AGE RELATED DIFFERENCES IN ADRIAMYCIN-INDUCED CARDIOTOXICITY: MECHANICAL, ULTRASTRUCTURAL AND BIOCHEMICAL CHANGES

A THESIS PRESENTED TO THE UNIVERSITY OF MANITOBA

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PHYSIOLOGY

BY

LEAH E. WEINBERG

DEPARTMENT OF PHYSIOLOGY

FACULTY OF MEDICINE

SEPTEMBER, 1985

AGE RELATED DIFFERENCES IN ADRIAMYCIN-INDUCED CARDIOTOXICITY: MECHANICAL, ULTRASTRUCTURAL AND BIOCHEMICAL CHANGES

BY

LEAH E. WEINBERG

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to Dr. P.K. Singal for his guidance, understanding, consistent support and friendship during the course of this research and for allowing me to further my scholastic career under his auspices.

I would also like to extend a special word of thanks to Dr. N.S. Dhalla, Dr. V. Panagia, Dr. R.E. Beamish and Dr. P. Ganguly for their interest, support and advice.

I am deeply grateful for the love and encouragement of my children, Lloyd, Garry and Shoshana in my pursuit of knowledge and academia.

I would like to express my warm appreciation to Marilyne Vandel for her patience and help in the preparation of this thesis.

I offer my thanks to my colleagues in the Department of Physiology and especially to those in the Experimental Cardiology Laboratory for their advice and assistance.

This study was supported by the Manitoba Heart Foundation through a grant extended to Dr. P.K. Singal.

ABSTRACT

Adriamycin, an anthracycline antibiotic, is an effective chemotherapeutic drug widely used in current oncology to treat leukemias, lymphomas and a variety of solid tumors. A toxic, life-threatening side effect induced by adriamycin is a dose-dependent, progressive cardiomyopathy and fulminating congestive heart failure. Therefore, appreciation of the dose-effect, structure-function relationship is fundamental to the use of adriamycin. In this regard, an empirical dose of 550 mg/m^2 has been suggested to minimize the cardiotoxicity due to adriamycin. However, risk factors other than total cumulative dose may also adversely influence the development of cardiomyopathy and heart failure. Some of these risk factors are: age, previous or concomitant radiation, liver disease, cardiovascular disease, and concurrent chemotherapy. In the present study, the effects of chronic administration of adriamycin (total cumulative dose 15 mg/kg) were investigated in two age groups of rats with regard to their differences in the developed force, ultrastructure and lipid peroxide changes in the myocardium. Animals in the older group treated with adriamycin (C_o + A group) showed hydroperitoneum, higher mortality and a greater decline in weight and feed consumption. Other symptoms of congestive heart failure were also noticeable in the C_o + A group. Studies on the developed force in papillary muscles isolated from hearts of animals treated with adriamycin showed a lesser increase in peak developed force as well as dp/dt in response to positive inotropic

interventions in the older C_o + A group compared to the younger C_y + A group. There was also a lesser decline in these force parameters in the C_o + A group in response to negative interventions. The reduced response to both positive and negative interventions in the C_o + A group appeared to be consistant with an intracellular Ca^{2+} overload in these hearts. Ultrastructural studies showed greater damage in the C_o + A group than the C_y + A group. The disruption and loss of myofibrils in conjuction with extensive mitochondrial damage seen in the older treated group reinforce the view that the older myocardium is at greater risk of developing cardiomyopathy. The 100% increase in myocardial malondialdehyde content in the C_o + A group indicated increased production of lipid peroxides. Lipid deposits in and around degenerating mitochondria observed in ultrastructural studies of the C_o + A group also supported the idea of greater lipid peroxidation occuring in these hearts. These data suggest that a chronic adriamycin administration may lead to the formation of increased lipid peroxides that can cause intracellular Ca^{2+} overload affecting mitochondria as well as high-energy phosphate stores. This can be seen to cause myocardial cell damage and failure. It is possible that with age the antioxidative capacity of the heart may be declining. However, further studies are needed to define the exact mechanism of increased cardiotoxicity in the older animals.

TABLE OF CONTENTS

	PAGE
LIST OF TABLES	1
LIST OF FIGURES	2
INTRODUCTION	3
REVIEW OF THE LITERATURE	
General Background	6
Source and Pharmacokinetics of Adriamycin	7
Cardiovascular Effects	9
a) Acute	9
b) Chronic	11
Suggested Mechanisms of Anthracycline Cardiotoxicity	15
Risk Factors in Adriamycin Cardiotoxicity	21
MATERIALS AND METHODS	
Animals	27
Adriamycin Treatment	27
Contractile Function Studies	28
Ultrastructural Studies	29
Myocardial Malondialdehyde Assay	30
Statistical Analysis	31
RESULTS	
General Observations	32
Effects on Body Weight and Feed Consumption	32

Effects on Heart Weight and Mortality 35

Papillary Muscle Studies 37

Morphological Studies 43

Lipid Peroxidation 56

DISCUSSION 58

REFERENCES 69

LIST OF TABLES

TABLE NUMBER:		PAGE NUMBER
1.	Comparison of effects of adriamycin on different parameters in two age groups of rats.	36
2.	Effects of different interventions on the maximum developed force in papillary muscles isolated from the hearts of control and adriamycin treated hearts.	38
3.	Effects of different interventions on the (+) and (-) dp/dt in papillary muscles isolated from the hearts of control and adriamycin treated rats.	41

LIST OF FIGURES

FIGURE:		PAGE NUMBER
1.	Effects of chronic adriamycin treatment on body weight and feed consumed in the younger group of rats.	33
2.	Effects of chronic adriamycin treatment on body weight and feed consumed in the older group of rats.	34
3.	Effects of different interventions on the peak developed force in isolated papillary muscles of C_y + A group.	39
4.	Effects of different interventions on the peak developed force in isolated papillary muscles of C_o + A group.	40
5.	Ultrastructure of the control heart from a rat treated with vehicle alone.	45
6.	Nuclear changes include mild perinuclear edema and dispersion of chromatin in C_y + A group.	47
7.	Ultrastructure of the heart from a different animal in the C_y + A group.	48
8.	Electron micrograph of C_y + A group.	49
9.	Intracellular vacuolization and electron dense inclusion bodies are apparent, C_o + A group.	51
10.	Ultrastructural changes in myocardial contractile elements in C_o + A group.	52
11.	Discruption and fragmentation of myofibrils with patches of myofibrillar dropout. Mitochondria show severe changes in C_o + A group.	53
12.	Swelling and degeneration of mitochondria in the C_o + A group.	54
13.	Effects of chronic adriamycin treatment on myocardial malondialdehyde content in two age groups of rats.	57

INTRODUCTION

Adriamycin (doxorubicin) is an anthracycline derivative used in the chemotherapy of a variety of tumors. It has been clearly established as one of the most efficaceous antineoplastic drugs used in current adult and pediatric oncology. Adriamycin exhibits the usual toxicities associated with antineoplastic drug therapy (myelosuppression, alopecia, nausea and vomiting), but the most serious side effect is manifested by the onset of an insidious, intractable and often fatal congestive heart failure. This limits the therapeutic potential of this important chemotherapeutic agent for a long term administration. Pathologic changes in the heart associated with chronic effects of anthracyclines consist of dilatation, degeneration and atrophy of muscle cells, interstitial edema and fibrosis. Characteristic morphological changes of cardiac cells produced by adriamycin involve the myofibrils, the nuclei, the mitochondria and the membrane systems of the T-tubules, the sarcoplasmic reticulum and the intercellular junctions.

The exact pathogenic mechanism of the delayed chronic cardiotoxicity of anthracyclines is still unknown although several suggestions have been postulated. These include its known ability to bind DNA and inhibit nucleic acid synthesis leading to interference with normal protein synthesis and protein turnover in the cell, release of vasoactive amines, free radical formation and lipid peroxidation; abnormalities in the mitochondria affecting myocardial respiration and electron transport; and increased calcium concentrations in the myocardial cell. A number of protective agents have been used in an attempt to

prevent the adriamycin-induced cardiomyopathy and these include the use of free radical scavengers, coenzyme Q_{10} and calcium antagonists. Because of conflicting *in vivo* as well as *in vitro* studies, none of these agents has been proven to be completely protective.

In a retrospective analysis of patient charts it was found that the chance of developing doxorubicin-induced heart failure increased with an increase in the total cumulative dose of doxorubicin administered. There was a continuum of increasing risk as the cumulative amount of administered drug increased beyond 550 mg/m^2 body surface area. Thus, an empirical dose of 550 mg/m^2 of body surface area has been recommended in order to reduce the incidence of developing congestive heart failure. Apparently, a routine compliance with this dosage can preclude the administration of doxorubicin to some patients who might benefit further from prolonged therapy with the drug. Conversely, there can be patients who develop fatal congestive heart failure well below the suggested total cumulative dose of 550 mg/m^2. The issue of adopting a standard empirical dose of adriamycin as an upper limit for therapeutic use is further complicated by the presence of other risk factors which may enhance the cardiotoxic effects of the drug. In this regard, a list of potential risk factors includes advanced age, previous mediastinal radiation, concomitant cyclophosphamide administration, previous liver disease, presence of cardiac hypertrophy, previous hypertension or cardiac disease and, more recently, use of calcium antagonists. Since dose limitation alone will not prevent development of the adriamycin-induced heart failure, it is important to

examine risk factors which may influence the anthracycline cardiomyopathic process or the associated heart failure.

In a study of patient records, a steady increase in the probability of developing congestive heart failure has been noted with increasing age. Reports are also available showing that age $\geq$ 70 is a risk factor predisposing this patient group to adriamycin-induced heart failure. However, in a clinical trial study no statistical significance of age as a risk factor was found when compared with subgroups by age and total dose of adriamycin. In view of the conflicting reports on age as a risk factor in potentiating drug-induced cardiomyopathy and heart failure, we undertook a study to describe age-related differences in chronic adriamycin-induced cardiotoxicity. Two age groups of rats defined as younger, 175 $\pm$ 25 grams and older, 450 $\pm$ 25 grams were utilized in this study. The major parameters examined to elucidate any age related differences included papillary muscle mechanical function studies, electron microscopic analysis of myocardial ultrastructure, and biochemical assay of myocardial malondialdehyde content to determine evidence of free radical activity and lipid peroxidation in the myocardium. In addition, the general condition of the animals, food consumption and body weight changes were monitored daily. Heart weight/body weight ratio and mortality were also recorded to determine any age-related differences in chronic administration of adriamycin.

REVIEW OF LITERATURE

I. General Background

Adriamycin and daunomycin are two anthracylcine antibiotics effective against a variety of leukemias, lymphomas and solid tumors (Di Marco et al, 1969; Blum and Carter, 1974). Adriamycin was isolated in 1967 at the Farmitalia Research Laboratories in Milan, Italy from cultures of Streptomyces (peucetius var. caesius) micro-organism (Arcamone et al, 1972) and clinical trials were begun in 1968 in Milan, Italy under the direction of Dr. G. Bonadonna (Ghione, 1975). Adriamycin has been studied experimentally and clinically in a variety of neoplastic conditions. Clinical trials have shown good results in reduction and control of the neoplasms being treated. Adriamycin today has been clearly established as one of the most effective and widely used antineoplastic drugs in adult and pediatric oncology. However, the use of adriamycin has been restricted because of a demonstrated clinical and histopathological dose-limiting cardiotoxicity (Bonadonna and Monfardini, 1969; Lefrak et al, 1973). A delayed cardiomyopathy may develop during or after a course of adriamycin therapy and is manifested by the onset of an insidious, intractable and sometimes fatal congestive heart failure (Lefrak et al, 1973). The cardiotoxic effects of these drugs are cumulative and dose dependent. In a retroactive study of patient charts, Lefrak et al (1973) reported that if the total amount of adriamycin administered increased above 550 mg/m^2 body surface area, congestive heart failure occurred in 30% of patients. These and many other workers have now suggested that the total cumulative dose administered to patients should be less

than 550 mg/m^2 if one is to minimize mortality due to cardiotoxicity (Lefrak et al, 1973; Minow et al, 1977; Von Hoff and Layard, 1979).

II. Source and Pharmacokinetics of Adriamycin

Adriamycin (also known as doxorubicin) is a glycosidic antibiotic obtained either by aerobic fermentation of Streptomyces Peucetius or by chemical synthesis from daunomycin, also known as daunorubicin (Arcamone et al, 1972). This drug is now commercially available in Canada. Adriamycin hydrochloride is readily soluble in water, physiological saline and methanol, but only slightly soluble or insoluble in less polar organic solvents (Arcamone et al, 1972). The drug, in the lyophilized form, is stable at room temperature for years without any loss of activity. Even in solution (pH 7.0), it is stable for more than a month.

Adriamycin is administered intravenously and is a drug of multipotential molecular characteristics. As a result of both polarity and charge, adriamycin may have a variety of interactions with nucleic acids, lipids, proteins and supportive matrices of various tissues (Bachur, 1975). Pharmacokinetic studies in mice and rats showed that adriamycin is rapidly cleared from the plasma and concentrates in different tissues including heart (Yesair et al, 1972; Bachur, 1974). It is predominantly metabolized in the liver and is approximately 40-50% excreted in the bile while only small amounts are excreted via the kidneys and urine (Benjamin, 1975; Bachur, 1974, 1979). Similarly, in man it has been observed that adriamycin is rapidly cleared from plasma and excreted in bile and urine (Bachur, 1975)

with biliary excretion being most important to its removal from the body (Bachur, 1979).

From experiments on rabbits, Arena et al (1972) concluded that adriamycin is not absorbed by the gastrointestinal tract. Following intravenous injection of adriamycin (8 mg/kg) to mice into which Sarcoma 180 had been transplanted, this drug was quickly transferred from the blood to all the tissues except tumor and brain (Arena et al, 1972). Ten minutes after the injection the maximum activity of the drug was noticed in the liver whereas other tissues such as kidney, spleen, lung, intestine and heart had lesser drug concentration in a decreasing order. Furthermore, in heart, 50% of the concentration found 10 minutes after treatment was still present at 25 hours but no drug was found in heart at 48 hours, at which time some of the drug was still present in liver, kidney, lung and intestine. About 12% of the administered adriamycin was eliminated from the body of rabbits at 5 hours and practically no urinary excretion of the drug was observed. Essentially similar rapid transfer of adriamycin from plasma to tissues has been reported in normal rats and dogs (Kimura et al, 1972; Yesair et al, 1972). Adriamycin has a relatively long half-life in tissues due to its histophilic properties and shows cumulative characteristics upon repetitive injections. Although adriamycin is slowly metabolized, its aglycone-like compounds have been reported in hamsters (Mhatre et al, 1971; Yesair et al, 1972). A lot of information is available on the pharmacokinetics of adriamycin, however it should be recognized that there is still a need to study drug metabolism to clarify the picture.

III. Cardiovascular Effects

Adriamycin induced changes can be categorized into short term (acute) and long term (chronic) effects. The acute cardiotoxic effects develop within minutes after the intravenous administration of the drug and are characterized by hypotension, tachycardia, and various arrhythmias (Herman et al, 1971; Arena et al, 1972; Lefrak et al, 1973; Zbinden and Brandle, 1975). In contrast, chronic effects often develop only after several weeks or months of treatment, and sometimes even after the completion of therapy. These chronic effects include insidious onset of cardiac myopathy often leading to congestive heart failure (Buja et al, 1973; Chalcroft et al, 1973; Lefrak et al, 1973; Jaenke, 1974). This, generally, is a delayed effect of cumulative doses of adriamycin with a high incidence of heart failure occurring usually at $\geq$ 550 mg/m^2 of the drug (Lefrak et al, 1973; Blum, 1975). Although it is generally believed that these cardiovascular effects are of both direct and indirect nature, no conclusive study concerning the mechanism underlying the development of cardiomyopathy due to anthracycline treatment has yet appeared in the literature.

IIIa. Acute Caridotoxicity

Electrocardiograms (EKG) of rabbits injected with 20 mg/kg adriamycin showed rapid tachycardia associated with the inversion of T-wave (Arena et al, 1972). Reduction in the amplitude of electrocardiographic waves in dogs administered with 50 mg adriamycin was also observed (Herman et al, 1972) Furthermore, adriamycin (1.5 mg/kg) at 10 minutes was shown to have a negative inotropic effect and caused a decrease in both aortic and coronary

flow in the dog heart (Arena et al, 1972). Negative inotropic effect of adriamycin (5 ug/ml) was also demonstrated in isolated heart preparations and the effect was not reversed by propranolol or quinidine (Arena et al, 1972). Pretreatment with either dl-propanolol (0.5 mg), atropine (1.0 mg), diphenhydramine (10 mg) or lysergic acid diethylamide (50 ug) did not alter the adriamycin-induced changes in the coronary perfusion pressure in dog hearts (Herman et al, 1972). These alterations in cardiac function due to adriamycin treatment may be associated with different subcellular effects (Myers et al, 1977; Azuma et al, 1981; Singal et al, 1983; Singal and Panagia, 1984; Singal, 1985)

The only morphological change observed within minutes of drug-administration was in the nucleus (Buja et al, 1973; Lambertenghi-Deliliers et al, 1976; Merski et al, 1976). The characteristic changes in the nucleolema as well as the nucleolar chromosomal component of the myocytic nuclei (Lambertenghi-Deliliers, 1976) were quite similar to those seen in the hepatocyte nucleolus (Lambertenghi-Deliliers, 1976). Although there is unanimity of opinion concerning the nucleolar changes of rapid onset after the drug injection, there is some controversy with respect to the reversibility of these changes. According to Lambertenghi-Deliliers et al (1976), the nuclear changes reverted to normal within 14 hours in rats injected with a single dose of adriamycin (10 mg/kg). The transient occurrence of nuclear changes is also supported by the fact that only a small percentage of myocardial cells in patients who died from chronic anthracycline-toxicity showed nuclear lesions (Buja et al, 1973). However, in rats injected with a single dose of adriamycin (40

mg/kg), the nucleolar changes did not revert to normal (Merski et al, 1976). These differences could be due to the age and/or dose of the drug employed.

Occurrence of reddish fluorescence due to adriamycin in the nuclei immediately after its administration indicated rapid transfer of this drug across the nuclear membrane (Buja et al, 1973; Egorin et al, 1974). Furthermore, the reported characteristic nucleolar changes have been attributed to the intercalation of the drugs into nuclear DNA which in turn inhibits nucleic acid and protein synthesis (Arena et al, 1974). It is interesting to note that these drugs do bind to mitochondrial DNA thus affecting the protein synthesis in these organelles.

IIIb. Chronic Cardiotoxicity

Patients treated for far-advanced carcinoma by repeated injections of adriamycin (total cumulative dose 505 to 1004 mg/m^2 body surface) over a period of several months showed marked hypotension (B.P.-70/50 mm Hg), tachycardia (150 beats/min) and a conspicuous decrease in the QRS voltage on the EKG, cardiac dilatation and ventricular failure, as well as refractoriness to inotropic drugs and mechanical ventricular assistance (Lefrak et al, 1973). Repeated administration of adriamycin (total cumulative dose of 20 mg/kg or higher) in rats caused a depression in cardiac output and the response of these animals to norepinephrine (2 and 10 ug/kg) or epinephrine (0.5 and 1 ug/kg) was less than in controls (Zbinden et al, 1978). In this regard, several in vitro studies on the effects of adriamycin have reported depressed contractile function (Azuma et al, 1981; Singal and Pierce, 1983). The widening of the QRS complex

in adriamycin treated rats was significant after cumulative doses of 8-12 mg/kg over seven days and the continued treatment with drug resulted in intraventricular block (Zbinden et al, 1978).

Determination of serum levels of certain enzymes such as glutamic oxaloacetic acid transaminase (GOT), lactic dehydrogenase (LDH) and creatine phosphokinase (CPK) have been widely used in the diagnosis and assessment of myocardial ischemia (Sobel and Shell, 1972; Wagner et al, 1973). The release of these enzymes, which are present in high concentration in myocardial cells is also indicative of changes in cell membrane permeability. Using histochemical and biochemical approaches, it has been established that these and many other enzymes are released by the diseased myocardium (Jennings et al, 1957; Bajusz and Jasmin, 1964; Lee et al, 1971). Release of GOT, LDH and CPK enzymes from the heart has also been demonstrated in the isolated rat heart preparations subjected to successive perfusion with calcium-free and calcium-containing medium (Zimmerman and Hulsmann, 1966). Indeed some of the patients treated with adriamycin for far-advanced carcinoma showed manyfold increase in GOT, LDH and CPK enzyme content in the serum (Lefrak et al, 1973). Serum enzymes can be seen to provide further evidence regarding myocardial cell membrane abnormality in cardiomyopathy due to anthracyclines but the cause-effect relationship of these alterations is not clear at present.

In chronic anthracycline cardiotoxicity, degeneration of cardiac muscle cells is characterized by loss of myofibrils and/or marked cytoplasmic vacuolization. These structural changes have been found to be common in humans (Buja et al, 1973;

Lefrak et al, 1973; Bristow et al, 1976), rabbits (Jaenke, 1974; Olson et al, 1974), mice (Rosenoff et al, 1975; Lambertenghi-Deliliers et al, 1976) and rats (Chalcroft et al, 1973; Zbinden et al, 1978) thus emphasizing the similarities between different animal models and humans. In these electron microscope studies, involvement of the nuclei, intercellular junctions and mitochondria have also been reported. There is a great deal of controversy with respect to changes in the intercellular junctions during anthracycline-induced cardiotoxicity. In a study on patients it has been reported that following adriamycin treatment the intercalated disc remained continuous, and the intercellular space retained its uniform character (Lefrak et al, 1973). However, rounding of the myocardial cells in the same study would indicate changes in the cell-cell contact. Buja et al (1975) in a study of heart biopsies of patients treated with antineoplastic drugs, reported "unusual junctional structures" present in the cytoplasm and referred to them as "intracytoplasmic junctions". It was suggested that these junctions were formed between the infolding of the plasma membrane of the same cell and not between two adjacent cells as a result of remodeling of the cell surface (Buja et al, 1975).

Of all the subcellular structures present in the myocardial cell, only mitochondrial effects of anthracyclines have been studied in a considerable detail in _in vitro_ as well as _in vivo_ preparations. These mitochondrial effects have been reviewed recently (Dhalla et al, 1980). Daunomycin and adriamycin have been shown to inhibit ATP production and oxygen consumption in state 3 respiration of rat heart mitochondria _in vivo_ and _in_

vitro (Bachmann et al, 1975). Other workers have reported that adriamycin and related compounds have an uncoupling effect on the mitochondrial oxidative phosphorylation (Gosalvez et al, 1974). Both adriamycin and rubidazone caused uncoupling of calcium translocation from electron transfer reactions. ATP-dependent calcium uptake into mitochondria was also inhibited by the addition of rubidazone in the in vitro system (Bachmann and Zbinden, 1979). In rats treated with adriamycin, the mitochondrial calcium/oxygen ratio fell from about 2.0 to 0.2 in 24 hours after 4 doses of 4 mg/kg (Bachmann and Zbinden, 1979). In this regard high energy phosphate stores are known to be reduced by adriamycin (Azuma et al, 1981; Singal and Pierce, 1983).

Drug-induced structural damage to the mitochondria includes disruption of cristae, swelling and appearance of myelin figures in this organelle (Chalcroft et al, 1973; Jaenke, 1974; Olson and Capen, 1977; Baljas and Ferrans, 1978; Ferrans, 1978; Zbinden et al, 1978). These studies indicate the involvement of mitochondrial defects in myocardial cell damage upon anthracycline treatment. Although these changes may be secondary to the lesions at other sites, their importance in cardiotoxicity cannot be ignored. Accordingly, changes in mitochondrial function will lead to an impairment in the process of energy production which will further aggravate the process of cell damage and contractile failure (Dhalla et al, 1980). Accumulation of Ca^{2+} containing granules in the mitochondria of a hypertrophied rabbit hearts exposed to adriamycin has been reported (Singal, 1983; Singal et al, 1984).

Accumulation of lipid droplets in the myocardium during the

occurrence of anthracycline-cardiotoxicity has been indicated (Ferrans, 1978). However, no detailed study in this model concerning this aspect, particularly the nature and distribution of lipid droplets, has yet been undertaken. The importance of such information is evident from the fact that lipids are the main substrates of cardiac metabolism (Opie, 1969). Furthermore, accumulation of lipids may also be important in the suggested formation of lipid peroxides during adriamycin-cardiotoxicity (Myers et al, 1977). Employing cytochemical and electron microscopic methods, Roy (1975) has shown that lipids in the heart are present mostly in the interstitium as free droplets of triglycerides and has suggested that this substrate pool is mobile and readily available for energy production. In view of the detergent effects of free fatty acids on cellular function, the accumulation of lipid peroxides in the heart due to adriamycin can be seen to play some role in the pathogenesis of myocardial cell damage.

In summary, the structural damage to the myocardial cell due to chronic administration and/or large single injection of anthracyclines include: focal myofibrillar lysis, intracellular edema, dilatation of the sarcoplasmic reticulum, mitochondrial changes and formation of myelin figures, nuclear changes, accumulation of lipid droplets, and changes in the cell-cell relationship. However, the sequence of the morphologic changes enumerated above, particularly with respect to the changes in the cyto-membranes such as sarcolemma, mitochondria and sarcoplasmic reticulum has not been fully elucidated thus far.

IV. Suggested Mechanisms of Anthracycline Cardiotoxicity

Several different subcellular effects of adriamycin have been reported and the list includes: a) binding of anthracyclines to nuclear and mitochondrial DNA, with subsequent inhibition of RNA and protein synthesis; b) inhibition of Na-K-dependent ATPase activity; c) inhibition of reactions utilizing coenzyme Q; d) alterations in calcium transport and in intracellular electrolyte balance; e) chelation of divalent cations; and f) promotion of lipid peroxidation by means of reactions mediated by free radicals. However, the mechanism(s) for the cardiac morphologic changes and other functional alterations enumerated in acute and chronic cardiotoxicity are likely the result of one or more of these different subcellular drug effects. Thus no single mechanism explaining the adriamycin-induced cardiomyopathy is available. Some of the postulated mechanisms include:

1) Release of Vasoactive Substances

Bristow et al (1980) have shown that the anthracyclines can cause the release of histamine, catecholamines and prostaglandins. The acute effects of anthracyclines, ie. arrhythmias and peripheral vascular effects, may be consequences of the drug induced release of histamine and catecholamines (Bristow et al, 1980). Morphologic changes related to the acute arrhythmias remain to be determined although it is known that in chronic toxicity of adriamycin the specialized conducting cells of the rabbit heart show lesions similar to those in ordinary working myocardium (Ferrans, 1978). The acute hemodynamic effects of adriamycin were evaluated by Bristow et al (1980) in the open-chest dog. Profound hemodynamic changes similar to those produced by histamine were observed and histamine release in

peripheral tissues was documented by a marked increase in venous histamine levels. In addition to these observations, secondary catecholamine release occurred in response to histamine, and immunoreactive prostaglandins E and F were increased in coronary sinus blood (Bristow et al, 1980). H_1- and H_2- receptor blockade prevented the early (2-30 minutes postinfusion) effects of doxorubicin and combined histaminergic and adrenergic blockade prevented the late effects. Bristow et al (1980) postulated that the release of vasoactive substances could also be part of the pathogenetic mechanism of anthracycline cardiomyopathy. This mechanism, however, cannot explain the morphologic changes typical of adriamycin-induced cardiomyopathy.

2) Myofibrillar Loss

a) Inhibition of nucleic acid and protein synthesis - It is now well established that adriamycin intercalates between the base pairs of DNA and its antitumor activity is the result of the inhibition of nucleic acid and protein synthesis (Arena et al, 1974). This mechanism may also be cytotoxic in the heart causing cumulative damage to DNA in cardiac muscle cells leading to severe interference with protein synthesis. Since the half-life of contractile proteins in cardiac muscle is in the range of 1-2 weeks, repeated doses of adriamycin could inhibit protein synthesis sufficiently to explain the loss of myofibrils resulting in generalized deterioration and eventual failure of the heart (Merski et al, 1976). This approach may or may not explain dilation of the tubular system seen in adriamycin cardiomyopathy.

b) Lysosomal alterations - More recently Singal et al (1985) have studied morphologic and enzymatic changes in heart lysosomes

as an additional mechanism of adriamycin cardiotoxicity. Following chronic treatment of animals with a cumulative dose of 15mg/kg of adriamycin, there were lysosomal changes in adriamycin-treated hearts which preceded as well as accompanied nonspecific permeability changes in the sarcolemma and increased malondialdehyde content indicating peroxidation of membrane lipids. Changes in lysosomal hydrolases have also been reported by Gebbia et al (1985). These reports (Singal et al 1985; and Gebbia et al, 1985) suggest that release of lysosomal enzymes could play a role in the pathogenesis of cardiotoxicity and be progressive during chronic adriamycin treatment. Release of lysosomal enzymes may be due to peroxidative changes in lysosomal membranes allowing release of lysosomal hydrolases thereby causing accelerated degradation of proteins and myofibrillar loss (Singal et al, 1985). Thus myofibrillar lysis and decreased protein synthesis may both be responsible for the myofibrillar drop-out seen in chronic adriamycin cardiomyopathy.

3) Lipid Peroxidation

The pathogenesis of changes which involve membrane systems of the cell may be related to interference with normal synthesis and turnover of proteins in the membranes but evidence also suggests that peroxidation of membrane lipids may also contribute to membrane damage.

Increased concentration of malondialdehyde in adriamycin-treated animals has been shown by several studies (Myers et al, 1977; Singal et al, 1985). Malondialdehyde is a product of fatty acid peroxidation and a good indicator of this change (May and McCay, 1968) caused by the reaction of free radicals with polyun-

saturated fatty acids in the membrane. In this regard, adriamycin because of its capability to convert reversibly to a free-radical semiquinone can shuttle electrons to oxygen and has been shown to stimulate the production of superoxide (Bachur, 1977; Thayer, 1977; Doroshow, 1983), which can lead to the production of highly toxic hydroxyl radicals (Fridovich, 1974). These oxygen radical species can initiate free radical chain reactions leading to the peroxidation of polyunsaturated fatty acids in the hydrophobic interior of the membrane (Plaa and Witsche, 1976; Chance et al, 1979). In addition, production of superoxide radicals by adriamycin and daunorubicin can be facilitated by the transfer of electrons from endogenous compounds such as NADPH to oxygen. These free radicals can then oxidize unsaturated fatty acids in membranes to lipid peroxides (Myers et al, 1976). Manifestation of lipid peroxidation would then depend upon factors which affect the pharmacological actions of the drugs as well as the presence and activity in heart tissue of biochemical and enzymatic defenses against reactive forms of oxygen. Relative to other organs, the heart has a low activity of superoxide dismutase and catalase - two important enzymatic defences against free radical reactions (Doroshow, 1980). In this regard, in rats, superoxide dismutase activity in the heart is about 1/5 of that found in the liver, and heart catalase activity is 1/45 of liver catalase activity (Mimnaugh et al, 1982). It appears that in heart muscle, membrane alpha-tocopherol may be the most important defense against toxic reactive oxygen metabolites and in protecting membranes against lipid peroxidation (Mimnaugh et al, 1982). In mice pretreated intraperitoneally with alpha-

tocopherol 24 hours prior to adriamycin administration (15 mg/kg), the mortality on the 16th day was less than 15% in comparison to 85% in untreated animals (Myers et al, 1976). Later it was shown that cardiotoxicity was also reduced whereas antitumor activity was unaffected or even increased (Myers et al, 1977). Furthermore, it has also been shown that alpha-tocopherol in ameliorating the lethal toxicity of adriamycin in these animals did not affect tissue distribution or metabolism of adriamycin (Mimnaugh et al, 1979). However, this protective effect of alpha-tocopherol was found to be short lived as the mortality in both treated and untreated mice was comparable after two months (Myers, 1977; Mimnaugh et al, 1979). Tocopherols are known to have antioxidant activity which prevents the auto-oxidation of highly unsaturated fatty acids. The reduction in adriamycin-induced cardiotoxicity by alpha-tocopherol has been attributed to a reduction in the formation of lipid peroxides (Myers et al, 1977). It is possible that chronic anthracycline treatment progressively depletes cardiac membrane alpha-tocopherol to levels which can no longer prevent peroxidation of lipids and since other defenses against reactive oxygen are low, subsequent doses of anthracycline could stimulate uncontrolled lipid peroxidation affecting different subcellular membrane systems such as sarcoplasmic reticulum, mitochondria and sarcolemma.

Furthermore, adriamycin has been shown to change membrane fluidity in the <u>in vitro</u> system indicating a direct effect of the drug on the membranes (Murphree et al, 1977). Changes in the calcium transport and intracellular electrolyte imbalance in rat and rabbit hearts due to adriamycin administration (Olson et al,

1974; Anghileri, 1977; Olson and Capen, 1977) would also indicate some change in membrane permeability due to the drug (Singal et al, 1985). Thus adriamycin-induced lipid peroxidation can be seen to explain a variety of subcellular changes seen in in vitro as well as in vivo systems.

4) Enzyme Changes

Enzyme systems are also known to be affected by anthracycline drugs. In this regard, adriamycin has been shown to produce an inhibitory effect on the Na^+, K^+-ATPase activity (Gosalvez et al, 1975, 1979) as well as on guanylate cyclase activity (Levey et al, 1979). Adenylate cyclase activity was found to be stimulated by 1 and 10 nM of adriamycin whereas this enzyme activity was inhibited at 100 uM and 1 mM concentrations (Singal and Panagia, 1984). Adriamycin concentrations known to inhibit adenylate cyclase activity were also found to induce a negative intotropic effect in rat papillary muscles (Singal and Pierce, 1983). Furthermore, Ca^{2+} ATPase activity was found to be stimulated by adriamycin but the drug had no effect on Mg^{2+} ATPase and 5'-nucleotidase activities (Singal and Panagia, 1984). These data suggest direct as well as specific effects of adriamycin which may be important in drug-induced cardiotoxicity, in addition to lipid peroxidation and membrane permeability changes.

V. Risk Factors in Adriamycin Cardiotoxicity

It is now quite clear that the development of adriamycin-cardiomyopathy may also be associated with certain risk factors listed below.

1) Radiation - previous or concomittant radiation has been suggested to potentiate the adriamycin-induced cardiomyopathy.

Prior mediastinal radiation appears to increase the adriamycin cardiotoxicity at doses well below the suggested dose limitation of 550 mg/m^2 (Minow et al, 1977; Praga, 1979; Billingham et al, 1977). Children having prior radiation therapy to the mediastinal area were also reported to have a higher incidence of adriamycin-induced cardiomyopathy (Gilladoga, 1976). The potentiating effects of radiation are dose-related in animals (Fajardo et al, 1976) and man (Billingham et al, 1977) and therefore the dose of radiation received by the left ventricle is important. Adriamycin may induce a "recall" phenomenon of latent acute radiation change in the myocardial small vessels even if the radiation was given years prior to adriamycin administration (Billingham, 1979). Thus there may be acute and chronic radiation effects superimposed on the anthracycline changes, a situation that proves disastrous for the heart (Bristow et al, 1978). Based on these observations Bristow et al (1978) recommend that the total cumulative dose of adrimycin should ideally remain well below 550 mg/m^2 and careful monitoring of developing cardiomyopathy be undertaken by endomyocardial biopsy as well as hemodynamic technique such as radionucleotide ejection fractions.

2) Liver Disease - Liver is the predominant site of adriamycin metabolism and excretion is primarily via the liver and biliary system (Bachur, 1975, 1979). Any impairment in the liver and biliary system can cause greater drug toxicity. The delayed excretion of the drug increases the plasma half-life of adriamycin, thus prolonging or increasing the toxic effects. Elevated and prolonged levels of adriamycin and its metabo-

lites have been seen in patients with liver disease (Benjamin, 1975). Serum bilirubin levels indicating the presence of liver disease are criterion for dosage reduction of adriamycin (Benjamin, 1975). Rats fed a high fat diet showed a significant augmentation of the cardiotoxic effects of adriamycin demonstrated by EKG changes and myocardial histopathology (Zbinden et al, 1977). The high fat diet led to fatty infiltration and cellular degeneration of the liver so that adriamycin metabolism and excretion by the liver was probably impaired. Zbinden et al (1977) suggests these experimental findings are in accord with clinical observations which have identified liver disease as one of the important risk factor in adriamycin-induced cardiomyopathy.

3) Concurrent Chemotherapy - Since adriamycin is a frequent component of several combination chemotherapy regimes, it is likely that some drugs administered with adriamycin may possibly increase the risk of developing heart failure. In this regard, Praga et al (1979) report a definite adriamycin cardiomyopathy with (a) vincristine when given both before and concomitantly with adriamycin, (b) bleomycin when given before adriamycin. Cyclophosphamide did not seem to influence the risk of adriamycin-cardiomyopathy (Bristow et al, 1978; Praga, 1979; Minow and Benjamin, 1977). Nevertheless, it is still recommended that a reduced cumulative dose of adriamycin be given with cyclophosphamide as well as with other chemotherapeutic agents (Minow and Benjamin, 1977). In addition, children treated with a combination of adriamycin and other chemotherapeutic drugs also showed an increased incidence of heart failure over the incidence with

adriamycin alone (Smith et al, 1977).

4) Previous Cardiovascular Disease - It is suggested that pre-existing hypertension or cardiac disease may cause development of congestive heart failure at doses much lower than the 550 mg/m^2 (Lefrak et al, 1973). Uncontrolled hypertension or aortic stenosis (both of which increase the afterload on the heart) may be implicated as risk factors in drug induced cardiomyopathy (Singal, 1985). The normal process of hypertrophy to compensate for the increased afterload can be compromised by the adriamycin effects on the protein synthesis and turnover. Von Hoff and associates (1979) in a retrospective study reported that the probability of developing adriamycin induced heart failure versus total dose of adriamycin was higher in patients with previous cardiac disease or hypertension or both, as compared to all other patients and this difference almost reached statistical significance (P=0.08). It is assumed in these retrospective studies that patients with hypertension or cardiovascular disease had cardiac hypertrophy but this was not confirmed. In this regard, in a rabbit model, hypertrophy was produced by causing pressure overload (banding the abdominal aorta) and was confirmed by heart weight/body weight ratio and left ventricular wall thickness (Singal et al, 1984; Singal, 1983). This study showed that adriamycin caused cardiotoxicity and structural damage to the hypertrophied heart at much lower doses of adriamycin. At the same low dose no damage was seen in sham controls (Singal, 1983). This indicates an increased sensitivity of the hypertrophied heart to adriamycin induced damage. Other myocardial diseases, such as diabetic cardiomyo-

pathy, valvular defects, and coronary heart disease can also be potential risks in adriamycin therapy.

5) Calcium Entry Blockers - Other supportive therapeutic approaches in the adriamycin-induced cardiotoxicity may in fact be potential risk factors as exemplified by calcium antagonists. The influence of calcium entry blockers in adriamycin-induced cardiomyopathy is currently a controversial subject. Daniels and associates (1976) provided evidence for protection by subcutaneously administered verapamil but no survival data were given. Studies by Young and associates (1976) and Klugman and associates (1981) have provided evidence for a reduction in survival following treatment with the calcium entry blockers verapamil or nifedipine, and no protective effect by these drugs on cardiac ultrastructural changes induced by adriamycin was seen. Rabkin and associates (1983) observed a decreased calcium accumulation with verapamil despite reduction in survival. Experimental studies suggest that calcium entry blockers may actually enhance adriamycin-induced myocardial injury (Tsuro et al, 1983) as they have been shown to potentiate the antitumor activities of several DNA-interacting drugs including adriamycin. Thus the use of calcium entry blockers may also be considered another factor exposing the heart to an increased risk of developing adriamycin cardiotoxicity.

6) Age - In a retrospective study of 4018 patient records, Von Hoff and associates (1979) reported on the effect of age in the probability of developing drug induced heart failure at various adriamycin dose levels. By comparing all age groups these observers noted a steady increase in the probability of

developing congestive heart failure with increasing patient age (P=0.0027). Thus patients of age $\geq$70 and increasing dose appear to synergistically increase the probability of developing congestive heart failure (Bristow et al, 1978). Although age has been suspected as one of the risk factors, no investigative report is readily available to support this contention. Other variables such as pre-existent cardiac disease in older age groups may further complicate the issue. The higher incidence of heart failure could not be attributed to a higher incidence of coronary artery disease in the older patient. Praga and associates (1979) in a study involving 1273 patients in clinical trials with adriamycin found no statistical significance in age as a risk factor after comparing subgroups by age and total dose of adriamycin, in contrast Bristow and associates (1978) reported their impression that $\geq$ 70 does appear to be a risk factor (P=0.50) and predisposes this age group to adriamycin-induced heart failure.

The agents which have been studied in an attempt to protect against anthracycline-induced cardiomyopathy and heart failure have not yet proven to give satisfactory results. The extensive use of adriamycin and its effectiveness as a chemotherapeutic drug in oncology makes it appropriate to study risk factors which may potentiate adriamycin-induced cardiotoxicity. In this regard we chose to study age-related differences in chronic drug-induced cardiotoxicity.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 175 ± 25 grams, approximate age 1 1/2 months and 450 ± 25 grams, approximate age 6-6 1/2 months were used. In the present study the lower weight animals were called the "younger" group and higher weight animals were called the "older" group. The animals were grouped as follows:

C_y= young control animals (vehicle-treated)

C_y + A= young treated with adriamycin

C_o= older control animals (vehicle-treated)

C_o + A= older adriamycin treated

Animals were housed three to a cage and kept in environmentally controlled rooms. All animals were provided with standard rat chow and water ad libitum. Animal weight as well as feed consumed by each animal was monitored daily.

Adriamycin Treatment

Adriamycin (Doxorubicin HCl) obtained from Adria Laboratories of Canada Limited was solubilized with double distilled water. The drug was administered intraperitoneally in 6 equal injections to animals in groups C_y + A and C_o + A over a period of two weeks. The total cumulative dose delivered was 15 mg/kg body weight. Control animals in groups C_y and C_o were injected with the vehicle alone (lactose 75 mg/kg) in the same regimen. Both treated and control animals were observed for up to 4 weeks after the last injection for their general condition and behaviour. Mortality data were also obtained.

For studies on the heart muscle, the animals were sacrificed

by decapitation and their hearts were removed and weighed. The myocardial tissue was examined for its contractile function, ultrastructure and malondialdehyde content.

General Pathology

The peritoneal cavity was exposed by means of a midline abdominal incision, and any fluid present was collected and the volume was measured. The peritoneal cavity was examined for any gross abnormalities such as adhesions or discolorations. Appearance of liver, kidneys and lungs was also recorded.

Contractile Function Studies

Contractile function studies were carried out on papillary muscles which were quickly dissected from the left ventricle. All handling and removal of the papillary muscle was done in a petri dish containing oxygenated Krebs-Henseleit solution. The medium consisted of (mM): NaCl, 124.9; KCl, 5.15; $CaCl_2$, 2.65; NaH_2PO_4, 1.26; $NaHCO_3$, 20.13; glucose 10, pH 7.4. The papillary muscle was tied vertically with silk thread in a jacketed 100 ml constant - temperature bath maintained at 37°C and containing the same solution. A 95% oxygen/5% carbon dioxide mixture was continuously bubbled through the solution in the tissue bath maintaining the pH at 7.4. One end of the muscle was tied to a fixed holder while the other end was tied to a grass FT-03 force displacement transducer through a moveable holder in such a way as to permit isometric contraction. The muscle was stimulated supramaximally with square wave stimuli of 5 msec duration at the rate of 30/min by a Grass S44 stimulator through platinum electrodes placed on either side of the muscle. In all the experiments, resting tension was adjusted to give maximum contraction.

The muscle was allowed to equilibrate in the solution for a minimum of one half hour before the effects of different interventions were studied.

Effect on papillary muscle function of frequency change (increasing the rate of stimulation from 30/min to 90/min), different Ca^{2+} concentration (50% of normal or 1 mM above normal) and epinephrine (10^{-5} M) were recorded. A steady state contraction was attained between the study of effects of different interventions. Muscle weight (2 to 9 mg) and length (2.5 to 4 mm) were measured at the end of each experiment. Contractile force (peak tension) and a positive (+) and negative (-) dp/dt were monitored on a Beckman Dynograph Recorder.

Ultrastructural Studies

For ultrastructural studies, 5-10 animals in each group were used. Hearts were washed in cold 0.1 M sodium phosphate buffer (pH 7.4). Tissue samples, 4 to 6 mm. in size, were taken from four different areas of the subendocardium, as well as subepicardium of the free left ventricle wall between the midregion and apex of the heart. The tissue pieces were immersed for 15 minutes in 0.1 M phosphate buffer (pH 7.4) containing 3 percent glutaraldehyde. This briefly fixed tissue was further cut into pieces smaller than 1-mm. cubes. Aldehyde fixation was continued for a total duration of 2 hours. The tissues were washed for 1 hour in the above phosphate buffer containing 0.05 M sucrose. Postfixation was done in 2 percent OsO_4 for 1.5 hours, after which the tissue pieces were dehydrated in a graded alcohol series. Tissue embedding was done in Epon (Luft, 1961). Ultrathin sections were cut on MT II Porter-Blum ultramicrotome. Thin

sections were placed on Formvar coated grids and stained with uranyl acetate and lead citrate (Reynolds, 1963). Electron micrographs of the subendocardial and subepicardial regions from the four groups were compared to establish qualitative anatomical differences. At least four hearts from each group were examined for the study of ultrastructural changes using a Carl Zeiss EM9 electron microscope. Two hearts in each group were fixed by perfusion method (Singal and Dhalla, 1984).

Malondialdehyde Assay

Measurement of lipid peroxidation by determining myocardial malondialdehyde (MDA) content was performed using a modified Thiobarbituric Acid (TBA) method (Hunter et al., 1963, Placer et al., 1966). Malondialdehyde bis (dimethyl acetal) 99 + % obtained from Aldrich Chemical Company Incorporated was used as a standard. Hearts were quickly excised and washed in buffered 0.9% KCl pH 7.4. After removing the atria, extraneous fat and connective tissue, the ventricles were homogenized in 10% W/V in the same buffer. The homogenate was incubated for one hour at 37^{o}C in a water bath. A 1-ml aliquot was withdrawn from the incubation mixture and pipetted into an 8 ml Pyrex tube. One ml of 40% trichloroacetic (TCA) acid and one ml of 0.2% thiobarbituric acid were promptly added. Tube contents were vortexed briefly, boiled for 15 minutes and cooled in a bucket of ice for 5 minutes. Two ml of 70% trichloroacetic acid was then added to all tubes and contents were again vortexed briefly. The tubes were allowed to stand for 20 minutes. This was followed by a centrifugation of the tubes for 20 minutes at 1500 RPM (table top centrifuge). The supernatant was carefully drawn off and centri-

fuged in RCII-B Sorval centrifuge at 3500 RPM for 20 minutes. The colour was read at 532 nanometers on a Zeiss spectrophotometer. The standard tube contained 1 micromolar MDA.

Statistical Analysis

Data was analysed statistically and expressed as the mean $\pm$ S.E.M. Differences with a P value < 0.05 were taken to be significant.

RESULTS

General Observations

The general appearance of all groups of animals was recorded during the time course of the study. After completion of adriamycin treatment, the animal fur became scruffy and developed a light yellow tinge in both C_y + A and C_o + A groups. The treated animals developed bloody noses, had a red exudate around their eyes, and soft watery dark feces. These changes were more pronounced in the C_o + A group. Animals in this (C_o + A) group also appeared to be sicker, weaker and lethargic compared to the C_y + A group. Both treated groups had a sick demeanor compared to their controls.

The most predominant feature in C_o + A group animals was the development of a grossly enlarged abdomen and ascites. This condition of the animals started becoming apparent within a week after the completion of treatment with adriamycin. At sacrifice, all C_o + A treated animals had a significant amount of peritoneal fluid. In addition in all C_o + A animals the liver was congested and enlarged with rounded edges. In the C_y + A group a majority of the animals developed congested livers but only a few developed overt ascites or an enlarged abdomen as compared to the C_o + A group.

Effects on Body Weight and Feed Consumption

Animal body weight and feed consumed by each animal in a day was monitored and the data are summarized in Figures 1 and 2. The animals in the C_y + A group stopped gaining but did not lose weight for the duration of the drug treatment (Fig. 1). Thereafter the daily gain in body weight in the young treated

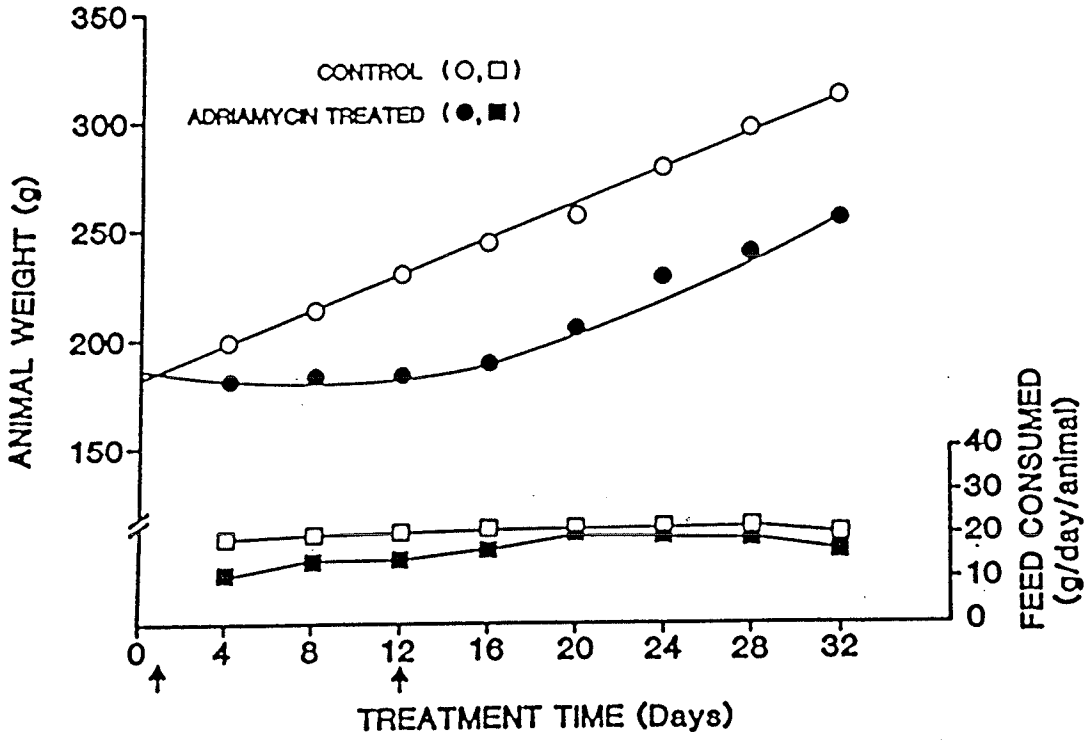

FIGURE 1) Effects of chronic adriamycin treatment on body weight and feed consumed in the younger group of rats. Adriamycin was administered during the period shown between the arrows. A marginal reduction in the feed consumed and no gain in the body weight is apparent during period of adriamycin administration in the treated animals.

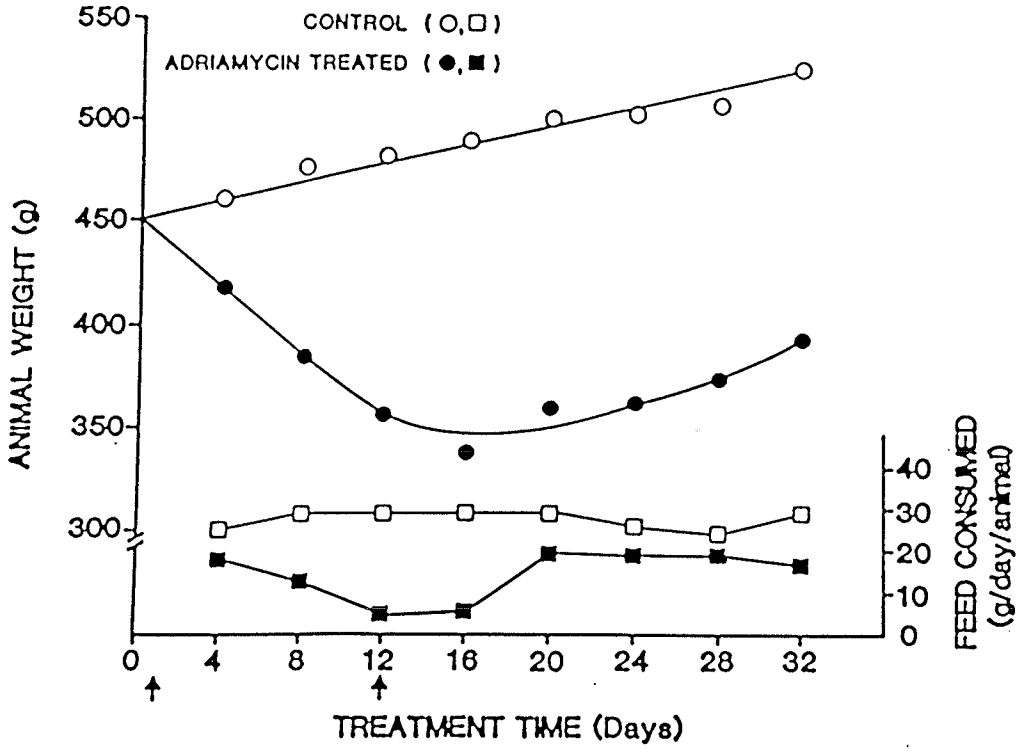

FIGURE 2) Effects of chronic adriamycin treatment on body weight and feed consumed in the older group of rats. Adriamycin was administered during the period shown between the arrows. A significant decrease in body weight and feed consumed during the period of adriamycin administration is apparent and post treatment recovery of these parameters was delayed by 4-6 days.

animals was parallel to the control group. In contrast, the older treated animals showed about 22% loss in body weight during the adriamycin treatment period (Fig. 2). In the post treatment period, these animals showed only a partial recovery in body weight. In both younger and older adriamycin treated groups, the animals showed a loss of appetite for the duration of the drug treatment. In the young-treated group, the decline in feed consumption per animal per day was about 20% and in the older group this decline was about 46%.

Effects on Heart Weight and Mortality

Data on heart weight, heart weight/body weight (HW/BW) ratio and mortality are summarized in Table 1. Treatment with adriamycin resulted in a difference in mean heart weight between the treated and age matched control animals. The hearts in the C_y + A group animals weighed about 15% less than controls. This difference, however, was not statistically significant. In the older groups, adriamycin treatment resulted in about 38% decrease in heart weight and the difference was statistically significant ($P<0.01$) in comparison to its age matched control. In the younger group the difference in HW/BW ratio between the drug treated and control group was not significant. This ratio in the C_o + A group was significantly ($P<0.01$) less as compared to C_o group.

Mortality, in the older adriamycin treated group was about 30% and in the younger drug treated group was about 17%. Most of these deaths were seen in the post-treatment period. There was no mortality in the C_o or C_y groups.

TABLE 1

Comparison of effects of adriamycin on different parameters in two age groups of rats.

Animal Group	Heart Weight (grams)	Heart Weight/ Body Weight Ratio (mg/gm)	Mortality
C_y (n=20)	0.78 ± 0.04	2.56 ± 0.19	0
C_y + A (n=34)	0.67 ± 0.03	2.63 ± 0.09	6/34
C_o (n=20)	1.47 ± 0.05	2.90 ± 0.14	0
C_o + A (n=33)	0.91 ± 0.07*	2.36 ± 0.18*	10/33

Data for heart weight and heart weight/body weight ratio are expressed as mean ± SE of 5 experiments. C_y, young control; C_y + A, young-treated with adriamycin. C_o, older control; C_o + A, older-treated with adriamycin. For treatment schedule and other conditions of the experiments see Materials and Methods.
*) Significantly different ($P<0.01$) from the control values in the C_o group.

Papillary Muscle Studies

Papillary muscles isolated from the hearts of control and adriamycin treated rats were examined to study the contractile force changes in the hearts. There was no significant difference between the maximum developed force in the muscles from C_y and C_o groups. Adriamycin depressed the peak developed force in the C_y + A and C_o + A groups but the differences were not significant. In order to further discern the differences in the maximum developed force due to adriamycin treatment, the papillary muscle preparations in the bath were exposed to different interventions.

Force-Frequency Response

It is generally known that an increase in stimulation frequency in a variety of mammalian species induces an increase in peak developed force. This positive force-frequency relationship is also termed as Bowditch staircase phenomenon (Bowditch, 1871). In the present study when the stimulation frequency was increased from 30 beats/minute to 90 beats/minute, the papillary muscles from C_y and C_o groups showed a comparable decline in their peak developed force (Table 2). Frequency-dependent decrease in the peak developed force was less in the adriamycin treated C_y + A (Fig. 3) and C_o + A (Fig. 4) groups and this difference was significant ($P<0.05$) in the older treated animals. Positive as well as negative dp/dt values were also depressed in C_y and C_o groups because of an increase in stimulation frequency. This depression in dp/dt, however, was less in C_o group as compared to the C_y group (Table 3). Adriamycin treated animals were not significantly different with regard to changes in dp/dt.

TABLE 2

Effects of different interventions on the maximum developed force in papillary muscles isolated from the hearts of control and adriamycin treated rats.

Animal Group	High Frequency Response	Low Ca^{2+}	High Ca^{2+}	Epinephrine
C_y	-25.5 ± 3.88	-31.06 ± 4.45	+32.66 ± 4.78	+54.57 ± 4.15
C_y+A	-19.98 ± 2.66	-21.11 ± 4.60	+22.47 ± 3.14	+27.75 ± 9.81
C_o	-23.14 ± 2.75	-35.15 ± 2.6	+17.7 ± 3.6	+17.8 ± 2.7
C_o+A	-15.56^{+} ± 1.55	- 8.4^{*+} ± 1.51	+ 8.9^{*+} ± 1.92	+ 8.74^{+} ± 1.96

Data are expressed as percent change from the steady state force obtained in the same papillary muscle immediately prior to the intervention. Each data point is a mean ± S.E. of 4-7 experiments. C_y, young control; C_y+A, young-treated with adriamycin; C_o, older control; C_o+A, older-treated with adriamycin. For treatment schedule and other conditions of the experiment see Materials and Methods. *) Significantly different ($P<0.05$) from the young-treated (C_y + A) group. +) Significantly different ($P<0.05$) from control (C_o) group.

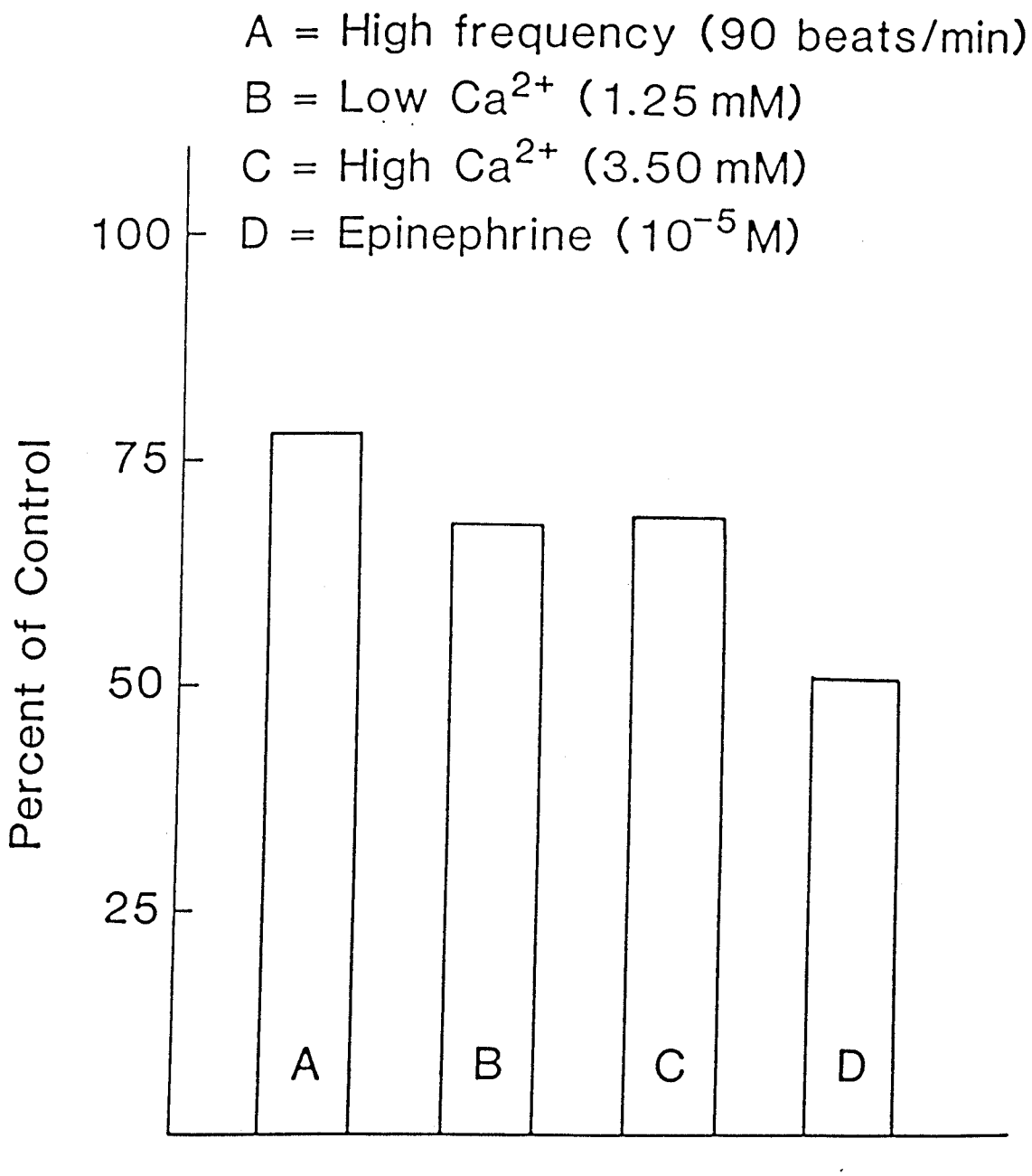

FIGURE 3) Effects of different interventions on the peak developed force in isolated papillary muscles of C_y + A group. The results are expressed as percent of the responses to interventions seen in the control papillary muscles. It should be noted that the negative response to high frequency (A) as well as low Ca^{2+} (B) was reduced because of adriamycin treatment. Similarly the positive response to high Ca^{2+} (C) and epinephrine (D) was also reduced in the C_y + A group as compared to the C_y group. For treatment schedule and other conditions of the experiments see Materials & Methods.

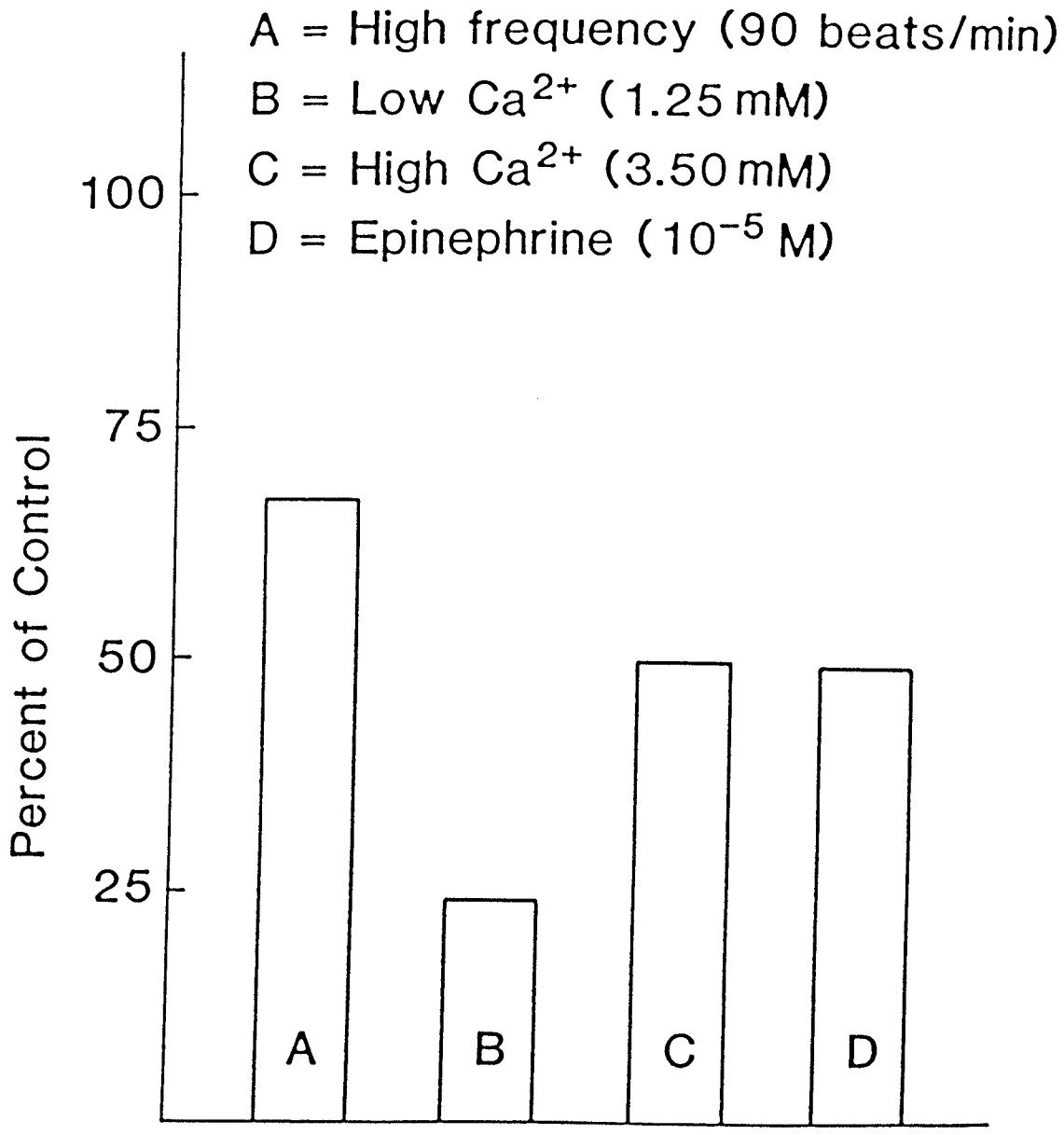

FIGURE 4) Effects of different interventions on the peak developed force in isolated papillary muscles of C_o + A group. The results are expressed as percent of the responses to interventions seen in the control papillary muscles. It should be noted that the negative response to high frequency (A) as well as low Ca^{2+} (B) was reduced because of adriamycin treatment. Similarly the positive response to high Ca^{2+} (C) and epinephrine (D) was also reduced in the C_o + A group as compared to the C_o group. For treatment schedule and other conditions of experiments see Materials & Methods. B and C significantly different ($P<0.05$) from the C_v + A group. A, B, C, and D significantly different ($P<0.05$) from control C_o group.

TABLE 3

Effects of different interventions on the (+) and (-) dp/dt in papillary muscles isolated from the hearts of control and adriamycin treated rats.

Animal	High Frequency Response		Low Ca^{2+}		High Ca^{2+}		Epinephrine	
	+dp/dt	-dp/dt	+dp/dt	-dp/dt	+dp/dt	-dp/dt	+dp/dt	-dp/dt
C_y	-20.59 ± 3.55	-19.77 ± 5.35	-25.31 ± 4.04	-36.16 ± 6.08	+18.32 ± 5.03	+28.65 ± 6.91	+77.25 ±11.90	+92.27 ±13.02
C_y+A	-13.86 ± 2.35	-16.13 ± 3.90	-15.84 ± 4.60	-15.84^{+} ± 3.81	+13.44 ± 1.84	+13.18^{+} ± 1.32	+30.48^{+} ± 7.22	+26.78^{+} ± 6.56
C_o	-12.66 ± 4.4	-13.69 ± 1.88	-29.47 ± 4.09	-31.77 ± 4.55	+16.95 ± 1.42	+35.77 ± 7.88	+32.39 ± 1.11	+32.54 ± 7.42
C_o+A	-10.70 ± 2.64	-13.13 ± 0.85	- 9.36^{+} ± 1.44	- 7.83^{+} ± 1.17	+ 7.62^{+*} ± 0.84	+ 7.72^{+*} ± 0.84	+25.68 ± 3.57	+16.11 ± 5.77

Data are expressed as percent change from the steady state values obtained in the same papillary muscle immediately prior to the intervention. Each data point is a mean ± S.E. of 4-7 experiments. C_y, young control; C_y+A, young-treated with adriamycin; C_o, older control; C_o+A, older-treated with adriamycin. For treatment schedule and other conditions of the experiments see Materials and Methods. *) Significantly different ($P<0.05$) from the younger treated (C_y + A) group. +) Significantly different ($P<0.01$) from the respective control values in C_y or C_o groups.

Effects of Calcium

Reducing the bath concentration of calcium by 50% as expected reduced the peak developed force by 30-35% in both C_y and C_o groups (Table 2). The depression in the developed force due to a reduction in extracellular calcium was markedly less in the adriamycin treated C_y + A (Fig. 3) and C_o + A (Fig. 4) groups. The depression in the older adriamycin treated group (C_o + A) was significantly ($P<0.05$) less than the younger treated (C_y + A) as well as untreated control groups (C_y and C_o). A similar pattern of changes was seen with regard to (+) and (-) dp/dt and the data are shown in Table 3. The differences in (-) dp/dt in C_y + A group and in (+) and (-) dp/dt in the C_o + A group were significant with a P value of < 0.01.

Increasing the calcium concentration in the bath by 1 mM resulted in a significant increase in peak developed force in the C_y and C_o groups (Table 2) whereas this increase in the adriamycin treated C_y + A and C_o + A groups was about 30 and 50% less than the increase seen in respective controls (Figs. 3 and 4). Both (+) and (-) dp/dt values were increased in the C_y and C_o groups (Table 3). Adriamycin treatment of the younger group resulted in a diminished response of (-) dp/dt whereas in the older group the adriamycin treatment attenuated both (+) and (-) dp/dt.

Epinephrine Response

Epinephrine (10^{-5}M) resulted in a significant increase in the peak developed force in the C_y and C_o groups (Table 2). In adriamycin treated animals in both groups the epinephrine-induced increase in the peak developed force was reduced to about 50% level of the respective control values (Figs. 3 and 4). Similar

pattern of changes were also noted in (+) and (-) dp/dt in all four group of animals.

In a preliminary study, it was found that the size of epinephrine-induced increase in peak developed force was dependent on the duration of the post-treatment period.

Morphological Studies

Electron microscopic analysis of left ventricular free wall, mid-portion, was conducted on heart tissue excised from all four groups of rats treated either with adriamycin or lactose (vehicle) solution. The morphological appearance of myocardial cells in hearts from control rats (C_y and C_o groups) treated with the vehicle alone was found to be normal (Fig. 5). A moderate dilation of the intercellular space and presence of 100-200 A^o size vesicles in this area as well as in the T-tubules appears to be a normal feature of the perfusion fixed hearts (Singal et al., 1979). In adriamycin treated C_y + A and C_o + A groups, morphological changes in the myocardial cells involved the mitochondria, the sarcoplasmic reticulum, the lysosomes, the nucleus and the myofibrils (Figs. 6-12).

Ultrastructural changes due to adriamycin treatment in the C_y + A group were not as marked as in the C_o + A group. In the C_y + A group, the dilatation of the sarcoplasmic reticulum and T-tubules was mild to moderate resulting in moderate vacuolization but this did not cause distortion of myofibrils or mitochondria (Figs. 6-8). The myofibrils maintained their characteristic parallel orientation and there was no loss of registry of Z-bands. Only a few cells exhibited a prevalent dilation of the sarcoplasmic reticulum (Fig. 8). There was swelling of the

FIGURE 5) Ultrastructure of the control heart from a rat treated with vehicle alone. Moderate dilation of the T-tubules and presence of small vesicles in this area appears to be a normal feature of perfusion fixed hearts as has been reported earlier (Singal et al, 1979). Bar indicates two microns.

FIGURE CAPTION ON
PAGE 44

FIGURES 6-8) Effects of chronic adriamycin treatment on the myocardial ultrastructure in younger group (C_y + A) of rats.

FIGURE 6) Nuclear changes include mild perinuclear edema and dispersion of chromatin. Mitochondria are swollen and there is moderate dilation of sarcoplasmic reticulum and T-tubules. Bar indicates one and one half microns.

FIGURE 7) Ultrastructure of the heart from a different animal. In addition to the subcellular changes seen in figure 6, lipid bodies are apparent. Collagen fibers are in abundance in the interstitial space. Bar indicates one and one half microns.

FIGURE 8) Electron micrograph shows more prevalent dilation of sarcoplasmic reticulum and T-tubules although there is still no loss of parallel orientation of myofibrils or registry of Z-lines. Bar indicates one and one half microns.

FIGURE CAPTION ON
PAGE 46

FIGURE CAPTION ON
PAGE 46

FIGURE CAPTION ON
PAGE 46

FIGURES 9-12) Ultrastructural changes in older group of rats (C_o + A) treated with adriamycin.

FIGURE 9) Intracellular vacuolization and electron dense inclusion bodies are apparent. Laminated myelin figures are present in and around degenerating mitocondria. Note normal appearance of intercalated disc. Bar indicates one and one half microns.

FIGURE 10) Upper panel: disruption of myocardial contractile elements with the formation of contraction bands. Lower panel: swelling of mitochondria, sarcoplasmic reticulum and T-tubules. Loss of registry of Z-bands and characteristic parallel orientation of myofibrils. A secondary lysosome can also be seen. Bar indicates one and one half microns.

FIGURE 11) Disruption and fragmentation of myofibrils with patches of myofibrillar dropout. Mitochondria show severe changes characterized by swelling, focal dilations, rupture of outer mitochondrial membrane, derrangement of cristae and clearing of mitochondrial matrix. Inclusion body is also apparent. Bar indicates two microns.

FIGURE 12) Swelling and degeneration of mitochondria. Presence of many electron dense lipoid bodies in and around mitochondria. Bar indicates two microns.

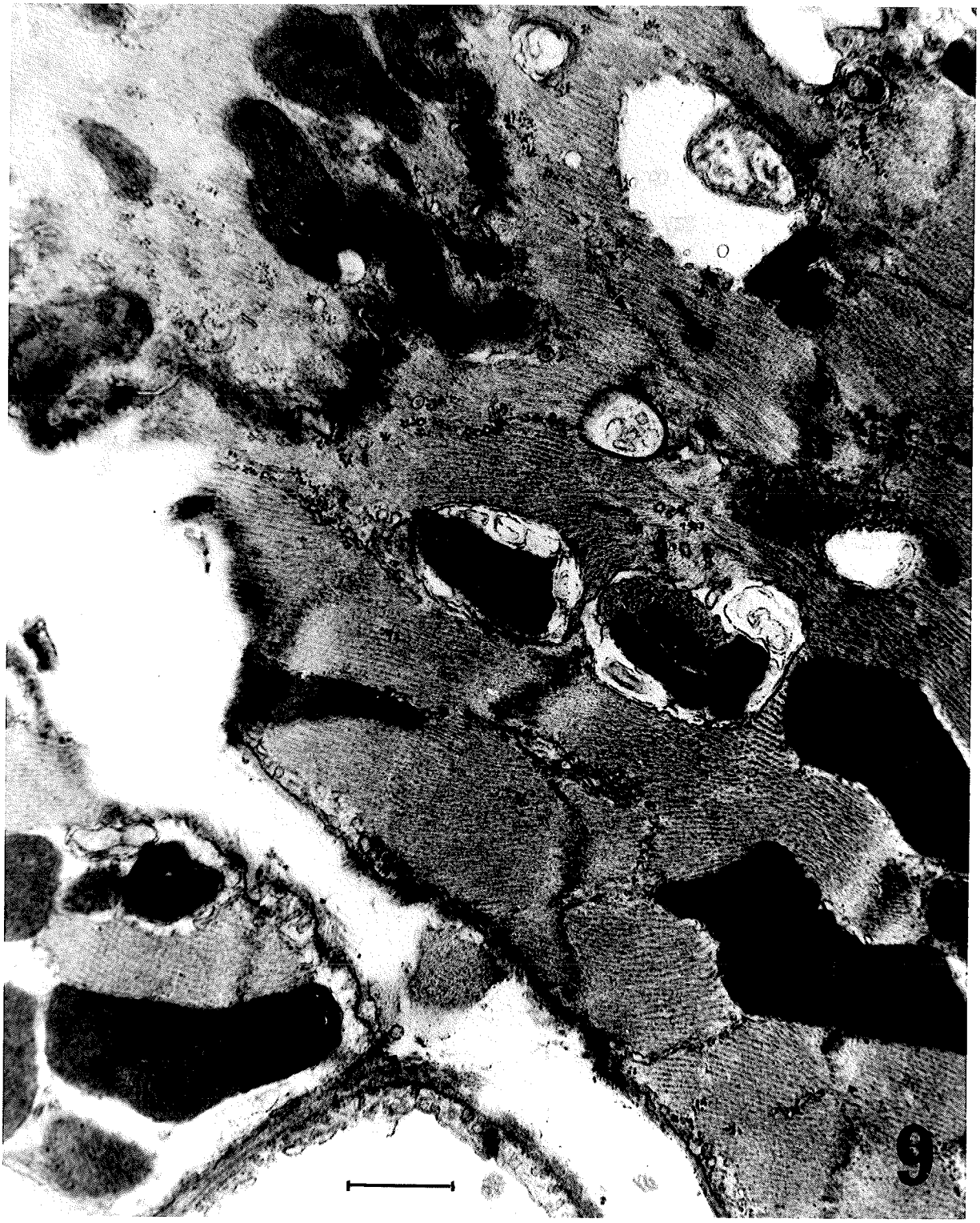

FIGURE CAPTION ON
PAGE 50

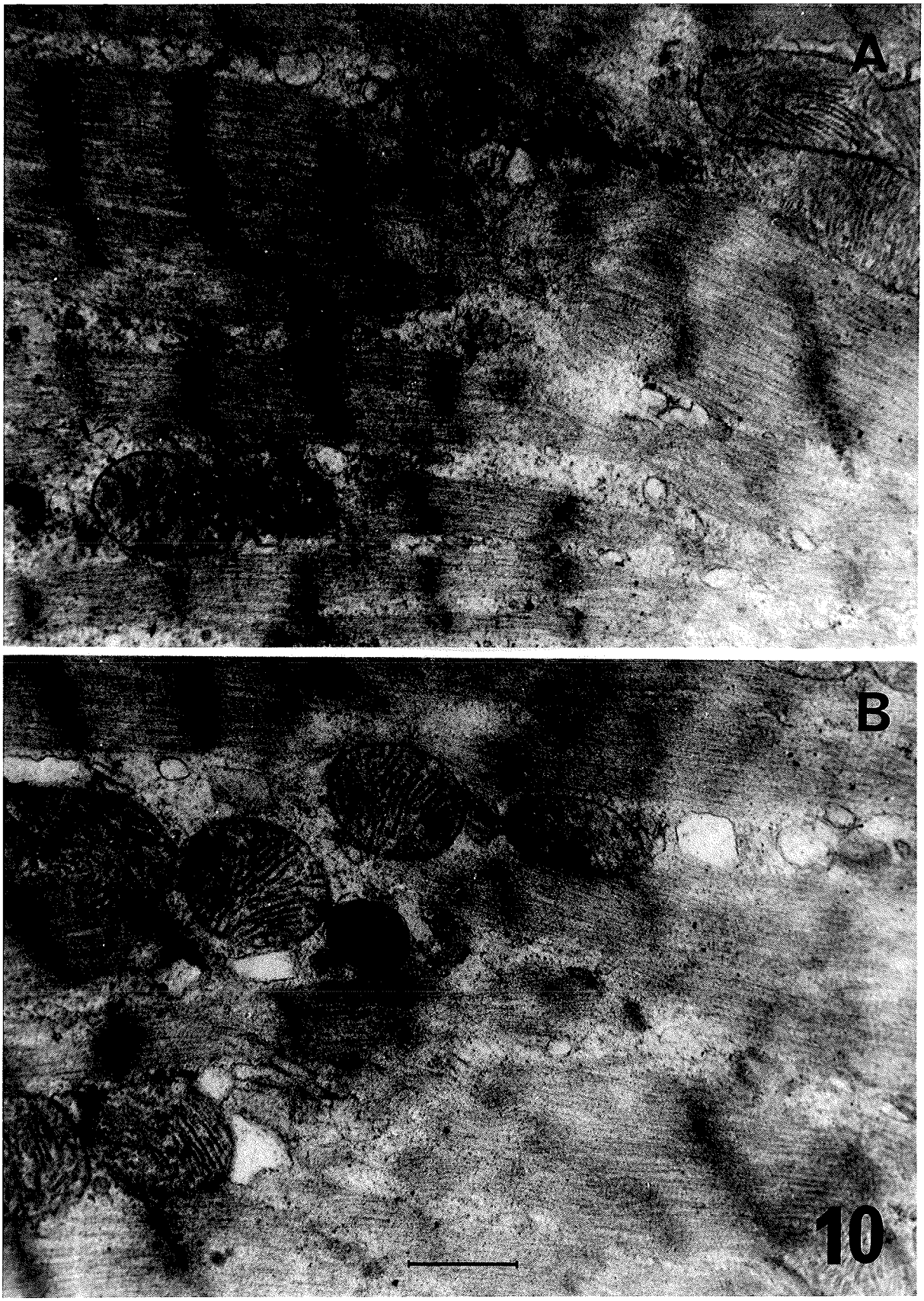

FIGURE CAPTION ON
PAGE 50

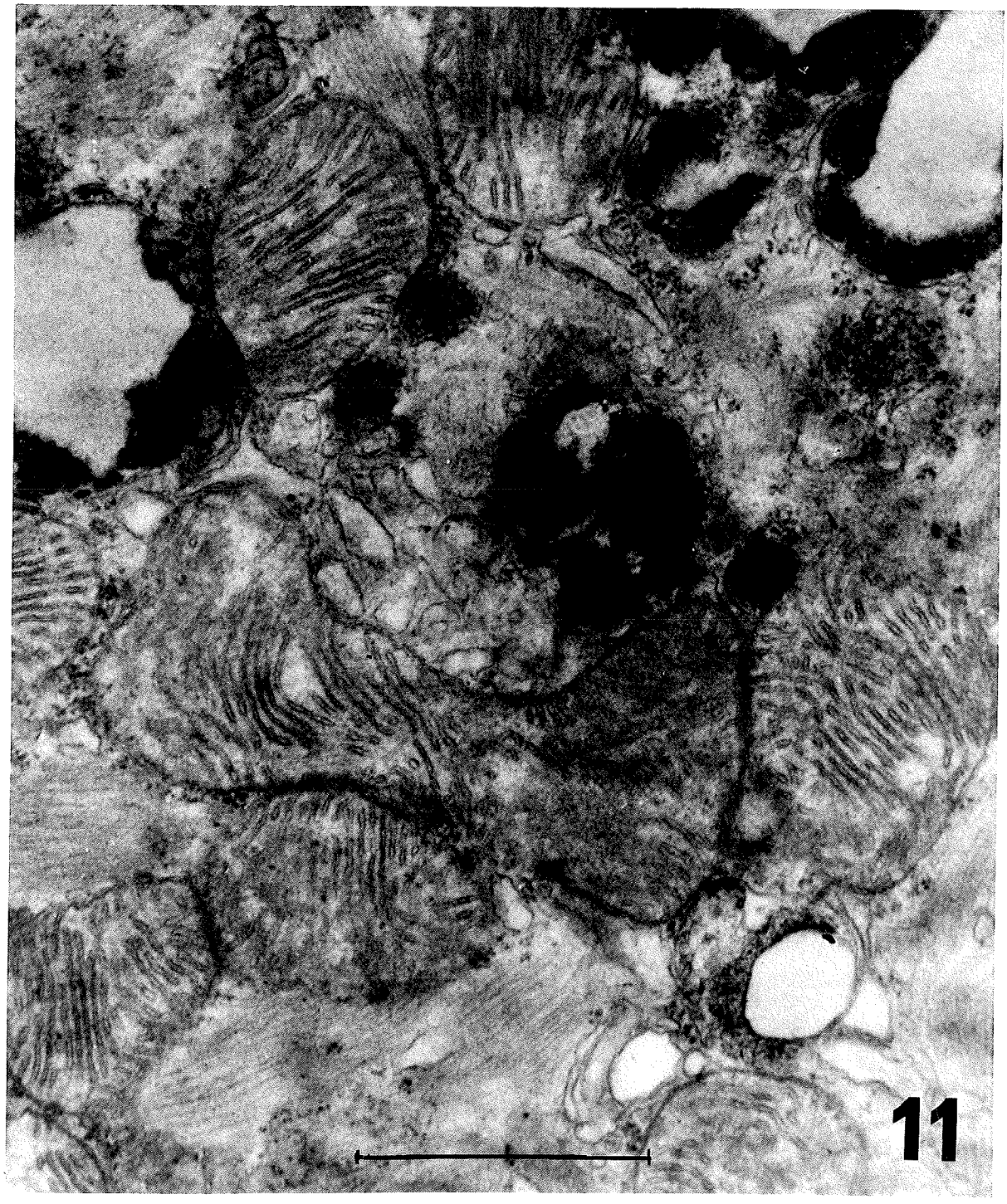

FIGURE CAPTION ON
PAGE 50

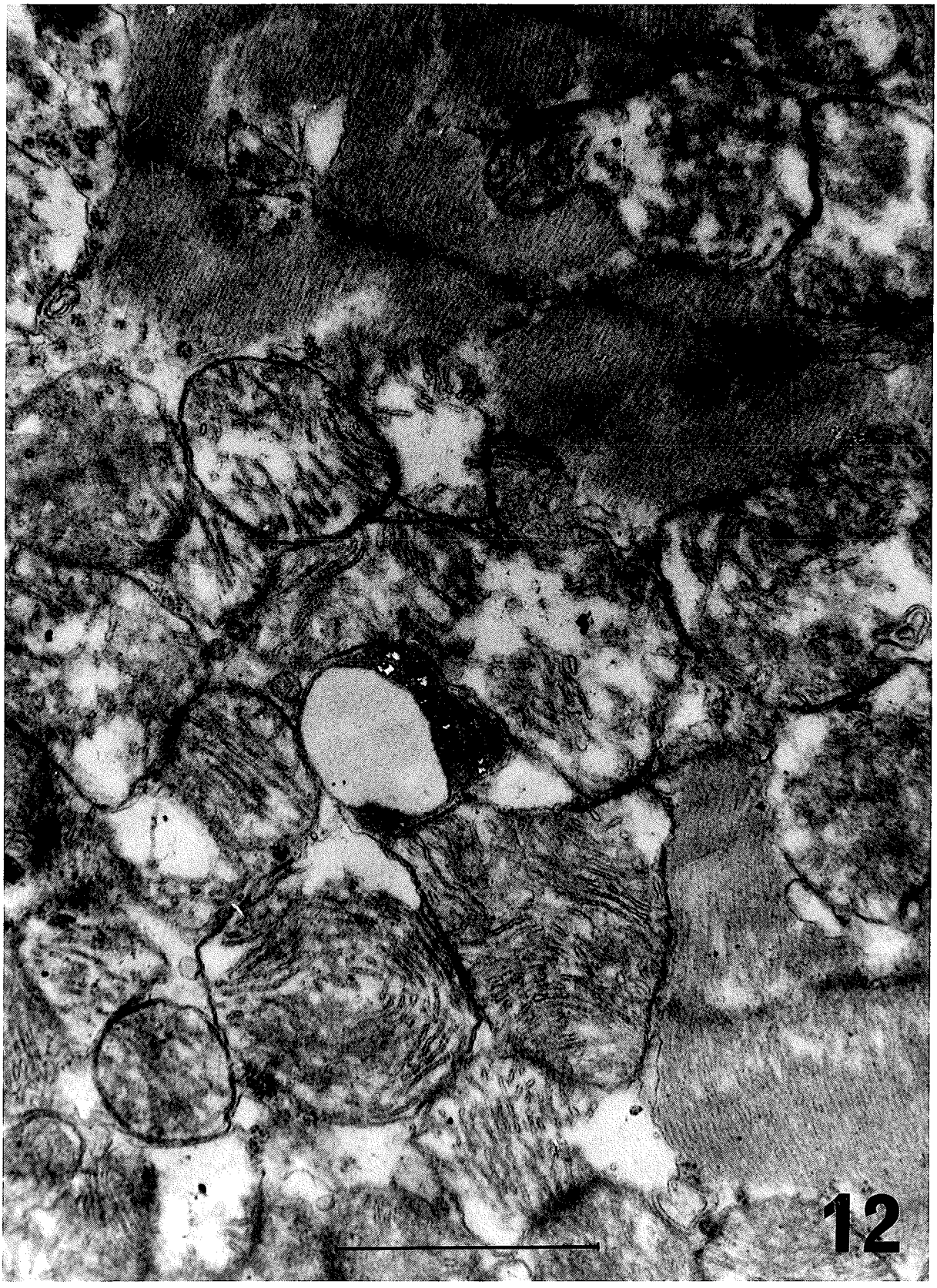

FIGURE CAPTION ON
PAGE 50

mitochondria with occasional changes in the cristae and few inclusion bodies or lysosomes (Fig. 6-8). Myelin figures or lipid deposits were not evident. Nuclear changes included dispersion of chromatin and the presence of a mild perinuclear edema (Figs. 6 and 7). It is important to note that there was no vacuolization due to the loss of myofibrils in the hearts of these adriamycin treated younger animals.

In the C_O + A treated group the most prominent myocyte ultrastructural changes were observed in the mitochondria and in the appearance of multiple intracellular vacuoles of varying sizes resulting from dilatation of the sarcotubular system (Figs. 9, 11 and 12). Histologic changes observed in mitochondria were characterized by swelling and focal dilations or loss of outer mitochondrial membrane, disarrangement of cristae, clearing of mitochondrial matrix and formation of numerous concentric membranous lamellae. Severely degenerated mitochondria contained electron-dense bodies and/or inclusion bodies (Fig. 12).

The severity of intracellular vacuolization varied among fibers and appeared to be due to a distention of the sarcoplasmic reticulum (SR) and the T-tubules. These vacuoles were of varying sizes. Slightly affected cells had few foci of mild distention of the SR and T-tubules while severely affected cells had numerous large vacuoles that were distributed throughout the sarcoplasm and produced distortion of adjacent myofibrils, nuclei and mitochondria. Some severely degenerating cells had large coalescent vacuoles with multilaminated concentric myelin figures and electron dense inclusion bodies. Accompaning the mitochondrial changes and intracellular vacuolization was disruption and

lysis of the myocardial contractile elements (Figs. 10 and 11). This was characterized by the loss of registry of Z-bands and loss of characteristic parallel orientation of myofibrils and appearance of contraction bands (Fig. 10). Some myofibrils were fragmented and there was progressive disorganization and disappearance of organized bundles of actin and myosin filaments.

In the C_o + A group, nuclear changes included dispersion of the chromatin and some change in shape of the nucleus. General morphologic changes included appearance of active lysosomes, residual bodies, and multilaminated myelin figures and lipid deposits in the sarcoplasm (Figs. 11 and 12). There was some interstitial edema which was accompanied by an increase in collagen fibers.

Lipid Peroxidation

Since free radical activity and lipid peroxidation has been suggested to play a role in adriamycin cardiotoxicity, lipid peroxide activity was studied by evaluating myocardial malondialdehyde (MDA) content and the results are shown in Figure 13. Control animals in the older vehicle treated group (C_o) showed a significantly less amount of MDA in the myocardium as compared to the C_y group. Adriamycin treatment in the older group resulted in a hundred percent increase in the MDA content as compared to age matched controls. There was no significant difference between the MDA contents of experimental and control animals in the younger group.

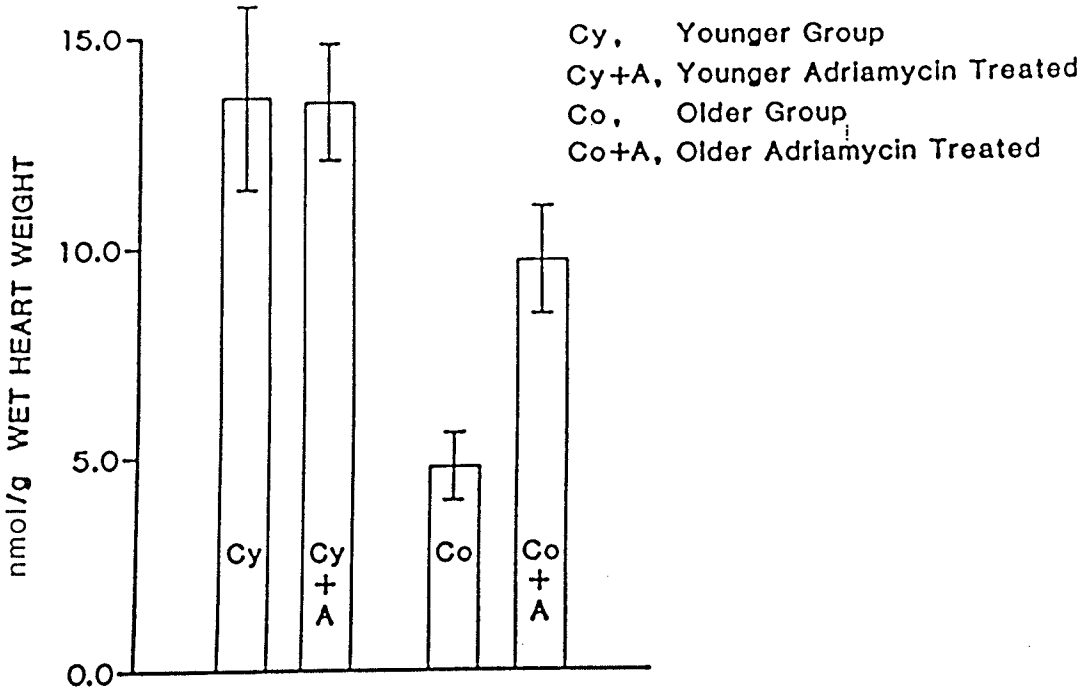

FIGURE 13) Effects of chronic adriamycin treatment on myocardial malondialdehyde content in two age groups of rats. MDA content of C_y + A compared to C_y group was not different. MDA content in C_o + A group was significantly higher ($P<0.01$) compared to C_o group. For details see Materials & Methods.

DISCUSSION

Although adriamycin is one of the most effective antineoplastic drugs in clinical oncology, its use is attenuated because of its ability to induce a progressive cardiomyopathy and fatal congestive heart failure. This situation is exacerbated by risk factors which may predispose a patient to developing myocardial damage and congestive heart failure. In the present study, animals in the older age group showed more myocardial cell damage and occurrence of hydroperitoneum. The study provides support to the view that age can be a risk factor in adriamycin-induced cardiomyopathy.

It is obvious that a human model cannot be employed in an experimental study like ours; however, the resemblance between the anthracycline-induced cardiomyopathy in an animal model and humans increases the level of confidence in the information obtained from the animal model thus making interpretations more relevant to a clinical situation. In this regard, several different animal models such as mice (Rosenhoff et al, 1975; Myers et al, 1977) rabbits (Jaenke, 1974; Olson et al, 1974; Young, 1975), and rats (Mettler et al, 1977; Zbinden et al, 1978) have been employed in the past for the study of anthracycline-induced cardiotoxicity. Repeated intravenous injections of adriamycin and daunomycin to rabbits at cumulative doses of 350-600 mg/m^2 body surface, resulted in congestive heart failure as indicated by subcutaneous edema, ascites, pleural effusions, elevated serum enzymes, and passive congestion of the liver. Histologically, marked focal lesions in the heart muscle were seen by light microscopy. Ultrastructural damage included loss

of myofibrils, vacuolization of myocytes and degeneration of mitochondria. Similar histopathologic lesions and clinical symptoms have been observed in rats receiving 10-20 mg/kg adriamycin intraperitoneally (Olsen and Capen, 1977). In the present study, however, most of these changes were seen only in the C_o + A group indicating a higher sensitivity of this group to adriamycin-induced cell damage. At any rate, it is important to note that changes seen in animals by us as well as others are similar to those seen in patients who died after high-dose treatments with anthracyclines (Ainger et al, 1971; Buja et al, 1973; Lefrak et al, 1973). Therefore, the information obtained in the rat model as employed in the present study can be seen to have relevance in understanding age-related differences in adriamycin-induced cardiomyopathy in patients.

The progressive decrease in body weight in the C_o + A group during the treatment period may be due to decreased consumption of feed with or without any change in the metabolic activity of the animal. In contrast, C_y + A group did not show any loss of their body weight during the treatment period. It appears that both treated groups showed a loss of appetite during the treatment period but the change was of more severe consequence in the older group. In the post treatment period the younger group was able to parallel the weight gain of its aged matched controls. The C_o + A group however, had only a partial recovery in body weight and this may not have been a true weight gain but instead reflected the accumulation of ascites secondary to congestive heart failure which was predominant in the C_o + A group. In this regard, only the C_o + A group exhibited overt ascites although both C_o + A and

C_y + A groups showed turgid livers. In addition, heart weight as well as heart to body weight ratio were decreased more in the C_o + A group compared to the control C_o group. Mortality was also greater in the older group. All of the above observations in C_o + A group are in keeping with previous findings in adriamycin-induced cardiotoxicity (Lefrak et al, 1973; Ferrans, 1976) and indicate that the older subjects may be less resistant to the toxic effects of adriamycin. The reasons for this difference are not immediately clear but may have to do either with the cardiovascular status of the animals at different ages or with differential metabolism and excretion of the drug in the two age groups with the older group having a longer tissue and plasma 1/2 life and thus exhibiting greater toxicity to the drug than the younger group. An age-related study with reference to metabolism and excretion of adriamycin would help to clarify the latter postulation.

The contractile properties and responsiveness of cardiac muscle to hormones can be evaluated in isolated, isometrically contracting papillary muscles (Sonnenblick, 1962; Hoffman et al, 1968). Changes in contractile state can be evaluated by measuring peak developed force and the rate of force development [(+) and (-) dp/dt]. Differences in maximum developed force due to adriamycin treatment were, therefore, examined in papillary muscle preparations exposed to different interventions in a jacketed 100 ml constant - temperature tissue bath. The effect of frequency change (increasing the rate of stimulation) caused a decline in the peak developed force in all four groups. In this regard, the rat heart has long been recognized to be anamalous.

The negative force-frequency relationship in the adult rat heart has been attributed to a reduced tendency to accumulate intracellular Na^+ and a reduced influx of Ca^{2+} through Na^+-Ca^{2+} exchange mechanism (Langer, 1978). This decrease was less in the treated groups but was significantly different only in the older treated animals probably indicating a reduced depression of the Na^+-Ca^{2+} exchange mechanisms in these hearts.

A reduction of the bath concentration of calcium by 50% reduced the peak developed force in all groups but this decline was less in the adriamycin treated C_o + A group as compared to the decline seen in the C_y + A group as well as C_o and C_y groups. These findings, also supported by similar changes in dp/dt, may indicate an increased reliability of the hearts in the C_o + A group on the intracellular stores of Ca^{2+}. In contrast, the effect of increased calcium concentration in the bath, as expected, resulted in a significant increase in peak developed force in the untreated C_o and C_y groups while the increase in the treated C_o + A and C_y + A groups was blunted; this blunting effect was greater in the older group. The C_o + A group also exhibited a diminished response of (+) and (-) dp/dt. Since increase of extracellular Ca^{2+} did not increase the peak force as well as dp/dt in the C_o + A group in amounts comparable to controls, the defect may also lie in subcellular Ca^{2+} uptake and release from other membrane systems, defective energy metabolism and a shortfall in the contractile elements. Based on the force data obtained after changing the bath concentration of Ca^{2+}, it would appear that there is an intracellular Ca^{2+} overload in adriamycin treated hearts which buffers the effects of lowering

the bath Ca^{2+} concentration and becomes detrimental in high Ca^{2+}. Since these responses were more marked in the C_o + A group, the condition of Ca^{2+} overload may be more severe in these hearts. In this regard, it should be noted that intracellular Ca^{2+} overload is known to depress high energy phosphate stores as well as cause myocardiual cell damage (Fleckenstein et al, 1983; Singal et al, 1985).

The response to epinephrine in the adriamycin treated groups resulted in an increase in peak developed force of only 50% of the respective control values and dp/dt changes also showed a similar pattern. These observations are supportive of observations that isoproterenol produces significantly smaller increase in peak force developed and dp/dt in papillary muscles in failing human hearts (Ginsburg et al, 1983). The results indicate that failing heart muscle is less sensitive than normal cardiac muscle to stimulation by isoproterenol, suggesting an alteration in a component of the beta-adrenergic pathway (Ginsburg et al, 1983). Elevated catecholamine levels are present in patients with heart failure presumably as a compensatory mechanism to support the failing myocardium (Chidsey et al, 1966; Thomas and Marks, 1978). This increased exposure to catecholamines would be expected to produce a decrease in beta-receptor density and an accompanying decrease in receptor agonist affinity. It is suggested that down regulation of beta-adrenergic receptors might occur in patients exposed to high levels of circulating catecholamines which may occur in congestive heart failure (Bristow et al, 1981). In this regard, it is also known that the aged myocardium loses its ability to be stimulated by isoproterenol (Lakatta et

al, 1975).

Extensive myocardial cell damage seen only in the C_o + A group when exposed to chronic adriamycin administration of 15 mg/kg which failed to elicit the same degree of structural change in the C_y + A group, further supports the view that older age myocardium is more sensitive to adriamycin-induced cell damage. Answer to the question, why an older age heart is more sensitive to adriamycin-induced cell injury may be a complex one. In this regard, drug uptake, excretion and metabolism may be prolonged in the C_o + A group. In addition binding characteristics of adriamycin with the lipoprotein components of cells may be different in the two age groups. Slower rates of drug metabolism can be seen to increase the concentration of the drug and/or its metabolites in the cell, prolong the tissue half-life, accounting for a higher activity in the older myocardium and ultimately resulting in greater myocyte damage than in the younger myocardium. At any rate, all these different possibilities must to be related with age. In order to understand these age related differences in drug response, it is imperative to understand the mechanism of adriamycin-induced cardiomyopathy.

One of the postulated mechanisms of adriamycin-induced myocardial damage is through the formation of free radicals reacting with polyunsaturated fatty acids and resulting in lipid peroxidation. During normal metabolism, molecular oxygen can be reduced to form superoxide radical (O_2^-) which can initiate the production of other toxic radicals and products such as hydroxyl radical ($OH^\cdot$) and hydrogen peroxide (Fridovich, 1974). Cellular defense against these free radical toxic substances is provided

by enzymes such as superoxide dismutase, catalase, glutathione peroxidase (Del Maestro, 1980) as well as by other antioxidants such as alpha-tocopherol (Myers et al, 1976).

Although the exact details of the processes involved in the formation of lipid peroxides from polyunsaturated fatty acids are not known, the end products of these processes are hydroperoxides (Barber and Bernheim, 1967; DeChatelet, 1975; Pryor, 1973). Some of the identified intermediates in lipid peroxidation include organic free radicals, diene conjugates and peroxide radicals (Barber and Bernheim, 1967). The occurrence of free radicals is considered as rate limiting in this process of lipid peroxidation. Although the breakdown pathways for peroxidized lipids are also not clearly understood, the reported stable end products are ethane, propane, pentane (Dillard et al, 1977; Konze and Elstner, 1978) and malondialdehyde (MDA) (Barber and Bernheim, 1967). The biochemical determination of MDA has been considered to be a good measure of the formation of lipid peroxides (Hunter et al, 1964). In the present study, lipid peroxidation as measured by myocardial malondialdehyde content was seen to double in the older myocardium compared to its age matched control as a result of adriamycin treatment. The MDA content of experimental and control animals in the younger group showed no significant difference. It is possible that the antioxidative capacity of the older myocardium may be decreased with age and ultimately overwhelmed by the increased generation of free radicals (Singal, 1983). Regardless of the specific mechanism, the present investigation suggests that the older myocardium is at greater risk of developing damage due to free

radical formation.

It should be noted that lipid peroxidation of membrane structures by chemicals and drugs has been associated with a variety of pathological situations (Plaa and Witschi, 1976). The introduction of hydrophilic functions due to the peroxidation of polyunsaturated fatty acids can be seen to produce perturbation of membrane microarchitecture, alteration of enzyme activities and pathological changes in the membrane permeability (Del Maestro, 1980). Because of the abundance of polyunsaturated fatty acid chains, lipid membranes are particularly sensitive to peroxidative changes (Plaa and Witschi, 1976) causing permeability changes in the membranes. This can be seen to cause disruption of the normal membrane ion gradients, an increase in intracellular calcium content and a decrease in high energy phosphate (Fleckenstein et al, 1983) causing heart muscle fibers to undergo severe functional and structural alterations (Fleckenstein et al, 1983). In this regard, the changes in $^{45}Ca^{2+}$ retention, morphology including increased mitochondrial swelling and intramitochondrial calcium phosphate crystals in the myocardium of rabbits treated with adriamycin suggest that this drug directly or indirectly affects Ca^{2+} flux across the sarcolemma. Singal (1984) has shown the occurrence of intramitochondrial Ca^{2+} granules in a hypertrophied rabbit heart exposed to adriamycin. It is likely that in the C_o + A group there is either increased production of free radicals and/or reduced antioxidant capacity which causes accumulation of more lipid peroxides and subsequent membrane injury. This is supported by the extensive ultrastructural damage seen in the C_o + A group coupled with an increase in

the MDA content as well as lipoid deposits in the cell.

In addition, the release of vasoactive substances (Bristow et al, 1980) which can also induce formation of reactive oxygen species (Singal et al, 1982) may exacerbate the production of free radicals, lipid peroxides, altered sarcolemmal permeability and Ca^{2+} overload. Since mitochondria are the major producer of ATP, there may exist a delicate balance between energy supply and demand in the hearts in C_o + A group. If mitochondrial membranes are extensively damaged due to lipid peroxidation, as well as due to a Ca^{2+} overload in the cell, the rate of oxidative ATP-resynthesis is critically diminished (Fleckenstein et al, 1983). In addition, Ca^{2+} overload greatly increases ATP consumption by Ca^{2+} - activated myofibrillar, sarcoplasmic and mitochondrial ATPases (Singal et al, 1985) further decreasing the high energy phosphate pools so that cardiac function and structural integrity can no longer be maintained. Such a chain of events may also contribute in adriamycin cardiomyopathy.

The increased influx of Ca^{2+} (Fleckenstein et al, 1983) owing to increased lipid peroxidation in the membrane (Singal et al, 1983), could be one of the mechanisms responsible for contraction band lesions seen in the C_o + A group. The large amount of lipid deposits, membrane whorls, inclusion bodies and extensive mitochondrial damage seen in the hearts of the C_o + A group would indicate that these older hearts are undergoing more peroxidative changes than the C_y + A group. Supporting this observation is the hundred percent increase in MDA content seen in the C_o + A group compared to the C_o group. Although the biochemical basis of adriamycin-induced cell damage in the older myocardium may be

complex, a tentative proposal can be put forward in which intracellular Ca^{2+} overload subsequent to a low energy state owing to mitochondrial defects as well as membrane permeability changes may have an important role. Furthermore, this mechanism appears to have greater consequences in the C_o + A group, indicating that the older heart is at greater risk of developing an adriamycin induced cardiomyopathy.

Congestive heart failure presumably results from an intrinsic abnormality in contractile function. While biochemical investigations have shown that a decrease in contractile function is not the result of an alteration in energy production or reserve alone, but may also involve a reduction in or an impairment of the delivery of calcium to the contractile apparatus (Sobel et al, 1967; Chandler et al, 1968). Characteristics of congestive heart failure induced by adriamycin in the C_o + A group may also involve abnormalities in energy production due to mitochondrial damage as well as alterations in calcium movements and availability. These mechanisms would also contribute to the depressed response to the positive inotropic agents seen in the older treated animals, in conjunction with primary changes to the beta-adrenergic pathway. It is known that the adriamycin-induced congestive heart failure can be refactory to normal drugs and regimes used in treatment of congestive heart failure and thereby result in fulminating heart failure and death (Lefrak et al, 1973). In addition, loss of myofibrils seen in C_o + A group ultrastructural studies would also contribute to a decreased response of myofibrils to different interventions.

In conclusion, our studies showed frequent incidence and

symptoms of heart failure characteristics, changes in the peak developed force, dp/dt, MDA content and myocardial ultrastructure of the C_o + A group as compared to the C_y + A group. Irrespective of the biochemical basis of adriamycin cardiomyopathy, it is suggested that age is a risk factor for adriamycin-induced myocardial cell damage and heart failure. As adriamycin induced cardiotoxicity is a dose-related disease process that results in cytolysis of individual myocardial cells, in older patients such degenrative changes may be more pronounced and caused by dosage much lower than the generally accepted recommendation of 550 mg/m^2. Compatible with this conclusion are previous survey studies which reported that older age $\geq$ 60-70 years may be a risk factor for adriamycin-induced cardiomyopathy and heart failure (Bristow et al, 1978; Von Hoff et al, 1979).

REFERENCES

REFERENCES

Ainger L.E., Bushore J., Johnson W.W. and Ito J. Daunomycin: A cardiotoxic drug. J. Natn. Med. Assoc. 63: 261-267, 1971.

Anghilieri L.J. Ca^{2+} transport inhibition by the antitumor agents adriamycin and daunomycin. Arzneimittal-Forsch 27: 1177-1180, 1977.

Arcamone F., Cassinelli G., Franceschi G., Penco S., Pol C., Redaelli S. and Selva A. Structure and physiochemical properties of adriamycin (Doxorubicin). In: Int. Symp. Adriamycin. Eds. Carter, S.K., DiMarco, A., Ghione, M. Krakoff, I.H. and Mathe, G. Springer-Verlag, pp 9-22, 1972.

Arena E., Alessandro N.D., Dusonchet L., Gebbia N., Gerbasi F., Sanguedolce R. and Rausa L. Influence of pharmokinetic variations on the pharmacological properties of adriamycin. In: Int. Symp. Adriamycin. Eds. Carter, S.K., DiMarco, A., Ghione, M., Krakoff, I.H. and Mathe, G. Springer-Verlag, pp 96-116, 1972.

Arena E., Biondo F., D´Alessandro N., Dusonchet L., Gebbia N. and Gerbasi F. DNA, RNA and protein synthesis in heart, liver, brain of mice treated with daunomycin or adriamycin. Int. Res. Commun. Sys. Med. Sci. 2: 1053-1061, 1974.

Azuma J., Sperelakis N., Hasegawa H., Tanimoto T., Vogel S., Ozura K., Awata N., Sawamura A., Harada H., Tshiyama T., Morita Y. and Yamamura Y. Adriamycin cardiotoxicity: possible pathogenic mechanisms. J. Mol. Cell. Cardiol. 13: 381-397, 1981.

Bachman E., Weber E. and Zbinden G. Effect of seven anthracycline antibiotics on electrocardiogram and mitochondrial function of rat hearts. Agents and Actions 5: 383-393, 1975.

Bachur N.R. Adriamycin (NSC - 123127) pharmacology. Cancer Chemother. Rep. (Part 3) 6(2): 153-158, 1975.

Bachur N.R. Anthracycline antibiotic pharmacology and metabolism. Cancer Treat. Rep. 63(5): 817-820, 1979.

Bachur N.R., Gordon S.L., Gee M.V. Anthracycline antibiotic augmentation of microsomal electron transport and free radical formation. Mol. Pharmacol. 13: 901-910, 1977.

Bachur N.R., Gordon S.L., Gee M.V. and Kon H. NADPH cytochrome P-450 reductase activation of quinone anticancer agents to free radicals. Proc. Nat. Acad. Sci. U.S.A. 76(2): 954-957, 1979.

Bachur N.R., Hildebrand R.C. and Jaenke R.S. Adriamycin and daunorubicin disposition in the rabbit. J. Pharmacol. Exp. Ther. 191: 331-340, 1974.

Bajusz E. and Jasmin G. Histochemical studies on the myocardium following experimental interference with coronary circulation in

the rat. Acta Histochemica 18: 222-237, 1964.

Baljas T. and Ferrans V.J. Cardiac lesions induced by chemicals. Environ. Health Persp. 26: 181-191, 1978.

Barber A.A. and Bernheim, F. Lipid Peroxidation: Its measurement, occurrence, and significance in animal tissues. Adv. Gerontol. Res. 2: 355-403, 1967.

Benjamin R.S. Clinical pharmacology of adriamycin (NSC-123127). Cancer Chemother. Rep. (Part 3) 6(2): 183-185, 1975.

Billingham M.E., Bristow M.R., Glatstein E., Mason J.W., Masek M.A. and Daniels J.R. Adriamycin cardiotoxicity: endomyocardial evidence of enhancement by irradiation. Am. J. Surg. Path. 1: 17-23, 1977.

Billingham M.E. Endomyocardial changes in anthracycline-treated patients with and without irradiation. Front. Radiat. Ther. Onc. 13: 67-81, 1979.

Blum R.H. and Carter S.K. Adriamycin. A new anticancer drug with significant clinical activity. Ann. Intern Med. 80: 249-259, 1974.

Blum R.H. An overview of studies with adriamycin (NSC-123127) in the United States. Cancer Chemother. Rep. (Part 3) 6(2): 247-251, 1975.

Bonadonna G. and Monfardini S. Cardiac Toxicity of daunorubicin. Lancet 1: 837, 1969.

Bowditch H.P. Uber die Eigenthumlichkeiten der Reizbarkeit, welche die Muskelfasern des Herzens Zeigen. Arb. Physiol. Leipzig 6: 139, 1871.

Bristow M.R. Anthracycline cardiotoxicity. In Drug-Induced Heart Disease. Ed. M.R. Bristow. Elsevier/North-Holland Biomedical Press, New York, pp. 191-215, 1980.

Bristow M.R. Toxic cardiomyopathy due to doxorubicin. Hosp. Prac. Dec.: 101-111, 1982.

Bristow M.R., Billingham M.E., Mason J.W. and Daniels J.R. Clinical spectrum of anthracycline antibiotic cardiotoxicity. Cancer Treat. Rep. 62(6): 873-879, 1978.

Bristow M.R., Ginsburg R., Sageman W.S., Billingham M.E. and Stinson E.B. Analysis of the beta-adrenergic receptor pathway in myocardial disease. Circ. 64(Suppl. II): IV-286, 1981.

Bristow M.R., Lopez M.B., Mason J.W., Billingham M.E. and Winchester M.A. Efficacy and cost of cardiac monitoring in patients receiving doxorubicin. Cancer 50: 32-41, 1982.

Bristow M.R., Mason J.W. and Billingham M.E. Doxorubicin Cardiomyopathy: Evaluation by phonocardiography, endomyocardial biopsy and cardiac catheterization. Ann. Intern Med. 88: 168-175, 1978.

Bristow M.R., Mason J.W., Billingham M.E. and Daniels J.R. Endomyocardial biopsy and systolic time intervals in detection of adriamycin cardiotoxicity. Circ. 5 (Suppl. II): II-80a, 1976.
Bristow M.R., Sageman W.S., Scott R.H., Billingham M.E., Bowden R.E., Kernoff R.S., Snidow G.H. and Daniels J.R. Acute and Chronic Cardiovascular Effects of Doxorubicin in the Dog: The cardiovascular pharmacology of drug-induced histamine release. J. Cardiovasc. Pharm. 2: 487-515, 1980.

Buja L.M. and Ferrans V.J. In: Recent Advances in Studies on Cardiac Structure and Metabolism. Eds. Fleckenstein A. and Rona G. Univ. Park Press, Baltimore Vol. 6: 487-497, 1975.

Buja M., Ferrans V.J., Mayer R.J., Roberts W.C. and Henderson E.S. Cardiac ultrastructural changes induced by daunorubicin therapy. Cancer 32: 771-788, 1973.

Chalcroft S.C.W., Gavin J.B. and Herdon P.B. Fine structural changes in rat myocardium induced by daunorubicin. Pathology 5: 99-105, 1973.

Chance B., Seiss H. and Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol. Rev. 59: 527-605, 1979.

Chandler B.M., Sonnenblick E.H. and Pool P.E. Mechanochemistry of cardiac muscle. Circ. Res. 22: 729-735, 1968.

Chidsey C.A., Sonnenblick E.H., Morrow A.G. and Braunwald E. Norepinephrine stores and contractile force of papillary muscle from failing heart muscle. Circ. 33: 43-51, 1966.

Daniels J.R., Billingham M.E., Gelbert A. and Bristowe M.R. Electrolyte and water alterations during the life course of the biol 14.6 syrian golden hamster representing a disease model of a hereditary cardiomyopathy. Circ. 54: 20 (Abstr.), 1976.

DeChatelet L.R. Oxidative bactericidal mechanisms of polymorphonuclear leukocytes. J. Infect. Diseases 131: 295-303, 1975.

Del Maestro R.F. An approach to free radicals in medicine and biology. Acta Physiol. Scand. (Suppl.) 492: 153-168, 1980.

Dhalla N.S., Das P.K. and Sharma G.P. Subcellular basis of cardiac contractile failure. J. Mol. Cell. Cardiol. 10: 363-385, 1978.

Dhalla N.S., Singal P.K. and Dhillon K.S. Mitochondrial functions and drug-induced heart disease. In: Drug-Induced Heart Disease. Ed. Bristow M.R. Elsevier/North-Holland Biomedical Press, New York, Vol. 5, pp 39-61, 1980.

Dhalla N.S., Ziegelhoffer A. and Harrow J.A.C. Regulatory role of membrane systems in heart function. Can. J. Physiol. Pharmacol. 55: 1211-1234, 1977.

Dillard C.J., Dumelin E.E. and Tappel A.L. Effect of dietary vitamin E on expiration of pentane and ethane by the rat. Lipids 12: 109-114, 1977.

Di Marco A. Adriamycin (NSC - 123127): Mode and mechanism of action. Cancer Chemother. Rep. (Part 3) 6(2): 91-106, 1975.

Di Marco A., Gaetani M. and Scarpinato B. Adriamycin (NSC-123127): A new antibiotic with antitumor activity. Cancer Chemother. Rep. 53: 33-37, 1969.

Domae N., Sawada H., Matsuyama E., Konishi T. and Uchino H. Cardiomyopathy and other chronic toxic effects induced in rabbits by Doxorubicin and possible prevention by coenzyme Q_{10}. Cancer Treat. Rep. 65(1-2): 79-91, 1981.

Doroshow J.H. Effect of anthracycline antibiotics on oxygen radical formation in rat heart. Cancer Res. 43: 460-472, 1983.

Doroshow J.H., Locker G.Y. and Meyers C.E. Experimental animal models of adriamycin cardiotoxicity. Cancer Treat. Rep. 63(5): 855-860, 1979.

Doroshow J.H., Locker G.Y. and Meyers C.E. Enzymatic defenses of the mouse heart against reactive oxygen metabolites. Alterations produced by doxorubicin. J. Clin. Invest. 65: 128-135, 1980.

Egorin M.J., Hildebrand R.C., Cimino E.F. and Bachur N.R. Cytofluorescence localization of adriamycin and daunorubicin. Cancer Res. 34: 2243-2245, 1974.

Fajardo L.F, Eltringham J.R. and Stewart, J.Jr. Combined cardiotoxicity of adriamycin and x-radiation. Lab. Invest. 34: 86-96, 1976.

Ferrans V.J. Overview of cardiac pathology in relation to anthracycline cardiotoxicity. Cancer Treat. Rep. 62(6): 955-961, 1978.

Fleckenstein A., Frey M. and Fleckenstein-Grun G. Consequences of uncontrolled calcium entry and its prevention with calcium antagonists. Eur. Heart. J. 4(Suppl. H): 43-50, 1983.

Fridovich I. Superoxide dismutase. Adv. Enzymol. 41: 35-97, 1974.

Gebbia N., Leto G., Gagliano M., Tumminello F.M. and Rausa L. Lysosomal alterations in heart and liver of mice treated with doxorubicin. Cancer Chemother. Pharmacol. 15: 26-30, 1985.

Ghione M. Development of adriamycin (NSC-123127). Cancer Chemother. Rep. 6(2): 83-89, 1975.

Gilladoga A.C., Manuel C., Tan T.C., Wollver N., Sternberg S.S. and Murphy M.L. The cardiotoxicity of adriamycin and daunomycin in children. Cancer 37: 1070-1078, 1976.

Ginsburg R., Bristow M.R., Billingham M.E., Stenson E.B., Schroeder J.S. and Harrison D.C. Study of normal and failing isolated human heart: Decreased response of failing heart to isoproterenol. Am. Heart J. 106: 535-540, 1983.

Gosalvez M. and Blanco M. Inhibition of Na-K ATPase by the antitumor antibiotic adriamycin. Abstr. p 4. In: Fifth International Biophysics Congress, Copenhagen, Aug. 1975.

Gosalvez M., Blanco M., Hunter J., Miko M. and Chance B. Effects of anticancer agents on the respiration of isolated mitochondria and tumor cells. Eur. J. Cancer 10: 567-574, 1974.

Gosalvez M., Van Rossum G.D.V. and Blanco M.F. Inhibition of sodium-potassium-activated adenosine 5´-triphosphate and ion transport by adriamycin. Cancer Res. 39: 257-261, 1979.

Gudbjarnason S., Doell B. and Oskarskattir G. Docosahexaenoic acid in cardiac metabolism and function. Acta Biol. Med. Germ. 37: 777-784, 1978.

Herman E.H., Mhatre R.M., Lee I.P., Vick J. and Waravdekar V.S. A comparison of the cardiovascular actions of daunomycin, adriamycin and N-acetyldaunomycin in hamsters and monkeys. Pharmacology 6: 230-241, 1971.

Herman E.H., Mhatre R.M., Leef I.P. and Waravdekar V.S. Prevention of the cardiotoxic effects of adriamycin and daunomycin in the isolated dog heart. Proc. Soc. Exp. Biol. Med. 140: 234-239, 1972.

Hoffman B.F., Bassett A.L. and Bartelstone H.J. Some mechanical properties of isolated mammalian cardiac muscle. Circ. Res. 23: 291-312, 1968.

Hunter F.E., Gebicki J.M., Hoffsten P.E., Weinstein J. and Scott A. Swelling and lysis of rat liver mitochondria induced by ferrous ions. J. Biol. Chem. 238: 828-835, 1963.

Hunter F.E., Scott A., Hoffsten P.E., Guerra F., Weinstein J., Schneider A., Schultz B., Fink J., Ford L. and Smith E. Studies on the mechanism of ascorbate-induced swelling and lysis of isolated liver mitochondria. J. Biol. Chem. 239: 604-613, 1964.

Iwamoto Y., Hansen I.L., Porter T.H. and Folkers K. Inhibition of coenzyme Q_{10}-enzymes, succinoxidase and NADH-oxidase by adriamycin and other quinones having antitumor activity. Biochem. Biophys. Res. Commun. 58: 633-638, 1974.

Jaenke R.S. An anthracycline antibiotic-induced cardiomyopathy in rabbits. Lab. Invest. 30: 292-304, 1974.

Jaenke R.S. Delayed and progressive myocardial lesions after adriamycin administration in the rabbit. Cancer Res. 36: 2958-2966, 1976.

Jennings R.B., Kaltenbach J.P. and Smetters G.W. Enzymatic changes in acute myocardial ischemic injury. A.M.A. Archiv. Pathol. 64: 10-16, 1957.

Kimura K., Fujita H. and Sakai Y. Blood levels, tissue distribution and clinical effects of adriamycin. In: Int. Symp. Adriamycin Eds. Carter S.K., Di Marco A., Ghione M., Krakoff I.H. and Mathe G. Springer-Verlag, pp 124-134, 1972.

Klugman S., Bartoli Klugmann F., Decorti G., Gori D., Silvestri F. and Camerini T. Adriamycin experimental cardiomyopathy in Swiss mice. Different effects of two calcium antagonistic drugs on ADR-induced cardiomyopathy. Pharm. Res. Commun. 13: 769-776, 1981.

Konze J.R. and Elstner E.J. Ethane and ethylene formation by mitochondria as indication of aerobic lipid degredation in response to wounding of plant tissue. Biochem. Biophys. Acta 528: 213-221, 1978.

Lakatta E.G. Alterations in the cardiovascular system that occur in advanced age. Fed. Proc. 36(2): 163-167, 1979.

Lambertenghi-Deliliers G., Zanon P.L., Pozzoli E.F. and Bellini O. Myocardial injury by a single dose of adriamycin: an electron microscopic study. Tumori 62: 517-528, 1976.

Langer G.A. Interspecies variation in myocardial physiology: the anomolous rat. Env. Health Persp. 26: 175-179, 1978.

Lee S.H., Dusek J. and Rona G. Electron microscopic study of glutamic oxalacetic transaminase activity in ischemic myocardium. J. Mol. Cell. Cardiol. 3: 103-109, 1971.

Lefrak E.A., Pitha J., Rosenheim S. and Gottlieb J.A. A clinicopathologic analysis of adriamycin cardiotoxicity. Cancer 32: 302-314, 1973.

Levey G.S., Levey B.A., Ruiz E. and Lehotay D.C. Selective inhibition of rat and human cardiac guanylate cyclase in vitro by doxorubicin (Adriamycin): possible link to anthracycline cardiotoxicity. J. Mol. Cell. Cardiol. 11: 591-599, 1979.

Luft J.H. Improvements in epoxy resin embedding methods. J. Biochim. Biophys. Cytol. 9: 409-414, 1961.

May H.E. and McCay P.B. Reduced triphosphopyridine nucleotide oxidase-catalyzed alterations of membrane phospholipids. J.

Biol. Chem. 243: 2296-2305, 1968.

Merski J.A., Daskal L. and Busch H. Effects of adriamycin on ultrastructure of nucleoli in the heart and liver cells of the rat. Cancer Res. 36: 1580-1584, 1976.

Mettler F.P., Young D.M. and Ward J.M. Adriamycin-induced cardiotoxicity (cardiomyopathy and congestive heart failure) in rats. Cancer Res. 37: 2705-2713, 1977.

Mhatre R.M., Herman E., Huidobro A. and Waravdekar V.S. The possible relationship between metabolism and cardiac toxicity of daunomycin and related compounds. J. Pharmacol. Exp. Ther. 178: 216-222, 1971.

Mimnaugh E.G., Ginsburg E. and Gram T.E. Differential effects of anthracycline drugs on rat heart and liver microsomal reduced nicotinamide adenine dinucleotide phosphate-dependent lipid peroxidation. Cancer Res. 42: 3574-3582, 1982.

Mimnaugh E.G., Siddik Z.H., Drew R., Sikic B. and Gram T.E. The effects of alpha-tocopherol on the toxicity, disposition and metabolism of adriamycin in mice. Tox. App. Pharm. 49: 119-126, 1979.

Minow R.A., Benjamin R.S., Lee E.F. and Gottlieb J.A. Adriamycin cardiomyopathy - risk factors. Cancer 39: 1397-1402, 1977.

Murphree A., Tritton T.R. and Sartorelli A.C. Differential effects of adriamycin on fluidity and fusion characteristics of cardiolipin containing liposomes. Fed. Proc. Fed. Am. Soc. Exp. Biol. 36: 167a, 1977.

Myers C.E., McGuire W.P., Liss R.H., Irfrim I., Grotsinger K. and Young R.C. Adriamycin - the role of lipid peroxidation in cardiac toxicity and tumor response. Science 197: 165-167, 1977.

Myers C.E., McGuire W. and Young R. Adrimaycin: Amelioration of toxicity by alpha-tocopherol. Cancer Treat. Rep. 60(7): 961-962, 1976.

Olson H.M. and Capen C.C. Subacute cardiotoxicity of adriamycin in the rat. Lab. Invest. 37: 386-394, 1977.

Olson H.M., Young D.M., Prieur D.J., Le Roy A.F. and Reagan R.L. Electrolyte and morphologic alterations of myocardium in adriamycin-treated rabbits. Am. J. Pathol. 77: 439-454, 1974.

Opie L.H. Metabolism of the heart in health and disease. Part II. Am. Heart J. 77(1): 100-122, 1969.

Plaa G.L. and Witsche H. Chemicals, drugs and lipid peroxidation. Ann. Rev. Pharmacol. 16: 125-141, 1976.

Placer Z.A., Cushman L.L and Johnson B.C. Estimation of product of lipid peroxidation (Malonyl dialdehyde) in biochemical systems. Anal. Biochem. 16: 359-365, 1966.

Praga C., Beretta G., Vigo P.L., Lenaz G.R., Pollini C., Bonadonna G., Canetta R., Castellani R., Villa E., Gallagher C.G., Von Melchner H., Hayat M., Ribaud P., De Wasch G., Mattsson W., Heinz R., Waldner R., Kolaric K., Buehner R., Bokkel-Huyninck T., Perevodchikova N.I., Manziuk L.A., Senn H.J. and Mayr A.C. Adriamycin cardiotoxicity: a survey of 1273 patients. Cancer Treat. Rep. 63(5): 827-834, 1979.

Pryor W.A. Free radical reactions and their importance in biochemical systems. Fed. Proc. 32(8): 1862-1869, 1973.

Rabkin S.W., Otten M. and Polimeni P.I. Increased mortality with cardiotoxic doses of adriamycin after verapamil pretreatment despite prevention of myocardial calcium accumulation. Can. J. Physiol. Pharmacol. 61: 1050-1056, 1983.

Revis N. and Marusic N. Sequestration of $^{45}Ca^{2+}$ by mitochondria from rabbit heart, liver, and kidney after doxorubicin or digoxin/doxorubicin treatment. Exp. Mol. Path. 31: 440-451, 1979.

Reynolds E.S. The use of lead citrate at high pH as an electronopaque stain in electron microscopy. J. Cell. Biol. 17: 208-212, 1983.

Rosenhoff S.M., Olson H.M., Young D.M., Bostick F. and Young R.C. Adriamycin-induced cardiac damage in the mouse: a single animal model of cardiotoxicity. J. Natl. Cancer Inst. 55: 191-194, 1975.

Roy P.E. Lipid droplets in the heart interstitium: Concentration and distribution. Recent Advances In Studies On Cardiac Structure And Metabolism. Vol. 10: 17-28, 1975.

Schewe T., Halangk W., Hiebsch C. and Rapoport S. Degredation of mitochondria by cytosolic factors in reticulocytes. Acta Biol. Med. Germ. 36: 363-372, 1977.

Singal P.K. Ultrastructural evidence for increased sensitivity of the hypertrophied heart to adriamycin. Persp. Cardiovasc. Res. 7: 197-209, 1983.

Singal P.K. and Dhalla N.S. Morphological methods for studying heart membranes. In "Methods in Studying Cardiac Membranes". Ed. Dhalla N.S., C.R.C. Press, Florida, Vol. II, pp 3-16, 1984.

Singal P.K., Forbes M. and Sperelakis N. Occurrence of intramitochondrial Ca^{2+} granules in hypertrophied heart exposed to adriamycin. Can. J. Physiol. Pharmacol. 62: 1239-1244, 1984.

Singal P.K. and Panagia V. Direct effects of adriamycin on the

rat heart sarcolemma. Res. Commun. Chem. Pathol. Pharmacol. 43(1): 67-77, 1984.

Singal P.K. and Pierce G.N. Adriamycin-induced changes in myocardial contractile function, sarcolemmal calcium binding and lipid peroxide activity. Fed. Proc. 42: 795, (Abstr.) 1983.

Singal P.K., Segstro R.J., Singh R.P. and Kutryk M.J. Changes in lysosomal morphology and enzyme activities during the development of adriamycin-induced cardiomyopathy. Exp. Cardiol. 1(2): 139-147, 1985.

Smith P.J., Ekert H., Waters K.D. and Matthews R.N. High incidence of cardiomyopathy in children treated with adriamycin and DTIC in combination chemotherapy. Cancer Treat. Rep. 61(9): 1736-1738, 1977.

Sobel B.E. and Shell W.E. Serum enzyme determinations in the diagnosis and assessment of myocardial infarction. Circ. 45: 471-482, 1972.

Sobel B.E, Spann J.F., Pool P.E. and Braunwald E.H. Normal oxidative phosphorylation in mitochondria from the failing heart. Circ. Res. 21: 355-363, 1967.

Sonnenblick E.H. Force-velocity relations in mammalian heart muscle. Am. J. Physiol. 202: 931-939, 1962.

Thayer W. Adriamycin stimulated superoxide formation in submitochondrial particles. Chem. Biol. Interact. 19: 265-278, 1977.

Thomas J.A. and Marks B.H. Plasma norepinephrine in congestive heart failure. Am. J. Cardiol. 41: 233-243, 1978.

Tong G.L., Cory M., Lee W.W., Henry D.W. and Zbinden G. Antitumor anthracycline antibiotics. Structure-activity and structure - cardiotoxicity relationships in rubidazone analogues. J. Medic. Chem. 21(8): 732-737, 1978.

Tsuro T., Fida H., Nojiri M., Tsukagoski S. and Sakurai Y. Circumvention of vinacristine and adriamycin resistance in vitro and in vivo by calcium influx blockers. Cancer Res. 43: 2905-2910, 1983.

Von Hoff D.D., Layard M.W., Basa P., Davis H.L., Von Hoff A.L., Rozencweig M. and Muggra F.M. Risk factors for doxorubicin-induced congestive heart failure. Ann. Intern. Med. 91: 710-717, 1979.

Wagner G.S., Roe C.R., Limbird L.E., Rosati R.A. and Wallace A.G. The importance of identification of the myocardial-specific isoenzyme of creatine phosphokinase (MB form) in the diagnosis of acute myocardial infarction. Circ. 47: 263-269, 1973.

Yesair D.W., Asbell M.A., Bruni R., Bullock F.J. and Schwartzbach.

Pharmacokinetics and metabolism of adriamycin and daunomycin. In: Int. Symp. Adriamycin. Eds. Carter S.K., Di Marco A., Ghione M., Krakoff I.H. and Mathe G. Springer-Verlag, pp 117-123, 1972.

Young D.M. Pathologic effects of adriamycin (NSC-123127) in experimental systems. Cancer Chemother. Rep. Part 3 6(2): 159-175, 1975.

Young D.M., Mettler F.P. and Fioravanti J.L. Adriamycin, verapamil and calcium metabolism. Proc. Am. Assoc. Cancer Res. 17: 90 (Abstr.), 1976.

Zbinden G., Bachmann E. and Holdiregger C. Model systems for cardiotoxic effects of anthracyclines. In: Fundamentals In Cancer Chemotherapy, Antibiotics Chemother. 23, pp 255-270, Karger, Basel, 1978.

Zbinden G. and Brandle E. Toxicologic screening of daunorubicin (NSC-82151), adriamycin (NSC-123127) and their derivatives in rats. Cancer Chemother. Rep. 59: 707-715, 1975.

Zbinden G., Brandle E. and Pfister M. Modification of adriamycin toxicity in rats fed a high fat diet. Agents and Actions 7(1): 163-170, 1977.

Zimmermann A.N.E. and Hulsmann W.C. Paradoxical influence of calcium ions on the permeability of the cell membranes of the isolated rat heart. Nature 211: 646-647, 1966.