OXYGEN RADICAL INJURY IN NORMAL AND HYPERTROPHIED RAT HEARTS

By

© MADHU GUPTA

Thesis submitted to the Faculty of Graduate studies of the University of Manitoba in partial fulfillment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

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Dedicated to my beloved daughter Minnie.

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SUMMARY

Although hypertrophied hearts have been shown to respond differently to drugs whose toxic effects are known to involve oxygen radical species , changes in oxygen radical processes during hypertrophy remain to be described. Furthermore, the sequence of subcellular changes underlying oxygen radical-induced myocardial dysfunction in a normal heart have not been defined. The aims of this research, therefore, were: 1) to define and characterize oxygen radical induced injury in normal and in hypertrophied hearts; 2) to examine important myocardial antioxidant enzymes and lipid peroxidation in normal and in hypertrophied hearts at different post-operative intervals.

In order to define the time-course of oxygen radical-induced myocardial injury, isolated rat hearts were perfused with Krebs Henseleit (KH) buffer containing xanthine (2 mM) - xanthine oxidase (10 U/L) (X-XO). Perfusion of rat heart with X-XO (an ex vivo oxygen radical source) resulted in decline in peak developed force as well as rate of force development and relaxation within 5 min and complete contractile failure was observed at 20 min. Resting tension was higher at 10 min and showed a maximum increase of 400% at 40 min.

The presence as well as the nature of different radical species responsible for X-XO induced contractile dysfunction was confirmed by the use of a variety of antioxidants including glutathione, methionine, superoxide dismutase, catalase and

mannitol. Glutathione (200 uM and 2 mM), methionine (1 and 20 mM), superoxide dismutase (1.2 x 10^5 U/L), catalase (2 and 4 x 10^4 U/L) and mannitol (10 and 20 mM), showed dose-dependent protection against X-XO induced-contractile failure and the rise in resting tension. These data suggested the participation of superoxide, hydrogen peroxide and hydroxyl radicals in producing X-XO-induced contractile failure. However, desferal (300 uM and 3mM), an iron chelating agent, failed to show any protection against X-XO-induced changes in contractile function indicating a lack of a role of iron in the production of activated oxygen species including hydroxyl radicals via X-XO interaction.

Myocardial malondialdehyde (MDA) content, which is an indirect indicator of lipid peroxidation, was significantly higher at 5 min and showed a continuous increase upon prolongation of X-XO perfusion for up to 40 min. A strong correlation ($r = 0.935$) was noted between increased MDA content and decrease in peak developed force. Creatine phosphate (CP) and adenosine triphosphate (ATP) showed a time-dependent decrease due to X-XO perfusion. A strong correlation was also observed ($r = 0.819$) between depletion of myocardial ATP content and loss of developed force. Adenosine diphosphate showed an increase at 5 min followed by a decrease at 20 and 40 min. Adenosine monophosphate, adenosine and creatine content increased with continued X-XO perfusion. Qualitative ultrastructural changes involved damage to sarcoplasmic reticulum,

mitochondria, interstitial edema formation and damage to these organelles became more severe upon prolongation of perfusion time with X-XO. In a semiquantitative morphometric study, significant ultrastructural changes became apparent only after 10 min of X-XO perfusion.

Cardiac hypertrophy was induced in rats by banding of the abdominal aorta in the subdiaphragmatic region proximal to its renal bifurcation. After 6, 12, 24 and 48 weeks of surgery, cardiac hypertrophy was confirmed by increased left ventricle/body weight ratio (25 - 30%). Myocardial RNA content showed a significant increase in hypertrophied hearts. Hemodynamically, these animals demonstrated increased blood pressure as well as left ventricular pressure (24 - 30%) and a corresponding increase in +dP/dt and -dP/dt. These hypertrophied hearts were not in failure as indicated by unchanged left ventricular end diastolic pressure, wet/dry weight ratios for liver and lung and absence of ascites or pleuritis.

All hypertrophied hearts showed a greater resistance to X-XO-induced contractile failure and rise in resting tension. Since 50% of contractile failure in hypertrophied hearts was observed at approximately 10 min, these hearts were also analysed for lipid peroxidation, high energy phosphates and qualitative and semiquantitative morphometric ultrastructural studies following X-XO perfusion for 10 min. Hypertrophied hearts revealed a lesser increase in lipid peroxidation and

less depletion of high energy phosphates relative to sham controls. Qualitative structural changes were less in hypertrophied hearts. The differences in ultrastructural changes became more evident in a semiquantitative morphometric analysis, which demonstrated the presence of a greater number of normal cells and relatively few severely damaged cells in the hypertrophied heart.

Control and hypertrophied hearts were analysed for antioxidative enzymes including superoxide dismutase and glutathione peroxidase, as well as for MDA content. The SOD activity was significantly higher in hypertrophied hearts at 6 and 12 weeks as compared to their respective controls. However, this difference did not remain significant at 24 and 48 weeks due to a marked increase in the SOD activity of control hearts in these age groups. During the time period studied, glutathione peroxidase activity remained unchanged in controls but was significantly elevated in hearts from all four hypertrophied groups. MDA levels were significantly lower in hypertrophied hearts.

In conclusion, this study demonstrates that the interaction of xanthine-xanthine oxidase results in the formation of activated oxygen radical species such as superoxide radicals, hydrogen peroxide and hydroxyl radicals and that the production of hydroxyl radical may not require the presence of iron. Perfusion of hearts with a source of such radical species results in contractile failure which correlates with lipid peroxidation and depletion of high

energy phosphates. Structural damage appears to be a delayed event contributing only to the later stages of injury. The stable heart hypertrophy up to 48 weeks is accompanied by a higher activity of antioxidative enzymes and a lower degree of lipid peroxidation. This adaptive change probably helps hypertrophied hearts to resist oxidative damage upon exposure to a radical generating system. It is proposed that antioxidative capacity in the heart is a dynamic phenomenon adjusting with changing physiological or pathophysiological conditions.

I. INTRODUCTION

Cardiovascular disease, especially congestive heart failure, is one of the leading cause of death in North America and the dominant etiologic factor for congestive heart failure is hypertension. Any pressure overload such as may be present during hypertension can be seen to accompany cardiac hypertrophy. Furthermore, patients with definite evidence of left ventricular hypertrophy have a significantly higher risk of developing congestive heart failure. The markedly increased risk of developing congestive heart failure in hypertensive subjects with left ventricular hypertrophy probably reflects the severity and duration of underlying hypertension and of the compensatory cardiac hypertrophy.

Cardiac hypertrophy is an adaptive mechanism which develops in response to chronic increase in work demands associated with conditions such as hypertensive heart disease, coronary defects, aortic stenosis, arteriovenous fistula and thyrotoxicosis. The increase in cardiac mass (hypertrophy) helps the heart to maintain its pump function in face of increased workload. This adaptive process is not a simple addition of identical units, rather it involves cellular and subcellular reorganization of myocytes. Some of the subcellular adjustments during hypertrophy include an increase in the relative volume of the mitochondria and the myofibrils, functional changes in mitochondria, an increase in the relative amount of the V_3 (low ATPase)

isoenzyme of myosin and a decreased rate of calcium cycling which probably results in more economical force development. The exact profile of the total reorganization is apparently determined by the type, intensity and duration of the specific stress imposed on the heart.

This condition of compensated hypertrophy can change into decompensated heart failure and patients with left ventricular hypertrophy show a 10 times greater risk of developing heart failure. Several theories, which may not be mutually exclusive, have been advanced to explain this transition. These include reduced energy supply, altered Ca^{2+} metabolism, down-regulation of receptors and relative hypoxia due to increased muscle mass. Recent data from this and other laboratories have raised a new possibility that the transition from a compensated hypertrophy to heart failure may also involve changes in free radical mechanisms. The role of free radicals has been documented in inflammation, pulmonary disorders, radiation injury, oxygen toxicity and ischemic injury to the brain, intestine, kidney and heart.

Activated oxygen species such as superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl ($OH^{\cdot}$) radicals can be generated <u>in vivo</u> by a number of cellular reactions. Although cytochrome oxidase catalyzes the tetravalent reduction of oxygen to H_2O without the release of these toxic oxygen species, the generation of $O_2^{\cdot -}$ in the mitochondria by the univalent reduction of oxygen was demonstrated.

Myeloperoxidase, NADPH oxidase and xanthine oxidase are other biologic sources of oxygen radicals. In normal healthy tissues, these toxic species are effectively scavenged by different enzyme systems including superoxide dismutase (SOD), glutathione peroxidase (GSHPx), catalase and other unspecified agents protecting the cell against toxic effects of $O_2^{\cdot -}$, H_2O_2 and $OH^{\cdot}$. However, in certain disease states, the cell may be predisposed to injury either due to increased production of free radicals or alterations in defense mechanisms against these highly reactive species. These active, inorganic radical species, if not scavenged effectively, can initiate oxidation of lipids in the lipid bilayer of membranes as well as of proteins especially those containing SH groups. Furthermore, oxidation of membrane lipids results in formation of peroxide clusters, that initiate further lipid peroxidation, thus setting up a chain reaction which can compromise membrane fluidity and permeability characteristics. Thus, the quantitation of lipid peroxidation in tissues would provide a useful basis for identifying radical-induced injury.

The potential role of oxygen radicals in the pathogenesis of myocardial injury has been the subject of many recent investigations. It is established that these radicals play an important role in ischemia-reperfusion-induced myocardial damage. A role for these radical species has also been suggested in stress-induced myocardial dysfunction. Catecholamine-induced cardiomyopathy and arrhythmias are suggested to be mediated by production of oxygen radical

species and lipid peroxidation. Cardiotoxic effects of adriamycin, an anticancer drug, have also been shown to involve free radical mechanisms. Evidence for the involvement of oxygen radicals in the above-mentioned heart diseases is derived from the fact that treatment with different radical scavengers and antioxidants has shown beneficial effects. However, details of the radical-induced cardiac injury with respect to function, high energy phosphates and structure remain to be delineated.

During heart hypertrophy, because of alterations in myocardial metabolism and plasma catecholamine content, the probability of greater oxygen radical production could be increased. In fact, in a recent investigation, increased radical production in the mitochondria isolated from a hypertrophied heart was shown (Guarnieri et al, 1985). Observations that adriamycin and catecholamines (radical producing agents) produced greater cardiotoxicity in the hypertrophied heart also supports the argument that radical metabolism is changed during cardiac hypertrophy. Various physiological as well as pathophysiological conditions such as age, exercise and beta-thalassemia minor are known to be associated with increased radical production and have also been shown to be accompanied by alterations in antioxidative enzyme levels. Since heart hypertrophy is accompanied by metabolic changes as well as altered protein synthesis, it is possible that these adaptations are also associated with changes in antioxidative enzymes in the hypertrophied hearts.

The present research was undertaken: 1) to examine in detail structural, functional and subcellular changes in normal hearts exposed to oxygen radicals in order to understand the pathogenesis of radical-induced injury; 2) to characterize differences in the response of control and hypertrophied hearts to ex vivo oxidative stress with respect to these parameters; and 3) to discern any changes in antioxidative enzymes and lipid peroxidation in hypertrophied hearts as compared to controls. For this purpose, isolated perfused hearts were exposed to xanthine-xanthine oxidase and the time- course of changes in contractile function was studied in relation to changes in the content of MDA and of high energy phosphates. Qualitative as well as a semiquantitative morphometric analyses of ultrastructural changes were also conducted. Heart hypertrophy was induced by banding of the aorta in the subdiaphragmic region proximal to the renal arteries. Hemodynamic assessments were done at 6, 12, 24 and 48 weeks after the surgery and the hearts were exposed ex vivo to the same oxidative stress. Superoxide dismutase, and glutathione peroxidase activities and MDA content of the sham control and hypertrophied hearts were monitored. It was hoped that the results of this study could extend our existing knowledge concerning the pathophysiology of oxygen radical-induced damage as well as changes in radical metabolism during cardiac hypertrophy due to pressure overload.

II. REVIEW OF LITERATURE

A. OXYGEN RADICALS

Molecular oxygen is both essential as well as destructive to biologic tissues. Many of the biologic reactions in which it participates generate a variety of activated oxygen intermediates, including superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\cdot}$) and singlet oxygen. For the sake of conciseness these will be collectively termed as oxygen radicals. Interest in oxygen radical involvement in various pathophysiological conditions dates from the late 1960's when erythrocuprin (SOD) was first discovered (McCord and Fridovich, 1969). This discovery provided an important tool with which biological systems could be probed for oxygen radical involvement. Oxygen radicals are highly reactive species which can injure and even kill the cell.

A free radical is any chemical species that has one or more unpaired electrons. Molecular oxygen, in the ground state, contains two unpaired electrons with parallel spins. Because of its unusual atomic configuration, it can undergo univalent reduction (for review see Green and Hill, 1984). Most organic compounds that might react with oxygen contain paired electrons; therefore, it becomes obvious that one electronic spin would have to be inverted in order to avoid the placement of two parallel spins in one orbital (Agner 1972). The inversion of electronic spin is slow relative to

the life time of collisional complexes and whenever energetically feasible, univalent pathways of oxygen reduction will be favored over divalent pathways. Spin restriction is more fully discussed by Taube (1965). However, in the presence of certain enzymes, it is possible to accomplish tetravalent reduction of O_2 to H_2O without the production of detectable intermediates. Cytochrome C oxidase, which accounts for most of the oxygen consumption by aerobes, produces H_2O as the first detectable product of oxygen reduction (Wharton et al 1968, Malkin and Malmstrom 1970). This is obviously advantageous in that it minimizes the load of reactive intermediates which must be scavenged. However there are a number of biological oxidations both enzymatic and spontaneous which do generate oxygen radicals and these mechanisms are discussed separately. It follows that oxygen radicals, which were formerly of interest primarily to radiation chemists, can be produced in biological systems and these have been shown to be injurious to tissues in various pathological processes.

B. BIOLOGICAL SOURCES OF OXYGEN RADICALS

There are multiple sources of oxygen radicals in a normal healthy cell (Forman and Boveris 1982, Freeman and Crapo 1982). During disease states, because of acceleration of exisiting pathways as well as appearance of new pathways,production of oxygen radicals is increased (Rao et al 1983, Garlick et al 1987). Intracellular presence of these radical species has been reported in vivo as well as in vitro

studies. Following are the potential in vivo sources of oxygen radicals.

1. **Mitochondria:**

Mitochondrial cytochrome oxidase accounts for greater than 95% of cellular oxygen consumption (Chance et al, 1979), yielding water without releasing $O_2^{\cdot}$ or H_2O_2 as intermediates, while 2-5% of O_2 undergoes univalent reduction and accounts for the production of radicals. A number of factors can enhance the univalent pathway of mitochondrial oxygen reduction. For example, superoxide radical generation by mitochondria is greatest when respiratory chain carriers located on the inner mitochondrial membrane are highly reduced (Turrens et al, 1982). The availability of NAD-linked substrates, succinate, ADP and oxygen (Freeman and Crapo, 1982), can also influence mitochondrial radical production. If oxygen is present in concentrations that limit its reduction to H_2O by cytochrome oxidase (1-3 mm Hg) increased respiratory chain reduction and an accumulation of reduced cofactors in cells may enhance $O_2^{\cdot}$ production by electron transport components in ischemic cells (Jobsis and LaManna, 1978).

2. **Enzymes:**

Other important intracellular sources of reactive oxygen species include various enzymes and the list includes oxidases and flavoproteins of peroxisomes, xanthine oxidase, aldehyde oxidase, tryptophan dioxygenase and dihydroorotate dehydrogenase (For reviews see Freeman and Crapo, 1982; Fridovich, 1982). Autooxidation of low molecular weight

of $O_2^{\cdot}$ and H_2O_2. These include thiols, hydroquinones, catecholamines, flavins and tetrahydroproteins. Cytochrome P450-dependent monooxygenases can also oxidize aromatic hydrocarbons (Griffin et al, 1981). Recently xanthine oxidase has been shown to be a major source of oxygen radicals during ischemia-reperfusion injury (McCord, 1985). In normal tissues, the enzyme exists as a dehydrogenase, utilizing NAD^+ instead of O_2 as electron acceptor. However, under ischemic conditions it is converted to the oxidase form (Chambers et al, 1985) which utilizes molecular oxygen as its electron donor resulting in production of radicals.

3. **Leukocytes:**

Activated phagocytic cells, including macrophages and neutrophils, produce $O_2^{\cdot}$, H_2O_2, $OH^{\cdot}$, hypochlorite and N-chloroamines (Thomas et al, 1983). Thus, active phagocytosis by leukocytes is associated with a burst of oxygen consumption (Patriarca et al, 1971) and a corresponding increase in the production of superoxide radicals (Babior et al, 1973). The mechanism of activated leukocyte-induced damage in inflammation involves infiltration of neutrophils and macrophages in the inflamed area. Although there are multiple stimuli for neutrophil migration, chemoattractants generated from the interaction of plasma lipids with oxygen free radicals derived from the action of cyclooxygenase or xanthine oxidase on their respective substrates may also be contributing to the accumulation of neutrophils (Petrone et al, 1980; Werns et al, 1986). Furthermore, activated

polymorphonuclear leukocytes possess NADPH oxidase on their plasma membrane that reduces molecular oxygen to the superoxide radical, with concomitant oxidation of cytosolic NADPH (Babior, 1978). This may enable polymorphs to digest phagocytosed microorganisms with a variety of oxidizing agents including oxygen radicals, hypochlorous acid (HOCL) and N-substituted chloramines (Thomas et al, 1983). The enzyme myeloperoxidase is required for the production of chloramine which is indirectly dependent on the NADPH oxidase activity for the supply of hydrogen peroxide via dismutation of superoxide. In addition to phagocytosis, oxidizing agents released from activated leukocytes have additional damaging effects on connective tissue (Greenwald et al 1976). Recently it has been recognized that superoxide radical can inactivate alpha-protease inhibitor (Schraufstatter et al, 1984), a protein which in healthy cells inhibits elastase and collagenase activity. The role of activated leukocytes in inflammation-induced tissue injury (Lee et al, 1981; Repine, 1985) and ischemia reperfusion injury (Engler et al, 1986; Parks et al, 1982) is becoming evident.

4. **Arachidonic Acid Metabolism:**

Enzymatic oxidation of arachidonic acid by membrane-bound cyclooxygenase involves free radical intermediates, one of which was shown by electron spin resonance to be a carbon-centered free radical. This radical is produced by cyclooxygenase-mediated abstraction of one of the methylene hydrogens of arachidonic acid, producing a fatty acid-free

radical species (Mason et al, 1980). Also during breakdown of the hydroperoxide to PGG_2, hemoprotein-derived radical is produced (Nobuchika et al, 1978). Thromboxane synthesis in platelets is inhibited by the radical scavengers imidazole and nordihydroguiaretic acid, suggesting that a free radical reaction is involved in conversion of prostaglandin endoperoxide (PGH_2) to thromboxanes (Salvador et al, 1977).

5. Toxic Agents:

A number of factors can modify the rate of $O_2^{\cdot -}$ and H_2O_2 production by living tissues. Xenobiotics such as paraquat (Fisher et al, 1973), adriamycin (Singal et al, 1987) and nitrofurantoin (Holtzman et al, 1981) are metabolically activated by cellular reductases and then cause cell damage via reduction of dioxygen to radical species. Exposure to hyperoxic conditions used to treat respiratory insufficiency also increases the rate of pulmonary $O_2^{\cdot -}$ and H_2O_2 generation (Freeman and Crapo, 1981, Yusa et al, 1984a).

C. DEFENSE MECHANISMS

Any organism that avails itself of the benefits of oxygen does so at the cost of maintaining an elaborate system of defenses against oxygen reduction intermediates, and possession of these defenses is a prerequisite for aerobic life.

One approach to defense is simply avoidance of the univalent pathway. This is achieved by enzymes with multiple electron carrying components which can accomplish the tetravalent reduction of dioxygen to water without the release

of reactive intermediates. Cytochrome oxidase is such an enzyme, and in actively respiring cells it is responsible for more than 90% of the observed dioxygen reduction (Wharton et al, 1968 Malkin and Malmstrom 1970). This avoidance of the univalent pathway reduces the burden of reactive intermediates the cell must face. Protection is also provided by metalloenzymes called superoxide dismutases, which catalyze the following reaction:

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \longrightarrow H_2O_2 + O_2 \qquad (1)$$

The superoxide dismutase found in the cytosol of mammalian cells is a homodimer with molecular weight 32,500 and contains both Cu(II) and Zn(II) at its active site (For reviews see Fridovich 1978, 1982, 1983). X-ray crystallography has provided a detailed view of the structure of this enzyme and has shown that copper and zinc are close together and are bridged by the imidazolate of histidine 61 (Tainer et al, 1982). During the catalytic cycle Cu(II) is alternately reduced to Cu(I) and then reoxidized, whereas Zn(II) does not change valence and plays a secondary role which has not yet been elucidated (Blackburn et al, 1984). It is clear, however, that the Zn(II) contributes to the structural stability of the enzyme (Cass et al, 1979, Forman and Fisher, 1981). Eukaryotic cells contain another superoxide dismutase which has manganese at its active site (Weisiger and Fridovich, 1973). This enzyme, a homotetramer of molecular weight ~ 95,000, is found in the matrix of the mitochondria. The mechanism of manganese superoxide dismutase involves alternate reduction and reoxidation of the active site Mn(III)

during successive encounters with $O_2^{\cdot -}$ (Pick et al, 1974).

Antioxidant defense is also provided by enzymes that eliminate the H_2O_2 produced either by dismutation of $O_2^{\cdot -}$ or directly by reoxidation of reduced flavoenzymes (Chance et al 1979, Flohe 1982). Catalase in mammalian cells is a hemoprotein; it dismutes H_2O_2 into H_2O and O_2 by catalyzing the following reaction:

$$H_2O_2 + H_2O_2 \longrightarrow 2H_2O + O_2 \qquad (2)$$

Mammalian catalase is a homotetrameric enzyme with a molecular weight of 240,000 (Deisseroth and Dounce, 1970). During the catalytic cycle the active site heme undergoes alternate divalent oxidation and reduction during successive encounters with H_2O_2. The oxidized state of the enzyme which is often called compound I is an Fe(IV) porphyrin pi cation radical (Dolphin et al, 1971). Compound I is a powerful oxidant and could be reduced by substances other than H_2O_2. In the case of catalase, the heme fits deeply and snugly into a crevice in the protein structure, and only small molecules can gain access to the heme iron (Reid et al, 1981). It is thus not surprising that catalase compound I can also be easily reduced by small molecules, such as methanol, ethanol, formate, and nitrite, and therefore that native catalase effectively acts as a peroxidase towards such small molecules (Chance, 1949, Keilin and Hartree, 1955). However, in the heart the concentration of catalase is reported to be low (Thayer, 1978, Doroshow et al, 1980) and catalase activity could not be detected in isolated rat cardiac myocytes (Jones

heart tissues (Herzog and Fahimi, 1974).

H_2O_2 can also be eliminated by peroxidases that catalyze reduction of H_2O_2 by a variety of electron donors. Glutathione peroxidase is a selenoenzyme and its activity in tissue depends on the nutritional state (Chaudiere and Tappel, 1983). When rats were maintained on selenium-deficient diet, glutathione peroxidase activity in the heart decreased substantially (Doroshow et al, 1980). The enzyme catalyzes the following reaction:

$$2GSH + H_2O_2 \longrightarrow GSSG + 2H_2O \qquad (3)$$

This reaction accomplishes the reduction of H_2O_2 to water at the expense of the oxidation of glutathione (GSH) to the corresponding disulfide. Although quite specific with respect to glutathione, glutathione peroxidase can also catalyze the reduction of fatty acid hydroperoxides (Forman and Fridovich, 1973) produced during the peroxidation of polyunsaturated fatty acids. Hydroperoxide reduction by glutathione peroxidase is accomplished at the expense of GSH. Glutathione reductase, which catalyzes the reduction of glutathione disulfide (GSSG) by NADPH (Mize and Langdon, 1962), prevents depletion of cellular glutathione. At a given concentration of GSH, the maximal velocity of this enzyme is independent of the type of peroxide. Activity appears to be limited by the ability to maintain adequate GSH levels in the cell, and this can be limited by the cellular supply of NADPH (Chance et al, 1979; Zimmer et al, 1981). NADPH supply is determined by the pentose phosphate pathway (Sies and Summer, 1975; Zimmer et

pentose phosphate pathway (Sies and Summer, 1975; Zimmer et al, 1981). Flux through this pathway has been shown to be dependent on the ratio of NADPH/$NADP^+$ as well as on the cellular content of GSSG (Zimmer et al, 1981, Fabregat et al, 1985). NADPH is a potent inhibitor of glucose-6-phosphate dehydrogenase, the rate limiting enzyme in the pathway. However, this inhibition can be overcome by GSSG (Zimmer et al, 1981) and in the heart this may be especially important since GSSG efflux is slow (Ishikawa et al, 1986). However, under conditions of oxidative stress, tissue glutathione content has been reported to be decreased (Viau et al, 1988) and the increased efflux of GSSH has been used as an index of oxidative stress in the heart (Guarnieri et al, 1987).

Catalase is located in peroxisomes, while glutathione peroxidase is distributed throughout the cytosol. This compartmentalization coupled with the lower K_m of glutathione peroxidase for H_2O_2 (Jones et al, 1981) and hydroperoxide suggests that in the heart catalase is less important than glutathione peroxidase in cellular H_2O_2 decomposition and in reducing lipid hydroperoxides. However, as intracellular rates of H_2O_2 generation are increased, catalase may become more important because the catalytic reaction, which has a greater rate constant, predominates over the peroxidative reaction.

Alpha-tocopherol is another important antioxidant which can interrupt radical-induced lipid peroxidation. It reacts rapidly with the chain-propagating fatty acid radicals to yield alpha-tochopherol radical, which is unable to further

propagate the chain reaction (Burton and Ingold, 1981, Burton et al, 1983). Tocopherol is particularly effective because of its hydrophobicity which allows it to partition into biological membranes, thus positioning it for maximum effectiveness. Alpha tocopherol is regenerated from its radical by Vitamin C which also serves as a water-soluble reductant and radical scavenger (Tappel 1969). Other non-enzymic antioxidants include B-carotene, and glutathione. These generally protect cells either by quenching singlet oxygen (Kearns 1971) or by donation of hydrogen atoms before the latter can initiate lipid peroxidation in membranes (Comporti, 1985; Arrigoni-Martelli, 1985).

Thus, in a healthy cell, the accumulation of these intermediate products of oxygen reduction such as superoxide, hydrogen peroxide and hydroxyl radicals is limited by the multi-layered defense system. However, if oxygen radicals are produced in excess of the tissue capacity to eliminate them, tissue damage can occur. For example, oxygen free radicals have been shown to kill bacteria, lyse cells, initiate lipid peroxidation of cell membranes and inactivate enzymes. The ability of oxygen radicals to damage a wide variety of cellular components has led to the suggestion that they may be important mediators of tissue damage in a variety of pathological states.

D. OXYGEN RADICALS AS MEDIATORS OF PATHOPHYSIOLOGIC PROCESSES

The precise mechanism of free radical-mediated cellular toxicity is uncertain. As previously noted, these radical

species are potent oxidizing agents that can damage a wide variety of organic molecules including lipids, proteins and nucleic acids.

1. **Lipid Peroxidation:**

It has been suggested that lipid peroxidation of cell membranes is a mechanism of free radical toxicity (For reviews see Tappel 1980, Aust and Svingen 1982) . Support for this concept comes from studies that demonstrate the ability of free radicals to initiate lipid peroxidation of cell membranes. The unsaturated bonds of membrane cholesterol and fatty acids can readily react with free radicals and undergo peroxidation. The chemical process of lipid peroxidation is defined as the reaction of an oxidant initiator with a polyunsaturated fat (LH) to form a lipid free radical intermediate ($L^{\cdot}$) (Mead, 1976; Pryor, 1976). A peroxy free radical ($LOO^{\cdot}$) is then formed when oxygen reacts with this free radical intermediate ($L^{\cdot}$).

$$L^{\cdot} + O_2 \longrightarrow LOO^{\cdot} \qquad (4)$$

$$LOO^{\cdot} + LH \longrightarrow LOOH + L^{\cdot} \qquad (5)$$

A relatively nonspecific hydrogen abstraction reaction is repeated when the unpaired electrons of the peroxy free radical react with another lipid molecule. Thus this process can become autocatalytic after initiation and will yield lipid peroxide, lipid alcohol and aldehyde by-products (Boveris et al 1981).

The biological sequelae of membrane lipid peroxidation depend on the fatty acid profile of membrane phospholipids

(and other oxidizable lipid components, for example cholesterol) and perhaps on the content of protein-bound iron which is able to decompose any generated hydroperoxides. Lipid peroxidation has been extensively reviewed (Gibson et al 1979, Donato 1981, Frankel 1980, Vladimisov et al 1980); these reviews summarize the hallmarks of cell damage that can be induced by lipid peroxidation. Plasma membrane and organelle membrane lipid peroxidation can be stimulated by oxygen radicals and are potentiated by the presence of metals (Aust and Svingen 1982). These metals can serve as redox catalysts and also catalyze the conversion of $O_2^{\cdot}$ and H_2O_2 to $OH^{\cdot}$ radical, a more potent oxidant (Svingen et al, 1979). Lipid peroxides and lipid peroxy radicals can exert their toxicity by reacting with many of the same cellular components as oxygen radicals. Because of the hydrophobic nature of lipid radicals most of the reactions take place with membrane fatty acids. The production of short chain fatty acids containing R-OOH, R-COOH, R-CHO, and R-OH groups within the bilayer may disrupt membrane architecture and function secondary to the introduction of hydrophilic moieties. (Plaa and Witsche, 1976). In vitro studies demonstrate abnormal membrane structure (Burton et al, 1984), permeability changes (Gupta et al, 1987) and functional loss and cell death (Gupta and Singal, 1987) after exposure of the tissue to oxygen radicals. Abnormal conditions in which lipid peroxidation has been implicated in some species include hemolytic anemia, reproductive dysfunction, lung damage, muscle atrophy, liver necrosis, encephalomalacia, ceroid epofuscinosis (McCay 1981),

iron overload (Gutteridge 1986) and ischemia-reperfusion injury,(Guarnieri et al 1980).

Lipid peroxidation can be detected by a variety of means. Increased absorbance of lipid extracts at 233 nm indicates conjugated diene formation, a consequence of hydrogen abstraction and bond migration in unsaturated fatty acids. Loss of cell membrane unsaturated fatty acids, production of volatile hydrocarbons such as ethane and pentane (Tappel and Dillard 1981), and oxygen uptake by membrane lipids all indicate peroxidation. Peroxidation of fatty acids containing three or more double bonds will produce malondialdehyde. The presence of this oxidation by-product can be measured with thiobarbituric acid, which, though not a specific or quantitative indicator of fatty acid oxidation, correlates with the extent of lipid peroxidation. This reaction has been critically discussed (Donato, 1981). Malondialdehyde produced by peroxidation can cause cross - linking and polymerization of membrane components (Nielsen, 1981). This can alter intrinsic membrane properties such as deformability, ion transport, enzyme activity and aggregation state of cell surface determinants (Freeman and Crapo, 1982). Because malondialdehyde is diffusible, it may also react with nitrogenous bases of DNA (Donato, 1981). All of these effects may explain why malondialdehyde is mutagenic (Mukai and Goldstein, 1976) and carcinogenic. Another useful estimate of extent of lipid peroxidation involves measurement of ethane and pentane gasses (Dillard et al, 1977). These

volatile hydrocarbons are the metabolic by-products of cellular hydroperoxide metabolism and can be detected by sensitive and noninvasive gas chromatographic methods.

2. **Protein Damage:**

Because of the presence of double bonds and sulfahydryl groups, (Pryor, 1976), amino acids such as tryptophan, tyrosine, phenylalanine, histidine, methionine and cysteine can undergo free radical-mediated amino acid modification. Enzymes which depend on these amino acids for activity will be inhibited by exposure to free radicals or radical-generating agents. Cytoplasmic and membrane proteins can also cross-link into dimers or larger aggregates after exposure to a number of oxidizing agents (Girotti, 1976). Reactions of free radicals with proteins may also generate by-products that would amplify the damage of the initial reactions. For example, oxidation of tryptophan will produce N-formyl kyneurenine and H_2O_2 as by-products (Meiners et al, 1977). N-formyl Kyneurenine can combine with amino-containing compounds by a Schiff's base reaction to form cross-linked species between lipids and proteins. Another way radical damage can be potentiated is by abstraction of an electron from molecules such as thiols, which can then form an intermediate capable of attacking other molecules.

The susceptibility of proteins to free radical damage depends on their amino acid composition. Oxygen radicals generated by xanthine-xanthine oxidase or activated phagocytes have been reported to alter Ca^{2+} ATPase activity

in cardiac sarcoplasmic reticulum, resulting in a depression and uncoupling of Ca^{2+} transport from ATP hydrolysis (Rowe et al 1983, Hess et al 1984). H_2O_2 and xanthine-xanthine oxidase are also capable of altering the ability of mitochondria to sequester and retain Ca^{2+} (Lotscher et al 1979, Harris et al 1982). The precise mechanism of these effects is not clear, but it is interesting to note that the Ca^{2+} ATPase contains a critical thiol group (Sarkadi et al 1980, Shalev et al 1981) and that the Ca^{2+} binding protein, calmodulin, has functionally important methionine groups (Walsh et al 1978). Ischemia-reperfusion-induced inhibition of Na^+K^+-ATPase is prevented by oxygen radical scavengers and antioxidants (Kim and Akera 1987). In this regard, Na^+K^+-ATPase is also shown to contain essential thiols necessary for its activity (Garner et al 1983). Addition of H_2O_2 to bovine lens has been shown to decrease K^+ influx and uncouple the ATPase.

3. **Nucleic Acid Damage:**

Radiations such as ultraviolet and visible light, heat and x-ray irradiation generate free radicals and excited molecules. Ionizing radiation produces radicals and electrons as primary species that decay to produce charged and neutral free radicals. Cell mutation and death from ionizing radiation is primarily due to free radical reactions with DNA. Cytotoxicity in large part is a consequence of chromosomal aberrations arising from either nucleic acid base modifications or DNA strand scission (Ward, 1986). Hydroxyl

radical is the main species responsible for DNA damage and all $OH^{\cdot}$ radical scavangers inhibit DNA strand scission. Superoxide dismutase, catalase and metal chelating agents also prevent strand scission. Enzymatic scavengers of $O_2^{\cdot -}$ and H_2O_2 protect DNA by decreasing the concentration of $OH^{\cdot}$ precursors, whereas metal chelating agents would prevent the metal catalyzed interaction of $O_2^{\cdot -}$ and H_2O_2 to form $OH^{\cdot}$ (Freeman and Crapo, 1982).

E. PATHOPHYSIOLOGICAL SIGNIFICANCE OF OXYGEN RADICALS

The role of free radicals in health and disease is becoming increasingly evident. The physiological role of these radicals in the bactericidal activity of leukocytes, drug metabolism in the liver, and their therapeutic role in radiation therapy is now well established.

In recent years medical scientists have begun to realise that oxygen radicals can impose a threat to tissue survival especially under conditions when certain radical- producing reactions are activated and/or their scavenging systems become ineffective. Extensive research during the last decade has provided evidence that oxygen radicals play some role in the development of many diseases (Cross et al, 1987; Marx, 1987). Much evidence is based on experimental data indicating increased rates of lipid peroxidation in diseased tissue, ameliorating effects of antioxidants, and the level of antioxidants in a variety of pathological conditions.

Furthermore, recently even direct radical measurement has been done in cardiac muscle subjected to ischemia (Rao et al, 1983) and ischemia-reperfusion induced rhythm disturbances (Garlick et al, 1987). Such studies often use experimental end-points of cell and tissue damage that may not reflect human disease processes and should be interpreted cautiously.

The brain is sensitive to oxygen radicals and their role is demonstrated in cerebral vascular reactivity and vascular wall damage in such conditions as hypertensive encephalopathy (Kontos et al 1981) and hyperbaric exposure to oxygen (Yusa et al, 1984 b). The lung is another target for oxygen radical attack and agents such as ionizing radiations, paraquat, or breathing 100% oxygen have been shown to exert their toxic effects through the generation of oxygen radicals (Brigham, 1986). The pathogenesis of the adult respiratory distress syndrome which is characterized by a diffuse acute inflammation and progressive damage to the alveolar capillary membrane may involve radicals released by activated polymorphonuclear cells (Lee et al, 1981, Repine, 1985). The neutrophils and macrophages that infiltrate inflamed tissue have several weapons that they can use to damage cells. These include, in addition to superoxide radicals, secreted enzymes such as elastase which breaks down connective tissue of the lung. The damage caused by elastase can lead to emphysema (Schraufstatter et al, 1984). Normally the enzyme elastase is held in check by $alpha_1$-protease

inhibitor but the reactions of superoxide and hydrogen peroxide can permanently inactivate this inhibitory protein, thus allowing elastase to work unchecked (Cochrane et al, 1983). This situation can be further excerbated by cigarette smoking, which has been linked to emphysema as well as to heart disease and lung cancer. Cigarette smoke contains free radicals that can also inactivate the protease inhibitor.

A popular current theory to explain gastrointestinal mucosal and endothelial lesions after even short term reduction in intestinal blood flow involves formation of radicals during reperfusion (Granger et al, 1981). Radicals may also be relevant to the pathogenesis of gastrointestinal tract lesions seen in patients after circulatory shock, and they might even play a role in gastrointestinal tract damage induced by alcohol and non-steroidal antinflammatory drugs.

Renal tubules have a high density of mitochondria, which show structural and functional defects in acute renal failure. In addition, both xanthine and arachidonate metabolisms are very active in renal tissues. Therefore, oxygen radicals might play a potential role in renal ischemia-reflow injury (Baud and Araillou, 1986), nephrotoxic nephritis (Rehan et al, 1984) and in the pathogenesis of immune-complex glomerulonephritis (Johnson et al, 1987). Oxygen radicals are important considerations in kidney preservation for transplantation and have also been implicated in aminoglycoside nephrotoxicity.

Injury to cutaneous structures by oxygen radicals is thought to occur in various conditions such as damage by ultraviolet radiations, the phototoxicity of porphyria, certain tetracyclines and benoxaprofen (Halliwell and Gutteridge, 1985). The role of oxygen radicals in such conditions as skin erythema, in skin graft implantation, contact dermatitis, age-related wrinkling and skin cancer is the subject of active investigation.

The role of oxygen radicals derived from activated polymorpholeukocytes, complement system and arachidonic acid metabolism is now believed to account for deleterious effects of inflammation both in animal models and humans (for reviews see Halliwell and Gutteridge, 1985; Fantone and Ward, 1982, Cross et al 1987). Inflammatory arthritis seems to represent the ideal condition in which induction of this disease process has been demonstrated by oxygen radical-producing reactions. Hyaluronic acid present in the cartilage pannus junction and in synovial fluid is easily degraded by oxygen radicals as are other proteoglycans and to some extent collagen and elastin. Oxidants can inactivate protease inhibitors and activate latent collagenase, thus potentiating damage. Other than arthritis, oxygen radicals have been found to be involved in chronic inflammation and DNA damage in other autoimmune diseases and in primary alloantigen-induced T-cell activation and proliferation (Chaudhri et al, 1986).

An antiinflammatory pharmaceutical preparation rich in SOD (Orgotein) is used in veterinary medicine and recently has been shown to be both effective and apparently safe in the treatment of various inflammatory lesions in humans. Catalase has also been used in the treatment of arthritic disease in humans with reported success (Proctor and Reynolds, 1984).

Diabetogenic actions of streptozotocin and alloxan may be mediated by oxygen radical mechanisms (Wahaieb and Godin, 1987) and use of SOD and catalase has been shown to prevent the deleterious effects of these agents on the pancreas. The implication of this experimental research in human patients remains far from clear, but certain complications observed in these diabetic patients can be explained on the basis of oxygen radical involvement. A few examples of such complications are retinopathy, greater incidence of ischemic heart disease and atherosclerosis. The use of antioxidants in preventing these complications may prove beneficial in the near future.

Much attention is currently focussed on a role for oxygen radicals in heart and blood vessel abnormalities and in myocardial changes secondary to the antitumor agent adriamycin (Singal et al, 1987), alcohol (Edes et al, 1986), and iron overload (Dillard et al, 1984). Oxygen radical reactions probably contribute to the production of ischemia-reperfusion injury (Werns et al, 1986), stress-induced arrhythmias (Singal et al, 1983) and atherogenesis (Heinecke et al, 1986).

A role for oxygen radicals in myocardial injury caused by ischemia-reperfusion is apparent. Activation of the complement system, generation of chemotactic peptides, migration and activation of polymorphonuclear cells, oxygen radical-induced membrane and lipid changes and depletion of intracellular scavengers of free radicals may all be involved in reperfusion injury. Agents that lower circulating polymorphonuclear cell counts modulate antioxidant enzyme levels, chelate iron or scavenge radicals may all prove therapeutically useful. The expanding clinical use of thrombolytic agents such as streptokinase, urokinase and tissue plasminogen activator as well as angioplasty for restoring the blood flow in acute myocardial infarction illustrates the potential clinical importance of this research. In addition to exacerbating the myocardial damage due to heart attacks, oxidative damage by free radicals may contribute to early changes that lead to arterial blockage. The involvement of superoxide radical and oxidized lipids in vessel wall abnormalities may bring new insights into atherogenesis. Oxygen radicals alter the kinetics of circulating lipoproteins, increase the permeability of endothelial cells and modulate uptake of lipoproteins by macrophages. Whether these radicals play a key role in the development of atheroma remains to be established.

F. OXYGEN RADICALS AND CARDIAC HYPERTROPHY

There are reasons to believe that due to altered cardiac

metabolism and increased levels of plasma catecholamines during cardiac hypertrophy and failure, oxygen radical metabolism may be altered in a hypertrophied heart. In this regard, increased formation of superoxide radicals has been reported in mitochondria isolated from hypertrophied hearts (Guarnieri et al, 1985). Furthermore, the hypertrophied heart is shown to be more sensitive to oxidative injury due to adriamycin (Singal et al, 1984) and ischemia-reperfusion injury (Guarnieri et al, 1985, Levitsky 1986). However, more studies are required to investigate the details of oxygen radical metabolism during cardiac hypertrophy and failure. In addition, pathogenesis of oxygen radical-induced injury in the heart, normal or otherwise, remains to be understood. It also remains to be seen if increased metabolism as well as protein synthesis in a hypertrophied heart is accompanied by a shift in antioxidant and/or peroxidant capacities. Following is a brief review of literature of cardiac hypertrophy.

The term "hypertrophy" can be defined as the growth of an organ or part due to an increase in size of its constituent cells as opposed to hyperplasia which involves nuclear division. Cardiac hypertrophy usually develops in response to increased workload on the myocardium and is manifest when the heart weight exceeds the accepted norms for age, sex and body weight. Study of this growth process in the heart is of great interest because clinically it has been

observed that heart failure may follow the increased heart size. Furthermore, cardiac hypertrophy is one of the major adaptive mechanisms in chronic cardiovascular diseases, such as hypertension and valvular defects.

G. CARDIAC HYPERTROPHY: AN ADAPTIVE OR A PATHOLOGICAL PROCESS

In an effort to cope with the stress of an imposed work overload, a common response of nearly every organ in the body is to increase its size (Julian et al, 1981). The heart is capable of dramatically illustrating this adaptive process by changing its mass in response to modified functional demands. Nonetheless, the presence of hypertrophy in various disease conditions and with strenuous exertion involving myocardial decompensation is strong evidence for the hypertrophy eventually being a pathological change. Where exactly the line should be drawn between the physiological and pathological phases of the same process is not clear. Various classifications have been put forward in an attempt to distinguish between different phases of the compensatory growth of the heart. For example, Meerson (1969) divided hypertrophy into three stages which corresponded to Selye's phases of the general stress syndrome (Zak, 1984). Under this classification, stage of damage (alarm reaction), stage of stable hyperfunction (resistance) and stage of progressive cardiosclerosis and gradual exhaustion were described.

Zak (1984) proposed an alternative classification in

which measurable indices of growth and their correlation with the different phases was emphasized: phase I - developing hypertrophy; phase II - compensatory hypertrophy; and phase III - heart failure. During phase I, imposed workload exceeds the mass of the heart and in phase II, induced growth has compensated for the increased workload established in phase I. In phase III, workload per unit of cardiac mass increases again due to progressively decreasing force generation and consequent dilatation of the heart. Emphasizing the functional status of the hypertrophied myocardium, Wikman-Coffelt et al (1979) categorized the hypertrophy process as physiological or pathological hypertrophy i.e. whether factors secondary to the process of hypertrophy have induced the heart to augment or depress its mechanical function. Jacob (1983) restated these two stages as "Physiological reactions" and "Pathological response."

Adaptational changes accompanying cardiac hypertrophy include modification of contractile proteins (for review see Swynghedauw 1986), membrane proteins (Lelievre et al 1986) and alteration of electrical properties of the membrane (Gulch 1980, Ten Eick and Bassel 1983). Chronic pressure overload is shown to modify gene expression for myosin heavy chain (Sinha et al 1983) resulting in predominent expression of fetal (V_3) isoform (Lompre et al 1979). During cardiac hypertrophy appearance of the fetal form of the isozyme has also been reported for creatine phosphokinase (Ingwell and Fossel

1983); however, the significance of this observation remains to be elucidated. V_3 isoform of myosin is reported to produce decreased velocity of shortening and rate of force development while time to peak tension was increased (Schwartz et al 1981). Heat measurments in pressure-overloaded hearts indicated that the economy of isometric shortening is increased (Alpert and Mulieri 1982).

H. CELLULAR BASIS OF CARDIAC HYPERTROPHY

During the last two decades considerable information has accumulated about biochemical and cellular processes that accompany cardiac hypertrophy. A variety of experimental procedures was developed in animals which induce a rapid or gradual increase in cardiac mass in response to increased work demand. These include constriction of the ascending/descending aorta or main pulmonary artery; renal or deoxycorticosteroid-induced systemic hypertension; damage to valves which results in aortic, pulmonic or tricuspid insufficiency; chronic hypoxia, anemia, arteriovenous fistula, administration of thyroxin or isoproterenol and severe prolonged exercise with a treadmill or an enforced swimming program. Cardiac hypertrophy secondary to genetically determined cardiomyopathy in the Syrian hamster has also been studied.

Cardiac growth stimulated by these procedures is usually due to (1) increase in size of the myocardial cells (Laks

et al, 1969; Carney and Brown, 1964) (2) increased number of connective tissue cells (Meerson et al, 1968; Morkin and Ashfold, 1968) and (3) longitudinal splitting of muscle cell (Grove et al, 1969; Linzbach, 1960). These conclusions are based on histological , radioautographic and quantitative cytological studies. Increased workload on the myocardium appears to activate the genetic apparatus of the myocardial cell, resulting in enhanced nucleic acid (Fanburg and Posner, 1968), and protein (Schreiber et al, 1966 Morgan et al 1986) synthesis.

The sequence of events leading to increased protein synthesis in stressed heart has been investigated both in vivo and in vitro and increased ribosomal RNA content and increased amino acid incorporation has been used to assess increased protein synthesis. It was shown that following aortic constriction, cardiac mass and protein content increase by 30 - 50 percent in 48 hrs (Zak and Fischman, 1971; Nair et al, 1968), representing the synthesis of 1 mg protein/hr/g tissue. This increased protein synthesis is the product of selective synthesis and did not include all cardiac proteins. For example, increase in mitochondrial and microsomal proteins has been shown to occur as early as 1 hr (Schreiber et al, 1967; Zak et al, 1976) and increased myosin biosynthesis occurs approximately 3 hrs following work overload (Schreiber et al, 1966). Therefore, independent control mechanisms exist for mitochondrial and myofibrillar

proteins and this may account for different patterns of subcellular accumulation following different physiological stimuli. In contrast, myoglobin and collagen synthesis appear unchanged at this time (Schreiber et al, 1970). Increased rate of protein synthesis has been demonstrated to be independent of cardiac contraction, intraventicular pressure development and oxygen consumption in isolated perfused hearts subjected to increased aortic pressure (Morgan et al 1986). Protein degradation, on the other hand, is reported to have slowed down (Zak et al, 1971, 1976). It is possible that during the early or compensated phase of cardiac hypertrophy there is proportionate synthesis of subcellular organelles so that the mitochondrial/ myofibrillar ratio remains constant, and asynchronous synthesis of these proteins in later stages may be responsible for cardiac failure (Wikman-Coffelt et al, 1979).

Although considerable information has accumulated describing the biochemical and cytological changes in the hypertrophied cardiac muscle cell, little is known about the mechanism of initiation of the integrated growth response by the physiological stimulus. In this regard, numerous ingenious theories of growth regulation in the heart by its functional load have been advanced : (a) increased work demand leads to a local tissue hypoxia (Norman, 1962) or depletion of high energy phosphates (Badeer, 1968) which in turn induce or repress the synthetic process; (b) increased wall tension results in a "wear

and tear effect" leading to the breakdown of macromolecules with the release of substances that stimulate growth (Meerson, 1969); (c) stretch of the muscle cell secondary to enhanced preload or afterload results in augmented synthesis (Eyster et al 1927, Morgan et al 1986); (d) humoral or hormonal factors such as catecholamines, cyclic AMP, and growth factors participate in hypertrophy (Seifert and Rudland 1974, Hnik 1962). However, none of the above-mentioned theories could entirely explain the induction of growth process. The recent development of cloned DNA probes for each specific protein might provide a solution to what is, as yet, an unresolved problem.

I. CONSEQUENCES OF CARDIAC HYPERTROPHY

1. Functional Changes in Cardiac Hypertrophy:

The contractile and pump functions of pressure-overloaded hearts have been subjects of recent controversy. It is clear that hypertrophy and other compensations maintain pump function in initial stages of pressure overload. It is equally clear that if overload is excessive and/or prolonged, pump function eventually fails. But whether systolic contractile function is normal or reduced in each hypertrophied muscle unit of the pressure-overloaded non-failing and failing heart has been critically debated. A number of recent studies make it clear that there is no universally applicable answer to this issue. Rather, the data indicate that within a group of pressure-overloaded

hypertrophied hearts, there is a pathologic continuum of systolic contractile muscle function which varies from normal in some hearts to depressed in other hearts. The continuum extends from one extreme of normally functioning units of hypertrophied muscle with excessive pump function, across the middle group of moderately dysfunctioning units which maintain basic pump function, to the other extreme of severely dysfunctioning units of hypertrophied muscle with poor pump function and congestive failure.

The presence and degree of myocardial contractile dysfunction in experimental animals vary in direct relation to the extent of the overload stress and correlate with degree of hypertrophy. For example, various degrees of acute pulmonary artery constriction have been used in a number of studies in which contractile function was analyzed in either in vivo hemodynamic measurements or in in vitro papillary muscle studies. Acute constriction (50%) of the pulmonary artery in cats for 4 - 24 weeks produced 38% right ventricular hypertrophy with no impairment in contractile function (maximum isotonic shortening and isometric tension development) and there was no heart failure in these animals (Pannier, 1971). Similarly, in dogs, acute constriction of the abdominal aorta for 17 days resulted in 36% left ventricular hypertrophy and myocardial contractile function (based on isovolumic and ejection phase index) was not impaired (Sasayama et al, 1975). A constriction of (80%) in the pulmonary artery for 3 - 12 weeks resulted in 90%

development of hypertrophy with only a 25% reduction in contractile function (isotonic velocity and isometric tension) (Spann et al, 1967). In another study a similar degree of pulmonary artery constriction was found to be associated with only transient impairment (at 6 weeks) in contractile function which returned to normal at 24 weeks (Williams and Potter, 1974). In this study it was concluded that the contractile abnormalities were a transient defect, probably related to a reversible injury produced by acute pressure overload. When the pulmonary artery was severely constricted (90%) in adult cats for 3 - 13 weeks, there was 142% development of hypertrophy and the contractile function (isotonic velocity and isometric tension) was severely (62%) impaired (Spann et al, 1967). Similarly, in dogs, severe acute aortic constriction was also reported to be associated with abnormal contractile function (Newman and Webb, 1980).

The importance of the degree of hypertrophy in determining contractile function is evidenced in a study on spontaneously hypertensive rats (Pfeffer et al, 1979). In 6 month old animals with 25% hypertrophy, left ventricular contractile function was normal as was diastolic volume and end-diastolic pressure. At age 18 months with 60% hypertrophy, there was a depression of left ventricular contractile function with an increase in diastolic volume and end diastolic pressure.

The development of acute injury due to sudden imposition of workload was avoided in a study (Cooper et al, 1981),

in which kittens were subjected to chronic pressure overload which increased gradually with the growth of the animal. After 25 and 60 weeks of pulmonary banding, *in vivo* pump function (cardiac output and ejection fraction) and *in vitro* muscle function (maximum isometric tension development and lightly preloaded shortening velocity) were measured in these animals. Both groups showed normal function but contractile function of papillary muscle was markedly reduced (Cooper *et al* 1981). A similar model of gradual and mild aortic constriction was produced in dogs (Carabello *et al*, 1981). In this study, a non-constricting subcoronary band was placed along the aorta in young puppies. After 37 weeks, the aortic lumen was 65% of normal size with 29 mm peak systolic gradient and development of 27% heart hypertrophy. This model showed normal pump function as judged by ejection fraction, dP/dt, stroke work and ventricular function at rest. However, *in vitro* contractile function was not measured in this study. Thus, models employed in these studies avoid non-pathophysiologic acute damage due to the sudden imposition of pressure overload and may be closer to the course of events seen in clinical situations. A similar approach of a gradual imposition of pressure overload in rats was adopted in the present study.

The presence of contractile abnormalities in clinical cardiac hypertrophy indicated that hypertrophy produces abnormal intrinsic contractile function which might contribute to the

eventual deterioration of initially compensating hypertrophy into a congestive heart failure state (McDonald 1975). The exact reason for abnormal intrinsic contractile function during cardiac hypertrophy is not clear but it is known that the contractile function of the heart is served by a number of key proteins that regulate the efficiency of excitation-contraction coupling which leads to tension development (Katz 1977). Components of sarcoplasmic reticulum and mitochondria also affect the tension development in the heart. A number of studies have shown that biochemical properties of each of these components may be altered during the process of hypertrophy (Sordahl 1979).

Myosin, a contractile protein, acts as an enzyme (myosin ATPase) which is essential for actin-myosin interaction and hence the force development by the heart. A number of reports suggest that the activity of myosin ATPase changes in the hypertrophied heart responding to hemodynamic overload (Swynghedauw et al 1973, Fizelova and Fizel 1970, Wikmen Coffelt et al 1975). Changes in enzyme activity appear to be dependent on severity and duration of stress (Swynghedauw et al, 1976; Wikman-Coffelt et al, 1979; Scheuer and Bhan, 1979). This may be related to the alterations in synthesis of the molecule and/or post synthetic modification such as phosphorylation (Adelstein and Eisenberg, 1980) or modification of sulfhydryl groups involved in the active site of the molecule (Thomas and Alpert, 1977). Most authors agree that myosin ATPase activity

is not altered in compensated hypertrophy (Wikman-Coffelt et al, 1975; Fizelova and Fizel, 1970) and is decreased in chronic hypertrophy (Maughan et al, 1979; Swynghedauw et al, 1973). Several authors found actomyosin ATPase to be depressed in chronic hypertrophy (Fizelova and Fizel, 1970; Hoar et al, 1971). Altered proportions of myosin isozyme have been demonstrated in pressure overload-induced and thyroxin-induced hypertrophy and have been related to altered cardiac performance in these models (Litten et al 1982, Lompre et al 1979). A defective sarcoplasmic reticular ATPase may be partially responsible for the attenuated contractility observed in the failing heart (Limas and Cohen, 1977, Suko et al, 1970). It is apparent that the amount of activity of the calcium-accumulating system in the heart may be altered in congestive failure, but whether this is a primary or secondary effect is yet to be established.

An interesting insight into a possible mechanism for eventual decline of abnormal unit muscle function toward reversibly abnormal pump function (Coulson et al, 1977) is provided by a model of progressive pressure overload producing very extensive hypertrophy (Rembert et al, 1978). It was found that when the demand for myocardial perfusion is high, there is a selective decrease in subendocardial perfusion. This might be expected to result in transiently abnormal ventricular function during stress and perhaps eventually to irreversible ventricular damage.

2. **Metabolic Alterations During Hypertrophy:**

A number of investigations have shown that cardiac metabolism changes in response to increased workload (for reviews see Meerson, 1969 a; Sordahl, 1979). Initially, there is an increase in oxygen consumption which is associated with increased coronary flow, increased uptake of glucose and conversion of a positive pyruvate balance to a negative one (Meerson, 1969a). However, in long standing cardiac hyperfunction that is secondary to hypertension, the oxygen consumption per unit mass of the myocardium returns to normal. It is interesting to note that this return is not due to a decrease in cardiac hyperfunction, rather, because of the development of hypertrophy, the functional level of each subcellular element is increased, resulting in greater energy production per unit mass. This adaptation plays an important role in providing stable, long lasting compensatory hyperfunction (Meerson, 1969 a). It has been reported that total oxygen consumption in humans with compensated cardiac defects is increased by an average of 12% under basal metabolic conditions. The greatest increase in oxygen consumption (18%) was observed in lesions of the aortic valve associated with intense hyperfunction of the left ventricle. These increases in oxygen consumption demonstrate that a clinically compensated state of aortic stenosis is associated with a considerable increase in oxygen requirements of the hypertrophied heart (for review, see Meerson, 1969 a).

Levine and Wagman (1962) studied myocardial oxygen consumption in patients with overt heart failure secondary to valvular lesions or hypertension. In spite of decreased efficiency of the heart as a pump, myocardial oxygen consumption was three times elevated in these patients. It remains to be seen whether or not some of the excess oxygen is converted into radical intermediates.

The glycogen concentration was found to be markedly reduced just after aortic stenosis in rabbits. During stable hyperfunction, there is a restoration of glycogen concentration to normal levels, while the failure stage was found to be associated with either normal or somewhat decreased levels of glycogen . The activity of phosphorylase was reported to be increased initially followed by a return to normal, and finally it is decreased during heart failure. On the other hand, the terminal products of glycolysis such as lactate and pyruvate were found to be increased (for review, see Meerson, 1969a). There are conflicting reports available in the literature regarding oxidative phosphorylation in hypertrophy and heart failure. The principal points made in various studies suggest either no significant change in oxidative phosphorylation (Chidsey et al, 1966; Pierce et al, 1984) or significant depression in mitochondrial energy-producing capacity due to hypertrophy-failure (Procita et al, 1965; Wollenberger et al, 1963; Argus et al, 1964; Lindenmayer et al, 1971). In the latter case, it was found that the degree of coupling of

oxidation to phosphorylation in the area of the respiratory chain between NAD and cytochrome c was only slightly different from control, while in the area between cytochrome c and oxygen, there was a marked reduction in coupling (Schwartz and Lee, 1962). This observation might have an important link in production of oxygen radicals, as has been discussed before in the section on oxygen radicals.

Creatine phosphate levels appear to be low in compensated hypertrophy (Alpert and Hamrell, 1976), while adenosine triphosphate levels are within the normal range in the non-failing hypertrophied heart (Katz, 1977; Alpert and Hamrell, 1976) falling only in the terminal phases of heart failure (Katz, 1977, Alpert and Harmell, 1976). In fact, it was proposed by Sordahl et al (1973) that in the later stages of hypertrophy when mitochondrial respiratory activity is at or below control levels, the energy produced is insufficient to meet the demands of the hyperfunctioning heart and thus contributes to the development of heart failure.

3.Sympathetic Nervous System in Hypertrophied and Failing Heart:

The sympathetic nervous system represents a major control mechanism in the moment-to-moment regulation of the normal circulatory responses to changing metabolic requirements of tissues. This system provides a sensitive mechanism for rapid alteration of myocardial contractility, heart rate and peripheral venous and arterial tone. Nerve terminals from the network of sympathetic nerves that supply the cardiovascular

system are found in arteries, veins and all chambers of the heart. Nerve terminals are the storage site of the neurotransmitter norepinephrine. Stimulation of the sympathetic nerves to the heart results in discharge of this norepinephrine which acts upon the cardiac cellular beta-receptor to exert effects dependent on the site of stimulation. Increases in heart rate occur with stimulation of the sinoatrial node, increased velocity of conduction occurs through the atrioventricular junctional tissues and augmentation of myocardial contractility occurs following interaction with beta-receptors which results in the activation of cyclic AMP-dependent protein kinase (Rinaldi et al, 1982). Hence, beta-adrenergic effects on cardiac function are explained in terms of cyclic AMP-dependent protein phosphorylation of either the contractile machinery (Perry, 1979) or membrane proteins involved in calcium movements (Kirchberger et al, 1974).

It is known that cardiac contractile proteins, namely troponin I and myosin light chains, undergo cyclic AMP-induced phosphorylation (Cole and Perry 1975, Rubio et al 1975, Morgan et al 1976). Phosphorylation of troponin I resulted in increased sensivitity of actomyosin ATPase for Ca^{2+} (Rubioet al 1975). The role of phosphorylation of myosin P light chain is not clear, but it is suggested to modulate cross-bridge cycling (Huxley 1972).

Synthesis of norepinephrine and its metabolism occurs

within cardiac sympathetic neurons and in the synaptic cleft. During norepinephrine synthesis, tyrosine hydroxylase is the major regulating enzyme and neuronal synthesis accounts for 80% to 90% of total catecholamine content of the heart while the remaining 20% is due to reuptake of norepinephrine by nerve terminals. It has now been established that catecholamines are inactivated under in vivo conditions by three pathways involving monoamine oxidase, catechol-O-methyl transferase and autooxidation of catecholamines. It is also known that autooxidation of catecholamine involves generation of oxygen radicals (Singal et al, 1981, 1982).

During hypertrophy and cardiac failure, the sympathetic nervous system is quite abnormal. A number of reports indicate that myocardial stores of norepinephrine are severely reduced in both clinical (Chidsey et al, 1963, 1965) and experimental (Spann et al, 1964, 1965) hypertrophy with or without congestive heart failure. Norepinephrine depletion occurred in both ventricles regardless of which ventricle was subjected to the primary hemodynamic burden. Reduced total cardiac content of norepinephrine proves that there is a true depletion of catecholamine, rather than dilution of the normal component of norepinephrine by hypertrophy of the heart. Depletion of cardiac norepinephrine content has been attributed to a reduction in norepinephrine uptake (Fischer et al, 1965) and a defect in the synthetic pathway for norepinephrine both in experimental hypertrophy with

congestive heart failure (Pool et al, 1967) and clinical hypertrophy (Dequattro et al, 1973) with congestive heart failure. Furthermore, these changes in norepinephrine metabolism have been found to be associated with decreased neurotransmitter release following sympathetic nerve stimulation in experimental ventricular hypertrophy and heart failure (Covell et al, 1966) as well as in the clinical situation (Goldstein et al, 1975). The number of cardiac alpha and beta adrenoceptors was shown to be increased in cardiac hypertrophy (Karliner et al, 1980; Limas, 1979). It was proposed that this increase might be a compensatory phenomenon in order to cope with the decline in tissue catecholamine levels (Limas, 1979). Nonetheless, it should be noted that depletion of tissue catecholamines is usually associated with an increase in the plasma levels of these hormones. Thus, high levels of circulating catecholamines in chronic heart hypertrophy may become available for oxidation into toxic substances such as free radicals and adrenochrome, which may then produce cell damage as well as defects in contractile properties of the myocardium.

From the foregoing review it is quite clear that differential protein synthesis during hypertrophy may induce differences in the total antioxidant capacity. Furthermore, increased cell metabolism as well as oxygen consumption during hypertrophy may provide for the formation of oxygen radicals. Together, these changes can result in an altered

balance between free radical production and scavenging mechanisms in hypertrophied heart. Thus, study of oxygen radical mechanisms in control as well as hypertrophied hearts as proposed here can be of great value in the understanding of pathophysiology of heart disease.

III. METHODS

A. ANIMALS:

Adult male Sprague-Dawley rats weighing 225 - 275 grams were used. Animals were housed (2 to 3 in a cage) in the Central Animal Care Facilities of the University, and were provided with standard rat chow and water ad libitum.

B. HEART PERFUSION:

Animals were sacrificed by decapitation, their hearts were rapidly excised and placed in an ice-cold oxygenated buffer solution. The hearts were mounted on a steel cannula and perfused according to the Langendorff method in a retrograde fashion through the coronary arteries at a flow rate of 8 ml/min. The perfusion medium was a modified Krebs-Henseleit (KH) solution containing (mM): NaCl, 120; $NaHCO_3$, 25.4; KCl, 4.8; KH_2PO_4, 1.2; $MgSO_4$, 0.86; $CaCl_2$, 1.25 and glucose 11.0. This solution was continuously gassed with a mixture of 95% O_2 and 5% CO_2 which resulted in a pH of 7.4. The temperature of the entire perfusion system was maintained at 37^{o}C. All atrial tissue and fat were trimmed off. Hearts were electrically paced with bipolar platinum electrodes attached to the ventricular muscle. Supramaximal stimuli (4V-6V) of 1.5 ms duration were delivered at the rate of 300 pulses/min. Myocardial contractile force was recorded after calibrating the channel in grams and data expressed as percent change relative to zero time data. Rate of change of developed tension

(instantaneous maximal dF/dt) was recorded on a Beckman recorder *via* a force displacement transducer (FT .03). A resting tension of 2 g was applied to provide a constant load on the hearts.

C. SOURCE OF OXYGEN RADICALS:

In order to generate partially reduced forms of oxygen, xanthine (2 mM at 40^{o}C) and xanthine oxidase (10 U/L) were dissolved in the above mentioned Krebs Henseleit solution (McCord *et al*, 1977, Burton *et al*, 1984) and mixed for 90 min. In order to obtain a stabilized force, all hearts were perfused with normal oxygenated KH buffer for 15 min before switching to the KH medium containing xanthine-xanthine oxidase (X-XO). Hearts showing instability in rhythm or force were not used.

Characterization of Oxygen Radical Species:

In order to characterize the nature of the radical species resulting from xanthine - xanthine oxidase (X-XO) interaction, effects of antioxidants and specific scavengers on X-XO-induced changes were studied. For this purpose, superoxide dismutase (3 x 10^4 and 1.2 x 10^5 U/L), catalase (2 and 4 x 10^4 U/L), mannitol (10 and 20 mM), GSH (200 uM and 2 mM) and methionine (1 and 20 mM), were employed. Effects of each intervention on developed force and instantaneous maximal rate of force development (+dF/dt) and rate of relaxation (-dF/dt) were studied in separate experiments. The role of iron in hydroxyl radical formation was studied by adding desferrioxamine (300 uM and 3 mM), an iron chelator, in Krebs

Henseleit buffer containing xanthine - xanthine oxidase and its effects on force parameters were evaluated. For control studies, effects of xanthine, xanthine oxidase, as well as of superoxide dismutase, catalase, mannitol, GSH, methionine and desferrioxamine in higher concentration were also individually tested.

D. TIME-COURSE STUDIES OF EFFECTS OF OXYGEN RADICALS:

In order to examine the sequence of events following xanthine - xanthine oxidase perfusion, lipid peroxidation, high energy phosphates and structural changes were studied at 5, 10, 20 and 40 min of perfusion with Krebs Henseleit solution containing X-XO.

1. Malondialdehyde Assay

Lipid peroxide formation was followed by the thiobarbituric acid method for the examination of malondialdehyde content (Hunter et al, 1963; Placer et al, 1966). Hearts were quickly washed in ice-cold buffered 0.9% KCl (pH 7.4). After removing atria, extraneous fat and connective tissues, the ventricles were weighed, homogenized in 10 vol of the above buffer. The homogenate was incubated for one hour at 37^{o}C in a water bath. After incubation, 2 ml aliquots were withdrawn from the homogenate and pipetted into 8 ml pyrex tubes. One ml of 40% trichloroacetic acid (TCA) and one ml of 0.2% thiobarbituric acid were added immediately. Tube contents were mixed, boiled for 15 minutes and cooled on ice for 5 min. Two ml of 70% TCA was then

added to all tubes and, after mixing contents were allowed to stand at room temperature (25°C) for 20 min. Following this, the tubes were centrifuged at 800 x g for 20 min in a table-top centrifuge. The supernatant was carefully drawn off and centrifuged again for 20 minutes in an RC II-B Sorvall centrifuge at 3500 rpm. The supernatent was read at 532 nm on a Spectronic 2000 spectrophotometer, and the tissue malondialdehyde (MDA) concentrations were calculated using 1 nmol standard MDA and expresssed as nmoles/g wet weight. Malondialdehyde bis (dimethyl acetal), obtained from Aldrich Chemical Company Inc. was used as a standard.

2. **High Energy Phosphates and their Metabolites**

Hearts were quickly frozen using Wollenberger clamps precooled in liquid nitrogen. The frozen tissue was pulverized in the presence of liquid nitrogen. The tissue powder thus obtained was homogenized in 6% perchloric acid (8 ml/g heart weight) with a Polytron homogenizer for 15 seconds. The homogenate was centrifuged at 6,000 rpm for 20 min. The supernatant was neutralized with 5 M K_2CO_3 and centrifuged (10,000 rpm for 15 min) to remove precipitated $KClO_4$. The neutralized supernatant was stored in liquid nitrogen until it was analysed by high performance liquid chromatography (HPLC).

Adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), adenosine, creatine phosphate (CP) and creatine were separated and analyzed using HPLC as described by Sellevold et al, (1987). This procedure allows a simultaneous analysis of various metabolites. The

HPLC analyses were performed using a Beckman system containing a 110B solvent delivery system, a 421A gradient controller, a 210A sample injector system, a 46 mm(id) x 25 cm reverse phase C-18 column and a 163 variable wavelength detector set at 206 nm. Flow rate was maintained at 1 ml/min. The mobile phase consisted of 215 mM potassium dihydrogen phosphate (KH_2PO_4), 2.3 mM tetrabutylammonium hydrogen sulphate (TBAHS), and 3.5% acetonitrile (pH = 6.25). With this method, creatine eluted first, followed by CP, AMP, ADP, adenosine and ATP, at their specific retention times. The peaks found at 206 nm in the tissue samples were identified by comparison of their retention times with known standards. The concentrations (umole/g dry weight) of nucleotides, nucleoside and creatine compounds were quantified by measuring the area under the peak and comparing it with a known standard. The extract from each heart was chromatographed in duplicate or triplicate and the mean value from each sample was calculated.

3. **Ultrastructural Studies**

The hearts were perfusion-fixed with 3% glutaraldehyde (8 ml/min) for 15 min and processed for electron microscopic examination using techniques previously described (Singal et al, 1981). Briefly, mid-myocardial pieces were obtained from four different areas of the left ventricular free wall between the mid-region and the apex of the heart. These pre-fixed tissue pieces were immersed in 0.1M phosphate buffer (pH 7.4) containing 3% glutaraldehyde and were further cut

into cubes < 1 mm in size. The aldehyde fixation was continued for an additional 2 hr. The tissues were washed for 1 hr in the above described phosphate buffer containing 0.05 M sucrose. Post-fixation was performed in 2% OsO_4 for 2 hr with a change of solution at 1 hour, after which the tissue pieces were dehydrated in a series of graded alcohol solutions. The dehydrated tissue was transferred into propylene oxide followed by an Epon-propylene mixture. The tissue was embedded in Epon (Luft, 1961). Ultra-thin sections were cut on an MT II Porter-Blum Ultramicrotome. Thin sections were placed on Formvar - coated grids and stained with uranyl acetate and lead citrate (Reynolds, 1963). The stained sections were examined under an electron microscope.

For a semiquantitative morphometric analysis, a minimum of 100 non-overlapping cells were counted from each heart (n=4) and damage was defined as mild, moderate or severe according to the structural abnormalities seen in the mitochondria, myofibrils, sarcoplasmic reticulum, increase in the number of lysosomes and the general appearance of the cell (Billingham et al, 1979). Mitochondria demonstrating swelling or loss of cristae or clear matrix were considered damaged, while formation of contraction bands and/or loss of filaments were the criteria for the damaged myofibrils. Based on this analysis, a score was assigned to each myocyte and the injury was categorized as mild, moderate or severe. The number of cells in each category were expressed as the percentage of total cells counted. The absolute number of cardiac mitochondria and myofibrils in 25 cell profiles, and 50 blood

vessels in cross-section were also examined and counted in normal and X-XO treated hearts (n=4). Vacuolation and/or blebbing of endothelial cells indicated vascular injury.

In order to study the permeability changes following 20 min perfusion with KH buffer containing X-XO, hearts were perfusion-fixed with 3% glutaraldehyde solution containing 1% lanthanum in collidine buffer (pH = 7.8) for 5 min at the perfusion rate of 8 - 10 ml/min (Singal and Dhalla, 1984). The midmyocardial region was then chopped into small cubes of approximately 1mm in size and fixation in the same solution was continued for another 2 hr. Tissues were given five gentle washings in the wash solution each of 30 min duration. Post-fixation was done with osmium tetroxide solution containing colloidal lanthanum for 1 to 2 hr. Tissues were then dehydrated in increasing concentrations of ethanol, embedded in epon, sectioned and stained for electron microscopic examination as described above. At least three different blocks from four hearts were examined and compared with control hearts perfused with KH buffer for 20 min.

E. HEART HYPERTROPHY PROCEDURE:

For this part of the study, rats weighing 100-140g were used. Pressure overload was produced by the narrowing of the abdominal aorta in anesthetized (Nembutal, 35 mg/kg i.p.) rats. Access to the aorta was gained through a midline abdominal incision and a 0.5 cm segment of this vessel in the subdiaphragmatic region was cleared from adhering tissue. To

produce a uniform degree of constriction in all animals, a blunt steel wire having a 1.15 mm diameter was placed along the aorta proximal to renal arteries and a 3-0 silk ligature was tied snugly around the wire and aorta. The wire was then withdrawn from the ligature and the abdominal incision was closed. Sham-operated rats were treated identically except that the aortic band was not placed. In this way, a gradual and progressive pressure overload developed. Relative reduction in the diameter of the aorta in the banded region was quantitated in a post-mortem study of aortic strips at different post-operative intervals.

1. **Experimental Protocol**

Following surgery, animals were sacrificed at four time intervals for up to 48 weeks. For this, animals were divided into 8 groups as follows: 1) 6 weeks sham control (6 WS); 2) 6W hypertrophy (6 WH); 3) 12W sham control (12 WS); 4) 12W hypertrophy (12 WH); 5) 24 W sham control (24 WS); 6) 24W hypertrophy (24 WH); 7) 48W sham control (48 WS); 8) 48W hypertrophy (48 WH). These animals were employed in further studies as follows.

2. **Hemodynamic Measurements:**

Following different postoperative periods, animals were anaesthetized with thiopental-sodium (35 mg/kg i.p.) and the hemodynamic function of the heart in closed chest rats was assessed. An ultraminiature catheter pressure transducer (model PR 249, Millar Instruments Inc. Houston, Texas) was inserted into the right carotid artery and then quickly advanced into the

left ventricle. Heart rate, left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), aortic systolic and diastolic pressures and maximal rate of rise and fall of left ventricular pressure ($\pm$ dP/dt) were continuously recorded on a precalibrated multichannel Beckman dynograph. Measurements were taken 15 min after catheterization of the left ventricle when steady state was reached.

3. **Tissue Weights**

After decapitation, the heart, lung and liver were immediately removed, freed from adhering tissues and weighed. The left ventricle (including the interventricular septum) was dissected and weighed. Dry weights for liver and lung were obtained by placing these organs in an oven (65^{o}C) for 48 hrs and wet/dry weight ratios for these tissues were estimated.

4. **Determination of Total RNA:**

In order to examine RNA content during cardiac hypertrophy, a modified method of Murno and Fleck, as described by Jamieson et al (1982) was followed. Briefly, the left ventricle was homogenized in 9 vol of sodium phosphate buffer (pH = 7.4) and 5 vol of 0.6 M perchloric acid was added to precipitate protein and nucleic acids. After cooling for 10 min on ice, samples were centrifuged at 8500 g for 20 min in a Sorval RC2B centrifuge; pellets were washed twice with 10 vol of 0.2 M perchloric acid, resuspended in 4 vol of 0.3 N KOH, and incubated at 37^{o}C for

one hour to hydrolyse RNA. After precipitating out protein and DNA (2.5 ml of 1.2 M perchloric acid), the RNA content of the supernatants was determined at 260 nm. Using standard RNA, the data were expressed as total RNA in mg.

5. **Superoxide Dismutase Assay:**

Autooxidation of 6-hydroxydopamine gives rise to breakdown products with an absorbance increase at 490 nm. The appearance of colour is promoted by superoxide anion and inhibition of this reaction has been used to assess superoxide dismutase (SOD) activity (Heikkila and Cabbat, 1976). Cytosolic concentration of SOD was determined by homogenizing the left ventricle in 9 vol of 0.05 M sodium phosphate buffer (pH = 7.4) and centrifuging the homogenate at 30,000 g for 45 min. A fresh 6-OH dopamine hydrobromide (Sigma Chemicals) solution (10^{-3} M) was prepared in distilled water bubbled with nitrogen. Autooxidation of dopamine was measured by addition of 50 ul of the above solution (final concentration 1.66×10^{-5} uM) to the buffer (final vol 3 ml) and increase in absorbance was recorded for 5 min at 490 nm, using a Spectronic 2000 spectrophotometer. The effects of various concentrations (100U - 2000U) of standard SOD (Sigma Chemicals) as well as various concentrations of tissue protein (0.2 - 1.2 mg) on this autooxidation were examined to assess heart SOD activity in different groups.

6. **Glutathione Peroxidase Assay:**

GSHPX activity was measured by the method of Paglia and Valentine (1967) and the activity was expressed as umol of NADPH oxidized to NADP per min per g wet tissue, using a molar extinction coefficient for NADPH at 340 nm of 6.22 x 10^6. Cytosolic concentrations of glutathione peroxidase were determined by homogenizing the left ventricle in 9 vol of 0.05 M sodium phosphate buffer and then centrifuging at 30,000 g for 45 min. GSHPX was assayed in a 3 ml cuvette containing 2.0 ml of 75 mM phosphate buffer pH 7.0. The following solutions were then added: 50 ul of 60 mM glutathione, 0.10 ml of glutathione reductase solution (30 units/ml), 50 ul of 0.12 M NaN_3, 0.1 ml of 15 mM Na_2EDTA, 0.1 ml of 3.0 mM NADPH, an aliquot of cytosolic fraction (100 ul) and H_2O was added to make up a total volume 2.9 ml. The reaction was started by the addition of 0.1 ml of 7.5 mM H_2O_2 and the conversion of NADPH to NADP was monitored by continuous recording of the change in absorbance of the system at 340 nm every minute for 5 min.

F. CHEMICALS

Xanthine, superoxide dismutase, catalase, ATP, ADP, AMP, adenosine, creatine and CP were obtained from Sigma Chemicals. KH_2PO_4, TBAHS and acetonitrile of HPLC grade were obtained from Fisher Scientific. Xanthine oxidase was obtained from Calbiochem. All other chemicals were obtained from commercial sources and were of highest purity (99%) available.

G. PROTEIN ESTIMATION AND STATISTICS:

For determination of protein concentration in the heart homogenate, bovine serum albumin (fraction V, Sigma Chemicals) was used as a standard (Lowry et al, 1951). The results were expressed as mean ± SEM. For normalization, percentages were employed on the assumption that parameters under study for both control and test conditions would vary in a linear fashion. A one-way analysis of variance was carried out to test for any differences between the mean values of all groups. Individual means were compared using unpaired Students 't' test. For comparisons involving more than two groups, Tukey's multiple comparison test was used. For semiquantitative morphological studies, the Kruskal-Wallis test was used to establish differences in various groups while individual means were compared by the Wilcoxen two sample rank test. p values below 0.05 were considered significant.

IV. RESULTS

A. MYOCARDIAL EFFECTS OF OXYGEN RADICALS IN CONTROL HEARTS

After obtaining stable contractions for 15 min with normal Krebs Henseleit (KH) buffer, hearts were perfused with the KH medium containing xanthine-xanthine oxidase (X-XO) for different time intervals (5, 10, 20, 40 min). Changes in contractile function (developed tension, resting tension and ± dF/dt), lipid peroxidation, high energy phosphate levels and ultrastructural damage were studied.

1. Contractile Force

A typical record of X-XO induced changes in contractile force as well as in instantaneous maximum rate of force development and relaxation (±dF/dt) is shown in Figure 1 and the data are summarized in Table 1 and Figure 2. After 5 min of perfusion, force declined to 56.2 ± 5.6% without any significant change in resting tension (+20.00 ± 10.16%). At 10 min, contractile force was reduced to 26 ± 2.4% of the control value while the rise in resting tension was 58 ± 26%. Complete failure with a marked increase in resting tension (357 ± 40.4%) was apparent within 20 min. Further perfusion with X-XO for 40 min caused only a small increase in resting tension (400 ± 56%). This decline in peak developed force was also accompanied by a depression of +dF/dt and -dF/dt (Table 1.). Hearts (n=4) perfused with KH medium containing only xanthine (2 mM) or xanthine oxidase (10 U/L) for 40 min

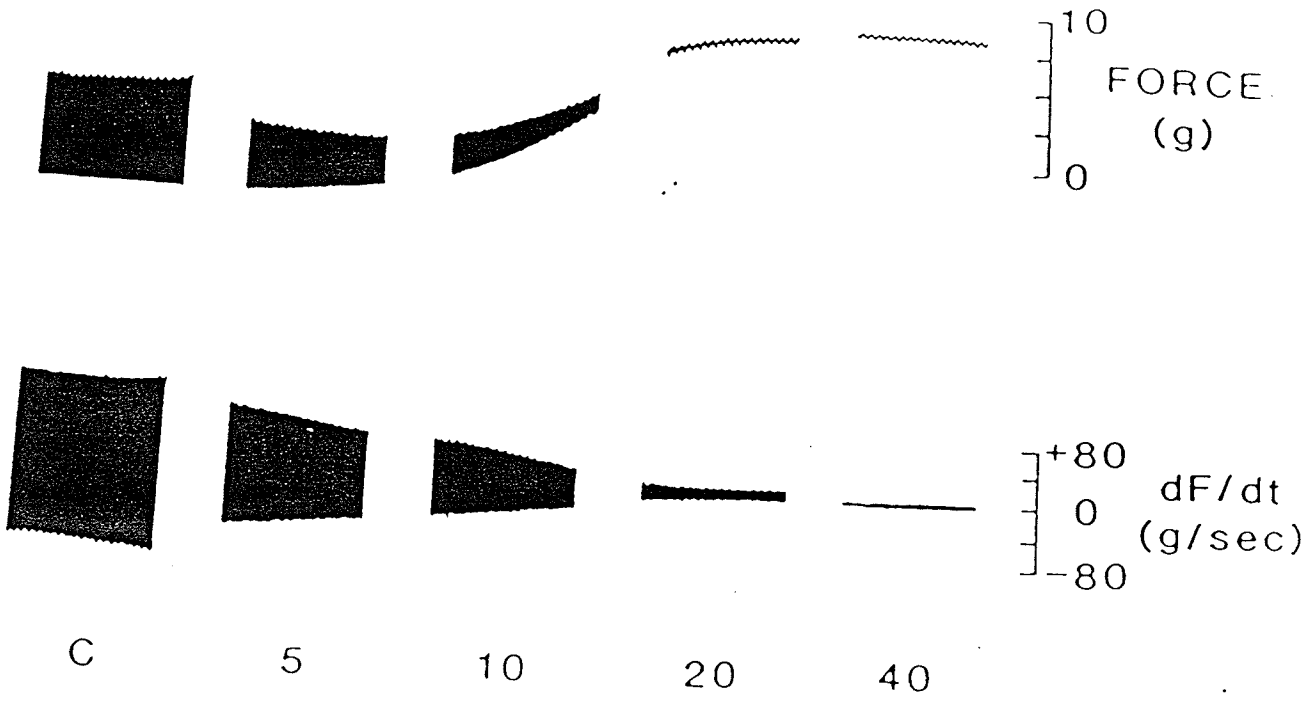


Figure 1: A typical recording showing effects of xanthine-xanthine oxidase (X-XO) on peak developed force (FORCE) and instantaneous maximal rate of force development and relaxation (dF/dt) in isolated perfused rat heart. C = before exposure to X-XO, 5, 10, 20 and 40 indicate time in min after perfusion with X-XO; paper speed = 0.1 mm/sec.

Table 1. Effect of xanthine-xanthine oxidase perfusion on rate of change of force (dF/dt) in isolated rat hearts perfused for different durations.

dF/dt (g/sec)	Control	Time (min)			
		5	10	20	40
positive	110.00 ± 14.8	68.58 ± 4.58*	34.22 ± 4.32*	5.33 ± 4.0*	0
negative	98.32 ± 8.9	63.22 ± 4.59*	40.18 ± 5.96*	10.32 ± 4.8*	0

Results are expressed as mean ± SEM of 8 experiments X-XO = xanthine (2 mM), xanthine oxidase (10 U/L). * Significantly different from control ($p < .01$; One Way ANOVA; Tukey's Test).

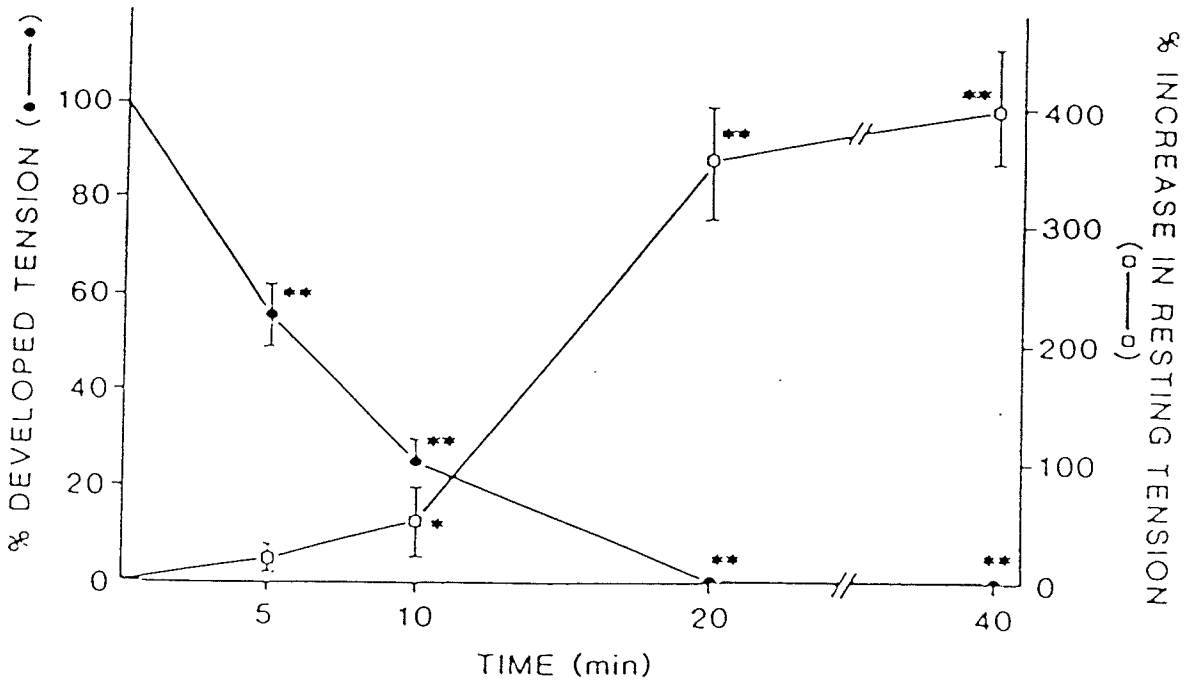


Figure 2: Effects of xanthine-xanthine oxidase (X-XO) on developed tension (●—●) and resting tension (□—□) in isolated perfused rat hearts. Values are % of control data and are expressed as mean ±SEM of 8 experiments. *p < .05, **p < .01 compared to zero min control data (one way ANOVA; Tukey's test). Basal values (pre X-XO) for developed tension and resting tension were 6.45 ± 1.01g and 2.2 ± 0.14g respectively.

did not show any change in these force parameters.

2. **Characterization of Radical Species in X-XO Medium:**

In order to confirm the presence as well as describe the nature of different oxygen metabolites, the effects of antioxidants and/or scavengers such as GSH, methionine, superoxide dismutase, catalase, and mannitol, on X-XO-induced contractile failure were examined (Figure 3 and Tables 2, 3 and 4). Superoxide dismutase (SOD) (1.2×10^5 U/L), catalase (2 and 4×10^4 U/L) and mannitol (10 and 20 mM) showed a protective effect against the X-XO-induced contractile failure and the rise in resting tension. SOD at the lower dose (3×10^4 U/L) had no protective effect (Figure 3) - if anything, there was a further decline in - dF/dt up to 8 min of perfusion with X-XO and SOD. Inclusion of GSH (200 uM and 2 mM) or methionine (1 and 20 mM) in KH medium containing X-XO also showed dose-dependent protective effects on developed tension (Table 3) and on the rate of development of force (+dF/dt) and on the rate of relaxation (-dF/dt) (Table 4). On the other hand, desferal (300 uM and 3mM), an iron chelator, did not show any protection against X-XO-induced contractile failure (Tables 3 and 4). In control experiments, hearts perfused with higher concentrations of SOD, catalase, mannitol, GSH, methionine or desferal alone did not show any change in developed force (data not shown).

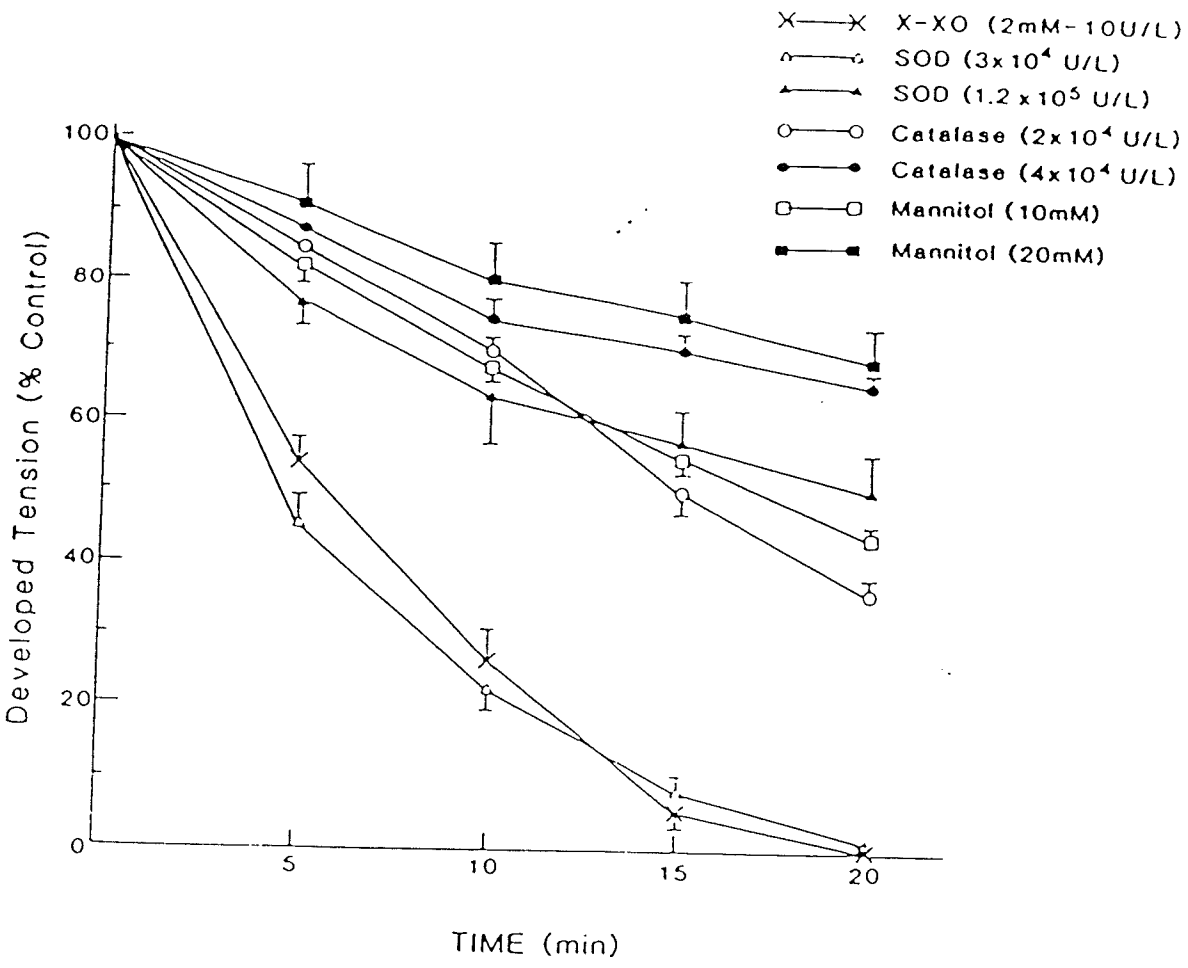


Figure 3: Effects of superoxide dismutase (SOD), catalase and mannitol on xanthine-xanthine oxidase induced contractile failure in isolated perfused rat hearts. Superoxide dismutase at a lower concentration had no protective effect but a significant protection was seen at higher concentration ($p < .05$). Both catalase and mannitol were protective at concentrations used ($p < .01$; One Way ANOVA; Tukey's Test). Values are % of control data and are expressed as mean $\pm$ SEM of 8 experiments.

Table 2. Effects of different radical scavengers on xanthine-xanthine oxidase (X-XO) induced changes in positive (+) and negative (-) dF/dt in isolated perfused rat hearts.

Agent	Concentration	Perfusion Time (min)									
		4		8		12		16		20	
		+	-	+	-	+	-	+	-	+	-
X-XO	2 mM; 10 U/L	50±6	51±5	27±4	23±4	12±3	10±2	5±1	0.5±0.5	0	0
X-XO + Superoxide dismutase	3×10^4 U/L	47±2	28±2*	22±1	9±2*	14±1	6±2	6±2	2±2	0	0
	1.2×10^5 U/L	84±2*	81±3*	77±5*	72±6*	71±7*	66±8*	68±8*	61±9*	58±9*	51±9*
X-XO + Mannitol	10 mM	81±1*	85±2*	68±3*	74±1*	64±1*	68±3*	55±1*	63±3*	52±3*	53±2*
	20 mM	93±3*	91±2*	91±5*	80±5*	85±6*	74±6*	76±13*	71±9*	69±12*	69±12*
X-XO + Catalase	2×10^4 U/L	90±2*	88±2*	78±4*	70±6*	57±2*	52±4*	43±3*	45±2*	32±1*	31±2*
	4×10^4 U/L	87±3*	77±7*	84±3*	63±3*	77±2*	62±4*	69±2*	54±6*	66±2*	52±6*

Values are mean ± SEM of 5 experiments expressed as percent of zero time control data.
* Significantly different ($p < 0.01$; Anova one way; Q test) from hearts perfused with Krebs Hanseleit solution containing X-XO. Both + and - dF/dt in the absence of X-XO were stable for more than one hour.

Table 3: Effects of various antioxidants on xanthine-xanthine oxidase (X-XO)-induced changes in peak developed force at different time intervals.

Agents	Concentration	4 min	8 min	12 min	16 min	20 min
X-XO	2 mM; 10 U/L	58.2 ± 6.4	29.8 ± 4.6	18.3 ± 3.6	8.7 ± 4.1	0
X-XO + GSH	200 uM	74.7 ± 3.3*	51.8 ± 4.6*	32.9 ± 2.1*	23.4 ± 3.3*	10.5 ± 1.8*
	2 mM	83.9 ± 3.1*	77.2 ± 3.3*	77.5 ± 2.8*	64.8 ± 2.7*	59.9 ± 2.7*
X-XO + Methionine	1 mM	80.0 ± 1.2*	56.9 ± 1.7*	43.7 ± 1.5*	23.2 ± 1.9*	15.7 ± 1.4*
	20 mM	70.5 ± 8.7*	65.6 ± 1.9*	65.6 ± 1.8*	63.8 ± 2.9*	62.5 ± 4.2*
X-XO + Desferal	300 uM	52.0 ± 2.9	20.2 ± 2.6	15.3 ± 2.2	6.9 ± 3.2	0
	3 mM	55.3 ± 4.6	26.2 ± 2.5	16.2 ± 1.6	8.3 ± 1.6	0

Values are mean ± SEM of 5 experiments expressed as zero time control data. * Significantly different ($p < .01$ ANOVA one way, Q test) from hearts perfused with Krebs Henseleit solution containing X-XO.

Table 4: Effects of different agents on xanthine - xanthine oxidase (X-XO) induced changes in positive (+) and negative (-) dF/dt in isolated perfused rat hearts.

Agents	Conc.	Rate of change in contractile force (dF/dt)									
		Perfusion time (min)									
		4		8		12		16		20	
		+	-	+	-	+	-	+	-	+	-
X-XO	2 mM, 10 U/L	50.0 ± 6.0	51.0 ± 5.0	27.0 ± 4.0	23.0 ± 4.0	12.0 ± 3.0	10.0 ± 2.0	5.0 ± 1.0	0.5 ± 0.5	0 0	0 0
X-XO + GSH	200 uM	77.0 ± 1.9*	48.7 ± 1.6	57.8 ± 3.9*	27.4 ± 2.3	35.4 ± 1.4*	21.1 ± 1.8*	21.6 ± 1.3*	16.2 ± 2.0*	9.03 ± 1.05*	14.6 ± 1.2*
	2 mM	77.2 ± 0.9*	73.8 ± 6.4*	71.6 ± 1.1*	60.4 ± 5.6*	65.4 ± 1.1*	50.5 ± 5.5*	54.4 ± 0.9*	44.2 ± 3.9*	42.6 ± 1.8*	40.2 ± 1.9*
X-XO + Meth	1 mM	88.4 ± 1.3*	62.7 ± 3.0*	71.5 ± 3.9*	42.7 ± 4.8*	49.6 ± 5.0*	30.5 ± 1.8*	23.0 ± 1.8*	20.2 ± 3.0*	14.0 ± 1.7*	17.2 ± 1.6*
	20 mM	85.2 ± 6.7*	86.6 ± 2.4*	83.1 ± 5.7*	62.5 ± 2.9*	79.6 ± 5.0*	41.4 ± 3.8*	76.6 ± 6.5*	41.3 ± 5.5*	75.8 ± 7.2*	34.9 ± 6.6*
X-XO + Desferal	300 uM	58.6 ± 9.7	39.2 ± 1.0	19.8 ± 1.9	20.4 ± 1.6	2.3 ± 1.5	12.2 ± 0.9	0	0	0	0
	3 mM	42.5 ±11.0	47.4 ± 7.2	28.7 ± 4.0	24.0 ± 3.5	12.3 ± 1.4	14.3 ± 4.1	1.7 ± 1.8	10.9 ± 5.9	0	0

Values are mean ± SEM of 5 experiments expressed as zero time control data. * Significantly different ($p < .01$; ANOVA one way; Q test) from hearts perfused with Krebs Henseleit solution containing x-xo. Meth. = Methionine. Conc. = Concentrations

3. **Lipid Peroxidation:**

Hearts perfused with normal KH buffer and KH buffer containing X-XO were examined for malondialdehyde MDA content, a relatively stable end-product of lipid peroxidation. X-XO perfusion for 5 min caused more than a 50% increase in MDA, while 10 min of perfusion resulted in almost a 90% increase in MDA content. At 20 and 40 min, the increase in MDA was 135% and 168% respectively. These changes in MDA content were significantly different from zero time control data (Figure 4). Since both developed force and MDA content showed time-dependent changes due to X-XO, a Scatchard analysis of correlation between percent decline in force and percent increase in MDA content was done. A signficant ($p<.001$) positive correlation ($r = 0.935$) was noted between these two variables (Figure 5).

4. **Myocardial High Energy Phosphates and their Metabolites:**

Analysis of cardiac high energy phosphates and their metabolite levels was performed using high performance liquid chromatography. Assays of cardiac tissue extracts from at least 6 hearts for each data point were performed and compared with known standards (Figure 6).

The characteristic response of myocardial adenine nucleotide and creatine compounds to X-XO perfusion for different times is shown in Table 5. Perfusion with X-XO for up to 20 min caused a progressive depletion of ATP, CP and

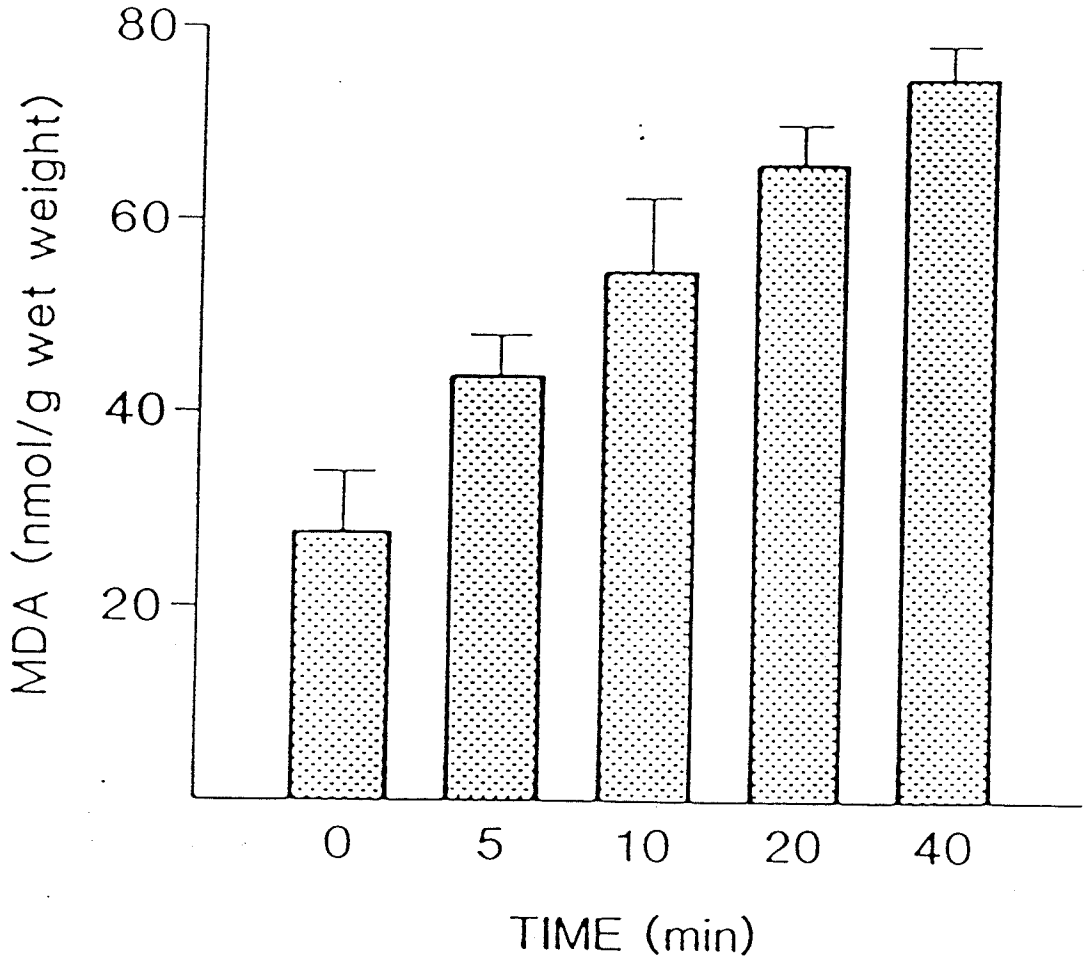


Figure 4: Time-related increase in malondialdehyde (MDA) content of hearts exposed to xanthine-xanthine oxidase for increasing time intervals. Zero min data represent the MDA content of heart perfused with control buffer for 40 min. Each bar indicates the mean $\pm$ SEM of 6 hearts and is significantly different from zero min data ($p < .05$).

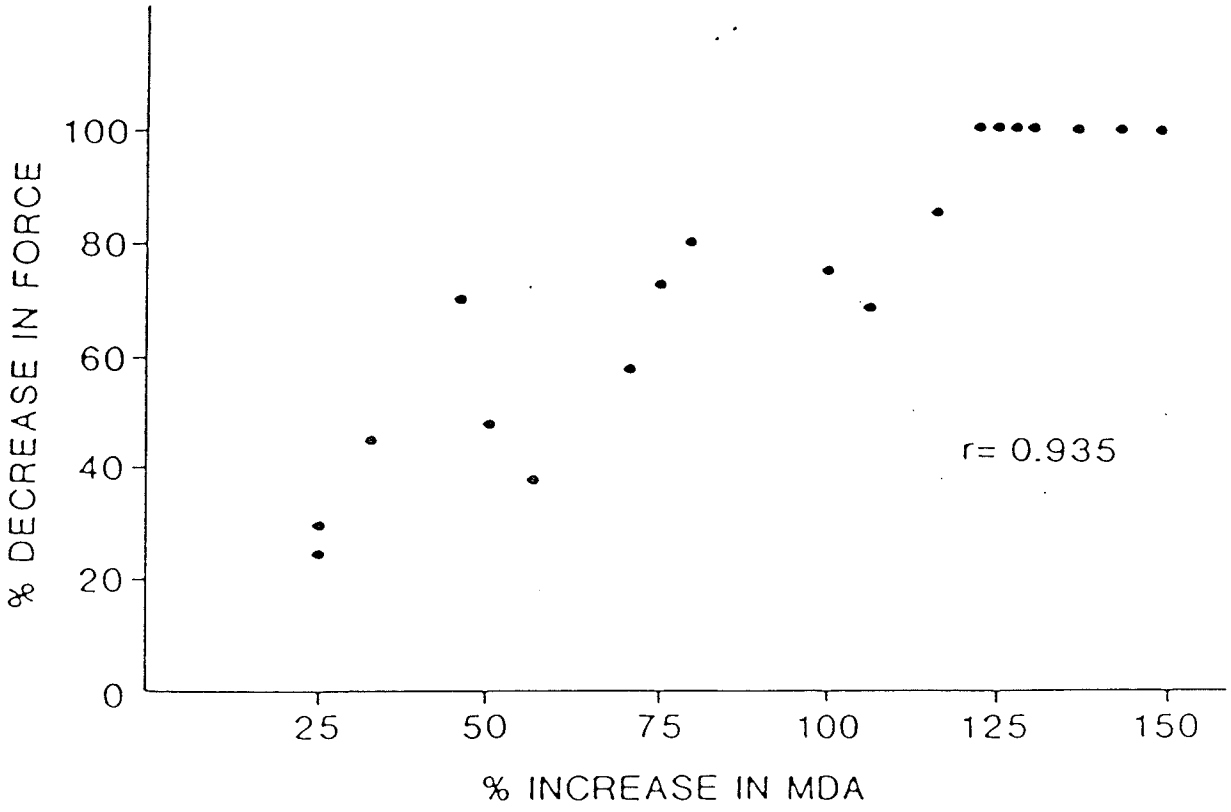


Figure 5: Contractile failure and malondialdehyde (MDA) content following exposure to X-XO. A correlation coefficient value of 0.935 was obtained between these two variables. $p<.001$; r^2(coefficient of determination, an index of linearity) = 0.874

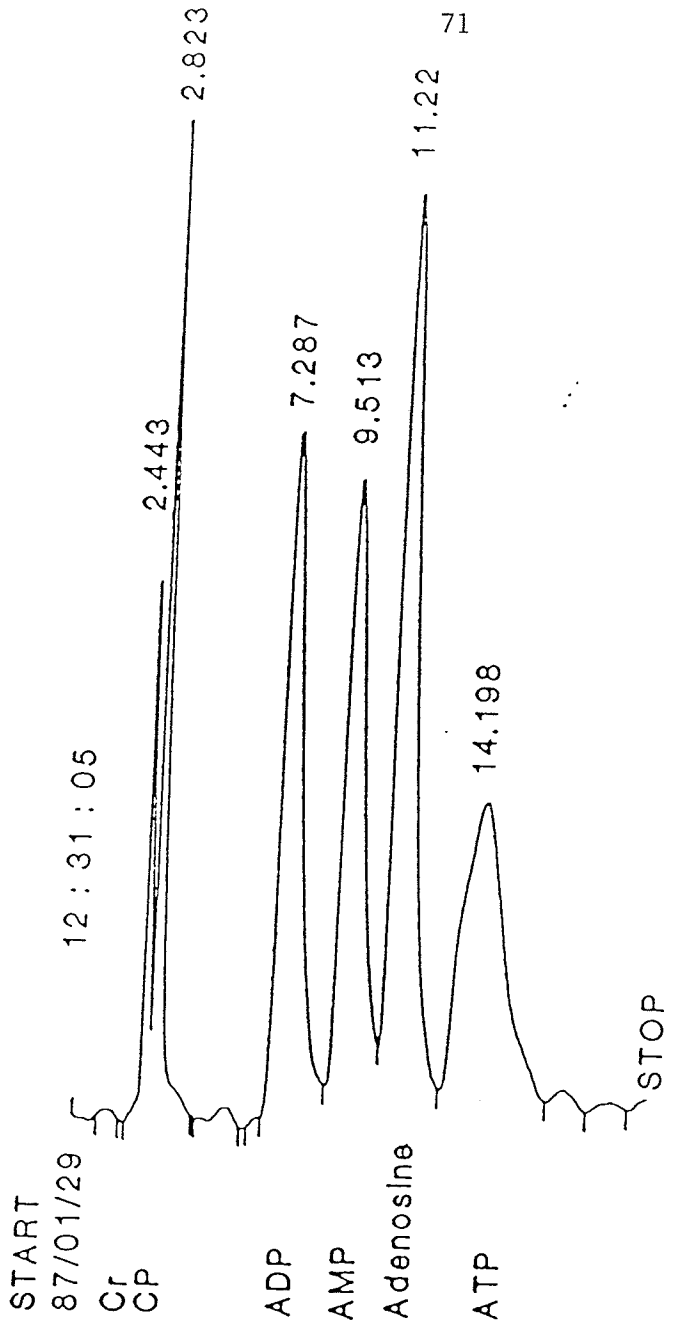


Figure 6: HPLC analysis of a standard mixture (1 nmole) of creatine (Cr), creatine phosphate (CP), adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine and adenosine triphosphate (ATP). Retention times (in min) are shown with the respective peaks. Areas under individual peaks represent the quantity of the constituents.

Table 5. Effects of X-XO perfusion for different durations on high energy phosphates in isolated rat hearts.

	umol/g dry weight				
	Control	5 min	10 min	20 min	40 min
THEP	64.90 ± 8.20	44.25 ± 4.90*	35.95 ± 1.80**	20.10 ± 4.90**	18.50 ± 1.80**
CP	42.50 ± 6.65	28.65 ± 3.30*	24.70 ± 1.45*	13.35 ± 5.00**	12.50 ± 2.30**
ATP	22.40 ± 3.25	15.60 ± 1.80*	11.25 ± 0.70**	6.75 ± 1.95**	6.00 ± 0.30**
ADP	5.05 ± 0.30	6.20 ± 0.65*	5.05 ± 0.20	3.85 ± 0.32*	3.25 ± 0.22**
AMP	0.96 ± 0.46	1.20 ± 0.16*	1.32 ± 0.10*	1.50 ± 0.14**	1.58 ± 0.14**
Adenosine	0.12 ± 0.01	0.14 ± 0.02	0.24 ± 0.03*	0.27 ± 0.04*	0.32 ± 0.01**
TAd	28.40 ± 2.90	23.00 ± 2.50*	17.62 ± 0.80*	12.10 ± 1.81**	10.83 ± 0.46**
Adenylate charge	0.88 ± 0.02	0.81 ± 0.01	0.78 ± 0.01*	0.71 ± 0.02*	0.70 ± 0.03*
Creatine	49.75 ± 4.55	56.00 ± 6.55	62.50 ± 3.30*	73.00 ± 9.50*	80.25 ± 4.80**
TCr	92.25 ± 10.20	84.65 ± 9.80	87.20 ± 4.75	86.35 ± 14.34	86.25 ± 4.90

Results are expressed as Mean ± SEM of 6 hearts. * $p < .05$. ** $p < .01$ compared to control value (ANOVA One Way, Tukey's Test for multiple comparison). THEP = Total High Energy Phosphates (ATP + CP); TAd = Total nucleotide pool (ATP + ADP + AMP); Adenylate charge = (ATP + 0.5 ADP/TAd); TCr = Total Creatine (CP + creatine).

total high energy phosphates (THEP). Continued perfusion with X-XO for up to 40 min did not result in any further decline in ATP, CP or THEP contents of the myocardium. ADP content increased (+ 22%) during the first 5 min of perfusion with X-XO followed by a significant decline with continued exposure to X-XO. Concentrations of AMP and adenosine increased significantly up to 20 min with no further change with continued perfusion with X-XO for 40 min. The total adenine nucleotide pool (TAd) showed a significant decline between 5-40 min perfusion with X-XO. Creatine (Cr) was increased at 10, 20 and 40 min of perfusion with X-XO while total creatine content (TCr=Cr + CP) did not change. Adenylate charge, defined as: ATP + 0.5 ADP/TAd (Atkinson, 1968), was decreased at 10, 20 and 40 min of perfusion with X-XO. Since the processes of contraction and relaxation are dependent upon availability of high energy phosphates, developed tension was also examined in relation to changes in ATP content at different levels of contractile failure. A good correlation (r=0.819) between developed force and ATP content of myocardium was seen (Figure 7).

5. **Ultrastructural Changes:**

Hearts perfused with control KH buffer for one hour showed normal ultrastructure including compact myocytes free of edema, myofibrils in register having typical appearance of A and I bands and normal T-tubules. Mitochondria in these control hearts were generally normal with moderately dense

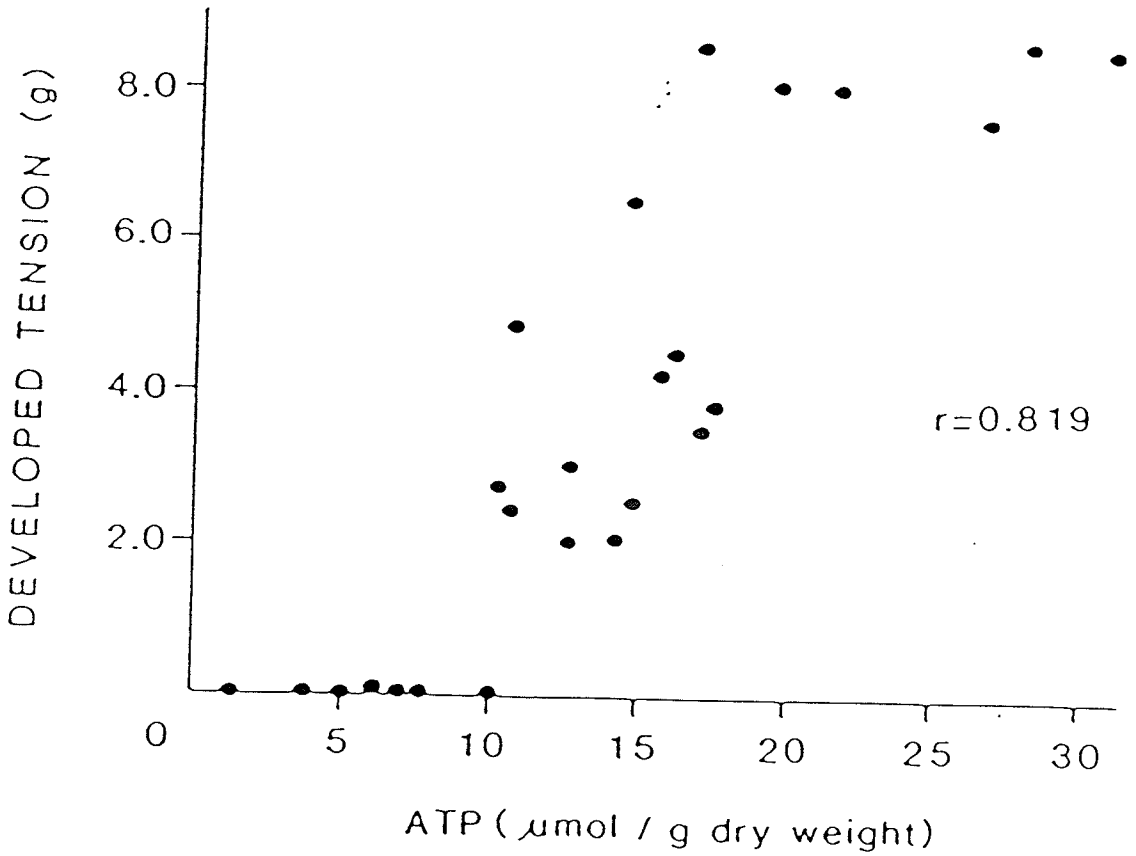
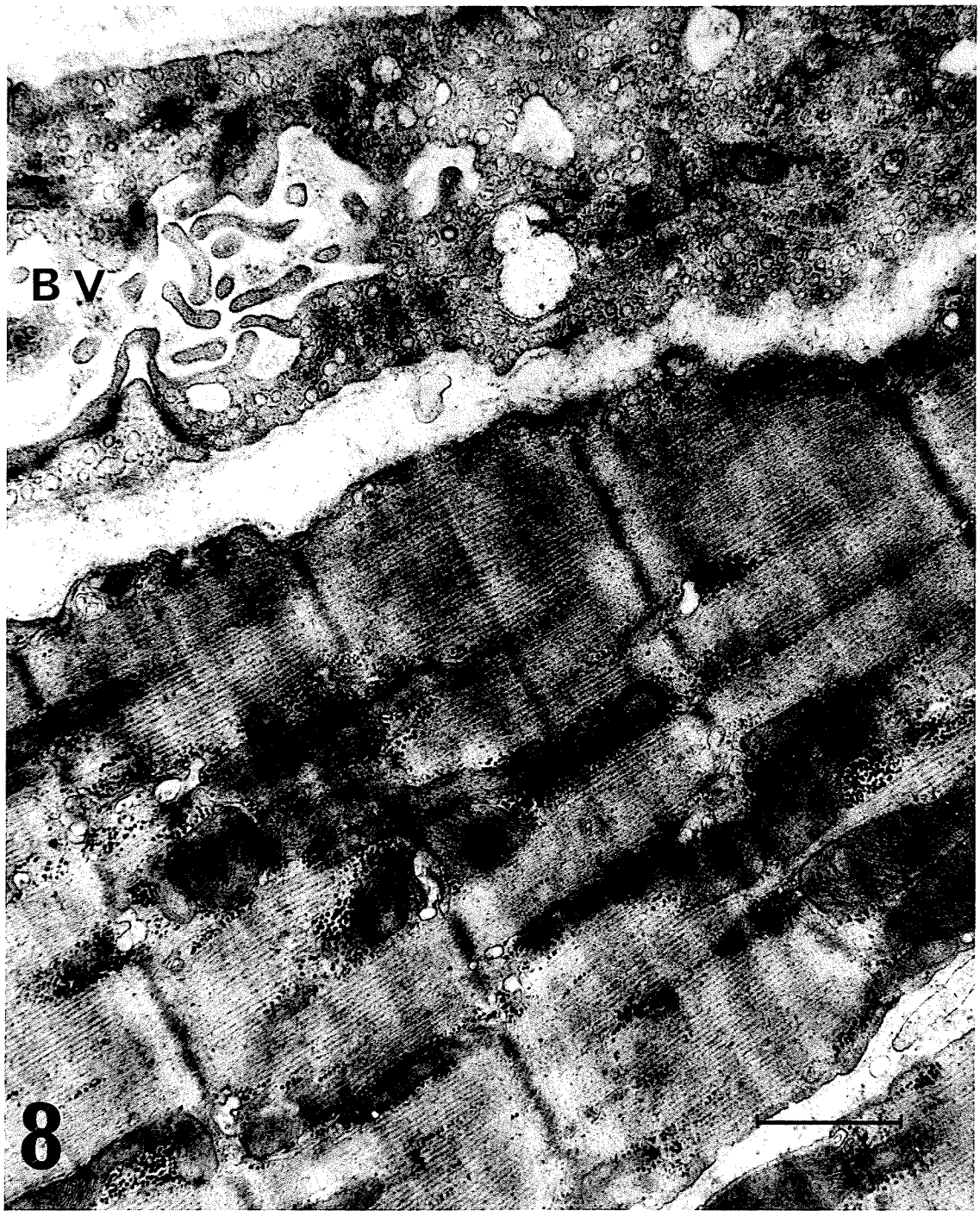


Figure 7: Demonstration of relationship between contractile force and ATP content of the myocardium following exposure to X-XO (0-40 min). A correlation coefficient value of 0.819 was obtained between these two variables. $p < .001$.

matrix (Figure 8). Endothelial cells of the vasculature were normal and showed cytoplasmic processes as well as small vesicles.

Qualitative ultrastructural changes seen at 5, 10, 20 and 40 min of X-XO perfussion are shown in Figures 9, 10, 11 and 12, respectively. At 5 min of X-XO perfusion, swelling of some of the mitochondria was apparent, while myofibrils, sarcoplasmic reticulum and nucleus appeared normal (Figure 9). At 10 min of X-XO perfusion, cells were edematous, showed disruption of the sarcoplasmic reticulum (SR) system and vacuolisation (Figure 10). Damage to the myofibrils in these hearts was minimal and the intercalated discs were intact. After 20 min of perfusion with X-XO most of the mitochondria exhibited marked swelling and loss of cristae and myofibrils were disrupted (Figure 11). At 40 min of perfusion, damage involved almost every organelle of the cell (Figure 12). Mitochondria appeared swollen and showed loss of cristae membranes, myofibrils showed loss of myofilaments; however, cell to cell contact in the intercalated disc was maintained even after 40 min of perfusion with X-XO. Because of a large variability in the type as well as extent of injury to the subcellular elements, a semiquantitative morphometric analysis of cell injury was also done. These data are shown in Figure 13 and Table 6. A small percentage of cells in normal hearts perfused with KH buffer was found to be abnormal because of sporadic mitochondrial swelling as well as vacuolisation in some endothelial cells (Table 6 and Figure 13).

Figure 8: Electronmicrograph demonstrating the ultrastructure of control myocardium. Note normal appearance of mitochondria, tubular system and sarcomeres. BV, blood vessel with endothelial cells showing cytoplasmic processes as well as pinocytotic vesicles. The magnification line represents 1 um.


BV
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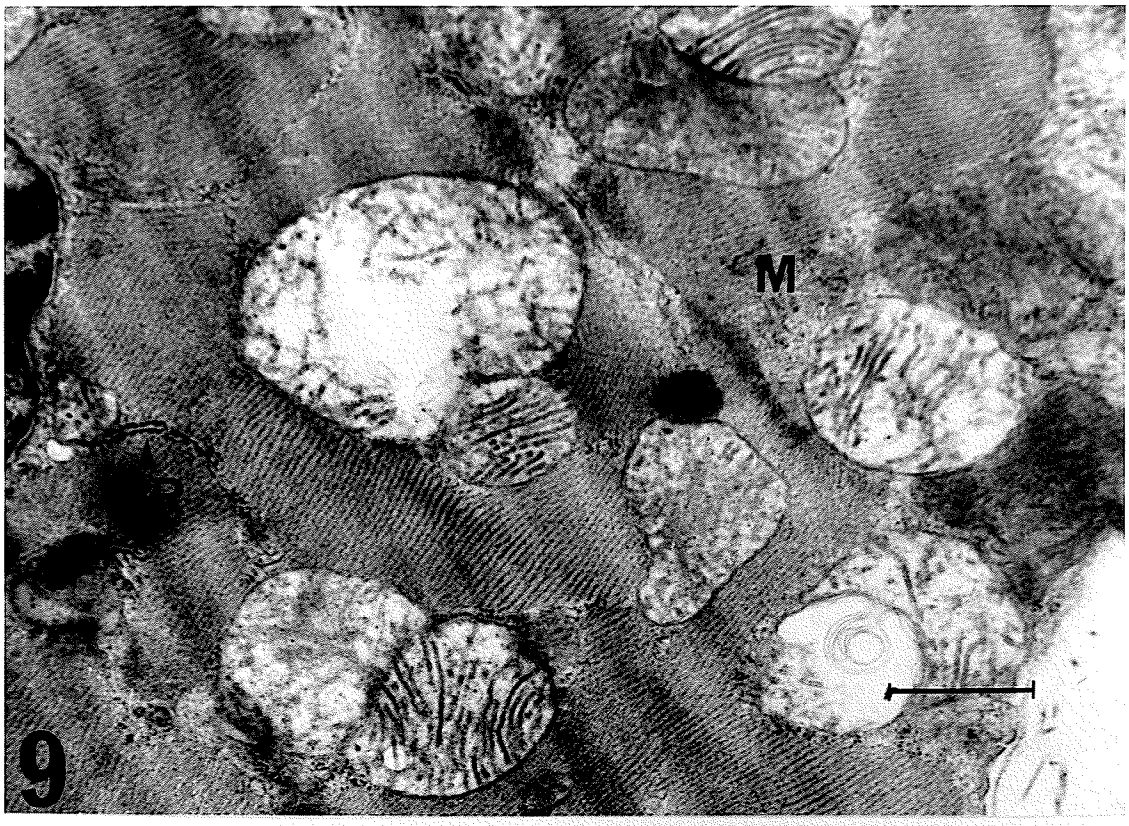
Figures 9 - 12: Qualitative ultrastructural changes due to xanthine-xanthine oxidase in an isolated perfused rat heart at different perfusion times. The magnification line in all micrographs represents one micron.

Figure 9: Electronmicrograph illustrates the maximum damage seen in only a limited number of mitochondria after 5 min of perfusion with xanthine-xanthine oxidase. Mitochondrial damage seen in this micrograph was present only in small percentage of cells examined (Table 6). Sarcoplasmic reticulum (SR) and myofibrils (M) are normal. No interstitial edema is evident.

Figure 10: Electronmicrograph shows the damage seen after 10 min of perfusion with xanthine-xanthine oxidase. The sarcoplasmic reticulum is damaged, interstitial edema between the myofibrils is evident (★) and mitochondria are swollen.


M
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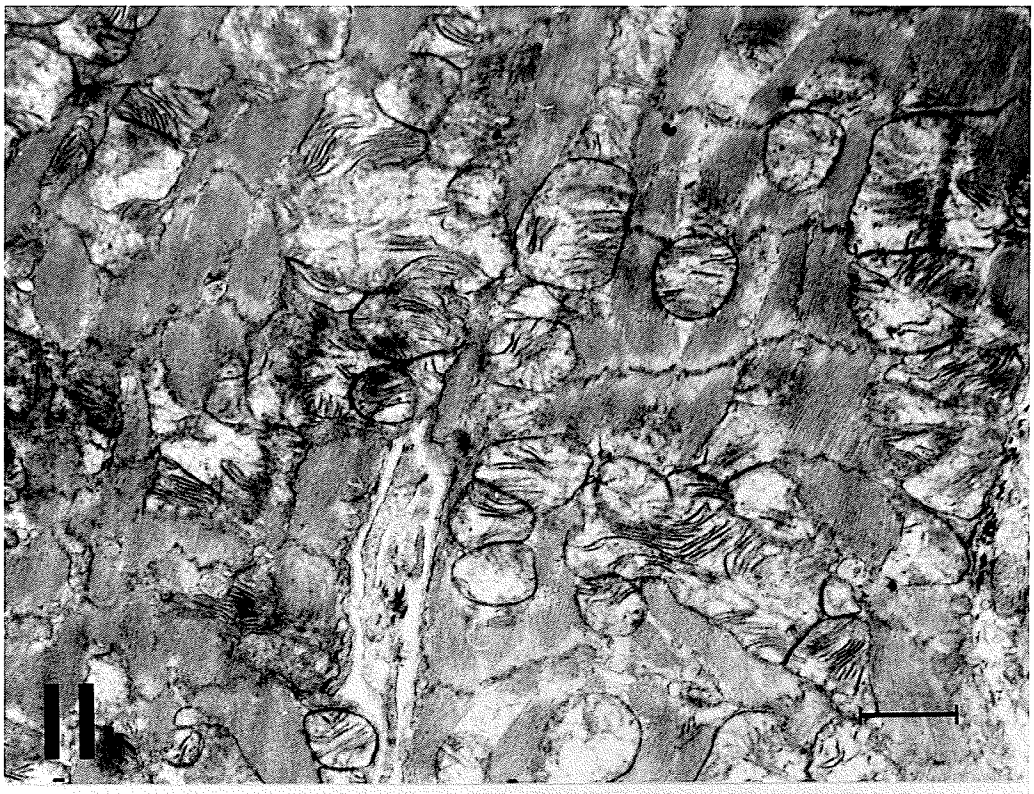
d

Figures 9 - 12 cont:

Figure 11: Electronmicrograph illustrates damage observed after 20 min of perfusion with xanthine-xanthine oxidase. Mitochondrial damage has progressed further as indicated by loss of cristae membrane and clearing of matrix in majority of mitochondria. The sarcoplasmic reticulum is damaged and myofibrils are in disarray.

Figure 12: Electronmicrograph shows advanced damage following 40 min of perfusion with xanthine-xanthine oxidase. Mitochondria are swollen and clearing of the matrix is frequent. The intercalated disc (ID) seen in this and other micrographs was always normal in appearance with respect to electron density and intercellular gap. L, lysosome.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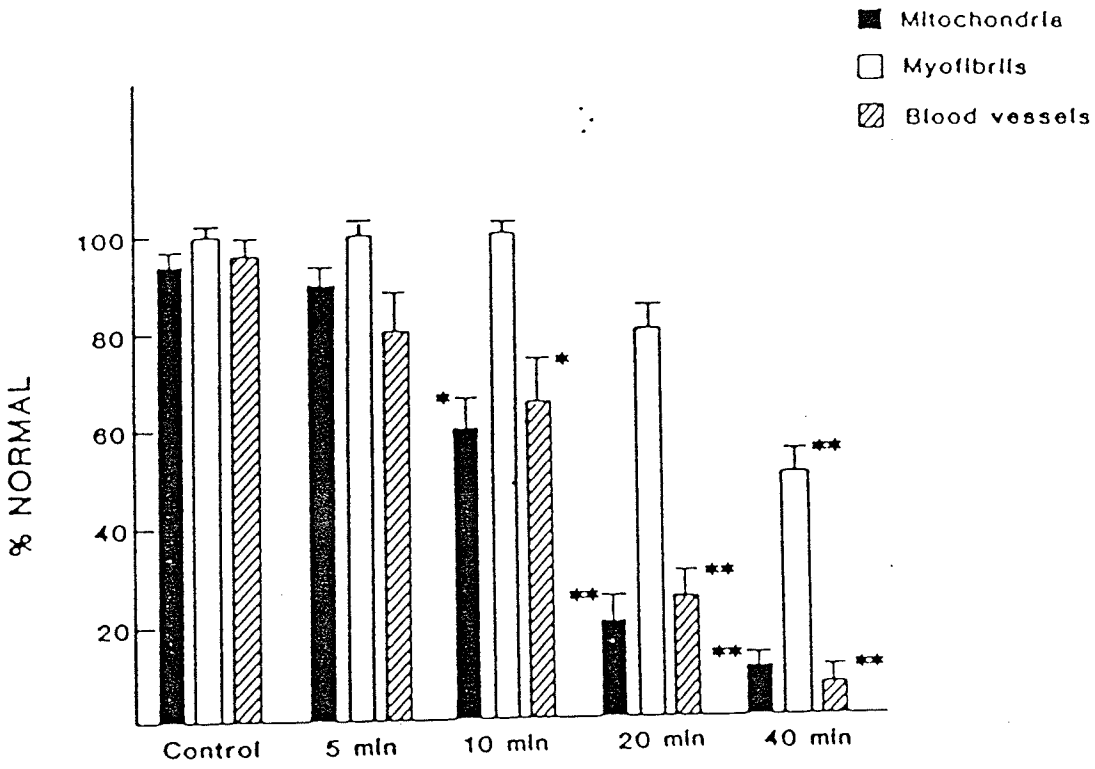


Figure 13: Semiquantitative analysis of myocardial injury due to xanthine-xanthine oxidase (X-XO). Percentages of normal and abnormal mitochondria, myofibrils and blood vessels at different times of perfusion with X-XO were counted. For each point, a minimum of 25 cells and 50 blood vessel cross-sections were examined from four hearts. * $p < .05$ compared to control; ** $p < .01$ compared to control value.

Table 6: Ultrastructural damage to the rat heart perfused with xanthine-xanthine oxidase for different time intervals.

Group	% Myocytes			
	Normal	Mild	Moderate	Severe
Control	92.0 ± 2.3	6.0 ± 5.0	2.0 ± 2.0	0
5 min	77.0 ± 5.0	18.0 ± 4.8*	5.0 ± 3.0	0
10 min	21.2 ± 3.8*	42.4 ± 4.2*	30.3 ± 2.8*	6.1 ± 2.5
20 min	8.5 ± 2.3*	25.5 ± 6.0*	38.3 ± 4.6*	27.6 ± 5.2*
40 min	3.4 ± 1.2*	17.2 ± 2.3*	24.1 ± 2.6*	55.2 ± 2.5*

Data expressed as mean ± SEM (n=4).* $p < .01$ compared to control value (Krauskal-Wallis Test; Wilcoxon Test).

Perfusion with X-XO for 5 min resulted in mild to moderate injury in 23% of myocytes. However, X-XO perfusion for 10 min and longer resulted in injury to a majority of cells with a progressive increase in the number of cells showing moderate to severe injury (Table 6). Examination and counting of different cell elements revealed that this shift towards severe damage followed injury to the mitochondria and blood vessels (Figure 13) accompanied by other ultrastructural changes.

Hearts were also perfusion-fixed in the presence of lanthanum, an extracellular marker, to test for any gross permeability changes. This electron-dense tracer was restricted to extracellular spaces in hearts perfused with control medium (data not shown). Hearts exposed to X-XO medium-fixed at 20 min showed intracytoplasmic localization of lanthanum (Figure 14). This tracer was specifically localized in the perimitochondrial region.

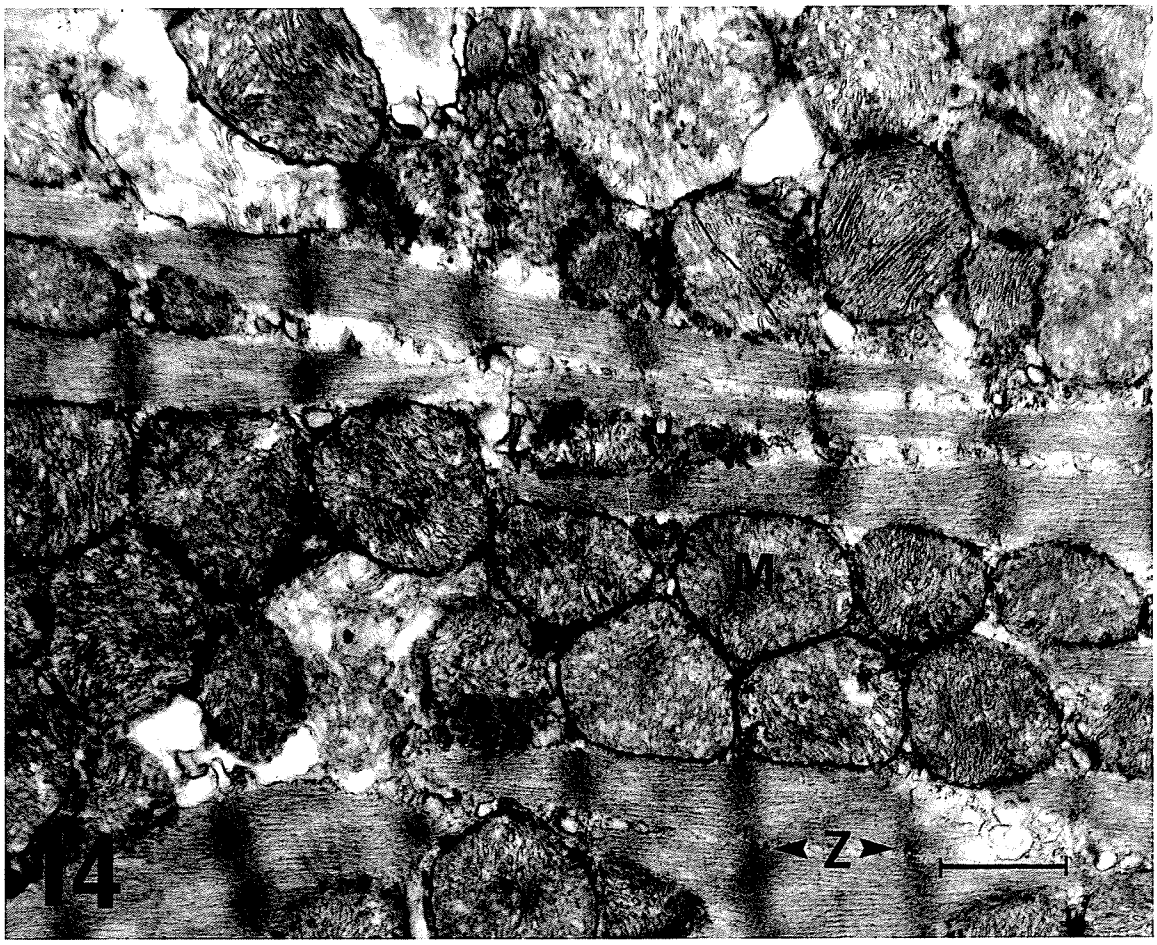
B. MYOCARDIAL EFFECTS OF OXYGEN RADICALS IN HYPERTROPHIED HEARTS

1. Characterization of the Hypertrophy Model:

a) General Observations

In this study, following 6, 12, 24 and 48 weeks of surgery, all rats in control and experimental groups remained in good condition and showed no apparent difference in their daily eating or drinking patterns. Throughout the post-

Figure 14: Electron micrograph from xanthine-xanthine oxidase-perfused heart. Lanthanum, an extracellular tracer, is seen intracellularly and around swollen mitochondria (M). Contracture of myofibrils is indicated by a reduced gap between Z lines (Z) which appear fuzzy. Magnification line indicates 1 um.


M
Z
914

operative period, both control and experimental animals showed weight gain for up to 12 weeks and thereafter not much change in their body weight was observed. Banded animals showed no change in their body weight compared to their respective sham-controls (Table 7). The circumference of the aortic lumen at the banded site in control at 6W and 48W duration was 5.0 $\pm$ 0.2 and 8.2 $\pm$ 0.3 mm respectively while in banded rats at 6W, it was 3.7 $\pm$ 0.25 mm and at 48W it was 3.75 $\pm$ 0.5 mm. Thus, the degree of aortic constriction at 6W duration was 26 $\pm$ 2% which gradually progressed to 54.26 $\pm$ 1.8% at 48 weeks duration. In pressure-overloaded animals, the increase in left ventricular weight was in the range of 23 - 31% following different durations of surgery and the increase in left ventricle/body weight ratios was also in a similar range. An increase in the heart weight to body weight ratio has been shown to indicate the presence of cardiac hypertrophy (Hamrell and Alpert, 1977; Lecarpentier et al, 1982). Increased rate of protein synthesis during developing hypertrophy appears to be related primarily to an increase in the number of myocardial ribosomes (Rabinowitz and Zak, 1972). Increased RNA content per gram of heart as well as increased total cardiac RNA content reflect the increased number of ribosomes and this has been observed in cardiac growth induced by a variety of experimental procedures (Grimm et al, 1966; Korecky and French, 1967; Meerson, 1969 b). In the present investigation, an increase

Table 7: Left ventricular weight, body weight and myocardial RNA content in rats from control and hypertrophied groups.

Groups	Left ventricle weight (mg)	Body weight (g)	Left ventricle/ Body weight (mg/g)	Total RNA Content (mg)
6 WS	804 ± 18.5 (12)	437 ± 7.9 (12)	1.83 ± 0.04 (12)	1.79 ± 0.09 (6)
6 WH	1010 ± 23.4** (12)	445 ± 6.9 (12)	2.27 ± 0.04** (12)	3.09 ± 0.08** (6)
12 WS	904 ± 29.7 (7)	533 ± 9.04 (11)	1.68 ± 0.04 (11)	1.82 ± 0.12 (6)
12 WH	1112 ± 39.5** (11)	525 ± 12.0 (11)	2.16 ± 0.08** (11)	2.45 ± 0.08* (6)
24 WS	1010 ± 46.5 (6)	568 ± 33.2 (6)	1.76 ± 0.06 (6)	2.02 ± 0.08 (5)
24 WH	1328 ± 53.0* (6)	579 ± 15.3 (6)	2.16 ± 0.06* (6)	2.63 ± 0.11* (5)
48 WS	1026 ± 38.4 (7)	607 ± 23.6 (7)	1.68 ± 0.03 (7)	2.16 ± 0.09 (5)
48 WH	1262 ± 32.1** (7)	580 ± 28.2 (7)	2.17 ± 0.04** (7)	2.53 ± 0.16* (5)

Data are expressed as mean ± SEM with no. of experiments in parenthesis. Each hypertrophied group is compared with its respective sham control (ANOVA; Unpaired 't' test). * $p < .01$, ** $p < .001$. W = weeks post-operation. S = Sham, H = Hypertrophy.

Table 8: Comparison of wet weight, dry weight and wet/dry weight ratios of liver and lung of sham operated and aortic constricted rats at different durations following the surgical procedure.

	Liver		Lung		Wet wt/Dry wt	
	Wet wt (g)	Dry wt (g)	Wet wt (g)	Dry wt (g)	Liver	Lung
6 WS	12.64 ± 0.50	3.95 ± 0.13	1.77 ± 0.06	0.31 ± 0.01	3.20 ± 0.32	5.71 ± 0.18
6 WH	12.81 ± 0.20	3.80 ± 0.14	1.80 ± 0.05	0.33 ± 0.02	3.36 ± 0.04	5.40 ± 0.20
12 WS	15.80 ± 0.3	4.52 ± 0.20	2.12 ± 0.03	0.42 ± 0.03	3.49 ± 0.03	5.00 ± 0.16
12 WH	15.70 ± 0.40	4.60 ± 0.16	2.20 ± 0.02	0.43 ± 0.02	3.41 ± 0.04	5.11 ± 0.12
24 WS	17.90 ± 1.02	5.45 ± 0.35	2.33 ± 0.24	0.48 ± 0.04	3.28 ± 0.03	4.77 ± 0.16
24 WH	19.89 ± 1.28	6.04 ± 0.39	2.80 ± 0.10	0.60 ± 0.03	3.29 ± 0.02	4.68 ± 0.17
48 WS	16.20 ± 1.60	4.89 ± 0.50	3.94 ± 0.35	0.86 ± 0.10	3.20 ± 0.04	4.60 ± 0.17
48 WH	18.37 ± 0.90	5.51 ± 0.22	3.89 ± 0.19	0.87 ± 0.05	3.32 ± 0.06	4.49 ± 0.18

Data are expressed as mean ± SEM of 6 experiments; Symbols are the same as in Table 7.

of 72.6 ± 4.2% was observed in total RNA content of LV in the 6 week hypertrophied group (Table 7), while total RNA content of the remaining groups of hypertrophied hearts showed an approximately 25% increase. Therefore, our experimental model does reflect an increase in protein synthesis in these hearts.

Animals with left ventricular hypertrophy did not show ascites, pleural effusion or any changes in dry to wet weight ratios for lungs or liver (Table 8).

b. **Hemodynamic Assessment**

In order to gain some information regarding the functional status of experimental animals following 6, 12, 24 and 48 weeks of pressure overload, sham-control as well as hypertrophy animals were examined for left ventricular function (LVSP, LVEDP, ±dP/dt), aortic pressures, electrocardiographic changes and heart rate. Changes in these parameters in experimental animals were compared with their respective sham-controls (Table 9). Typical recordings of these parameters in sham-control, 6 week hypertrophy and 48 week hypertrophy animals are shown in Figure 11. Electrocardiographic changes revealed an increase in QRS amplitude and occasionally ST depression in standard Limb lead II ECG (Figure 15), further suggesting the presence of hypertrophy in experimental animals. Pressure overload for different durations resulted in a 24 - 30% increase in left

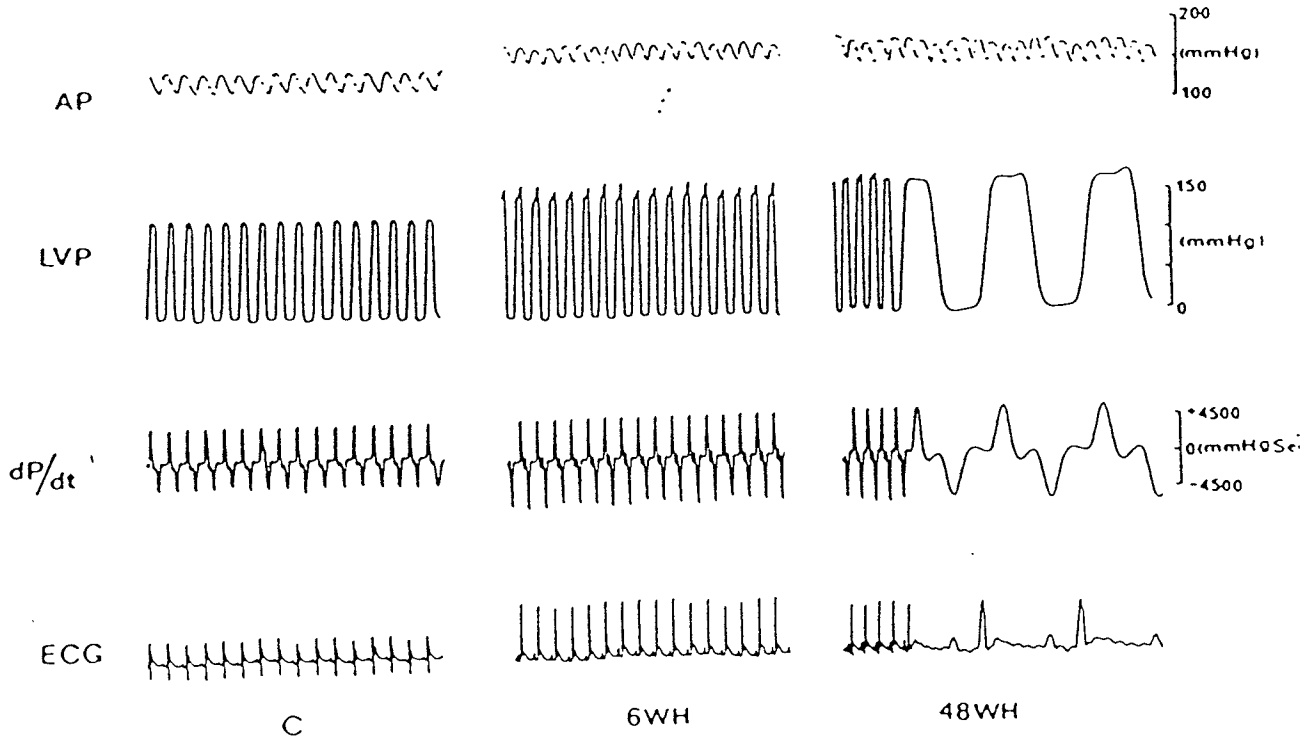


Figure 15: Comparison of systolic and diastolic left ventricular pressures (LVP) and their rate of rise (+ dP/dt) and fall (- dP/dt), aortic pressures (AP) and electro-cardiographic recordings in anaesthetised sham-control (C), 6 (6WH) and 48 (48WH) week hypertrophy rats.

Table 9: Hemodynamic studies on sham control and hypertrophied hearts subjected to pressure overload for different durations.

Groups	ASP (mmHg)	ADP (mmHg)	LVSP (mmHg)	LVEDP (mmHg)	+dP/dt ($mmHgsec^{-1}$)	-dP/dt ($mmHgsec^{-1}$)	Heart rate (beats/min)
6 WS (6)	136.4 ± 2.9	113.4 ± 3.1	138.0 ± 2.9	3.91 ± 0.30	4945 ± 180	4558 ± 185	340 ± 8
6 WH (7)	170.8 ± 8.9*	131.6 ± 4.7*	170.8 ± 8.9*	3.74 ± 0.89	5629 ± 188*	5514 ± 385*	360 ± 10
12 WS (7)	134.6 ± 4.0	113.0 ± 3.0	133.5 ± 4.6	2.75 ± 0.20	5207 ± 142	5221 ± 306	360 ± 10
12 WH (7)	168.0 ± 5.2**	130.8 ± 5.0*	170.8 ± 4.6**	2.83 ± 0.45	5871 ± 268*	6143 ± 420*	397 ± 13
24 WS (8)	138.0 ± 6.8	107.0 ± 5.3	135.0 ± 5.5	3.26 ± 0.59	4541 ± 202	4133 ± 180	323 ± 14
24 WH (8)	175.5 ± 4.6**	134.5 ± 5.7*	177.9 ± 3.8**	3.42 ± 0.48	6335 ± 257**	5735 ± 316**	356 ± 17
48 WS (7)	130.0 ± 6.2	115.0 ± 2.8	138.0 ± 5.8	3.82 ± 0.80	5067 ± 130	4922 ± 310	341 ± 15
48 WH (7)	168.0 ± 3.2**	138.0 ± 5.2*	177.0 ± 4.8**	3.75 ± 0.90	5810 ± 300*	5910 ± 300*	348 ± 12

Data are expressed as mean ± SEM with no. of experiments in parenthesis. S = sham control; H = Hypertrophy. W = weeks post-operation. Hypertrophied groups are compared with their respective sham control (ANOVA; unpaired 't' test). * $p < .05$, ** $p < .001$. ASP = Aortic Systolic Pressure; ADP = Aortic Diastolic Pressure, LVSP = Left Ventricle Systolic Pressure, LVEDP = Left Ventricle End Diastolic Pressure, dP/dt = Rate of change of left ventricular pressure.

ventricular systolic pressure, an increase in +dP/dt and -dP/dt while LVEDP showed no change. Aortic systolic pressure also showed a similar degree of increase as LVSP while aortic diastolic pressure showed a 15 - 25% increase in hearts subjected to different durations of pressure overload as compared to their respective sham-controls. Heart rate in different groups of hypertrophied animals was not significantly different from their sham-controls. There was no difference in these parameters when sham-controls were compared with each other.

2. RESPONSE OF HYPERTROPHIED HEARTS TO OXIDATIVE STRESS

Oxidative stress was induced by perfusing the hearts with xanthine-xanthine oxidase (X-XO) in an isolated Langendorff's perfusion set up. Following a 15 min equilibration period, the responses of hypertrophied hearts to X-XO perfusion were examined by comparing contractile function (developed tension, resting tension, $\pm$ dF/dt), lipid peroxidation, high energy phosphates and ultrastructural changes in control and experimental hearts.

Following 6, 12, 24, and 48 weeks of surgery, sham-control and hypertrophied hearts were exposed to X-XO. Complete contractile failure was seen after about 20 min in all of the sham control and hypertrophied hearts, with the exception of 6 week hypertrophied hearts which showed failure after 24 min. Typical recordings of changes in developed tension and $\pm$ dF/dt in control, 6W and 48W hypertrophied

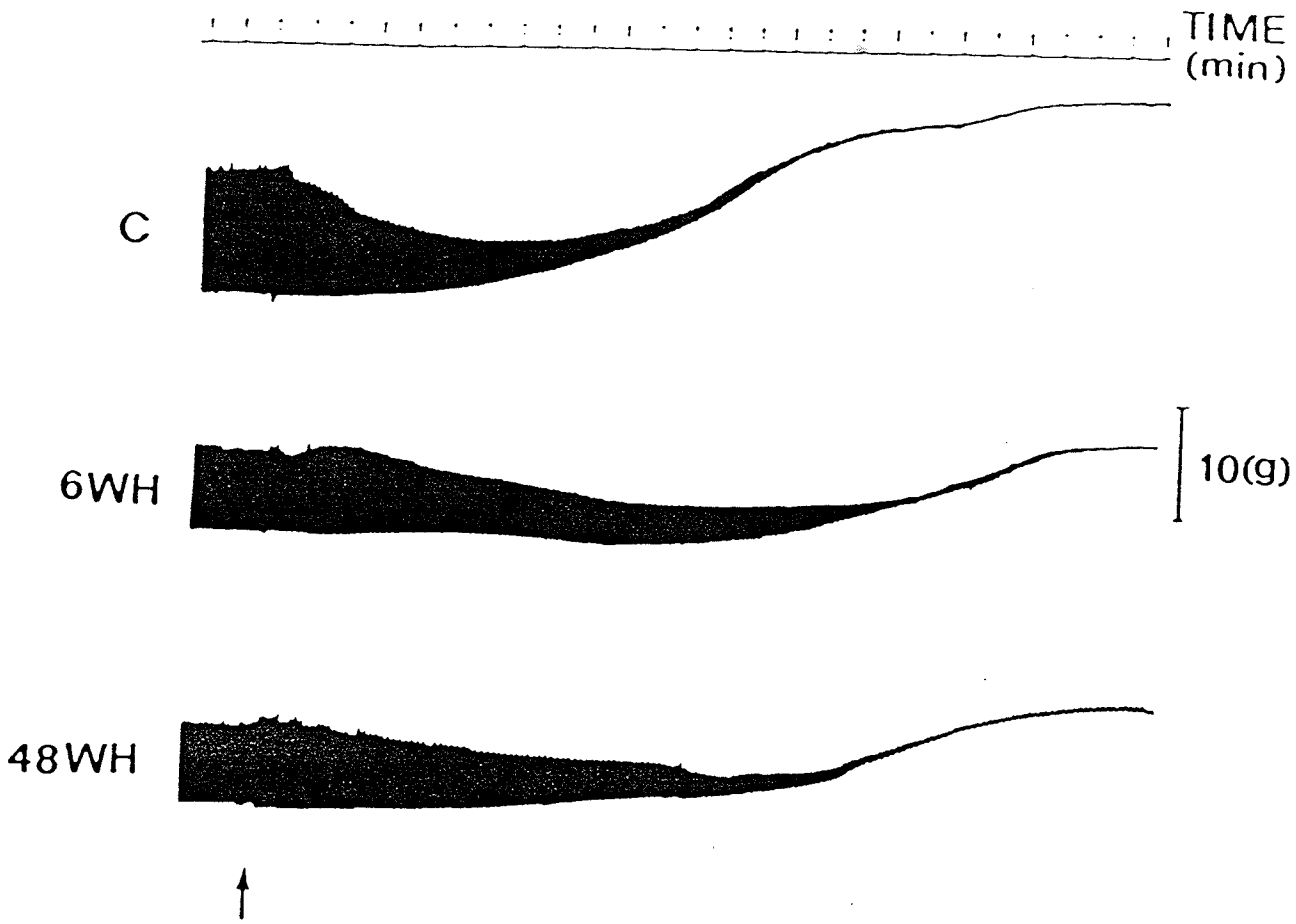


Figure 16: Typical polygraph recordings showing effects of xanthine-xanthine oxidase perfusion on peak developed force in control (C), 6 week hypertrophied (6WH) and 48 week hypertrophied (48WH) hearts. Note the greater force retention in hypertrophied hearts. The arrow indicates time of introduction of oxidative stress. Paper speed is shown on top.

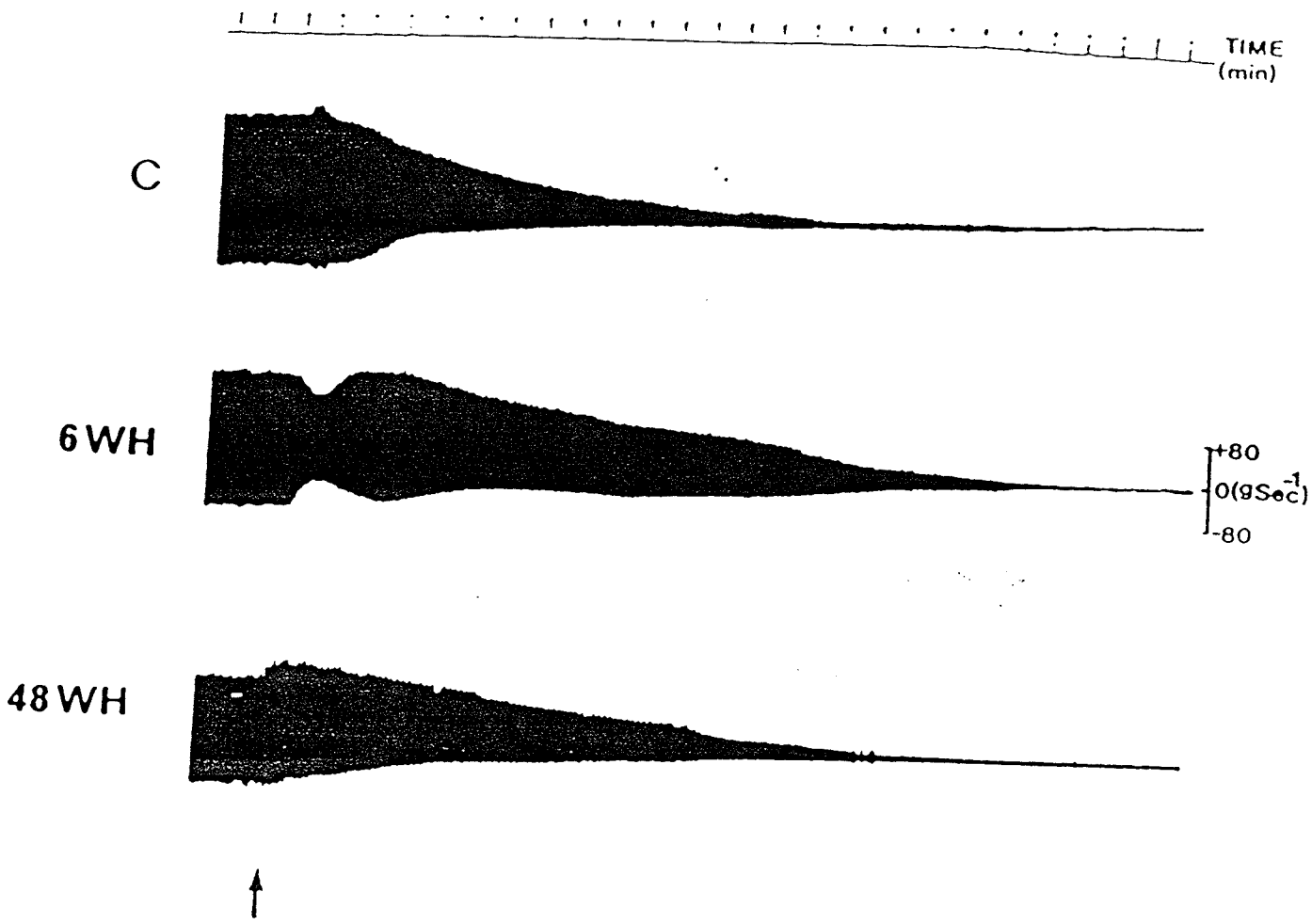


Figure 17: Typical polygraph recordings demonstrating the effects of xanthine-xanthine oxidase (X-XO) perfusion on rate of force development (+dF/dt) and rate of relaxation (-dF/dt). C = Control, 6WH = 6 week and, 48WH = 48 week hypertrophied hearts. Arrow indicates time of introduction of X-XO. Paper speed is shown on top.

hearts are presented in Figure 16 and Figure 17, respectively. At each time point between 4 to 24 min, 6 week hypertrophied hearts showed significantly greater force retention as compared to sham-control hearts (Table 10). Retention of developed force was also higher in 12, 24 and 48 week hypertrophy groups but these differences were statistically significant only up to 8 min of X-XO perfusion. Similarly, following exposure to X-XO, both +dF/dt and -dF/dt showed a relatively lesser drop in all of the hypertrophied hearts as compared to controls (Table 11), the differences being significant up to 8 min. The rise in resting tension was significantly less in different groups of hypertrophied hearts at a majority of time points (Figure 18). The mean value for rise in resting tension for the four hypertrophy groups at 28 min of X-XO perfusion was 327 $\pm$ 19% as compared to the control groups mean value of 480 $\pm$ 30%.

Since 10 min perfusion with X-XO caused approximately 50% contractile failure in hypertrophied hearts, analyses of lipid peroxidation, high energy phosphates and ultrastructural changes were performed at this time . Lipid peroxidation was estimated from the formation of malondialdehyde in control and hypertrophied hearts. Data on MDA content for sham-control hearts were pooled as there was no difference in this parameter in various groups of control hearts (Table 12). Hypertrophied hearts showed a lesser degree (range 45 - 50%) of lipid peroxidation in response to X-XO perfusion for 10 min as compared to an 80.8 $\pm$ 4.2% increase seen

Table 10: Xanthine - xanthine oxidase induced changes in developed tension in different groups of isolated perfused hearts.

Group	Perfusion Time (min) 4	8	12	16	20	24
6 WS	65.0 ± 2.8	40.8 ± 6.8	15.5 ± 10.2	3.8 ± 2.8	0	0
6 WH	80.5 ± 8.2*	60.5 ± 3.9*	52.8 ± 9.8*	25.9 ± 7.2*	8.2 ± 5.0*	2.2 ± 1.8
12 WS	77.2 ± 3.5	52.3 ± 9.4	30.9 ± 10.7	14.5 ± 9.5	3.2 ± 3.2	0
12 WH	90.2 ± 4.4*	69.3 ± 2.1*	43.3 ± 3.7	17.6 ± 2.5	4.2 ± 2.0	0
24 WS	70.4 ± 3.1	49.7 ± 5.8	34.9 ± 4.5	16.9 ± 3.8	3.6 ± 2.5	0
24 WH	93.0 ± 4.6*	78.2 ± 10.1*	44.9 ± 9.6	19.1 ± 8.2	2.9 ± 2.8	0
48 WS	73.0 ± 4.8	49.6 ± 6.8	38.6 ± 3.8	15.3 ± 3.5	0	0
48 WH	84.6 ± 3.0*	70.8 ± 5.6*	41.8 ± 6.4	22.0 ± 4.5	0	0

Values are mean ± SEM of 5 to 6 experiments, expressed as percent of zero time control data. W = weeks post-operation, H = Hypertrophy. * Significantly ($p < .05$) different compared to respective sham controls (S).

Table 11: Xanthine - xanthine oxidase induced changes in positive (+) and negative (-) dF/dt in different groups of isolated perfused hearts.

Groups	Rate of change in contractile force (dF/dt)									
	Perfusion time (min)									
	4		8		12		16		20	
	+	-	+	-	+	-	+	-	+	-
6 WS	57.40 ±10.2	55.40 ± 9.1	32.40 ± 6.3	30.50 ± 8.8	10.80 ± 2.5	8.50 ± 4.4	2.80 ± 1.9	2.20 ± 2.1	0	0
6 WH	77.30 ± 2.0*	69.00 ±10.4*	59.90 ± 2.9*	49.20 ± 7.8*	45.90 ± 2.1*	32.20 ± 5.2*	23.20 ± 2.6*	18.80 ± 3.4*	10.50 ± 2.5*	9.4* ± 1.20
12 WS	62.00 ± 2.7	51.89 ± 2.3	48.31 ± 5.7	38.67 ± 1.0	25.61 ± 2.6	23.03 ± 0.9	8.16 ± 4.3	8.49 ± 2.2	0.83 ± 0.72	0
12 WH	86.91 ± 4.1*	67.95 ± 2.7*	64.07 ± 3.7*	49.31 ± 2.5*	29.57 ± 2.0	25.21 ± 1.7	10.32 ± 2.8	9.91 ± 2.1	0.71 ± 0.42	2.46 ± 1.61
24 WS	71.89 ± 11.2	54.42 ±15.1	40.61 ± 9.4	35.21 ± 9.8	26.87 ± 8.3	26.13 ± 8.7	11.31 ± 4.5	16.60 ± 4.6	1.52 ± 0.67	4.42 ± 3.85
24 WH	88.41 ± 3.0*	79.51 ± 4.6*	64.48 ± 5.5*	54.53 ± 5.5*	32.34 ± 6.1	30.70 ± 5.4	15.26 ± 1.8	18.76 ± 3.9	5.03 ± 0.91	6.07 ± 3.75
48 WS	78.83 ± 8.9	56.20 ± 8.2	42.91 ± 8.5	32.80 ± 7.9	28.70 ± 5.5	24.50 ± 5.1	12.50 ± 3.9	14.20 ± 5.2	0.91 ± 0.80	0
48 WH	88.21 ± 4.8*	72.10 ± 6.8*	51.82 ± 6.1*	48.50 ± 8.8*	33.85 ± 4.9	32.80 ± 4.9	19.48 ± 6.2	20.90 ± 4.8	2.10 ± 1.20	0

Values are mean ± SEM of 6 experiments, expressed as percent of zero time control data. * Significantly ($p < .05$) different compared to respective sham controls (S), H = Hypertrophy, W = weeks post-operation.

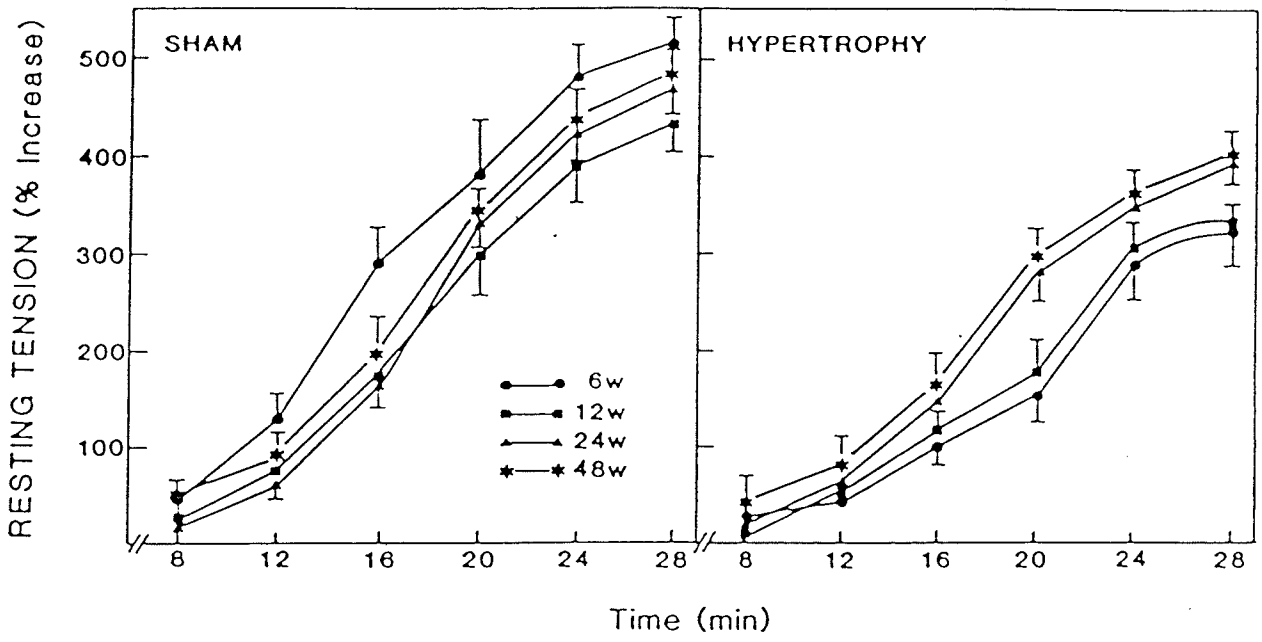


Figure 18: Comparison of effect of xanthine-xanthine oxidase perfusion on rise in resting tension in sham control (SHAM) and hypertrophied (HYPERTROPHY) hearts. W = weeks of post-operative duration. Each value represents mean $\pm$ SEM of 6 experiments.

in sham-control hearts.

High energy phosphates were measured in the form of myocardial content of ATP and CP. Sham-control and hypertrophied hearts at different post-surgery intervals did not differ from each other with respect to their ATP (range 21 - 25 umol/g dry weight) or CP (range 41 - 46 umol/g dry weight) contents. Perfusion with X-XO for 10 min resulted in a decline in high energy phosphates in both control as well as in hypertrophied hearts. However, the degree of depletion of ATP and CP was more in control hearts as compared to hypertrophied hearts (Table 12).

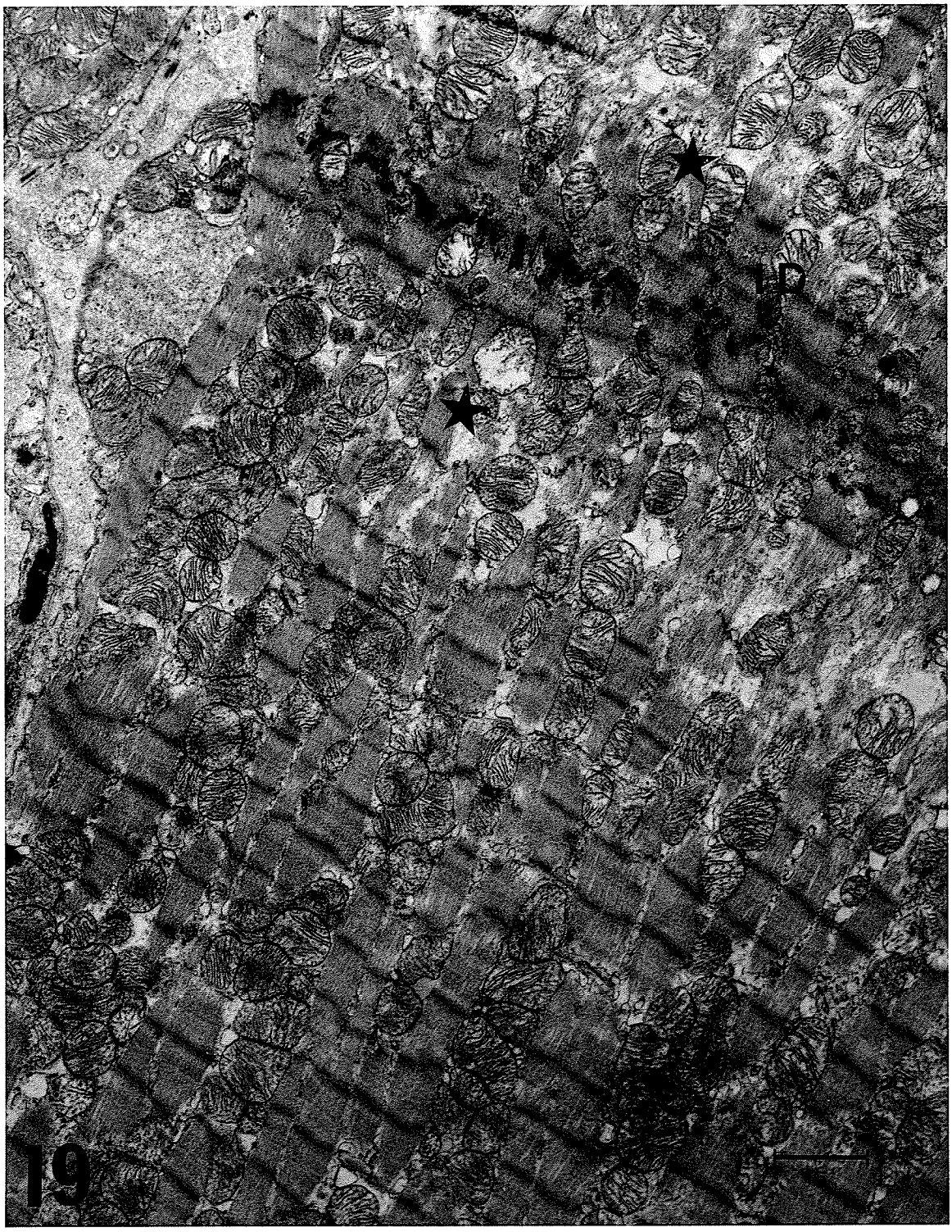
Ultrastructural changes following 10 min of perfusion with X-XO revealed lesser qualitative damage in hypertrophied hearts (Fig. 19) compared to control hearts (Fig. 10). There was less damage to sarcoplasmic reticulum and mitochondria and interstitial edema was absent in hypertrophied hearts exposed to X-XO for 10 min. Structural differences became more evident when semiquantitative morphometric analysis was performed in these hearts. Based on damage to different cell organelles and general appearance of cells , the cells were categorized as normal, mildly, moderatly or severely damaged. Sham-control hearts demonstrated typical damage (Table 6) following a 10 min exposure to X-XO. The percent of cells showing normal appearance was 21.2%. Hypertrophied hearts showed less damage and percent of normal cells was significantly more in different groups of hypertrophied hearts

Table 12: Effect of xanthine-xanthine oxidase (X-XO) perfusion on myocardial lipid peroxidation, high energy phosphates and ultrastructure of sham control and hypertrophied hearts.

Groups	MDA (% increase)	High energy phospates (% decline)		Myocytes (% Normal)
		ATP	CP	
Control	80.8 ± 4.2	45.7 ± 2.8	38.8 ± 4.1	21.2 ± 3.8
6 WH	45.2 ± 3.8*	30.0 ± 3.5*	27.2 ± 3.1*	30.6 ± 2.6*
12 WH	48.6 ± 5.2*	33.9 ± 2.9*	30.2 ± 2.1*	29.3 ± 2.8*
24 WH	47.8 ± 3.6*	32.6 ± 3.0*	29.8 ± 3.2*	31.8 ± 3.2*
48 WH	49.6 ± 4.8	33.4 ± 3.2*	30.0 ± 2.9*	29.6 ± 4.8*

Hearts were perfused for 10 min with Krebs Henseleit (KH) alone or KH + X-XO. Data expressed as mean ± SEM of 5 experiments and is percent change from hearts exposed to KH solution for 10 min. For control, data from different groups of sham control hearts was pooled together. * Significantly different ($p < .01$) from control (ANOVA; Students 't' test), for ultrastructural studies Kruskal-Wallis test and Wilcoxen test were used MDA = Malondialdehyde, ATP = Adenosine Triphosphate, CP = Creatine Phosphate.

Figure 19: Electronmicrograph demonstrating the effect of 10 min perfusion with Xanthine - Xanthine Oxidase in a hypertrophied heart. Note less damage to sarcoplasmic reticulum and interstitial edema localized on either side of the intercalated disc (ID) as compared to 10 min. damage in controls (Fig. 10.). Myocardial injury is apparent from the development of contraction bands, some degree of swelling in mitochondria and stress damage () indicated by disrupted sarcomeres as well as Z lines on either side of the intact ID. Magnification line indicates 2 um.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significantly more in different groups of hypertrophied hearts (Table 12). Furthermore, none of the cells exhibited severe damage while there was a shift of moderately damaged cells (11 - 16% versus 30% in control) to the mild (53 - 58% in hypertrophied hearts versus 42% in control) category. The process of hypertrophy (up to 48 weeks) was not accompanied by any apparent cell damage.

C. ANALYSIS OF ANTIOXIDATIVE ENZYMES AND LIPID PEROXIDATION IN NORMAL AND HYPERTROPHIED HEARTS

In order to further elucidate the differences in susceptibilty to free radical damage in control and hypertrophied hearts, superoxide dismutase and glutathione peroxidase activities and MDA content were monitored in these hearts.

1. Superoxide Dismutase:

The increase in absorbance due to autooxidation of dopamine was followed at 490 nm and in the absence or presence of various concentrations of standard SOD (100 U -2000 U) and different concentrations of tissue proteins (0.2 - 1.2 mg). Addition of 100 U of SOD did not produce any change while 500 - 2000 U of SOD showed a dose-dependent inhibition of autooxidation of dopamine (Figure 20a). Similarly, different concentrations of tissue protein inhibited dopamine autooxidation in a concentration-dependent manner, although the linear range of inhibition

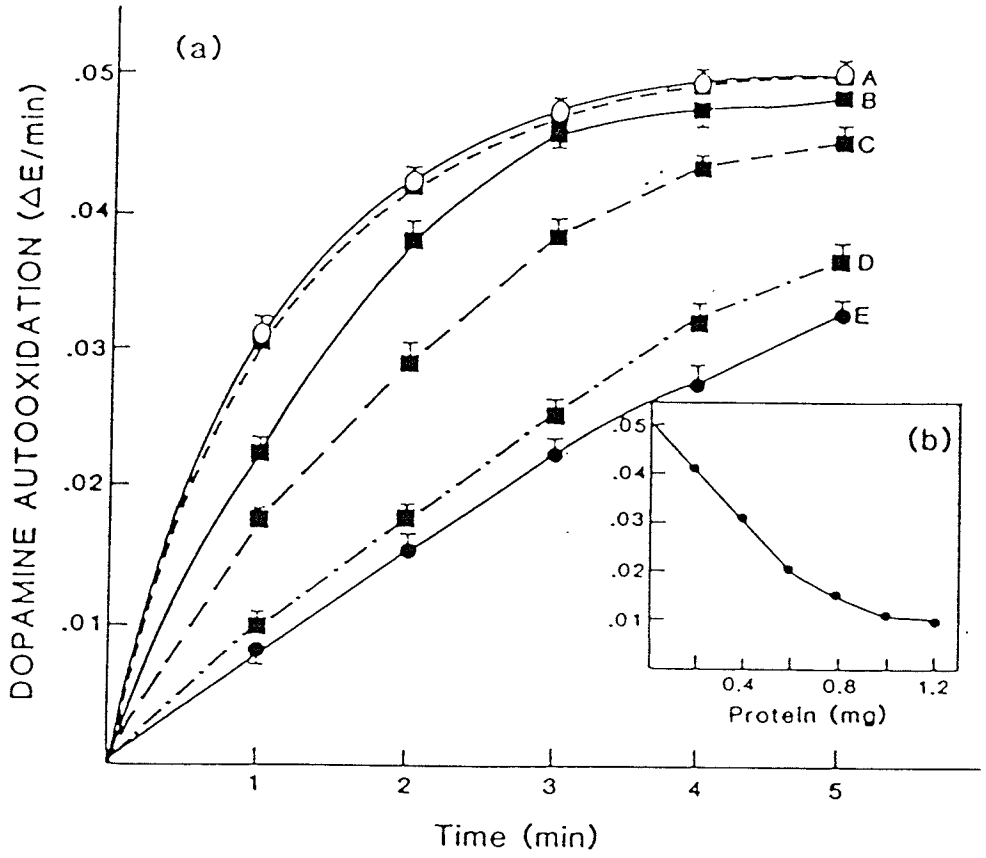

Figure 20 : Effect of various interventions on time-related increases in dopamine autooxidation (DAA) (o-o). Control DAA in absence of SOD or heart homogenate. (A-D) DAA in presence of different concentrations of SOD, (A) 100 U, (B) 500 U, (C) 1000 U and (D) 2000 U. (E). DAA in presence of 400 ug of homogenate protein. The inset (b) demonstrates the effect of various concentrations of tissue protein (0.2 - 1.2 mg) on DAA at 5 min.

was found to be between 0.2 -0.6 mg of tissue protein (Figure 20b). Therefore, for analysing the SOD activity in heart homogenates, 400 ug of tissue protein was used and data expressed as E/min/mg protein due to autooxidation of dopamine. Heart homogenates from hypertrophied hearts showed a greater degree of inhibition (range 56 - 70%) of autooxidation of dopamine over a period of 5 min (Figure 21B) as compared to the degree of inhibition (35 - 68%) observed with homogenates from different sham-controls (Figure 21A). It is interesting to note that sham control hearts demonstrate an age-related variation in inhibition of dopamine autooxidation i.e. 6W sham controls showed 35% inhibition (less SOD) compared to 68% inhibition (more SOD) in 48 W sham controls. When individual hypertrophied groups were compared with their respective sham controls, the greatest stimulation (greater than 170%) of SOD activity was observed at 6W, with 130% at 12W and no significant difference in 24 and 48 week hypertrophied hearts (Figure 21C).

2. **Glutathione Peroxidase:**

The glutathione peroxidase (GSHPx) activity was measured by following the rate of glutathione oxidation by H_2O_2 at 240 nm (Fig. 22). There was an increase (19.7 $\pm$ 1.2%) in GSHPx activity in 6 weeks hypertrophied hearts compared to sham controls, and this elevated activity was maintained in 12, 24 and 48W hypertrophied hearts. There was no significant difference in GSHPx activity when different sham control groups were compared with each other.

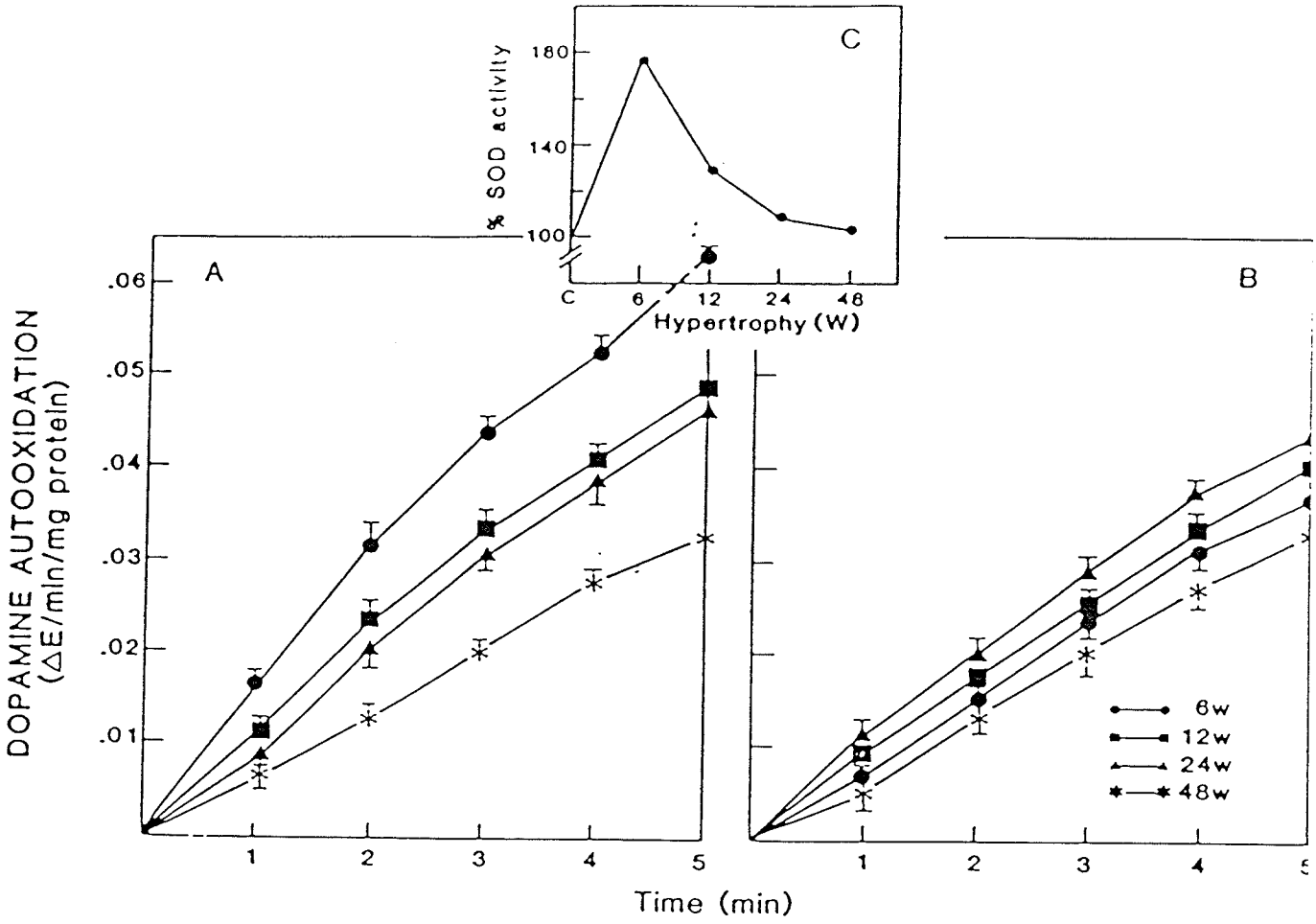

Figure 21: SOD activity examined as inhibition of autooxidation of dopamine by sham control (A) and hypertrophied (B) heart homogenates. 400 ug of tissue protein was used with the standard dopamine solution, and data were calculated as increase in absorbance/min/mg tissue protein. Each value represents mean ± SEM of duplicates of 6 experiments. The inset (C) demonstrates % SOD activity in various hypertrophied hearts, in relation to respective sham-controls. W = weeks of post operative duration.

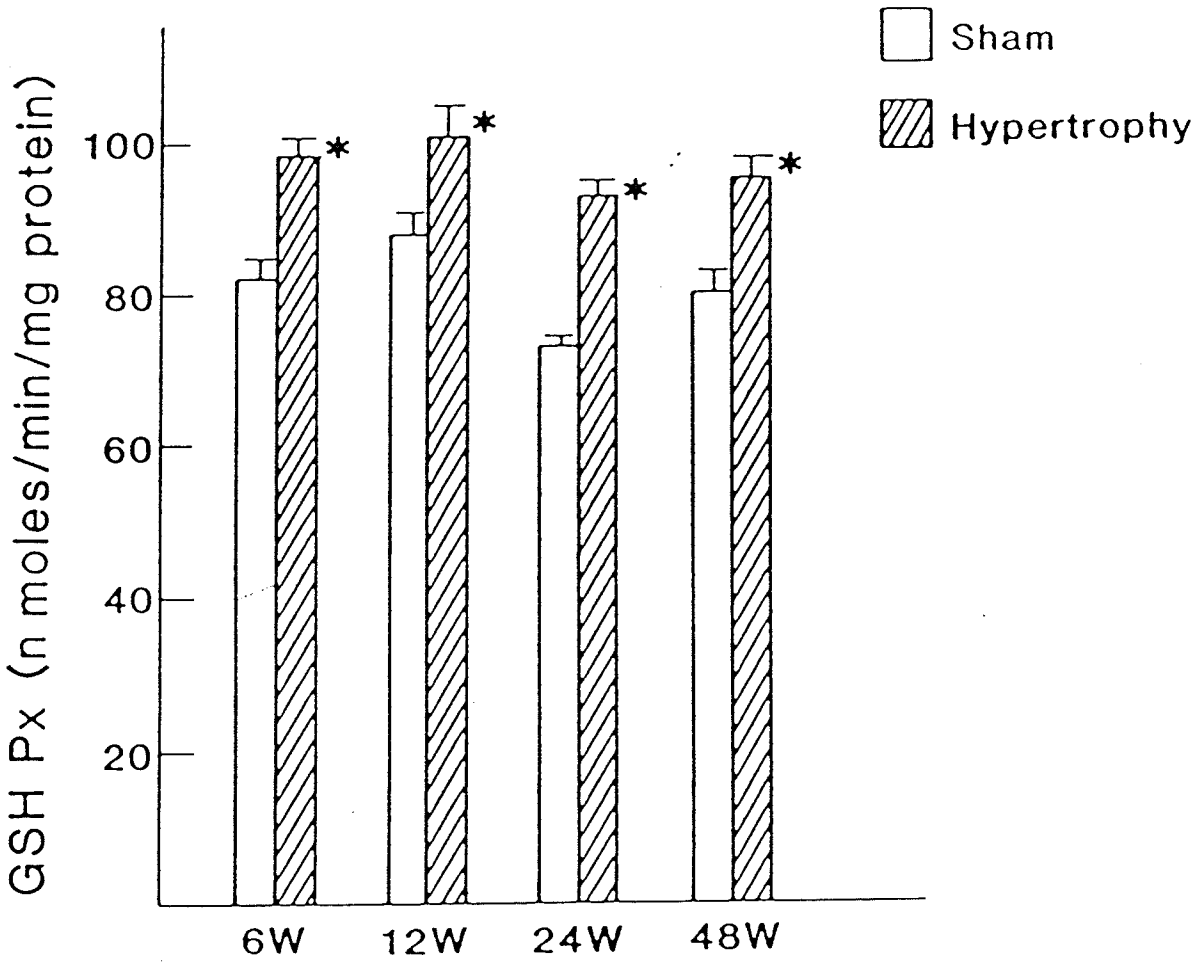

Figure 22: Glutathione peroxidase activity in different groups of hearts. W = weeks of post operative duration. * Significantly different from respective sham-control ($p < .05$; unpaired Students t test)

3. **Lipid Peroxidation:**

Hypertrophied and sham-control hearts were analyzed for malondialdehyde (MDA), a relatively stable end product and an indicator of lipid peroxidation and the results are shown in Table 13. Hypertrophied hearts at each post-operative duration showed a lesser degree of MDA formation. The range of decreases in MDA formation varied from 20 - 27%. The slight drop in MDA content in sham-controls with age did not approached the level of significance.

Table 13: Malondialdehyde content in different groups of hypertrophied and sham control hearts.

Post operative Duration (weeks)	Malondialdehyde (nmoles/g wet weight)	
	Sham	Hypertrophy
6	29.04 ± 2.24 (6)	21.82 ± 2.02* (6)
12	27.07 ± 1.54 (7)	20.74 ± 0.86* (7)
24	27.51 ± 1.43 (6)	20.05 ± 0.26* (7)
48	26.54 ± 0.94 (7)	21.32 ± 0.53* (6)

Data are expressed as mean ± SEM with number of experiments in parenthesis. * Significantly different ($p < .05$) when compared to sham control.

V. DISCUSSION

A. MYOCARDIAL EFFECTS OF OXYGEN RADICALS IN NORMAL HEARTS

Oxygen radicals are produced *in situ* as part of several normal essential processes as well as during various pathological conditions. Their role in health and disease is fairly well established. In order to fully understand the role of oxygen radicals in various diseases, it is important to delineate the subcellular basis of their toxicity. Although direct measurement of these radicals in living tissue has been reported (Rao *et al*, 1983, Garlick *et al*, 1987), because of their high reactivity and short half life, accuracy of such measurements in biological tissues remains to be established. At present, therefore, contribution of oxygen radicals to tissue injury is often inferred from knowledge of the inciting stimulus or by studying the protective action of different radical scavengers, or, by monitoring lipid peroxidation products of oxygen radical injury. In the present study, all three approaches were adopted to understand the pathogenesis of oxygen radical-induced myocardial injury.

In this study, xanthine-xanthine oxidase was utilized as a source of oxygen radicals. Although oxygen radicals can be generated by a number of sources such as Fe^{3+}- ADP complex (Kramer *et al*, 1984), dihydroxyfumarate (Mak *et al*, 1976), cumene hydroperoxide buffer (Koster *et al*, 1985) and hydrogen peroxide (Fliss *et al*, 1988), the use of xanthine-xanthine

oxidase is advantageous because this system is chemically well characterized (Fridovich and Handler, 1962; Fridovich, 1970). Furthermore, the amount of radicals generated by the concentration of xanthine-xanthine oxidase employed in this study is in the concentration range of radicals shown to be generated by activated neutrophils (Tate et al, 1982). Even though controversial in humans, one of the demonstrated sources of oxygen radicals during ischemia-reperfusion damage in a variety of experimental models, was shown to be the enzyme xanthine oxidase (Chambers et al, 1985; Charlat et al, 1987). The xanthine-xanthine oxidase system may serve as an experimental model for a vascular source of various oxygen radicals such as may be the case in activated neutrophils (Jolly et al, 1984, Rowe et al, 1983, Wei et al, 1985) and /or autooxidation of catecholamines (Singal et al, 1982, 1983). At any rate, in the present study the xanthine-xanthine oxidase system was used as a source of oxygen radicals.

Data presented in this study demonstrate that perfusion of the rat heart with xanthine-xanthine oxidase resulted in contractile failure and a rise in resting tension. Since xanthine or xanthine oxidase alone had no effect on heart function, it indicates that the interaction of the two agents is responsible for the observed contractile effects. Because antioxidants such as methionine (Matheson et al, 1975) and glutathione, a reducing agent (Winterbourn, 1979), showed a protective effect in our study, we infer that oxidant species were generated due to xanthine-xanthine oxidase interaction.

This is in agreement with an earlier observation in which the production of oxidant species was demonstrated chemically (Fridovich, 1970). Significant protection with the use of superoxide dismutase (a specific scavenger of superoxide radicals), catalase (a scavenger of hydrogen peroxide) and mannitol (a $OH^{\cdot}$ radical scavenger) demonstrates that superoxide, hydroxygen peroxide and hydroxyl radicals might have participated in contractile effects of xanthine-xanthine oxidase perfusion. The detrimental effect of SOD at low concentration on - dF/dt remains unexplained at this time.

Burton et al (1984) using an isolated septal preparation showed significant protection by SOD and concluded that only superoxide radicals are generated by X-XO interaction. However, our data show that superoxide alone is not responsible for the contractile failure seen here. If it was, then catalase or mannitol would not prevent contractile failure because these agents do not have any effect on superoxide radicals. Furthermore, the scavenging action of mannitol was shown to be independent of its osmotic effect (Magovern et al, 1984). The discrepancy between our results and those reported by Burton et al could be due to the differences in the mode of xanthine oxidase administration. While xanthine was being perfused through the septal artery they administered XO separately so that the time allowed for X-XO interaction was less. We used a buffer reservoir for the enzymatic reaction which probably allowed the buildup of hydrogen peroxide as well as

permitting the interaction of hydrogen peroxide with superoxide to produce hydroxyl radicals. Since tissue injury in biological systems often involves the participation of more than one radical species, the medium used in this study which permits an examination of the collective effects of all three radical species is very relevant to the *in vivo* situation.

Radical production *via* the xanthine-xanthine oxidase system has been shown to depend on O_2, pH and xanthine concentration (Fridovich, 1970). We observed that it might also depend on the time allowed for xanthine-xanthine oxidase interaction with O_2 prior to perfusion. It was found that contractile failure occurred early if this time was prolonged from 5 - 120 min (data not shown). In this study, this period was controlled to 90 min. The mechanism by which these radicals are produced is fairly well understood in the case of xanthine-xanthine oxidase. It is known that in the presence of oxygen, xanthine oxidase oxidizes xanthine to uric acid and, in the process, molecular oxygen is reduced to superoxide radical according to the following reaction:

$$\text{Xanthine} + H_2O + O_2 \xrightarrow{\text{Xanthine Oxidase}} \text{Uric acid} + 2O_2^{\cdot -} + 2H^+ \quad (1)$$

The superoxide radicals thus generated have the potential to react with a variety of biologically relevant organic and inorganic substrates (Fridovich, 1978; 1983). For example, superoxide can reduce ferricytochrome c, quinones, transition metal complexes and can oxidize ascorbic acid, fatty acids, alpha-tocopherol, catechols, catecholamines,

hemoproteins and thiols (McCord, 1983; McCord and Roy, 1982). Although reactivity of $O_2^{\cdot -}$ may appear limited based on cell-free *in vitro* studies, our observation on the protective action of superoxide dismutase indicates that the participation of superoxide is at least partially responsible for the observed contractile failure. In a recently published report, Blaustein *et al* (1986) failed to show any protection against an exogenous source of oxygen radicals with superoxide dismutase; this negative finding may have been due to the low concentration of the enzyme used. Since at a lower dose we also did not see any protection, it apparently is a dose-dependent phenomenon. Superoxide radicals are also capable of undergoing spontaneous dismutation according to the following reaction:

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \longrightarrow H_2O_2 \qquad (2)$$

Hydrogen peroxide thus generated is considered to be a powerful oxidant but it reacts only slowly with most organic substrates (Chance *et al*; 1979, Fee and Valentine, 1979). However, H_2O_2 reacts with transition metal ions and their inorganic or organic complexes at rapid rates to generate powerful oxidants such as hydroxyl radicals. Therefore, protection offered by catalase against X-XO-induced contractile failure may not only involve faster reduction of H_2O_2 to H_2O but might also result in a diminished production of hydroxyl radicals. It is known that interaction of hydrogen peroxide with superoxide radicals can result in the formation of hydroxyl radicals via the Haber Weiss (1934) reaction:

$$H_2O_2 + O_2^{\cdot -} \longrightarrow OH^{\cdot} + OH^{-} + O_2 \qquad (3)$$

This reaction is thermodynamically favorable and has been documented as a source of hydroxyl radicals (Beauchamp and Fridovich, 1970; Kellog and Fridovich, 1975; Koppenol, 1976). Under chemically defined conditions, the reaction has been suggested to be kinetically slow or even negligible (Rigo et al, 1977; McCord and Day, 1978) and may be a combination of the following two half reactions requiring iron as a catalyst (McCord and Day, 1978).

$$O_2^{\cdot -} + Fe^{3+}\text{- EDTA} \longrightarrow O_2 + Fe^{2+}\text{- EDTA} \qquad (4)$$

$$Fe^{2+}\text{- EDTA} + H_2O_2 \longrightarrow Fe^{3+}\text{- EDTA} + OH^{-} + OH^{\cdot} \qquad (5)$$

$$2O_2 + O_2^{\cdot -} \longrightarrow OH^{-} + OH^{\cdot} + O_2$$

The requirement for iron in reaction 4 and 5 for the production of $OH^{\cdot}$ (reaction 3) has also been demonstrated by in vitro hydroxylation studies (Halliwell, 1978). Hydroxylation of aromatic compounds with xanthine-xanthine oxidase, at pH 7.4, could not occur until low concentrations (uM range) of $FeSO_4$ or $FeCl_3$ were provided in the medium (Halliwell, 1978). The combination of purine and xanthine oxidase in the perfusion medium was found to cause a significant depression of contractile function and myocardial cell damage in isolated myocardial septal preparations only upon the addition of 2.4 uM Fe^{3+}-loaded transferrin into the perfusion system (Burton et al, 1984).

In the present study, xanthine-xanthine oxidase-induced contractile failure could occur even in the presence of desferal (desferrioxamine), a specific iron chelating agent, used

clinically for treating iron toxicity (Cooper et al, 1977). The concentration of desferrioxamine used in the present study (300 uM and 3mM) cannot be argued to be suboptimal, because 15 and 150 uM concentrations have been shown to protect against reperfusion-induced arrhythmias (Bernier et al, 1986). These observations indicate that OH· radicals may arise independently of the presence of iron. It should be noted that desferal complexes with iron to form a stable chelate ferrioxamine which does not possess catalytic activity in the generation of OH· radicals (Cumming et al, 1969). Although iron was not added to the system used in the present study, it may have been present as a contaminant in chemical ingredients of the perfusion buffer in trace amounts and in vivo in heart cells. Our data support the work of Fridovich and coworkers (1970, 1975) and Peters and Foote (1976) who also demonstrated that the interaction of superoxide and hydrogen peroxide to produce hydroxyl radicals is possible in the absence of metal ions and addition of iron to the xanthine-xanthine oxidase may only be enhancing the rate of this interaction. The possibility of a direct toxic effect of desferrioxamine was ruled out in experiments employing this drug alone.

The time-course study revealed that the decrease in contractile force at 5 min due to active oxygen species was associated with an increase in lipid peroxidation and a loss of high energy phosphates (THEP) without any significant change in myocyte structure. Significant structural damage to myocytes and coronaries became apparent only after 10 min of

perfusion. Both increased lipid peroxidation and loss of THEP may result in a heterogenous cascade of metabolic alterations collectively causing a depression of function and subsequent cell damage (Fleckenstein et al, 1974, Plaa and Witsche, 1976).

Several reports suggest that unsaturated fatty acids of the membrane lipid bilayer are primary targets of free radical attack resulting in formation of lipid peroxy radicals and lipid peroxidation (Plaa and Witsche, 1976, Singal et al, 1983). Formation of peroxidation products in the membrane has been suggested to modify its permeability characteristics (Chance et al, 1979, Plaa and Witsche, 1976). The time-related increase in lipid peroxidation in the X-XO perfused heart was demonstrated by an increase in thiobarbituric acid reactive material. Here it should be noted that although the formation of MDA does not equal the amount of fatty acid oxidized, the relationship between substrate utilized and product formed is stoichiometric within limits (May and McCay, 1968). The increase in lipid peroxidation in whole heart (Gupta et al, 1987) and in sarcolemma and microsomal membrane preparations (Kramer et al, 1984) in response to oxygen radicals generated by X-XO and dihydroxyfumarate Fe^{3+}- ADP complex has been reported earlier. Furthermore, increased lipid peroxidation has also been observed during oxidative stress caused by ischemia-reperfusion injury (Gauduel and Duvelleroy, 1984), and adriamycin (Singal et al, 1987). The strong positive correlation between the increase in lipid peroxidation and decrease in force provides evidence that the enhanced lipid

peroxidation could be one important factor in producing myocardial dysfunction. However, damage to membrane-bound proteins containing oxidizable amino acids cannot be ruled out. In this regard, it was reported that oxygen radicals can oxidize cysteine molecules in several proteins (Misra, 1974).

The significance of the loss of ATP in contractile failure was indicated by a close correlation between these two variables. A drop in high energy phosphate content clearly indicates that oxygen radicals in the perfusion medium are affecting mitochondrial oxidative phosphorylation. Depressed adenylate charge observed in the present study has been suggested to favour ATP regeneration (Atkinson, 1968); however, this can happen only if proper conditions for synthesis are available. Although alterations in mitochondrial structure were not found to accompany the early myocardial dysfunction due to oxygen radicals, a continued exposure to radicals did result in mitochondrial damage. Thus, in later stages, damage to the mitochondrial ultrastructure can also contribute in oxygen radical injury. The exact nature of the mitochondrial defect remains unclear but it may concern lack of substrates and/or cofactors. The presence of excess cytoplasmic calcium due to sarcolemmal permeability change can result in intramitochondrial accumulation of Ca^{2+} which impairs ATP production. Altered permeability of sarcolemma produced by oxygen radicals in the present study was evidenced by intracellular localization of lanthanum - an extracellular tracer. The decrease in high energy phosphates was accompanied

by net loss of total adenine nucleotides and an increase of adenosine and creatine indicating an accumulation of metabolic breakdown products. It has been reported that hydrogen peroxide and hydroxyl radicals depress canine cardiac sarcoplasmic reticulum Ca^{2+} transport through inhibition of ATPase activity (Rowe et al, 1983) and this hydroxyl radical effect is promoted by a drop in pH (Hess et al, 1981). In this regard, ATP degradation is known to be associated with proton generation (Gevers, 1977). Depressed sarcoplasmic reticular function, intracellular build up of Ca^{2+} and loss of ATP can all be seen to impede relaxation and this might explain the dramatic rise in resting tension seen in this study. Patent mechanical coupling between cells seen in the ultrastructural examination would help support the rise in resting tension.

Vascular damage due to X-XO has been demonstrated in cerebral arterioles (Wei et al, 1985) and in cardiac vessels (Burton et al, 1984). In the present study, extensive damage to coronary vessels was observed only during the later stages of perfusion. Therefore, vascular injury may not be the cause of the early myocardial changes noted in this study. It should be emphasized that perfusion with X-XO has not been found to impede rat coronary flow (Ytrehus et al, 1986) excluding hypoxia or ischemia as the underlying cause for myocardial injury in the present model.

Based on the present time-course study of effects of xanthine-xanthine oxidase on myocardial function, cell structure, sarcolemmal permeability, high energy phosphates and lipid peroxidation, a "simplified" scheme for the

pathogenesis of oxygen radical-induced myocardial injury is proposed in Figure 23. The presence of active oxygen species was established by use of antioxidants. These toxic agents result in accumulation of lipid peroxides causing changes in membrane function. Two systems found to be affected in the present model are mitochondria and sarcolemma. These changes in myocytes are suggested to result in the loss of high energy phosphates as well as occurrence of Ca^{2+} overload. Both of these may lead to contractile failure, a rise in resting tension and the loss of structural integrity as seen here.

B. CARDIAC HYPERTROPHY AND OXYGEN RADICALS

Banding of the abdominal aorta in rats increased the left ventricle to body weight ratio by about 25% within 6 weeks, reflecting the presence of left ventricular hypertrophy. This hypertrophic state remained stable as there was no further significant increase in left ventricle to body weight ratio beyond 6 weeks until 48 weeks. Hemodynamically, animals with aortic banding demonstrated a stable hyperfunction as reflected by elevated systolic pressures, and increased rate of force development and relaxation. Lack of changes in left ventricular end diastolic pressure, wet to dry weight ratio for liver and lungs as well as absence of pleural effusions and ascites, clearly excluded cardiac decompensation in experimental animals. Furthermore, no changes in high energy phosphate levels were observed in hypertrophied hearts. Similar findings have been reported during stable cardiac hypertrophy while high energy

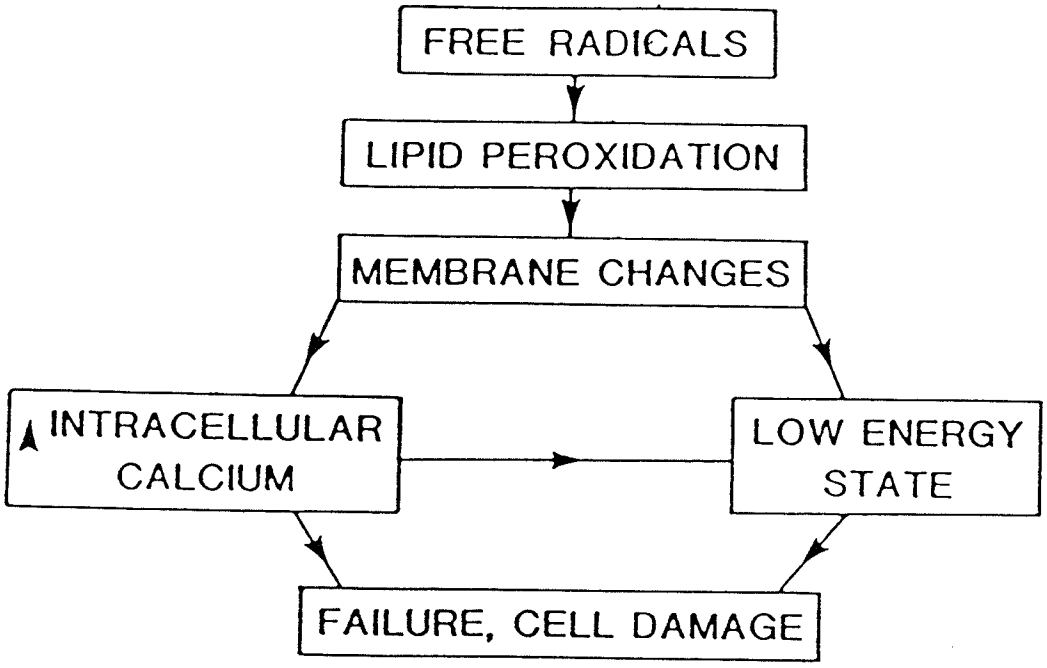

Figure 23: A tentative scheme of events of oxygen radical induced damage in rat heart. Perfusion of oxygen radicals results in lipid peroxidation and increased membrane permeability to ions including calcium in the heart cell. These changes coupled with a low energy state may be responsible for contractile failure and cell damage seen in the present study.

phosphates have been shown to be low in a failing heart (Katz, 1977; Alpert and Hamrell, 1976). These results indicate that in this animal model, there was a stable compensated cardiac hypertrophy which did not show any sign of transition into a decompensated heart failure state.

Although the exact initiating stimulus for the increased cardiac mass is not clear, the developing phase of cardiac hypertrophy is usually considered to be due to increased protein synthesis occurring as a result of activation of nucleic acid synthesis. In this regard, several reports indicate that the RNA content of the hypertrophying myocardium is increased (Fizelova and Fizel, 1970; Moroz, 1967). We also observed a 73% increase in left ventricular RNA content following 6 weeks of pressure overload. However, during the later course of hypertrophy, total left ventricular RNA content increased only in proportion to the increase in heart weight. Therefore, it seems that following aortic constriction RNA content first shows an increase and then returns to normal upon prolongation of the hypertrophy. Similar changes in RNA content were also reported by other investigators (Meerson, 1962 Grimm et al, 1966; Fanburg and Posner, 1968). In a preliminary study using two animals from each group, the wet to dry weight ratio of the left ventricle from control (4.2) animals was found to be similar to that in experimental (4.1) animals, as has also been reported by others (Movroudis et al, 1979; Polimeni et al, 1983). Since myocardial water content was not increased in the experimental model reported here and protein content of the

hypertrophied heart was also not elevated, it is possible that the unchanged protein concentration could have been due to a rapid rate of protein turnover. In this regard, a faster rate of total protein degradation in hypertrophying skeletal muscle was reported (Laurent and Millward, 1980) and increased myosin degradation has been observed in cardiac hypertrophy (Morkin et al, 1972). Our data support the idea that during the stable phase of cardiac hypertrophy an augmented protein synthesis is accompanied by a parallel increase in protein degradation (Schreiber et al, 1981).

Data obtained in this study show that increased antioxidative capacity is supported by differential (specific) changes in SOD and glutathione peroxidase activities (Gupta and Singal, 1988). In this regard, increased SOD activity was significantly higher 12 weeks post-operatively. As the duration of hypertrophy was prolonged further this increase did not remain significant because of an age-related increase in the SOD activity of controls. Glutathione peroxidase activity, on the other hand, remained elevated throughout the duration of study. An increase in GSHPx in response to increased pressure load for 60 days has recently been reported in rabbits (Guarnieri et al, 1987). Our data show that both SOD and GSHPx respond to pressure overload and that the change in SOD activity may not remain as great as that of GSHPx, when the duration of hypertrophy is prolonged. Decreased malondialdehyde formation, even at the time when the increase in SOD was no longer apparent further supports the view that

glutathione peroxidase may be playing an important role in maintaining a low level of lipid peroxidation in hypertrophied hearts. In this regard, GSHPx has been shown to be a significant factor in the stabilization of polyunsaturated membrane lipids (Christophersen, 1969). In addition, glutathione peroxidase activity was shown to be crucial for the prevention of liver membrane damage associated with alpha-tocopherol deficiency (Machado et al, 1971). Furthermore, a glutathione-dependent cytosolic factor has been demonstrated to inhibit lipid peroxidation in microsomal and mitochondrial membranes of rat heart and lung tissues (Gibson et al, 1980). This inhibition was found to be due to the prevention of peroxidative attack on the polyunsaturated fatty acids in the membrane lipids even under conditions that would otherwise promote rapid lipid peroxidation (Gibson et al, 1980). Since rat heart is rich in selenium-dependent glutathione peroxidase activity and has very little non-selenium-containing (glutathione S-transferase) activity (Lawrence and Burk, 1978), the increased glutathione peroxidase activity in the present study may be of the selenium-dependent type. At any rate, an increase in antioxidative capacity in form of cytosolic SOD and glutathione peroxidase activity during stable hypertrophy is strongly suggested by the present study.

The exact stimulus for increased activity of these enzymes is not known; however, one possibility is that increased radical formation itself during increased metabolic activity due to pressure overload may be a signal. In this regard, glutathione peroxidase activity was reported to be

increased in intestinal mucosa and liver after oral administration of peroxidized lipids (Reddy and Tappel, 1974) and in lung following peroxidation due to ozone or 90% oxygen for two weeks (Kimball et al, 1976; Chow and Tappel, 1972). Furthermore, a number of studies have reported that antioxidative enzymes may respond to a wide range of physiological and pathological conditions of increased oxidative stress. Such conditions include age (Nohl and Hegner, 1979), exercise (Kanter et al, 1985; Higuchi et al, 1985) and beta-thalassaemia minor (Gerli et al, 1980) where compensatory increase in antioxidative enzyme activity has been demonstrated. In this study, an age-related increase in SOD activity in sham-control hearts was also apparent. Here, it should also be noted that antioxidative enzymes may also undergo radical-induced inactivation (Hodgson and Fridovich, 1975; Searle and Wilson, 1980; Guarnieri et al, 1980). A complex pattern of enzyme increase and decrease was observed in different organs during diabetes (Wohaieb and Godin, 1987). It is suggested here that during hypertrophy the increase in enzyme activity is a compensatory response, which helps the heart maintain its function in spite of possible increases in radical production.

A number of factors can be contributing to increasing the radical production in a hypertrophied heart. Since myocardial oxygen consumption is increased during hypertrophy (Meerson, 1969), it is possible that a higher oxygen metabolism may partially be responsible for the increased radical formation.

Autooxidation of catecholamines could be another source of oxygen radicals (Singal et al, 1983). It is known that during cardiac hypertrophy there is depletion of cardiac norepinephrine (Spann et al, 1967) and a decrease in norepinephrine binding and uptake (Fischer et al, 1965). This coupled with the observation that plasma norepinephrine levels are high (Limas, 1979), makes it possible to state that increased radical formation could occur via autooxidation of catecholamines in a hypertrophied heart. Furthermore, increased formation of oxygen radicals has been demonstrated in mitochondria isolated from hypertrophied hearts (Guarnieri et al, 1985). Our data suggest that during cardiac hypertrophy, one of the adaptive mechanisms against such an increase in radical formation is the development of an increased antioxidative capacity.

It was interesting to note that all hypertrophied hearts were relatively more resistant to injury caused by an ex vivo perfusion with oxygen radicals. Radical-induced contractile failure was delayed in 6 weeks hypertrophied hearts, while the remaining hypertrophied hearts exhibited no change in duration of failure. The reason for the different behavior of 6 week hypertrophied hearts is not clear, but data presented here show that the greatest stimulation of SOD activity occurred at this period. Nonetheless, a lesser degree of oxidative damage in all the hypertrophied hearts was also demonstrated by a lower level of MDA formation, a lesser decline in high energy phosphates, and a lower degree of ultrastructural damage. The correlation between less contractile failure and reduced loss of high

energy phosphates as well as reduced lipid peroxidation further supports the scheme for oxygen radical-induced myocardial injury shown in Figure 23.

The increased tolerance to oxidative stress of the hypertrophied heart may have been due to increased activity of antioxidative enzymes, superoxide dismutase and glutathione peroxidase, as well as the lower level of lipid peroxidation in the hypertrophied hearts. Participation of other factors such as increased contractile mass, changes in the permeability of coronary vessels (for review see Wikman-Coffelt, 1979), such that the free radical injury can not extend into the hypertrophied myocardium with the same speed and intensity, can not be ignored at this time. However, the lesser degree of radical-induced damage in hypertrophied hearts can not be argued on the basis of suboptimum dose (on tissue weight basis) of X-XO because in our preliminary study a 20% increase in the flow rate did not affect the pattern of failure due to X-XO. Furthermore, no relationship was apparent between the heart weight and failure time in control or hypertrophied hearts. The increased flow rate (20%) itself did not have any effect on the heart function.

The hypertrophied heart has been shown to be more sensitive to the cardiotoxic effects of adriamycin (Singal et al, 1984) as well as to ischemia-reperfusion injury (Levitsky 1986), two conditions known to involve oxygen radicals in producing cardiotoxic effects. These observations may be contrary to the findings of the present study. The discrepancy

may be due to differences in the intensity of imposed workload, different degree of hypertrophy produced and animal models used. Since these investigators (Singal et al, 1984; Levitsky et al, 1986) did not assess the functional status of the heart, it is possible that, to begin with, the contractile function of these hearts may have been impaired. In this regard, it is known that contractile functional abnormalities depend on the degree of hypertrophy (Spann et al, 1984). Nevertheless, the present study clearly indicates that during the stable phase of cardiac hypertrophy, an elevated antioxidative capacity associated with a maintenance of heart function.

In conclusion, it is proposed that myocardial adaptation to increased pressure load is accompanied by higher activity of radical scavenging enzymes. This greater antioxidative capacity of the heart reduces lipid peroxidation and probably helps to maintain the heart function against higher oxidative stress due to increased metabolism. This study emphasizes that antioxidant status in the heart is a dynamic function adjusting to the physiological and/or pathophysiological conditions imposed.

VI. CONCLUSION

In this study, experiments were designed to evaluate the time-course of changes in the effect of oxygen radicals on rat heart function, lipid peroxidation, high energy phosphate levels and structural damage. Experiments were also designed to study antioxidative enzyme levels in the non-failing hypertrophied heart resulting from chronic pressure-overload due to abdominal aortic banding in rats. The response of hypertrophied hearts to oxidative stress was also evaluated in terms of contractile function, lipid peroxidation high energy phosphates and structural damage. From the results obtained, the following conclusions are made:

1) Perfusion of the heart with xanthine-xanthine oxidase results in contractile failure, increased lipid peroxidation and structural damage.

2) Contractile failure due to oxidative stress appears to be mediated by an increase in lipid peroxidation and depletion of high energy phosphates while structural damage may be a delayed event.

3) Some of the oxidant species identified in xanthine-xanthine oxidase-induced contractile failure are superoxide radicals, hydrogen peroxide and hydroxyl radicals.

4) Iron may not be a necessary requirement for the production of toxic oxygen radical species by xanthine-xanthine oxidase.

5) The aortic banded rats exhibit a stable left ventricular hypertrophy between 6 - 48 weeks with no signs of heart failure.

6) The stable hypertrophy phase is accompanied by an elevated but sustained hemodynamic function.

7) Hypertrophied hearts show greater resistance to oxidative stress which may be due to a lower level of lipid peroxidation and a higher antioxidative capacity.

8) Increase in antioxidative enzyme activities of superoxide dismutase and glutathione peroxidase during hypertrophy is suggested to be one of the adaptive mechanisms during pressure overload.

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