models, mitochondrial oxidative phosphorylation in a severe heart failure stage was depressed. Early studies using overloaded guinea pig hearts demonstrated diminished ATP and creatine phosphate (CP) stores (Feinstein, 1962). Furthermore, significant depressions in creatine

phosphate concentrations in the right ventricle of cats with right ventricular failure secondary to pulmonary artery constriction was also reported (Pool et al., 1967). A deficit in high-energy phosphate stores in the failing human heart has also been documented (Ingwall, 1993). Myocardial high-energy phosphate levels, as indexed by the CP/ATP ratio, were significantly depressed in patients suffering from congestive heart failure (CHF) secondary to dilated cardiomyopathy (Hardy et al., 1991). Therefore, a defect in myocardial energy production was viewed as the major problem. However, it is now generally accepted that depressed mitochondrial oxidative phosphorylation accompanies only severe end-stage heart failure, whereas during the compensated stage, mitochondrial respiration may be normal or only slightly depressed (Newman, 1983). Thus, abnormalities in high energy phosphate metabolism by itself, may not be the inciting stimulus for pump dysfunction associated with cardiac failure.

Profound structural abnormalities of myocardial mitochondria have been identified in both the experimental and clinical setting of heart failure. Decrease in mitochondrial size and structural injury (loss of matrix granules, disruption of cristae) of these energy producing organelles have been reported in dogs with CHF (Sabbah et al., 1992a). Similar alterations in mitochondrial morphology have been reported in CHF patients (Hatt, 1988; Perennec and Hatt, 1988). These ultrastructural abnormalities may be responsible, in part, for the reductions in high energy phosphate production that reportedly occurs in the late stages of heart failure. However, more specific studies are needed to establish a cause and effect relationship between mitochondrial structural defects and deficient energy production during the development of heart failure.

B.2. Defects in Intracellular Calcium Handling

Appropriate release and uptake of intracellular calcium is necessary for the normal systolic and diastolic function of the mammalian heart. Intracellular calcium homeostasis is maintained by sarcolemmal calcium transport mechanisms including sodium-calcium exchanger, an energy-dependent calcium pump and calcium channels. In addition, the sarcoplasmic reticulum (SR), a highly specialized network of subcellular membranes, is also believed to participate in the regulation of cytoplasmic calcium levels. SR vesicles isolated from cardiac muscle have been shown to accumulate calcium in an energy-dependent manner (Dhalla et al., 1982; Levitsky et al., 1976).

A progressive decrease in SR calcium uptake in the viable left ventricle during the development of CHF subsequent to left ventricular myocardial infarction (MI) in rats has been reported (Afzal and Dhalla, 1992) and similar findings of reduced calcium sequestration by the SR have also been reported in the myocardium of humans with terminal heart failure (Beuckelmann et al., 1992). At the level of the sarcolemma, sodium-calcium exchange and calcium pump activities have also been shown to be depressed during heart failure subsequent to MI as well as during ischemia-reperfusion (Dixon et al., 1992; Makino et al., 1988). These abnormalities are thought to partly account for the occurrence of intracellular calcium overload and prolonged myocardial relaxation associated with heart failure. These studies provide support for the hypothesis that the handling of intracellular calcium is abnormal in the failing heart (Morgan, 1991).

The result of these alterations in both hypertrophied and failing hearts is a prolonged relaxation due to decreased velocity of lengthening, which has been shown to be

accompanied by abnormal calcium handling involving the sarcoplasmic reticulum (Lompre et al., 1991). In experimental cardiac hypertrophy, there is a decrease in calcium transport by the sarcoplasmic reticulum, with a further decrease seen in the failing heart (Schwartz et al., 1993; De La Bastie et al., 1990). Severe hypertrophy of the left ventricle of rat heart resulted in a decrease in the number of functionally active calcium-ATPase molecules, leading to a decrease in the density of the calcium pumps (De La Bastie et al., 1990). This results in an alteration in the function of the sarcoplasmic reticulum and impaired calcium movements in the hypertrophied myocardium. A decrease in the myocardial level of the mRNA encoding the calcium-ATPase of the sarcoplasmic reticulum has been shown during experimental cardiac hypertrophy and failure as well as in the human ventricle during end-stage heart failure (Mercadier et al., 1990). A causative role of these abnormalities in heart failure is not established.

B.3. Contractile Proteins and Gene Expression Alterations

In the heart, the basic unit of contraction is the sarcomere, which is composed of a diverse set of proteins working in a well coordinated fashion to generate force of contraction. Two major components of the sarcomere are the thick and thin filaments. The thick filaments are made up of myosin molecules each containing two heavy chains and four light chains. The thin filaments are composed predominantly of actin, tropomyosin and troponin complex. The thick filament may contain two possible myosin heavy chain (MHC) isoforms, α-MHC and β-MHC, while the thin filament may contain two actin isoforms, α-skeletal actin and α-cardiac actin.

Cardiac hypertrophy and subsequent heart failure induced by hemodynamic overload have been shown to be accompanied by a process of gene reprogramming. The latter is characterized by both qualitative and quantitative changes in the cardiomyocyte gene expression. The qualitative changes involve a differential expression of multigene families of contractile proteins involving myosin and actin. These changes presumably represent an adaptive response to the new functional demands placed upon the viable myocardium. In a chronic increase in hemodynamic overload on the heart, an analysis of the mRNA levels indicated activation of the β-MHC gene while the α-MHC gene was deactivated (Schwartz et al., 1993). This results in a slower rate of ATP cycling by myosin, which is believed to account for the slower velocity of contraction in the hypertrophied heart. With respect to actin, there was increased expression of α-skeletal actin mRNA during hypertrophy (Schwartz et al., 1986). Since β-MHC and α-skeletal actin mRNA are predominant at birth in the rat ventricles, the idea that reactivation of a fetal gene program occurs with cardiac hypertrophy was developed. With respect to human heart, isomyosin composition in the ventricles does not change during hypertrophy because they normally contain almost exclusively high levels of β-MHC. The atria however did show changes in isomyosin composition (Schwartz et al., 1993).

Until recently, isoactins were less well studied and it was unclear whether cardiovascular diseases in humans were accompanied by isoactin expressions. Cardiac tissue samples obtained from patients undergoing cardiac transplantation due to advanced stages of heart failure showed that the percentage of α-skeletal actin mRNA of all pathological hearts was the same as controls (Schwartz et al., 1993; Boheler et al., 1991). Thus, it can be

seen that myosin heavy chain and actin multigene families are independently regulated in human heart and are expressed in a specific fashion. In addition to a modulation of genes responsible for contractile properties of the myocardium, as discussed earlier there are changes in the expression of genes influencing myocardial relaxation through sarcoplasmic reticular function. These studies demonstrate that cardiac hypertrophy as well as failure are accompanied by a complex modulation of a battery of genes responsible for the relaxation and contractile proteins of the heart muscle and that characteristic changes in isogene expression of hypertrophied myocardium during relaxation and contraction may also contribute to heart failure following myocardial hypertrophy.

B.4. Chronic Overactivation of the Sympathetic Nervous System

CHF has been shown to be accompanied by overactivation of the sympathetic nervous system. In the initial stages of CHF, sympathetic activation provides an immediate means of supporting blood pressure in face of a low cardiac output by virtue of its ability to stimulate myocardial contractility, heart rate and systemic vasoconstriction (Packer et al., 1987; Francis et al., 1984). Plasma concentrations of norepinephrine have been documented to reflect the degree of activity of the sympathetic nervous system (Leimbach et al., 1986; Lake et al., 1976).

In patients with chronic, clinically stable CHF, there is a trend for plasma norepinephrine to gradually increase over time (Francis et al., 1988). Plasma norepinephrine has also been shown to increase progressively in an experimental pacing model of CHF in dogs (Riegger et al., 1984). In addition, it has also been reported that plasma norepinephrine levels are elevated in patients with asymptomatic left ventricular dysfunction, with a further

increase seen with the progression to overt CHF (Francis et al., 1990). Chronic sympathetic overactivation may exert deleterious effects on cardiac function through toxic effects of catecholamines (Singal et al., 1982) as well as by exacerbating any preexisting imbalance between myocardial oxygen supply and demand. Prolonged sympathoadrenergic activation may also directly provoke spontaneous and lethal ventricular tachyarrhythmias (Lown and Verrier, 1976; Sabbah et al., 1992b). The magnitude of sympathetic activation in CHF has been suggested to independently predict survival. In this regard, the annual mortality rate of patients with venous plasma norepinephrine concentrations > 800 pg/ml may exceed 70% (Floras, 1993; Cohn et al., 1984; 1991). Furthermore, data from the Studies of Left Ventricular Dysfunction (SOLVD) Trial indicated that sympathetic excitation actually precedes the development of clinically recognized heart failure (Ferguson, 1993; Francis et al., 1990). Taken together, these observations suggest that sympathetic activation plays an important pathogenetic role in this disorder and in addition, appears to provide important prognostic information regarding outcome for CHF patients.

Although in heart failure, plasma concentrations of norepinephrine have been shown to be markedly elevated, myocardial levels have been reported to be profoundly depressed. In guinea pigs with left ventricular failure and in dogs with right ventricular failure, a striking reduction of norepinephrine concentration was observed in all chambers of the heart (Spann et al., 1964; Chidsey et al., 1965). Total norepinephrine content in both the left and right ventricles of these animals were reduced to values of less than 20% of normal (Chidsey et al., 1964). The reduction in myocardial norepinephrine stores in dogs with CHF was found to be so severe that the response to tyramine administration, which acts by releasing

norepinephrine, was virtually absent in the isolated papillary muscle (Chidsey et al., 1964). The mechanism responsible for this reduction in myocardial norepinephrine stores has not been elucidated. However, it has been suggested that a prolonged increase in cardiac sympathetic nerve activity may exhaust the norepinephrine stores in the heart (Chidsey et al., 1964). Taking both the experimental and clinical findings together, it is clear that CHF is associated with substantial reductions in cardiac norepinephrine stores.

B.5. Augmentation of Adrenergic Receptors

The hallmark of heart failure is decreased myocardial inotropic function. During periods of increased cardiac workload (such as following myocardial infarction), initial response is increased contractility. This is facilitated by the release of norepinephrine from sympathetic nerves and epinephrine from the adrenal gland. These catecholamines enhance the contractile response by interacting with a transmembrane signalling system located within the sarcolemma of myocytes which consists of adrenergic receptors, signal transduction complex, G proteins and an effector enzyme. In the heart, the stimulatory guanine nucleotide-binding protein (G_s) couples β-adrenergic receptors with activation of the effector enzyme adenylyl cyclase. In contrast, the inhibitory guanine nucleotide-binding regulatory protein (G_i) couples inhibitory receptors with inhibition of the effector enzyme. Stimulation of adenylyl cyclase leads to the synthesis of the intracellular second messenger, cAMP. cAMP activates protein kinase A which results in phosphorylation of calcium channels and subsequent enhancement of cardiac contractility. Increased activation of the β-adrenergic system is an important compensatory mechanism which supports cardiac function and meets circulatory needs.

In the 1960's, it was recognized that heart failure was associated with a diminished response to adrenergic stimulation (Covell et al., 1966). Subsequent studies demonstrated that in patients with CHF, myocardial β-adrenergic receptor density and responsiveness are severely diminished (Bristow et al., 1982; 1986; Denniss et al., 1989; Murphree and Saffitz, 1989), which in turn confers a subsensitivity to β-adrenergic receptor-mediated contractile events. Investigations on membranes derived from human left and right ventricular tissue showed that these tissues contain two types of β-adrenergic receptor subtypes: β_1 and β_2. In the non-failing heart, the predominant receptor is the β_1 subtype (Bristow et al., 1986). However, in the failing heart, there was a reduction in the β_1-receptor density while β_2-receptor density remained unchanged (Asano et al., 1988), resulting in a shift in the ratio of β_1/β_2 receptors from 80:20 in non-failing to 60:40 in a failing heart. Thus, there appears to be a selective downregulation of β_1-adrenergic receptors and the β_2-receptor subpopulation becomes relatively more important in mediating the inotropic support in response to nonselective β-agonist stimulation as well as being a prime target for inotropic stimulation by selective β_2-agonists (Bristow et al., 1986).

More recently, it has been documented that the marked alteration in β_1:β_2 receptor density ratio does not occur in a spatially uniform distribution in patients with CHF. Rather, it results primarily from loss of receptors in the subendocardium (Beau et al., 1993). This important finding suggests that a substantial transmural gradient may exist in the distribution of β-adrenergic receptor subtypes in the failing myocardium (Beau et al., 1993). These desensitization changes in β-adrenergic receptors limit the functional capacity of the failing heart because of the blunted inotropic response to any given number of agonist-occupied

receptors (Bristow, 1993). These findings provide evidence for the key role played by the β-adrenergic nervous system in mediating the natural history of heart failure.

In addition to downregulation of β-adrenergic receptors, uncoupling of the receptor from adenylyl cyclase has also been reported. Considerable evidence in support of this phenomenon stems from studies that have shown an increase in the activity of G_i and a decrease in the activity of G_s in human heart failure (Feldman et al., 1988; Karliner and Scheinmen, 1988; Denniss et al., 1989; Bohm et al., 1990). Although the pathophysiological mechanisms responsible for this receptor uncoupling remain undefined, these abnormalities clearly contribute to the insensitivity of the failing heart to adrenergic stimulation.

Myocardial responsiveness to adrenergic stimulation is also mediated by α-adrenergic receptors. However, in contrast to the two subtypes of β-receptors, only one subtype of α-adrenergic receptors (α_1) are found in human ventricular myocardium (Bohm et al., 1988; Braunwald et al., 1988). These receptors are also coupled by a G-protein. Stimulation of the α_1 receptor activates phospholipase C, which mediates the hydrolysis of phosphotidylinositol 4,5-biphosphate into diacyglycerol (DAG) and inosital triphosphate (IP_3). DAG activates protein kinase C which results in the subsequent phosphorylation of cellular proteins, whereas IP_3 mobilizes calcium from the sarcoplasmic reticulum, thereby increasing the amount of calcium available to interact with contractile myofilaments. α_1-adrenergic receptors account for a very minute proportion of the total adrenergic receptor pool in human ventricular myocardium (Feldman, 1993). It has been shown that α_1 receptor density remains unaltered in the failing human heart (Bristow et al., 1988). However, subsequent studies have demonstrated that stimulation of α_1 receptors exerts a positive inotropic effect in both

normal and failing hearts (Landzberg et al., 1991; Colucci, 1993), though the magnitude of the α_1-mediated contractile response is significantly lower in heart failure patients (Colucci, 1993). These observations suggest that the α_1-adrenergic receptor pathway is also attenuated in CHF.

B.6. Ventricular Remodeling

The term "ventricular remodeling" refers to global changes in ventricular chamber size, shape and mass that develop in a complex and coordinated fashion in response to altered myocardial loading conditions (Grossman and Lorell, 1993). More recently, the term has also included changes occurring at the cellular level in both myocyte and the extracellular matrix compartments (Sabbah and Goldstein, 1993). The clinical setting in which ventricular remodeling occurs in a prominent fashion is after myocardial infarction (Grossman and Lorell, 1993). Following myocardial infarction, there are major alterations in the topography of both infarcted and noninfarcted ventricular regions (Pfeffer and Braunwald, 1990). In the infarct region, viable myocardium is replaced with non-contractile connective tissue and "infarct expansion" occurs which is defined as acute dilation and thinning of the area of infarction not explained by additional myocardial necrosis (Hutchins and Bulkley, 1978). Infarct expansion has been demonstrated to serve as the basis for aneurysm formation and subsequent cardiac rupture (Hochman and Bulkley, 1982; Schuster and Bulkley, 1979) and is an important substrate for left ventricular dilation as shown by echocardiography (Eaton et al., 1979; Erlebacher et al., 1984).

A significant body of evidence from experimental and clinical studies have demonstrated a loss of intrinsic contractility in the surviving myocardium after transmural

myocardial infarction (Mill et al., 1990). As a result, intense interest regarding the remodeling of the noninfarcted myocardium, remote from the zone of infarction, has begun to emerge. Myocyte cell lengthening with a disproportionate increase in cell diameter leads to a reduction in the wall thickness-to-chamber radius ratio (Micheletti et al., 1993). As a result, myocardium remote from the area of infarction is subjected to an increased diastolic wall stress (Weisman et al., 1985). This increased stress can lead to myocyte slippage, resulting in a decreased number of myocytes across the wall (Weisman et al., 1988) and concomitant ventricular wall thinning. These architectural rearrangements of myocytes in the unaffected regions of the infarcted ventricle contribute in the ventricular dilation (Micheletti et al., 1993).

Increasing evidence shows that dilatation of the remnant left ventricle plays a central role in the development of chronic heart failure after myocardial infarction (Gaudron et al., 1993a). Ventricular dilation during the post-myocardial infarction period has been demonstrated to be accompanied by increased left ventricular end-systolic volume (White et al., 1987; Hammermeister et al., 1979) and this derangement has been shown to be associated with depressed ventricular function and poor survival in both animals and patients (Anversa et al.,1993; Pfeffer et al., 1988a; Warren et al., 1988). Thus, ventricular chamber enlargement, when left unattended for a prolonged period of time, results in progressive cardiac dysfunction and ultimately heart failure.

B.7. Extracellular Matrix

Alterations in the extracellular matrix (ECM) have also been suggested to contribute to the development of heart failure subsequent to myocardial infarction (Pelouch et al.,

1993a; Ju and Dixon, 1995; Dixon et al., 1996a) and cardiomyopathy (Dixon et al., 1997). In the case of myocardial infarction, recent studies have revealed abnormal collagen deposition in the scar region as well as in the remaining, viable myocardium remote from the site of infarction (Dixon et al., 1996b; Ju et al., 1997). The cardiac ECM acts to direct the contractile force produced by myocytes and in addition, contributes to the passive stretch characteristics of the ventricular chamber (Pelouch et al., 1993b). The cardiac ECM is predominantly composed of collagen, with collagen types I and III being the most abundant forms within the heart (Speiser et al., 1991; Bashey et al., 1992). Concentration of collagen in the cardiac interstitium is dependent on the relative balance between synthetic pathways and degradation of collagen mediated by matrix metalloproteinases (MMP's) (Dixon et al., 1996a). Results from experimental studies (Pelouch et al., 1993a; McCormick et al., 1994; Cleutjens et al., 1995) and those from clinical investigations (Volders et al., 1993; Weber, 1989) have provided incremental evidence for increased deposition of collagen proteins in regions of the left ventricle remote from the zone of infarction. This abnormal increase in myocardial collagen concentration results in myocardial fibrosis (Weber and Brilla, 1992) which manifests itself as reactive interstitial and perivascular fibrosis (in the surviving myocardium) and replacement fibrosis for necrosed cardiac muscle (Weber and Brilla, 1991). Development of cardiac fibrosis may be attributed to either increased collagen synthesis, reduced collagen degradation, or both (Dixon et al., 1997). Excessive collagen deposition may lead to diastolic dysfunction by increasing cardiac muscle stiffness and reducing ventricular chamber compliance (Litwin et al., 1991). Presence of excessive cardiac collagen has also been reported in human dilated cardiomyopathy (Bishop et al., 1990; Schaper et al.,

1991), in the Syrian hamster with genetic cardiomyopathy (Dixon et al., 1997), and pressure-induced hypertrophy (Dhalla et al., 1996).

Recent studies suggest that the renin-angiotensin system plays an important role in the stimulation of myocardial fibrosis (Brilla et al., 1990). Upregulation of angiotensin type 1 receptors (AT_1) has been shown to occur in cardiomyopathic hamsters (Lambert et al., 1995). Administration of losartan, an AT_1 blocker, resulted in a significant decrease in cardiac fibrosis in post-MI rat hearts and this reduction was associated with an improved left ventricular function in these experimental animals (Ju et al., 1997). These studies provide further evidence linking cardiac ECM remodeling in the development of heart failure.

B.8. Excessive Activity of the Renin-Angiotensin System

Chronic heart failure is characterized by elevated intracardiac and venous pressures and low cardiac output (Hodsman et al., 1988). Activation of the renin-angiotensin system (RAS) results in salt and water retention and an increase in peripheral vascular resistance in an effort to increase blood volume, blood pressure and organ perfusion. These effects are mediated by the generation of an effector peptide, angiotensin (Ang) II. Ang II formation is initiated by renin, a proteolytic enzyme that is stored and secreted by the juxtaglomerular apparatus within the kidney. Upon appropriate stimulation, renin is secreted and acts on angiotensinogen to form the decapeptide Ang I. Ang I is cleaved by the action of angiotensin converting enzyme (ACE) which leads to the generation of the effector peptide Ang II. Ang II is one of the most potent pressor substances and it exerts a direct effect on smooth muscle cells and also stimulates the release of aldosterone from the adrenal gland. The effects of Ang II are mediated by two types of specific receptors: AT_1 and AT_2.

In addition to circulating renin-angiotensin (Campbell et al., 1991), it has now been firmly established that there is the existence of local RAS in several organs, including the heart (Lee et al., 1993; Dzau, 1988; Campbell and Habener, 1986; Dzau et al., 1987). Whereas the circulating RAS appears to be responsible for acute effects, the tissue RAS seems to participate in mediating more chronic processes such as secondary structural changes and may therefore contribute to a variety of cardiovascular disorders, including CHF (Lee et al., 1993).

Examination of the role of the RAS in CHF was neglected until the early 1980's. At this time, the first studies to document a pathophysiological role for RAS in the development of CHF were from surviving patients with myocardial infarction. Plasma renin and aldosterone values were not found to be elevated in patients with uncomplicated myocardial infarction (Michorowski and Ceremuzynski, 1983; Dzau et al., 1981). However, in contrast, significant elevations in plasma renin and Ang II levels were observed in acute myocardial infarction patients in whom left ventricular failure developed (Michorowski and Ceremuzynski, 1983; Brivet et al., 1981; Vaney et al., 1984). Furthermore, an increase in myocardial mRNA for angiotensinogen and ACE was observed in patients with asymptomatic left ventricular dysfunction (Francis et al., 1993). These findings suggest some involvement of RAS in the pathogenesis of the syndrome of CHF.

Evidence for enhanced activity of RAS during CHF has also been obtained from various animal studies. Banding of rat aorta has been shown to be associated with significant upregulation of angiotensin mRNA during the transition from hypertrophy to heart failure (Baker et al., 1990). Volume-overload induced by aortocaval fistula in rat resulted in an

elevation in left ventricular renin mRNA levels (Boer et al., 1994). In addition, heart failure due to mitral regurgitation in the dog (Dell' Italia et al., 1995) and pacing-induced tachycardia in the rat (Finckh et al., 1991) was associated with substantial increases in intracardiac Ang II and angiotensinogen mRNA.

Use of ACE inhibitors in the treatment of heart failure patients has resulted in an improvement in hemodynamic indexes as well as clinical symptoms and quality of life (Gavras et al., 1978; Davis et al., 1979; Turini et al., 1979; Ader et al., 1980; Dzau et al., 1980). An improvement in the severity of heart failure and its other clinical manifestations have been shown to occur in patients treated with ACE inhibitors (Dzau et al., 1980; Levine et al., 1980). The results of these clinical trials demonstrate that increased activity of the RAS contributes directly to the hemodynamic and clinical abnormalities characteristic of heart failure.

B.9. Hyporesponsiveness to Atrial Natriuretic Peptide

Atrial natriuretic peptide (ANP) was first described more than a decade ago (De Bold et al., 1981) and since then, it has become one of the most familiar and thoroughly studied natriuretic peptides of cardiac origin. ANP is secreted in response to atrial distention or stretch (De Bold et al., 1981). The biological actions of ANP are mediated by binding of the peptide to two classes of specific membrane receptors, ANP_a and ANP_b. Amongst its many actions, ANP has been shown to relax vascular smooth muscle, inhibit activity of RAS and modulate the release of vasopressin (Brenner et al., 1990). These multiple biological actions indicate the importance of ANP in the regulation of body fluid and cardiovascular homeostasis.

Perturbations in the ANP system have been documented in a variety of pathophysiological conditions, particularly those of edematous disorders (Brenner et al., 1990). CHF is one of the most common pathological entities that is associated with aberrations in the ANP system (Winaver et al., 1995). Circulating levels of ANP have been reported to be markedly elevated in patients with CHF (Nakaoka et al., 1985; Shenker et al., 1985; Burnett et al., 1986). It has been postulated that the significant rise in circulating ANP in CHF patients occurs in response to the marked atrial distension that accompanies the elevation of left and right atrial pressures (Packer et al., 1987). Consequently, plasma ANP levels are thought to bear a direct correlation to the severity of cardiac failure (Nakaoka et al., 1985).

Increases in circulating ANP have been viewed as an important adaptive mechanism that aids the failing heart by virtue of reducing intravascular blood volume and venous return while at the same time, producing systemic vasodilation. However, the occurrence of salt and water retention despite high circulating levels of ANP in patients with CHF suggested that there may be derangements in the ANP system during CHF. Initial studies aimed at examining the pathophysiological alterations of the ANP system demonstrated that the natriuretic response to exogenous ANP administration was attenuated in rats with experimental heart failure due to A-V fistula (Abassi et al., 1990; Winaver et al., 1988). The actions of ANP on the kidney in particular, have been demonstrated to be markedly attenuated in patients with CHF. Infusion of ANP produced little change in urinary sodium or water excretion in these patients (Cody et al., 1986; Winaver et al., 1995). The blunted renal response to ANP is now thought to be part of a more generalized target organ

hyporesponsiveness to the hormone in CHF. Proposed mechanisms that may contribute to the development of end-organ resistance to ANP include downregulation of ANP receptors in response to high circulatory ANP levels and increased activity of RAS which opposes the actions of ANP within the kidney (Winaver et al., 1995; Koepke et al., 1987). Thus, markedly elevated ANP levels carry an unfavourable long-term prognosis for CHF patients and serves to underscore its key homeostatic role in preserving fluid balance and cardiovascular function.

B.10. Apoptosis

Apoptosis was first described in 1972 as a cell death mechanism that was truly distinct from necrosis (Kerr et al., 1972). In contrast to necrosis, which is a type of cell death characterized by loss of membrane integrity and inflammatory responses secondary to severe trauma or injury to the cell, apoptosis is an active, tightly regulated, energy requiring process in which cell death proceeds in an orderly and controlled manner and follows a defined program (Kerr et al., 1972; Wyllie et al., 1980; Searle et al., 1982; Barr and Tomei, 1994). Thus, apoptosis is frequently referred to as "programmed cell death" (Buttke and Sandstrom, 1994). Unlike necrosis, apoptosis is generally of a non-inflammatory nature due to the fact that the apoptotic cell does not swell or rupture prior to its being engulfed by macrophages and this process of phagocytosis also occurs in a rapid fashion (James, 1993). Fragmentation of chromosomal DNA is the biological hallmark of apoptosis (Arends et al., 1990; Bursch et al., 1990). This process has been shown to be associated with the abnormal expression of such genes as p53 (Yonish-Rouach et al., 1991) and c-*myc* (Evan et al., 1992) or a deficiency of other genes such as bcl-2 (Hockenbery et al., 1990).

Apoptosis has been viewed as a valuable process for removing cells that are no longer useful or are potentially harmful, e.g. benign and malignant tumors. However, as is the case with many biological processes that go awry, the end result is disease. Apoptosis is no exception, with both acute and chronic disregulation of cell death contributing to a variety of diverse human diseases (Barr and Tomei, 1994). Due to the complex integration of multiple apoptotic signals, there is a multitude of control points where normal control of cell death may be lost. As a result, ongoing investigation to define the role of apoptosis in the decline of myocardial function is a particularly active area.

Recent reports have detected the occurrence of programmed cell death in ischemic/reperfused rabbit myocardial tissue but not in normal or ischemic-only rabbit hearts (Gottlieb et al., 1994). This suggested that apoptosis may be unique to reperfusion injury. This finding spurred on further studies to assess the contribution of apoptosis to infarct size. Programmed cell death was found to be the major initial form of myocardial damage produced by occlusion of the left main coronary artery in rats as confirmed by electrophoretic analysis of the DNA extracted from ischemic myocardium (Kajstura et al., 1996). Acute myocardial infarction in patients has also been shown to be characterized by activation of programmed cell death in the surviving portion of the left ventricular free wall, with apoptosis affecting nearly 12% of the myocardial population in the region bordering on the infarct and approximately 1% of myocytes remote from the area of necrotic tissue (Olivetti et al., 1996). Furthermore, it has also been reported that a subset of myocytes undergo apoptosis after acute-MI in patients with patent infarct-related arteries (Saraste et al., 1997). These studies demonstrate that apoptosis may act as a confounding factor in acute

myocardial infarction by increasing the magnitude of myocyte cell death. Size of the infarct has been reported to be an important determinant of prognosis in humans, with acute infarcts comprising ≥ 40% of the left ventricular mass being associated with intractable cardiogenic shock and resultant heart failure (Caulfield et al., 1976).

The role of apoptosis in the development of CHF has been further suggested by the occurrence of this phenomenon in patients with end-stage heart failure. Examination of explanted hearts from patients with idiopathic dilated cardiomyopathy (IDCM) obtained during cardiac transplantation revealed histochemical evidence of DNA fragmentation (Narula et al., 1996; Yao et al., 1996).

C. FREE RADICALS, ANTIOXIDANTS AND OXIDATIVE STRESS

In recent years, considerable evidence has accumulated to suggest that changes in free radical production and antioxidant status may play a role in the pathogenesis of cardiac dysfunction and heart failure (Kaul et al., 1993). Although the involvement of free radicals in various types of tissue injury has been known for some time, their role in cardiac abnormalities was first documented by early studies dealing with catecholamine-induced cardiomyopathy (Singal et al., 1982), adriamycin-cardiomyopathy (Singal et al., 1987) and ischemia-reperfusion injury (Bolli, 1988). These early studies have been followed by a series of elegant and detailed experiments which have provided incremental evidence supporting free radical involvement in cardiac injury (Kaul et al., 1993).

C.1. Free Radicals

In the simplest terms, a free radical is any atom or molecule that has an unpaired electron in their outer orbit making that atom or molecule a highly reactive species. Free

radical production occurs via the addition of an electron or by it's removal in a reduction/oxidation reaction. Due to its unique diradical configuration, oxygen is a major intracellular source of radical species. A sequential univalent reduction of oxygen gives rise to reactive intermediate products (Kaul et al., 1993; Singal et al., 1988). A single electron reduction of oxygen gives rise to superoxide anion, which can act as both a reducing and an oxidizing agent. The relatively short half life of superoxide anion limits its diffusion away from the site of its generation. Two electron reduction of oxygen yields the nonradical species, hydrogen peroxide. Hydrogen peroxide has a relatively long half life and therefore can travel significant distances, causing damage at sites distant from its origin. A three electron reduction of oxygen yields hydroxyl radical, which is the most reactive and potent of all the free oxygen radicals. Addition of a fourth electron results in the formation of water. All of these reactive oxygen intermediates are called *activated oxygen species* and are collectively termed as *partially reduced forms of oxygen* (PRFO) (Singal and Kirshenbaum, 1990). Endogenous sources of free radicals are the numerous enzyme and non-enzyme systems located in the subcellular membranes, plasma membrane and blood cell elements.

One mechanism of tissue damage induced by PRFO is via lipid peroxidation, in which there is formation of lipid peroxides within myocardial cell membranes. This process is initiated when one hydrogen is abstracted from polyunsaturated fatty acids (PUFA's) by a free radical to form fatty acid radicals (Kaul et al., 1993). In addition, PRFO can cause oxidation of the thiol groups in proteins and can directly interact with nucleic acids. Thus in these chemical reactions, there are structural as well as functional modifications in the macromolecules leading to modifications/alterations in cell and organ function.

C.2. Antioxidants

Due to a continuous generation of PRFO during cardiac cell metabolism, a number of protective enzymatic as well as nonenzymatic antioxidants have evolved constituting an *antioxidant reserve* (Singal and Kirshenbaum, 1990) that act to limit the tissue concentration of these highly reactive species. A proper balance between the generation of PRFO and the antioxidant defense system is critical for the maintenance of a normal myocardial cell structure and function.

Three of the most important endogenous enzymatic antioxidants commonly present in most cells are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx). SOD catalyzes the dismutation of superoxide anion to hydrogen peroxide. Both catalase and glutathione peroxidase catalyze the reduction of hydrogen peroxide to water, thereby preventing the formation of the potent hydroxyl radical.

C.3. Oxidative Stress and Heart Failure

Any time there is a relative increase in PRFO, it can result in increased oxidative stress and cell injury. One of the most important endogenous nonenzymatic antioxidants is glutathione. Glutathione is present in high concentrations as GSH with minor fractions being GSSG (Reed, 1990). GSH as a co-substrate of glutathione peroxidase, plays an important role in the removal of hydrogen peroxide as well as other organic peroxides, thus preventing the peroxidation of membrane lipids (Ferrari et al., 1986). Study of changes in glutathione status provides important information on cellular oxidative events and tissue accumulation and/or release of GSSG.

The redox ratio (GSH/GSSG) is used as a sensitive index of oxidative stress (Ferrari et al., 1985; Curello et al., 1985). An increase in the redox ratio indicates reduced oxidative stress while a decrease in the redox ratio suggests increased oxidative stress. Depletion of total glutathione (GSH) levels to about 20-30% can impair the cell's defense against free radical attack and may lead to cell injury and death (Reed, 1984). There are many myocardial disease conditions under which the GSH levels are compromised and the maintenance of GSH levels reduce myocardial vulnerability to oxidative stress (Verma et al., 1997).

Under normal physiological conditions, the tissue concentration of oxygen radicals is limited due to the existence of a delicate balance between the generation of PRFO and the antioxidant defense system (Singh et al., 1995). However, if this balance is disturbed in favour of more PRFO, either through an enhanced production or via a reduction in the endogenous antioxidant defense system or both, the heart is at risk for a PRFO-mediated myocardial cell damage. Thus, changes in myocardial antioxidant status and oxidative stress may have a profound effect on cardiac structure and function (Gupta and Singal, 1989a; Kaul et al., 1993).

One of the most clearly documented conditions of increased myocardial oxidative stress has been reported in an animal model of acute heart failure involving hypoxia-reoxygenation and ischemia-reperfusion. Acute failure in rat hearts due to 10 min hypoxia at 37°C was accompanied by a decrease in SOD and GSHPx activities. Functional recovery upon reoxygenation was poor (Dhaliwal et al., 1991). Same duration of hypoxia conducted at a lower temperature (22°C) maintained antioxidant recovery and afforded full recovery

of function upon reoxygenation (Dhaliwal et al., 1991). Studies on the cardiac myocytes isolated from normal adult hearts exposed to hypoxia for 15 minutes resulted in a reduction in SOD and GSHPx activities (Kirshenbaum and Singal, 1992). Upon 15 minutes of reoxygenation, there was a significant increase in lipid peroxidation accompanied by changes in cell morphology and function. Addition of catalase to the medium modulated the hypoxic injury, suggesting that a decreased antioxidant reserve during hypoxia did contribute to the injury upon reoxygenation (Dhaliwal et al., 1991). These changes were also accompanied by a decrease in tissue redox state (GSH/GSSG), indicating increased oxidative stress. Free radical mediated increases in oxidative stress has also been documented in myocardial stunning (post-ischemic myocardial dysfunction). Indirect evidence implicating oxidative stress stemmed from the finding that administration of antioxidants SOD and CAT resulted in a significantly enhanced recovery of function after reperfusion (Myers et al., 1985). More direct evidence was obtained by detecting the production of free radicals using spin trap. In the open-chest dog model of post-ischemic dysfunction (15 minutes coronary occlusion), a burst of free radical production was detected in the stunned myocardium (Bolli et al., 1988). Subsequent studies involving antioxidant therapy begun at the time of reperfusion demonstrated that a substantial portion of the damage responsible for stunning develops in the initial seconds of reperfusion and can be attenuated by administration of free-radical scavengers just before reinstitution of blood flow (Bolli et al., 1989). These elegantly performed studies provide solid evidence in support of a role for free radical mediated oxidative stress in the genesis of myocardial stunning (Bolli, 1996).

In animal models with chronic cardiac dysfunction and heart failure, myocardial oxidative stress has been reported to increase while the antioxidant reserve is depressed (Dhalla and Singal, 1994; Siveski-Iliskovic et al., 1994; 1995). For example, pressure-overload induced heart failure in guinea pigs via banding of the ascending aorta resulted in heart failure at 20 weeks of post-surgery duration (Dhalla and Singal, 1994). Hemodynamic data revealed decreases in aortic systolic pressure (ASP), left ventricular systolic pressure (LVSP) and increases in left ventricular end-diastolic pressure (LVEDP) along with other indications of heart failure such as dyspnea, ascites and tissue congestion. These hemodynamic alterations were accompanied by decreases in myocardial SOD and GSHPx activities as compared to controls. Redox state (mainly due to an increase in GSSG accumulation) was also depressed, indicating increased oxidative stress (Dhalla and Singal, 1994). Thus, in this animal model of chronic heart failure, a depression in the endogenous antioxidant reserve was seen. Depressed antioxidant reserve and increased oxidative stress have also been reported in congestive heart failure due to adriamycin (Siveski-Iliskovic et al., 1994; 1995). In the cardiomyopathic hamster model of heart failure, myocardial lipid peroxidation was also reported to increase (Kobayashi et al., 1987).

Clinical studies of heart failure patients have corroborated the findings obtained from the experimental animal models. Malondialdehyde (MDA) levels have been reported to be significantly higher in patients with CHF (Belch et al., 1991; McMurray et al., 1990). Excessive quantities of pentane, a byproduct of lipid peroxidation, have been found to be significantly elevated as compared with healthy age-matched subjects (McMurray et al., 1990; Weitz et al., 1991; Sobotka et al., 1993). In addition, lipid peroxidation has been

shown to increase in proportion to the severity of heart failure (Diaz-Velez et al., 1996; Sobotka et al., 1993).

D. EXPERIMENTAL MODELS OF HEART FAILURE

Over the years, a number of models have been developed to produce CHF in animals which contain the primary clinical features of this disorder such as increased filling pressures, low cardiac output and neurohormonal activation. One of the earliest models developed to study heart failure was the coronary-artery ligation model. Experimental coronary occlusion with consequent myocardial infarction is produced by the surgical ligation of one or more coronary arteries (Selye et al., 1960; Johns and Olson, 1954). Initially, this surgical procedure was performed on dogs. However, coronary occlusion in the dog produced infarct sizes that were extremely variable due to the presence of collateral vessels. This made evaluation of therapeutic interventions extremely difficult (Johns and Olson, 1954). As a result, coronary occlusion was also tried in other animals. These studies demonstrated that the rat model produces infarct sizes that were more consistent than in the dog and this was shown to be largely due to the absence of collateral vessels (Fishbein et al., 1978). Following surgical induction of myocardial infarction, rats have been shown to develop clinical signs of heart failure consisting of dyspnea, pulmonary edema, hepatomegaly and ascites of varying severity at different post-surgical durations (Dixon et al., 1990; Hill and Singal, 1996). Since this closely mimics the human situation, the rat model is extensively used for studying heart failure.

More recently, low cardiac output CHF has also been produced by rapid ventricular pacing in the rabbit (Masaki et al., 1993; Shannon et al., 1993). Paced animals develop signs

and symptoms of CHF similar to those in humans (appetite loss, fatigue) as well as characteristic hemodynamic derangements (Masaki et al., 1993). In addition, this model also produces chronic biventricular CHF. Pressure-overload induced by coarctation of the ascending aorta has also been established as a good model for studying low output CHF, particularly the transition from compensatory hypertrophy to decompensatory hypertrophy and heart failure. Banding of the ascending aorta results in sustained hemodynamic functioning at 10 weeks of post-surgical duration followed by depressed hemodynamic function and heart failure at 20 weeks post-surgical duration (Dhalla and Singal, 1994).

Chronic arteriovenous (AV) fistula is a high cardiac output model of heart failure characterized by low total peripheral resistance (TPR) (Huang et al., 1992). This model creates a volume-overload on the heart with the subsequent development of heart failure. This model allows for the studying of blood flow and cardiac output distribution. This in turn allows for the examining of the basic circulatory alterations that are known to occur with such diseases as anemia and hyperthyroidism-induced heart failure.

E. PHARMACOLOGICAL TREATMENTS FOR HEART FAILURE

E.1. Digitalis

More than 200 years has passed since digitalis was first introduced as a therapeutic agent to treat heart failure. Since that time, considerable controversy has existed regarding the therapeutic efficacy of digitalis as the drug of choice in the treatment of chronic heart failure. The clinical benefits observed in CHF patients treated with digitalis have traditionally been attributed to the hemodynamic improvement produced by this agent (Krum et al., 1995a; Guyatt et al., 1988). However, therapy with other positive inotropic agents has

also resulted in similar hemodynamic improvements without any influence on long-term outcomes in patients with heart failure (Uretsky et al., 1990; Packer et al., 1991). As a result, considerable attention has recently been focussed on the additional pharmacologic actions of digitalis that are independent of its direct positive inotropic effects.

Short-term administration of digitalis in patients with chronic heart failure have demonstrated acute peripheral vasodilation and reduced plasma norepinephrine levels (Gheorghiade et al., 1989). These effects were sustained during long-term therapy with digitalis (Krum et al., 1995a). These anti-adrenergic effects may be of important prognostic significance given the fact that the degree of sympathetic activity has been found to be directly related to patient prognosis in patients with heart failure (Cohn et al., 1984). These newly documented effects of digitalis on autonomic function may explain why digitalis glycosides are the only group of positive inotropic drugs that persistently increase ejection fraction during long-term administration in patients with heart failure (Erdmann, 1995). These studies support the clinical use of digitalis as a mainstay in the treatment of heart failure.

E.2. Diuretics

Administration of diuretics has long been accepted as a first-line of treatment for patients with CHF. This is due principally to the substantial improvement in congestive symptoms experienced by these patients. Amongst the most studied of diuretics is furosemide, a member of the "loop" diuretics. The predominant effect of furoesmide has been to reduce left ventricular filling pressure by reducing preload (Taylor et al., 1982;

Nelson et al., 1983). This has been shown to result in an improved hemodynamic profile along with rapid relief of symptoms of breathlessness.

Although diuretics, particularly loop diuretics, improve the hemodynamic profile and congestive symptoms in patients with CHF, there is no valid information at the present time concerning their ability to reduce morbidity and mortality (Taylor, 1995). This may be due to the fact that diuretics induce an increase in systemic vascular resistance (Nelson et al., 1983) as a result of activation of the renin-angiotensin system. When such excitation is suppressed by concomitant administration of ACE inhibitors, a dramatic reduction in morbidity and mortality is seen (Taylor, 1995). Thus, although diuretics remain the cornerstone of treatment for relieving symptoms of CHF, administration of diuretics in combination with ACE inhibitors produces a clinical benefit far beyond that achieved by diuretics alone.

E.3. Angiotensin Converting Enzyme (ACE) Inhibitors

In 1984, it was demonstrated for the first time in an animal model of CHF caused by rapid ventricular pacing that chronic ACE blockade retards the progression of pump failure (Riegger et al., 1984) and this beneficial effect was shown to be accompanied by a blunting of neurohormonal activation as evidenced by a decrease in peripheral vascular resistance. Subsequent animal studies involving experimental myocardial infarction demonstrated that long-term therapy with the ACE inhibitor captopril not only improved left ventricular function and lessened dilation in the chronic phase of infarction, but also improved survival (Pfeffer et al., 1985; Jugdutt, 1995). Prevention of deterioration of cardiac performance and attenuation of left ventricular remodeling has also been reported in spontaneously

hypertensive rats (SHR) (Pfeffer and Pfeffer, 1988b) as well as in rats with myocardial infarction (Khaper and Singal, 1997).

The first clinical trial that demonstrated a clear reduction in mortality among patients with severe CHF after administration of ACE inhibitors was the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). The results of the CONSENSUS trial suggested that the reduction in mortality afforded by enalapril amongst New York Heart Association (NYHA) class IV patients was due in part to its antagonistic effects on RAS (The CONSENSUS Trial Study Group, 1987). Since that time, numerous clinical studies have demonstrated that administration of ACE inhibitors alone (Magnani, 1988; Newman et al., 1988) or as an add-on therapy to that of existing therapy with digitalis and diuretics (The Captopril-Digoxin Multicenter Research Group, 1988; Riegger, 1993) improves survival, left ventricular function as well as maximal exercise performance.

Although ACE inhibitors have been shown to be effective therapeutic agents in the clinical setting of heart failure, ACE inhibition is a relatively non-specific approach to blockade the RAS (Regitz-Zagrosek et al., 1995). Furthermore, side effects of ACE inhibitors, resulting from high plasma kinin levels due to their inhibition of bradykinin degradation, contraindicate high-dose treatment which may limit their beneficial effects (Yeo and Ramsey, 1990; Yeo et al., 1991). Thus, a great deal of attention has focussed on finding an alternative approach to inhibit RAS.

The effects of Ang II are mediated by binding of the ligand to two types of specific receptors: AT_1 and AT_2. Antagonism of Ang II receptors have been extensively studied as a more specific blocker of RAS. AT_1 receptor antagonists have been developed and these

agents have already proved useful in the management of hypertension (Delacretaz et al., 1995). The first clinical study involving human heart failure demonstrated that a single dose of the AT_1 blocker losartan resulted in an improved left ventricular ejection fraction concomitant with vasodilation and modulation of the neurohormonal response (Gottlieb et al., 1993). Following the single dose experiment, a multi-dose study of losartan in heart failure for a period of 12 weeks was found to produce favourable hemodynamic effects and improved clinical symptoms (Crozier et al., 1995). Further impetus for the use of AT_1 receptor antagonists stems from recent findings reporting that in addition to ACE, an Ang II forming serine proteinase (chymase) has been identified as an additional Ang II forming pathway (Urata et al., 1995). These findings suggest the use of ACE inhibitors in combination with AT_1 blockers for a more complete blockade of the RAS.

E.4. Beta-Blockers

The rationale for the use of beta-blockers as part of the therapeutic regimen for heart failure relates to several factors. Firstly, a poor prognosis for CHF patients remains despite the use of ACE inhibitors. Secondly, greater the degree of heightened adrenergic activity, worse is the outlook for CHF patients. Thirdly, downregulation of myocardial beta-adrenergic receptors in response to increased sympathetic activity has been documented in heart failure. As a result, introduction of beta-blockers as part of a combined treatment therapy has emerged.

Traditionally, beta-blockers have been considered contraindicated in heart failure due to their negative inotropic effects, which in turn may exacerbate the severity of cardiac dysfunction in the already failing heart. However, upon recognition that elevated plasma

levels of norepinephrine, a surrogate marker for adrenergic activity, increase in proportion to the severity of heart failure, the idea of applying beta-blocking agents as a means of counteracting the chronic overactivation of the sympathetic nervous system was examined. Beta-adrenoreceptor blockade as a treatment for heart failure was first described in a 1975 report which showed that congestive cardiomyopathy patients treated with alprenolol or practolol for five months experienced hemodynamic improvement (Waagstein et al., 1975). Later, it was reported that prolongation of survival among congestive cardiomyopathy patients could be achieved with beta-blocker administration (Swedberg et al., 1979). These initial positive findings led to the evaluation of beta-blocker use in patients with acute myocardial infarction. In 1981, three large-scale studies examining the value of beta-blocker therapy in the clinical setting of myocardial infarction were completed (Norwegian Multicenter Study Group, 1981; Hjalmarson et al., 1981; Beta-Blocker Heart Attack Trial Research Group, 1982). The findings from all three studies demonstrated significant reductions in mortality with the use of propranolol, metaprolol or timolol. Beneficial effects of beta-blockers have also been shown in experimental animals. Administration of propranolol following coronary occlusion resulted in significantly reduced infarct sizes (Braunwald et al., 1983; Pierce et al., 1973; Rasmussen et al., 1977). It was also demonstrated that the beneficial effect of beta-blocker administration on infarct size decreased with the delay in drug administration (Miura et al., 1979). Although many mechanisms of action have been proposed, the prevailing and most widely held view is that beta blockade reduces myocardial oxygen demands by virtue of blocking beta-receptors and

sympathetic nervous system activity (Braunwald et al., 1983) as evidenced by a decrease in heart rate and blood pressure.

New data have recently emerged from experimental animal and patient studies which have documented the therapeutic efficacy of carvedilol, a novel multiple action vasodilating beta-blocker, in the treatment of heart failure. In addition to being a beta-adrenoreceptor antagonist, it also acts as an α_1-adrenoreceptor blocker (Feuerstein and Ruffolo, 1995; Raftery, 1995). These combined actions of carvedilol produce systemic arterial vasodilation in the absence of reflex tachycardia. Addition of carvedilol to conventional therapy has been shown to decrease mortality among patients with chronic heart failure in a large double-blind, placebo-controlled clinical trial (Packer et al., 1996a). Carvedilol administration has also been shown to produce important hemodynamic and clinical benefits in patients with mild to moderate CHF (Packer et al., 1996b; Krum et al., 1995b). An unusually high degree of efficacy has been observed for carvedilol in experimental animal studies exploring its cardioprotective effects. Carvedilol has been shown to reduce infarct size following coronary occlusion in rat, dog and pig. This was especially so in the pig model where nearly complete protection against myocardial necrosis was observed (i.e. 91% reduction in infarct size) (Hamburger et al., 1991; Feuerstein et al., 1993; Bril et al., 1992). It has since been shown that carvedilol has potent antioxidant properties (Yue et al., 1992) and this may explain the dramatic salvage of myocardial tissue in this model that is known to involve oxygen free radical mediated injury. Because carvedilol exerts pharmacologic effects that are atypical of and in addition to other beta-blockers, this makes carvedilol an attractive and leading beta-blocking agent of choice for the treatment of heart failure.

F. MYOCARDIAL INFARCTION AND HEART FAILURE

During the last few decades, there has been a major focus on improving the early and late outcomes of myocardial infarction. Improvements in thrombolytic therapy have reduced short-term mortality of patients with myocardial infarction by about 20-30% in the last two decades (Breithardt et al., 1995). However, this has not been accompanied by a similar decline in long-term mortality (Gersh, 1995). Reasons for this diverging trend are due to the fact that survivors of myocardial infarction have an increased susceptibility of recurrent ischemia, cardiac rupture, arrhythmias and pump failure. Despite this array of post-myocardial infarction complications, heart failure continues to be the prominent clinical problem after myocardial infarction (Armstrong and Moe, 1994). Irreversible loss of contracting myocardium due to MI results in a chronic increase in the work load of the remaining viable myocardium. The heart responds with an increase in muscle mass and this process of heart hypertrophy represents a fundamental compensatory mechanism that permits the ventricle to sustain normal perfusion pressure. However, if this increased load on the heart is allowed to continue for a prolonged period, cardiac pumping function may become ineffective and heart failure supervenes. As a result, research efforts have intensified with regards to the pathogenesis and management of post-MI heart failure. In this regard, an improved and expanded knowledge of the pathophysiological mechanisms that ultimately contribute to progressive cardiac dysfunction and failure have led to a preventative approach to heart failure treatment following myocardial infarction.

G. VITAMIN E

Vitamin E is an essential micronutrient that also functions as one of the major components of the body's antioxidant defense system. It is essential, by definition, because the body cannot synthesize it on its own and therefore it must be provided for by food and supplements. Vitamin E was first discovered and isolated from wheat germ in the 1930's (Evans et al., 1936). The antioxidant activity of vitamin E was later discovered in 1937 (Olcott and Emerson, 1937).

Vitamin E is a generic term that encompasses a group of eight structurally related compounds. These eight vitamin E-like compounds are divided into two groups: tocopherols and trienols. Each of these have a chromanol head group and a phytyl side chain (Packer, 1994). The side chain of tocopherols are saturated while those of tocotrienols are unsaturated. The antioxidant property resides in the chroman head group (Burton et al., 1980) while the phytyl side chain is thought to retain the molecule and anchor it in the membrane (van Acker et al.,1993). Of these eight substances, d-alpha-tocopherol (Figure 1) has the highest biopotency and is the most common type of vitamin E absorbed from the human diet. Approximately 20-40% of dietary vitamin E in a normal diet is absorbed. Upon being absorbed, it is bound to lipoproteins while circulating in the blood and is stored principally in adipose tissue, liver and muscle.

Vitamin E reacts directly with free radicals. During this process, vitamin E donates a hydrogen atom, resulting in the generation of a hydroperoxide and vitamin E radical, both of which are less reactive. This effectively terminates lipid peroxide mediated chain

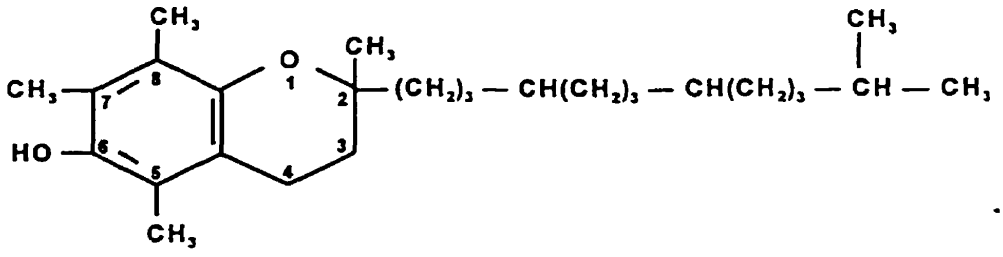


Figure 1: Chemical structure of vitamin E.

reactions. As a result, vitamin E is referred to as a "chain-breaking" antioxidant (Packer, 1994). Since vitamin E is lipid-soluble, it represents the major defense mechanism against oxidation of polyunsaturated fatty acids (PUFA's).

The resultant vitamin E radical is less reactive and incapable of any further free radical scavenging activity. Thus, it must be regenerated back to a functional vitamin E molecule. It has been shown that vitamin C regenerates vitamin E to the reduced form and therefore acts to spare the functional form of vitamin E (Chan, 1993). This synergistic interaction between vitamins C and E is an efficient and critical mechanism for the recycling of functional vitamin E.

Recent studies have demonstrated a new role for vitamin E that involves a non-antioxidant mechanism as the basis for its action. Evidence in support of this new role for vitamin E stems in large part from studies examining the mechanism of its inhibitory effects on smooth muscle cell proliferation (SMC) and growth during atherosclerosis. During the pathogenesis of atherosclerosis, stimulation of SMC proliferation by low density lipoproteins

is associated with an increase in protein kinase C activity and the process is inhibited by d-alpha-tocopherol, the most biologically active form of vitamin E (Ozer et al., 1995; Stauble et al., 1994; Chatelain et al., 1993). This effect of d-alpha-tocopherol was shown to be independent of its free radical scavenging properties, occurring mainly as a result of its ability to activate the cellular release of transforming growth factor-beta (TGF-β), a SMC growth inhibitor. Additional mechanisms of action by which vitamin E is known to function include reductions in platelet adhesion and aggregation (Steiner, 1991) and inhibition of vitamin K-dependent clotting factors (Dowd and Zheng, 1995).

Evidence supporting a protective role for vitamin E in cardiovascular disease has begun to accumulate. Studies of middle-age men from different European populations have demonstrated a high inverse correlation between mortality from ischemic heart disease and lipid-standardized plasma vitamin E levels (Gey et al., 1989). Furthermore, another European study has reported that low plasma concentrations of vitamin E were correlated with an increased risk of angina pectoris in men even after adjusting for age, blood pressure and total cholesterol (Riemersma et al., 1991). Very recently, two large prospective studies of association between vitamin E intake and risk of coronary heart disease demonstrated a substantially reduced risk in both men and women who used vitamin E supplements (Stampfer et al., 1993; Rimm et al., 1993). Data from animal studies have shown that toxicity of vitamin E is very low. In human studies of oral vitamin E therapy, few side effects have been reported, even at doses as high as 3200 mg/day (3200 IU/day) (Bendich and Machlin, 1988). These findings support a safe and protective role for vitamin E supplementation against the development and progression of cardiovascular disease.

The data reported from patient studies as well as animal experiments provide a strong indication of the involvement of increased free radical production and oxidative stress in the pathogenesis of cardiac dysfunction and failure. However, no information is available with respect to antioxidants and oxidative stress in the left and right ventricles of the heart. Furthermore, direct information on the cause and effect relationship between antioxidant changes and heart failure is lacking.

III. METHODS

A. ANIMALS

Male Sprague-Dawley rats weighing 150 ± 10g were used in this study. Animals were purchased from Central Animal Care Services at the University of Manitoba and housed in metal wire cages (two rats/cage). Feed and water was provided *ad libitum*, unless specified otherwise. All of the animals used in this study were maintained and treated in accordance with the policies and procedures of the Canadian Council on Animal Care (CCAC).

B. EXPERIMENTAL MODEL

Myocardial infarction was produced via occlusion of the left coronary artery (Hill and Singal, 1996). In this procedure, the animals were anesthetized with 5% isoflurane in an induction chamber and a left intercostal thoracotomy was performed under aseptic conditions. The skin was incised along the left sternal border and the third and fourth ribs were cut proximal to the sternum. The pericardial sac was perforated and the heart was exteriorized through the intercostal space. The left coronary artery was ligated about 1-2 mm from its origin with a 6-0 silk thread. Following the left coronary artery occlusion, the heart was repositioned in the chest. Closure of the thoracic cavity was accomplished by reapproximation of the ribs along with a purse-string suture of the incised muscles. Prior to suturing of the skin, air in the chest was removed using a syringe. Throughout this surgical procedure, a maintenance dose of anaesthesia (1.5-3% isoflurane) was delivered using a

positive pressure ventilation system consisting of 95% O_2. The mortality among the coronary ligated animals was 35% within the first 24 hours.

It should be pointed out that with the surgical procedure used in this study, most of the experimental animals develop an infarct size ranging between 20-50% of the left ventricular mass. Rats with infarcts comprising <20% of the left ventricular mass were not included for further investigations. Control animals were treated in a similar fashion with the exception that the suture around the left coronary artery was not tied. No mortality was observed in the sham control animals within 24 hours of the operation. Following the operation, animals were placed in a chamber in which an oxygen atmosphere was maintained and the animals were allowed to recover. Analgesia (Buprenorphine, .01-.05 mg/kg body weight) was given subcutaneously, every 12 hours for up to 48 hours post-operatively. Animals were utilized at 1, 4, 8 and 16 weeks post-MI for different studies.

C. VITAMIN E SUPPLEMENTATION

Animals in the control and infarcted group were divided into 2 subgroups. One subgroup in each category received vitamin E in the diet while the other received standard rat chow (basal diet, PMI Feeds, St. Louis, MO). Dietary supplementation consisted of 1545 mg/kg of vitamin E and basal diet contained 40 mg/kg tocopherol acetate. Diet supplementation of these animals was initiated 2 weeks prior to induction of MI and was continued until 16 weeks post-MI. Untreated animals were maintained on the regular rat chow basal diet that was not enriched with vitamin E.

D. HEMODYNAMIC MEASUREMENTS

Animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.). A miniature pressure transducer (Millar Micro-Tip) was inserted into the right carotid artery and then advanced into the left ventricle. Left ventricular end-diastolic (LVEDP) and left ventricular systolic (LVSP) pressures were recorded on a computer using an Axotape Data Acquisition Program. For recording of right ventricular pressures, the miniature pressure transducer was inserted into the right jugular vein and then advanced into the right ventricle. After hemodynamic recordings, animals were sacrificed and the heart and other organs were removed for further studies.

E. TISSUE WEIGHTS

Pieces of tissues from the lungs as well as liver were removed and weighed. For the determination of dry weight, these tissue pieces were placed in the oven at 65°C until a constant weight was reached. Wet/dry weight ratios were calculated for lungs as well as liver.

F. BIOCHEMICAL ASSAYS

Connective tissue, atria and the scar tissue were carefully removed from all hearts. The viable portion of the left ventricle with the septum were separated from the right ventricle. Scar tissue was distinct from viable myocardium due to it's white colour appearance and thin texture as opposed to the reddish-brown colour and thick texture appearance of the surviving myocardium. These right and left ventricles were separately analyzed for antioxidant and oxidative stress changes. Prior to homogenization, hearts were placed in a 0.2 mol/L Tris and 0.16 mol/L KCl buffer, pH=7.4. The hearts were allowed to

beat for a short period of time in the buffer, thereby allowing for perfusion of the myocardium as well as to wash out blood to minimize the extent of contamination by blood-derived elements on antioxidant enzyme measurements.

F.1. Catalase

Catalase activity was determined by the method described elsewhere (Clairborne, 1985). Tissue was homogenized in (1:10) 50 mmol/L potassium phosphate buffer, pH 7.4 and the homogenate was centrifuged at 18,000 g for 45 min. Supernatant (50 µl) was added to a 3-ml cuvette which contained 2.95 ml of 19 mmol/L hydrogen peroxide in 50 mmol/L potassium phosphate buffer, pH 7.4. Changes in absorbency at 240 nm were continuously followed for 5 minutes. Catalase activity was expressed as Units/milligram protein.

F.2. Glutathione Peroxidase (GSHPx)

GSHPx activity was determined by the method described elsewhere (Paglia and Valentine, 1967). Tissue was homogenized 1:10 in 75 mmol/l phosphate buffer, pH 7.0. Homogenate was centrifuged at 18,000 g for 45 minutes and the supernatant was aspirated and assayed for total cytosolic GSHPx activity. GSHPx activity was assayed in a 3-ml cuvette containing 2.0 ml of 75 mmol/L phosphate buffer, pH 7.0. The following solutions were then added: 50 µl of 60 mmol/L glutathione, 100 µl glutathione reductase solution (30 U/ml), 50 µl of 0.12 mol/L NaN_3, 100 µl of 15 mmol/L Na_2 EDTA, 100 µl of 3.0 mmol/L NADPH, and 100 µl of cytosolic fraction. The reaction was started by the addition of 100 µl of 7.5 mmol/L H_2O_2 and the conversion of NADPH to NADP was monitored by a continuous recording of the change of absorbency at 340 nm at one minute intervals for 5 minutes. GSHPx activity was expressed as nanomoles of reduced nicotinamide adenine

dinucleotide phosphate (NADPH) oxidized to nicotinamide adenine dinucleotide phosphate (NADP) per minute per milligram protein, using a molar extinction coefficient for NADPH at 340 nm of 6.22×10^6.

G. VITAMIN E ANALYSIS

α-Tocopherol was measured in myocardial tissue and in the food using a modification of the extraction procedures and reverse phase HPLC detection method (Palace and Brown, 1994). Briefly, 100 mg of myocardial tissue or 300 mg of food were homogenized in 20 volumes of ice cold, double distilled water. Proteins were then precipitated in the homogenates by adding an equal volume of ice cold methanol. In the case of myocardial tissue extractions, the methanol contained 60 μg/ml tocopherol acetate, which was used as an internal standard to correct for extraction efficiency in each sample. Because tocopherol acetate was the primary source of vitamin E in the food, retinol acetate was used as an internal standard for these extractions. Tocopherol as well as the retinol acetate and tocopherol acetate internal standards were extracted from the homogenates by mixing with 2 ml of ice cold 3:2 (v/v) ethyl acetate: hexane. After standing on ice, capped and shielded from light for 15 min, the two phases were separated by centrifugation at 3000Xg for 5 min and a 1 ml aliquot of the top ethyl acetate:heaxane layer was retrieved. The aliquot was dried under vacuum in a rotary evaporator to complete dryness, reconstituted in 60 μl of HPLC mobile phase and injected directly into the HPLC system for analysis. A standard curve constructed with commercially prepared tocopherol and tocopherol acetate standards allowed quantification of each analyte from area under the peak.

The HPLC mobile phase contained 70:20:10 (v/v/v) acetonitrile:dichloromethane:methanol and was delivered at 1 ml/min through an Adsorbosphere HS C18 (250 X 4.6 mm, 5 μm pore size) preceded by a 5 mm guard column with the same packing material. Ultraviolet detection of peaks was accomplished by monitoring at 325 nm until 7 min for retinol acetate and then switching to 292 nm for detection of tocopherol and tocopherol acetate. Total run time was 30 min, with typical retention times for retinol acetate, tocopherol and tocopherol acetate of 5.1, 10.7 and 12.5 min, respectively.

H. GLUTATHIONE

Concentrations of total glutathione (GSSG + GSH) were measured in the myocardium by the glutathione reductase/5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) recycling assay (Anderson, 1985). The rate of TNB formation, being proportional to the sum of GSH and GSSG present, was followed at 412 nm. Myocardial tissue was homogenized in 5% sulfosalicylic acid. The tissue homogenate was centrifuged for 10 min at 10,000 g. Supernatant was stored at 4°C until assayed. GSSG alone was measured by treating the sulfosalicylic acid supernatant with 2-vinylpyridine and triethanolamine. The solution was vigorously mixed and final pH of the solution was adjusted to between 6 and 7. After 60 min, the derivatized samples were assayed as described above in the DTNB-GSSG reductase recycling assay. GSH values were calculated as the difference between total (GSSG + GSH)

and GSSG concentrations. Values are reported in GSH equivalents and expressed as μmol per gram tissue weight.

I. THIOBARBITURIC ACID REACTING SUBSTANCES (TBARS)

Lipid peroxide content in myocardium was determined by quantitating the thiobarbituric acid reactive substances as described previously (Singal and Pierce, 1986). Tissue was homogenized in (10% wt/vol) 0.2 mol/L Tris-0.16 mol/L KCl buffer, pH 7.4 and incubated for 1 hour at 37°C in a water bath. A 2 ml aliquot was withdrawn from the incubation mixture and pipetted into a 12 ml Corning culture tube. This was followed by the addition of 2.0 ml of 40% trichloroacetic acid and 1.0 ml of 0.2% thiobarbituric acid (TBA). In order to minimize peroxidation during the assay procedure, 100 µl of 2% butylated hydroxy-toluene was added to the TBA reagent mixture. Tubes were then boiled for 15 min and cooled on ice for 15 min. Two ml of 70% trichloroacetic acid were added and tubes were allowed to stand for 20 min, at which time the tubes were subsequently centrifuged at 800 g for 20 min. The developed color was read at 532 nm on a spectrophotometer. Commercially available malondialdehyde was used as a standard.

J. PROTEINS AND STATISTICAL ANALYSIS

Proteins were determined by the method described elsewhere (Lowry et al., 1951). Data were expressed as the mean ± SEM. For a statistical analysis of the data, group means were compared by one-way ANOVA and ANOVA followed by Bonferroni's test was used to identify differences between groups. Values of $p<0.05$ were considered significant. In cases where Bonferroni's test returned marginal significant differences ($p<0.1$) and the power

of the test was <0.8, type II errors were minimized by reanalyzing the groups with student's t-test ($p<0.05$).

IV. RESULTS

A. GENERAL OBSERVATIONS

Following coronary-artery ligation, rats in all groups were observed for 16 weeks on a daily basis for their food intake, general behaviour and presence of any clinical signs of heart failure. There was a nominal decline (≈7%) in the food consumed by the coronary-artery ligated animals as compared to their sham controls. Food consumption in the coronary-artery ligated animals supplemented with vitamin E was not different as compared to their vitamin E treated sham controls. In both the vitamin E treated and untreated sham-operated animals, nothing unusual was noted throughout the experimental period. However, in the untreated coronary-artery ligated rats, lethargy, dyspnea and cyanosis of peripheral extremities were present, appearing at about 12 weeks after the surgery. At autopsy, congested lungs as well as hepatomegaly associated with a black cherry colour appearance of the liver were noted in these animals. In contrast, coronary-artery ligated animals supplemented with vitamin E did not display any of the above mentioned clinical signs at any time point throughout the course of the study. Lung congestion as well as hepatomegaly were not noticeable in the coronary-artery ligated vitamin E supplemented animals.

B. HEMODYNAMIC STUDIES

Animals were assessed for left and right ventricular functions (peak systolic and end-diastolic pressures) at 1, 4, 8 and 16 weeks post-MI and these data are shown in Table 1. There was no change in either the LVEDP or LVSP in the coronary-ligated animals at 1 week post-MI. However, there was a significant increase in the LVEDP and a significant

Table 1: Left and right ventricular pressures (mmHg) in rats at 1, 4, 8 and 16 weeks post-MI as compared to respective sham controls.

Post-Surgical Duration	LVEDP		LVSP		RVEDP		RVSP	
	Cont	Expt	Cont	Expt	Cont	Expt	Cont	Expt
1 Week	2.4 ± .26	2.0 ± .6	125 ± 1.0	133.0 ± 2.3	1.1 ± .6	1.7 ± .2	31.8 ± .7	43.8 ± 1.1*
4 Weeks	1.5 ± .29	8.2 ± .52$^{\theta}$	125.9 ± 1.2	112.0 ± 1.57*	0.8 ± .4	1.6 ± .4	29.7 ± .3	31.4 ± .8
8 Weeks	3.2 ± .60	17.4 ± 1.7*	130.9 ± 4.2	99.9 ± .52*	2.6 ± .2	6.1 ± .5*	28.5 ± 1.6	24.6 ± .9
16 Weeks	3.7 ± .33	31.4 ± 1.5*	130.0 ± 5.0	89.2 ± 1.4*	2.1 ± .2	10.8 ± .8*	30.5 ± .5	19.9 ± .5*

Values are mean ± S.E. of 6-8 rats. LVEDP) Left ventricular end-diastolic pressure; LVSP) Left ventricular systolic pressure; RVEDP) Right ventricular end-diastolic pressure; RVSP) Right ventricular systolic pressure. Significant differences ($p < 0.05$) from respective control groups are indicated by θ) ANOVA and *) ANOVA followed by Bonferroni's test.

decrease in the LVSP in the infarcted animals relative to their controls at 4, 8 and 16 weeks post-MI. These changes were progressive and became more pronounced as the period of post-MI increased from 4 to 16 weeks. With respect to right ventricular function, no change in the RVEDP was observed at 1 and 4 weeks in the infarcted animals as compared to their respective controls. RVEDP was elevated in the post-MI animals at 8 and 16 weeks. In the infarcted animals, a significant elevation in the RVSP was observed at 1 week post-MI. This pressure was near control levels in the 4 and 8 week groups and a significant depression in the RVSP was seen at 16 weeks.

Effects of vitamin E treatment on left and right ventricular function (peak systolic and end-diastolic pressures) were assessed in the animals at 16 weeks post-MI and these data are shown in Table 2. Vitamin E treatment of coronary artery-ligated animals resulted in the complete normalization of RVEDP and RVSP while a significant improvement was observed in the LVEDP and LVSP. However, LVEDP and LVSP in these vitamin E treated infarcted animals still remained significantly different from the respective control values.

C. TISSUE WEIGHTS

The lung and liver wet/dry weight ratios of infarcted animals were no different from their respective controls at 1 and 4 weeks post-MI (Table 3). However, this ratio for lungs was significantly higher at 8 and 16 weeks while in the liver the ratio was significantly increased only at 16 weeks post-MI. Vitamin E treatment of coronary-artery ligated animals resulted in a significant reduction in the wet to dry weight ratio for both the lungs and liver at 16 weeks post-MI, such that these values were not statistically different from their respective controls (Table 4).

Table 2: Effects of vitamin E treatment on left and right ventricular pressures (mmHg) in rats at 16 weeks post-MI.

Animal Group	LVEDP	LVSP	RVEDP	RVSP
Basal Diet:				
CONT	2.3 ± 0.6	128.3 ± 1.8	1.5 ± 0.5	30.4 ± 1.7
MI	28.1 ± 0.8*	83.6 ± 2.3*	9.1 ± 0.9*	17.4 ± 0.4*
Vitamin E Supplemented Diet:				
CONT	3.8 ± 1.7	130.8 ± 2.2	2.6 ± 0.8	38.3 ± 3.4
MI	13.5 ± 1.7*†	108.5 ± 6.8*†	2.8 ± 1.3†	34.1 ± 2.6†

Values are mean ± SEM of 6-8 rats. LVEDP - left ventricular end-diastolic pressure; LVSP - left ventricular systolic pressure; RVEDP - right ventricular end-diastolic pressure; RVSP - right ventricular systolic pressure. *) Significantly different ($p<0.05$) from respective control group by ANOVA followed by Bonferroni's test. †) Significantly different ($p<0.05$) from untreated MI group by ANOVA followed by Bonferroni's test.

Table 3: Lung and Liver wet/dry weight ratios in the animals at 1, 4, 8 and 16 weeks of post-surgery duration.

Post-Surgical Duration	Lung		Liver	
	Cont	Expt	Cont	Expt
1 Week	4.6 ± .01	4.8 ± .17	2.8 ± .5	2.7 ± .10
4 Weeks	4.8 ± .06	5.0 ± .04	3.1 ± .04	3.1 ± .02
8 Weeks	4.8 ± .05	5.6 ± .3*	3.0 ± .12	3.3 ± .04
16 Weeks	4.7 ± .03	6.8 ± .17*	2.9 ± .10	3.9 ± .05$^{\theta}$

Values are mean ± S.E. of 6-8 rats. Significant differences ($p < 0.05$) from respective control groups are indicated by θ) ANOVA and *) ANOVA followed by Bonferroni's test.

Table 4: Lung and liver wet/dry weight ratios in rats at 16 weeks post-MI with and without vitamin E treatment.

Animal Group	Lung	Liver
Basal Diet:		
CONT	4.5 ± 0.1	2.9 ± 0.1
MI	7.1 ± 0.8*	4.5 ± 0.5*
Vitamin E Supplemented Diet:		
CONT	4.8 ● 0.2	3.2 ± 0.1
MI	5.1 ± 0.3†	3.2 ± 0.1†

Values are mean ± SEM of 6-8 rats. *) Significantly different ($p<0.05$) from respective control group by ANOVA followed by Bonferroni's test. †) Significantly different ($p<0.05$) from untreated MI group by ANOVA followed by Bonferroni's test.

D. MYOCARDIAL ENDOGENOUS ANTIOXIDANT ENZYMES

Myocardial catalase and glutathione peroxidase activities were examined in the viable left and right ventricles separately, at 1, 4, 8 and 16 weeks post-MI and compared with their respective controls. At 1 week, catalase activity in the left ventricle was maintained near that of its respective controls while in the right ventricle it was 134% as compared to the controls and this increase was statistically significant (Figure 2). At 4, 8 and 16 weeks, catalase activity in the left ventricle was 71, 48 and 28% of the respective control values. In contrast, catalase activity in the right ventricle was no different from the respective controls at 4 and 8 weeks post-MI, while a significant decrease was observed at 16 weeks. Catalase activity in the right ventricle remained significantly higher as compared to the corresponding left ventricle at all time points. Values for glutathione peroxidase activity in the left ventricle at 1, 4, 8 and 16 weeks post-MI were 102%, 74%, 60% and 55% respectively as compared to their controls (Figure 3). In the right ventricle, the values in the post-MI groups at 1, 4, 8 and 16 weeks were 125%, 103%, 89% and 77% respectively as compared to their controls.

Treatment with vitamin E resulted in a significant increase in catalase activities in both the viable left and right ventricles of infarcted animals at 16 weeks post-MI. However, catalase activity in the left ventricle of these vitamin E treated infarcted animals remained lower than their respective control values (Figure 4). In contrast, no difference in catalase activity was observed in the right ventricle of vitamin E treated infarcted animals as compared to respective controls (Figure 4).

With respect to GSHPx activity, vitamin E supplementation increased the values for GSHPx in the left ventricle, though they remained significantly different from their

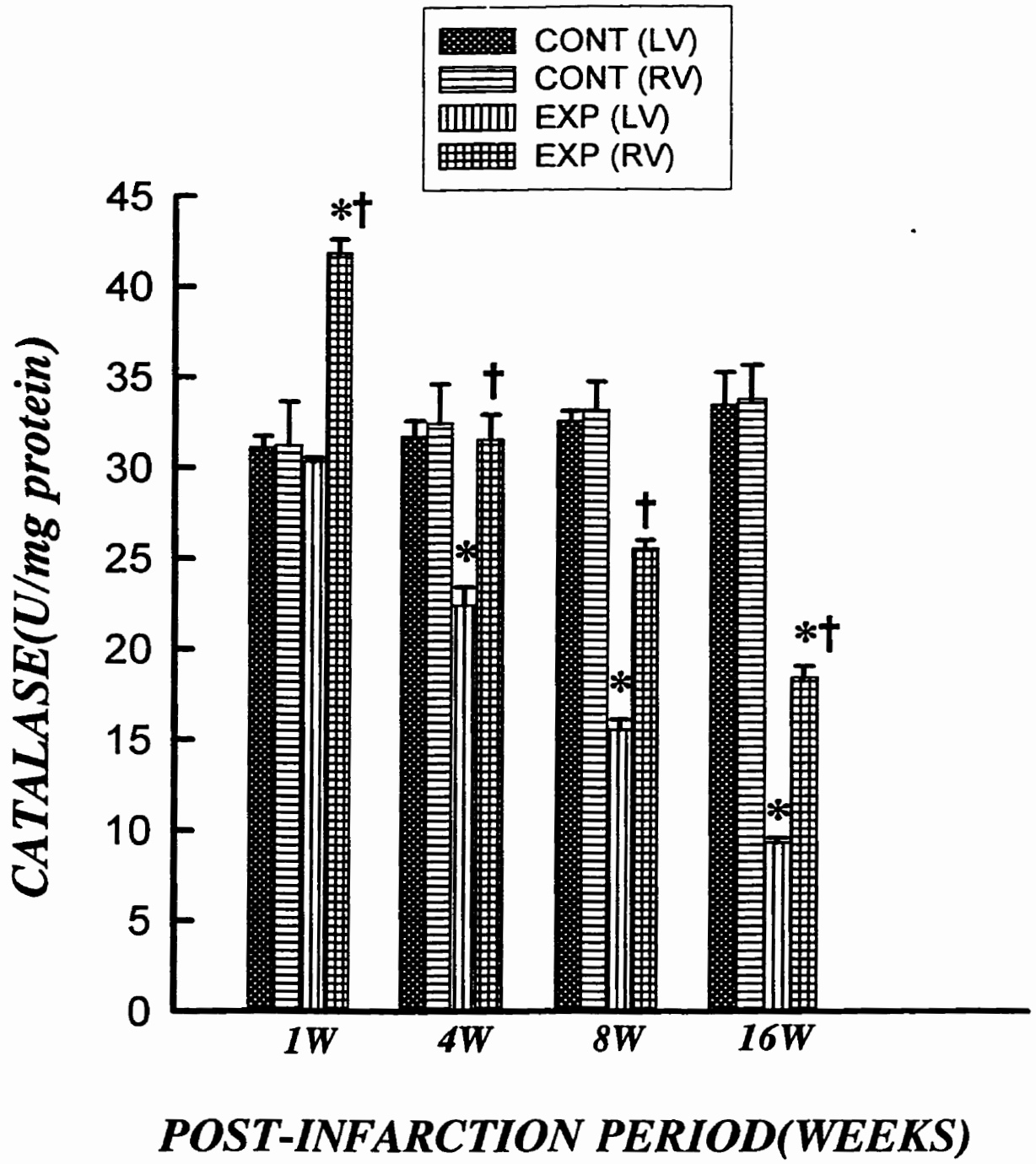


Figure 2: **Myocardial catalase activity in the left and right ventricles of control and experimental hearts at 1, 4, 8 and 16 weeks post-infarction. Data are mean ± S.E. from 8-10 rats. *) Significantly different ($p < 0.05$) from respective controls. †) Significantly different ($p < 0.05$) from corresponding experimental left ventricular tissue.**

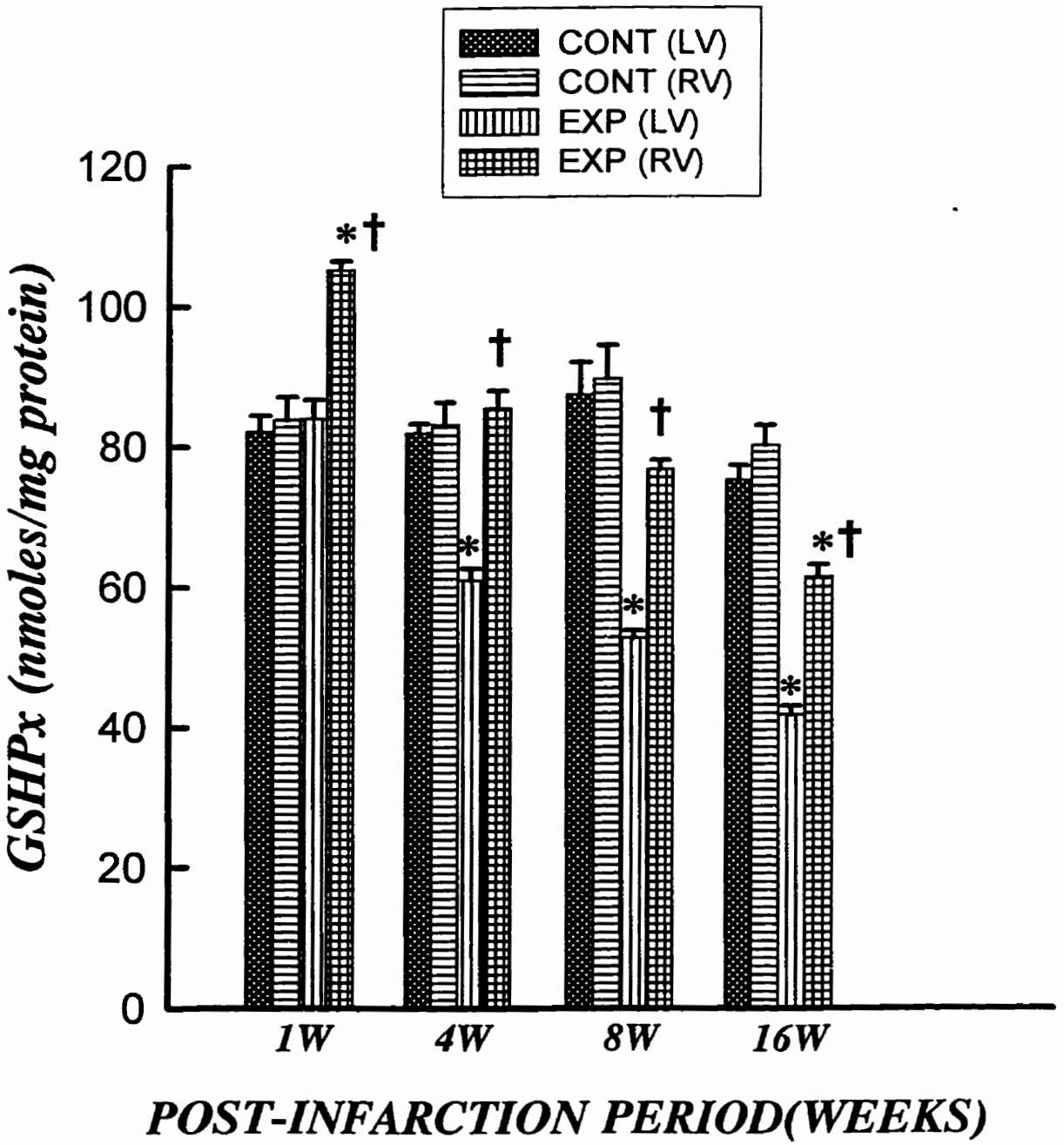


Figure 3: Myocardial glutathione peroxidase (GSHPx) activity in the left and right ventricles of control and experimental hearts at 1, 4, 8 and 16 weeks post-infarction. Data are mean ± S.E. from 8-10 rats. *) Significantly different ($p < 0.05$) from respective controls. †) Significantly different ($p < 0.05$) from corresponding experimental left ventricular tissue.

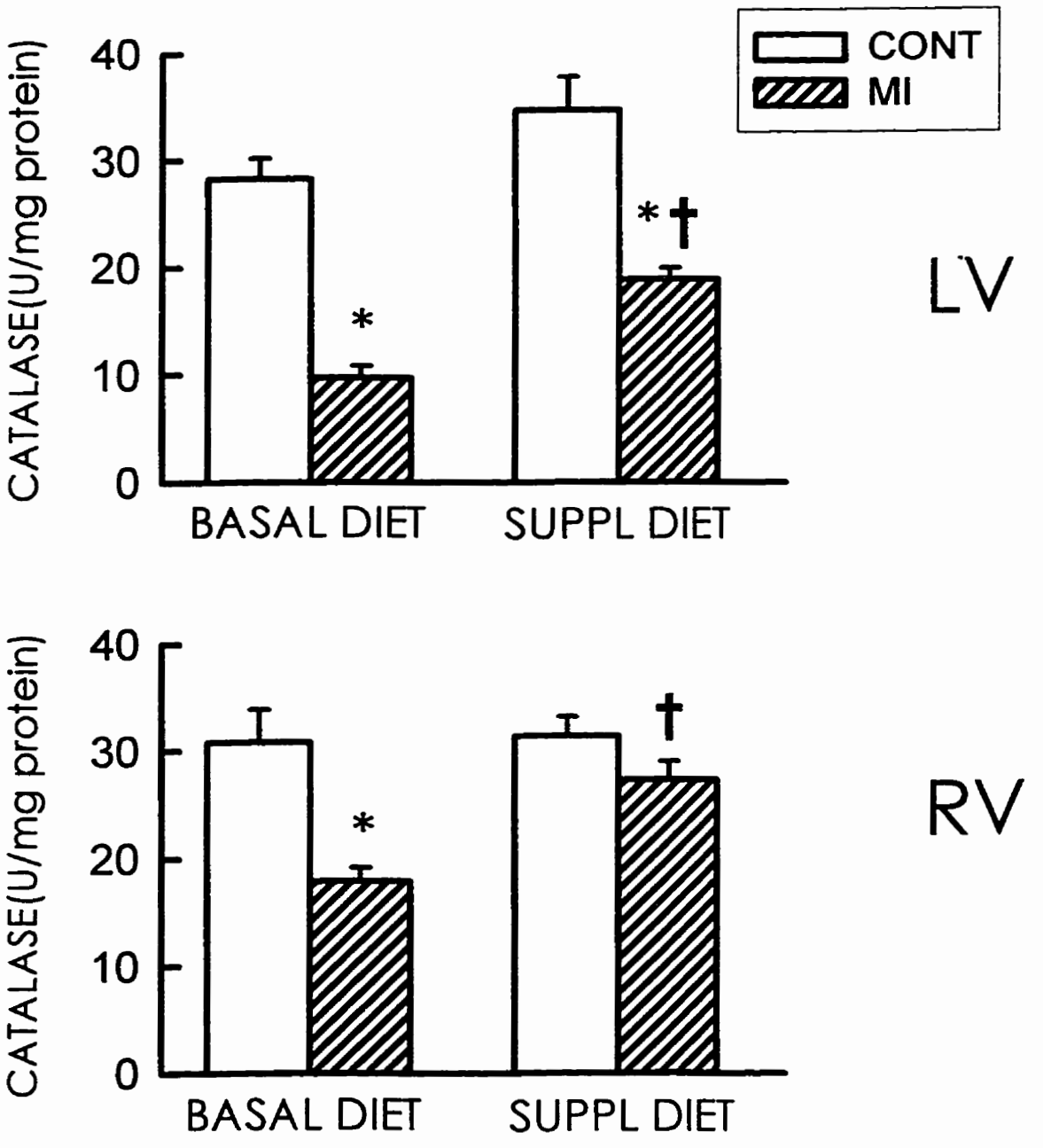


Figure 4: Myocardial catalase activity at 16 weeks after surgery in the viable LV and RV of control (CONT) and MI animals maintained on a basal or vitamin E supplemented (Suppl) diet. Data are mean ± SEM from 6-8 rats. *) Significantly different ($p<0.05$) from respective controls. †) Significantly different ($p<0.05$) from corresponding ventricular tissue of untreated MI hearts.

respective control values (Figure 5). No difference in GSHPx activity was observed in the right ventricle of the vitamin E treated infarcted animals as compared to their respective controls (Figure 5).

E. MYOCARDIAL VITAMIN E CONTENT

Myocardial vitamin E content was analyzed by quantitating α-tocopherol by the HPLC method and these data are shown in Figure 6. A 33% decrease in vitamin E content was seen in the viable left ventricle of untreated infarcted animals relative to respective sham controls at 16 weeks post-MI. In contrast, no change in vitamin E content was observed in the viable right ventricle of infarcted animals as compared to respective controls. Vitamin E supplementation resulted in a substantial increase in vitamin E content in the left and right ventricles of both sham controls and infarcted animals. However, in the vitamin E supplemented animals, the left ventricles in the MI group still had a significantly lower value. The content of vitamin E in the right ventricle of vitamin E treated infarcted animals was not statistically different from that of vitamin E treated sham controls.

F. GLUTATHIONE

Myocardial reduced (GSH) and oxidized (GSSG) glutathione levels were also examined in the surviving left and right ventricular tissue of MI animals and compared with their respective controls (Table 5). At 1 week, GSH levels in the left ventricle were unchanged from controls. At 4, 8 and 16 weeks, GSH levels in the left ventricle were 70, 60 and 44% of control values. GSH levels in the right ventricle at 1 week were significantly increased above that of controls. GSH levels in the right ventricle showed no statistically significant changes until 8 and 16 weeks, when a 19% and 35% decrease was observed,

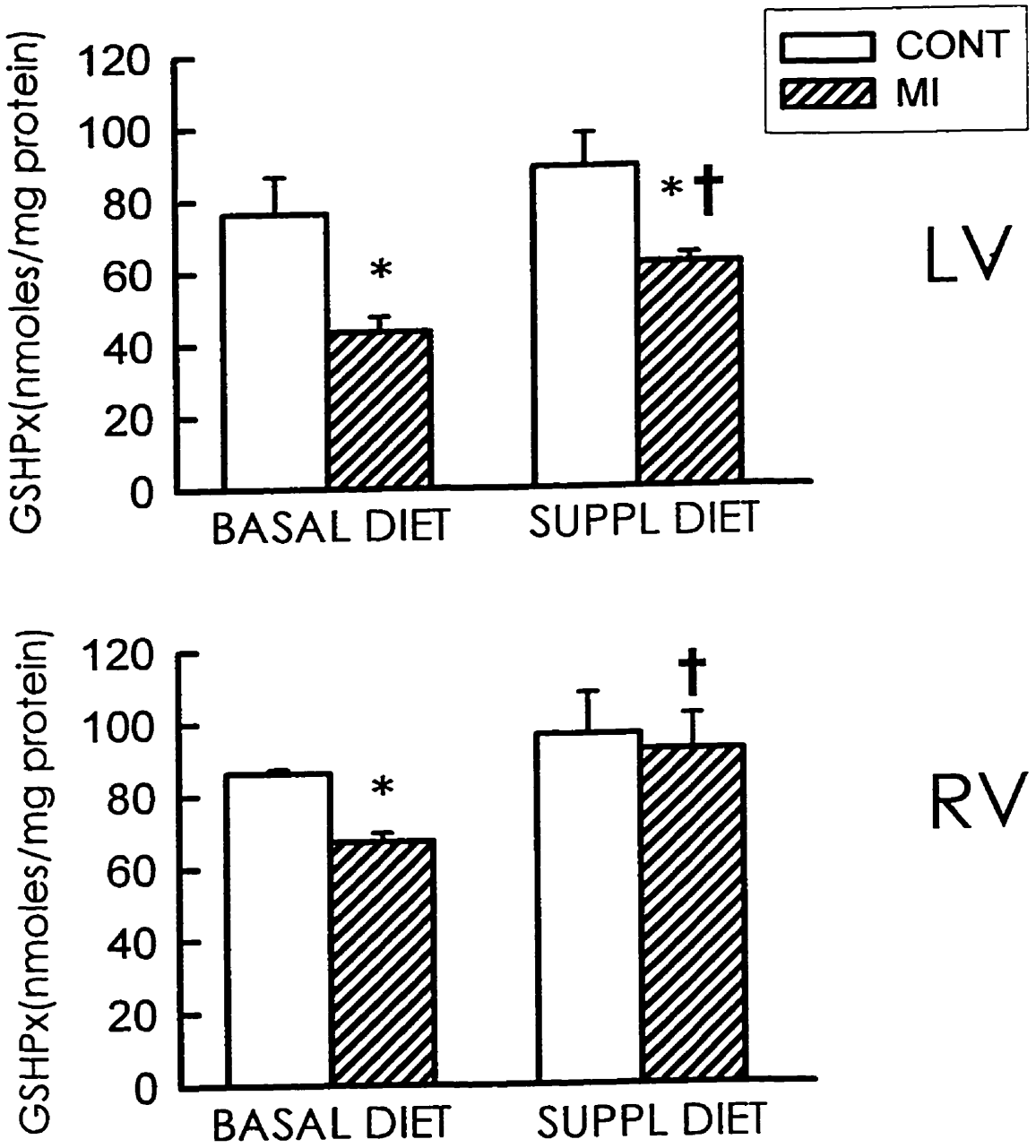


Figure 5: Myocardial GSHPx activity at 16 weeks after surgery in the viable LV and RV of control (CONT) and MI animals maintained on a basal or vitamin E supplemented (Suppl) diet. Data are mean ± SEM from 6-8 rats. *) Significantly different ($p<0.05$) from respective controls. †) Significantly different ($p<0.05$) from corresponding ventricular tissue of untreated MI hearts.

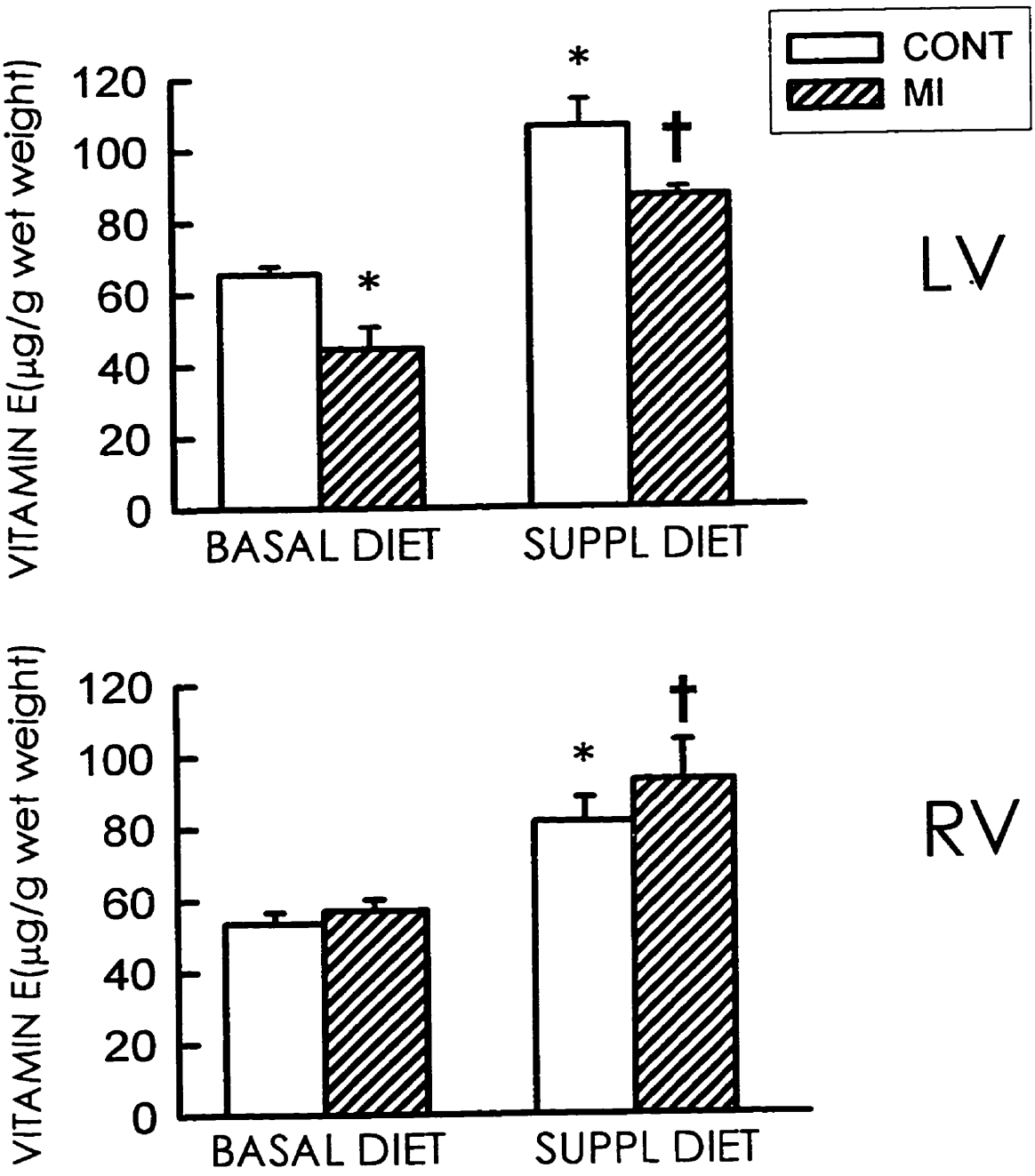


Figure 6: **Myocardial vitamin E levels at 16 weeks after surgery in the viable LV and RV of control (CONT) and MI animals maintained on a basal or vitamin E supplemented (Suppl) diet. Data are mean ± SEM from 6-8 rats. *) Significantly different ($p<0.05$) from respective controls. †) Significantly different ($p<0.05$) from corresponding ventricular tissue of untreated MI hearts.**

Table 5: Myocardial reduced (GSH) and oxidized (GSSG) glutathione levels in the left and right ventricles of control and experimental rats at 1, 4, 8 and 16 weeks of post-surgical duration.

Post-Surgical Duration	GSH (μmol/g tissue wt)				GSSG (μmol/g tissue wt)			
	LV		RV		LV		RV	
	Cont	Expt	Cont	Expt	Cont	Expt	Cont	Expt
1 Week	78.0 ± 1.9	77.3 ± 0.8	81.5 ± 4.5	91.1 ± 0.9θ†	5.9 ± 0.5	8.9 ± 0.3	6.1 ± 0.9	5.1 ± 0.3
4 Weeks	86.9 ± 4.1	60.6 ± 0.9*	83.0 ± 1.2	81.5 ± 1.4†	4.6 ± 0.2	12.4 ± 0.5θ	5.1 ± 0.6	6.1 ± 0.5†
8 Weeks	81.9 ± 3.4	49.5 ± 1.7*	82.6 ± 2.5	67.0 ± 0.9*†	4.9 ± 0.7	21.5 ± 0.7*	5.5 ± 0.5	9.6 ± 0.6†
16 Weeks	79.2 ± 1.3	35.0 ± 2.2*	82.7 ± 2.3	53.4 ± 1.9*†	7.7 ± 0.7	28.8 ± 2.2*	7.0 ± 0.3	17.2 ± 1.3*†

Values are mean ± S.E. of 8-10 rats. Significant differences ($p<0.05$) from respective control groups are indicated by θ) ANOVA. *) ANOVA followed by Bonferroni's test. †) Significantly different from corresponding experimental left ventricular tissue.

respectively. However, GSH levels in the right ventricle remained significantly higher than those of the left ventricle.

GSSG content in both the left and right ventricles were unchanged from their respective controls at 1 week. However, GSSG content was significantly elevated at 4, 8 and 16 weeks in the left ventricle as compared to the values in left ventricular controls and those of the right ventricle in MI animals. The GSSG levels in the right ventricle were increased significantly only at 16 weeks post-MI.

The GSH/GSSG ratio was also analyzed and these data are shown in Figure 7. Base line values for this ratio in control right and left ventricles were not different from each other. This ratio was marginally increased in the right ventricle of infarcted animals but these differences were not statistically different relative to controls at 1 week. A significant depression in the GSH/GSSG ratio in the left ventricle was seen at 1, 4, 8 and 16 weeks relative to controls whereas a significant decrease in this ratio in the right ventricle was observed only at 8 and 16 weeks. In the MI animals, the ratio in the right ventricle was higher than the left ventricle at all time points.

Treatment of infarcted animals with vitamin E increased GSH levels in both the left and right ventricles at 16 weeks post-MI such that these values were not significantly different from respective control values (Table 6). Vitamin E treatment of MI animals also resulted in the restoration of GSSG levels back to control values in each of the respective ventricles (Table 6). Supplementation with vitamin E increased the GSH/GSSG ratio in both the left and right ventricles of 16 week post-MI animals such that these values were no different from respective control values (Table 6).

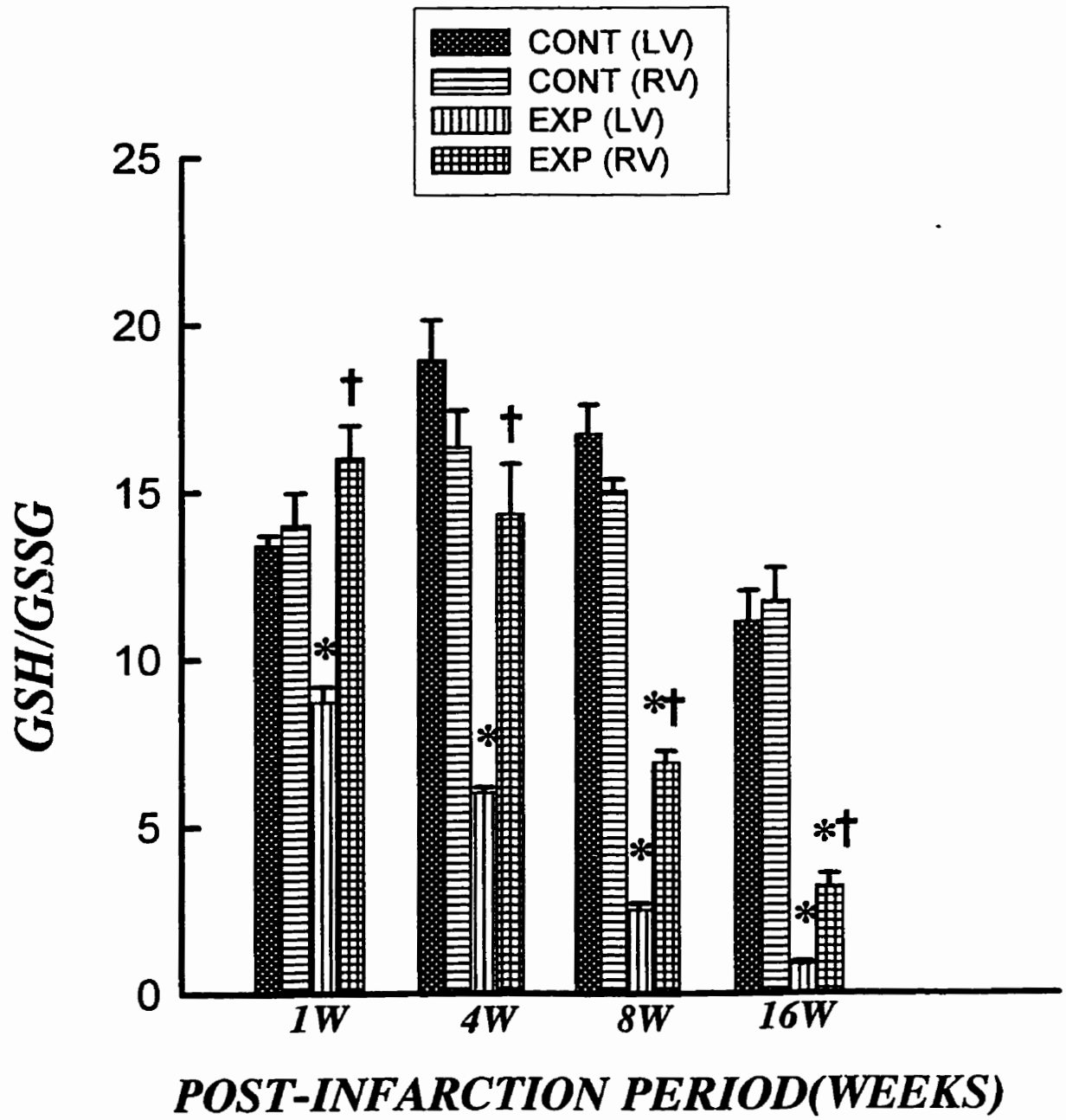


Figure 7: Reduced and oxidized (GSH/GSSG) glutathione ratio in the left and right ventricles of control and experimental hearts at 1, 4, 8 and 16 weeks post-infarction. Data are mean ± S.E. from 8-10 rats. *) Significantly different ($p < 0.05$) from respective controls. †) Significantly different ($p < 0.05$) from corresponding experimental left ventricular tissue.

Table 6: Myocardial reduced (GSH) and oxidized (GSSG) glutathione content in rats at 16 weeks post-MI with and without vitamin E treatment.

Animal Group	GSH (μmole/g tissue wt)		GSSG (μmole/g tissue wt)		GSH/GSSG	
	LV	RV	LV	RV	LV	RV
Basal Diet:						
CONT	70.0 ± 2.6	70.7 ± 1.8	6.0 ± 0.7	6.3 ± 1.1	11.7	11.2
MI	37.4 ± 3.4*	59.6 ± 3.1ϕ	30.5 ± 2.2*	16.3 ± 2.2*	1.2*	4.0*
Vitamin E Supplemented Diet:						
CONT	71.1 ± 2.9	68.5 ± 3.0	5.9 ± 0.4	5.9 ± 0.3	12.1	11.6
MI	65.3 ± 5.0†	65.5 ± 1.6	8.0 ± 1.1†	5.9 ± 0.7†	8.2†	11.1†

Values are mean ± SEM of 6-8 rats. *) Significantly different ($p<0.05$) from respective control group by ANOVA followed by Bonferroni's test. †) Significantly different ($p<0.05$) from untreated MI group by ANOVA followed by Bonferroni's test. ϕ) Significantly different ($p<0.05$) from respective control group by Student's t-test.

G. LIPID PEROXIDATION

The amount of lipid peroxidation was determined by evaluating myocardial thiobarbituric acid reactive substances (TBARS) and these data are shown in Figure 8. At 1 week, no change was observed in the amount of TBARS in either the left or right ventricle relative to respective controls. However, at 4, 8 and 16 weeks, myocardial TBARS in the left ventricle were elevated by 40, 51 and 100% respectively, as compared with their controls. In contrast, levels of myocardial TBARS were not changed in the right ventricle until 8 weeks. At 16 weeks, a significant increase (53%) was observed in the right ventricle relative to the respective control value.

Myocardial TBARS in the left ventricle of vitamin E treated 16 week post-MI animals remained elevated as compared with their respective controls (Figure 9). However, the magnitude of TBARS elevation in the left ventricle was far less in the vitamin E treated group as compared to the untreated group. Myocardial TBARS in the right ventricle of the vitamin E treated group were no different from respective sham controls (Figure 9).

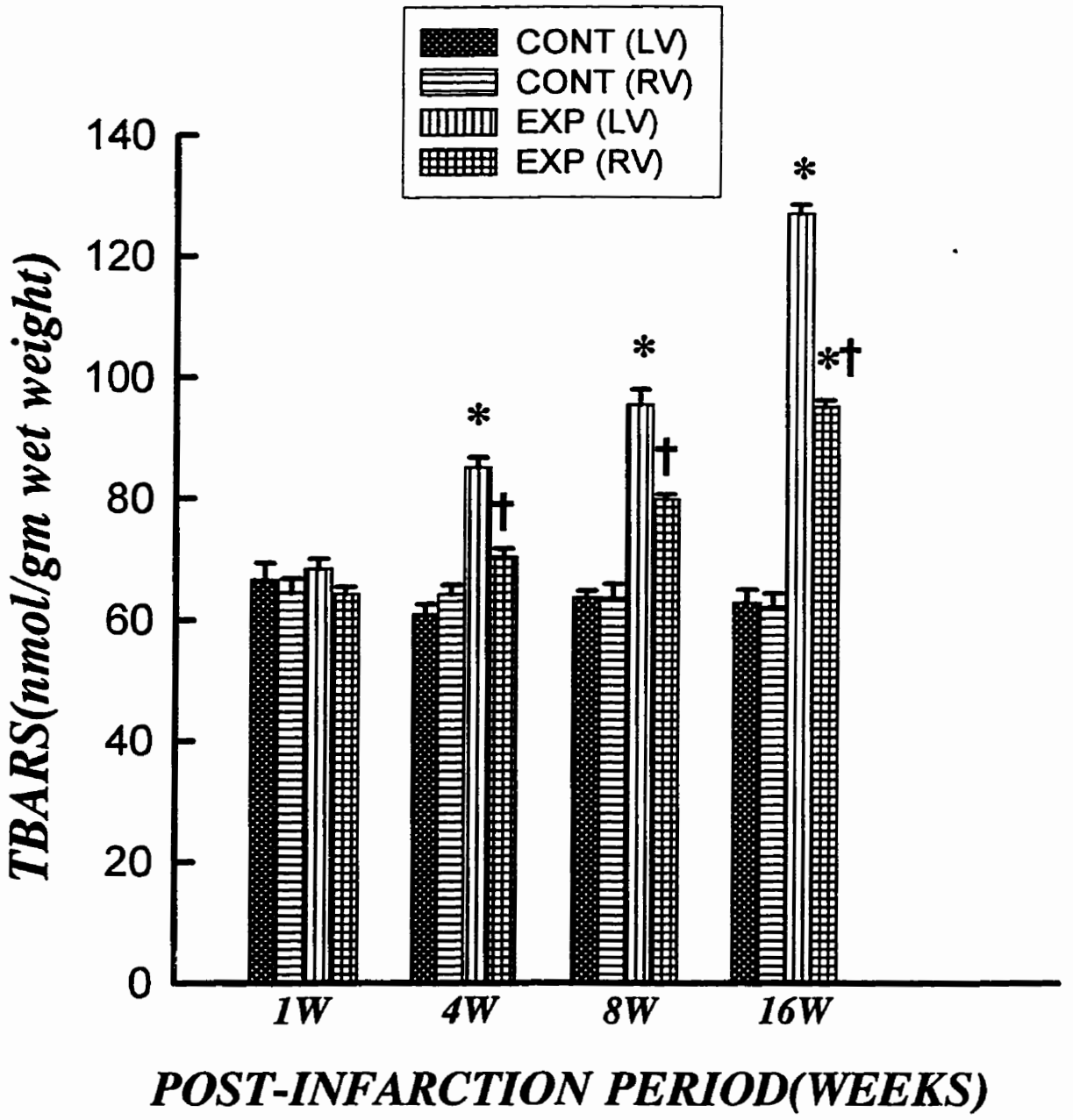


Figure 8: Lipid peroxidation as indicated by thiobarbituric acid reacting substances (TBARS) in the left and right ventricles of control and experimental hearts at 1, 4, 8 and 16 weeks post-infarction. Data are mean ± S.E. from 8-10 rats. *) Significantly different ($p < 0.05$) from respective controls. †) Significantly different ($p < 0.05$) from corresponding experimental left ventricular tissue.

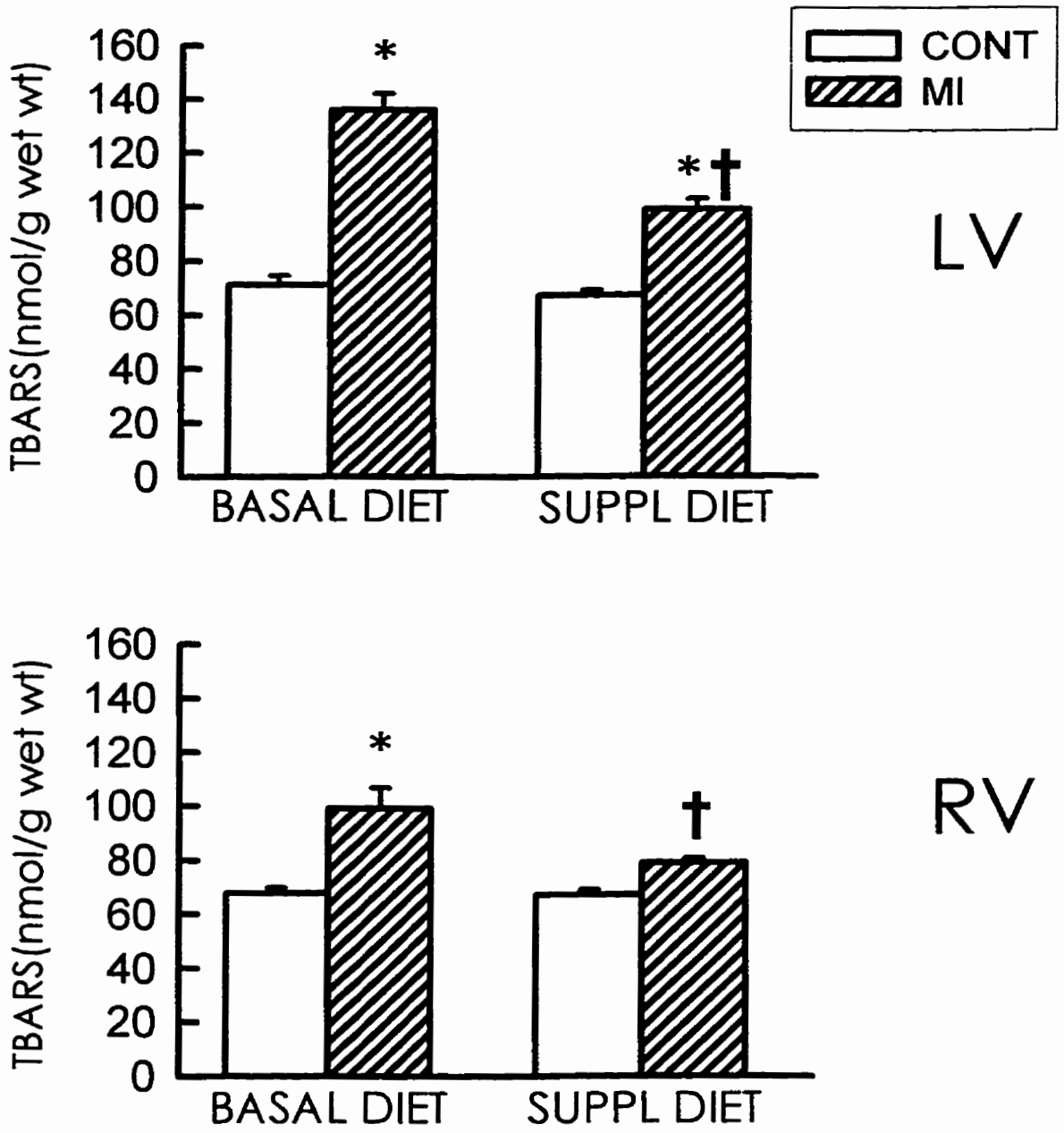


Figure 9: Lipid peroxidation as indicated by TBARS at 16 weeks after surgery in the viable LV and RV of control (CONT) and MI animals maintained on a basal or vitamin E supplemented (Suppl) diet. Data are mean ± SEM from 6-8 rats. *) Significantly different ($p<0.05$) from respective controls. †) Significantly different ($p<0.05$) from corresponding ventricular tissue of untreated MI hearts.

V. DISCUSSION

Surviving patients with myocardial infarction (MI) are at an increased risk for the occurrence of an array of cardiovascular complications involving congestive heart failure (CHF), reinfarction, arrhythmias and sudden cardiac death (Woo and White, 1994; Rutherford et al.,1994; Pfeffer et al., 1993). However, CHF continues to be a prominent clinical problem, with 15-25% of surviving patients with MI ultimately developing chronic overt CHF in the subsequent 5 years (Francis et al., 1993). It has been reported that the only kind of heart disease that is on the rise is heart failure (Beamish, 1994). There are two main reasons for this increase in the number of heart failure patients: A) a significant improvement in the survival rate from heart attacks has resulted in these patients living long enough to go on to develop CHF; B) the prevalence of heart failure is age-dependent and the number of people over the age of 65 is steadily growing. This adverse outcome has resulted in a refocussing of our attention towards developing a clearer understanding of the pathogenesis of CHF. Subsequent treatment measures which may prevent or retard its progression, particularly in these two growing subsets of patients, are also being sought.

During the past decade, experimental and clinical research efforts have resulted in the development of different therapeutic approaches towards the management of MI and CHF patients. In the acute setting, thrombolytic agents help restore patency of infarct-related arteries and limit infarct size (Sharpe, 1993). Addition of ACE inhibitors to standard conventional therapy for clinical CHF has led to demonstrable improvements in reducing the distressing symptoms accompanying this syndrome. Despite these therapeutic advances,

long-term prognosis for heart failure patients remains grave. Thus, further investigations are needed to delineate mechanisms that may be operative in mediating the development of heart failure.

Findings from the present study demonstrate the occurrence of an antioxidant deficit and increased oxidative stress in the surviving myocardium during the development of heart failure subsequent to MI. Induction of MI in the present study produced typical features of CHF consisting of fluid retention and hemodynamic impairment. The next logical step in our research process was to examine the therapeutic efficacy of antioxidant therapy in modulating the severity of heart failure. Data reported in the present study has significantly advanced our understanding of the pathogenesis of heart failure as well as providing a novel treatment therapy for preventing/mitigating its progression.

Although involvement of free radicals in tissue injury has been known to radiation and cancer biologists for some time, attention of cardiac biologists was drawn to this fact by early studies dealing with catecholamine-induced cardiomyopathy (Singal et al., 1982), adriamycin-cardiomyopathy (Singal et al., 1987) and ischemia-reperfusion injury (Bolli, 1988). These early studies have been followed by a series of elegant and detailed experiments which have provided incremental evidence in support of the concept that free radicals do mediate cardiac injury (Kaul et al., 1993). However, direct information on the cause and effect relationship is lacking. Data presented in this thesis show that an increase in oxidative stress mediated by free-radicals plays a causal role in the development of heart failure.

The occurrence of postinfarction heart failure in patients (Kannel et al., 1979; Nicod et al., 1988; Greene et al., 1989; Pfeffer et al., 1993) and its reproduction in different animal models (Fletcher et al., 1981; Pfeffer et al., 1985; Dixon et al., 1990; Hill and Singal, 1996) has been firmly established. Characteristic hemodynamic as well as neurohormonal changes associated with CHF have been documented in humans (Krum et al., 1995b; McMurray et al., 1992; Packer, 1992) as well as in a variety of animal species, including rat (Siveski-Iliskovic et al., 1994; Hill and Singal, 1996; Watkins et al.,1976; Hodsman et al, 1988). Some of the clinical features in common to both patients and different animal models include fluid retention as well as ventricular dilation and neurohormonal activation (Pfeffer and Braunwald, 1990; Gaudron et al., 1993b; Francis et al., 1993). Since coronary artery ligation in the rat is an excellent model of chronic left ventricular failure that closely mimics the structural, functional and symptomatic features of the human condition, its use as an animal model has been extensive and reliable (Hodsman et al.,1988). The use of this model in our study is also one that is highly reproducible.

A significant amount of research has been devoted to understanding the cellular and subcellular mechanisms underlying the development of cardiac dysfunction and progression to overt heart failure. These efforts have resulted in several postulates being put forth which include abnormalities in the production and utilization of high energy phosphates (Bing, 1983), excitation-contraction coupling and calcium metabolism defects (Bing, 1983; Gwathmey et al., 1987), adrenergic nervous system alterations (Bristow et al., 1982; Vatner et al., 1985; Chidsey et al., 1964), ventricular remodeling (Gaudron et al., 1993a; Pfeffer and Braunwald, 1990; McKay et al., 1986), free radical formation and lipid peroxidation (Dhalla

and Singal, 1994; Siveski-Iliskovic et al., 1994; Belch et al., 1991; McMurray et al., 1990; Sobotka et al., 1993) and apoptosis (Narula et al., 1996; Yao et al., 1996). Hence, the pathogenesis of heart failure, irrespective of etiology, appears to be multifactorial and each of the above listed mechanisms may not be mutually exclusive.

In the present study, maintenance of LVSP and LVEDP in the 1-week post-MI group indicated sustained left ventricular functioning in these animals. Maintenance of RVEDP and a significant increase in RVSP indicated enhanced functioning of the right ventricle. Clinical signs of heart failure were not evident and wet/dry weight ratio of lungs and liver also did not suggest any heart failure. Thus, these animals were considered to be in a "nonfailure" stage. In the 4-week post-MI group, LVEDP was elevated while LVSP was depressed. RVEDP and RVSP were both maintained. Absence of lung or liver congestion indicated that these animals had some functional abnormalities without any influence on the tissues upstream. This stage was designated as "mild heart failure". At 8 weeks, a further reduction in LVSP and an increase in LVEDP was seen while RVEDP was elevated and RVSP sustained. Pulmonary edema without liver congestion was apparent and thus these animals were considered to be in a stage of "moderate heart failure". At 16 weeks, the greatest elevation in LVEDP and depression in LVSP was also accompanied by a significant increase in the RVEDP and decrease in the RVSP. Pulmonary edema as well as liver congestion were both present. In addition, animals from the 16 week post-MI group displayed overt clinical signs of heart failure consisting of dyspnea, abdominal enlargement and ascites, cyanosis of peripheral extremities and markedly lethargic behavior. Thus, the 16 week post-MI group was considered to be in a stage of "severe heart failure". The

proposed segregation of experimental MI animals into nonfailure and mild, moderate and severe stages of heart failure at 1, 4, 8 and 16 weeks, respectively, is being adopted here for a more objective comparison of the hemodynamic function with the biochemical changes in antioxidants and oxidative stress in each of the ventricles. It is emphasized that this classification is strictly arbitrary. Although deterioration of contractile function occurred in both ventricles over a 16 week period, changes in the right ventricle did not appear until 4-8 weeks after the changes in the left ventricle. Thus, right side heart failure follows left side heart failure in the more chronic stages of post-MI (Hill and Singal, 1997).

Although heart failure subsequent to MI has been reported to be associated with an antioxidant deficit as well as increased oxidative stress (Hill and Singal, 1996), the present study demonstrates for the first time that these changes are regionally specific and also occur in a characteristic fashion, first in the left ventricle, later on followed by the right ventricle. Furthermore, these regionally specific changes correlated with the severity of dysfunction and failure in each of the ventricles. In a variety of experimental studies, the presence of an antioxidant deficit has been reported to be one of the mechanisms mediating the development of heart failure (Dhalla et al., 1996; Gupta and Singal, 1989b; Singal and Kirshenbaum, 1990). One week after MI, sustained cardiac function in the left ventricle was accompanied by the maintenance of antioxidants while the hyperfunctioning right ventricle showed a significant increase in the antioxidant status. An increase in the myocardial antioxidant status in the right ventricle at this stage was more clearly evidenced by a significantly higher redox ratio as compared to the left ventricle, indicating reduced oxidative stress in the right ventricle (Kaul et al., 1993; Ferrari et al, 1991; Curello et al., 1986). An increase in the

myocardial redox state has been reported in various conditions affecting the heart and the change is associated with maintained or improved hemodynamic function (Dhalla et al., 1996; Gupta and Singal, 1989b; Ferrari et al.,1991; Shan et al., 1990).

Previous studies have shown that left ventricular failure after chronic MI is associated with improved or sustained right ventricular function (Fletcher et al., 1981; Pfeffer et al., 1979). The precise mechanism by which left ventricular MI leads to sustained or hyperfunctioning of the right ventricle is currently unknown. Postulates that have been put forth to explain this phenomenon include: 1) Pulmonary hypertension arising from medial hypertrophy of the muscular branches of the pulmonary artery following infarction (Turek et al., 1978); 2) altered gradient across the pulmonary vascular bed during left ventricular failure (Pfeffer et al., 1979) and 3) sustained systemic arterial pressure in face of reduced cardiac output as a consequence of left ventricular failure (Turek et al., 1978). However, the current study demonstrates for the first time that maintenance of the myocardial endogenous antioxidant status in the right ventricle following left ventricular MI may serve to sustain right ventricular function, while a deficit in the antioxidant defense system may predispose the right ventricle to oxidative damage and subsequent myocardial dysfunction.

Mild failure at 4 weeks post-MI was accompanied by significant depressions in catalase and GSHPx activities in the left ventricle, but not in the viable right ventricle. An increase in oxidative stress in the left ventricle at this stage was evidenced by a significant decrease in GSH/GSSG ratio and a significant increase in TBARS. Increases in TBARS have been reported in heart failure secondary to adriamycin cardiotoxicity (Siveski-Iliskovic

et al., 1994) and chronic pressure overload of the heart (Dhalla and Singal, 1994). In the present study, moderate failure was associated with a further reduction in catalase and GSHPx activities as well as a further increase in TBARS in the left ventricle, but not in the right ventricle. The severe failure stage was not only accompanied by the greatest deficit in antioxidant status and the most pronounced increase in oxidative stress in the left ventricle but involvement of the right ventricle with respect to antioxidant deficit and elevated oxidative stress was also found to occur. These findings suggest that a relative deficit in myocardial endogenous antioxidants and higher oxidative stress may play a pathophysiological role in heart failure subsequent to left ventricular MI. Furthermore, in each of the ventricles, the increase in oxidative stress precedes the depressed function. Thus, these data provide strong evidence of a tight correlation between the myocardial antioxidant status and cardiac function/dysfunction in each of the respective ventricles subsequent to left ventricular MI.

In the present study, enzymatic antioxidant measurements consisted of GSHPx and catalase activities. Superoxide dismutase (SOD) activity was not measured. Although SOD is the first line of defense against free-oxygen radical mediated damage, it acts to increase the levels of hydrogen peroxides by virtue of catalyzing the dismutation of superoxide anion to hydrogen peroxide (Kaul et al., 1993). As a result, catalase and GSHPx become the most crucial antioxidant enzymes as they both act to detoxify the elevated levels of peroxides generated by the enzymatic action of SOD. Thus, we deliberately chose to measure GSHPx and catalase activities only.

Recent clinical studies of heart failure patients have corroborated the findings obtained from the various animal models of heart failure. In patients with CHF, pentane, a product of lipid peroxidation, was found to be significantly elevated as compared to healthy age-matched subjects (McMurray et al., 1990; Weitz et al., 1991; Roberts et al., 1990; Sobotka et al., 1993). Furthermore, it has also been demonstrated that in CHF patients, lipid peroxidation increases in proportion to the severity of heart failure as assessed in the exhaled air or in the plasma (Diaz-Velez et al., 1996; Sobotka et al., 1993). In patients, plasma levels of vitamin E, a "chain-breaking antioxidant", were found to be progressively decreased by 26% during the first 48 hours after the onset of acute MI (Scragg et al., 1989) and these reductions in vitamin E coincided with a period of increased risk for subsequent reinfarction (Street et al., 1994). Thus, the concept that "a relative deficit in the antioxidant reserve" may mediate the pathogenesis of heart failure (Singal and Kirshenbaum, 1990) is further supported by these studies.

Molecular mechanisms for the depressed activities of these antioxidants are not known. Antioxidant enzymes have been shown to be substrate-stimulated as well as inactivated under oxidative stress (Reddy and Tappel, 1974; Kimball et al., 1976; Chow and Tappel, 1972; Cowan et al., 1993). Sympathectomy (Toleikis and Godin, 1995) and a subchronic β-blockade (Khaper et al., 1997) have also been shown to modify myocardial antioxidant enzyme activities. Alterations in antioxidant enzymes under a wide range of physiological and pathological conditions such as age (Nohl and Hegner, 1979), exercise (Kanter et al., 1985; Higuchi et al., 1985), beta-thalassaemia (Gerli et al., 1980), cardiac hypertrophy (Gupta and Singal, 1989b; Dhalla and Singal, 1994; Kirshenbaum and Singal, 1993), heart

failure (Dhalla and Singal, 1994) and hypoxia (Dhaliwal et al., 1991) have been reported. Irrespective of the mechanism, these studies clearly document a dynamic nature of the endogenous antioxidant status which adjusts to the physiological and pathophysiological conditions imposed.

Although the above mentioned findings suggested that increased oxidative stress may contribute to the development of CHF, they were largely correlative in nature and did not establish whether these antioxidant changes were a "cause" or an "effect" of CHF. Pathological tissue damage can result in a secondary decrease in the activity of antioxidant enzymes and elevations in lipid peroxides. Thus, in an effort to resolve this issue, we decided to examine the effects of long-term antioxidant therapy involving vitamin E on the development of CHF subsequent to MI. The rationale behind vitamin E as the therapeutic antioxidant agent of choice was based on the fact that vitamin E has been shown to be cardioprotective in patients with ischemic heart disease (IHD) (Stephens et al., 1996) as well as in a pressure-overload model of CHF in guinea pigs (Dhalla and Singal, 1994). Data from animal studies indicate that vitamin E toxicity is low and is not associated with any mutagenicity or carcinogenicity (Bendich and Machlin, 1988). Furthermore, human studies involving oral vitamin E therapy have documented few side effects, even at high doses (>3000 mg/day) (Bendich and Machlin, 1988). For these reasons, we chose vitamin E as our therapeutic antioxidant.

The route of vitamin E administration was via dietary supplementation so as to mimic as closely as possible the way humans obtain their vitamin E. The dosage of vitamin E used in our study was 1545 mg/kg basal diet. This amount was determined on the basis of the

average amount of food consumed by the rats per day and a relative absorption efficiency of 20-40%, which makes this dose of vitamin E comparable to that which has been shown to be efficacious in both the human condition and experimental animal models. Vitamin E treatment of coronary-artery ligated animals with dietary vitamin E resulted in the complete normalization of RVSP and RVEDP in the 16 week post-MI animals. An improvement in left ventricular hemodynamic parameters was also seen in this treatment group, though complete normalization of function was not achieved as LVEDP remained mildly elevated and LVSP remained mildly depressed. However, absence of lung or liver congestion in these animals indicates that the residual functional abnormalities were not severe enough to exert any influence on the tissues upstream. In addition, no other clinical signs of heart failure were present.

Restoration of normal right ventricular function with vitamin E treatment occurring in association with left ventricular infarction may have important prognostic implications. It has been reported that there is a high frequency (40%) of right ventricular dysfunction accompanying inferior left ventricular infarction (Shah et al.,1985). Some clinical studies have shown that patients with acute inferior MI and right ventricular involvement have a poorer short-term prognosis than patients in whom right ventricular function is unaffected (Bueno et al.,1997; Zehender et al.,1993; Shah et al., 1985). Poorer outcome in these subset of patients is related primarily to the development of low cardiac output shock (Lloyd et al.,1981; Goldstein et al.,1982). Furthermore, presence of postinfarction right ventricular dysfunction is associated with a higher incidence of major cardiac complications (Bueno et al., 1997).

Enhanced left and right ventricular hemodynamic function in the 16 week post-MI animals supplemented with dietary vitamin E was associated with reduced oxidative stress, as indicated by an increased GSH/GSSG ratio and reduced formation of TBARS in each of the ventricles. Furthermore, an improvement in the myocardial catalase and GSHPx activities along with an increase in the myocardial content of vitamin E was also seen in each of the respective ventricles of this treatment group. These findings demonstrate for the first time a causal role for oxidative stress in the pathogenesis of heart failure.

It should be pointed out that in this study, we examined the effects of vitamin E therapy on left and right ventricular function specifically in the 16 week post-MI group. The reasons for selecting this particular post-MI period were two-fold. The first was to allow for an adequate amount of time for these animals to accumulate vitamin E from their diet in levels sufficient for an effect to be seen. Since the uptake and absorption of vitamin E is rather low from diet, we felt that a more chronic vitamin E treatment therapy would allow for these animals to build up their tissue levels of vitamin E. Hence, we analyzed the effects of vitamin E therapy only in the 16 week post-MI animals. The second reason for selecting this particular post-MI period was due to the fact that at 16 weeks post-MI, animals are in severe heart failure. Since the primary objective of instituting a chronic antioxidant therapy involving vitamin E was to assess whether this treatment regimen could modulate the development/severity of heart failure, we wanted to establish the failure and then evaluate the effects of vitamin E on mitigating its progression. By selecting the 16 week post-MI time period, it allowed for this analysis to be performed.

The myocardial content of vitamin E was shown to be significantly reduced in the viable left ventricle of the 16 week post-MI animals while no change was observed in the right ventricle. Dietary supplementation with vitamin E increased the myocardial content of the vitamin in both the left and right ventricles of the 16 week post-MI animals, though the levels in the left ventricle remained lower as compared to their respective sham controls supplemented with vitamin E. However, the fact that the MI animals supplemented with vitamin E showed a significant improvement in both left and right ventricular hemodynamic function, indicates that the relative increase in oxidative stress in each of the respective ventricles contributes to the pathogenesis of ventricular dysfunction and failure. Due to its lipid solubility, vitamin E remains the most important and effective antioxidant in protecting against free-radical mediated lipid peroxidation and subsequent damage of biological membranes. Maintenance of sufficient levels of myocardial vitamin E has been reported to be cardioprotective in preventing the transition from compensatory hypertrophy to decompensatory hypertrophy and heart failure in a pressure-overload model of heart failure in guinea pigs (Dhalla et al., 1996).

A recent surge in patient studies have begun to document the clinical value of vitamin E treatment on prevention of cardiovascular complications in patients who are at an increased risk for such events. In patients with angiographically proven symptomatic coronary atherosclerosis, oral administration of 400-800 IU of vitamin E daily resulted in a substantial reduction in the rate of non-fatal MI (Stephens et al., 1996), although no effects on cardiovascular death was observed. Substantial decreases in plasma α-tocopherol levels with reperfusion after percutaneous transluminal coronary angioplasty (PTCA) in patients with

acute MI has been reported (Lafont et al., 1996). A similar reduction in α-tocopherol levels after reperfusion during coronary artery bypass graft (CABG) surgery has also been shown to occur in the plasma as well as myocardium (Weisel et al., 1989). Moreover, preoperative oral administration of vitamin E, alone or in combination with vitamin C, for 5 days prior to coronary bypass prevented the reductions in blood vitamin E levels associated with revascularization (Ferreira et al., 1991).

Previous research has demonstrated an inverse correlation between antioxidant vitamin intake and incidence of acute MI. Daily administration of vitamin E in combination with vitamins A, C and beta-carotene in patients with suspected acute MI resulted in a significantly lower average infarct size in the antioxidant supplemented group as compared to the placebo group (Singh et al., 1996). Furthermore, a significant decline in total cardiac end points, including total cardiac death and nonfatal acute MI, was also observed (Singh et al.,1996).

Clinical studies examining the effects of vitamin E in patients with CHF have been sparse. Plasma levels of malondialdehyde have been shown to progressively increase with the advancing severity of NYHA functional class (Charney et al., 1997; Ghatak et al., 1996) while the levels of plasma enzymatic antioxidants superoxide dismutase, catalase and glutathione peroxidase have all been shown to progressively decline (Ghatak et al.,1996). The addition of vitamin E in doses of 400 mg once a day orally for a period of 4 weeks substantially reduced the plasma levels of malondialdehyde and in addition, produced a significant elevation in the plasma enzymatic antioxidants (Ghatak et al., 1996).

Normalization of these oxidative indices was accompanied by a markedly improved response in these vitamin E supplemented patients.

Epidemiological studies have indicated that death from cardiovascular disease is inversely proportional to the plasma levels of vitamin E (Gey et al., 1987). Furthermore, evidence of an association between a high intake of vitamin E and a subsequent lower risk for coronary artery disease has been reported in both men and women (Rimm et al.,1993; Stampfer et al., 1993). The exact biological mechanisms responsible for this protection are unclear. Although the present study does not directly address the mechanism by which vitamin E modulates the development of CHF, the observation that vitamin E supplemented infarcted rats had reduced oxidative stress and an improved antioxidant reserve concomitant with enhanced hemodynamic functioning suggests that the protection afforded by vitamin E may be attributable in part to its antioxidant properties. Vitamin E is a lipid-soluble, naturally occurring antioxidant and its presence in biological membranes is thought to represent the major defense system against peroxidation of component lipids. Vitamin E acts to stabilize biological membranes by interrupting the chain of free radical reactions as well as by reducing lipid peroxidation (Lucy, 1972). A decline in myocardial vitamin E levels, as seen in this study, may comprise antioxidant protection, leaving the myocardium vulnerable to free-radical injury. Because vitamin E treatment produced an improvement in myocardial enzymatic antioxidants in the present study, its beneficial effect may be due to a combination of its direct antioxidant properties as well as its ability to spare the remaining endogenous myocardial enzymatic antioxidants.

A protective mechanism of vitamin E independent of direct free-radical scavenging may exist. α-Tocopherol has been shown to decrease platelet adhesion and aggregation (Steiner, 1991), promote the inhibition of vitamin K-dependent clotting factors (Dowd and Zheng, 1995) and inhibit the production of nitric oxide (Boulanger et al., 1992). In animals, vitamin E supplementation has been shown to reduce the susceptibility of low-density lipoprotein (Jialal and Grundy, 1992), thereby exerting antiatherogenic properties. Vitamin E is also thought to have a non-oxidant effect in suppressing atherosclerosis by inhibiting protein kinase C activity that is associated with the stimulation of smooth muscle cell proliferation by low density lipoprotein (Ozer et al., 1995; Stauble et al., 1994; Chatelain et al.,1993). Recently, vitamin E has been shown to have antiarrhythmic properties, preventing lethal ventricular arrhythmias in dogs subjected to MI (Sebbag et al.,1994). These additional operative mechanisms of action may explain why use of vitamin E in primary prevention of coronary heart disease has been successful (Rimm et al.,1993; Stampfer et al., 1993).

In conclusion, the present study reveals that heart failure subsequent to MI is associated with an antioxidant deficit and increased oxidative stress first in the left ventricle, followed in the more chronic stages, by the right ventricle. Improved ventricular function with vitamin E treatment was accompanied by a reduction in myocardial oxidative stress and an increased antioxidant reserve in each of the respective ventricles. These findings support a causal role for oxidative stress in mediating the pathogenesis of heart failure. The study suggests that surviving patients with MI may benefit from vitamin E therapy. Large scale clinical trials will be required to establish the efficacy of this approach as novel treatment strategy for preventing/retarding the progression of heart failure among post-MI patients.

VI. REFERENCES

Abassi Z, Haramati A, Hoffman A, Burnett JC Jr, Winaver J: Effect of converting-enzyme inhibition on renal response to ANF in rats with experimental heart failure. Am J Physiol (Regulatory Integrative Comp Physiol 28) 259:R84-R89, 1990

Ader R, Chatterjee K, Ports T, Brundage B, Hiramatsu B, Parmley W: Immediate and sustained hemodynamic and clinical improvement in chronic heart failure by an oral angiotensin-converting enzyme inhibitor. Circulation 61:931-937, 1980

Afzal N, Dhalla NS: Differential changes in left and right ventricular SR calcium transport in congestive heart failure. Am J Physiol 262 (Heart Circ Physiol 31): H868-H874, 1992

Anderson ME: Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol 113:548-555, 1985

Anversa P, Li P, Zhang X, Olivetti G, Capasso JM: Ischaemic myocardial injury and ventricular remodelling. Cardiovasc Res 27:145-157, 1993

Arends MJ, Morris RG, Wyllie AH: Apoptosis: the role of endonuclease. Am J Pathol 136:593-608, 1990

Armstrong PW, Moe GW: Medical advances in the treatment of congestive heart failure. Circulation 88: 2941-2952, 1994

Asano T, Kamiya N, Marishita R, Kato K: Immunoassay for the βγ-subunits of GTP-binding proteins and their regional distribution in bovine brain. J Biochem 103: 950-953, 1988

Baker KM, Chernin MI, Wixon SK, Aceto JF: Renin-angiotensin system involvement in pressure-overload cardiac hypertrophy in rats. Am J Physiol (Heart Circ Physiol) 259:H324-H332, 1990

Barr PJ, Tomei LD: Apoptosis and its role in human disease. Bio Technology 12:487-493, 1994

Bashey RI, Martinez-Hernandez A, Jiminez SA: Isolation, characterization and localization of cardiac collagen type VI. Associations with other extracellular matrix components. Circ Res 70:1006-1017, 1992

Beamish RE: Heart failure: the ironic failure of success. Can J Cardiol 10:603, 1994

Beau SL, Tolley TK, Saffitz JE: Heterogenous transmural distribution of β-adrenergic receptor subtypes in failing human hearts. Circulation 88:2501-2509, 1993

Belch JJ, Bridges AB, Scott N, Chopra M: Oxygen free radicals and congestive heart failure. Br Heart J 65(5):245-248, 1991

Bendich A, Machlin LJ: Safety of oral intake of vitamin E: Am J Clin Nutr 48:612-619, 1988

Beta-Blocker Heart Attack Trial Research Group: A randomized trial of propranolol in patients with acute myocardial infarction. I. Mortality results. JAMA 247:1707-1713, 1982

Beuckelmann DJ, Nabauer M, Erdmann E: Intracellular calcium handling in isolated ventricular myocytes from patients with terminal heart failure. Circulation 85:1046-1055, 1992

Bing RJ: The biochemical basis of myocardial failure. Hosp Pract Off Ed 18:93-97,101-107,110-112, 1983

Bishop JE, Greenbaum R, Gibson DG, Yacoub M, Laurent GJ: Enhanced deposition of predominantly type I collagen in myocardial disease. J Mol Cell Cardiol 22:1157-1165, 1990

Boer PH, Ruzicka M, Lear W, Harmsen E, Rosenthal J, Leenen FHH: Stretch-mediated activation of the cardiac renin gene. Am J Physiol (Heart Circ Physiol 36) 267:H1630-H1636, 1994

Boheler KR, Carrier L, De La Bastie D, Allen PD, Homajda M, Mercadier JJ, Schwartz K: Skeletal actin mRNA increases in the human heart during ontogenic development and is the major isoform of control and failing human hearts. J Clin Invest 88: 323-330, 1991

Bohm M, Diett F, Feiler G, Kemkes B, Erdmann E: α-Adrenoceptors and α-adrenoceptor-mediated positive inotropic effects in failing human myocardium. J Cardiovasc Pharmacol 12:357-364, 1988

Bohm M, Gierschik P, Jakobs K-H, Pieske B, Schnabel P, Ungerer M, Erdmann E: Increase of G_i in human hearts with dilated but not ischemic cardiomyopathy. Circulation 82:1249-1265, 1990

Bolli R: Oxygen-derived free radicals and postischemic myocardial dysfunction ("stunned myocardium"). J Am Coll Cardiol 12(1):239-249, 1988

Bolli R, Patel BS, Jeroudi MO, Lai EK, McCay PB: Demonstration of free radical generation in "stunned" myocardium of intact dogs with the use of the spin trap A-phenyl N-tert-butyl nitrone. J Clin Invest 82:476-485, 1988

Bolli R, Jeroudi MO, Patel BS, Aruoma OI, Halliwell B, Lai EK, McCay PB: Marked reduction of free radical generation and contractile dysfunction by antioxidant therapy begun at the time of reperfusion. Evidence that myocardial "stunning" is a manifestation of reperfusion injury. Circ Res 65:607-622, 1989

Bolli R: Myocardial "stunning" 20 years later: a summary of current concepts regarding its pathophysiology, pathogenesis, and clinical significance. Dialogues in Cardiovascular Medicine 1:5-26, 1996

Boulanger CM, Tanner FC, Bea ML, Hahn AW, Werner A, Luscher TF: Oxidized low density lipoproteins induce mRNA expression and release of endothelin from human and porcine endothelium. Circ Res 70:1191-1197, 1992

Braunwald E, Muller JE, Kloner RA, Maroko PR: Role of beta-adrenergic blockade in the therapy of patients with myocardial infarction. Am J Med 74:113-123, 1983

Braunwald E, Sonnenblick EH, Ross J: Mechanisms of cardiac contraction and relaxation. In: Braunwald E (ed), Heart Disease, A Textbook of Cardiovascular Medicine. Philadelphia, WB Saunders, pp 383-425, 1988

Breithardt G, Borggrefe M, Fetsch T, Bocker D, Makijarvi M, Reinhardt L: Prognosis and risk stratification after myocardial infarction. Eur Heart J 16(Suppl G):10-19, 1995

Brenner BM, Ballerman BJ, Gunning ME, Zeidel ML: Diverse biological action of atrial natriuretic peptide. Physiol Rev 70:665-699, 1990

Bril A, Slivjak M, DiMartino M, Feuerstein GZ, Linee P, Poyser RH, Ruffolo RR Jr, Smith EF: Cardioprotective effects of carvedilol, a novel β-adrenoceptor antagonist with vasodilating properties, in anesthetised minipigs: comparison with propranolol. Cardiovasc Res 26:518-525, 1992

Brilla CG, Pick R, Tan LB, Janicki JS, Weber KT: Remodelling of the rat right and left ventricles in experimental hypertension. Circ Res 67:1355-1364, 1990

Bristow MR, Ginsburg R, Minobe WA, Cubicciotti RS, Sageman WS, Lurie K, Billingham ME, Harrison DC , Stinson EB: Decreased catecholamine sensitivity and β-adrenergic receptor density in failing human hearts. N Engl J Med 307:205-211, 1982

Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R, Zera P, Menlove R, Shah P, Jamieson S, Stinson EB: β_1- and β_2-adrenergic receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective β_1-receptor downregulation in heart failure. Circ Res 59: 297-309, 1986

Bristow MR, Minobe W, Rasmussen R, Hershberger RE, Hoffman BB: Alpha-1 adrenergic receptors in the nonfailing and failing human heart. J Pharmacol Exp Ther 247:1039-1045, 1988

Bristow MR: Changes in myocardial and vascular receptors in heart failure. J Am Coll Cardiol 22(Suppl A):61A-71A, 1993

Brivet F, Delfraissey J-F, Guidicelli J-F, Richer C, Legrand A, Dormont J: Immediate effects of captopril in acute left ventricular failure secondary to acute myocardial infarction. Eur J Clin Invest 11:369-373, 1981

Bueno H, Lopez-Palop R, Bermejo J, Lopez-Sendon JL, Delcan JL: In-hospital outcome of elderly patients with acute inferior myocardial infarction and right ventricular involvement. Circulation 96:436-441, 1997

Burnett JC, Kao PC, Hu DC, Heser DW, Heublein D, Granger JP, Opgenorth TJ, Reeder GS: Atrial natriuretic peptide elevation in congestive heart failure in the human. Science 231:1145-1147, 1986

Bursch W, Kleine L, Tenniswood M: The biochemistry of cell death by apoptosis. Biochem Cell Biol 68: 1071-1074, 1990

Burton GW, Le Page Y, Gabe EJ, Ingold KU: Antioxidant activity of vitamin E and related phenols. Importance of stereoelectronic factors. J Am Chem Soc 102:7791-7792, 1980

Buttke TM, Sandstrom PA: Oxidative stress as a mediator of apoptosis. Immunology Today 15:7-10, 1994

Campbell DJ, Habener JF: Angiotensinogen gene is expressed and differentially regulated in multiple tissues of the rat. J Clin Invest 78:31-39, 1986

Campbell DJ, Kladis A, Skinner SL, Whitworth JA: Characterization of angiotensin peptides in plasma of anephric man. J Hypertens 9:265-274, 1991

Captopril-Digoxin Multicenter Research Group, The: Comparative effects of therapy with captopril and digoxin in patients with mild to moderate heart failure. JAMA 259:539-544, 1988

Caulfield JB, Leinbach R, Gold H: The relationship of myocardial infarct size and prognosis. Circulation 53 (Suppl 1):141-145, 1976

Chan AC: Partners in defense, vitamin E and vitamin C. Can J Physiol Pharmacol 71:725-731, 1993

Charney RH, Levy DK, Kalman J, Buchholz C, Ocampo ON, Eng C, Kukin ML: Free radical activity increases with NYHA class in congestive heart failure. J Am Coll Cardiol 102A: Abstract, 1997

Chatelain E, Boscoboinik DO, Bartoli GM, Kagan VE, Gey FK, Packer L, Azzi A: Inhibition of smooth muscle cell proliferation and protein kinase C activity by tocopherols and tocotrienols. Biochim Biophys Acta 1176(1-2): 83-89, 1993

Chidsey CA, Kaiser GA, Sonnenblick EH, Spann JF, Braunwald E. Cardiac norepinephrine stores in experimental heart failure in the dog. J Clin Invest 43:2386-2393, 1964

Chidsey CA, Braunwald E, Morrow AG. Catecholamine excretion and cardiac stores of norepinephrine in congestive heart failure. Am J Med 39:442-451, 1965

Chidsey CA, Weinbach EC, Pool PE, Morrow AG: Biochemical studies of energy production in the failing human heart. J Clin Invest 45:40-50, 1966

Chow CK, Tappel AL: An enzymatic protective mechanism against lipid peroxidation damage to lungs of ozone exposed rats. Lipids 7:518-524, 1972

Clairborne A: Catalase activity. In: Greenwald RA (ed), Handbook of Methods for Oxygen Radical Research. Boca Raton, CRC Press, pp. 283-284, 1985

Cleutjens JPM, Verluyten MJA, Smits JFM, Daemen MJAP: Collagen remodeling after myocardial infarction in the rat heart. Am J Pathol 147:325-338, 1995

Cody RJ, Atlas SA, Laragh JH, Kubo SH, Covit AB, Ryman KS, Shaknovitch A, Pondolfino K, Clark M, Camargo MJF, Scarborough RM, Lewicki JA: Atrial natriuretic factor in normal subjects and heart failure patients: plasma levels and renal, hormonal, and hemodynamic responses to peptide infusion. J Clin Invest 78:1362-1374, 1986

Cohn JN, Levine TB, Olivari MT: Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. N Engl J Med 311:819-823, 1984

Cohn JN, Simon A, Johnson G for the V-HeFT Study Group: Relationship of plasma norepinephrine and plasma renin activity to mortality in heart failure (abstr). Circulation 84(Suppl II):II-310, 1991

Colucci WS: In situ assessment of α- and β-adrenergic responses in failing human myocardium. Circulation 87(Suppl VII):VII-63–VII-67, 1993

Consensus Trial Study Group, The: Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). N Engl J Med 316:1429-1435, 1987

Covell JW, Chidsey CA, Braunwald E: Reduction in the cardiac response to postganglionic sympathetic nerve stimulation in experimental heart failure. Circ Res 19: 51-66, 1966

Cowan DB, Weisel RD, Williams WG, Mickle DAG: Identification of oxygen responsive elements in the 5'-flanking region of the human glutathione peroxidase gene. J Biol Chem 268:26904-26910, 1993

Crozier J, Ikram H, Awan N: Losartan in heart failure: hemodynamic effects and tolerability. Circulation 91:691-697, 1995

Curello S, Ceconi C, Bigoli C, Ferrari R, Albertini A, Guarnieri C. Change in the cardiac glutathione after ischemia and reperfusion. Experimentia 41: 42-43,1985

Curello S, Ceconi C, Medici D, Ferrari R: Oxidative stress during myocardial ischemia and reperfusion: experimental and clinical evidence. J Appl Cardiol 1:311-327, 1986

Davis R, Ribner HS, Keung E, Sonnenblick EH, LeJemtel TH: Effect of captopril in heart failure. N Engl J Med 301:117-121, 1979

De Bold AJ, Borenstein HB, Veress AT, Sonnenberg H: A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Life Sci 28:89-94, 1981

De La Bastie D, Levitsy D, Rappaport L, Mercadier JJ, Marotte F, Wisnewsky C, Brovkovich V, Schwartz K, Lompre AM: Function of the sarcoplasmic reticulum and expression of its calcium ATPase gene in pressure overload induced cardiac hypertrophy in the rat. Circ Res 66: 554-564, 1990

Delacretaz E, Nussberger J, Biollaz J, Waeber B, Brunner HR: Characterization of the angiotensin II receptor antagonist TCV-116 in healthy volunteers. Hypertension 25:14-21, 1995

Dell'Italia LJ, Meng QC, Straeter-Knowlen IM, Hankes G, Oparil S, Elton T: Increased angiotensin converting enzyme and chymase-like activity in cardiac tissue of dogs with chronic mitral regurgitation. Am J Physiol (Heart Circ Physiol 38) 269:H2065-H2073, 1995

Denniss AR, Marsh JD, Quigg RJ, Gordon JB, Colucci WS: β-Adrenergic receptor number and adenylate cyclase function in denervated transplanted and cardiomyopathic human hearts. Circulation 79:1028-1034, 1989

Dhaliwal H, Kirshenbaum LA, Randhawa AK, Singal PK: Correlation between antioxidant changes during hypoxia and recovery upon reoxygenation. Am J Physiol 261:H632-H638, 1991

Dhalla AK, Singal PK: Antioxidant changes in hypertrophied and failing guinea pig hearts. Am J Physiol 266(Heart Circ Physiol 35):H1280-H1285, 1994

Dhalla AK, Hill MF, Singal PK: Role of oxidative stress in transition of hypertrophy to heart failure. J Am Coll Cardiol 28:506-514, 1996

Dhalla NS, Pierce GN, Panagia V, Singal PK, Beamish RE. Calcium movements in relation to heart function. Basic Res Cardiol 77: 117-139, 1982

Diaz-Velez CR, Garcia-Castineiras S, Mendoza-Ramos E, Hernandez-Lopez E: Increased malondialdehyde in peripheral blood of patients with congestive heart failure. Am Heart J 131:146-152, 1996

Dixon IMC, Lee S-L, Dhalla NS: Nitrendipine binding in congestive heart failure due to myocardial infarction. Circ Research 66:782-788, 1990

Dixon IMC, Hata T, Dhalla NS: Sarcolemmal calcium transport in congestive heart failure due to myocardial infarction in rats. Am J Physiol 262 (Heart Circ Physiol 31): H1387-H1394, 1992

Dixon IMC, Pelouch V, Sethi R, Dhalla NS: Collagenous proteins in scar tissue subsequent to myocardial infarction. In: Dhalla NS, Singal PK, Takeda N, RE Beamish (eds), Pathophysiology of Heart Failure. Boston, Kluwer Academic Publishers, pp. 401-414, 1996a

Dixon IMC, Ju H, Jassal DS, Peterson DJ: Effect of ramipril and losartan on collagen expression in right and left heart after myocardial infarction. Mol Cell Biochem 165:31-45, 1996b

Dixon IMC, Ju H, Reid NL, Scammell-La Fleur T, Werner JP, Jasmin G: Cardiac collagen remodeling in the cardiomyopathic Syrian hamster and the effect of losartan. J Mol Cell Cardiol 29:1837-1850, 1997

Dowd P, Zheng ZB: On the mechanism of the anticlotting action of vitamin E quinone. Proc Natl Acad Sci USA 92:8171-8175, 1995

Dzau VJ, Colucci WS, Williams GH, Curfman G, Meggs L: Sustained effectiveness of converting enzyme inhibition in patients with severe congestive heart failure. N Engl J Med 302:1373-1379, 1980

Dzau V, Colucci WS, Hollenberg NK, Williams GH: Relation of the renin-angiotensin-aldosterone system to clinical state in congestive heart failure. Circulation 63:645-651, 1981

Dzau VJ, Ellison KE, Brody T, Ingelfinger J, Pratt RE: A comparative study of the distributions of renin and angiotensin messenger ribonucleic acids in rat and mouse tissues. Endocrinology 120:6:2334-2338, 1987

Dzau VJ: Cardiac renin-angiotensin system. Molecular and functional aspects. Am J Med 84(Suppl 3A):22-27, 1988

Eaton LW, Weiss JL, Bulkley BH, Garrison JB, Weisfeldt ML: Regional cardiac dilatation after acute myocardial infarction: recognition by two-dimensional echocardiography. N Engl J Med 300:57-62, 1979

Erdmann E: Digitalis — friend or foe? Eur Heart J 16(Suppl F):16-19, 1995

Erlebacher JA, Weiss JL, Weisfeldt ML, Bulkley BH: Early dilation of the infarcted segment in acute transmural myocardial infarction: role of infarct expansion in acute left ventricular enlargement. J Am Coll Cardiol 4:201-208, 1984

Evan GI, Wyllie AH, Gilbert CS, Littlewood TD, Land H, Brooks M, Waters CM, Penn LZ, Hancock DC. Induction of apoptosis in fibroblasts by c-myc protein. Cell 69:119-128, 1992

Evans HM, Emerson OH, Emerson GA: The isolation from wheat germ oil of an alcohol, α-tocopherol, having the properties of vitamin E. J Biol Chem 113:319-332, 1936

Feinstein BB: Effects of experimental congestive heart failure, Oabain and asphyxia on the high energy phosphate and creatine content of guinea pig heart. Circ Res 10:333-346, 1962

Feldman AM, Cates AE, Veazey WB, Hershberger RE, Bristow MR, Baughman KL, Baumgartner WA, Van Dop C: Increase of the 40,000-mol wt pertussis toxin substrate (G protein) in the failing human heart. J Clin Invest 82:189-197, 1988

Feldman AM: Modulation of adrenergic receptors and G-transduction proteins in failing human ventricular myocardium. Circulation 87(Suppl IV):IV-27–IV-34, 1993

Ferguson DW: Sympathetic mechanisms in heart failure: pathophysiological and pharmacological implications. Circulation 87(Suppl VII):VII-68–VII-75, 1993

Ferrari R, Ceconi C, Curello S, Guarnieri C, Caldarera CM, Albertini A, Visioli O: Oxygen-mediated myocardial damage during ischemia and reperfusion: role of the cellular defenses against oxygen toxicity. J Mol Cell Cardiol 17: 937-945, 1985

Ferrari R, Ceconi C, Curello S, Cargnoni A, Medici D: Oxygen-free radicals and reperfusion injury: The effects of ischemia and reperfusion on the cellular ability to neutralize oxygen toxicity. J Mol Cell Cardiol 18: 67-69, 1986

Ferrari R, Ceconi C, Curello S, Cargnoni A, Alfieri O, Pardini A, Marzollo P, Visioli O: Oxygen free radicals and myocardial damage: Protective role of thiol-containing agents. Am J Med 91(Suppl 3C):955-1055, 1991

Ferreira RF, Milei J, Llesuy S, Flecha BG, Hourquebie H, Molteni L, de Palma C, Paganini A, Scervino L, Boveris A: Antioxidant action of vitamins A and E in patients submitted to coronary artery bypass surgery. Vascular Surgery 25:191-195, 1991

Feuerstein GZ, Yue T-L, Cheng H-Y, Ruffolo RR: Myocardial protection by the novel vasodilating beta-blocker carvedilol: potential relevance of antioxidant activity. J Hypertens 11(Suppl 4):541-549, 1993

Feuerstein GZ, Ruffolo RR Jr: Carvedilol, a novel multiple action antihypertensive agent with antioxidant activity and the potential for myocardial and vascular protection. Eur Heart J 16(Suppl F):38-42, 1995

Finckh M, Hellmann W, Ganten D, Furtwangler A, Allgeier J, Boltz M, Holtz J: Enhanced cardiac angiotensinogen gene expression and angiotensin converting enzyme activity in tachypacing-induced heart failure in rats. Basic Res Cardiol 86:303-316, 1991

Fishbein MC, Maclean D, Maroko PR: Experimental myocardial infarction in the rat. Qualitative and quantitative changes during pathologic evolution. Am J Pathol 90:57-66, 1978

Fletcher PJ, Pfeffer JM, Pfeffer MA, Braunwald E: Left ventricular diastolic pressure-volume relations in rats with healed myocardial infarction. Circ Res 49:618-626, 1981

Floras JS: Clinical aspects of sympathetic activation and parasympathetic withdrawal in heart failure. J Am Coll Cardiol 22(Suppl A): 72A-84A, 1993

Francis GS, Goldsmith SR, Levine TB, Olivari MT, Cohn JN: The neurohumoral axis in congestive heart failure. Ann Intern Med 101:370-377, 1984

Francis GS, Rector TS, Cohn JN: Sequential neurohumoral measurements in patients with congestive heart failure. Am Heart J 116: 1464-1468, 1988

Francis GS, Benedict C, Johnstone DE, Kirlin PC, Nicklas J, Liang C-S, Kubo SH, Rudin-Toretsky E, Yusuf S, for the SOLVD Investigators: Comparison of neuroendocrine activation in patients with

left ventricular dysfunction with and without congestive heart failure: A substudy of the Studies of Left Ventricular Dysfunction. Circulation 82:1724-1729, 1990

Francis GS, McDonald KM, Cohn JN: Neurohumoral activation in preclinical heart failure. Remodeling and the potential for intervention. Circulation 87(Suppl IV):IV-90 — IV-96, 1993

Gaudron P, Eilles C, Kugler I, Ertl G: Progressive left ventricular dysfunction and remodeling after myocardial infarction. Potential mechanisms and early predictors. Circulation 87:755-763, 1993a

Gaudron P, Blumrich M, Ertl G: Aggravation of left ventricular dilatation and reduction of survival by a calcium channel blocker in rats with chronic myocardial infarction. Am Heart J 125:1226-1233, 1993b

Gavras H, Faxon DP, Berkoben J, Brunner HR, Ryan TJ: Angiotensin converting enzyme inhibition in patients with congestive heart failure. Circulation 58:770-776, 1978

Geltman EM: Current treatment of congestive heart failure. Coronary Artery Disease 4: 1-3, 1993

Gerli GC, Beretta L, Bianchi M, Pellegatta A, Agostini S: Erythrocyte superoxide dismutase, catalase and glutathione peroxidase activities in β-thalassaemia (major and minor). Scand J Haematol 25:87-92, 1980

Gersh BJ: The changing late prognosis of acute myocardial infarction. Implications and mechanisms. Eur Heart J 16(Suppl E):50-53, 1995

Gey KF, Brubacher GB, Stalinn HB: Plasma levels of antioxidant vitamins in relation to ischemic heart disease and cancer. Am J Clin Nutr 45:1368-1377, 1987

Gey KF, Puska P: Plasma vitamins E and A inversely correlated to mortality from ischemic heart disease in cross cultural epidemiology. Ann NY Acad Sci 570:268-282, 1989

Ghatak A, Brar MJ, Agarwal A, Goel N, Rastogi AK, Vaish AK, Sircar AR, Chandra M: Oxy free radical system in heart failure and therapeutic role of oral vitamin E. Int J Cardiol 57:119-127, 1996

Gheorghiade M, Hall V, Lakier JB, Goldstein S: Comparative hemodynamic and neurohormonal effects of intravenous captopril and digoxin and their combinations in patients with severe heart failure. J Am Coll Cardiol 13: 134-142, 1989

Goldstein JA, Vlahakes GJ, Verrier ED, Schiller ND, Tyberg JV, Ports TA, Parmley WW, Chatterjee K: The role of right ventricular systolic dysfunction and elevated intrapericardial pressure in the genesis of low cardiac output in experimental right ventricular infarction. Circulation 65:513-522, 1982

Gottlieb RA, Burleson KO, Kloner RA, Babior BM, Engler RL: Reperfusion injury induces apoptosis in rabbit cardiomyocytes. J Clin Invest 94:1621-1628, 1994

Gottlieb SS, Dickstein K, Fleck E, Kostis J, Levine TB, LeJemtel T, DeKock M.: Hemodynamic and neurohormonal effects of the angiotensin II antagonist losartan in patients with congestive heart failure. Circulation 88:1602-1609, 1993

Greene HL, Richardson DW, Hallstrom AP, McBride R, Capone RJ, Barker AH, Roden DM, Echt DS, and the CAPS investigators: congestive heart failure after acute myocardial infarction in patients receiving antiarrhythmic agents for ventricular premature complexes (Cardiac Arrhythmia Pilot Study). Am J Cardiol 63:393-398, 1989

Grossman W, Lorell BH: Hemodynamic aspects of left ventricular remodeling after myocardial infarction. Circulation 87(Suppl VII):VII-28–VII-30, 1993

Gupta M, Singal PK: Time course of structure, function and metabolic changes due to an exogenous source of oxygen metabolites in rat heart. Can J Physiol Pharmacol 67: 1549-1559, 1989a

Gupta M, Singal PK: Higher antioxidant capacity during chronic stable heart hypertrophy. Circ Res 64:398-406, 1989b

Guyatt GH, Sullivan MJ, Fallen EL, Tihal H, Rideout E, Halcrow S, Nogradi S, Townsend M, Taylor DW: A controlled trial of digoxin in congestive heart failure. Am J Cardiol 61:371-375, 1988

Gwathmey JK, Capelas L, MacKinnon R, Schoen FJ, Feldman MD, Grossman W, Morgan JP: Abnormal intracellular calcium handling in myocardium from patients with end-stage heart failure. Circ Res 61:70-76, 1987

Hamburger SA, Barone FC, Feuerstein G, Ruffolo RR: Carvedilol (Kredex) reduces infarct size in a canine model of acute myocardial infarction. Pharmacology 43:113-120, 1991

Hammermeister KE, Derouen TA, Dodge HT: Variables predictive of survival in patients with coronary artery disease: Selection by univariate and multivariate analyses from the clinical, electrocardiographic, exercise, arteriographic and quantitative angiographic evaluation. Circulation 59: 421-430, 1979

Hardy CJ, Weiss RG, Bottomley PA, Gerstenblith G: Altered myocardial high-energy phosphate metabolites in patients with dilated cardiomyopathy. Am Heart J 122: 795-801, 1991

Hatt PY: Morphological approach to the mechanism of heart failure. Cardiol 75:3-7, 1988

Higuchi M, Cartier LJ, Chen M, Holloszy JO: Superoxide dismutase and catalase in skeletal muscle: Adaptive response to exercise. J Gerontol 40:281-286, 1985

Hill MF, Singal PK: Antioxidant and oxidative stress changes during heart failure subsequent to myocardial infarction in rats. Am J Pathol 148:291-300, 1996

Hill MF, Singal PK: Right and left myocardial antioxidant responses during heart failure subsequent to myocardial infarction. Circulation 96:2414-2420, 1997

Hjalmarson A, Elmfeldt D, Herlitz J, Holmberg S, Malek I, Nyberg G, Ryden L, Swedberg K, Vedin A, Waagstein F, Waldenstrom A, Waldenstrom J, Wedel H, Wilhelmsen L, Wilhelmsson C: Effect on mortality of metoprolol in acute myocardial infarction. A double-blind randomised trial. Lancet II:823-827, 1981

Hochman JS, Bulkley BH: Pathogenesis of left ventricular aneurysms: an experimental study in the rat model. Am J Cardiol 50:83-88, 1982

Hockenbery DM, Nunez G, Milliman C, Schreiber RD, Korsmeyer SJ: Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 348:334-336, 1990

Hodsman GP, Kohzuki M, Howes LG, Sumithran E, Tsunoda K, Johnston CI: Neurohumoral responses to chronic myocardial infarction in rats. Circulation 78:376-381, 1988

Huang M, Hester RL, Guyton AC: Hemodynamic changes in rats after opening an arteriovenous fistula. Am J Physiol 262 (Heart Circ Physiol 31): H846-H851, 1992

Hutchins GM, Bulkley EH: Infarct expansion versus extension: Two different complications of acute myocardial infarction. Am J Cardiol 41: 1127-1132, 1978

Ingwall JS: Is cardiac failure a consequence of decreased energy reserve? Circulation 87(Suppl VII):VII58-VII62, 1993

James TN: Normal and abnormal consequences of apoptosis in the human heart: from postnatal morphogenesis to paroxysmal arrhythmias. Trans Am Clin Climatol Assoc 105:145-178, 1993

Jialal I, Grundy SM: Effect of dietary supplementation with alpha-tocopherol on the oxidative modification of low density lipoprotein. J Lipid Res 33:899-906, 1992

Johns TNP, Olson BJ: Experimental myocardial infarction: I. A method of coronary occlusion in small animals. Ann Surg 140:675-682, 1954

Ju H, Dixon IMC: The cardiac extracellular matrix and its role in the development of heart failure In: Singal PK, Dixon IMC, Beamish RE, Dhalla NS (eds), Mechanisms of Heart Failure. Boston, Kluwer Academic Publishers, pp. 75-90, 1995

Ju H, Zhao S, Jassal DS, Dixon IMC: Effect of AT_1 receptor blockade on cardiac collagen remodeling after myocardial infarction. Cardiovasc Res 35:223-232, 1997

Jugdutt BI: Effect of captopril and enalapril on left ventricular geometry, function and collagen during healing after anterior and inferior myocardial infarction in a dog model. J Am Coll Cardiol 25:1718-1725, 1995

Kajstura J, Cheng W, Reiss K, Clark WA, Sonnenblick EH, Krajewski S, Reed JC, Olivetti G, Anversa P: Apoptotic and necrotic myocyte cell deaths are independent contributing variables of infarct size in rats. Lab Invest 74:86-107, 1996

Kannel WB, Sorlie P, McNamara PM: Prognosis after initial myocardial infarction: The Framingham Study. Am J Cardiol 44:53-59, 1979

Kanter MM, Hamlin RL, Unverferth DV, Davis HW, Merola AJ: Effect of exercise training on antioxidant enzymes and cardiotoxicity of doxorubicin. J Appl Physiol 59:1298-1303, 1985

Karliner JS, Scheinmen M: Adenylate cyclase activity coupled to the stimulatory guanine nucleotide binding protein in patients having electrophysiologic studies and either structurally normal hearts or idiopathic myocardial disease. Am J Cardiol 62:1129-1139, 1988

Kaul N, Siveski-Iliskovic N, Hill M, Slezak J, Singal PK: Free radicals and the heart. J Pharmacol Toxicol Meth 30: 55-67, 1993

Kerr JFR, Wyllie AH, Currie AR: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239-257, 1972

Khaper N, Rigatto C, Seneviratne C, Li T, Singal PK: Chronic treatment with propranolol induces antioxidant changes and protects against ischemia-reperfusion injury. J Mol Cell Cardiol 29:3335-3344, 1997

Khaper N, Singal PK: Effects of afterload-reducing drugs on pathogenesis of antioxidant changes and congestive heart failure in rats. J Am Coll Cardiol 29:856-861, 1997

Kimball RF, Reddy K, Pierce TH, Schwartz LW, Mustafa MG, Cross CE: Oxygen toxicity: Augmentation of antioxidant defense mechanisms in rat lung. Am J Physiol 230:1425-1431, 1976

Kirshenbaum LA, Singal PK: Antioxidant changes in heart hypertrophy: significance during hypoxia-reoxygenation injury. Can J Physiol Pharmacol 70:1330-1335, 1992

Kirshenbaum LA, Singal PK: Increase in endogenous antioxidant enzymes protects hearts against reperfusion injury. Am J Physiol 265(Heart Circ Physiol 34):H484-493, 1993

Kobayashi A, Yamashita T, Kaneko M, Nishiyama T, Hayashi H, Yamazaki N: Effects of verapamil on experimental cardiomyopathy in the bio 14.6 Syrian hamster. J Am Coll Cardiol 10:1128-1134, 1987

Koepke JP, Jones S, DiBona GF: Renal nerves mediate blunted natriuresis to atrial natriuretic peptide in cirrhotic rats. Am J Physiol 252:1019-1023, 1987

Krum H, Bigger JT, Goldsmith RL, Packer M: Effect of long-term digoxin therapy on autonomic function in patients with chronic heart failure. J Am Coll Cardiol 25:289-294, 1995a

Krum H, Sackner-Bernstein JD, Goldsmith RL, Kukin ML, Schwartz B, Penn J, Medina N, Yushak M, Horn E, Katz SD, Levin HR, Neuberg GW, DeLong G, Packer M: Double-blind, placebo-controlled study of the long-term efficacy of carvedilol in patients with severe chronic heart failure. Circulation 92:1499-1506, 1995b

Lafont A, Marwick TH, Chisolm GM, Van Lente F, Vaska KJ, Whitlow PL: Decreased free radical scavengers with reperfusion after coronary angioplasty in patients with acute myocardial infarction. Am Heart J 131:219-223, 1996

Lake CR, Ziegler MG, Kopin IJ: Use of plasma norepinephrine for evaluation of sympathetic neuronal function in man. Life Sci 18: 1315-1325, 1976

Lambert C, Massillon Y, Meloche S: Upregulation of cardiac angiotensin II AT_1 receptors in congenital cardiomyopathic hamsters. Circ Res 77:1001-1007, 1995

Landzberg JS, Parker JD, Gauthier DF, Colucci WS: Effects of myocardial α-1-adrenergic receptor stimulation and blockade on contractility in humans. Circulation 84:1608-1614, 1991

Lee MA, Bohm M, Paul M, Ganten D: Tissue renin-angiotensin systems. Their role in cardiovascular disease. Circulation 87(Suppl IV): IV-7–IV-13, 1993

Leimbach WN, Wallin BG, Victor RG, Aylward PE, Sundlof G, Mark AL: Direct evidence from intraneural recordings for increased central sympathetic outflow in patients with heart failure. Circulation 73:913-919, 1986

Levine TB, Franciosa JA, Cohn JN: Acute and long-term response to an oral converting-enzyme inhibitor, captopril, in congestive heart failure. Circulation 62:35-41, 1980

Levitsky DO, Aliev MK, Kuzmin AV, Levchenko TS, Smirnov VN, Chazov EI: Isolation of calcium pump system and purification of calcium ion dependent ATPase from heart muscle. Biochim Biophys Acta 443:468-484, 1976

Lindenmayer GE, Harigaya S, Bajusz E, Schwartz A: Oxidative phosphorylation and calcium transport of mitochondria isolated from cardiomyopathic hamster hearts. J Mol Cell Cardiol 1:249-259, 1970

Litwin SE, Litwin CM, Raya TE, Warner AL, Goldman S: Contractility and stiffness of noninfarcted myocardium after coronary ligation in rats. Circulation 83;1028-1037, 1991

Lloyd EA, Gersh BJ, Kennelly BM: Hemodynamic spectrum of "dominant" right ventricular infarction in 19 patients. Am J Cardiol 48:1016-1022, 1981

Lompre AM, Lambert F, Lakatta EG, Schwartz K: Expression of sarcoplasmic reticulum calcium -ATPase and calsequestrin genes in rat heart during ontogenic development and aging. Circ Res 69: 1380-1388, 1991

Lown B, Verrier RL: Neural activity and ventricular fibrillation. N Engl J Med 294: 1165-1170, 1976

Lowry OH, Rosenbrough NT, Farr AL, Randall AT: Protein measurements with the Folin phenol reagent. J Biol Chem 193:265-275, 1951

Lucy JA: Functional and structural aspects of biological membranes: a suggested structural role of vitamin E in control of membrane permeability and stability. Ann N Y Acad Sci 203:4-11, 1972

Magnani B: Converting enzyme inhibition and heart failure. Am J Med 84(Suppl 3A):87-91, 1988

Makino N, Panagia V, Gupta MP, Dhalla NS: Defects in sarcolemmal Ca^{2+} transport in hearts due to induction of calcium paradox. Circ Res 63:313-321, 1988

Masaki H, Imaizumi T, Ando S-i, Hirooka Y, Harada S, Momohara M, Nagano M, Takeshita A: Production of chronic congestive heart failure by rapid ventricular pacing in the rabbit. Cardiovasc Res 27:828-831, 1993

McCormick RJ, Musch TI, Bergman BC, Thomas DP: Regional differences in LV collagen accumulation and mature cross-linking after myocardial infarction in rats. Am J Physiol 266:H354-H359, 1994

McKay RG, Pfeffer MA, Pasternak RC, Markis J, Come PC, Nakao S, Alderman JD, Ferguson JJ, Safian RD, Grossman W: Left ventricular remodeling after myocardial infarction: a corollary to infarct expansion. Circulation 74:693-702, 1986

McMurray J, McLay J, Chopra M, Bridges A, Belch JJF: Evidence for enhanced free radical activity in chronic congestive heart failure secondary to coronary artery disease. Am J Cardiol 65:1261-1262, 1990

McMurray JJ, Ray SG, Abdullah I, Dargie HJ, Morton JJ: Plasma endothelin in chronic heart failure. Circulation 85:1374-1379, 1992

Mercadier JJ, Lompre AM, Duc P, Boheler KR, Fraysse JB, Wisnewsky C, Allen PD, Komajda M, Schwartz K: Altered sarcoplasmic reticulum calcium ATPase gene expression in human ventricles during end-stage heart failure. J Clin Invest 85: 305-309, 1990

Micheletti R, Di Paola ED, Schiavone A, English E, Benatti P, Capasso JM, Anversa P, Bianchi G: Propionyl-L-carnitine limits chronic ventricular dilation after myocardial infarction in rats. Am J Physiol 264(Heart Circ Physiol 33): H1111-H1117, 1993

Michorowski B, Ceremuzynski L: The renin-angiotensin system and the clinical course of acute myocardial infarction. Eur Heart J 4:259-264, 1983

Mill JG, Stefanon I, Leite CM, Vassalo DV: Changes in performance of the surviving myocardium after left ventricular infarction in rats with chronic myocardial infarction. Cardiovasc Res 24:748-753, 1990

Miura M, Thomas R, Ganz W, Sokol T, Shell WE, Toshimitsu T, Kwan AC, Singh BN: The effect of delay in propranolol administration on reduction of myocardial infarct size after experimental coronary artery occlusion in dogs. Circulation 59:1148-1157, 1979

Morgan JP, Erny RE, Allen PD, Grossman W, Gwathmey JK: Abnormal intracellular calcium handling, a major cause of systolic and diastolic dysfunction in ventricular myocardium from patients with heart failure. Circulation 81(Suppl III):III-21-III-32, 1990

Morgan JP: Abnormal intracellular modulation of calcium as a major cause of cardiac contractile dysfunction. N Engl J Med 325:625-632, 1991

Murphree SS, Saffitz JE. Distribution of β-adrenergic receptors in failing human myocardium: implications for mechanisms of downregulation. Circulation 79:1214-1225, 1989

Myers ML, Bolli R, Lekich RF, Hartley CJ, Roberts R: Enhancement of recovery of myocardial function by oxygen free-radical scavengers after reversible regional ischemia. Circulation 72:915-921, 1985

Nakaoka H, Imataka K, Amano M, Fujii J: Plasma levels of atrial natriuretic factor in patients with congestive heart failure (letter). N Engl J Med 313:892, 1985

Narula J, Haider N, Virmani R, DiSalvo TG, Kolodgie FD, Hajjar RJ, Schmidt U, Semigran MJ, Dec GW, Khaw B-A: Apoptosis in myocytes in end-stage heart failure. N Engl J Med 335:1182-1189, 1996

Nelson GIC, Ahuja RC, Silke B, Taylor SH: Haemodynamic effects of furosemide and its influence on repetitive rapid volume loading in acute myocardial infarction. Eur Heart J 4:706-711, 1983

Newman TJ, Maskin CS, Dennick LG, Meyer JH, Hallows BG, Cooper WH: Effects of captopril on survival in patients with heart failure. 84(Suppl 3A):140-144, 1988

Newman WH: Biochemical, structural and mechanical defects of the failing myocardium. Pharmac Ther 22:215-247, 1983

Nicod P, Gilpin E, Dittrich H, Chappuis F, Ahnve S, Engler R, Henning H, Ross J Jr: Influence on prognosis and morbidity of left ventricular ejection fraction with and without signs of left ventricular failure after acute myocardial infarction. Am J Cardiol 61:1165-1171, 1988

Nohl H, Hegner D: Responses of mitochondrial superoxide dismutase, catalase and glutathione peroxidase activities to aging. Mech Ageing Dev 11:145-151, 1979

Norwegian Multicenter Study Group, The: Timolol-induced reduction in mortality and reinfarction in patients surviving acute myocardial infarction. N Engl J Med 304:801-807, 1981

Olcott HS, Emerson OH: Antioxidants. Antioxidation of fats. 9. The antioxidant properties of the tocopherols. J Am Chem Soc 59:1008-1009, 1937

Olivetti G, Quaini F, Sala R, Lagrasta C, Corradi D, Bonacina E, Gambert SR, Cigola E, Anversa P: Acute myocardial infarction in humans is associated with activation of programmed myocyte cell death in the surviving portion of the heart. J Mol Cell Cardiol 28:2005-2016, 1996

Ozer NK, Boscoboinik D, Azzi A: New roles of low density lipoproteins and vitamin E in the pathogenesis of atherosclerosis. Biochem Mol Biol Int 35(1): 117-124, 1995

Packer L: Vitamin E is nature's master antioxidant. Scientific American Science and Medicine 54-63, 1994

Packer M, Lee WH, Kessler PD, Gottlieb SS, Bernstein JL, Kukin ML: Role of neurohormonal mechanisms in determining survival in patients with severe chronic heart failure. Circulation 75(Suppl IV):IV-80— IV-92, 1987

Packer M, Carver JR, Rodeheffer RJ, Ivanhoe RJ, DiBianco R, Zeldis SM, Hendrix GH, Bommer WJ, Elkayam U, Kukin ML, Mallis GI, Sollano JA, Shannon J, Tandon PK, DeMets DL for the PROMISE Study Research Group: Effect of oral milrinone on mortality in severe chronic heart failure. N Engl J Med 325:1468-1475, 1991

Packer M: The neurohormonal hypothesis: a theory to explain the mechanism of disease progression in heart failure. J Am Coll Cardiol 20:248-254, 1992

Packer M, Bristow MR, Cohn JN, Colucci WS, Fowler MB, Gilbert EM, Shusterman NH for the U.S. Carvedilol Heart Failure Study Group: The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. N Engl J Med 334:1349-1355, 1996a

Packer M, Colucci WS, Sackner-Bernstein JD, Liang C-s, Goldscher DA, Freeman I, Kukin ML, Kinhal V, Udelson JE, Klapholz M, Gottlieb SS, Pearle D, Cody RJ, Gregory JJ, Kantrowitz NE, LeJemtel TH, Young ST, Lukas MA, Shusterman NH for the Precise Study Group. Double-blind, placebo-controlled study of the effects of carvedilol in patients with moderate to severe heart failure. The PRECISE Trial. Circulation 94:2793-2799, 1996b

Page E, Earley J, Power B: Normal growth of ultrastructures in rat left ventricular myocardial cells. Circ Res 34(Suppl II):12-16, 1974

Paglia DE, Valentine WN: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70:158-169, 1967

Palace VP, Brown SB: HPLC determination of tocopherol, retinol, dehydroretinol and retinyl palmitate in tissues of lake char (*Salvelinus namaycush*) exposed to coplanar 3,3',4,4',5-pentachlorobiphenyl. Environ Toxicol Chem 13:473-476, 1994

Pelouch V, Dixon IMC, Sethi R, Dhalla NS: Alteration of protein profile in congestive heart failure secondary to myocardial infarction. Mol Cell Biochem 129:121-131, 1993a

Pelouch V, Dixon IMC, Golfman L, Beamish RE, Dhalla NS: Role of extracellular matrix proteins in heart function. Mol Cell Biochem 129: 101-120, 1993b

Perennec J, Hatt PY: Myocardial morphology in cardiac hypertrophy and failure: electron microscopy in man. In: Swynghedauw B (ed), Cardiac Hypertrophy and Failure. London, John Libbey and Company, pp. 267-276, 1988

Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E: Myocardial infarct size and ventricular function in rats. Circ Res 44:503-512, 1979

Pfeffer MA, Pfeffer JM, Steinberg C, Finn P: Survival after an experimental myocardial infarction: beneficial effects of long-term therapy with captopril. Circulation 72:406-412, 1985

Pfeffer MA, Lamas GA, Vaughan DE, Parisi AF, Braunwald E: Effect of captopril on progressive ventricular dilatation after anterior myocardial infarction. N Engl J Med 319:80-86, 1988a

Pfeffer JM, Pfeffer MA: Angiotensin converting enzyme inhibition and ventricular remodeling in heart failure. Am J Med 84(Suppl 3A):37-44, 1988b

Pfeffer MA, Braunwald E: Ventricular remodeling after myocardial infarction: experimental observations and clinical implications. Circulation 81:1161-1172, 1990

Pfeffer MA, Pfeffer JM, Lamas GA: Development and prevention of congestive heart failure following myocardial infarction. Circulation 87(Suppl IV):IV-120 — IV-125, 1993

Pierce WS, Carter DR, McGarran MH, Waldhausen JA: Modification of myocardial infarct volume: an experimental study in the dog. Arch Surg 107:682-686, 1973

Pool PE, Spann JF, Buccino RA, Sonnenblick EH, Braunwald E: Myocardial high energy phosphate stores in cardiac hypertrophy and heart failure. Circ Res XXI:365-373, 1967

Raftery EB: Vasodilating beta-blockers in heart failure. Eur Heart J 16(Suppl F):32-37, 1995

Rasmussen MM, Reimer KA, Kloner RA, Jennings RB: Infarct size reduction by propranolol before and after coronary ligation in dogs. Circulation 56:794-798, 1977

Reddy K, Tappel AL: Effect of dietary selenium and autooxidized lipids on the glutathione peroxidase system of gastro-intestinal tract and other tissues in the rat. J Nutr 104:1069-1078, 1974

Reed DJ: Glutathione depletion and susceptibility. Pharmacol Rev 36: 25S-33S, 1984

Reed DJ: Glutathione: Toxicological implications. Annu Rev Pharmacol Toxicol 30: 603-631, 1990

Regitz-Zagrosek V, Neuss M, Fleck E: Effects of angiotensin receptor antagonists in heart failure: clinical and experimental aspects. Eur Heart J 16(Suppl N):86-91, 1995

Riegger GAJ, Liebau G, Holzschuh M, Witkowski D, Steilner H, Kochsiek K: Role of the renin-angiotensin system in the development of congestive heart failure in the dog as assessed by chronic converting-enzyme blockade. Am J Cardiol 53: 614-618, 1984

Riegger GAJ: ACE inhibitors in early stages of heart failure. Circulation 87(Suppl IV):IV-117—IV-119, 1993

Riemersma RA, Wood DA, MacIntyre CCA, Elton RA, Gey KF, Oliver MF: Risk of angina pectoris and plasma concentrations of vitamins A, C, E, and carotene. Lancet 337:1-5, 1991

Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC: Vitamin E consumption and the risk of coronary heart disease in men. N Engl J Med 328:1450-1456, 1993

Roberts MJD, Young IS, Trouton TG, Trimble ER, Khan MM, Webb SW, Wilson CM, Patterson GC, Adgey AAJ: Transient release of lipid peroxides after coronary artery balloon angioplasty. Lancet 336:143-145, 1990

Rutherford JD, Pfeffer MA, Moye LA, Davis BR, Flaker GC, Kowey PR, Lamas GA, Miller HS, Packer M, Rouleau JL, Braunwald E, on behalf of the SAVE investigators: Effects of captopril on ischemic events after myocardial infarction: results of the Survival and Ventricular Enlargement Trial. Circulation 90:1731-1738, 1994

Sabbah HN, Sharov V, Riddle JM, Kono T, Lesch M, Goldstein S: Mitochondrial abnormalities in myocardium of dogs with chronic heart failure. J Mol Cell Cardiol 24:1333-1347, 1992a

Sabbah HN, Goldberg AD, Schoels W, Kono T, Webb C, Brachmann J, Goldstein S: Spontaneous and inducible ventricular arrhythmias in a canine model of chronic heart failure: relation to haemodynamics and sympathoadrenergic activation. Eur Heart J 13:1562-1572, 1992b

Sabbah HN, Goldstein S: Ventricular remodeling: Consequences and therapy. Eur Ht J 14(Supppl C): 24-29, 1993

Saraste A, Pulkki K, Kallajoki M, Henriksen K, Parvinen M, Voipio-Pulkki L-M: Apoptosis in human acute myocardial infarction. Circulation 95:320-323, 1997

Sardahl LA, McCollum WB, Wood WG, Schwartz A: Mitochondria and sarcoplasmic reticulum function in cardiac hypertrophy and failure. Am J Physiol 224: 497-502, 1973

Schaper J, Froede R, Hein S, Buck A, Hashizume H, Speiser B, Friedl A, Bleese N: Impairment of the myocardial ultrastructure and changes of cytoskeleton in dilated cardiomyopathy. Circulation 83:504-514, 1991

Schuster EH, Bulkley BH: Expansion of transmural myocardial infarction: a pathophysiologic factor in cardiac rupture. Circulation 60:1532-1538, 1979

Schwartz A, Lee KS: Study of heart mitochondria and glycolytic metabolism in experimentally induced heart failure. Circ Res 10:321-332, 1962

Schwartz K, De La Bastie D, Bouveret P, Oliviero P, Alonso S, Buckingham ME: α-skeletal muscle actin mRNA's accumulate in hypertrophied adult rat hearts. Circ Res 59: 551-555, 1986

Schwartz K, Carrier L, Mercadier JJ, Lompre AM, Boheler K: Molecular phenotype of the hypertrophied and failing myocardium. Circulation 87:V11-5-V11-10, 1993

Scragg R, Jackson R, Holdaway I, Woollard G, Woollard D: Changes in plasma vitamin levels in the first 48 hours after onset of acute myocardial infarction. Am J Cardiol 64:971-974, 1989

Searle J, Kerr JFR, Bishop CJ: Necrosis and apoptosis: distinct modes of cell death with fundamentally different significance. Pathol Annu 17:229-259, 1982

Sebbag L, Forrat R, Canet E, Renaud S, Delaye J, de Lorgeril M: Effects of dietary supplementation with alpha-tocopherol on myocardial infarct size and ventricular arrhythmias in a dog model of ischemia-reperfusion. J Am Coll Cardiol 24:1580-1585, 1994

Selye H, Bajusz E, Grasso S, Mendell P: Simple techniques for the surgical occlusion of coronary vessels in the rat. Angiology 11:398-407, 1960

Shah PK, Maddahi J, Berman DS, Pichler M, Swan HJC: Scintigraphically detected predominant right ventricular dysfunction in acute myocardial infarction: clinical and hemodynamic correlates and implications for therapy and prognosis. J Am Coll Cardiol 6:1264-1272, 1985

Shan X, Aw TY, Jones DP: Glutathione dependent protection against oxidative injury. Pharmacol Ther 47:61-71, 1990

Shannon RP, Komamura K, Shen Y-T, Bishop SP, Vatner SF: Impaired regional subendocardial coronary flow reserve in conscious dogs with pacing-induced heart failure. Am J Physiol 265 (Heart Circ Physiol 34): H801-H809, 1993

Sharpe N: Myocardial infarction and heart failure. The common ground. Circulation 87:1037-1039, 1993

Shenker Y, Sider RS, Ostafin EA, Grekin RJ: Plasma levels of immunoreactive atrial natriuretic factor in healthy subjects and in patients with edema. J Clin Invest 76:1684-1687, 1985

Singal PK, Kapur N, Dhillon KS, Beamish RE, Dhalla NS: Role of free radicals in catecholamine-induced cardiomyopathy. Can J Physiol Pharmacol 60:1390-1397, 1982

Singal PK, Pierce GN: Adriamycin stimulates low affinity Ca^{2+}-binding and lipid peroxidation but depresses myocardial function. Am J Physiol 250:H419-H425, 1986

Singal PK, Deally CMR, Weinberg LE: Subcellular effects of adriamycin in the heart: a concise review. J Mol Cell Cardiol 19: 817-828, 1987

Singal PK, Petkau A, Gerrard JM, Hrushovetz S, Foerster J: Free radicals in health and disease. Mol Cell Biochem 84: 121-122, 1988

Singal PK, Kirshenbaum LA: A relative deficit in antioxidant reserve may contribute in cardiac failure. Can J Cardiol 6:47-49, 1990

Singh N, Dhalla AK, Seneviratne C, Singal PK: Oxidative stress and heart failure. Mol Cell Biochem 147: 77-81,1995

Singh RB, Niaz MA, Rastogi SS, Rastogi S: Usefulness of antioxidant vitamins in suspected acute myocardial infarction (the Indian Experiment of Infarct Survival-3). Am J Cardiol 77:232-236, 1996

Siveski-Iliskovic N, Kaul N, Singal PK: Probucol promotes endogenous antioxidants and provides protection against adriamycin-induced cardiomyopathy in rats. Circulation 89: 2829-2835, 1994

Siveski-Iliskovic N, Hill M, Chow DA, Singal PK: Probucol protects against adriamycin cardiomyopathy without interfering with its antitumor effect. Circulation 91: 10-15, 1995

Sobotka PA, Brottman MD, Weitz Z, Birnbaum AJ, Skosey JL, Zarling EJ: Elevated breath pentane in heart failure reduced by free radical scavenger. Free Radic Biol Med 14:643-647, 1993

Spann JF, Chidsey CA, Braunwald E. Reduction of cardiac stores of norepinephrine in experimental heart failure. Science 145:1439-1441, 1964

Speiser B, Riess CF, Schaper J: The extracellular matrix in human myocardium: Part I: Collagens I, II, IV and VI. Cardioscience 2:225-232, 1991

Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willett WC: Vitamin E consumption and the risk of coronary disease in women. N Engl J Med 328:1444-1449, 1993

Stauble B, Boscoboinik D, Tasinato A, Azzi A: Modulation of activator protein-1 (AP-1) transcription factor and protein kinase C by hydrogen peroxide and D-alpha-tocopherol in vascular smooth muscle cells. Eur J Biochem 226(2): 393-402, 1994

Steiner M: Influence of vitamin E on platelet function in humans. J Am Coll Nutr 10:466-473, 1991

Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, Mitchinson MJ, Brown MJ: Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). Lancet 347:781-786, 1996

Street DA, Comstock GW, Salkeld RM, Schuep W, Klag MJ: Serum antioxidants and myocardial infarction. Are low levels of carotenoids and α-tocopherol risk factors for myocardial infarction? Circulation 90:1154-1161, 1994

Swedberg K, Hjalmarson A, Waagstein F, Wallentin I: Prolongation of survival in congestive cardiomyopathy by beta-receptor blockade. Lancet 1:1374-1377, 1979

Taylor SH, Silke B, Nelson GIC: Principles of treatment of left ventricular failure. Eur Heart J 3(Suppl D):19-43, 1982

Taylor SH: Refocus on diuretics in the treatment of heart failure. Eur Heart J 16(Suppl F):7-15, 1995

Toleikis PM, Godin DV: Alteration of antioxidant status following sympathectomy: Differential effects of modified plasma levels of adrenaline and noradrenaline. Mol Cell Biochem 152:39-49, 1995

Turek Z, Grandtner M, Kubat K, Ringnalda BEM, Kreuzer F: Arterial blood gases, muscle fiber diameter and intercapillary distance in cardiac hypertrophy of rats with an old myocardial infarction. Pflugers Arch 376:209-215, 1978

Turini GA, Brunner HR, Gribic M, Waeber B, Gavras H: Improvement of chronic congestive heart failure by oral captopril. Lancet 1;1213-1215, 1979

Urata H, Nishimura H, Ganten D: Mechanisms of angiotensin II formation in humans. Eur Heart J 16(Suppl N):79-85, 1995

Uretsky B, Jessup M, Konstam M, Dec G, Leier C, Benotti J, Murali S, Herrmann HC, Sandberg JA: Multicenter trial of oral enoximone in patients with moderate to moderately severe congestive heart failure. Lack of benefit compared with placebo. Circulation 82:774-780, 1990

van Acker SABE, Koymans LMH, Bast A: Molecular pharmacology of vitamin E: structural aspects of antioxidant activity. Free Radic Biol Med 15:311-328, 1993

Vaney C, Waeber B, Turini GA, Margalith D, Brunner HR: Renin and the complications of myocardial infarction. Chest 86:40-43, 1984

Vatner DE, Vatner SF, Fujii AM, Homay CJ: Loss of high affinity cardiac beta adrenergic receptors in dogs with heart failure. J Clin Invest 76:2259-2264, 1985

Verma A, Hill M, Bhayana S, Pichardo J, Singal PK: Role of glutathione in acute myocardial adaptation. In: Sharma BK, Takeda N, Ganguly PK, Singal PK (eds), Adaptation Biology and Medicine, India, Narosa Publishing House, Vol. 1, 399-408, 1997

Volders PAG, Willems IEMG, Cleutjens JPM, Arends J-W, Havenith MG, Daemen MJAP: Interstitial collagen is increased in the non-infarcted human myocardium after myocardial infarction. J Mol Cell Cardiol 25:1317-1323, 1993

Waagstein F, Hjalmarson A, Varnauskas E, Wallentin I: Effect of chronic beta-adrenergic receptor blockade in congestive cardiomyopathy. Br Heart J 37:1022-1036, 1975

Warren SE, Royald HD, Markis JE, Grossman W, McKay RG: Time course of left ventricular dilatation after myocardial infarction: influence of infarct-related artery and success of coronary thrombolysis. J Am Coll Cardiol 11:12-19, 1988

Watkins L, Burton JA, Haber E, Cant JR, Smith FW, Barger AC: The renin-angiotensin-aldosterone system in congestive heart failure in conscious dogs. J Clin Invest 57:1606-1617, 1976

Weber KT: Cardiac interstitium in health and disease: The fibrillar collagen network. J Am Coll Cardiol 13:1637-1652, 1989

Weber KT, Brilla CG: Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. Circulation 83:1849-1865, 1991

Weber KT, Brilla CG: Factors associated with reactive and reparative fibrosis of the myocardium. Basic Res Cardiol 87(Suppl I):291-301, 1992

Weisel RD, Mickle DAG, Finkle CD, Tumiati LC, Madonik MM, Ivanov J, Burton GW, Ingold KU: Myocardial free-radical injury after cardioplegia. Circulation 80(Suppl III):III-14-III-18, 1989

Weisman HF, Bush DE, Mannisi JA, Bulkley BH: Global cardiac remodeling after acute myocardial infarction: a study in the rat model. J Am Coll Cardiol 5:1355-1362, 1985

Weisman HF, Bush DE, Mannisi JA, Weisfeldt ML, Healy B: Cellular mechanisms of myocardial infarct expansion. Circulation 78: 186-201, 1988

Weitz Z, Birnbaum AJ, Sobotka PA, Zarling EJ, Skosey JL: Elevated pentane levels during acute myocardial infarction. Lancet 337:933-935, 1991

White HD, Norris RM, Brown MA, Brandt PWT, Whitlock RML, Wild CJ: Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction. Circulation 76: 44-51, 1987

Winaver J, Hoffman A, Burnett JC Jr, Haramati A: Hormonal determinants of sodium excretion in rats with experimental high-output heart failure. Am J Physiol 254(Regulatory Integrative comp Physiol 23):R776-R784, 1988

Winaver J, Hoffman A, Abassi Z, Haramati A: Does the heart's hormone, ANP, help in congestive heart failure? News in Physiological Sciences 10:247-253, 1995

Woo KS, White HD: Factors affecting outcome after recovery from myocardial infarction. Annu Rev Med 45:325-339, 1994

Wyllie AH, Kerr JF, Currie AR: Cell death: the significance of apoptosis. Int Rev Cytol 68:251-306, 1980

Yao M, Keogh A, Spratt P, dos Remedios CG, Kiebling PC: Elevated DNase I levels in human idiopathic dilated cardiomyopathy: an indicator of apoptosis? J Mol Cell Cardiol 28:95-101, 1996

Yeo WW, Ramsey LE: Persistent dry cough with enalapril: incidence depends on method used. J Human Hypertens 4:517-520, 1990

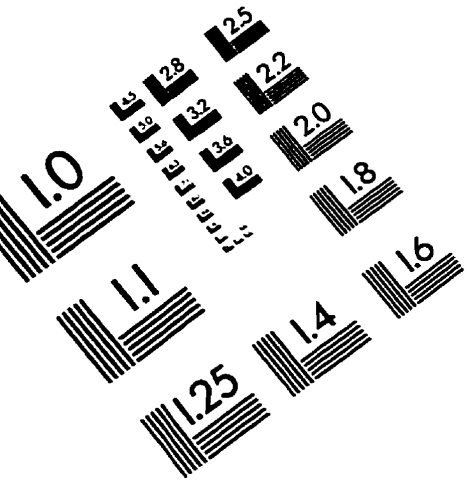
Yeo WW, Maclean D, Richardson PJ, Ramsay LE: Cough and enalapril: assessment by spontaneous reporting and visual analogue scale under double-blind conditions. Br J Clin Pharmacol 31:356-359, 1991

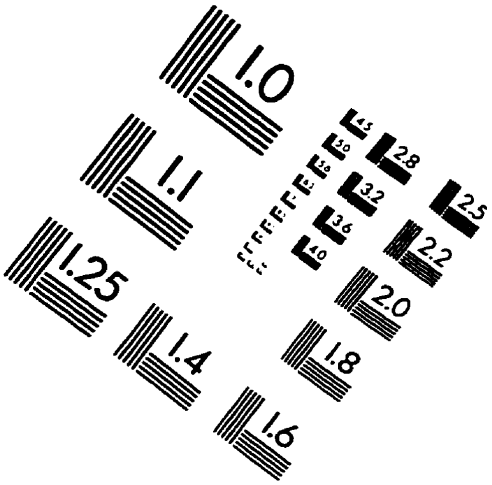
Yonish-Rouach E, Resnitzky D, Lotem J, Sachs L, Kimchi A, Oren M: Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interluekin-6. Nature 352:345-347, 1991

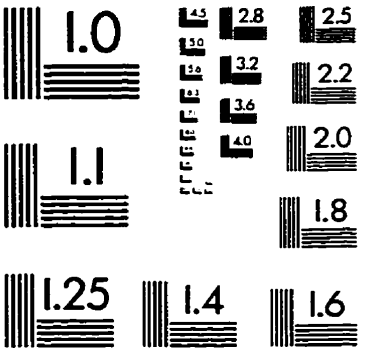
Yue T-L, Cheng H-Y, Lysko PG, McKenna PJ, Feuerstein R, Gu J-L, Lysko KA, Davis LL, Feuerstein G: Carvedilol, a new vasodilator and beta adrenoceptor antagonist, is an antioxidant and free radical scavenger. J Pharmacol Exp Therap 263:92-98, 1992

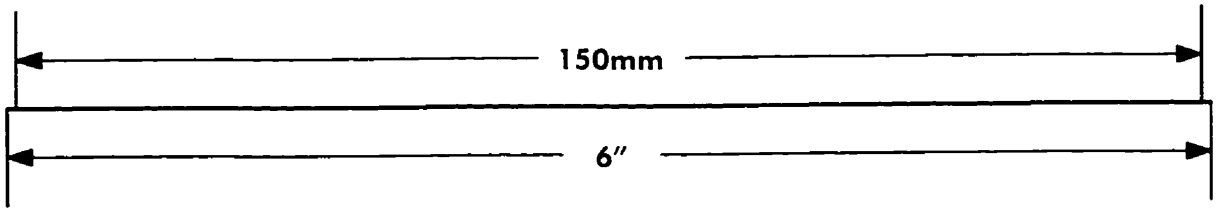
Zehender M, Kasper W, Kauder E, Schonthaler M, Geibel A, Olschewski M, Just H: Right ventricular infarction as an independent predictor of prognosis after acute inferior myocardial infarction. N Engl J Med 328:981-988, 1993

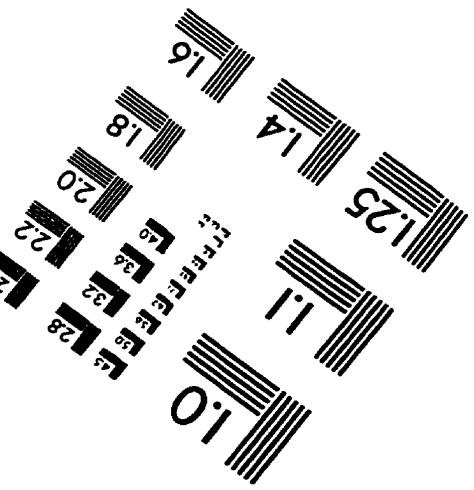
IMAGE EVALUATION
TEST TARGET (QA-3)

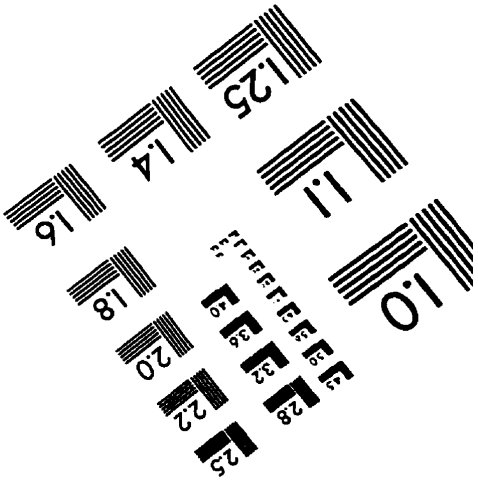












APPLIED IMAGE . Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989