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THE EFFECTS OF CHRONIC AEROBIC EXERCISE TRAINING
ON HYDROPEROXIDE ENZYME LEVELS AND THE CARDIOTOXIC EFFECTS
OF ADRIAMYCIN ADMINISTRATION IN MICE

Dissertation

Presented in Partial Fulfillment of the
Requirements for the Degree Doctor of Philosophy
In the Graduate School of The Ohio State University

by

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The Ohio State University
1984

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DEDICATION:

To my mother and father,
for instilling in me the value
of an education.
I would like to express my deepest appreciation to Dr. A. John Merola, for taking me "under his wing" and teaching and advising me, and to Dr. Robert Hamlin, for taking time out of his busy schedule to encourage and advise me throughout the length of the study.

I would also like to thank Dr. Robert Bartels for serving on my reading committee, as well as for his help and guidance during my years at Ohio State. In addition, I would like to acknowledge Dr. Edward Fox, whose untimely death most certainly made the completion of this project much more difficult.

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God bless all of you and thanks again.

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<td>Adriamycin</td>
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<td>A.T.P.</td>
<td>Adenosine Triphosphate</td>
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<td>Bathophenanthroline sulphonate</td>
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CHAPTER 1
INTRODUCTION

The human body possesses a remarkable ability to adapt to the various external and internal stresses that are constantly being placed upon it. If a particular stress is habitually introduced to the body a number of mechanisms will be called into play, and eventually strengthened, as they help the body to deal with the stress. Chronic exercise constitutes such a stress, and the research that deals with the adaptive mechanisms the body utilizes, though sometimes contradictory, tend to show that these adaptations have an overall positive effect on the body in general.

Some of the commonly reported training adaptations include an increased maximal cardiac output, increased stroke volume, decreased resting and submaximal heart rate and an increase in the amount of oxygen extracted from circulating blood (83). On a biochemical level, training induces increases in resting levels of various anaerobic substrates (ATP, CP and glycogen) (69), as well as an enhancement of various glycolytic (121,131) and oxidative (62) enzyme levels. The sum total of these various adaptations is, among other things, an augmentation of the skeletal muscle respiratory capacity with increases in the ability to oxidize pyruvate, fatty acids and ketones. The rise in muscle respiratory capacity results from an increase in muscle mitochondria, as well as from an alteration in
mitochondrial composition that makes skeletal muscle mitochondria more like heart mitochondria in their enzyme pattern (63).

This brief overview of exercise training effects is typical of those presented in various physiology and biochemistry texts and classrooms. In making the presentation, the author or instructor usually shows how oxygen utilization is tied to the oxidation of energy substrates by the terminal component of the respiratory chain, cytochrome oxidase. As a result of this process, oxygen accepts four electrons and is reduced to form water (87). The increased oxygen consumed during exercise provides a "sink" for these electrons, resulting in an increased ATP production and a slow down in the production of fatiguing by-products of metabolism (i.e., lactic acid). This presentation, however, does not fully explain the fate of the inspired oxygen. Scientists in general, and exercisephysiologists in particular, have been guilty in a sense of presenting only half of "the oxygen story".

It is now well understood that there are hazards of life in an oxygen rich environment, including toxicity of molecular oxygen and its derivatives, superoxide anion (O$_2^-$), singlet oxygen, hydrogen peroxide and the hydroxyl radical (78). It is, as Fridovich (46) states, "frightening to think that these free radicals of oxygen, long associated with the effects of ionizing radiation, should be normal products of aerobic metabolism." The potency of this oxygen toxicity was vividly shown when rats were placed in an atmosphere of 100% oxygen (only five times the normal amount) and died within 60
hours due to progressive dyspnea and profound hypoxemia resulting from pulmonary edema (85).

Aerobic organisms, however, possess a cellular defense mechanism consisting of the metalloenzymes superoxide dismutase, (which removes O$_2^-$), catalase and various peroxidases (which remove H$_2$O$_2$), thereby preventing the generation of the damaging hydroxyl radical (OH$^*$) (47). So powerful are the effects of these enzymes in preventing the toxic damage of oxygen radicals that anaerobic microorganisms (which lack the enzymes) were killed upon exposure to an oxygen atmosphere (85). In addition, elevated oxygen tensions have been shown to induce superoxide dismutase in microorganisms (51).

Adriamycin, an anthracycline derivative, is an important antineoplastic agent which has proven to be very effective in the treatment of a variety of neoplasms. However, as with most anti-cancer drugs, it is not without its toxic side effects. These include, among other things, myelosuppression, stomatitis, nausea, vomiting and alopecia (20). A unique (and most dangerous) side effect often seen with this class of drug is an irreversible cardiotoxicity, which often leads to cardiomyopathy and congestive heart failure. One of the proposed mechanisms for the development of this cardiotoxicity is the generation of potentially harmful free radicals by Adriamycin induced redox cycling, which could initiate the peroxidation of lipid membranes (96). The susceptibility of the heart to peroxidative damage caused by anthracycline administration may, in part, be explained by the fact that the heart contains lower
amounts of the antioxidant enzymes (particularly SOD and catalase) than do the other major tissues of the body (37).

It has recently been reported that unfit individuals have significantly less skeletal muscle activity of catalase and superoxide dismutase than do trained individuals (67). The same report also stated that when rats were placed on a treadmill running program the activity of these enzymes was increased in various tissues. In addition, it has been found that Adriamycin administration resulted in a dose dependent decrease in cardiac glutathione peroxidase activity.

In light of these findings it may be reasonable to assume that if an individual were pretrained and then administered an anthracycline drug he may be better prepared to detoxify the reactive oxygen metabolites. This hypothesis does not seem unreasonable if one stops to think about the remarkable adaptive capabilities of the body, in addition to the fact that superoxide production within a cell increases proportionally as oxygen concentration increases (28). Exercise, of course, produces a marked increase in oxygen consumption and presents the tissues of the body with alternating hypoxic and hyperoxic conditions.

In addition to looking at the effects of exercise training on the cardiotoxicity of anthracycline administration, this study may have other implications as well. It is not presently known if Adriamycin does, in fact, produce its toxic effects by redox cycling and subsequent lipid peroxidation. The results of this report may provide one more piece of the puzzle which might aid in solving this
controversial question. In addition, the human body is constantly exposed to various xenobiotic toxins (cigarette smoke, asbestos, carbon monoxide, etc.) which may be metabolized by radical mediated pathways (110). Should the exercise training induce enzyme changes, an interesting hypothesis may be generated; that is, is it possible that training may offer protection against pollutants that are encountered daily?

Purpose of the Study

It is the purpose of this study to look at the effects of aerobic exercise training on tissue (heart, liver and blood) levels of the antioxidant enzymes catalase, superoxide dismutase and glutathione peroxidase, as well as on the degenerative myocardial changes often seen with anthracycline administration, as measured histochemically and electrocardiographically.
Adriamycin—An Overview

Adriamycin (Adr), generically referred to as doxorubicin, is an anthracycline antibiotic originally isolated from the aerobic fermentation of Streptomyces peucetius (20). The compound was called Adriamycin because of the proximity of the parent strain source to the Adriatic Sea (134). Adriamycin and daunorubicin (rubomycin), a closely related antibiotic, are effective anti-tumor agents for various malignancies (59). Clinically tested in the United States since 1969, Adriamycin is used to treat hematological malignancies and a wide range of solid tumors (109). Some of the cancers which respond to Adriamycin administration include bladder carcinoma, breast adenocarcinoma, soft tissue and bone carcinomas, testicular carcinoma and acute leukemias (20). In fact, Adriamycin is currently recognized as having the broadest range of clinical usefulness of all of the anticancer drugs in routine clinical use (109).

The structure of Adriamycin (which differs from daunorubicin only in the hydroxylation of C14) is shown in Figure 2.1.
It can be seen that Adriamycin is a tetracyclic structure containing a quinone-like ring with an aminoglycoside, daunosamine, attached via a glycosidic bond. As will be explained in more detail later, the structure of the drug plays an important role in producing its cytotoxic effects. For the time being, it may be stated that an intact molecule is necessary for activity, as is an amino group on the sugar moiety (59).

Adriamycin and daunorubicin are given perenterally, since the glycosidic bond joining the daunosamine and the anthracycline nucleus is split in the gastrointestinal tract, rendering the drug inactive by the oral route (2). Doxorubicin is most commonly given as a single intravenous injection, at a dose of 30-60 mg/m² every three
weeks. This schedule is used primarily because of the prolonged half life of the drug (134).

It appears that the drug is metabolized primarily by the kidneys and liver (1), and that the liver represents the main route of elimination. Riggs, et al. (114) have shown that 40% of the administered dose of Adriamycin appeared in the bile in seven days. In contrast, approximately 10% was accounted for in the urine. Consequently, it appears that a large amount of the original dose (which is unaccounted for in the feces and urine) is retained in the tissues over a long period of time. This has been confirmed by various researchers (4, 55, 91). Bachur, et al. (2) found the highest levels of drug and metabolites in the kidney and spleen. Others have reported lower Adriamycin concentrations in the lungs, liver, heart, intestine and skeletal muscle, with negligible amounts found in the brain. It is of interest to note that, despite the relatively small concentration of drug found in the heart, myocardial cells are extremely sensitive to the effects of Adriamycin.

It is not presently known exactly how Adriamycin produces its cytotoxic effects on tumor cells. Among other things, the drug is known to interfere with oxidative phosphorylation, alter electrolyte composition and movement in the cells, generate free radical compounds and intercalate with D.N.A. (134). Any or all of these factors may be important in producing the antineoplastic activity of the drug. It is the latter effect (intercalation with D.N.A.), however, which has been researched most extensively and is implicated most often as the cause of the cytotoxicity. This association seems
feasible when one takes into account the fact that 60% of intracellular doxorubicin is found bound to the nucleus (103). DiMarco (34) has demonstrated that Adriamycin binds to D.N.A., causing fragmentation of D.N.A. and inhibition of D.N.A. synthesis. In human chromosome preparations, the anthracycline-D.N.A. complex can be observed as well defined orange-red fluorescent bands (76). When an Intercalating compound such as Adriamycin becomes associated with D.N.A., there is a local uncoiling of the double helix as a result of the separation of the stacked bases by the Intercalating moiety. Because of the topological restrictions inherent in the structure of closed circular D.N.A., the supercoiled double helix is changed greatly by the effects of the local uncoiling events (109).

As mentioned previously, the structure of the anthracycline is important for producing cytotoxic activity. It has been shown that the amino sugar of the antibiotic resides in the major groove of the D.N.A., allowing drug and base pair to interact (108). Intercalative binding produces elongation and stiffening of the D.N.A. helix, which presumably follows relaxation of the phosphodiester bond angles (124).

The consequences of the deformation of Intercalated D.N.A. are not well understood. Schwartz, et al. (124) feel that the affinities of at least some of the D.N.A. dependent enzymes (polymerases, nuclease, lligases, etc.) are altered at the drug binding sites. Other researchers (93, 94) feel that enzyme activity is not altered, and that the inhibition of the D.N.A. synthesis is accounted for by the loss of template activity by D.N.A. In the presence of the drug.
It is the feeling of various researchers that D.N.A. binding of intercalative anthracyclines, in and of itself, may not be a necessary or sufficient action to account for anti-tumor activity (124). In addition to the inhibition of nucleic acid synthesis, chromosome damage has been demonstrated as well by a drug-D.N.A. interaction (137); both actions may partially be due to the participation of reactive oxygen species (123). This theory bears greater weight in light of the fact that DiMarco, et al. (34) found little correlation between D.N.A. binding and decreased rates of cell proliferation. Bachur, et al. (3) feel that the drug is activated intracellularly, and produces radicals which lead to breaks in glycosidic linkages. They state that binding and distortion of D.N.A. occurs in *in vitro* systems, but never results in damage or breakage of the D.N.A. strands. In living systems, however, D.N.A. damage has been seen; they attribute this to free radical production in living cells.

As mentioned previously, Adriamycin, as is the case with every anti-neoplastic agent, produces various toxic side effects. Nausea and vomiting occur frequently with the drug, and patients may experience anorexia and diarrhea (109). Local necrosis at the site of injection can be a serious complication if care is not taken to avoid extravasation (118). Myelosuppression, manifested primarily as leukopenia, is common, as are symptoms of anemia, alopecia and thrombocytopenia. The anthracyclines are, in fact, immunosuppressive as well as mutagenic and carcinogenic agents (81).
The most commonly researched and reported side effect of the drug is a dose related and potentially lethal cardiotoxicity. In this respect the anthracycline antibiotics differ from many other anti-cancer drugs which tend to confine their toxic effects to tissues with high mitotic rates, such as bone marrow, the gastrointestinal mucosa and hair follicles (13). Cumulative doses higher than 5.50 mg/m² increase the risk of developing congestive heart failure considerably, and in most cases this dosage is not exceeded (73).

Pathologically, the cardiac changes that occur with Adriamycin toxicity may be subdivided into acute and chronic effects (40). The acute changes consist of early, transient electrocardiographic changes, while the chronic changes generally result in a delayed progressive cardiomyopathy (6). The EKG changes, most of which are reversible, include ST and T wave changes, a prolonged QT interval and a decrease in QRS voltage (134). Although most of the EKG changes are not predictive of cardiomyopathy, a total decrease in the QRS voltage is recognized as useful evidence of myocardial damage (6). Buyniski (18) saw delayed, severe decreases in the QRS voltage of rats administered Adr, and attributed this to underlying cardiac pathologic changes consisting of myocardial cell degeneration and atrophy leading to deterioration of ventricular function. The use of EKG changes as indicators of drug induced myocardial damage has recently been questioned, however, as other factors, such as hematocrit and body build can also affect EKG voltage (134). This line of reasoning provides a rationale for observing EKG voltage
changes in the present study. In some instances, trained individuals have been shown to have a greater QRS voltage than untrained individuals, possibly due to the cardiac hypertrophy commonly reported in training studies (39). It may be of interest to see if this training adaptation acts to "dampen" the decreased QRS voltage generally seen with Adriamycin administration.

The morphological changes observed with chronic toxicity of Adr consists of cardiac dilatation and, less frequently, mural thrombosis, degeneration and atrophy of cardiac muscle cells and interstitial edema fibrosis (40). Loss of myofibrils, distortions of the Z line substance and swollen mitochondria with dense inclusion bodies are observed upon electron microscopic examination (73). In addition, tubular structures coalesce to form large vacuoles and, after a large cumulative dose, myocytes are lost and replaced by connective tissue (134).

Theories abound as to the cause (or causes) of this cardiomyopathy. Olsen, et al. (105) found electrolyte alterations in the myocardium of rabbits receiving Adriamycin. Animals which developed cardiomyopathy had elevations in ventricular Ca, Na and H2O concentrations. The authors concluded that the increased calcium might be instrumental in the development of Adr-induced cardiomyopathy. To test this hypothesis, Young et al. (141) administered verapamil, a cardiovascular active calcium antagonist, to rabbits receiving Adriamycin. Their attempt to alleviate the problem was unsuccessful; in fact, the combination of the two drugs acted synergistically to produce greater cardiac depression.
Adriamycin has a glycosidic structure and is known to inhibit Na-K activated ATPase. This suggests that the Adr effect might occur at the same site as digitalis activity or that a common receptor for the anthracycline and the cardiac glycosides might be necessary for the uptake of the drugs into the heart (13). However, Phillips et al. (107) did not see changes in the severity of the cardiac lesions in rabbits after digitalization with ouabain, digitoxin or strophanthin K. Rosenoff et al. (117) suggested that alterations of D.N.A. synthesis may be responsible for the degenerative myocardial lesions. However, in light of the fact that cardiac cell proliferation ceases after the age of two months (142), this mechanism may not play as large a role in the development of cardiotoxicity as was once anticipated.

Adriamycin has been shown to inhibit CO-Q10 (ubiquinone) enzyme systems in isolated mitochondria (13), and Ogura (104) has shown that ubiquinone administration partially decreased Adr-induced myocardial damage. The bulk of the current research dealing with Adriamycin cardiotoxicity points to the drug induced development of free radicals (and subsequent lipid peroxidation) as the primary cause of damage in the heart; this adds greater meaning to the findings of the aforementioned study, in light of the fact that ubiquinone acts as a free radical scavenger. Unverferth et al. (132) have attributed an observed decrease in cardiac cyclic GMP levels to a free radical inhibition of guanylate cyclase. Several researchers have seen increases in peroxidative end products after Adriamycin administration. Mimnaugh et al. (90) have seen a five-to-seven fold
Increase in malondialdehyde formation, and Mullawan (96) has determined the formation of alkanes (ethane and n-pentane) in rat hepatic microsomes, which is a reliable indicator of microsomal lipid peroxidation. Although Mullawan et al. (97) in a related study did not see an increase in expired pentane in rats administered Adriamycin, they did concede that this was subsequent to an acute dose of the drug, and that chronic treatment might result in higher lipid peroxidation rates.

Probably the most highly quoted studies dealing with Adriamycin administration and lipid peroxidation were performed by Myers et al. (99, 100). In one study, the authors compared survival time of rats injected with 15 mg. of Adriamycin to those pre-treated with 85 units of alpha tocopherol (a known antioxidant) prior to drug administration. The vitamin E treated animals survived a significantly longer period of time than did the untreated animals. The results of a second study of Myers et al. (99) also showed a reduction in lipid peroxidation and cardiac toxicity in vitamin E treated animals, and they further found that the free radical scavengers did not alter the Adr-induced suppression of D.N.A. synthesis or the anti-tumor responsiveness of P-388 ascites tumor cells. These results suggest a dual mechanism of Adriamycin-induced cardiotoxicity and cytotoxicity, and tend to diminish the importance of the aforementioned results of DiMarco (34) and Bachur (2), which suggested that free radical production was, in part, responsible for the cytotoxicity of the drug.
Lipid Peroxidation, Redox Cycling and Free Radical Production

Lipid peroxidation is a continuous physiological process occurring in cell membranes. The process acts as a membrane renewal factor, as well as in the synthesis of prostaglandins and leukotrienes (87). Excessive activation of the process, however, has been implicated as a mechanism in the development of various disease states. Lipid peroxidation has been broadly defined by Tappel (129) as the oxidative deterioration of polyunsaturated lipids. It involves the reaction of oxygen and polyunsaturated lipids to form lipid free radicals and semi-stable hydroperoxides, which then promote free radical chain oxidations (17).

The free radical chain reaction occurs in three distinct steps (17). The first stage is an initiation process, at which time free radicals are generated. The second, or propagation stage involves the conservation of existing free radicals as the peroxidation reactions proceed. The third and final stage, termination, results in the destruction of free radicals. Figure 2:2 schematically depicts the various stages of lipid peroxidation.

\[
\text{Initiation: } \text{Polyunsaturated lipid} \rightarrow \text{Free radical}
\]

\[
\text{Propagation: } R^* + O_2 \rightarrow RO_2^* \\
RO_2^* + RH \rightarrow ROOH + R^*
\]

\[
\text{Termination: } 2 R^* \rightarrow R-R \\
2 ROO^* \rightarrow O_2 + ROOR \\
ROO^* + R \rightarrow ROOR
\]

Figure 2:2: Schematic Representation of Lipid Peroxidation
The presence of double bonds in polyunsaturated fatty acids make them particularly susceptible to peroxidation; a double bond weakens the carbon-hydrogen bond, making allylic hydrogens susceptible to abstraction by small amounts of initiators (31). Once formed, these fatty free radicals enter the propagation phase of lipid peroxidation, and undergo approximately 8 to 14 propagation cycles (140). It is during this propagation cycling period that the bulk of the membrane damage occurs. As can be seen in figure 2:2, chain termination involves the union of two reactive particles to yield stable non-radical products.

There are two important pathways for the production of radicals in living systems; the enzymatically controlled, one electron reduction of molecular oxygen, and reactions initiated by xenobiotics (110). The latter mechanism will be discussed in more detail later in the chapter; the role of molecular oxygen as a source of reactive oxygen species will presently be discussed. Molecular oxygen is paramagnetic because it contains two unpaired electrons with parallel spin states. These unpaired electrons may not reside in the same orbital, as two electrons must have antiparallel spins in order to do so. Thus, in order to reduce oxygen fully by addition of a pair of electrons it is necessary for inversion of one electron spin to occur. As this is a slow process, the problem can be obviated by the successive addition of single electrons. This univalent pathway of oxygen reduction is favored by living systems; however, the products of this pathway are an oxidized organic compound and two superoxide radicals (85). The oxygen molecule can maximally accept four
electrons (and thus form water). Addition of one, two or three electrons leads to the production of superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH*) respectively.

It is obvious that any system which produces O$_2^-$ will soon contain H$_2$O$_2$ as well. This is not to say, however, that H$_2$O$_2$ is formed solely as a result of O$_2^-$ formation. On the contrary, mitochondria have been shown to generate H$_2$O$_2$ at rates which depend on their metabolic state (77). Peroxisomes, organelles which have wide tissue distribution, contain enzymes whose catalytic activity lead to H$_2$O$_2$ formation as well. The aforementioned superoxide radical is also generated in vivo by a number of different enzyme systems and normal cellular processes. Among these are the xanthine oxidase system (48), as well as the autoxidation of catecholamines (25), flavins (89) and the conversion of oxyhemoglobin to methemoglobin (92).

Oxidative enzymes have evolved that circumvent the spin restriction and accomplish divalent and tetravalent reduction of O$_2$ without the release of intermediates. Thus, most of the oxygen consumed by respiring cells is utilized by cytochrome oxidase, which reduces O$_2$ to water without releasing either O$_2^-$ or H$_2$O$_2$ (46). This is not to say that no reactive intermediates are formed during respiration. Britton et al. (14) have estimated that in organisms which had superoxide dismutase activity inhibited, 17% of the oxygen consumption resulted in O$_2^-$ production. Boverls (11) has estimated that in intact organisms between two and five percent of the total electron flux through the chain leaks off to generate superoxide.
The role of $O_2^-$ in the initiation of peroxidation reactions is currently a subject of extensive investigation, as the radical in and of itself does not appear to be directly involved in the initiation of lipid peroxidation (17). In 1934, Haber and Weiss (52) proposed the generation of the highly reactive hydroxyl radical by the following reaction.

$$O_2^- + H_2O_2 \rightarrow OH^* + OH^- + O_2$$

Although this reaction is thermodynamically feasible, kinetically it is so slow that it is practically non-existent in living systems (85). More recent studies have indicated that the basic assumption of the Haber-Weiss reaction is correct, although the presence of a catalyzing agent (most likely iron) is necessary for activity. McCord and Day (86) proposed that $OH^*$ was generated via a Fenton-type reaction as described below.

$$O_2^- + Fe^{+++} \rightarrow O_2 + Fe^{++}$$

$$Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + OH^- + OH^*$$

Net $$O_2^- + H_2O_2 \rightarrow O_2 + OH^- + OH^*$$

Halliwell (53) confirmed the presence of the "Iron catalyzed Haber-Weiss reaction," showing that the above reactions could be inhibited by the iron chelators D.E.T.A.P.A.C. and B.P.S.

Another highly reactive and potentially significant species that has been postulated to be formed as a product of the Haber-Weiss
reactions is singlet oxygen (85). In the singlet state outer electrons are spin paired, eliminating the spin restriction against reactivity, and making singlet oxygen an extremely potent oxidant. Although solid proof of its existence has never been substantiated, Kellogg and Fridovich (71) have postulated that the oxygen molecule appearing as a product in the net Haber-Weiss reaction is actually electronically excited singlet oxygen.

The generation of moderately reactive products from very reactive products and vice versa may have some ominous implications in living systems. It may be possible for the moderately reactive products to diffuse from their site of production and cause damage at distant sites (127). McCay et al. (84) reported the generation of longer lived radicals from hydroxyl radicals in tris buffer, and indicated that these radicals had the capacity for diffusion and delayed reactivity.

What are the cellular consequences of free radical formation? Aside from the deterioration of membrane lipids radical production has been shown to cause changes in membrane protein structure, which may lead to alterations in enzyme activity (31). Mitochondrial and sarcoplasmic reticulum membranes contain high proportions of unsaturated fats, as well as a preponderance of iron associated with their cell membranes. Consequently both organelles appear to be highly susceptible to peroxidative damage (129). Swelling and lysis of mitochondrial membranes, as well as alterations of the activity of NADH-cytochrome C reductase and the succinoxidase system of heart and liver mitochondria have been associated with lipid peroxidation.
damage (17). In addition, breakdown of lysosomal membranes, which leads to the release of proteolytic enzymes (42), erythrocyte hemolysis in vitamin E deficiency (16) and edema seen with anthracycline toxicity (40) have all been attributed to peroxidative destruction.

It was stated earlier that free radicals may be produced in living systems not only by the one electron reduction of molecular oxygen, but by reactions initiated by xenobiotics as well. Adriamycin, a quinoid antibiotic, appears to be such a xenobiotic in that it may be enzymatically reduced and subsequently autoxidize, resulting in the generation of oxygen radicals and hydrogen peroxide (50). Various studies (2, 3) have established that quinone agents in general are catalytically activated to a free radical state by a microsomal system requiring NADPH as an electron donor; Handa and Sato (54) initially demonstrated that anthracycline antibiotics in particular enhanced oxygen consumption (indicative of free radical production) in the presence of NADPH. A scheme of the proposed mechanism is shown in figure 2:3.

Figure 2:3: Proposed mechanism of quinone reduction and autoxidation
It may be seen that microsomal NADPH cytochrome P-450 reductase acts as an electron shuttle between NADPH and the quinone moiety of the anthracycline drug leading to the formation of an anthracycline semiquinone, or free radical, state. Under aerobic conditions, the unshared electron of the anthracycline semiquinone is donated to molecular oxygen to form superoxide anion \((\mathcal{O}_2^-)\).

Further evidence of this P-450 reductase-stimulated redox cycling system was put forth by Goodman and Hochstein (50) who reported oxygen consumption in excess of the amount of drug present in a system containing NADPH, purified P-450 reductase and Adriamycin. Substitution of NADH for NADPH produced no significant oxidation; these phenomena are highly suggestive of redox cycling. In addition, oxidation was reduced by the addition of catalase and superoxide dismutase, suggesting the involvement of \(\text{H}_2\text{O}_2\) and \(\text{O}_2^-\) in the process. Mimnaugh et al. (90) saw a five- to seven-fold increase in malondialdehyde production in rat liver and heart microsomes after Adriamycin administration; they attributed this to the single electron transfer from NADPH to Adriamycin produced semiquinone radicals.

Not all studies are in complete agreement with the above description of redox cycling-induced peroxidative damage purportedly produced by Adriamycin administration. Mullawan et al. (96) suggests that redox cycling itself is not responsible for the \textit{in vitro} lipid peroxidation observed, but that iron-induced lipid peroxidation is stimulated by Adriamycin. Myers et al. (98) showed evidence of a doxorubicin-iron complex in human erythrocyte ghost membranes, with
subsequent binding and damage mediated by both superoxide and \( \text{H}_2\text{O}_2 \) production by the complex. The authors cited a recent study performed by Herman et al. (60) which showed that ICRF-187, an iron chelating agent, lessened the cardiotoxicity seen with Adriamycin as evidence of a drug-iron complex.

Despite these differences in interpreting the phenomena which occur, most researchers tend to conclude that lipid peroxidation is somehow stimulated by anthracycline administration. The theories which state that this is the case, and may be responsible for the cardiotoxicity associated with Adriamycin treatment, are strengthened by the results of a study by Thayer et al. (130) which showed that electron transfer after treatment with doxorubicin \textit{In vitro} is significantly enhanced and leads to an increase in \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) formation in cardiac mitochondria and sarcoplasmic reticulum, two major sites of damage by doxorubicin.

**Role of Scavenger Enzymes**

From the above discussion, it is obvious that aerobic organisms are continually exposed to many potentially damaging oxidative products, both endogenously (i.e., respiration) and exogenously (pollutants, drugs, etc.) produced. Millions of years ago, as the atmosphere was transformed from an anaerobic environment to a largely aerobic environment, organisms were forced to evolve mechanisms not only for the utilization of oxygen but also for defense against its toxic by-products (46). As a result, aerobic organisms possess several different mechanisms which provide protection against
uncontrolled free radical reactions (17). The enzymes superoxide dismutase, glutathione peroxidase and catalase are part of this elaborate defense system present in mammalian cells.

Superoxide dismutase scavenges the superoxide radical by catalyzing the following reaction.

\[ \text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2 \]

Three main types of superoxide dismutases have been isolated and characterized; they are distinguished by the type of metal present in their structure. Enzymes containing copper and zinc, manganese and iron have been described. In eucaryotes, the predominant enzyme is of the copper-zinc type, but a mitochondrial manganese enzyme is also present in many mammalian cells (35).

The importance of SOD as a defense mechanism was demonstrated in a number of studies. McCord et al. (85) showed that aerobic organisms exhibited greater enzyme activity than did aerotolerant organisms (bacteria which had an anaerobic metabolism even when grown in air), and that strict anaerobes, which could not tolerate oxygen, contained no SOD activity. Hassan et al. (56) grew *E. coli* in an aerated rich medium containing glucose, amino acids, purines, pyrimidines and vitamins. The energy needs of the cells were first met by fermentation of the glucose and respiration was low. As the glucose became exhausted the cells relied more on the amino acids and accumulated lactic acid as energy sources, and respiration rose. Superoxide dismutase activity was shown to increase as the fuel
source of the bacteria changed toward amino acid utilization, with a concomitant rise in respiration. The rationale for this observation may be that increased respiration brings about an increased production of $\text{O}_2^-$, which stimulated the increased SOD activity.

From the reaction seen on the previous page, it can be seen that the dismutation of superoxide produces $\text{H}_2\text{O}_2$ which, as mentioned previously, is itself a dangerously reactive substance. However, the cells contain catalases and peroxidases which scavenge the $\text{H}_2\text{O}_2$. Obviously, the enzymes tend to work in concert with one another to maintain low steady state levels of $\text{O}_2^-$ and $\text{H}_2\text{O}_2$. The likelihood that $\text{O}_2^-$ or $\text{H}_2\text{O}_2$ will participate in deleterious reactions, or collaborate in the production of even more reactive species such as $\text{OH}^*$ or singlet oxygen is diminished in proportion to the decrease in their concentrations (125).

Glutathione peroxidase, which consists of both a selenium dependent and non-selenium-dependent enzyme, catalyzes the reduction of hydrogen peroxide to water, and a large range of lipid hydroperoxides to hydroxy acids; the hydrogen donor in this process is uniquely glutathione (35). Catalase reduces $\text{H}_2\text{O}_2$ to water and dioxygen.

\[
\text{H}_2\text{O}_2 \xrightarrow{\text{GSH peroxidase}} 2\text{H}_2\text{O} \\
\text{ROOH} \xrightarrow{\text{GSH peroxidase}} \text{ROH} + \text{H}_2\text{O} \\
2\text{H}_2\text{O}_2 \xrightarrow{\text{catalase}} 2\text{H}_2\text{O} + \text{O}_2
\]
Cohen and Hochstein (26) stated that two H$_2$O$_2$ scavenging enzymes are necessary, as the rate constant for glutathione peroxidase favors low concentrations of H$_2$O$_2$, whereas the rate constant for catalase favors high concentrations of H$_2$O$_2$. Others (41) have disputed this conclusion. Diplock (35) feels that various enzymes are necessary as they are located in different intracellular compartments in eucaryote cells; SOD is cytosolic, catalase is largely located in peroxisomes and glutathione peroxidase is distributed between cytoplasm and the mitochondria.

The role of the various antioxidant enzymes in lessening the toxic effects often seen with anthracycline administration has been demonstrated. Bozzi et al. (12) found that Yoshida ascites cells, which have high glutathione and glutathione peroxidase contents, were more resistant to oxidative damage than were Ehrlich ascites cells, which had low enzyme levels after daunomycin administration. The authors stated that an inverse relationship is apparent between the extent of cellular damage brought about by reactive oxygen metabolites and the efficiency of the glutathione mediated H$_2$O$_2$ detoxifying system.

Revis et al. (112) administered Adriamycin Intravenously to rabbits and observed a decrease in the selenium concentration and glutathione peroxidase activity in the hearts of the Adr treated animals. In a related study Doroshow et al. (37) not only saw a decrease in glutathione activity, but found that the depression of enzyme activity was relatively long lived, leaving the heart with limited defenses subsequent to Adriamycin administration. Mimnaugh
et al. (90) found that superoxide dismutase administration effectively inhibited Adriamycin stimulated microsomal lipid peroxidation. Goodman and Hochstein (50) reported that SOD partially inhibited daunomycin augmented sulfite oxidation, and that the addition of catalase resulted in a decrease in drug-induced oxygen consumption.

Hein et al. (58) and Doroshow et al. (37) have demonstrated that superoxide dismutase and catalase levels in the heart are lower than in many well perfused organs such as kidney and liver. It has been suggested that this could well be the basis for the selected cardiotoxic effects of anthracycline agents (50). If this is, in fact, the case, inducing increases in the activity of these enzymes might possibly lessen the toxic side effects of these drugs. While reports of specific changes in cardiac tissue do not appear in the literature, it has certainly been demonstrated that inducibility of the enzymes is possible under a wide range of physiological and environmental conditions. Frank (45) found that adult rats pre-exposed to 10% O₂ for three days had marked tolerance to hypoxia-induced lung damage and lethality. He attributed this to the fact that hypoxic pre-exposure produced significant elevations in rat lung SOD, glutathione peroxidase and catalase. Conversely, Housset and Junot (64) found that cultures of endothelial cells from pig pulmonary artery and aorta exposed to hyperoxic conditions showed increased superoxide dismutase activity after five days. Gregory et al. (51) obtained similar results in anaerobic bacteria grown under 20 atmospheres of O₂ and attributed induction of the enzyme to a
response to oxygen rather than to a pressure increase, as 20 atmospheres of nitrogen did not stimulate enzyme changes. Gerl et al. (49) found that SOD, catalase and glutathione peroxidase levels were elevated in erythrocytes of patients with beta Thalassaemla minor, an anemic state which has been shown to increase the risk of oxidant stress and may subsequently lead to increased cell membrane peroxidation (111).

**Exercise and Free Radical Proliferation: Exercise and Adriamycin**

It is not unreasonable to assume that a bout of exercise would tend to promote lipid peroxidation within the cells. After all, McCord (85) estimated that for every twenty-five oxygen molecules reduced by cytochrome oxidase, one oxygen molecule picks up an electron from ubisemiquinone. Loschen et al. (77) have shown that the rate of hydrogen peroxide formation in mitochondria is linked directly to the energy coupling mechanism. Exercise, of course, increases the rate of oxygen utilization and the flux of electrons through the mitochondrial electron transport chain and may thus accelerate the rates of free radical formation. In addition, the increases in body temperature, catecholamine levels and substrate cycling, and faster rates of hemoglobin autooxidation during and after exercise may increase the rate of free radical formation. It is also of interest to note that Meerson et al. (87) have determined that lipid peroxidation is enhanced when the terminal component of the respiratory chain is inhibited, leading to the reduction of NAD to NADH, which eventually results in the reduction of molecular
oxygen dissolved in the lipid matrix of the membrane. They further state that the strongest activation of the peroxidative process may be expected when a relative anoxia or hypoxia (with its concomitant accumulation of reduced carriers) is followed by a period of reoxygenation. That is, a phenomenon which produces an excess of electron donors is followed by an event which leads to an excess in electron acceptors.

The preceding chain of events is analogous to a bout of strenuous exercise, and may explain why various studies have shown that exercise does, in fact, appear to increase the production of peroxidative by-products. Ulrey et al. (132) found elevated blood malondialdehyde in horses immediately after ten minutes of exercise. In addition, various components of the erythrocyte glutathione peroxidase system (glutathione and glutathione reductase) were increased as well. In a similar study using human subjects, Dillard et al. (32) found that exercise performed on a bicycle ergometer at 50% of the subjects' maximal oxygen consumption promoted increases in expired pentane concentration. Davies et al. (29) reported a two- to three-fold increase in the free radical concentration of muscle and liver following exercise to exhaustion. They also noted a decrease in mitochondrial respiratory control, loss of sarcoplasmic and endoplasmic reticulum integrity and increased levels of peroxidative end products. The damage seen was akin to the changes produced in non-exercising, vitamin E deficient animals. The authors speculated that the low level membrane damage induced by exercise may be one factor which causes fatigue during prolonged sub-maximal work.
Interestingly, they further theorized that the free radical-induced mitochondrial damage may be the initiating stimulus to mitochondrial biogenesis and the subsequent endurance enhancement often seen with aerobic training.

If Davies and co-workers are correct in their assumptions, they may confirm what various researchers have believed to be true for a long time; that is, exercise (one bout) in and of itself is not beneficial to an organism, and may in fact produce deleterious effects. Chronic training, on the other hand, promotes adaptations to offset the negative effects of an exercise bout, and eventually leads to a stronger and more resistant organism. It is this very hypothesis which provides the basis of this dissertation. Results of the aforementioned study performed by Jenkins et al. (67), which showed that trained individuals had increased levels of the enzymes catalase and superoxide dismutase in various tissues, add strength to the belief that the body has the ability to "make good out of bad" when habitually confronted with a potentially damaging set of circumstances.

There is a paucity of information in the literature dealing with the effects of exercise on the toxic side effects of Adriamycin administration. Combs et al. (27) theorized that an acute exercise stress, which tends to increase myocardial energy demand, might possibly increase the toxicity of Adriamycin. However, forced swimming following the administration of 18 or 23 mg/kg of Adriamycin did not appear to increase the toxicity of the drug, as measured by survival rate. One of the few chronic exercise studies dealing with
Adriamycin cardiotoxicity was performed by Hassler et al. (57), who placed rhesus monkeys on an exercise program following long term Adriamycin administration. The authors reached essentially the same conclusion as Combs et al.; that is, exercise did not enhance cardiotoxicity in rhesus monkeys which had previously been treated with Adriamycin. The mode of exercise used in this study involved isometric strength conditioning; it is doubtful that this exercise, however, which forced the animals to pull on an elaborate weighted pulley system in order to receive food, constituted much of an aerobic stress.

Experimental Model

Similar pathological changes have been reported in a variety of species, including rabbits (65), dogs (136), rats (88), mice (7) and monkeys (57). In all of the studies listed, the authors stated that the particular species in question would be a good model for studying drug induced cardiotoxicity. There are other factors involved, however, which tend to point to the mouse as possibly the best model to use (especially when one is working with a limited budget). Early studies conducted on dogs (36) failed to report cardiac damage. Studies performed with rats and rabbits have demonstrated that in many cases these animals develop kidney lesions (i.e., glomerular nephritis) which are not generally seen in humans (107). In addition, larger animals require a greater amount of drug than do smaller animals, which makes the use of larger animals more costly (88).
CHAPTER 3

METHODOLOGY

Animals:

One hundred and five male Swiss white mice weighing between 30 and 32g were purchased from the Charles River Company, Wilmington, Massachusetts. The mice were approximately 35 days old at commencement of the study. Animals were housed 10 or 11 to a cage in a controlled environment of 22°C and 30° relative humidity. A standard diet consisting of Purina Rat Chow and water were given ad libitum, while a cycle of 12 hours daylight and 12 hours darkness was maintained throughout the duration of the study.

Randomization:

Upon arrival, the animals were randomly assigned to either an exercise or non-exercise group. After nine weeks, the animals were further subdivided in a random fashion into drugged and non-drugged groups. A schematic view of the randomization process appears below.
Identification:

The mice had their ears pierced for identification purposes. A hole in the right ear indicated that the animal was a swimmer; a hole in the left ear indicated that the animal was receiving Adriamycin.

Exercise: Mode and Protocol:

The mice were swum five times per week in rust proof metal drums 36 cm. high and 70 cm. wide. Approximately 25 mice were placed in each drum simultaneously. Water temperature was maintained at 26°C (86°F). The animals initially were forced to swim for 15 minutes daily, and after two weeks they had progressed to the point where they were swimming for 50 minutes per day, five times per week. The animals were maintained on this schedule throughout the duration of the study. Non-swimming controls were placed in a 3cm. depth of water three times per week in an attempt to offset as many thermal and psychological variables as possible.
The rationale for this training protocol is based on the findings of various studies. Dawson and Horvath (30) presented data which showed that time to exhaustion in laboratory mice is approximately one hour when the water temperature is maintained at 28°C. Most animals show a decline in swimming capacity in water of a temperature much higher or lower than body temperature (5). In addition, Wilbur and Hunn (138) have demonstrated that group swimming greatly reduces time to exhaustion in swimming mice.

**Drug Dosing Regimen:**

Adriamycin hydrochloride was supplied by Adria Laboratories, Columbus, Ohio. The dosing schedule used was that of Bertazzoli et al. (7). Adriamycin, dissolved in saline, was injected intravenously through a tail vein twice a week (Tuesday and Friday) ten times at a dose of 4mg./kg./day. The animals were not treated for two weeks between the first four injections and the last six injections to allow recovery of bone marrow depression. Four weeks after the last injection the mice were sacrificed. Non-drugged controls were injected with saline in an identical manner.

<table>
<thead>
<tr>
<th></th>
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</tbody>
</table>

*Figure 3:2: Drug Dosing Schedule*
The total cumulative dose of 40 mg./kg. corresponds to Bertazzoli's calculation of a median cumulative cardiotoxic dose of 36.4 mg/kg.

**Excision and Storage of Tissue:**

After nine weeks of swim training, nine exercising and nine non-exercising mice were sacrificed; at the conclusion of the eleven week drug dosing schedule the remainder of the mice were killed. Animals were not exercised for 24 hours prior to sacrifice. Tissue was removed and stored in a similar manner during both sacrifice periods, with one modification to be described below.

The mice were injected interperitoneally with .05 to .1 cc (3-6 mg) pentobarbital. After EKG's were taken, the mice were decapitated and exsanguinated. Blood was collected into heparinized tubes and immediately placed in liquid nitrogen. Livers were removed and sectioned into thirds, then wrapped in foil and placed in liquid nitrogen as well. The mouse's chest cavity was transected, and the heart was removed and immediately washed free of erythrocytes in physiologic saline, blotted dry, weighed and placed in liquid nitrogen. After all of the tissue had been excised and frozen, the hearts were removed from the liquid nitrogen, pulverized with a mortar and pestle and divided into thirds. During the second sacrifice period, hearts were sectioned in half after they were weighed by making a medial sagittal cut at right angles through the intraventricular septum with a sharp scalpel blade. The left half of the heart was frozen in liquid nitrogen for enzyme analysis; the
right half was placed in paraformaldehyde, so that it could later be
stained and fixed for histological analysis.

During the first sacrifice period the right hindlimb skin was
reflected and the quadricep muscle was excised and frozen for enzyme
analysis. It was not removed during the final kill after the
twentieth week. The entire procedure took approximately four to five
minutes per animal. At the conclusion of each sacrifice period all
of the tissue was removed from the liquid nitrogen and stored in a
freezer at -70°C until it was analyzed.

Chemicals:

The following chemicals were purchased from the Sigma Chemical
Company, St. Louis, Missouri: Cytochrome C, xanthine oxidase,
glutathione reductase, NADPH, polyvinylpyrolidone. Other chemicals
used were of reagent grade and were supplied by various laboratories
at the Ohio State University.

General Enzyme Assay Procedures

Superoxide Dismutase

Cardiac, hepatic and whole blood superoxide dismutase activity
was assessed by the method of Keele, McCord and Fridovich (70). This
indirect assay procedure depends on the ability of superoxide
dismutase to compete with ferricytochrome C for superoxide anions
generated by the aerobic xanthine oxidase system.

Heart samples, which consisted of the pooled cardiac tissue of
three mice after the first kill and of four mice after the second
kill, and liver samples consisting of approximately 500 mg. replicates of hepatic tissue were used to prepare a single sample for the determination of enzymatic activity. Whole blood, lysed in one ml. of ice cold distilled water, then further diluted 1:40 was used to determine blood levels of the enzyme.

Tissue samples were suspended in 10 volumes of iced 0.05M potassium phosphate buffer, pH 7.8, which contained 100µM E.D.T.A. These suspensions were homogenized on ice with three strokes of a Polytron homogenizer. The crude homogenate was centrifuged for 30 minutes at 750xg in a Sorvall RC-Z model centrifuge at 4°C. The supernate was decanted at the end of centrifugation and used directly to determine SOD activity.

The spectrophotometric procedure was performed in a Guilford recording spectrophotometer (Guilford Instruments Laboratories, Oberlin, Ohio). The standard assay was performed in 3 ml. of a solution containing 1X10⁻⁵M ferricytochrome C, 5X10⁻⁵M xanthine and 1X10⁻⁵KCN. Approximately 8 µl. xanthine oxidase was necessary to produce a rate of reduction of cytochrome C of 0.025 absorbance per minute at 550 nm. and 25°C. The absorbance decay was recorded for three minutes following the addition of xanthine oxidase, and the mean absorbance change per minute was used. Under these specified conditions one unit of superoxide dismutase activity was that amount which halved the rate of reduction of cytochrome C. Activity was expressed as units per milligram protein.
Glutathione Peroxidase

Glutathione peroxidase activity was assayed by a modification of the method of Paglia and Valentine (106) as used by Lawrence and Burk (72). The method measures the rate of glutathione oxidation by H$_2$O$_2$ as catalyzed by the glutathione peroxidase present in the homogenate. Rather than measuring progressive loss of glutathione, however, this substrate is maintained at a constant concentration by the addition of exogenous glutathione reductase and NADPH, which immediately convert any oxidized glutathione (GSSG) produced to reduced form:

\[
2\text{GSH} + \text{H}_2\text{O}_2 \xrightarrow{\text{GSH-Px}} \text{H}_2\text{O} + \text{GSSG} \xrightarrow{\text{GSSG-R}} 2\text{GSH}
\]

The rate of GSSG formation is then measured by following the decrease in absorbance of the reaction mixture of 340 nm as NADPH is converted to NADP.

Liver and heart tissue were pooled in an identical manner as that described for SOD analysis. The tissue was homogenized on ice in a Polytron homogenizer set at low speed in a buffer containing 10 mM tris, pH 7.4, 250 mM sucrose, 1 mM EDTA and 1 mM dithiothreitol (125). The crude tissue homogenate was centrifuged for 30 minutes at 750 X g in a refrigerated centrifuge at 4°C. The 750 xg homogenate was centrifuged again for 15 minutes at 1000 xg and the resulting homogenate was used for enzyme activity determination. Whole blood samples were prepared by combining 0.05 ml. blood, 0.05 ml. saline,
1.0 ml. of 0.02 M phosphate buffer, pH 7, and .1 ml. Drabkins reagent (0.0016M KCN, 0.0012 M K$_2$Fe(CN)$_6$ and 0.0238 M NaHCO$_3$).

The reaction mixture consisted of 0.025 ml. of 50 mM KH$_2$PO$_4$, pH 7, 0.20 ml. of 1 mM EDTA, 0.10 ml. of 1 mM NaN$_3$, 0.10 ml. of 0.25 mM NADPH, 0.10 ml. of 1 EU GSSG reductase/ml. reaction mixture and 0.10 ml. of 10 mM GSH. 0.05 ml. of the sample enzyme source was added to the mixture to bring the volume up to 0.9 ml. After incubating for five minutes at room temperature, 0.1 ml. of 0.25 mM H$_2$O$_2$ was added to initiate the reaction. Enzyme activity was recorded for three minutes, and the mean absorbance change per minute was used. Activity was calculated from the extinction coefficient of 6.2 X 10$^6$. One unit of glutathione peroxidase activity represented the oxidation of 1 μmole NADPH/min. at 25° C. Enzyme activity was expressed as units per milligram protein.

**Catalase**

Catalase activity was assayed by the method of Cohen et al. (24). The assay measures catalase activity by titration of residual H$_2$O$_2$ with permanganate. The method provides linear first order kinetics and a linear dose response relationship.

Cardiac, hepatic and whole blood samples were obtained as previously described for superoxide dismutase, except for the use of an isotonic buffer containing NaCl and 0.01 M sodium phosphate buffered at pH 7.4, 10% Triton X-100 and ethanol for the preparation of the homogenate. Blood samples (0.2 ml) were lysed in 1.0 ml. of ice cold distilled water and diluted 1:40 in cold distilled water.
The reaction mixture consisted of 0.01 M phosphate buffer, pH 7.0, 6 mM H₂O₂, 6 N H₂SO₄ and 0.01 N KMnO₄.

Duplicate catalase samples were prepared by mixing 0.5 ml. tissue with 5.0 ml. cold H₂O₂ to initiate the reaction. After exactly three minutes the reaction was stopped by the addition of 1.0 ml. H₂SO₄. The H₂O₂ was measured by reacting it with a standard excess (7 ml.) of KMnO₄ and then measuring the residual KMnO₄ spectrophotometrically at 480 nm. Duplicate blanks were prepared simultaneously.

Under the conditions described, the decomposition of H₂O₂ by catalase follows first order kinetics as given by the equation:

$$ K = \log \left( \frac{S_0}{S_3} \right) \times 2.3t $$

- $K$ = first order reaction rate constant.
- $S_0$ = substrate concentration at zero time.
- $S_3$ = substrate concentration at three minutes.
- $t$ = time interval over which reaction is measured.

Enzyme activity was expressed as K per mg. protein, or, in the case of the blood samples, K per ml. blood.

**Succinic Oxidase**

Succinic oxidase activity of whole homogenate preparations of quadriceps muscle was measured by a modification of the method of Chance and Williams (22). The assay medium contained 0.25 M sucrose, 5 mM potassium succinate, 4 mM potassium phosphate, 4 mM MgCl₂ and 10 µM rotenone, to which 0.25 ml. of homogenate was added. The final
volume was 2 ml. The final pH 7.4. State 3 respiration was measured by the addition of 0.5 μMoles ADP. In addition, exogenous cytochrome C (7.5 μM) was added, as endogenous cytochrome C is easily leached off the mitochondrial membrane by salt solutions, with predictable effects on respiratory capacity. Finally, S13 (1 μM), a respiratory uncoupler, was added to the assay mixture in an effort to further stimulate respiration.

The rate of oxygen uptake was followed by using a recording Yellow Springs oxygen polarograph (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio) with a Clark type electrode at 25°C. All measurements were made in duplicate and the average of the two runs was used for the determination of respiratory capacity. The rate of oxygen consumption was calculated on the assumption that the O2 concentration in an aqueous solution at 25°C is 480 nanoatoms of oxygen per ml. (127). Enzyme activity is expressed as nanoatoms oxygen per min. per mg. protein. A representative trace of succinate supported respiration can be seen in Figure 3.3.

Protein Analysis

The protein concentrations of the homogenate of hepatic and cardiac tissues were determined by a variation of the Folin Phenol Reagent method of Lowry et al. (79). Two reagents were prepared; reagent A consisted of 1.5 ml. of 0.5% CuSO4·5H2O, 1.5 ml. of 1% K-tartrate and 30 ml. of 10% Na2CO3 in 5N NaOH. Reagent B consisted of 2N phenol reagent in distilled water. A protein standard of
bovine serum albumin (B.S.A) was prepared (5 mg./25 ml.) and a standard curve range of 0-160 μg. protein was used for each analysis.

0.05 ml. of the homogenate was diluted in 0.95 ml. distilled water, mixed with 1 ml. reagent A and allowed to stand at room temperature for 10 minutes. At that time, 3.0 ml. reagent B was added to each tube, vortexed and incubated in a water bath at 50°C for 15 minutes. Samples were read at a wavelength of 600 nm in a Guilford Spectrophotometer, model 240, at 25°C. A standard curve was plotted each day, and the unknown protein concentrations were calculated from it. A representative sample analysis can be seen in Figure 3:4.

**Electrocardiography**

Electrocardiographic records were performed with a Honeywell E for M model photographic oscillillograph. Recordings were made prior to sacrifice at the end of the ninth and twentieth week of the study. EKG's were calibrated using 1 mV amplitude (vertical) calibration signals derived from the EKG amplifier. Three leads (I, AVF and V_{10}) were recorded on each animal after they had been anesthetized by administration of 3-6 mgs. pentobarbital. Heart rate and total peak to peak QRS amplitude (measured in lead AVF) were calculated for each animal, as well as the P wave in lead I, Q_{10}, R in I and the S/R ratio in leads I and AVF.
SW - swimming mice
SED - sedentary mice
CON - control, succinate free

Figure 3.3: Oxygen polarograph traces for succinate oxidase activity in exercised and sedentary animals and a control trace.
Figure 3:4: Representative Standard Protein Curve
Histology: Tissue Preparation and Cardiomyopathic Analysis

Half of the heart tissue was fixed in 4% paraformaldehyde in a 0.1 M phosphate buffer, pH 7.3, and stored at 4°C. The tissue was washed in phosphate buffer for one hour and dehydrated in an alcohol solution for a total of 4.5 hours (50% alcohol for 30 min., 70% alcohol for one hour, 95% alcohol for two hours, 100% alcohol for one hour). Tissue was placed in an embedding medium of glycol methacrylate. Tissue embedded in this media offer superior ultrastructural preservation and semi-thin sections for high resolution light microscopy (80).

After infiltration for a period of 48 hours the tissue was embedded in molds with catalyzed methacrylate, covered, and stored overnight. 1.5 to 2 micron sections were made with a H/I Hacker microtome. One section was stained with 0.2% toluidine blue in 1% Borax solution for 1/2 hour. A second section was stained using Masson's Trichrome method, which results in collagen turning blue, nuclei black and cytoplasm, keratin and fibers staining red. The two sections were used, respectively, to measure vacuolization and myocyte damage (toluidine blue) as well as diffuse and focal fibrosis (trichrome).

Adriamycin cardiotoxicity was quantitatively evaluated by the method of Bertazzoli et al. (7). The heart lesions were evaluated on one median section of tissue and scored according to severity and extension, as shown in Figure 3:5. The product of severity and the extent of the damage observed in each heart gave a single score used for the measurement of cardiotoxicity.
### Degree Definition

#### Severity

1. Sarcoplasmic microvacuolizations and/or inclusions and interstitial or cellular edema.

2. Same as 1 plus sarcoplasmic macrovacuolizations or atrophy, necrosis, fibrosis, endocardial lesions and thrombi.

#### Extension

0. No lesions.

0.5. < 10 single altered myocytes in the whole heart section.

1. Scattered single altered myocytes.

2. Scattered small groups of altered myocytes.

3. Widely spread small groups of altered myocytes.

4. Confluent groups of altered myocytes.

5. Most cells damaged.

---

**Figure 3:5:** Morphologic evaluation of cardiac lesions as measured by Bertazzoli, et al.

---

**Statistical Analysis**

The data were analyzed using t-tests for independent means or one and two way analyses of variance (ANOVA) as appropriate.
CHAPTER 4
RESULTS

Mortality

At the onset of the drug dosing regimen (after nine weeks of swim training) there were twenty mice in three of the four experimental groups. The sedentary drug group had 22 mice. At the conclusion of the study, the swimming group contained 20 mice, the swim drug group had 16 mice, the sedentary group had 18 mice and the sedentary drug group had 22 mice. Table 4.1 summarizes the cause of death of the animals in each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Swim</th>
<th>Swim Drug</th>
<th>Sedentary</th>
<th>Sedentary</th>
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<tr>
<td>Original N</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>Death by Drowning</td>
<td>--</td>
<td>4</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Death during drug admin.</td>
<td>--</td>
<td>--</td>
<td>1</td>
<td>--</td>
</tr>
<tr>
<td>Natural causes</td>
<td>--</td>
<td>--</td>
<td>1</td>
<td>--</td>
</tr>
<tr>
<td>Final N</td>
<td>20</td>
<td>16</td>
<td>18</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 4.1: Mortality In the Various Experimental Groups
Enzyme adaptations following chronic aerobic exercise training

Succinic Oxidase

In order to biochemically determine if the mode of exercise employed was in fact producing a "training effect" in the animals, it was necessary to measure the activity of an enzyme involved in aerobic metabolism. The tissue chosen for analysis was the right quadriceps muscle, a mixed (i.e., containing both fast and slow twitch fibers) muscle located in the hind limb of the mouse. As the animals appeared to use their hind quarters almost exclusively while executing the swimming motion, the choice of muscle used to determine the efficacy of the training program appears justified.

The ability of the whole cytochrome chain to handle reducing equivalents ($H^+$ and $e^-$) gives a precise measure of the respiratory capacity of mammalian tissue. A simple and accurate method is to use succinate as a substrate, block endogenous NADH oxidation by rotenone and then follow the rate of oxygen uptake as it is reduced to water at the terminal cytochrome "a3". This method has been used by various researchers (62, 63) to measure succinic oxidase activity in a variety of tissues.

As can be seen in Figure 4.1, the exercised animals had greater quadriceps succinic oxidase activity than did the sedentary animals after nine weeks of aerobic swim training (17.32 nanoatoms O$_2$ min$^{-1}$ mg$^{-1}$ vs. 12.43 nanoatoms O$_2$ min$^{-1}$ mg$^{-1}$). The difference was significant at the 0.05 level. Addition of ADP, cytochrome C and S13 did not significantly affect oxygen uptake.
Figure 4.1: Succinic oxidase activity in mouse quadriceps muscle after nine weeks of aerobic training.

** significantly greater than sedentary control group (p < 0.05)
Catalase

Catalase activity was measured in the livers, blood and hearts of animals after nine weeks of swim training and again at the conclusion of the study. As catalase is involved in the detoxification of potentially harmful H₂O₂, increases were viewed as a positive adaptation. After nine weeks of swimming, liver levels of the enzyme did increase, although the change was not statistically significant (92.5 K/mg protein vs. 86.7 K/mg protein). Catalase activity in the heart did not change significantly either (3.81 K/mg protein vs. 3.27 K/mg protein). However, blood levels of the enzyme did rise dramatically (125.2 K/ml blood vs. 74.9 K/ml blood). The difference was statistically significant at the 0.05 level. See figures 4.2 and 4.3.

At the conclusion of the study, the swimming animals displayed significantly greater liver catalase activity than did the sedentary animals. The difference persisted in the animals that received Adriamycin as well, indicating that the exercise, and not the drug, was responsible for promoting significant changes in enzyme activity. This assertion was statistically demonstrated by means of a two way ANOVA, which showed that exercise greatly effected mean catalase levels in liver (PR > F = 0.0001), whereas the drug did not display a significant effect (PR > F = .22). It is of interest to note, however, that the swim-drug group had greater enzyme levels than did the swim-non drug group, and that the sedentary drug group had higher liver catalase than did the sedentary non drug group, In spite of the fact that the results were not statistically significant.
Exercise promoted a significant rise in heart catalase levels as well at the 0.05 level (least squares mean (LSM) 5.03 K/mg pro vs. LSM 3.39 K/mg protein). Adriamycin did not have a significant effect on heart enzyme levels (LSM 4.72 without drugs vs. 3.70 with drug). A point of interest worth noting was the mean heart catalase level of the swimmers, which was higher than that of the swim-drug animals. It may be possible that an oxidant stress produced by Adriamycin administration was responsible for lessening the rise in enzyme levels seen with exercise alone.

As was the case with liver catalase, exercise promoted a significant increase in mean blood catalase levels, regardless of drug status. These findings were consistent with the changes reported after nine weeks of aerobic training, and again point to the fact that exercise, and not Adriamycin administration, was responsible for the rise in blood catalase levels. See figures 4.4 and 4.5.

In addition, liver and blood catalase levels were significantly greater in the swim trained mice at the end of twenty one weeks of training than after nine weeks of training ($\bar{X} = 139.7$ K/mg protein vs. 92.5 K/mg protein). Obviously, a training adaptation occurred ($\bar{X}$ blood = 126.2 K/ml vs. 107.4 K/ml) as the length of the training period was increased.
Figure 4.2: Catalase Activity in Liver and Heart of Mice After Nine Weeks of Aerobic Training.
Figure 4.3: Catalase Activity in Blood of Mice After Nine Weeks of Aerobic Training

** significantly different from sedentary control group (p < 0.01)
Figure 4.4: Catalase Activity in Liver and Heart of Mice After 21 Weeks of Swimming and/or 40 mg/kg Cumulative Dose of Adriamycin.

Swimming produced significant increases in both tissues.
Figure 4.5: Catalase Activity in Blood of Mice After 21 Weeks of Swimming and/or 40 mg/kg Cumulative Dose of Adriamycin

** significantly different from sedentary animals (p < 0.05)
Glutathione Peroxidase

As was the case with catalase, glutathione peroxidase activity was measured in liver, blood and heart tissue after nine and 21 weeks of training. Significant increases in blood (Figure 4.7) and liver enzyme levels were recorded following nine weeks of swimming. Swim trained animals had a mean liver glutathione peroxidase level of 108.9 U/mg protein as opposed to 53.8 U/mg protein for sedentary controls (p < 0.01). Swim trained mice had mean blood levels of 10.88 U/ml blood versus 8.28 U/ml blood for sedentary mice (p < 0.01). As seen in figure 4.6, heart enzyme levels were greater in the swimmers as well (49.7 U/mg protein vs. 40.7 U/mg protein) although the difference was not statistically significant. This may be partially attributable to the small sample size (N=3).

After twenty one weeks of training, mean blood glutathione peroxidase levels were significantly greater in swimmers than non-swimmers (figure 4.9). The difference was attributed to the exercise regimen, and not Adrlamycin administration, as both the drugged and non drugged swimmers displayed higher enzyme levels than did the sedentary drugged and non drugged animals. In addition, blood enzyme levels were significantly higher in swimmers after 21 weeks of training than after nine weeks, indicative of an additional training effect as the exercise period was prolonged.

A significant exercise drug interaction was shown to take place in the case of glutathione peroxidase liver levels after 21 weeks of training (PR > F = 0.03). The swim non drugged mice had greater enzyme levels than did the swim drugged animals; however, the drugged
sedentary group had greater levels than did the non drugged sedentary animals. Despite this paradox in enzyme activity, both exercised groups had elevated glutathione peroxidase levels when compared with the sedentary groups, which may be viewed as a positive adaptation to the training in and of itself.

A statistically significant difference was shown to exist in the levels of glutathione peroxidase in the hearts of drugged and non drugged animals. These results are in agreement with those of Doroshow et al. (37), and point to a possible link between Adriamycin administration and the weakening of an important system necessary for combating free radical damage. Exercise alone increases glutathione peroxidase in the heart; in conjunction with Adriamycin treatment, however, enzyme levels were below control values.

**Superoxide Dismutase**

Superoxide dismutase levels were measured after nine and 21 weeks of training in blood, liver and heart tissue. The enzyme is involved in the scavenging of superoxide radicals by catalyzing the reaction:

\[ \text{O}_2 + \text{O}_2 + 2\text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2 \]

After nine weeks of swimming, blood levels of the enzyme were significantly increased \( (p < 0.01) \) in trained animals (247.1 U/ml blood vs. 164.2 U/ml blood) (see Figure 4.11). Heart and liver levels, however, did not change significantly as shown in figure 4.10. Swim trained animals had mean heart and liver SOD levels of
Figure 4.6: Glutathione Peroxidase Activity in Liver and Heart of Mice After Nine Weeks of Aerobic Training

** Significantly different from sedentary control group (p < 0.01).
Figure 4.7: Glutathione Peroxidase Activity in Blood of Mice After Nine Weeks of Aerobic Training

** Significantly different from sedentary control group \( p < 0.01 \).
Figure 4.8: Glutathione Peroxidase Activity in Liver and Heart of Mice after 21 Weeks of Swimming and/or 40 mg/kg Cumulative Dose of Adriamycin

Swimming produced a significant increase in liver. A significant exercise drug interaction occurred in heart. See text for details.
Figure 4.9: Glutathione Peroxidase Activity in Blood of Mice After 21 Weeks of Swimming and/or 40 mg/kg Cumulative Dose of Adriamycin.

* Significant greater than sedentary animals (p < 0.05)

Enzyme Units/ml. blood

**
12.06 U/mg protein and 34.24 U/mg protein respectively, while sedentary controls displayed mean heart levels of 13.15 U/mg and mean liver levels of 33.54 U/mg.

At the conclusion of the study, blood SOD levels remained significantly higher in the swimming animals, despite their drug status. Swimmers displayed a least square mean of 269.8 U/ml blood, as opposed to a mean of 176.1 U/ml blood for the sedentary mice. As previously stated, these results lead one to conclude that the exercise regimen, and not the drug, was responsible for producing the difference seen. See figure 4.13.

A significant exercise drug interaction was shown to exist in assessing the effects of exercise and Adriamycin on SOD liver levels. As seen in figure 4.12, the two non drugged groups had greater SOD liver levels than did the drugged groups; however, the trained non drugged mice had the highest recorded enzyme levels, while the trained drugged animals had the lowest SOD levels. While these seemingly contradictory results are somewhat difficult to explain, it appears safe to say that Adriamycin administration tends to lower SOD levels in the liver. Exercise, on the other hand, seems to increase liver enzyme levels; in conjunction with drug administration, however, this apparently beneficial adaptation is lost.

As illustrated in figure 4.12, heart levels of superoxide dismutase did not change significantly in response to exercise or drug administration. It should be noted, however, that swim trained animals had significantly greater SOD levels in all three tissues after 21 weeks of training than after nine weeks of training. While
the implications of these findings will be discussed further in the
following chapter, they are pointed out at this time to illustrate
the importance of the selected training durations in studies designed
to assess the adaptability of various enzymes.

A summary of the various enzymatic changes which occurred with
training and drug administration are listed in tables 4.2 - 4.4.

EKG changes with training and/or drug administration

Electrocardiographic measurements were made prior to sacrifice
after 21 weeks of training. Three leads (I, AVF, and V10) were
recorded after the animals had been anesthetized by administration of
0.05-1.0 cc pentobarbital.

P wave in lead I

Both drug and exercise significantly (p < 0.05) affected the
height of the P wave in lead I. As demonstrated in Figure 4.14,
swimming mice displayed the longest P waves, followed by sedentary
control mice, swim-drugged mice and sedentary drugged mice.
Obviously, exercise served to increase P wave amplitude, while
Adriamycin diminished voltage. A combination of drug and exercise
significantly reduced voltage below control levels; however, the
decrease was not nearly as great as that seen in the sedentary
drugged animals.
Figure 4.10: Superoxide Dismutase Activity in Liver and Heart of Mice After Nine Weeks of Aerobic Training
Figure 4.11: Superoxide Dismutase Activity in Blood of Mice After Nine Weeks of Aerobic Training.

** significantly different from sedentary control group (p < 0.01).
Figure 4.12: Superoxide Dismutase Activity in Liver and Heart of Mice After 21 Weeks of Swimming and/or 40 mg/kg cumulative Dose of Adriamycin

A significant exercise drug interaction occured in liver. No significant change was seen in heart. See text for details.
Figure 4.13: Superoxide Dismutase Activity in Blood of Mice After 21 Weeks of Swimming and/or 40 mg/kg Cumulative Dose of Adriamycin

** significantly greater than sedentary animals (p < 0.05)
Table 4.2
Enzyme Activities in Various Tissues Following Nine Weeks of Exercise Training

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Group</th>
<th>Catalase</th>
<th>Glutathione Peroxidase</th>
<th>Superoxide Dismutase</th>
<th>Succinic Oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Swim</td>
<td>126.2*</td>
<td>10.88*</td>
<td>247.1*</td>
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</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>± 20.97</td>
<td>± 0.61</td>
<td>± 21.2</td>
<td>---</td>
</tr>
<tr>
<td>Liver</td>
<td>Swim</td>
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<td>108.92*</td>
<td>34.24</td>
<td>---</td>
</tr>
<tr>
<td></td>
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<td>± 19.75</td>
<td>± 2.67</td>
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</tr>
<tr>
<td>Heart</td>
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</tr>
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<td>± 15.5</td>
<td>± 1.36</td>
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</tr>
<tr>
<td>Quadriceps</td>
<td>Swim</td>
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<td>---</td>
<td>17.32*</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>± 4.32</td>
</tr>
</tbody>
</table>

Values are means ± S.O. expressed as enzyme units/mg. protein for heart, liver and quadriceps tissue. Blood levels are expressed as units/ml. blood.

* significantly different from sedentary control group p < 0.01.
### Table 4.3

Enzymatic Activities in Various Tissues Following 21 Weeks of Exercise Training and/or 40 mg/kg Cumulative Dose of Adlamycin

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Group</th>
<th>Catalase</th>
<th>Glutathione Peroxidase</th>
<th>Superoxide Dismutase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Swim</td>
<td>107.35*</td>
<td>811.02*</td>
<td>273.28*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 46.2</td>
<td>± 0.45</td>
<td>± 9.93</td>
</tr>
<tr>
<td></td>
<td>Swim-Drug</td>
<td>98.79*</td>
<td>10.30**</td>
<td>266.31*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 6.99</td>
<td>± 0.42</td>
<td>± 12.16</td>
</tr>
<tr>
<td></td>
<td>Sedentary-Drug</td>
<td>75.37</td>
<td>8.97</td>
<td>175.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 4.92</td>
<td>± 0.29</td>
<td>± 9.39</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>75.36</td>
<td>9.55</td>
<td>176.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 4.46</td>
<td>± 0.36</td>
<td>± 14.84</td>
</tr>
<tr>
<td>Liver</td>
<td>Swim</td>
<td>139.70*</td>
<td>94.51*</td>
<td>42.41*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 17.38</td>
<td>± 3.36</td>
<td>± 1.27</td>
</tr>
<tr>
<td></td>
<td>Swim-Drug</td>
<td>157.80*</td>
<td>88.01*</td>
<td>32.58*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 16.18</td>
<td>± 1.99</td>
<td>± 1.25</td>
</tr>
<tr>
<td></td>
<td>Sedentary-Drug</td>
<td>97.98</td>
<td>62.11</td>
<td>32.95*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 12.83</td>
<td>± 1.81</td>
<td>± 0.92</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>81.73</td>
<td>57.72</td>
<td>38.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 5.00</td>
<td>± 1.97</td>
<td>± 0.93</td>
</tr>
<tr>
<td>Heart</td>
<td>Swim</td>
<td>86.14*</td>
<td>55.60**</td>
<td>17.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.58</td>
<td>± 2.12</td>
<td>± 0.96</td>
</tr>
<tr>
<td></td>
<td>Swim-Drug</td>
<td>83.93</td>
<td>b51.18</td>
<td>15.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.58</td>
<td>± 2.86</td>
<td>± 0.90</td>
</tr>
<tr>
<td></td>
<td>Sedentary-Drug</td>
<td>3.48</td>
<td>b46.00</td>
<td>15.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.85</td>
<td>± 1.73</td>
<td>± 0.59</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>3.30</td>
<td>52.00</td>
<td>15.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.79</td>
<td>± 2.58</td>
<td>± 0.40</td>
</tr>
</tbody>
</table>

- * values are linear means ± SEM expressed as enzyme units/mg protein for heart and liver, and unit/ml blood for blood.
- * significantly different from sedentary animals (p < 0.05).
- ** significantly different from sedentary drugged animals (p < 0.05).
- a combined swim group means significantly greater than combined sedentary group means (p < 0.05), using two-way ANOVA.
- b combined drugged group means significantly lower than combined non-drugged group means (p < 0.05), using two-way ANOVA.
Table 4.4

Least Square Means (L.S.M.) and Standard Error of the Mean (S.E.M.) for Various Groups used In Computing Two Way Analysis of Variance

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Group</th>
<th>Catalase</th>
<th>Glutathione Peroxidase</th>
<th>Superoxide Dismutase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Exercise</td>
<td>103.07 ± 3.73</td>
<td>10.66 ± 0.28</td>
<td>269.80 ± 8.52</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>75.36 ± 3.47</td>
<td>9.26 ± 0.26</td>
<td>176.13 ± 7.93</td>
</tr>
<tr>
<td></td>
<td>Drug</td>
<td>87.08 ± 3.66</td>
<td>9.63 ± 0.28</td>
<td>220.88 ± 9.76</td>
</tr>
<tr>
<td></td>
<td>No Drug</td>
<td>91.36 ± 3.55</td>
<td>10.29 ± 0.27</td>
<td>225.05 ± 11.65</td>
</tr>
<tr>
<td>Liver</td>
<td>Exercise</td>
<td>148.75 ± 10.20</td>
<td>91.30 ± 1.80</td>
<td>37.50 ± 0.81</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>89.86 ± 9.50</td>
<td>59.92 ± 1.67</td>
<td>35.64 ± 0.75</td>
</tr>
<tr>
<td></td>
<td>Drug</td>
<td>127.89 ± 10.00</td>
<td>75.06 ± 1.80</td>
<td>32.77 ± 0.98</td>
</tr>
<tr>
<td></td>
<td>No Drug</td>
<td>110.72 ± 9.71</td>
<td>76.12 ± 1.71</td>
<td>40.38 ± 1.16</td>
</tr>
<tr>
<td>Heart</td>
<td>Exercise</td>
<td>5.03 ± 0.51</td>
<td>53.41 ± 1.62</td>
<td>16.19 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>3.39 ± 0.51</td>
<td>49.00 ± 1.62</td>
<td>17.25 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>Drug</td>
<td>3.70 ± 0.51</td>
<td>48.59 ± 1.62</td>
<td>15.24 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>No Drug</td>
<td>4.72 ± 0.51</td>
<td>53.82 ± 1.62</td>
<td>16.19 ± 0.54</td>
</tr>
</tbody>
</table>
R wave in lead I

Exercise significantly increased R wave voltage in lead I. Mean voltage for the swimming animals was 18.63 m.m., the swim-drugged animals 16.87 m.m., the sedentary controls 8.11 and the sedentary drugged mice 7.9 m.m. This was obviously a "training" effect of the swimming, the magnitude of which was not diminished by Adriamycin administration.

Q wave in lead V10

A significant \( p < .001 \) decrease in Q wave voltage was demonstrated in lead V10 in the drugged animals. The sedentary drugged animals displayed a more profound decrease in voltage than did the swim-drugged animals. According to the statistical analysis, this decrease was attributable to the administration of the drug, and was not an exercise effect. Results may be seen in figure 4.14.

QRS in aVF

No statistically significant differences were found between the various groups and their total QRS voltage in lead aVF. Swimming mice displayed a mean QRS complex of 1.1 mv., followed by .95 mv for the sedentary drug group, .93 mv for the sedentary controls and .81 mv for the swim-drug animals. These results differ from those of others (18) who have seen decreased QRS voltage in rats administered Adriamycin. In many clinical settings, diminished QRS voltage is used as an indicator of Adriamycin induced cardiotoxicity.
**S/R ratio in lead I**

A significant exercise-drug interaction was shown to effect the S/R ratio of mice in lead I. It appears that exercise as well as Adriamycin produce a decrease in the S/R ratio. As seen in figure 4.15, sedentary control animals had an S/R of 4.29; treatment with Adriamycin or exercise led to a diminution in the ratio. Consequently, the use of the S/R ratio in lead I as a diagnostic tool for measuring Adriamycin cardiotoxicity appears to be a possibility.

**S/R ratio in lead aVf**

As was the case with the S/R in lead I, significant changes were seen in the S/R of mice in lead aVf as well. However, the changes in aVf were attributable to Adriamycin administration, according to the statistical procedures used. Both drugged groups had lower S/R ratios than did the non-drugged animals, indicating that the exercise was not responsible for the changes seen. See figure 4.15.

**Heart Rate**

A highly significant (p < .0001) exercise-drug interaction clouds the data somewhat, and makes it difficult to "weed out" drug effects from training effects. A training bradycardia did take place; however, when a least square mean is used to measure differences between the trained and untrained animals the differences are obliterated (481.1 beats per minute for the sedentary animals vs.
Figure 4.14: Comparison of the Various Experimental Groups and the P wave in lead I, QV10, and R wave in Lead I.

* significantly greater than sedentary animals (p < 0.05).
** significantly different from sedentary control group (p < 0.05).
*** significantly lower than non-drugged animals (p < 0.05).
Figure 4.15: S/R Ratio in Leads I and aVF

* significantly lower than non-drugged animals (p < 0.05).
** significantly lower than sedentary control group
468.1 beats per minute for the swimmers). This is due to the fact that the sedentary drugged mice had a significant decrease in resting heart rate. It is safe to say, however, that both treatments, exercise as well as drug administration, produced a bradycardia in mice. See figure 4.16.

In addition, after 21 weeks of training, exercised mice had significantly lower heart rates than they did after nine weeks of training. At nine weeks, no significant differences were found between the swimming and sedentary groups. Obviously, the prolonged training duration was necessary to produce the resting bradycardia seen in the trained animals.

Adaptations of Body Weight, Heart Weight, Heart Weight to Body Weight Ratios and Food Consumption

The initial body weights of the mice at the onset of the study was between 28-33.5 grams (mean = 32.2). Weights were recorded weekly throughout the duration of the study. Although the animals were given food and water ad libitum, the mean body weight of the swim-drugged mice during the drug dosing period (i.e., weeks 9-21) (37.04 gms) was significantly lower (p. < 0.05) than that of the other groups. The swimming non drugged ($\bar{x} = 39.3$ grams) and sedentary drug treated groups (39.6 grams) had significantly lower means than did the sedentary control group ($\bar{x} = 44.1$) as well. The final mean body weight of the drugged swimmers represented a value which was 17% lower than that of the sedentary control group. The
Figure 4.16: Resting heart rates of the various experimental and control groups taken at weeks 9 and 21

A significant exercise drug interaction makes it difficult to make definitive statements about the treatments' effects on heart rate. Swim group after 21 weeks of training had a significantly lower heart rate than swim group after 9 weeks of training (p < 0.05). See text.


Table 4.5

**EKG Data Following 21 Weeks of Aerobic Exercise and/or Adriamycin Administration**

<table>
<thead>
<tr>
<th>Group</th>
<th>P1(mm)</th>
<th>Qv10(mm)</th>
<th>RI(mm)</th>
<th>ORS-AVF(mv)</th>
<th>S/R</th>
<th>S/R-AVF</th>
<th>HR(BPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swim</td>
<td>3.05**</td>
<td>16.60</td>
<td>18.63*</td>
<td>1.06</td>
<td>0.751**</td>
<td>1.12***</td>
<td>454.25**</td>
</tr>
<tr>
<td>±0.15</td>
<td>± 1.15</td>
<td>± 1.81</td>
<td>±0.06</td>
<td>± .19</td>
<td>±0.17</td>
<td>± 9.27</td>
<td></td>
</tr>
<tr>
<td>Swim-Adria</td>
<td>1.76**</td>
<td>0.14.33</td>
<td>16.87*</td>
<td>0.813</td>
<td>0.649**</td>
<td>0.790</td>
<td>482.00**</td>
</tr>
<tr>
<td>±0.18</td>
<td>± 1.92</td>
<td>± 2.13</td>
<td>±0.05</td>
<td>±0.29</td>
<td>±0.18</td>
<td>± 8.50</td>
<td></td>
</tr>
<tr>
<td>Sedentary-Drug</td>
<td>0.947**</td>
<td>0.10.19**</td>
<td>7.90</td>
<td>0.954</td>
<td>1.58**</td>
<td>0.454</td>
<td>432.38**</td>
</tr>
<tr>
<td>±0.16</td>
<td>± 1.19</td>
<td>± 1.45</td>
<td>±0.09</td>
<td>±0.55</td>
<td>±0.11</td>
<td>± 12.76</td>
<td></td>
</tr>
<tr>
<td>Sedentary-Control</td>
<td>2.36</td>
<td>17.22</td>
<td>8.12</td>
<td>0.930</td>
<td>4.29</td>
<td>0.886</td>
<td>529.72</td>
</tr>
<tr>
<td>±0.19</td>
<td>± 1.06</td>
<td>± 1.84</td>
<td>±0.06</td>
<td>±1.1</td>
<td>±0.16</td>
<td>± 16.14</td>
<td></td>
</tr>
</tbody>
</table>

- Values represent means ± SEM for the total group.
- Exercise significantly affected PI & RI. Drug administration produced changes in PI, Qv10 and S/R in AvF. See text for details.
- * significantly different from sedentary animals (p < 0.05)
- ** significantly different from sedentary control animals (p < 0.05)
- *** significantly different from sedentary drugged animals (p < 0.05)
- a combined drugged group mean significantly lower than combined non-drugged group mean (p < 0.05), using two-way ANOVA.
Table 4.6

Least Square Means and Standard Errors for EKG Data

<table>
<thead>
<tr>
<th>Group</th>
<th>P1(mm)</th>
<th>Qvl0(mm)</th>
<th>RL(mm)</th>
<th>ORS-AVF(mm)</th>
<th>S/RI</th>
<th>S/R-AVF</th>
<th>HR(PM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise</td>
<td>2.41</td>
<td>15.47</td>
<td>17.75</td>
<td>0.93</td>
<td>0.70</td>
<td>0.96</td>
<td>468.1</td>
</tr>
<tr>
<td></td>
<td>±0.12</td>
<td>± 0.96</td>
<td>± 1.3</td>
<td>±0.05</td>
<td>±0.42</td>
<td>±0.11</td>
<td>± 9.0</td>
</tr>
<tr>
<td>No Exercise</td>
<td>1.65</td>
<td>13.74</td>
<td>8.01</td>
<td>0.94</td>
<td>2.93</td>
<td>0.67</td>
<td>481.1</td>
</tr>
<tr>
<td></td>
<td>±0.12</td>
<td>± 0.91</td>
<td>±1.2</td>
<td>±0.05</td>
<td>±0.42</td>
<td>±0.11</td>
<td>± 8.5</td>
</tr>
<tr>
<td>Drug</td>
<td>1.36</td>
<td>12.26</td>
<td>12.38</td>
<td>0.86</td>
<td>1.12</td>
<td>0.62</td>
<td>457.2</td>
</tr>
<tr>
<td></td>
<td>±0.12</td>
<td>± 0.95</td>
<td>± 1.3</td>
<td>±0.05</td>
<td>±0.41</td>
<td>±0.11</td>
<td>± 8.9</td>
</tr>
<tr>
<td>No Drug</td>
<td>2.71</td>
<td>16.91</td>
<td>13.37</td>
<td>0.99</td>
<td>2.52</td>
<td>1.0</td>
<td>492.0</td>
</tr>
<tr>
<td></td>
<td>±0.12</td>
<td>± 0.92</td>
<td>± 1.3</td>
<td>±0.05</td>
<td>±0.43</td>
<td>±0.11</td>
<td>± 8.6</td>
</tr>
</tbody>
</table>
Table 4.7
Total Body Weights at 9 and 21 Weeks

<table>
<thead>
<tr>
<th>Time of Measurement</th>
<th>Swim- No Drug</th>
<th>Sedentary No Drug</th>
<th>Swim- Drug</th>
<th>Sedentary Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 weeks</td>
<td>36.4</td>
<td>39.5</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>21 weeks</td>
<td>41.0*</td>
<td>45.6</td>
<td>36.0*</td>
<td>37.9*</td>
</tr>
</tbody>
</table>

Values are means expressed as grams.

* significantly different from the sedentary control group (p < 0.05)
Figure 4.17: Week by Week Body Weight Chart

- Data points represent mean body weights for each group.
Table 4.8

Heart Weights and Heart Weight to Body Weight Ratios Following Chronic Exercise and/or Adriamycin Administration

<table>
<thead>
<tr>
<th></th>
<th>Swim-</th>
<th>Swim-</th>
<th>Sedentary-</th>
<th>Sedentary Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Drug</td>
<td>Drug</td>
<td>Drug</td>
<td>Control</td>
</tr>
<tr>
<td>Heart Weight</td>
<td>0.199 ±0.0049</td>
<td>0.202 ±0.0089</td>
<td>0.206 ±0.0071</td>
<td>0.211 ±0.0044</td>
</tr>
<tr>
<td>Body Weight at Sacrifice</td>
<td>41.0*</td>
<td>36.0*</td>
<td>37.9*</td>
<td>45.6</td>
</tr>
<tr>
<td>Heart Weight to Body Weight</td>
<td>0.486 ±0.012</td>
<td>0.561** ±0.026</td>
<td>0.543** ±0.017</td>
<td>0.463 ±0.009</td>
</tr>
</tbody>
</table>

Values are means ± SEM expressed in grams.

* Significantly different from sedentary control group (p < 0.05)
** Significantly greater than non drug groups (p = 0.05).
swim non drug and the sedentary drug treated animals were 5% lighter on an average than the controls. Obviously, both treatments (exercise and Adriamycin) tended to lead to a decreased body weight, and in conjunction produced the greatest weight loss of all.

At the end of the initial nine week training period, swimmers had mean body weights of 36.4 grams as opposed to a mean of 39.5 grams for the sedentary mice. At the conclusion of the drug dosing regime twelve weeks later the trained drugged animals weighed an average of 36 grams (a net loss of 0.4 grams). In addition, the sedentary drugged animals at sacrifice had a mean weight of 37.9 grams. This represents a 1.6 gram weight loss from the onset of the drug dosing at week nine to the end of the study. When contrasted with the final sedentary control mean weight of 45.6 grams (a net gain of 6.1 grams from weeks 9 to 21) one can see the profound effects of exercise and Adriamycin administration on body weight. The results are shown in table 4.7 and figure 4.17.

Heart Weights

No differences were found in any of the mean heart weights at the time of analysis. Results shown in table 4.8.

Heart Weight to Body Weight Ratios

The ratio of the heart weight to the body weight of each mouse at the time of sacrifice was computed. The two drug treated groups demonstrated significantly greater heart weight to body weight ratios than did the non drugged groups. Exercise did not appear to be a
factor in producing these results. Values for each group are shown in Table 4.8. The fact that the drug produced a significant weight loss in both the trained and sedentary groups, coupled with the fact that no significant heart weight changes were reported between groups, leads one to conclude that body weight changes, rather than a selective cardiac hypertrophy, was responsible for the results.

Food Consumption

For a two week period during the study, average food consumption per group was computed by giving pre-weighed rations to the animals. As shown in Figure 4.18, both exercise and drug administration led to a decreased caloric intake. Swim trained animals consumed 65.1 percent as much food as the sedentary controls; Adriamycin treated mice ate 59.5 percent as much as controls. The combination of swimming and drug administration produced the most profound decline in food consumption; mice in this group consumed only 34.9 percent as much food as did the sedentary controls. The significant differences in body weights reported between the drugged and non-drugged groups, as well as in the swim trained animals, were undoubtedly due in large part to this decrease in caloric consumption.

Histological Analysis

Microscopic examination was made of sections of the heart to determine the effect of exercise on the development of the histomorphologic changes of Adriamycin induced cardiotoxicity. The observations in the heart of each mouse according to treatment groups
Figure 4.18: Average daily food consumption in mice during a two week period.

Results are expressed as a percentage of food consumed by the sedentary control group.
Lesions consistent with the cardiotoxicity of Adriamycin in the mouse model were seen in both the sedentary drug group and the swim drug group. The lesions were characterized by variable amounts of degeneration of myocardial fibers. Many of the affected cells had single, large or multiple, very small intracytoplasmic vacuoles within the myocardial fibers. Also, the fibers within the myocardium showed some disorganization and variation in size. In a few of the drugged mice there were large arterial thrombi, variable amounts of multifocal chronic interstitial myocarditis and focal or multifocal myocardial fibrosis in the area of inflammation. In addition, it is of interest to note that in the hearts examined, three out of fifteen (20%) swim-drug hearts were considered to be normal; one out of 22 (4.5%) of the sedentary-drugged animals had hearts which appeared normal upon microscopic examination.

Upon statistical analysis, it was determined that, although both drugged groups had significantly more damage than did the non-drugged groups ($p < 0.001$), the sedentary drugged mice had greater damage than did the swim-trained drug mice ($p < 0.05$). These findings were based on the numerical value assigned to the damage in the heart of each mouse, based on Bertazzoli's criteria for cardiotoxicity (see Chapter 3). According to this criteria, sedentary-drugged mice had a mean cardiotoxicity score of 3.05, as compared to a swim-drugged score of 1.53. Sedentary controls and swim-non drug mice had scores of 0.056 and 0.00 respectively. These findings lead one to conclude...
that the incidence and severity of the vascular degeneration, focal myocarditis and fibrosis were increased in the mice given Adriamycin without exercise. A summary of the histomorphological observations is shown in table 4.10.
KEY TO HISTOMORPHOLOGIC OBSERVATIONS

- = No change (within normal histologic limits or indicated change not present).
* = Tissue not available, insufficient tissue in plane of section, or artifact precludes evaluation.
P = Indicated change or lesion present.
1 = Very slight degree or very small amount of indicated change.
2 = Slight degree or small amount of indicated change.
3 = Moderate degree or amount of indicated change.
4 = Marked degree or amount of indicated change.

CRITERIA FOR GRADING OF "VACUOLAR DEGENERATION" IN THE HEART

Grade 1 = Very slight degree or very small amount of the indicated change (scattered, single myocardial fibers with vacuolization or degenerative changes).

Grade 2 = Slight degree or small amount of indicated change (scattered small groups of altered myocardial fibers throughout the atrial and ventricular myocardium).

Grade 3 = Moderate degree or amount of the indicated change (disseminated myocardial fiber vacuolization or degeneration with only occasional focal unaffected areas).

Grade 4 = Marked degree or amount of the indicated change (confluent groups of affected myocardial fibers - most myocardial fibers affected).
Table 4.9

Histomorphological Observations of Adriamycin Induced Cardiotoxicity

<table>
<thead>
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Table 4.10
Summary of Histomorphologic Observations

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- vacuolar degeneration, Grade 1 0 9 0 6
- vacuolar degeneration, Grade 2 0 3 0 9
- vacuolar degeneration, Grade 3 0 0 0 6
- multifocal myocarditis 0 2 0 7
- focal myocardial fibrosis 0 1 0 5
- multifocal myocardial fibrosis 0 2 0 1
- intrafiber vacuoles 0 0 1 0
- atrial thrombus 0 4 0 6

Group I = Swimming/No Adriamycin
Group II = Swimming/4 mg/kg Adriamycin
Group III = No exercise/No Adriamycin
Group IV = No exercise/4 mg/kg Adriamycin
Mode and Intensity of Training

Prior to evaluating the results and implications of the study, a pertinent question may be asked; that is, were the animals in fact trained? Were the mode and intensity of the exercise program powerful enough to elicit the responses seen? Upon reviewing the data, the answer to this question must be an unequivocal yes. Numerous studies (62, 63) have determined that two-fold increases in the levels of mitochondrial respiratory chain enzymes involved in the oxidation of NADH and succinate of various skeletal muscles is not uncommon following a period of aerobic training. The 30% increase in succinoxidase activity which was seen in quadriceps muscle after nine weeks of training tend to confirm that the exercise regimen was stressful enough to produce a "training" effect. In addition, a resting bradycardia existed in the swim trained animals. Scheuer and Tipton (122), in reviewing the work of various investigators, concluded that chronic exercise, possibly by altering autonomic nervous system function, will usually cause a resting bradycardia in most species. The decreased body weight and food consumption seen in the exercised animals are also consistent with the findings of others who have conducted training studies (82).
This is not to say, however, that the mode of exercise used was ideal, and not without its intrinsic problems. Updyke (135) and others have seen the presence of pneumonia and acute respiratory disease in mice who swam daily for four week. As no post mortem studies were performed on lung tissue, it is difficult to assess the damage that may have been done due to swimming. The fact that very few animals died or exhibited symptoms of severe disease leads one to believe that the swimming did not produce too many undesirable changes.

It should be pointed out that toward the end of the study, four Adriamycin treated swimmers drowned, and a few others had difficulty swimming for fifty minutes daily. At first it was thought that the drug was affecting the animals' swim performance; however, Richter (113) found that clipping of the vibrissae caused rats to die within one to five minutes when placed in water. It was noted that many of the drug treated animals tended to lose their whiskers, which may have been responsible for the decline in swim performance. Future researchers may make note of this problem. In general, however, swimming proved to be a favorable way to train the mice. No special equipment was needed, many animals could be exercised simultaneously and the mortality rate was relatively low.

Antioxidant Enzyme Changes

Based on prior research, many of the changes that occurred in the antioxidant enzyme levels in the hearts, livers and blood of mice should have been predictable, although other changes were paradoxical
and not as easily explained. As pointed out previously, various researchers have demonstrated the adaptability of the antioxidant enzymes under a wide range of physiological and environmental conditions. Varying conditions such as hyperoxic exposure (45) and beta-Thalassaemia minor (49), a form of anemia which increases the risk of oxidant stress, lead to increases in tissue levels of the enzymes. Exercise has been shown to increase peroxidative by-products, as demonstrated in studies by Dillard et al. (32), Ullrey et al. (132) and Davies et al. (29), and enhanced levels of superoxide dismutase and catalase subsequent to chronic aerobic training has been demonstrated by Jenkins et al. (67). Consequently, the findings of the present study, including increases in blood levels of catalase, SOD and GP and liver levels of GP after nine weeks of training, as well as the enhanced blood and liver levels of all three enzymes, in addition to increased heart catalase levels after 21 weeks of training were not too surprising. The data dealing with the swim trained-drug treated animals presents some conflicting results which are worth discussing, however. As mentioned previously, catalase activity in liver, heart and blood was elevated in the trained mice after 21 weeks; liver and blood increases persisted in the drug treated swimmers. In the heart, however, the drugged swimmers had significantly lower catalase levels than did the non drugged swimmers. A similar situation existed in the liver concentration of SOD. It is difficult to assess the reason for this; if Adriamycin does, in fact, produce toxic free radical intermediates, it is not unreasonable to think that chronic
administration of the drug might actually enhance enzyme levels. Therefore, one might reason that exercise training in conjunction with drug administration would tend to increase enzyme levels above those of the non drugged swimmers. That this did not occur is not easy to explain, although it is not surprising in light of the findings of other investigators. For example, Revis et al. (112) and Doroshov et al. (37) (as well as the present study) found decreased levels of cardiac glutathione peroxidase following Adrlyamcin administration.

A possible explanation for the diminution in cardiac enzyme activity was provided by Willis (139), who has shown that lipid peroxides may inhibit the activity of selected enzymes, probably by oxidation of reduced thiol groups and Rister and Bachner (116), who showed that incubation of glutathione peroxidase in vitro with a source of superoxide anion will significantly diminish enzyme activity. That Adrlyamcin, as a possible source of free radicals, would lead to diminished enzyme activity while exercise tends to increase enzyme activity is somewhat of a mystery, and further clarification is necessary. It is possible that the enhanced protein synthesis which is often times seen with exercise (62) may account for these apparently contradictory results.

The results of the present study are in close agreement with those of Doroshov et al. (37) and Hein et al. (58), who have demonstrated that SOD and catalase levels in the heart are lower than in well perfused organs such as the liver. Sedentary control mice had liver catalase levels of 75.4 K/mg protein and heart levels of 3.3 K/mg
protein. Therefore, heart levels were only 4.4% of that in liver. SOD in heart are 15.18 U/mg protein as opposed to liver levels of 38.34 U/mg protein, or only 39.5% as great. Glutathione peroxidase levels were not appreciably different in the two organs. Heart levels were 52.00 U/mg protein, liver levels were 57.72 U/mg protein (90% as great). Thus, cardiac tissue, in contrast to liver, has appreciably lower levels of two of the three antioxidant enzymes. As mentioned in an earlier chapter, this in part may account for the selective cardiotoxicity seen with Adriamycin administration.

The overall increases in liver and blood enzyme levels seen with exercise most certainly may be looked upon as a positive adaptation to training. Although the majority of the damage due to Adriamycin administration occurs in the heart, detoxification of reactive free radical intermediates at others sites in the body may lessen the cardiotoxicity. Swartz (128) stated that radicals of intermediate reactivity, such as the semiquinone radical purportedly produced during the metabolism of Adriamycin, may live long enough to travel to critical sites, and have sufficient reactivity to cause damage when they get there. Consequently, it may not be unreasonable to assume that enhanced antioxidant enzyme levels in liver and blood may work to detoxify these reactive metabolites before they ever reach the heart. This assertion bears greater weight in light of the fact that the liver is the primary site of Adriamycin metabolism.

Finally, it should be pointed out the catalase activity in the liver and blood, as well as SOD activity in the heart, liver and blood of swim trained animals was significantly greater after 21
weeks of training than after 9 weeks of training. (Liver catalase increased by 33%, blood catalase by 15%, heart SOD by 30%, liver SOD by 18% and SOD blood by 6%). These findings may have implications to training studies which look at enzymatic adaptations in various organs. If the duration of the training period is not sufficient to produce enzyme changes, an investigator may wrongfully assume that exercise promotes no such adaptation. Longer training durations may alleviate this problem.

Electrocardiographic Changes

Voltages of components of the EKG are determined by various cardiac or extracardiac factors. Cardiac determinants include the magnitude of the action potential, magnitude of the waves of depolarization and the degree of asynchronicity (uncancellation) of simultaneous waves of depolarization. Extracardiac determinants include the volume and resistivity of blood within chambers (Brody Effect (15)), volume and resistivity of pericardial sac effusates and interfaces between eccentric rings of tissues of various volumes of resistivity (Rudy and Plonsey, eccentric sphere model (119)). Not only do the above determinants modify amplitudes of body surface potentials, but they may alter potentials at varying positions on the torso surface in opposite directions. That is, increased left ventricular volume may increase potentials and regions monitoring spread radial to the LV lumen, but may decrease potentials at regions monitoring spread tangential to the lumen.

In the study, statistically significant voltage changes were
registered among the groups of mice. According to Unverferth (133), these changes may have resulted from a decrease in body weight with constancy of heart weight (i.e., swim drug group vs. sedentary controls), or from changes in resistivity of blood resulting from a changing hematocrit. In addition, alterations in percentages of fat and/or muscle, according to the Rudy-Plonsey model, could change voltages as well.

From observations of the EKG data, it is clear that swim trained mice had longer P and R waves in lead I than did the sedentary animals. This was undoubtedly a "training" effect. Drug administration produced a decreased voltage in Qv10 and the P wave in lead I, as well as diminution in the S/R ratio in lead aVF. The sedentary drugged animals displayed more marked changes than did the swim-drug mice, which may indicate a more severe effect of Adriamycin on sedentary than on swimming mice. Such apparently discordant changes (i.e., increased amplitude in lead I, decreased amplitude in Qv10) are consistent with the Brody effect and should not be disturbing. In fact, it is possible that if subsequent research bears out the results of this study in other species, the above alterations may possibly be used as a diagnostic tool for the acute toxicity which generally accompanies Adriamycin administration. Characteristic EKG tracings may be seen in figure 5.1.

Finally, it is clear from this study that either mice differ from other mammals given Adriamycin, or the more systematic lead system used in this study better characterized the EKG effects of
Figure 5.1: Characteristic EKG Tracings in Leads I, aVF and V10
Adriamycin, as the animals displayed no significant QRS voltage changes after drug administration. Buyniski (18) has stated that a total decrease in QRS voltage may be used as evidence of myocardial damage produced by Adriamycin administration in the rat.

**Body Weight, Heart Weight Changes**

As mentioned previously, drug treated and exercised mice had significantly lower body weights than did the sedentary control group. These findings are consistent with prior data which has shown that Adriamycin produces appetite suppression in a variety of species (20), as does moderate exercise. Mayer et al. (82) reported that exercise performed for less than one hour leads to decreased food consumption with a concomitant loss of body weight. Therefore, it was not surprising to find that the group which received Adriamycin and exercised had the lowest body weight throughout the course of the study.

Many investigators have mistakenly assumed that an increased heart weight to body weight ratio is a prime indicator of cardiac hypertrophy. This is not necessarily true; as Sheuer and Tipton (122) have indicated, true hypertrophy may only be assumed when absolute heart weight is increased and enlargement of myocardial cells occur. This has been a controversial issue; studies by Morgenroth et al. (95) and Nutter et al. (102); among others, have demonstrated cardiac hypertrophy in human athletes. However, as no pre-post tests were applied in these studies, it is difficult to assess the effects of exercise on the development of the hypertrophy.
Other factors, such as lifestyle or genetics, may have come into play.

The increased heart weight to body weight ratio seen in the drugged animals in this study is likely due to a decrease in body weight, rather than a selective hypertrophy due to the drug. Exercise, on the other hand, produced no such increase in the heart weight to body weight ratio. In fact, the absolute heart weights of the two swimming groups were actually lower than that of the sedentary animals. Consequently, it was concluded that fifty minutes of daily swimming was not a sufficient stimulus to induce cardiac hypertrophy in mice.

**Histological Alterations**

Adriamycin cardiotoxicity was quantitatively evaluated using the method of Bertazzoli, et al. (7). Heart lesions were scored according to severity and extension; lesions observed included vacuolar degeneration (grades 1-3), myocarditis, fibrosis, and atrial thrombus.

The heart sections of the non-drugged mice appeared to be normal; this observation held true for both the swimming and sedentary animals. In fact, of the 38 non-drugged mice examined only one (a sedentary animal) exhibited any sign of a histological abnormality. On the other hand, only four of 37 Adriamycin treated mice (11%) were considered to be normal. These findings were not at all surprising, as the cardiotoxic effects of the drug are well documented and universal among species. What was somewhat surprising, and until
this point unpublished in the literature, were the differences between the drugged swimmers and the drugged sedentary mice. As stated earlier, 20% of the swim trained mice had hearts which were considered histologically normal, as opposed to 4.5% of the sedentary mice hearts. Based on the Bertazzolli rating scale, the sedentary drug treated animals had significantly greater damage than did the swim trained drug treated mice. The implications of these findings are potentially very important in the treatment of patients receiving chemotherapy. As mentioned in a previous chapter, researchers have administered a variety of agents in an attempt to alleviate or diminish the cardiotoxic threat of Adriamycin administrations. Among these are vitamin E (100, 136), CoQ (104), verapamil (141), digitoxin (107), and, most recently, N-acetylcysteine (133). All have produced varying and, in most cases, ultimately unsuccessful results. The use of exercise, or a related type of therapy, opens up new avenues of research which may eventually yield positive results in the battle against the potentially lethal side effects of chemotherapy.

Practical Implications

Obviously, it is not always practical for a patient receiving Adriamycin to suddenly begin an exercise program. The drug produces a variety of side effects which generally leave the individual too tired or sick to engage in strenuous activity. How, then, may the results of this study be interpreted in a practical manner?

The overall increase in the oxidant stress enzymes with exercise is obviously a positive adaptation. We encounter daily a variety of
toxins which ultimately produce free radicals in the tissues; these include carbon monoxide, cigarette smoke, ultraviolet rays, etc. Research has linked a variety of disease states, as well as the process of aging, with the production of harmful free radical intermediates. It may be reasonable to assume, therefore, that any stimulus which increases our ability to deal with these potentially damaging radicals is a step toward alleviating their toxic effects.

In this regard, aerobic exercise may be viewed in yet another manner as a healthful act. The literature is full of data dealing with the effects of training on the cardiovascular system, obesity, etc. Most investigators conclude that exercise is necessary and helpful in the promotion of a healthier lifestyle. The results of the present study tend to bear this out.

The results of the study have some practical implications for dealing with the toxic effects of Adriamycin administration as well. If it is not feasible for patients receiving the drug to exercise, it may be feasible to do more research with synthetic enzyme analog administration in conjunction with chemotherapy treatment. A few studies dealing with this possible treatment have been done. Mimnaugh et al. (90) found that superoxide dismutase administration effectively inhibited Adriamycin stimulated microsomal lipid peroxidation in rat liver and heart microsomes. Bozzi et al. (12) found that Toshida ascites cells, which have high glutathione and glutathione peroxidase contents were more resistant to oxidative damage than were Ehrlich ascites cells, which have low enzyme levels,
after daunomycin administration. It appears that more research needs to be done in this area.

Finally, this study, as well as those done by Combs et al. (27) and Hassler et al. (57) have possible implications for the way in which physicians advise patients receiving Adriamycin in the future. It is generally accepted in the medical community that individuals treated with Adriamycin should do very little strenuous activity, primarily because of the drug's cardiotoxic side effects. The two aforementioned studies demonstrated that exercise did not exacerbate the toxicity; the present study has actually shown a beneficial effect of exercise. Therefore, increasing activity in patients receiving Adriamycin may not be as dangerous as once thought; it may actually provide physiological benefits and at the same time improve the quality of the patients' life as well.
SUMMARY AND CONCLUSIONS

The study was undertaken to look at the effects of chronic aerobic exercise training on the levels of the antioxidant enzymes catalase, superoxide dismutase and glutathione peroxidase in various tissues, as well as upon the cardiotoxicity often seen with Adriamycin administration.

Following nine weeks of swim training it was determined that:
- swimming significantly increased succinic oxidase levels in mouse quadricep muscle.
- trained mice displayed significantly greater blood catalase levels than did sedentary animals.
- training produced a significant rise in blood and liver glutathione peroxidase activity.
- superoxide dismutase activity was elevated in the blood of the trained animals.

At the conclusion of the study after twenty-one weeks of training and a twelve week drug dosing regimen:
- trained animals, regardless of drug status, had elevated blood catalase and superoxide dismutase activities.
- swim trained mice displayed significantly greater blood glutathione peroxidase activity than did sedentary animals; trained-drug treated mice had greater blood glutathione peroxidase activity than did untrained-drug treated animals.
- swimming produced elevated liver levels of all three enzymes.
- Adriamycin decreased S.O.D. liver levels significantly.
- Swimming led to an increase in heart catalase activity; the increase was diminished in trained mice receiving Adriamycin.
- Trained mice demonstrated increased P and R wave amplitude in lead I.
- Adriamycin diminished P wave amplitude in lead I and Q wave voltage in V_{10}.
- Both exercise and Adriamycin produced a resting bradycardia, as well as a diminution in body weight.
- The degree of cardiotoxicity was significantly greater in the sedentary drug treated animals than in the swim trained drug treated animals, as measured histochemically.

The results tend to point toward a correlation between antioxidant enzyme levels and the degree of damage caused by an anthracycline drug. These findings strengthen the belief of various other investigators who feel that the cardiotoxicity produced by drugs such as Adriamycin is in fact due to free radical generation during the metabolism of the drug.

Therapy with a variety of antioxidants administered in conjunction with Adriamycin have provided tentative results at best. The use of vitamin E has been shown to lessen the toxicity of the drug in one study (100), but provided no relief in another (136). While the present study implicates free radicals as the culprit responsible for the severe toxicity of the drug, the discovery of a suitable compound to deal with these potentially lethal intermediates has, until this point, proven to be unsuccessful.
It may be possible that the answer to this puzzling problem lies in the antioxidant enzyme system itself. Many studies have shown that a variety of stimuli induce increases in enzyme activity. The results of the present study suggest that exercise is one such stimulus. Although it might not be feasible (or beneficial) for people receiving chemotherapy to suddenly begin an exercise program, it might be possible to increase antioxidant enzyme levels by other means. Glutathione, selenium or synthetic enzyme analog supplementation are three possible approaches which come to mind. In any case, it appears that investigators who attempt to alleviate the toxicity of Adriamycin by administration of various antioxidants are on the right track, and further research will likely provide a cure in time.

As for the implications of the results on exercise training, it has once again been shown that chronic strenuous activity produces beneficial adaptations. The increased enzyme levels seen in the trained animals may provide protection from various xenobiotics that we encounter in our daily lives, such as carbon monoxide and ultraviolet radiation. In addition, with the staggering incidence of cancer in this country, it might just be possible that an individual who has maintained an active lifestyle and is suddenly forced to undergo chemotherapy treatment may have an advantage over a sedentary individual in dealing with the toxic side effects of the treatment. Future research in the area will undoubtedly provide us with answers to many of these puzzling questions.


60. Herman, E. and Ferrans, V.J. (1981) Reduction of chronic doxorubicin cardiotoxicity in dogs by pretreatment with (±)-1, 2-bis(3,5-dioxopiperazinyl - 1-yl) propane (ICRF-187) Cancer Res. 41:3436.


