THE EFFECTS OF HIGH CHOLESTEROL DIET ON SOME STRUCTURAL AND FUNCTIONAL PROPERTIES OF RAT MYOCARDIUM

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To My Family

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ABSTRACT

The effects of cholesterol on myocardial structure and function were studied in male rats fed either control or 2% cholesterol diets for different periods. In one set of experiments, morphological examination of hearts from these animals was carried out to determine changes in coronary vessels as well as in the myocardium. The second set of experiments concerned the monitoring of electrocardiographic changes before and after a subcutaneous injection of 250 mg/kg isoproterenol. For the third series of experiments, the sarcolemmal membranes were prepared by the hypotonic shock-LiBr method and studies of their chemical composition, ATP hydrolyzing activities and ATP-independent calcium binding were performed. In some experiments isolated hearts from the control and cholesterol fed animals were perfused in the absence or presence of glucose and some indices of contractile function were recorded on a polygraph recorder. As well, the effect of hypoxia was studied. These experiments were designed to investigate whether or not a high intake of cholesterol in the diet has any detrimental effects on the myocardium.

At 6 weeks, animals from both groups had histologically normal coronary arteries and normal myocardial ultrastructure. After 12 weeks, the morphological appearance of coronary vessels remained normal in both control and cholesterol fed groups. However, electron microscopic examination revealed widespread

evidence of myocardial cell damage in cholesterol fed rats. Degenerative changes included the presence of large numbers of lipid droplets and vacuoles, swollen mitochondria, disruption of normal geometry of myofibrils, alterations in the normal contour of the nucleus and increased complexity of the intercalated disc. As well, large numbers of leucocytes were observed and many had penetrated into the myocardial cells. By 24 weeks there was still no morphological evidence of atherosclerosis but the myocardium of the cholesterol fed animals showed lysosomal activation. These organelles were found intimately associated with cellular membrane systems including mitochondria, sarcoplasmic reticulum and sarcolemma. Regression of lipid deposition as well as resolution of some abnormalities was noted at this time. However, destruction of the mitochondria was indicated by the formation of myelin figures and the presence of membrane bound structures in the extracellular space which may have represented the end products of autolysis of these organelles.

Studies of the electrocardiograms from both groups of animals suggested that a number of differences existed between them. A variety of ST changes were noted in cholesterol fed rats from all of the feeding periods. As well, conduction abnormalities were indicated by the appearance of a high amplitude S wave in lead II at 12 weeks and a significant prolongation of the PR interval at 24 weeks. Administration of isoproterenol produced a variety of arrhythmias in both diet groups, however, cholesterol fed animals of the 12 and 24 week

groups were refractory to the positive chronotropic action of catecholamine. While overall mortality due to isoproterenol did not differ between diet groups only animals fed cholesterol supplemented diets died within the first 5 min following drug administration.

Biochemical studies of the isolated sarcolemmal membranes revealed a significant stimulation of the Na^{+}-K^{+}ATPase in cholesterol fed rats of the 6 week group as well as an increase in the calcium binding capacity in the presence of 1.25 mM $CaCl_2$. By 12 weeks Na^{+}-K^{+}ATPase, Mg^{2+}ATPase and Ca^{2+}ATPase activities were all significantly increased when compared to control. Calcium binding in the presence of 0.05 mM $CaCl_2$ was also elevated and may reflect the increase in Ca^{2+}ATPase activity observed at this time. As well, sarcolemmal cholesterol levels were elevated and a significant increase in the cholesterol/phospholipid molar ratio was noted. As an increase in this ratio is regarded as indicative of a decrease in membrane fluidity, sarcolemmal membranes from rats fed normal diets were treated with concanavalin A, a lectin which also is believed to decrease membrane fluidity. Stimulation of divalent cation ATPase activities were achieved by this treatment and resembled that observed in the sarcolemma from cholesterol fed rats. Concanavalin A did not alter the activity of Na^{+}-K^{+}ATPase, however, suggesting that some of the observed differences in sarcolemmal activities did not correlate well with changes in membrane order. At 24 weeks there was a generalized depression in

the sarcolemmal ATPase activities and both Mg^{2+}ATPase and Ca^{2+}ATPase were significantly less than in control.

A study of some indices of cardiac function was carried out in isolated rat hearts, perfused in the presence or absence of glucose, from animals fed control or 2% cholesterol diets for 12 weeks. In the absence of glucose, hearts from the control rats exhibited an increased spontaneous rate of contraction as well as a decrease in time to peak tension. Anaerobic-substrate free perfusion produced a greater degree of contracture in the cholesterol group following 30 min of hypoxia, however, only the control group developed significantly greater coronary pressure under these conditions. The contractile force development was greater in the treated group in the presence of glucose but was decreased significantly when substrate was absent in the perfusion buffer. The findings of this study indicate that hearts from the cholesterol fed animals may rely to a greater extent on glycolysis.

It is evident from these data that cholesterol can mediate myocardial cell damage directly. Alteration in structural, electrophysiological, biochemical and functional parameters were noted, however, the specific changes were strongly related to the length of time on diet. It appears that some changes represent compensation while others are pathological in nature. It is likely some of the observations were a result of a decrease in membrane fluidity, however, concommitant changes in sarcolemmal

permeability and lipid metabolism by mitochondria may also have contributed to cell damage. The results of the present investigation tend to support the hypothesis that high cholesterol diets are capable of initiating a cardiomyopathic process independent of atherosclerosis, ischemia or hypertrophy in rats. Furthermore, these results can be seen to have significant implications in understanding the nutritional basis of heart disease in man.

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I. INTRODUCTION

Despite the general acceptance of the hypothesis that elevated serum cholesterol levels are a major risk factor in the development of atherosclerosis (Albrink et al,1961; Kannel et al,1971; Shurtliff et al,1974; Jackson and Gotto,1976), the contribution of cholesterol from dietary sources remains controversial (Mann,1977; Oliver,1981b). Experimental atherosclerosis has been described for a number of species however, the heterogeneity of the response in different animals is indicative of the complexity of the diet-disease relationship (Mahley, 1978). It seems that the ability of animals to adjust rates of endogenous cholesterol synthesis and degradation to dietary cholesterol intake determines their susceptibility to vascular disease. In man, where the disease process is undoubtedly multifactorial (Shurtliff et al,1974) it is even more difficult to assess the contribution of dietary cholesterol. Therefore, the relevance of studies of experimental atherosclerosis in animals to the human cardiovascular disorder is debatable (Sabine,1977).

Experimental studies have demonstrated alterations in the structural and functional properties of the myocardium in response to cholesterol in both atherosclerotic and non-atherosclerotic models. Some of these have suggested pathological changes might be due to direct effects of cholesterol, whereas others have indicated these may be due to

the occurrence of atherosclerosis. The pathological changes reported include degeneration of myocardial cells and specialized conducting tissues (Melax and Leeson,1975; David et al,1978; Lee et al,1978; Senges et al,1981), decreased contractility (Peterson et al,1979), and alterations in electromechanical coupling (Pfeiffer et al,1978) and electrophysiological parameters (Lee et al,1978; Senges et al, 1981; Alivisatos et al,1977) Structural abnormalities of the myocardium as a result of hypercholesterolemic diets have been reported in the presence and absence of vascular involvement (Melax and Leeson,1975; David et al,1978; Lee et al, 1978; Senges et al,1981). Similarities exist in the ultrastructural findings in the atherosclerosis-prone rabbit (David et al,1978) and the atherosclerosis-resistant rat (Melax and Leeson,1975). The occurrence of such structural abnormalities in the absence of impaired coronary circulation in some studies suggests cholesterol itself may be responsible for deleterious changes. Similarly, in man, an idiopathic cholesterol pericarditis has been reported (Stanley et al,1981). Therefore, there is some evidence in both experimental studies and clinical studies that cholesterol may mediate myocardial tissue damage directly. In view of this, the loss of myocardial structural and/or functional integrity occurring in response to cholesterol enriched diets, deserves closer examination.

Cholesterol is a major constituent of biomembranes (Quinn and Chapman, 1980) and is thought to be involved in the regulation of membrane fluidity (Shinitzky and Henkart,1979) and

permeability (Demel and DeKruyff,1976). Disturbances in cholesterol metabolism are found to be associated with a number of pathological disorders in man (Jain,1975) some of which are characterized by alterations in the normal cholesterol content of the membranes of affected cells (Cooper et al, 1975; Hughes,1972). Changes in the cholesterol content of myocardial membrane systems have been reported in cardiomyopathic hamsters (Slack et al,1980) and in dogs with myocardial ischemia as a result of coronary ligation (Rouslin et al,1980). The consequences of dietary cholesterol supplementation for myocardial membrane structure and function have not been studied extensively. Alterations in the composition and characteristics of membrane systems such as sarcolemma, sarcoplasmic reticulum and mitochondria are known to be associated with cardiac contractile failure (Dhalla et al,1978; Katz and Messineo, 1981). In view of the fact that structural and functional integrity of subcellular membrane systems is essential for maintenance of electrical, mechanical and biochemical integrity in the heart, the direct effects of diet on these parameters is of interest.

The present study was designed to investigate the effects of cholesterol supplementation on some structural and functional parameters of the myocardium and the sarcolemma. It was hoped that the choice of a species resistant to atherosclerosis would facilitate elucidation of diet related damage in the absence of myocardial ischemia or hypertrophy. Ultrastructural, electrocardiographic, biochemical and functional data were

collected in order to determine which of these parameters would be altered by increased dietary cholesterol as well as to determine if a relationship between any changes in them could be deduced. The existence of a cardiomyopathic process related to cholesterol would add another dimension to predicting susceptibility to heart disease on the basis of diet.

II. REVIEW OF THE LITERATURE

The role of cholesterol in the genesis of cardiovascular disease remains controversial despite a large number of studies conducted both in humans and a wide variety of other species. The mechanisms by which increased consumption of cholesterol and elevated serum cholesterol levels augment the incidence of coronary heart disease in man remain unresolved. Nevertheless hyperlipidemic states, including hypercholesterolemia, are consistently found to be associated with atherosclerosis as well as ischemic heart disease and have been cited as a major risk factor in the development of these disorders (Albrink et al,1961; Gofman et al,1966; Kannel et al, 1971; Carlson and Bottiger,1972; Shurtliff,1974; Fisher and Truitt,1976). Prevention trials involving diet modifications with increased polyunsaturated to saturated fatty acid ratios have demonstrated a decreased incidence of non-fatal myocardial infarction with a concomitant reduction in serum cholesterol levels (Dayton et al, 1969; Miettinen et al,1972; Hjermann et al,1980). Only modest reductions in serum cholesterol were achieved in these studies,however. Treatment with hypolipemic agents including clofibrate, niacin and nicotinic acid alone or in combination, has produced inconsistent results (Coronary Drug Project Research Group,1975; Rosenhammer et al,1980; Oliver,1981a). It would appear, from these epidemiological studies, the efficacy of cholesterol lowering therapies is marginal in patients with pre-existing atherosclerosis and coronary heart disease.

Dietary factors, altered lipid metabolism and a variety of genetic and pathophysiological disorders have been shown to alter the characteristic composition of both serum lipoproteins and membranes in man (Skipski,1972; Jain,1975; Cooper et al,1975; Fisher and Truitt, 1976; Mahley,1978; Katz and Messineo,1981). Cholesterol/phospholipid ratios of biomembranes are determined by cellular regulatory mechanisms and serum lipoprotein profiles which are maintained within fairly narrow limits under normal circumstances (Skipski,1972; Sabine, 1977). The composition of the various lipid constituents is essentially characteristic for each species and cell type (Rouser et al,1968; Jain, 1975; Quinn and Chapman,1980; Boggs,1980). The heterogeneity in the content of plasma membranes from various tissues as well as that of the subcellular membranes within the same tissue implies sterols influence specific functions differently in various membranes (Jain,1975). It would seem that the normal cholesterol content of a particular membrane is not only characteristic but optimal for cell function as alterations have been found associated with a wide range of disorders (Jain,1975). In addition, experimental enrichment of human red cells and platelets, in vitro, has been found to mimic some of the membrane changes associated with spur cell anemia and increased thrombus formation, respectively, in vivo (Cooper et al,1975; Stuart et al,1980). These types of observations have led to the suggestion that levels of cholesterol accepted as "normal" in atherosclerosis-prone western societies may be excessive and capable of causing subtle changes

in membrane function prior to the development of vascular disease (Cooper,1977; Cooper and Shattil,1980). Nonetheless, the causitive relationship between diet, elevated serum cholesterol and heart disease in man remains an enigma (Mann,1977; Horrobin,1980; Oliver,1981b).

A. Models of Experimental Hypercholesterolemia and Atherosclerosis

Studies of experimental hypercholesterolemia and atherosclerosis have been carried out in animals in an attempt to elucidate the mechanisms through which cholesterol exerts its deleterious effects on the cardiovascular system. These have utilized spontaneously hypercholesterolemic models, animals in which other metabolic disorders are accompanied by cardiovascular symptoms and animals which have been subjected to dietary manipulation. To what extent any or all of these experimental models resemble the human disorder is speculative (Sabine,1977).

Spontaneously hypercholesterolemic models fall into two categories. The first is represented by animals which have elevated serum cholesterol levels when fed a normal diet. Such a strain of rats was developed by Boissel et al (1981) by selectively breeding animals fed a normal chow diet which nonetheless exhibited a significant elevation of blood cholesterol. A spontaneously low cholesterol strain of rats was developed at the same time. When both were fed a hyperlipidemic diet for one month, the increase in cholesterol levels, expressed as a percentage of basal values was the same, suggesting there

may be a separate genetic basis for spontaneous hypercholesterolemia and the dietary response. A similar model has been developed in pigeons (Patton et al,1974). The second genetic model involves animals who hyperrespond to cholesterol supplemented diets. Such models have been developed in several different rat strains (Adel et al,1969; Imai and Matsumara,1973; Van Zupphen and Den Bieman,1981; Yamori et al,1981). In rats, the development of spontaneously hypercholesterolemic models was necessitated by the fact that this species is resistant to both severe hypercholesterolemia and to atherosclerosis when fed diets supplemented with cholesterol alone. Only further measures, such as addition to the diet of saturated fatty acids, conjugated bile acids or either surgical or propylthiouracil (PTU) induced hypothyroidism, produce significant serum cholesterol elevation and atherosclerosis (Mahley and Holcombe,1977; Lasser et al, 1973). Addition of other variables has made interpretation of results difficult in terms of assessing effects due solely to cholesterol.

Other species studied include swine (Tall et al,1977; Mahley et al,1975), monkey (Mahley et al,1976), rabbit (Ross et al,1978; Kushwaha et al,1978a), baboon (Kushwaha et al,1978b) and dog (Mahley et al,1974). The relative sensitivity amongst the various species studied, including man, has been assessed (Mahley,1978). This must be taken into account when discussing the various studies, as some species are highly susceptible (rabbit), some intermediate (dog) and others relatively resistant (rat and man)

to the deleterious effects of increased dietary cholesterol(Mahley,1978).

The rabbit has been studied extensively as a model of hypercholesterolemia and atherogenesis. It has been shown this species is exquisitely sensitive to cholesterol supplemented diets, developing aortic lesions as early as seven weeks after commencement of feeding, with disseminated plaque development involving the coronary vasculature at eleven weeks (Lee et al,1978). These changes are accompanied by electrocardiographic evidence of myocardial ischemia, histopathological findings of cell damage in the myocardium and other organs and a dramatic increase in serum cholesterol levels (Lee et al,1978). Such findings are only observed in humans suffering from one of the primary hyperlipoproteinemias (Fisher and Truitt,1976) or severe coronary atherosclerosis (Geer et al,1980). In neither case do these appear rapidly as development occurs gradually, over a period of many years. It would appear the rabbit is an appropriate model for examining the sequelae of the most severe forms of atherosclerotic heart disease but is less suitable for understanding the membrane and cellular changes which may initiate the disease process and which may prove to be more responsive to prevention and treatment. Nevertheless, animal studies have given many valuable insights into cholesterol related cardiovascular disease despite the difficulties discussed previously. Alterations in metabolic, electrophysiological, structural and functional parameters have been cited.

B. Cholesterol and Lipoprotein Metabolism in Animals and Man

Both animals and humans consuming high levels of cholesterol in their diets have altered cholesterol metabolism reflected by characteristic changes in plasma lipoprotein patterns. Common features of these changes include an increase in a beta migrating very low density lipoprotein (beta-VLDL), the appearance of an intermediate density lipoprotein (IDL) containing apolipoprotein E and a specific high density lipoprotein (HDL_c) containing apolipoprotein E and deficient in apolipoprotein B. These alterations appear to represent a common response to increased dietary cholesterol (Mahley,1978). As plasma cholesterol levels increase, beta-VLDL, IDL, LDL and HDL_c become enriched in esterified cholesterol and apolipoprotein E and tend to displace HDL (normal). The source of these abnormal lipoproteins has been investigated and there appears to be controversy as to whether they originate from synthesis in the intestinal or hepatic compartments, or both, or from catabolic processes occurring in the plasma compartment (Camejo et al,1973; Frnka and Reiser, 1974; Roth and Patsch,1974; Ross and Zilversmit,1977; Swift et al,1980; Riley et al,1980). Swift et al (1980) presented convincing evidence for the synthesis of abnormal nascent VLDL in the hepatic Golgi apparatus of hypercholesterolemic rats. The study of Riley et al (1980) demonstrated alterations in mesenteric lymph lipoproteins particularly in the IDL and LDL subfractions. Mahley et al (1980) have shown that beta-VLDL from

dogs, monkeys, rabbits and rats fed cholesterol diets of varying composition is capable of stimulating cholesteryl ester synthesis in macrophages. They suggest further that this phenomenon results from high affinity binding of beta-VLDL to the macrophage LDL receptor and may represent the mechanism of cholesterol deposition in vessel walls. Whether cholesterol deposition occurs in other tissues by this mechanism is not known. However, in hypercholesterolemic rabbits, cholesterol is deposited in the heart (Ho and Taylor,1968; Ho et al,1974) with some of the storage within the cell membranes (Sabine,1977) and some in the form of intracellular lipid droplets (Jackson and Gotto,1976). It is of interest that only small increments in membrane cholesterol are required for alterations in membrane function to occur (Bloj et al,1973b). In this regard, increases in the cholesterol content of various cardiac membrane systems is found in cardiomyopathic hamsters (Owens et al,1974; Slack et al,1980) and dogs with myocardial ischemia as a result of coronary ligation (Rouslin et al,1980). Increased membrane cholesterol is regarded as a marker of myocardial injury in these studies.

Changes in cellular cholesterol levels may result from alterations in "de novo" synthesis, internalization of lipoproteins and liberation of free cholesterol by lysosomal enzymes (Brown and Goldstein,1979) or exchange of free cholesterol between plasma membranes and lipoproteins (Hageman and Gould,1951). Rapid exchange of free cholesterol between the different classes of lipoproteins and between lipoproteins and

cell plasma membranes is well documented (Portman et al,1980; Gottlieb, 1980). In addition, lipoprotein lipase activity has been found associated with isolated rat cardiocytes (Vahouny et al,1980) and heart muscle contains higher levels of free cholesterol than any other muscle type in this species (Okano et al,1980). Due to its insolubility, it is likely the majority of cholesterol is stored within membrane systems. Furthermore, intracellular membranes have a relatively low cholesterol content, therefore the plasma membrane is probably the major storage site (Ashworth and Green,1966; Boggs,1980).

Cholesterol feeding in rats produces a series of metabolic responses which may be responsible for its efficiency in balancing dietary cholesterol with rates of endogenous synthesis and degradation. Several investigators have reported suppression of hepatic cholesterol synthesis by cholesterol feeding (Frantz et al,1954; Dietchy and Siperstein,1967; Mathe and Chevallier,1979). This suppression is believed to be mediated through a feedback mechanism on the activity of the rate controlling enzyme of cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase (EC 1.1.1.34). Recent studies suggest dietary cholesterol induces a decrease in the activity of this enzyme which is accompanied by an increase in endoplasmic reticular free cholesterol (Balasubramaniam et al,1978; Mitropoulos et al,1980). The liver is the main site of cholesterol storage in the rat. Cholesterol is stored in esterified form and this reaction is catalyzed by the enzyme,

acyl-coenzyme A; cholesterol acyltransferase (EC 2.3.1.26). Balasubramaniam et al (1978) found increases in the activity of this enzyme associated with an increase in free cholesterol in the hepatic endoplasmic reticulum. The pivotal position of cholesterol in the modulation of membrane fluidity and the influence of this property on the activity of a wide range of membrane bound enzymes (see reviews by Coleman,1973; Demel and De Kruyff, 1976; Farias et al,1975; Lenaz,1979) suggests this may form the basis for feedback regulation of cholesterol biosynthesis and storage by dietary lipids in rats. Other metabolic responses to increased dietary cholesterol are increased synthesis of bile acids and an enhanced fecal excretion of sterols (Wilson,1962; Mathe and Chevallier,1979).

The liver and intestine account for approximately 90% of sterol synthesis in the rat but only hepatic synthesis is inhibited by dietary cholesterol (Dietchy and Siperstein,1967). Mathe and Chevallier (1979) found small increases in carcass cholesterol under these conditions. Ho and Taylor (1968) concluded cholesterol feeding increased concentrations of the sterol principally in the liver and to a lesser extent in the plasma. Increments of 1-3% in the cholesterol content of red cell membranes of rats fed cholesterol supplemented diets has been reported (Bloj et al,1973b).

In summary, all species so far studied appear to manufacture specific abnormal lipoproteins in response to increased dietary

cholesterol. The development of subsequent hypercholesterolemia and/or atherosclerosis is strongly species dependent and may be related to the ability to adjust the rates of endogenous synthesis, catabolism and storage to the rate of dietary intake.

C. Cholesterol and Myocardial Structure and Function

The role of cholesterol in cardiac membranes and myocardial cell function is of considerable interest although few studies have been conducted. One of the functions of cardiac sarcolemma is to confer the property of excitability to the cell by developing and maintaining an appropriate membrane potential. The membrane possesses differential permeability to the different ion species and as a consequence determines their concentration gradient between the extracellular and intracellular compartments. Concentration gradients across the membrane are maintained both by active and passive processes (Katz et al,1982). As the structure of the membrane is itself responsible for the phenomenon, changes in the nature or quantity of any of the component molecules can be seen to bring about changes in ionic permeability. Transient changes in permeability, in of themselves, underlie the process of excitation through the opening and closing of a number of time and/or voltage dependent ion channels located in the membrane (Coraboeuf,1978; Reuter, 1979; Katz et al,1982).

Studies on model black lipid membranes have shown membrane capacitance varies as a function of cholesterol content (Hanai et

al,1965; Ohki,1969; Papahadjopoulos et al,1971). As well, it has been shown that either cholesterol enrichment or depletion of model membranes and natural membranes changes their permeability to numerous electrolytes and non-electrolytes (McElhaney et al,1973; Grunze and Deuticke,1974; Deuticke,1977; Grunze et al,1980). In model membranes, cholesterol was found to inhibit cation conductance and to enhance anion conductance (Szabo,1974; Anderson,1978). Reports concerning ionic permeability and electrophysiological properties of the sarcolemma in response to cholesterol supplementation or depletion are not numerous. Pfeiffer et al (1978) studied cholesterol fed rabbits and reported an increase in sodium permeability accompanied by an increase in the sodium content of the myocardium. This study suggested, but did not demonstrate, that calcium levels might also be elevated. In this regard, it is interesting that enrichment of sarcoplasmic reticulum with cholesterol results in inhibition of Ca^{2+}ATPase activity (Madden et al,1979; 1981). David et al (1978) noted vesiculation of the sarcoplasmic reticulum from rabbits fed cholesterol rich diets which might suggest a loss in the integrity of this membrane system. A decrease in calcium pump activity might result in intracellular calcium overload and depression in this activity is associated with several forms of cardiac failure (Dhalla et al,1978). In addition, increases in intracellular sodium are thought to be accompanied by increases in intracellular calcium via sodium-calcium exchange at the sarcolemma (Nayler,1967; Glitch et

al,1970). The observation regarding increased intracellular sodium levels in cholesterol fed rabbits by Pfeiffer et al (1978) suggests the increase in intracellular calcium levels they postulate could be mediated through either or both of the above mechanisms. One study utilizing puppy Purkinje fibre superfused with Tyrode's buffer saturated with cholesterol demonstrated marked changes in intracellular microelectrode recordings. These included hyperpolarization, increased action potential amplitude and an increase in spontaneous action potentials (Alivisatos et al,1977). These in vitro observations are consistent with the findings of Senges et al (1981) in hearts from hypercholesterolemic rabbits which showed an increased incidence of supraventricular tachycardias. Ultrastructural analysis of the atrioventricular nodal tissue in this study indicated degenerative changes may have been responsible for the electrophysiological abnormalities. Lee et al (1978), demonstrated electrocardiographic evidence for altered conduction in hearts from atherosclerotic rabbits. Their study showed spontaneous depression of the S-T segment which was enhanced by the isoproterenol. They concluded their findings were consistent with the presence of subendocardial ischemia due to atherosclerosis. Interestingly, S-T depression in response to exercise has been reported in asymptomatic hyperlipoproteinemia (Olsson,1977). Melax and Leeson (1975) showed separation and tortuosities of the intercalated disc in rats fed cholesterol supplemented diets. Such findings also would be consistent with impaired conduction.

Isolated rat heart myocytes subjected to cholesterol depletion show an increased rate of depolarization and a decrease in spontaneous activity in response to changes in sodium and calcium concentrations (Hasin et al,1981). It would appear from the foregoing that alterations in membrane permeability and thus in the excitability and conduction properties of cardiac tissue occur with changes in cholesterol content in membranes both in vivo and in vitro. As changes were noted in both atherosclerotic and non-atherosclerotic models it seems likely that some alterations may be due to cholesterol and not necessarily related to atherosclerosis and ischemia. In addition, the ultrastructural evidence presented in many of the studies discussed support the contention that deleterious changes are occurring within components of the myocardium. These include cellular necrosis (Pfeiffer et al,1978), fat deposition and a decline in mitochondrial volume (David et al,1978), disruption of myofibrils (Melax and Leeson,1975) and degenerative changes in cardiac nerves and specialized conducting tissues (Senges et al,1981).

A few studies which have examined the functional deficit in the myocardium indicate that subtle changes in myocardial contractile performance occur in response to cholesterol feeding in experimental animals. Peterson et al (1979) found an increase in time to peak tension and lower maximum shortening velocities in isolated papillary muscle from atherosclerotic rabbits. These findings however did not correlate well with the development of atherosclerosis and the authors concluded that the loss of

contractility was probably due to a lipid induced defect in cellular calcium homeostasis. Pfeiffer et al (1978), in contrast, found no difference in shortening velocity of isometrically contracting papillary muscle from hypercholesterolemic rabbits. David et al (1978) found a non- significant decrease in the contractility index of isolated trabeculae in the same model. While decreased myocardial contractility as a result of coronary artery disease in man is well documented (Hamby et al,1973; Moraski et al,1975; Rackley and Russel,1975) there is no convincing evidence of gross impairment, unrelated to atherosclerosis, following ingestion of cholesterol enriched diets in either humans or experimental animals.

It has been reported that exposure of coronary vessels to cholesterol either in vitro (Yokoyama and Henry,1979) or to diet induced hypercholesterolemia in vivo (Rosendorff et al,1981) causes increased coronary vascular reactivity in the absence of vessel disease. Yokoyama and Henry (1979) perfused isolated canine coronary vessels with nanomolar concentrations of highly purified cholesterol and observed potent constrictor responses which could be abolished by verapamil. Neither alpha nor beta adrenergic blockade abolished the response leading these investigators to conclude that acquisition of cholesterol by membranes altered the contractile properties of vascular smooth muscle. In addition, prevention of the response with a calcium channel blocker coupled with an increase in the response to graded elevations of calcium in vessels exposed to cholesterol in

this study, suggests that cholesterol alters calcium homeostasis under these conditions. Rosendorff et al (1981) found that hypercholesterolemic dogs had an increased responsiveness to norepinephrine which involved both dilator and constrictor mechanisms. Dilator responses were enhanced in the presence of low doses of norepinephrine while constrictor responses were potentiated at higher doses. In this regard, it is interesting that isolated rat hearts exposed to cholesterol show a potent inhibition of extraneuronal catecholamine uptake (Salt and Iverson, 1972). These authors suggest that catecholamine levels, principally norepinephrine, might be enhanced in hypercholesterolemic states. In contrast to these studies, Johannsen et al (1981) concluded there were no differences in the vascular responsiveness of hypercholesterolemic and normal dogs to either the constrictor effects of sympathetic nerve stimulation or phenylephrine or in the dilator response to verapamil. The reason for these differences is not known as both studies were carried out in vivo, on the same species and both reported that coronary vessels were histologically normal.

It would appear that the effect of cholesterol on myocardial structure and function, unrelated to vascular disease, is not well understood. However, some of the foregoing studies do suggest that alterations in some myocardial and vascular functions do occur in direct response to elevated cholesterol levels and it is likely these changes result from membrane related phenomena.

D. Cholesterol and Membrane Structure and Function

The primary role of cholesterol in the cell would appear to be the modulation of membrane fluidity (Shinitzky and Henkart,1979; Shinitzky et al,1980) which is considered to exert profound effects on membrane function (Quinn and Chapman,1980). Changes in fluidity associated with with alterations in cholesterol content have been reported to influence membrane permeability and transport characteristics (McElhaney et al, 1975; Grunze and Deuticke,1974; Cooper et al,1975; Wiley and Cooper,1975; Deuticke and Ruska,1976; Claret et al,1978; Grunze et al,1980). Incorporation of cholesterol into either phospholipid vesicles or biomembranes has been reported to decrease chloride (Papahadjopoulos and Watkins,1967), glucose (Demel et al,1968), glycol (DeGier et al, 1968), sugar (DeGier et al,1969) and monovalent cation (Cooper et al,1975) permeabilities. The mechanisms by which cholesterol modulates permeability are related to interactions of sterols with membrane phospholipids. These physical effects of cholesterol in phospholipid membranes include condensation of the area per molecule in monolayers, inhibition of acyl chain motion in the outer segment of phospholipid bilayers, an increase in the bilayer width and an increase in the perpendicular orientation of the fatty acyl chains (Papahadjopoulos et al,1973). Other effects of cholesterol are related to temperature sensitive phenomena. Cholesterol has been shown to induce an intermediate fluidity

state and to abolish temperature sensitive phase transitions in pure phospholipid vesicles (Chapman,1973; Lenaz,1979). The role of sterols in the biomembranes of differentiated cells is more complex. However, changes in cholesterol content of cell membranes have been shown to alter both their permeability as well as the activities and cooperativity characteristics of associated enzyme proteins (for review see Coleman,1973; Farias et al,1975; Lenaz,1979). The sarcolemmal membrane of the cardiac cell contains a number of enzyme systems which play a major role in the regulation of cell function (Dhalla et al,1977). Although little is known regarding the effects of cholesterol on these myocardial proteins, similar enzyme activities have been studied in other cell types. These investigations have demonstrated modulation of enzyme activity by cholesterol supplementation, depletion or substitution. One of the most extensively studied enzymes in this regard is the Na^+-K^+ATPase, considered to be a marker enzyme for cell plasma membranes (DePierre and Karnovsky,1973). This enzyme is found in a wide variety of cell types and in the myocardium is believed to be responsible for the maintenance of membrane potential (Schwartz,1975). Na^+-K^+ATPase may be the binding site for digitalis glycosides and inhibition of the enzyme is widely regarded as the basis for the positive inotropic action these drugs exert in the myocardium (Akera and Brody,1978). It has been shown that Na^+-K^+ATPase, both purified and membrane associated, is sensitive to changes in cholesterol content however, the relationship of the sterol to enzyme

activity is far from clear. While some investigators have found cholesterol necessary for activation of the enzyme (Noguchi and Freed,1971; Jarnefelt,1972) many others have concluded the contrary (for review see Roelofson,1981). A number of studies have shown that cholesterol modulates either the activity of the enzyme or its allosteric regulatory properties (for review see Coleman,1973; Farias et al,1975; Lenaz,1979).

Delipidation and reconstitution experiments with Na^+-K^+ATPase from a variety of sources have yielded conflicting results regarding the specificity of its lipid requirement for activity. Noguchi and Freed (1971), using dissociated Na^+-K^+ATPase from rat brain, demonstrated that reconstitution of the enzyme without the cholesterol fraction resulted in a substantial decrease in the ability of the remainder of extracted lipids to restore activity. They also found the ability of ouabain to inhibit enzyme activity was reduced in the absence of cholesterol but the sensitivity to the glycoside was restored in its presence. This study concluded that cholesterol was necessary for reconstitution and probably for activation of the enzyme and that inhibition by ouabain, which contains a sterol structure similar to cholesterol, might be due to competition between the two molecules at the same allosteric site on the enzyme. Jarnefelt (1972), studying Na^+-K^+ATPase from the electric organ of the eel, found that selective removal of cholesterol resulted in a 20-30% decrease in enzyme activity while removal of phospholipid caused an 80% decline. Reactivation of the enzyme,

however, with either cholesterol alone or in combination with phospholipid was greater than that achieved by the phospholipid fraction. The study concluded that while removal of phospholipid was responsible for inactivation, reconstitution required the presence of cholesterol (Jarnefelt,1972). This apparent contradiction can be resolved if restoration of the correct microenvironment for optimal activity is achieved by inclusion of the cholesterol fraction in the reconstitution medium. Many other studies have demonstrated reactivation of Na^+-K^+ATPase by a variety of phospholipids following delipidation by phospholipases and detergents (Tanaka and Strickland,1965; Fenster and Copenhaver,1967; Tanaka and Sakamoto,1969; Wheeler and Whittam,1970; Palatini et al,1972; Kimelberg and Papahadjopoulos,1972; Wheeler et al,1975; Walker and Wheeler,1975). The reason for these differences in lipid specificity is not known but a recent review by Roelofson (1981) suggests that the conditions for delipidation may influence the nature of reactivation. This review concludes that the preponderance of evidence suggests the enzyme requires negatively charged phospholipids for activity. This does not imply, however, that neutral lipids, such as cholesterol, do not exert influence on enzyme activity. Kimelberg and Paphadjopoulos (1974) studied a purified Na^+-K^+ ATPase from rabbit kidney and found that addition of cholesterol inhibited phospholipid stimulated activity of the enzyme. These authors concluded the effect on enzyme activity was a result of decreased fluidity of the microenvironment resulting

from cholesterol incorporation. Similarly, Kimelberg (1975) found that activation of the same preparation by phosphatidylserine was inhibited in the presence of equimolar amounts of cholesterol. In contrast, the ouabain insensitive Mg^{2+}ATPase component of the enzyme was stimulated by cholesterol incorporation. Kinetic analysis demonstrated cholesterol increased V_{max} for Mg^{2+}ATPase while reducing V_{max} for Na^{+}-K^{+}ATPase, although the latter was not statistically significant. K_m values were not changed reliably for either enzyme by the addition of cholesterol. This study suggested that cholesterol may act as a modulator of enzyme activity by influencing the physical state of membrane phospholipids. It is difficult to assess the relevance of results obtained on delipidated reconstituted enzyme systems to the influence cholesterol might exert in natural membranes but these studies do indicate that cholesterol is capable of modifying Mg^{2+} dependent - Na^{+}-K^{+}ATPase activity under some conditions.

Cooper et al (1975) found that experimental depletion or supplementation of erythrocytes altered sodium and potassium fluxes as well as cellular content of these cations. Cholesterol poor membranes (Cholesterol/Phospholipid molar ratio or C/P =.42) displayed an increase in both total and ouabain sensitive sodium efflux, increased potassium influx and a twofold increase in cellular sodium at the expense of cellular potassium. Cholesterol enriched membranes (C/P=1.65) showed no changes in any of these parameters. In contrast, Pfeiffer et al (1978) found increased sodium efflux and an increase in cellular sodium in hearts from

hypercholesterolemic rabbits. As the rabbit myocardium is in a cholesterol enriched state under these conditions (Ho and Taylor,1968; Ho et al,1974), the differences in the findings of the two studies may represent a tissue specific response. Nevertheless, as Na^+-K^+ATPase activity is regulated by the internal sodium concentration, changes in the intracellular concentration of this cation can be seen to influence the enzyme activity.

Claret et al (1978) studied the effect of cholesterol depletion in erythrocytes and found an increase in maximal sodium efflux accompanied by a reduction in the apparent affinity for internal sodium. However, these authors did indicate that at critical sodium concentrations the cholesterol effect is no longer observed. Thus it is difficult to compare results of studies in which internal sodium concentrations may have been different. This may account for the fact that both decreases in pump mediated potassium influx (Poznansky et al,1973) and increases in sodium efflux (Cooper et al,1975) have been reported for cholesterol depleted erythrocytes, although one would expect these functions to be stoichiometrically coupled. It should be noted that the stimulation of pump mediated sodium efflux by cholesterol depletion observed in erythocytes does not occur in the enzyme derived from rabbit kidney (Peters et al,1981).

Although cholesterol is generally considered to exert its effects through alterations in membrane fluidity, the possibility

that it might be interacting directly with the pump was considered in two further studies. Giraud et al (1980) studied the effect of five fluidizing agents in addition to cholesterol depletion on erythrocyte Na^+-K^+ ATPase and obtained inconsistent results with respect to changes in the apparent affinity constant for sodium and maximal flux rates. While all treatments produced a comparable decrease in microviscosity of the membrane, only some exerted the cholesterol effect on the kinetic parameters of the pump. A subsequent investigation (Giraud et al,1981) indicated that the amphiphiles distributed differently in the outer and inner leaflets of the membrane and this assymetric distribution was a function of charge. As only the affinity for the internal sodium site was altered by cholesterol depletion and those agents mimicing its effect it was concluded that these treatments selectively altered the fluidity of the inner leaflet. Agents which acted preferentially on the outer leaflet were not effective. The authors concluded that the inner and outer cation binding sites are surrounded by different lipid environments and that the inner site alone was sensitive to changes in membrane lipid order.

One report concerning cholesterol enrichment of rat and dog heart homogenates by incubation in cholesterol containing buffers demonstrated a concentration dependent increase in ATPase activity in the presence of magnesium, sodium and potassium. Similar observations were noted in dog brain homogenates and synaptosomal plasma membranes (Alivisatos et al, 1977). In

contrast, a study of cholesterol incorporation, in vitro, into rat liver plasma membranes showed no effect on the activity of Na^+-K^+ ATPase (Leoni et al,1982). However, fluidization of the membranes with cis-vaccenic acid caused a significant decrease in Na^+-K^+ATPase activity.

Thus it would seem that both cholesterol depletion and enrichment are capable of producing an increase in cellular sodium content, rate of sodium efflux and Mg^{2+}-dependent Na^+-K^+ATPase activity in cells. In this regard, the erythrocyte may represent an anomaly as its membrane has a much higher cholesterol/phospholipid ratio in comparison to the plasmalemma of most other cell types (Ashworth and Green,1966).

Reports concerning replacement of cholesterol in membranes by desmosterol or its oxidation to 4-cholesten-3-one have produced conflicting results. Feeding of rats with 20,25-diazacholesterol increased the activity of sarcolemmal and erythrocyte Na^+-K^+ATPase by replacing membrane cholesterol with desmosterol (Peter and Fiehn,1973; Fiehn and Seiler,1975). Conversion of cholesterol in erythrocytes into its oxidized product 4-cholesten-3-one was reported to inhibit enzyme activity by Seiler and Fiehn (1976) however Peters et al (1981) reported Na^+-K^+ATPase activity from rabbit kidney was not appreciably altered by cholesterol oxidation. These discrepancies are similar to those noted in regard to cholesterol supplementation and depletion and may be explained on the basis that the different

studies were conducted on enzymes which were derived from tissues whose normal lipid environments were substantially different.

It has been reported that cholesterol supplemented diets result in an increase in the Hill coefficient for inhibition by fluoride of Na^+-K^+ ATPase (Bloj et al,1973a). This modulation of the allosteric behaviour of the enzyme was associated with an increase in membrane cholesterol too small to be reflected in a change in the cholesterol/phospholipid molar ratio.

From the foregoing it is apparent that Na^+-K^+ATPase is lipid requiring enzyme. While the role of cholesterol in either confering or modulating enzyme activity is not resolved it has been amply demonstrated that this sterol is capable of altering the characteristics of the enzyme derived from a variety of different tissues. The nature of these effects would appear to be influenced both by the tissue from which the enzyme is derived and the experimental conditions under which the studies were conducted. This probably underlies the many apparent contradictions in the literature. Insofar as the cardiac cell is concerned, the study of Alivisatos et al (1977) demonstrating stimulation of membrane ATPase by cholesterol, is consistent with electrophysiological observations showing hyperpolarization in response to cholesterol superfusion. Because the pump is electrogenic (Thomas,1972), removing sodium from the cell in excess of potassium gained, it is capable of hyperpolarizing the membrane when its activity is stimulated (Gadsby and

Cranefield,1979). The mechanism underlying the phenomenon is not known but it could result from an increase in intracellular sodium content such as that reported by Pfeiffer et al (1978) in hypercholesterolemic rabbits. The consequences of cholesterol enrichment for the Na^{+}-K^{+}ATPase of cardiac sarcolemma remains to be investigated.

Another group of enzymes whose interrelationship with the cholesterol content of membranes has been studied are the divalent cation ATPases stimulated by magnesium and calcium. These enzymes include the magnesium stimulated ATPase from rat liver membrane (Mg^{2+}ATPase), the calcium stimulated-magnesium dependent [(Ca^{2+}-Mg^{2+})ATPase] enzymes of the erythrocyte plasmalemma and muscle sarcoplasmic reticulum (Drabikowski et al,1972; Bloj et al,1976; 1979; Madden et al,1979; 1981). The function of these proteins is thought to be the regulation of the intracellular concentrations of magnesium and calcium (Dhalla et al,1977; 1978). Enzymes with comparable function are found associated with myocardial membrane systems (Dhalla et al,1977). The function of divalent cation ATPases with respect to regulation of calcium movements in cardiac cells has been described by several investigators however, the relationship to magnesium homeostasis has not been studied in any detail. Both a calcium and a magnesium stimulated ATPase have been described for the sarcolemma (Anand et al,1977). The Ca^{2+}ATPase has been isolated from rat heart and it is believed that the enzyme may function to regulate calcium entry into the cell (Dhalla et

al,1981). Whether this protein is similar to the Na^{+}-Ca^{2+} antiporter described for bovine heart is not known (Miyamoto and Racker,1980). Ca^{2+}-Mg^{2+}ATPase activity associated with the sarcolemma is thought to be involved in extruding calcium from the cell (Morcos and Drummond,1980; Caroni and Carafoli, 1980; 1981) and strongly resembles the enzyme found associated with the erythrocyte membrane (Carafoli et al,1980). (Ca^{2+}-Mg^{2+})ATPase of the sarcoplasmic reticulum is thought to sequester calcium, lowering the intracellular concentration of this cation and allowing relaxation to occur (Tada et al,1977). All of these enzymes are vital to cell function (Dhalla et al,1977) and changes in their activities have been found associated with cardiac contractile failure of varying etiology (Dhalla et al,1978).

Mg^{2+}ATPase activity of liver plasma membrane is stimulated by decreasing membrane fluidity (Riordan,1980). This activation was most effectively achieved by treatment of membranes with concanavalin A. As well, stimulation of the enzyme after some treatments could be acheived by the addition of cholesterol. This study concluded that increased membrane order enhanced enzyme activity either by displacing more of the enzyme from the lipid into the aeqeous phase or by stabilizing the enzyme itself. Concanavalin A has been shown by others to increase the activities of both calcium and magnesium stimulated ATPases (Carraway et al, 1980; Averdunk and Gunther,1980). As both concanavalin A (Mak and Wong,1980) and cholesterol (Kroes et

al,1972) decrease membrane fluidity, it is possible that their similar effects on Mg^{2+}ATPase activity may be related to increased membrane order. In contrast to these findings, Leoni et al (1982) found cholesterol had no effect on Mg^{2+} ATPase from rat liver membranes. Both studies of Riordan (1980) and Leoni et al (1982) demonstrated inhibition of enzyme activity following fluidization with cis-vaccenic acid. The reason for the differences in the effects observed for cholesterol are not clear. Studies of Ca^{2+}- Mg^{2+}ATPase from muscle sarcoplasmic reticulum indicate the enzyme is dependent on phospholipids for activity (Dean and Tanford,1978). Moreover, cholesterol is normally excluded from the phospholipid annulus surrounding the enzyme (Warren et al,1975). Madden et al (1979) reported increased incorporation of cholesterol into sarcoplasmic reticular vesicles resulted in a proportionate decrease in enzyme activity. A recent study by these investigators, using tightly coupled vesicles, demonstrated unmasking of the inhibitory influence of cholesterol by collapse of the calcium gradient with an ionophore (Madden et al,1981).

The effects of cholesterol loading, in vivo and in vitro, on the allosteric properties of (Ca^{2+}-Mg^{2+})ATPase of erythrocytes has been studied (Bloj et al,1973a; 1973b; 1976; 1979). Rats fed cholesterol supplemented diets exhibited a decrease in the Hill coefficient for inhibition by fluoride of the enzyme (Bloj et al,1973a). Furthermore, although increments of 1-3% in membrane cholesterol were found in these studies, these relatively small

increases altered neither membrane fatty acid composition nor the cholesterol/phospholipid molar ratio (Bloj et al,1973b; 1976). These observations indicate that very small increases in membrane cholesterol are required to change the allosteric properties of membrane bound enzymes. Changes in the degree of cooperativity for activation of the enzyme by calcium and magnesium (Bloj et al,1976) as well as alterations in Arrhenius plot profiles have also been cited (Bloj et al,1979). The latter study demonstrated these effects were rapid in onset (24 hrs) but reversal to control values relatively slow (4 days) upon eliminating cholesterol from the diet.

It is of interest to note that the increases in membrane cholesterol observed in hereditary muscular dystrophy (Owens et al,1974; Slack et al,1980) were accompanied by alterations in calcium transport (Owens et al,1974). Verapamil, a calcium blocking agent was extremely effective in preventing the necrosis occurring in this kind of cardiomyopathy (Jasmin and Solymoss,1975; Bhattacharya et al,1982). Treatment of dystrophic hamsters with this drug, however, does not prevent the increase in myocardial membrane cholesterol indicating this change in membrane composition is not secondary to cellular necrosis (Slack et al,1980). These results support the idea that changes in membrane fluidity and permeability due to cholesterol may lead to myocardial calcium overload and necrosis in this disorder.

From the foregoing it is apparent that cholesterol exerts a

profound influence on the structure and function of both the cells and cell membranes of many tissues from a variety of species. In the myocardium, it is likely that some of these effects are a consequence of atherosclerosis. Nevertheless, there is increasing evidence that cholesterol may mediate damage directly. It would be of interest to examine this possibility under conditions in which atherosclerosis is not a factor. The selection of a species which is resistant to the development of vessel disease when fed cholesterol supplemented diets would seem to best serve this objective. Under these conditions it should be possible to examine the influence of dietary cholesterol on the myocardium and address the question of whether cholesterol itself can initiate a cardiomyopathic process exclusive of atherosclerosis.

III. METHODS AND MATERIALS

A. Chemicals

Isoproterenol, ouabain, concanavalin A, Tris-ATP, alpha-methyl-d-mannoside and cholesterol assay kits were obtained from Sigma (St.Louis,MO). $^{45}CaCl_2$ was supplied by New England Nuclear (Boston,MA).

B. Animals and Diets

Male Sprague Dawley rats, weighing on average 100 grams (4 weeks), were obtained from Bio-Laboratories, St. Paul,MN,USA and placed immediately on either control rat chow or chow supplemented with 2% cholesterol (ICN Biochemicals, Cleveland, Ohio, USA) ad libitum for 6,12 or 24 weeks. Adequate water was available at all times. The animals were housed in a controlled environment, 2-3 to a cage, and maintained on a 13 hour light- 11 hour dark photoperiod until sacrifice. The animals were sacrificed by decapitation and their hearts were quickly removed.

C. Morphological Procedures

One millimeter cubes were cut from the free wall of the left ventricle and immersed in 2% glutaraldehyde in phosphate buffer for 2 hrs. The tissue was washed in successive changes of phosphate buffer for a further period of 2 hrs and post fixed in 1% osmium tetroxide for 1 hr at 4 C. The tissue blocks were dehydrated in a graded alcohol series and embedded in Epon 812

(Luft,1961). Thick and thin sections were cut on a Porter-Blum ultramicrotome. Thin sections were placed on Formvar coated grids and some were stained with uranyl acetate and lead citrate (Reynolds,1963). Both stained and unstained sections were examined in a Zeiss EM 9 electron microscope. Thick sections were placed on clean glass slides, stained with toluidine blue and examined in a Zeiss photomicroscope.

D. Electrocardiographic Measurement

Control and cholesterol fed rats from the 6,12 and 24 week groups were anesthetized with 48 mg/kg sodium pentobarbital. Animals were placed in the ventral position and subcutaneous needle electrodes were inserted in both forelimbs and the left hindlimb. Standard lead II electrocardiogram (ECG) was recorded on a Beckman R-611 dynograph recorder. After recording the resting ECG the animals were injected subcutaneously with 250 mg/kg isoproterenol and further recordings were made 5,15,30 and 60 min following administration. The sensitivity of the recorder was set at 0.02 mv/mm and amplitude measurements were made by increasing the chart speed to 50 mm/sec.

E. Biochemical Analysis

a. Isolation of Rat Heart Sarcolemma

Following decapitation, hearts were removed from rats fed diets for 6,12 or 24 weeks and placed in ice-cold buffer. The sarcolemmal fraction was isolated by the hypotonic shock-LiBr

procedure according to the method of McNamara et al (1974) as modified by Dhalla et al (1977).

Hearts were washed in ice cold 10 mM Tris-HCl, pH 7.4, containing 1 mM ethylenediaminetetraacetate (EDTA) and the atria and connective tissues were dissected away. The ventricles were minced finely with scissors in the above buffer and the material was homogenized in 10 vol (w/v) with 2-30 sec bursts in a Waring blender at speed 5. The homogenate was filtered through four layers of gauze and centrifuged at 1000 g for 10 min. The pellet was suspended in 10 vol of 10 mM Tris-HCl, pH 7.4, and stirred for 15 min. Following centrifugation at 1000 g for 10 min the pellet was resuspended in 10 vol of 10 mM Tris-HCl, pH 8.0, stirred for 15 min and centrifuged at 1000 g for 10 min. This step was repeated in 10 mM Tris-HCl, pH 7.4. The washed particles were suspended in 20 vol Tris- HCl, pH 7.4, containing 0.4 M LiBr, stirred for 30 min and centrifuged at 1000 g for 10 min. The pellet was suspended in 10 mM Tris-HCl, pH 7.4, stirred for 15 min and centrifuged at 1000 g for 10 min. The sarcolemma enriched pellet was further extracted in 10 mM Tris-HCl, containing 0.6 M KCl, pH 6.8, for 15 min and centrifuged at 1000 g for 10 min. The pelleted sarcolemma was washed for 15 min in 10 mM Tris-HCl, 1 mM EDTA, pH 7.4, and centrifuged at 1000 g for 10 min. The final pellet was suspended in 1 mM Tris HCl, pH 7.0. All isolation steps were carried out at 4 C. Membranes were frozen for lipid analysis and sialic acid determination. Fresh membranes were used for enzyme analyses and calcium binding determinations.

b. Treatment of Membranes with Concanavalin A

Sarcolemma was isolated from rats fed normal chow diets and the effects of concanavalin A on membrane enzyme activities were determined. Sarcolemmal membranes (30-50 ug/ml) were incubated in a medium containing either 10,100 or 1000 ug/mg protein concanavalin A in 50 mM Tris-HCl (pH 7.4) for 20 min at 37 C prior to assaying enzyme activities as described below. In some experiments 50 mM alpha-methyl-d-mannoside was included in the incubation medium both in the presence and absence of concanavalin A.

c. Enzyme Assays

Adenosine triphosphatase activities of the isolated sarcolemmal membranes were studied by suspending 30-50 ug/ml of membrane protein in a medium containing 50 mM Tris-HCl, pH 7.4. For determination of the total enzyme activity the incubation medium contained 100 mM NaCl, 10 mM KCl, 4 mM $MgCl_2$, 1 mM EDTA. The activity of Ca^{2+}ATPase was studied in the presence of either 1.25 mM or 4.00 mM $CaCl_2$ and the activity of Mg^{2+}ATPase was studied in the presence of 4.00 mM $MgCl_2$, 1 mM EDTA. The difference between the total activity and the Mg^{2+} ATPase activity was taken to represent the Na^{+}-K^{+}ATPase. Membranes were preincubated for 3 min at 37 C and the reaction was started by the addition of 4 mM Tris ATP, pH 7.4. The reaction was terminated by the addition of 1 ml of 12% ice cold

trichloroacetic acid. Phosphate liberated was determined according to the procedure of Taussky and Shorr (1953). Protein was estimated by the method of Lowry et al (1951) using bovine serum albumin as a standard. Ouabain sensitivity of the Na^+-K^+ATPase was determined by studying the activity of this enzyme in the presence of 2 mM ouabain. All the enzyme reactions were linear under the experimental conditions employed here.

d. Calcium Binding

For the study of ATP-independent calcium binding membrane protein (0.10-0.15 mg/ml) was suspended in 50 mM Tris HCl, pH 7.4 and preincubated for 3 min at 37 C. The reaction was initiated by the addition of 100 ul $^{45}CaCl_2$ at a concentration of 5×10^{-5} or 1.25×10^{-3} M. The reaction was terminated by millipore filtration after 5 min (Sulahke and Dhalla,1971). Blanks, without protein, were incubated under conditions identical to those described above. Samples of the filtrate (100 ul) were added to 10 mls of Beckman Ready-Solv scintillation cocktail and vials were counted in a Beckman liquid scintillation counter.

e. Sialic Acid Determination

Membranes were washed and hydrolyzed in 0.1 N H_2SO_4 for 1 hr at 80 C to release sialic acid (Cook,1976). The sialic acid content was determined using the thiobarbituric acid assay of Warren (1963).

f. Phospholipid Analysis

Membrane protein (2.0 mg) was suspended and extracted overnight in a solution of chloroform/methanol (Folch et al,1957). The non lipid contaminants were removed by repeated washings in chloroform:methanol: HCl. Following drying over nitrogen, the extracted lipids were spotted on activated thin layer chromatography plates and the plates were run in a chromatography tank containing chloroform:methanol:NH_3 for 2 hrs. Following drying, the plates were placed in a second tank containing chloroform:methanol:glacial acetic acid:H_2O at a ninety degrees respective to their position in the first solvent and run for a further 2 hrs (Pumphrey,1969). Spots were visualized by spraying with H_2SO_4 (5%) and drying briefly at 160 C. The plates were examined under ultraviolet light and the location of the spots was marked. Spots were removed from the plates into labelled tubes and hydrolyzed in 0.7 ml perchloric acid for 2 hrs at 150 C. Phosphate evaluation was made according to the procedure of Bartlett (1969).

g. Cholesterol Analysis

For determination of plasma cholesterol blood was collected in heparinized tubes from the neck vessels at the time of sacrifice. Samples were centrifuged at 1000 rpm for 20 min and total plasma cholesterol was determined using a kit from Sigma. For determination of membrane cholesterol 200 ul samples were taken from the organic lipid extract of the phospholipid procedure described previously and analyzed using the same kit.

F. Langendorff Heart Perfusion

For heart perfusion studies the animals (12 weeks on diets) were sacrificed by decapitation and their hearts were rapidly excised and placed in ice cold buffer. The hearts were mounted on a steel cannula and perfused in a non-circulated fashion through the coronary arteries at a constant flow rate of 15 ml/min with a Watson-Marlow peristaltic pump according to the Langendorff method. The perfusion medium was Krebs-Hensleit buffer consisting of (mM): NaCl,120; $NaHCO_3$,20; KCl,4.63; KH_2PO_4,1.17; $CaCl_2$,1.25; $MgCl_2$,1.20 and glucose 8. In some experiments glucose was replaced with equimolar concentrations of mannitol. The perfusion system was maintained at 37 C. For normoxic perfusion the buffer was gassed with 95% O_2-5% CO_2 mixture (pO_2> 600 mm Hg). To induce hypoxia, the oxygenated buffer was replaced by buffer gassed with 95% N_2 and 5% CO_2 mixture (pO_2 less than 30 mm Hg). Buffer pH was 7.4. Myocardial contractile force (peak tension) was recorded by attaching the apex of the heart to a Grass Instrument Co. (Quincy,MA) FT.03 force displacement transducer which was located to produce an initial resting tension of 1 g. The hearts were allowed to contract spontaneously and the intrinsic heart rate was determined by increasing the chart speed of the recorder. Coronary perfusion pressure was obtained by attaching a side arm off the cannula to a Statham P23ID pressure transducer. Since the hearts were perfused at a constant flow, changes in pressure were regarded as indicative of changes in coronary vascular

resistance. Hearts were allowed to equilibrate for 10 min before recordings were made.

G. Statistical Analysis

All results of these studies were subjected to a multifactorial analysis of variance using a Duncan's New Multiple Range test to locate group differences. All values are expressed as the mean $\pm$SEM. Minimum statistical significance was taken as alpha = 0.05.

IV. RESULTS

A. General Data

Tissue data from rats fed either control or 2% cholesterol diets for 6,12or 24 weeks is summarized in Table I. Body weights did not differ in the 6 or 24 week groups, however, the weight of rats fed the cholesterol diet for 12 weeks was significantly less than that of their control diet cohorts. Ventricular weights as well as ventricular weight/ body weight ratios did not differ between diet groups for all feeding periods studied. Liver weights were significantly greater in animals fed a cholesterol diet for 24 weeks. Plasma cholesterol levels were elevated 11-14% throughout the feeding period in animals fed 2% cholesterol diets but this elevation was statistically significant only at 6 weeks.

B. Morphological Data

Hearts of rats fed a normal diet as well as those fed a cholesterol diet for 6 weeks displayed normal histological appearance. Figure IA shows a representative cross sectional view of the myocardium from a 12 week cholesterol fed rat. The appearance of a large number of leucocytes was a major feature in this treatment group. Coronary vessels were found to be patent and there was no evidence of atherosclerosis. Figure 1B shows a typical longitudinal section of the myocardium from a rat fed a cholesterol rich diet for 24 weeks. As was seen in the 12 week group coronary vessels were histologically normal, however, small

Table 1. Some Characteristics of Rats Fed Control or 2% Cholesterol Supplemented Diets.

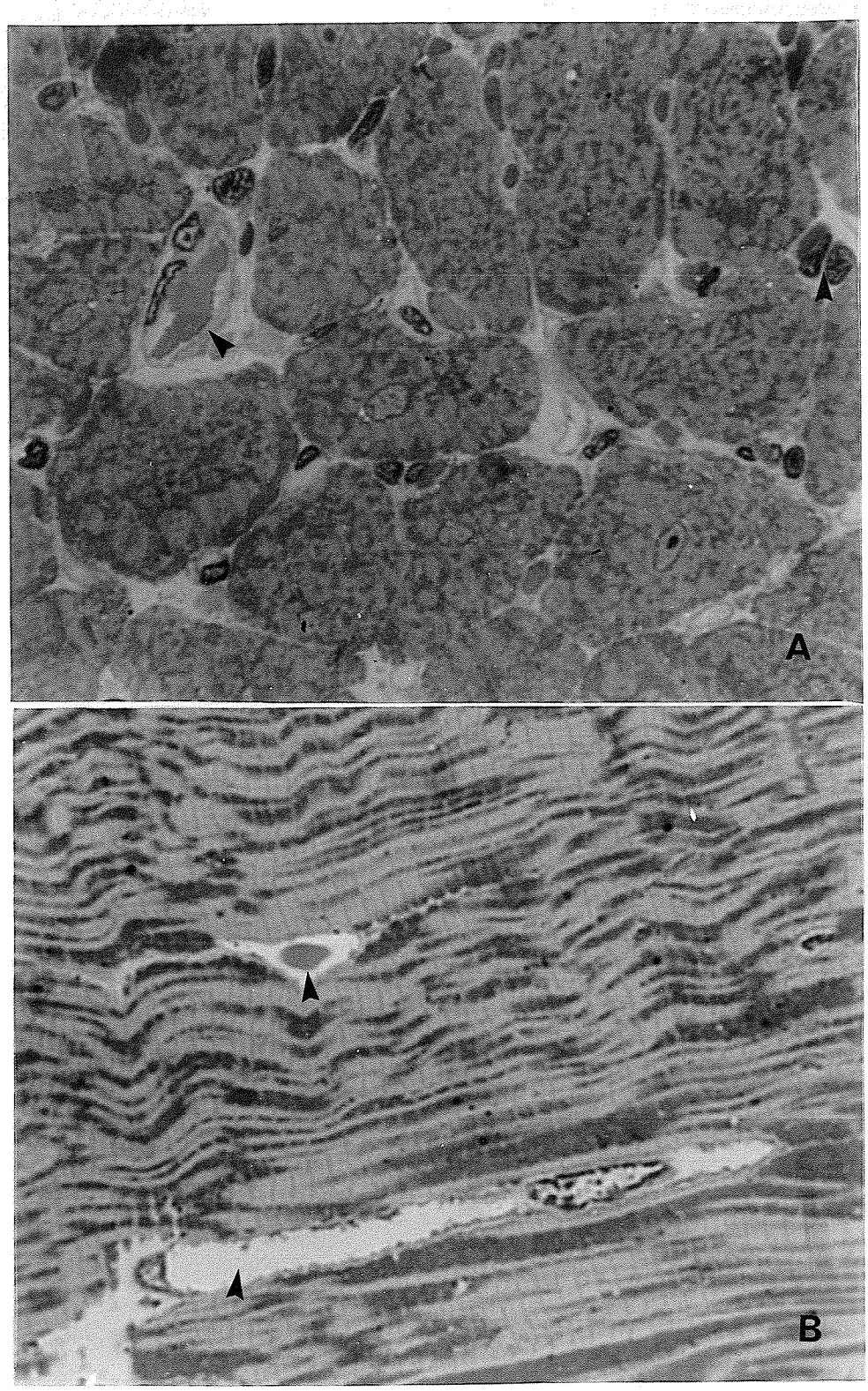
Time on Diet (weeks)	Body Weight (g)		Ventricular Weight (VW) (mg)		Liver Weight (g)		VW/BW (mg/g)		Plasma Cholesterol (mg/dl)	
	C	CF	C	CF	C	CF	C	CF	C	CF
6	420 ± 19	369 ± 24	1254 ± 51	1189 ± 40	15.6 ± 1.3	15.4 ± 1.2	3.00 ± 0.13	3.24 ± 0.11	90.1 ± 2.24	101.3 ± 3.67*
12	457 ± 8	414 ± 9**	1304 ± 33	1246 ± 29	14.9 ± 1.1	16.0 ± 1.4	2.86 ± 0.07	3.01 ± 0.06	81.0 ± 8.74	91.0 ± 5.41
24	480 ± 29	511 ± 7	1256 ± 52	1352 ± 37	15.6 ± 1.0	19.1 ± 0.4*	2.65 ± 0.15	2.65 ± 0.06	90.7 ± 5.40	103.7 ± 5.37

*P < 0.05, ** P < 0.01, C = Control rats, CF = Cholesterol fed rats. Each value is a mean ± S.E. of 10 experiments.

FIGURE 1: Photomicrograph of ventricular myocardium from rats fed a 2% cholesterol diet:

A) 12 weeks: large numbers of leucocytes are seen in the extracellular and vascular spaces. Coronary vessels are patent.(arrows) x 140

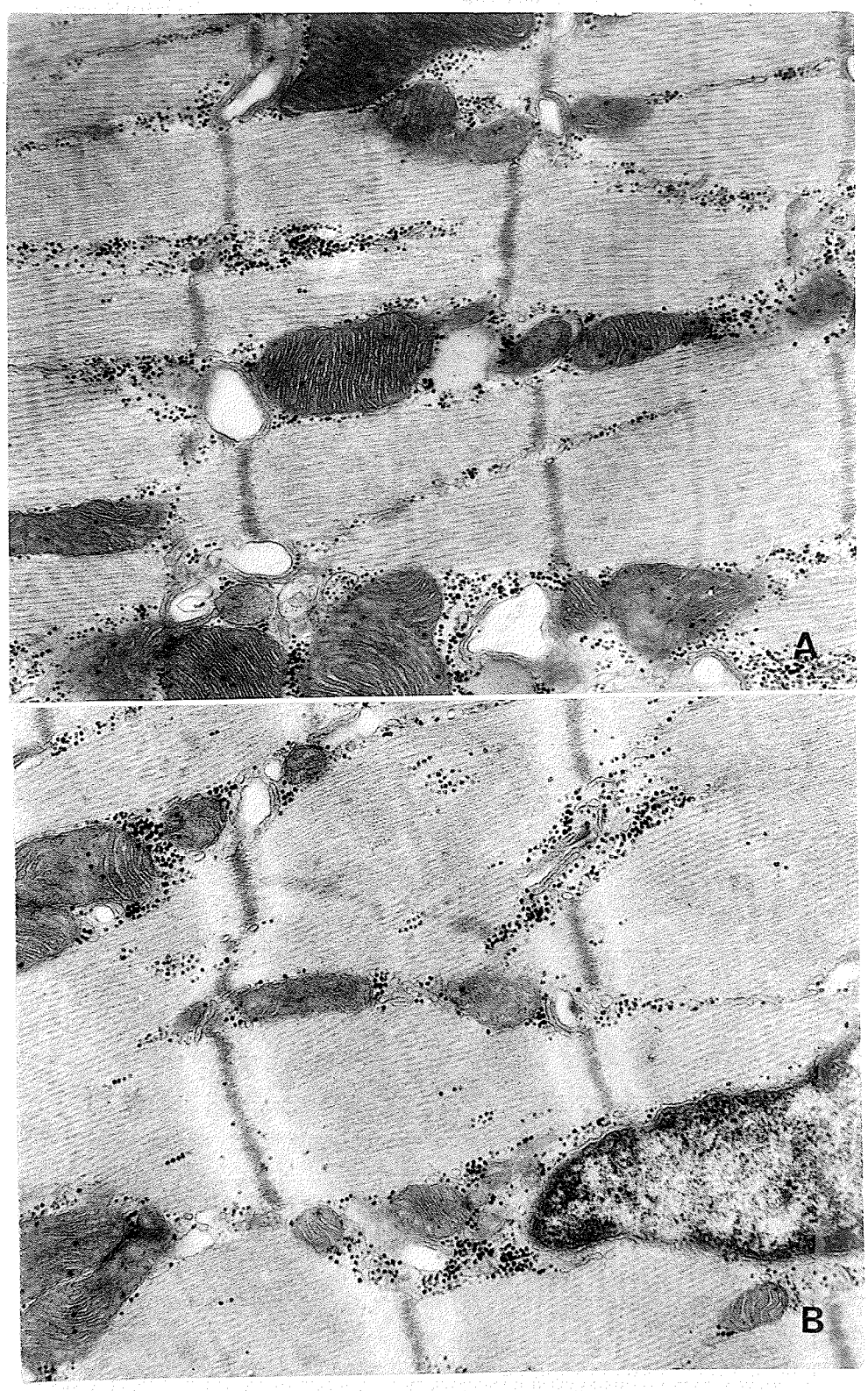
B) 24 weeks: small electron dense bodies are scattered throughout the myocardium. Coronary vessels are free of plaque. (arrows) x 75


A
B

electron dense bodies were seen scattered throughout the myocardium as well as in the vasculature. The histological appearance of hearts from cholesterol fed rats of the 6 week group was not different from the control.

In all rats which were fed a normal diet as well as those fed a cholesterol supplemented diet for 6 weeks no ultrastructural abnormalities were observed (Figure 2A and 2B). On the other hand, rats which were maintained on the cholesterol diet for 12 or 24 weeks showed considerable degeneration and many structural abnormalities. At 12 weeks many of the mitochondria appeared to be swollen and often were surrounded by vacuoles and vesiculations (Figure 3).In some cells the Z line had lost its normal appearance and had developed a wavy outline. As well the myofibrils appeared to have lost their normal geometric arrangement. A few small electron dense bodies were also observed at this time. Figure 4 shows similar details including abnormal arrangement (whorls) of myofibrils. In some sections contracture and clumping of large numbers of mitochondria were observed (Figure 5). The intercalated disc had assumed a complex and tortuous arrangement (Figure 6). Such a derangement was reported to precede disintegration of this structure by Melax and Leeson (1975). The contour of the nucleus was observed to change from its normal smooth, round to fusiform shape and was lobulated (Figure 7). As well it appeared to interdigitate with the surrounding myofibrils and pseudopodial processes were noted. At

FIGURE 2: Electronmicrograph of ventricular myocardium of rats fed either control (A) or 2 % cholesterol diet (B) for 6 weeks. Normal ultrastructure of sarcomeres, mitochondria, sarcoplasmic reticulum, transverse tubules and nuclei were observed in all control groups and in the 6 week treated group.
1A - 24,500 1B - 26,500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B

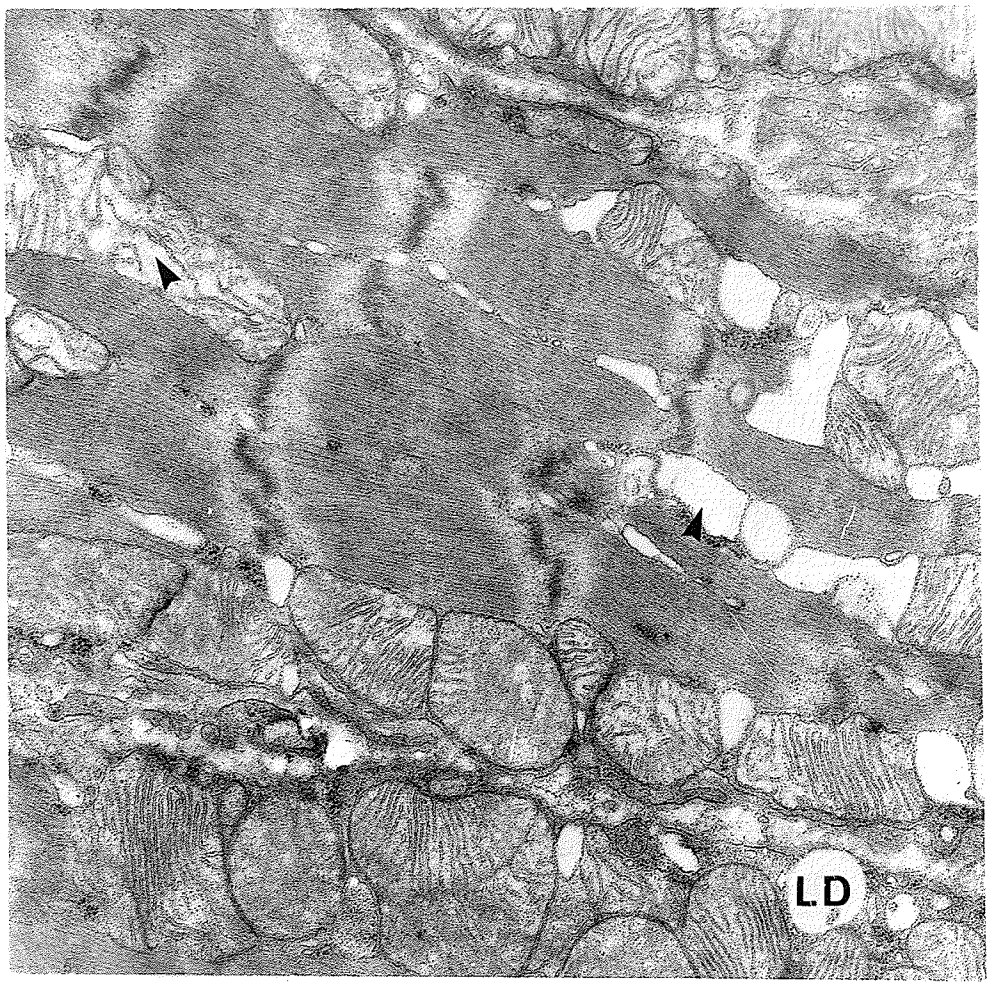


FIGURE 3: Electronmicrograph of the ventricular myocardium of a rat fed 2% cholesterol diet for 12 weeks. Note vacuoles, lipid droplets (LD) and swollen mitochondria. x 16,800.

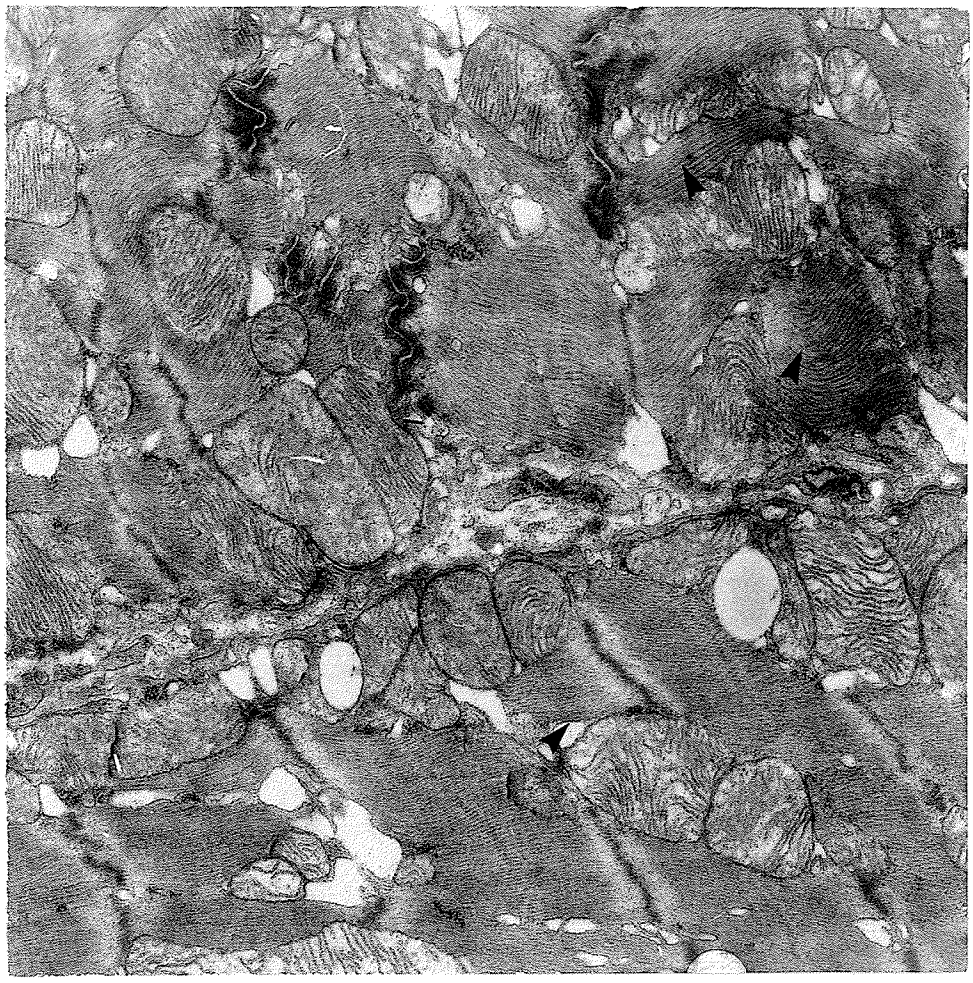


FIGURE 4: Electronmicrograph of the ventricular myocardium of a rat fed a 2% cholesterol diet for 12 weeks. Abnormal arrangement of the myofilaments (arrows) is observed. x 16,500.

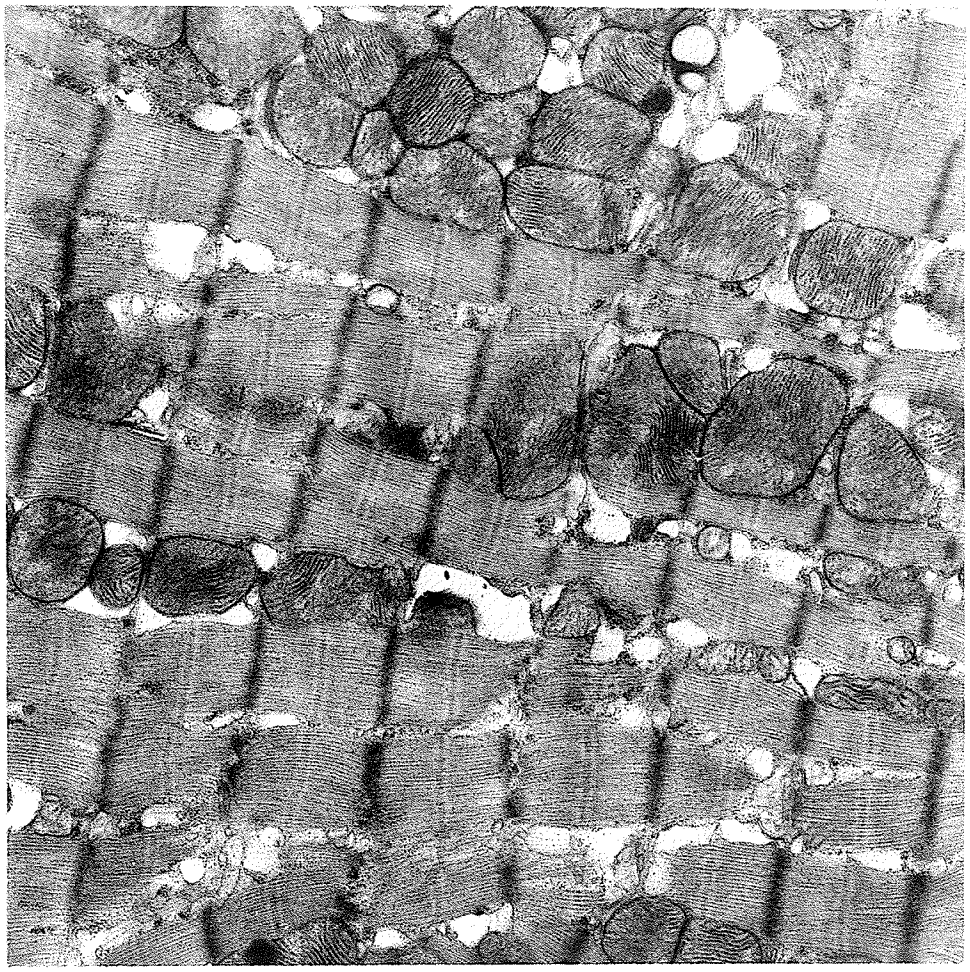


FIGURE 5: Electronmicrograph of the ventricular myocardium of a rat fed a 2% cholesterol diet for 12 weeks. Contracture bands and clumping of large numbers of mitochondria are shown. x 10,500.

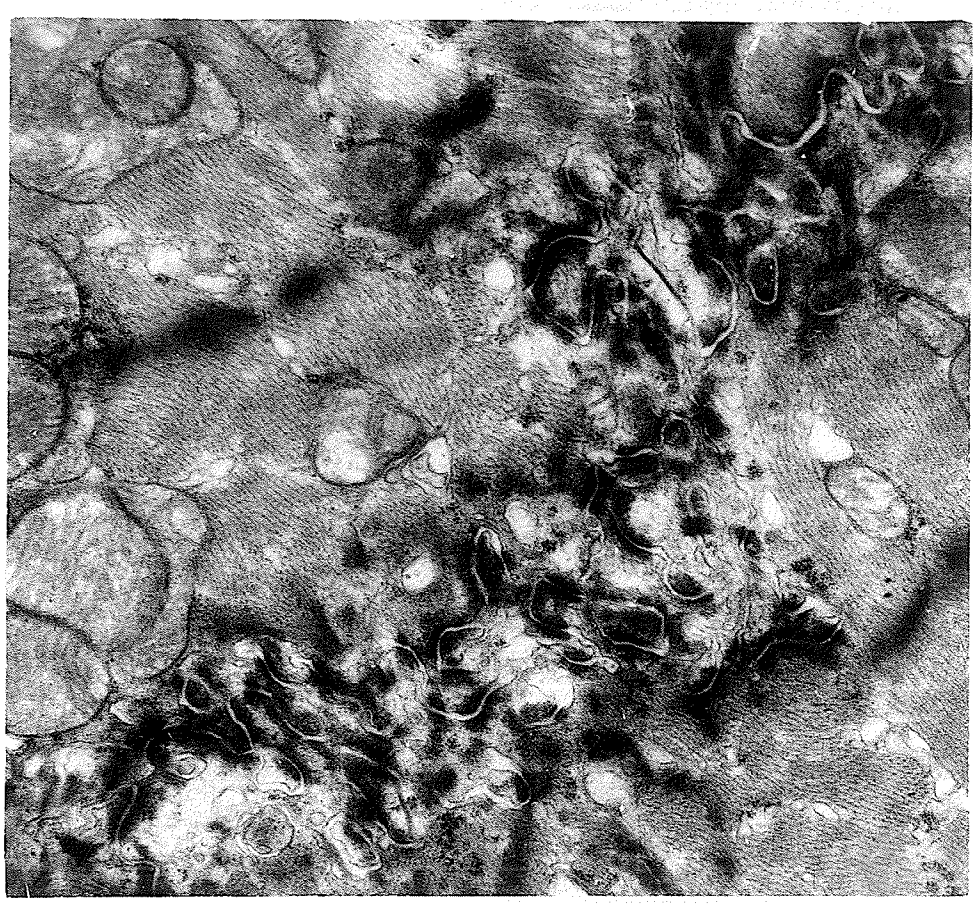


FIGURE 6: Electronmicrograph of the ventricular myocardium of a rat fed a 2% cholesterol diet for 12 weeks. Note the increased complexity of the intercalated disc region. x 26,500

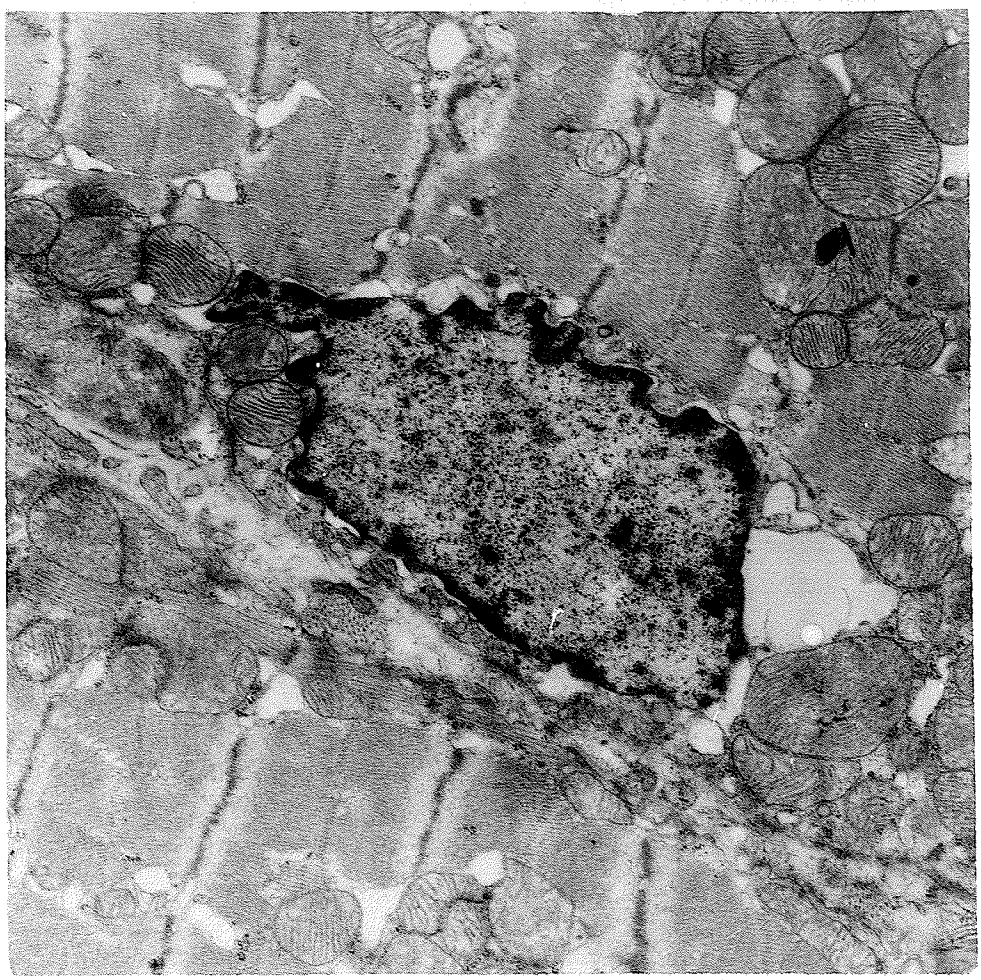


FIGURE 7: Electronmicrograph of the ventricular myocardium of a rat fed a 2% cholesterol diet for 12 weeks. Lobulation of the nuclear membrane is illustrated. x 16,500.

12 weeks leucocyte invasion of myocardial cells was widespread. An example of this invasive process is shown in Figure 8.

By 24 weeks the most prominent feature observed in the myocardium of cholesterol fed animals was lysosomal activity and destruction of mitochondria and other membrane systems by these inclusions. Many features seen at 12 weeks were no longer present, however, some abnormalities persisted. Figure 9 is a representative micrograph from the myocardium of a rat fed a 2% cholesterol diet for 24 weeks. Large numbers of electron dense bodies were found scattered throughout the cells, however, the association was primarily with membrane systems and relatively few of these structures were found associated with the contractile proteins. Figure 10 demonstrates the presence of electron dense lysosomal structures adjacent to the sarcolemma. Blebs of this membrane system were observed in areas where mitochondria were located directly beneath the sarcolemma. In addition, partially disintegrated membranous structures were found in the extracellular space indicating that damaged mitochondria may have been extruded from the cell at this location. Figure 11 shows lysosomal invasion of the sarcoplasmic reticulum and the transverse tubular system The nucleus showed an abnormal appearance (Figure 12) and lysosomes were observed in close association with the nuclear membrane as well as within this organelle . Figure 13 shows some mitochondrial abnormalities present after 24 weeks of cholesterol supplementation. Lysosomes were found in close association with mitochondrial membranes,

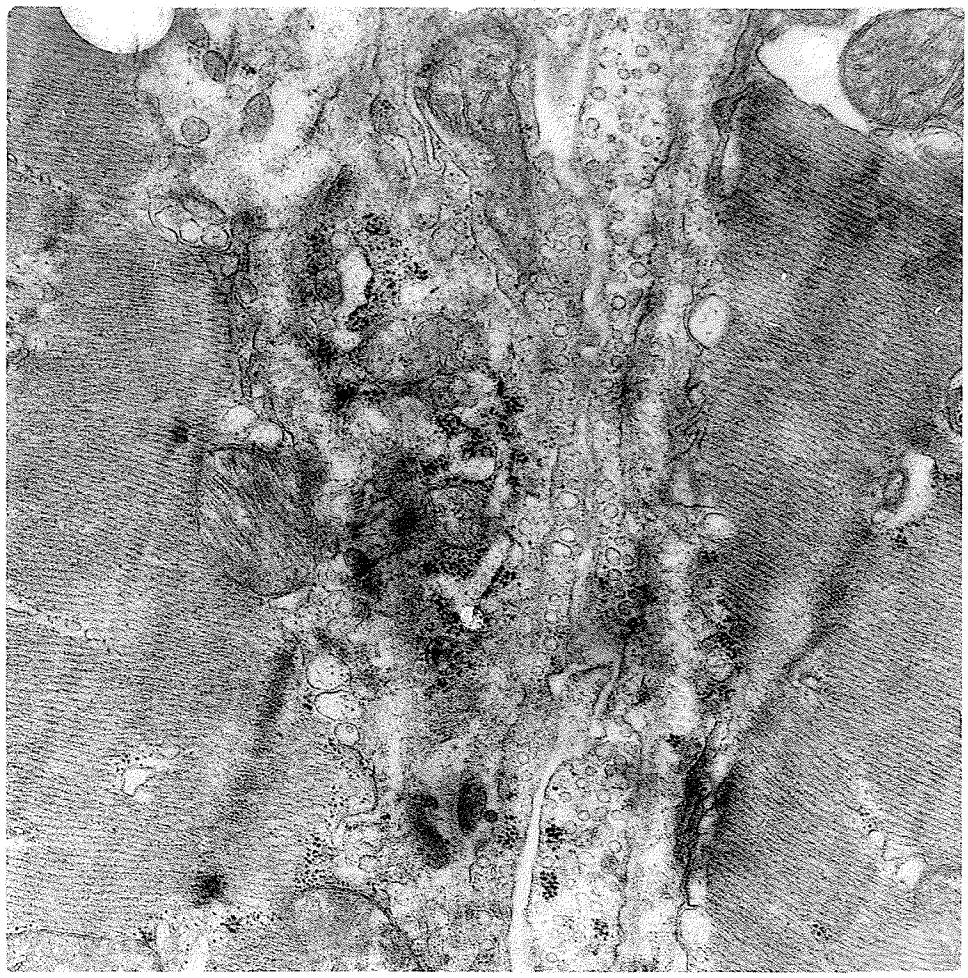


FIGURE 8: Electronmicrograph of the ventricular myocardium of a rat fed a 2% cholesterol diet for 12 weeks. Leucocyte penetration of myocardial cells can be seen. x 24,500.

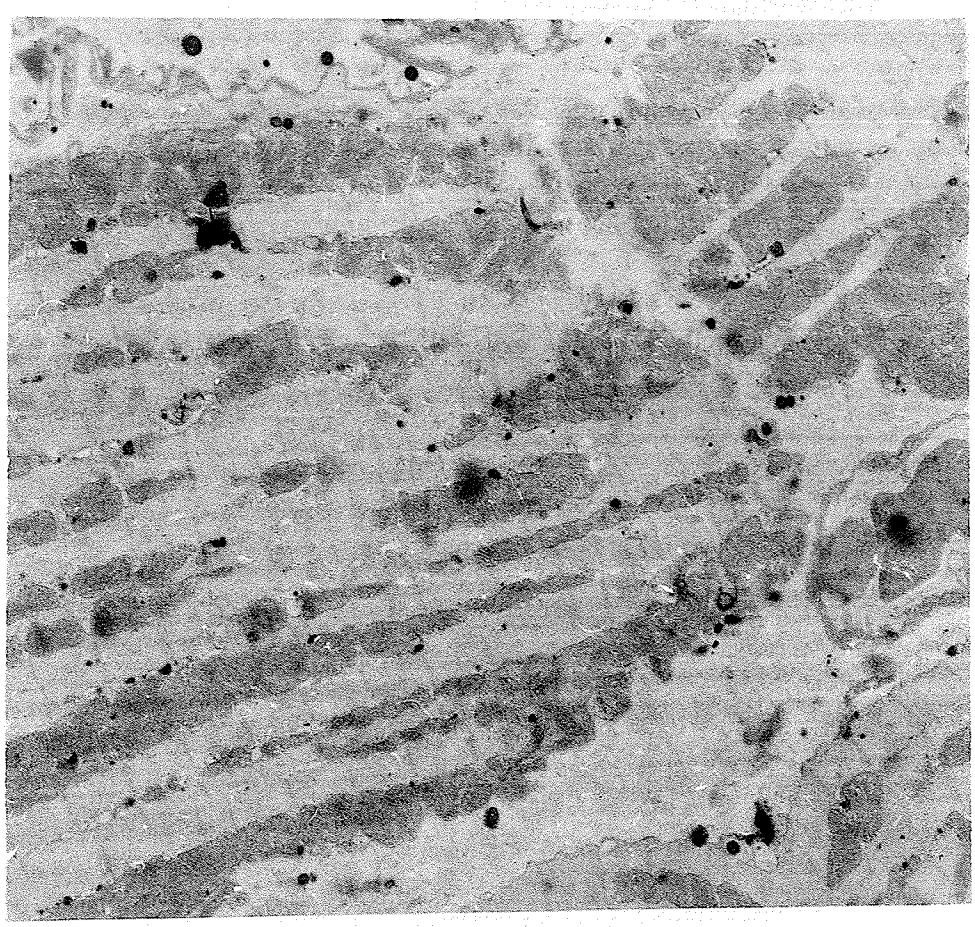
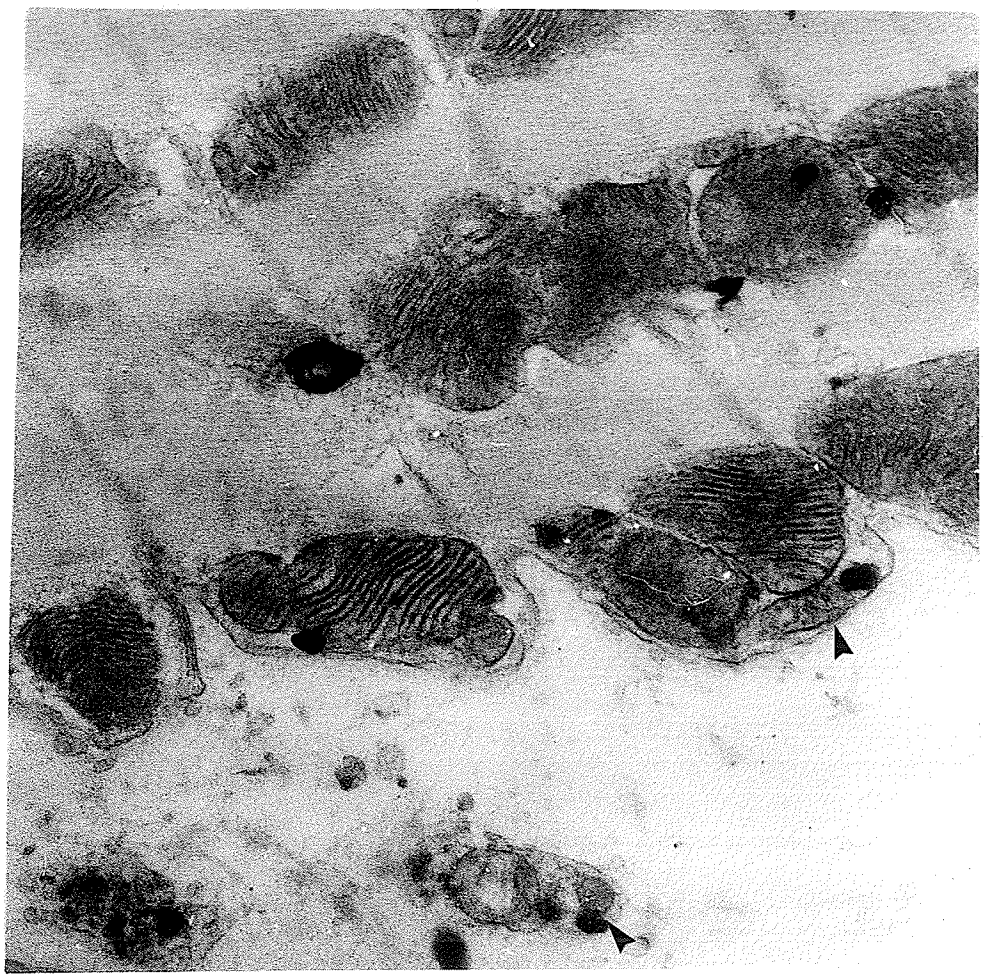


FIGURE 9: Electronmicrograph of the ventricular myocardium of a rat fed a 2% cholesterol diet for 24 weeks (unstained). Note the presence of a large number of electon dense bodies in the myocardial cells.

FIGURE 10: Electronmicrograph of the ventricular myocardium of a rat fed a 2% cholesterol diet for 24 weeks. Blebs of the sarcolemmal membrane as well as membrane bound structures in the extracellular space were noted. x 21,000.



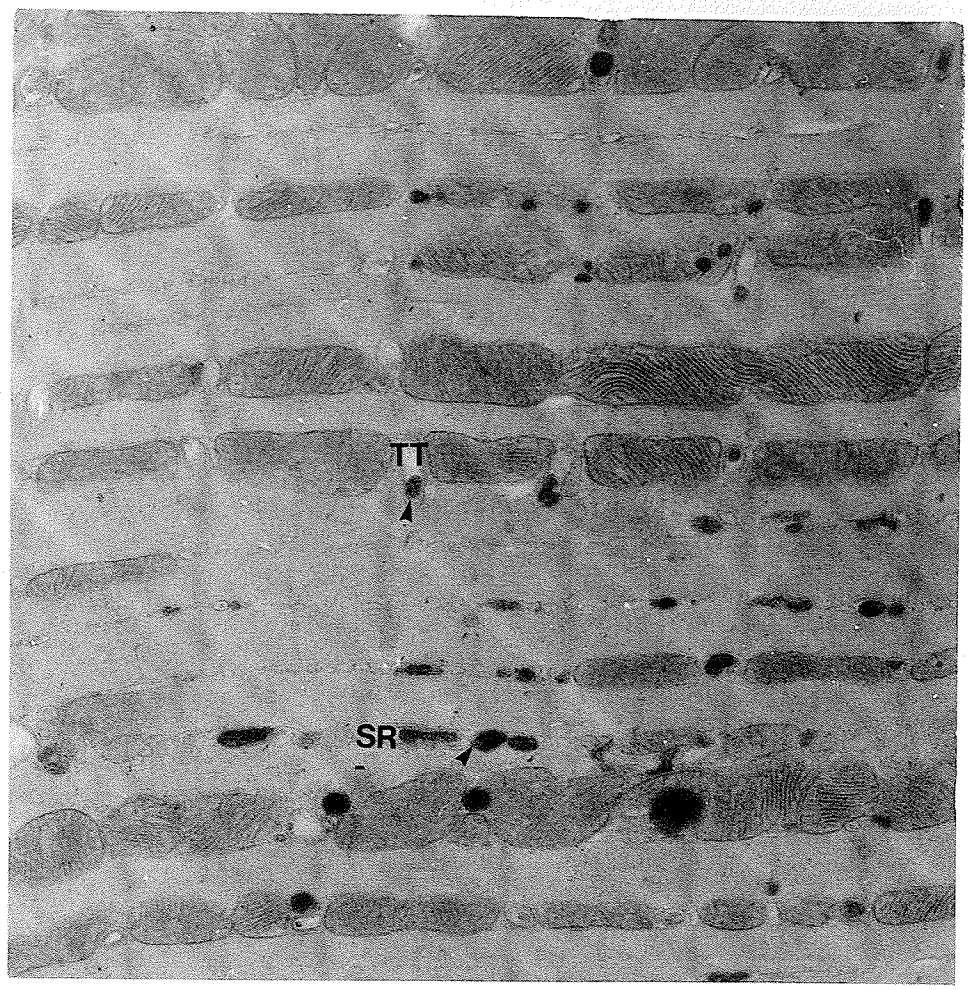


FIGURE 11: Electronmicrograph of the ventricular myocardium of a rat fed a 2% cholesterol diet for 24 weeks. Lysosomes were observed within the sarcoplasmic reticulum (SR) and transverse tubules (TT). x12,800.

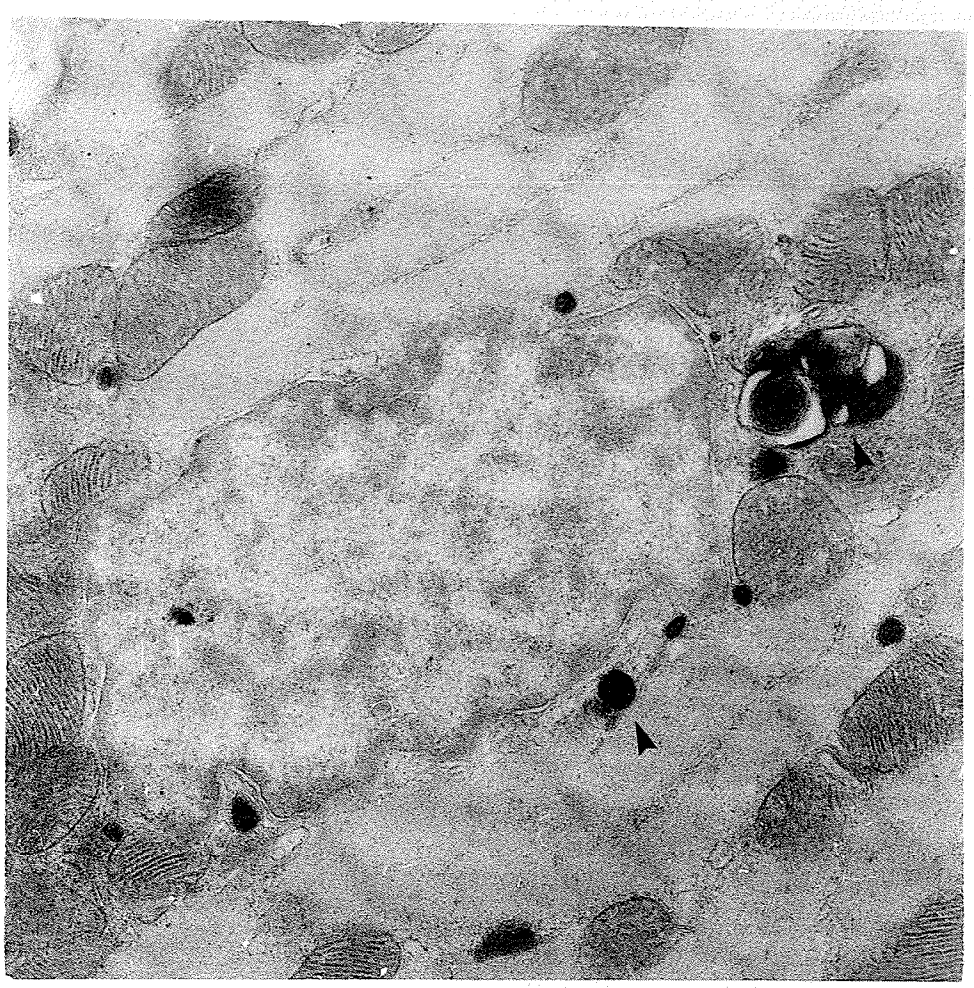


FIGURE 12: Electronmicrograph of the ventricular myocardium of a rat fed a 2% cholesterol diet for 24 weeks. Note the lobulation of the nuclear membrane and the formation of a myelin figure at one nuclear pole (arrows). Also, lysosomes are closely apposed to the nuclear membrane. x20,400.

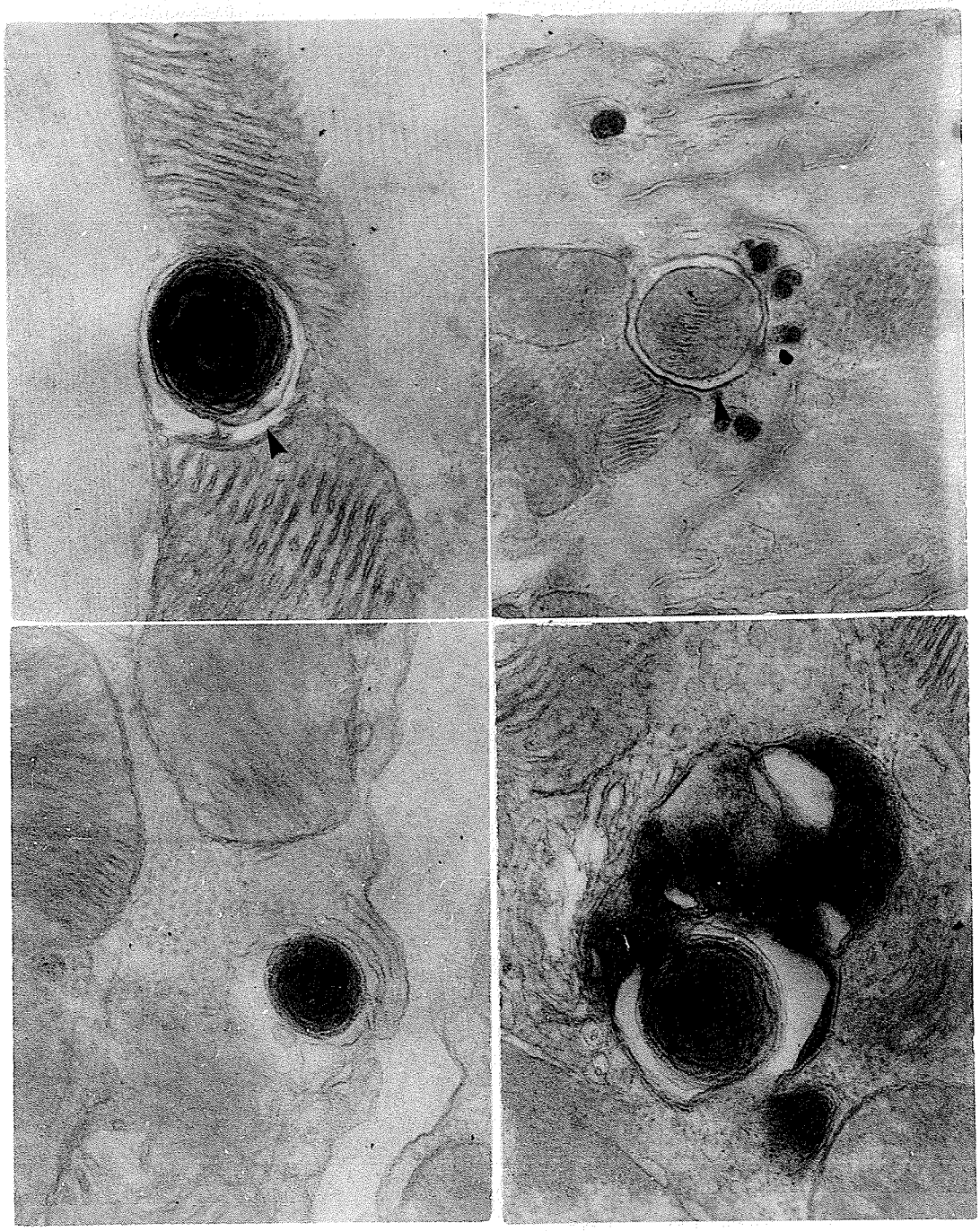


FIGURE 13: Electronmicrograph of the ventricular myocardium of a rat fed a 2% cholesterol diet for 24 weeks. Note destruction of mitochondria by lysosomes. 13 A, C, D x 58,000 13 B x 17,500.

sometimes appearing to invade these structures (Figure 13B). Some mitochondria were enclosed in a membranous sheath (Figure 13 B) which in turn was surrounded by a large number of lysosomes. Elements of the intercalated disc are also present in this micrograph and this structure was often found to have the abnormal appearance described for the 12 week group. Occasionally separation of the low resistance pathways between cells was noted although this was not a frequent observation. Leucocyte activity was considerably less at this time than that seen at 12 weeks and it seemed as if some repair process had occurred in the interim.

The ultrastructural findings described here suggest that disruption and disintegration of many of the elements of the myocardial cells occur in response to high cholesterol diets and that some inflammatory processes occur simultaneously under these conditions.

C. Electrocardiographic Data

Normal heart rate, interval duration and wave amplitude as measured from the electrocardiographs obtained from rats fed either control or cholesterol enriched diets is presented in Tables 2 and 3 whereas representative electrocardiogram tracings are shown in Figure 14-19. Heart rates did not differ between the two diet groups at any time. The PR interval was similar at 6 and 12 weeks in animals on both diet regimens, however, this time was prolonged significantly following 24 weeks in the cholesterol group. QRS duration did not differ at any period irrespective of

Table 2. Measurements from the Normal Electrocardiogram of Rats Fed Control or 2% Cholesterol Supplemented Diets.

Time on Diet (weeks)	Rate (beats/min)		PR Interval (sec)		QRS Interval (sec)		QT Interval (sec)	
	C	CF	C	CF	C	CF	C	CF
6	388 ± 23	400 ± 12	.0450 ± 0.0010	.0428 ± 0.0008	.0233 ± .0014	.0239 ± 0.0040	±.0650 0.0022	±.0733 0.0020
12	405 ± 34	415 ± 22	.0438 ± 0.0024	.0461 ± 0.0015	.0250 ± 0.0022	.0233 ± 0.0010	±.0733 0.0049	±.0908 0.0081
24	360 ± 53	380 ± 0	.0417 ± 0.0017	.0489 ± 0.0034*	.0239 ± 0.0027	.0228 ± 0.0039	±.0837 0.0067	±.0857 0.0059

C = Control rats, CF = Cholesterol fed rats. Each value is a mean ± S.E. of 5 experiments.
* $P < 0.05$.

Table 3. Wave Amplitude From the Normal Electrocardiogram of Rats Fed Control or 2% Cholesterol Supplemented Diets.

Time on Diet (weeks)	P wave (mv)		R wave (mv)		T wave (mv)	
	C	CF	C	CF	C	CF
6	.0575 ± 0.0025	.0800 ± 0.0115*	.2225 ± 0.0202	.2733 ± 0.0696	.0725 ± 0.0111	.0933 ± 0.0176
12	.0700 ± 0.0100	.0625 ± 0.0063	.2125 ± 0.0221	.1775 ± 0.0375	.1050 ± 0.0065	.1475 ± 0.0652
24	.0433 ± 0.0033	.0633 ± 0.0145	.2867 ± 0.0371	.2733 ± 0.0521	.1267 ± 0.0133	.0867 ± 0.0371

Each value is a mean ± S.E. of 5 experiments. C = Control rats, CF = Cholesterol fed rats. * $P < 0.05$.

diet. Although the QT interval was consistently longer in cholesterol fed rats at 6 and 12 weeks (Table 2) this was not statistically significant. Wave amplitudes did not differ between groups except at 6 weeks when the P wave was significantly greater in cholesterol fed animals (Table 3). It should be noted however that an S wave, whose amplitude exceeded that observed in all other groups studied was found in the rats fed cholesterol diets for 12 weeks (data not shown).

Figures 14A, 16A and 18A represent normal ECG (lead II) patterns recorded from rats fed a control diet for 6,12 or 24 weeks respectively. All three exhibit similar characteristics with respect to pattern. The spiked P wave is followed by a shallow downward deflection which represents the Q wave. In this study, this wave was usually small and often absent under normal conditions. The S wave was seen infrequently in these recordings and the T wave was often inscribed before the downstroke of the R wave returned to baseline. The T wave appears to have a fast initial component with a gradual return to baseline. Figure 15A is a representative recording taken from a rat fed a cholesterol rich diet for 6 weeks. A higher take off of the T wave than that observed in control was noted. Inscription of the T wave on the downstroke of the R wave was found frequently in the control group, however, this was pronounced in the cholesterol group and may represent an elevation of the S-T component. In other respects the ECG pattern in this group did not differ from control illustrated in Figure 14A.

Figure 17A is a recording taken from a rat fed 2% cholesterol diet for 12 weeks. A prominent S wave was consistently found in this group. This feature was rudimentary or absent in the control group (Figure 16A). In the cholesterol group it either equaled or exceeded the amplitude of the R wave. The T configuration was a small low amplitude wave, as shown here, or it appeared as a high peaked wave exceeding the amplitude of the preceding R wave. Lead aVR was run on some recordings and a small sharp q and a prominent tall positive deflection were recorded. These findings would be consistent with some form of conduction disturbance in these animals. Figure 19A was taken from an animal fed 2% cholesterol diet for 24 weeks. A prominent P wave was followed by a broad Q wave. T wave configuration was similar to that seen at 12 weeks. The wave was either normal in appearance or a tall broad peak as illustrated here. The prominent S wave present in the 12 week group was absent at 24 weeks. Prolongation of the PR interval in this group (Table 2) suggests a conduction disturbance is probably present at this time.

Control and cholesterol fed groups were administered with an acute dose of isoproterenol (250 mg/kg) and ECG recordings were made 5,15,30 and 60 min following injection. This dosage has been shown to produce 60% mortality and myocardial infarction in this species (Wexler et al,1968). Table 4 illustrates the effect on heart rate response in both diet groups. At 6 weeks the chronotropic response was essentially the same. However, at 12

Table 4. Heart Rate Response to an Acute Dose of Isoproterenol in Rats Fed Control or 2% Cholesterol Supplemented Diets.

Min Post Isoproterenol Injection	6 weeks		12 weeks		24 weeks	
	C	CF	C	CF	C	CF
0	100	100	100	100	100	100
5	122	120	119	101	130	116
15	113	118	115	96	122	109
30	116	115	110	100	102	92
60	119	110	111	96	103	-

Each value is an average of 5 experiments and is represented as % of normal heart rate in each group. C = Control rats, CF = Cholesterol fed rats. Isoproterenol (250 mg/kg) was injected subcutaneously.

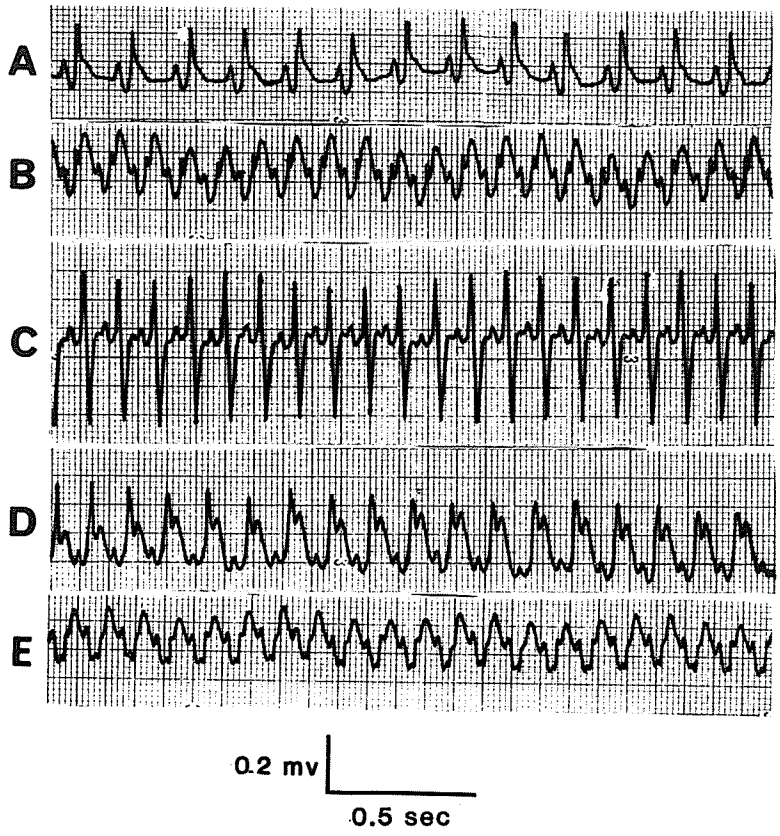


FIGURE 14: Electrocardiogram of a control rat (6 weeks) before (A) isoproterenol (250mg/kg) and 5 (B), 15 (C), 30 (D) and 60 (E) min following drug administration.

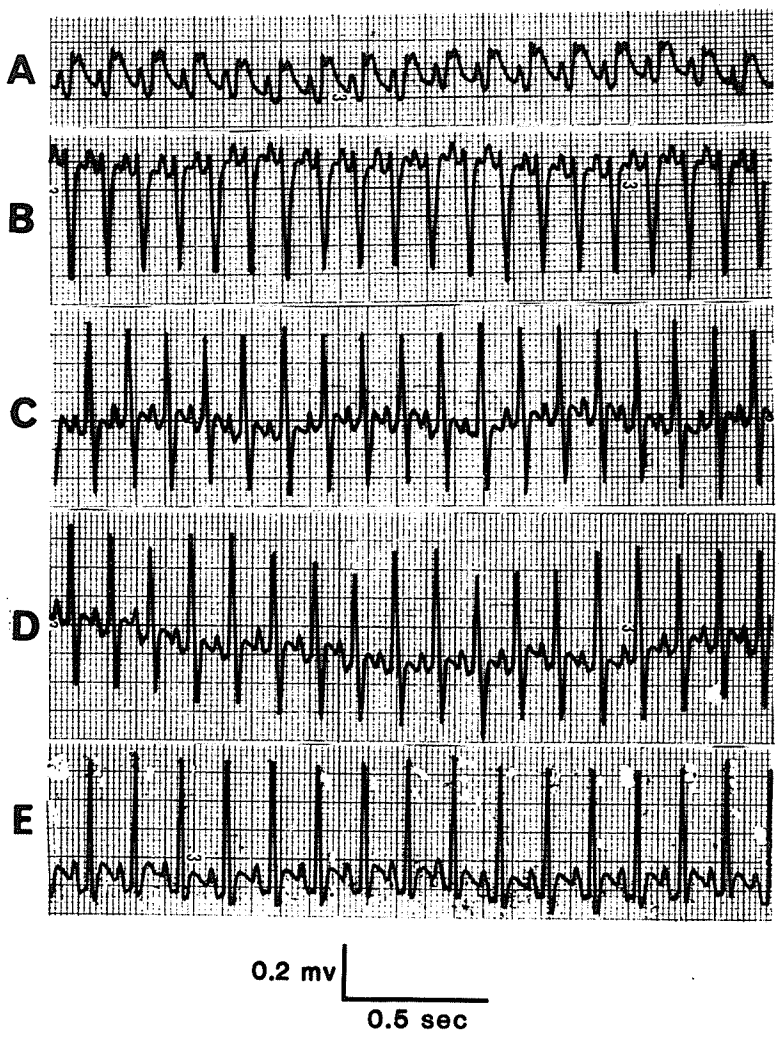


FIGURE 15: Electrocardiogram of a cholesterol fed rat (6 weeks) before (A) isoproterenol (250mg/kg) and 5 (B), 15 (B), 30 (D) and 60 (E) min following drug administration.

and 24 weeks the increase in heart rate was attenuated in the cholesterol fed animals although statistical quantitative analysis of the data was not possible due to the high incidence of rhythm disturbance present in both groups. Figure 14 B-E and Figure 15 B-E show the isoproternenol response in animals fed control or cholesterol diets respectively for a period of 6 weeks. At 5 min post isoproternol injection (Figure 14B) ECG's from control animals displayed a broad Q wave and a high amplitude T wave which in some respects resembled that seen in the cholesterol fed group prior to catecholamine administration. Other responses in some animals in the control group at this period included the appearance of a prominent S wave. At 15 min (Figure 14C) conduction block, arrhythmias and brief runs of ventricular fibrillation were observed in all animals although they did recover normal rhythm at this time, as illustrated here. At 60 min (Figure 14E) the 50% of animals surviving had all regained regular rhythm though abnormalities in ECG pattern persisted. In the cholesterol group (Figure 15B) at 5 min the ECG of all animals studied had a prominent S wave. This pattern persisted at 15 min (Figure 15C) and 30 min (Figure 15D) and evidence of conduction block was seen in some animals at this time. At all times following isoproterenol administration the apparent elevation of the ST component observed initially (Figure 15A) had been converted to an apparent depression of this component. At 60 min (Figure 15E) surviving animals (50%) all displayed abnormalities of ECG pattern and periodic rhythm

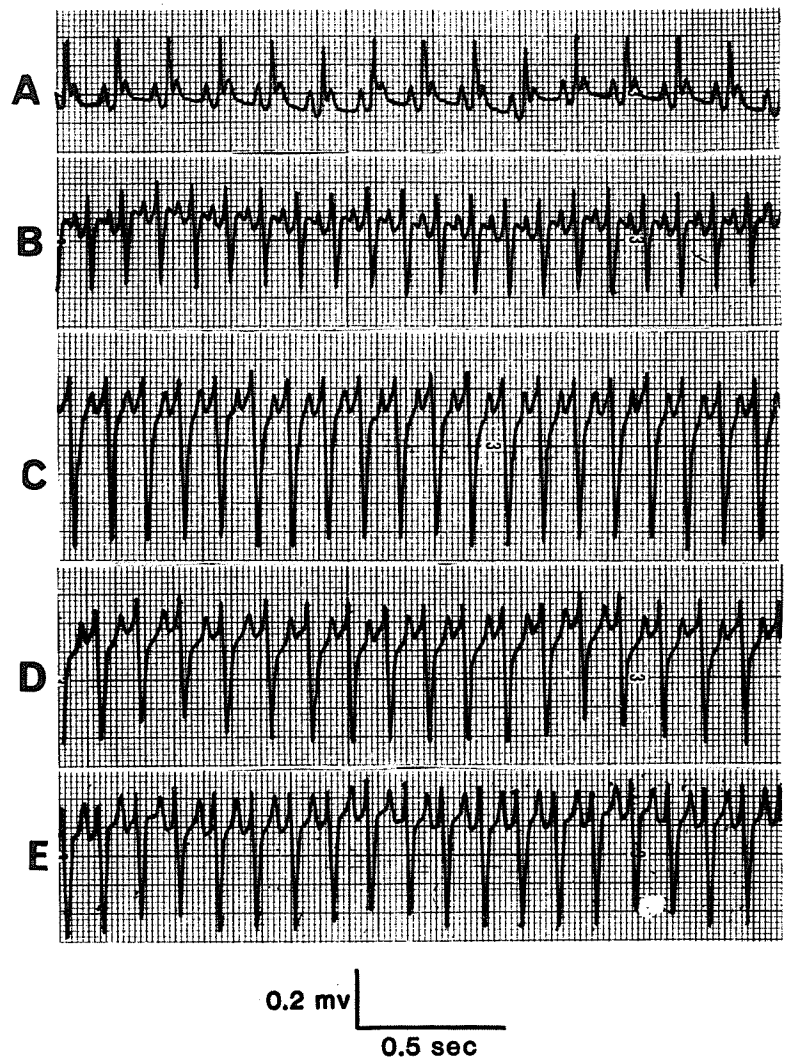


FIGURE 16: Electrocardiogram of a control rat (12 weeks) before (A) isoproterenol (250mg/kg) and 5 (B), 15 (B), 30 (D) and 60 (E) min following drug administration

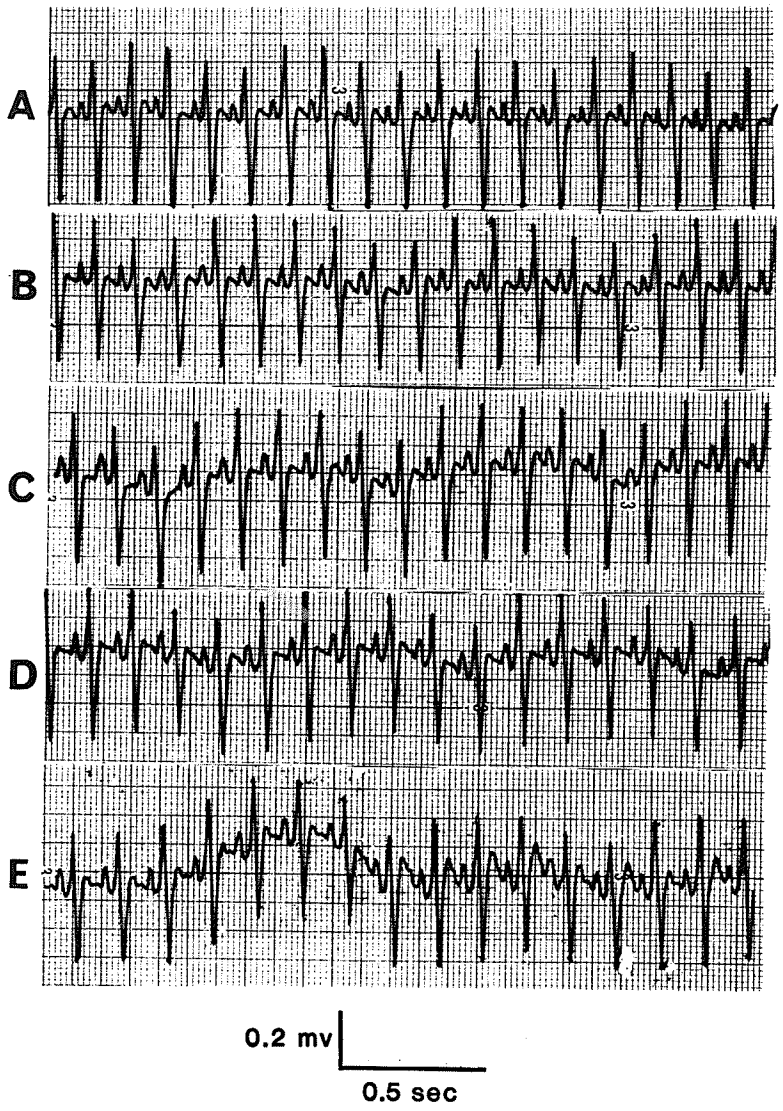


FIGURE 17: Electrocardiogram of a cholesterol fed rat (12 weeks) before (A) isoproternenol (250mg/kg) and 5 (B), 15 (C), 30 (D) and 60 (E) min following drug administration.

disturbances.

Figure 16 B-E and Figure 17 B-E are ECG recordings from animals fed control or cholesterol enriched diets for 12 weeks. At 5 min following isoproterenol injection (Figure 16B) the control group exhibited a prominent S wave which was present in the cholesterol group in the absence of catecholamine (Figure 17B). At 15 min (Figure 16C) this feature was more pronounced and arrhythmias were noted in one animal. Recordings were made using lead aVR and a positive tall deflection was found indicating the pattern irregularities under these conditions resemble those present in the cholesterol group prior to isoproterenol administration as noted earlier. At 30 min (Figure 16D) abnormalities persisted including prominent S waves, QRS changes and atrioventricular block. Patterns were essentially similar at 60 min (Figure 16E) with a survival rate of 50%. Figure 17B is an ECG recording taken from a cholesterol fed rat 5 min post injection. All animals displayed a pattern similar to that observed prior to isoproterenol however, one went into complete heart block and expired. This pattern of deep S waves and low amplitude T waves continued throughout the monitoring period (Figure 17 C-E) with all animals developing occassional arrhythmic activity. Survival at 60 min was 75% in this group.

The response of animals fed a control diet for 24 weeks is shown in Figure 18 B-E. At 5 and 15 min post injection (Figure 18 B-C) this group also developed prominent S waves. At 30 min

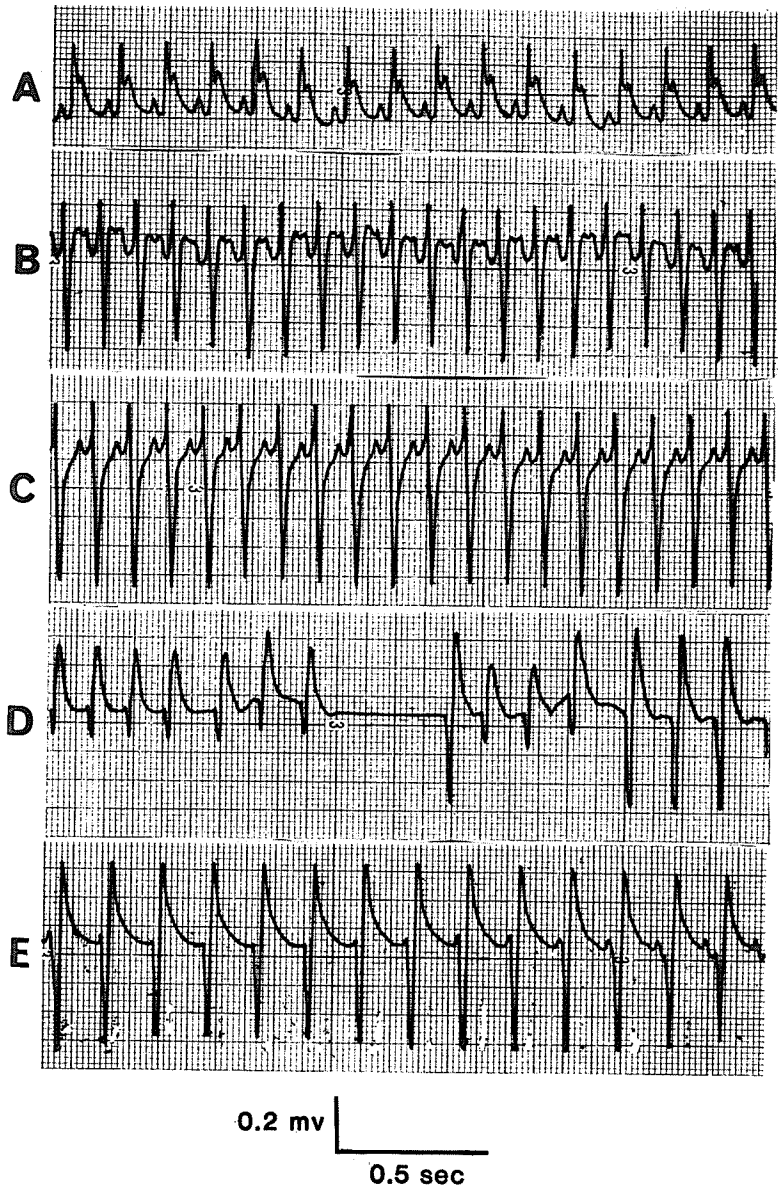


FIGURE 18: Electrocardiogram of a control rat (24 weeks) before (A) isoproterenol (250mg/kg) and 5 (B), 15 (C), 30 (D) and 60 (E) min following drug administration.

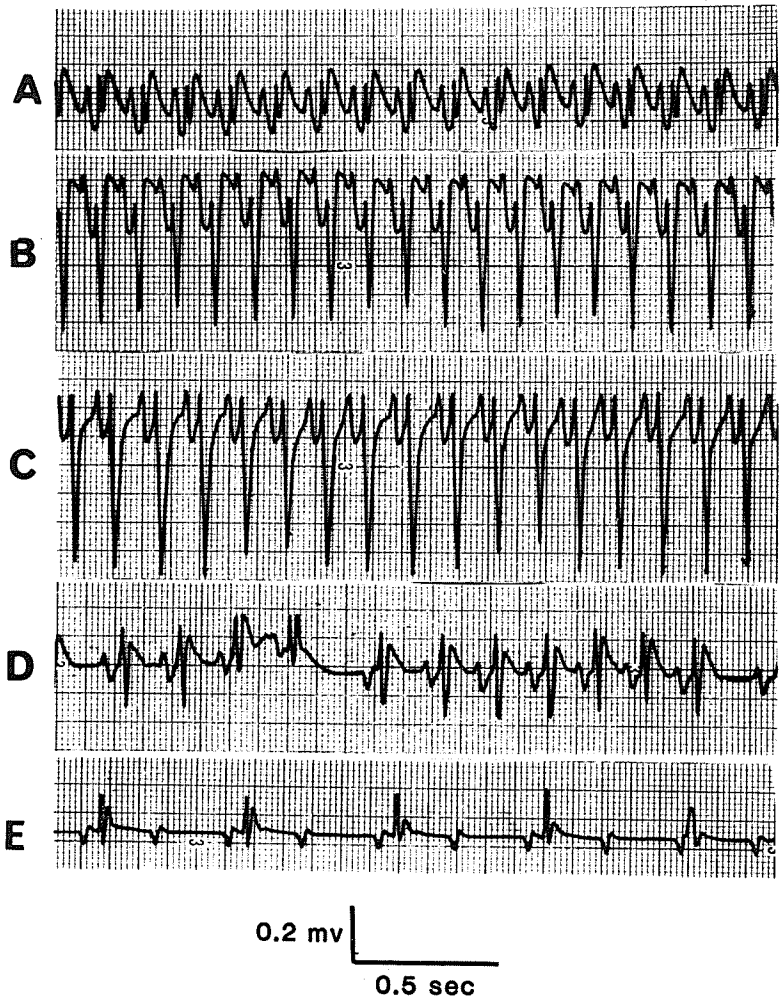


FIGURE 19: Electrocardiogram of a cholesterol fed rat (24 weeks) before (A) isoproterenol (250mg/kg) and 5 (B), 15 (C), 30 (D) and 60 (E) min following drug administration.

(Figure 18D) this pattern as well as arrhythmias and heart block were observed. Arrthymic activity was present in all surviving animals at 60 min (Figure 18E). Figure 19 B-E are recordings taken from a rat fed 24 weeks on the cholesterol diet. At 5 min (Figure 19B) prominent S waves and high amplitude P and T waves were seen. One animal went into complete heart block followed by ventricular fibrillation and death. The same pattern was observed at 15 min (Figure 19C). AT 30 min (Figure 19D) all animals had persistent arrhythmias. This pattern continued at 60 min (Figure 19E) in all surviving animals.

In general, these results indicated some ECG abnormalities observed in the cholesterol fed rats at rest could be produced in control animals by administration of an acute dose of isoproterenol. These findings are consistent with the conclusion of ventricular conduction defects in the treated group. Responses to catecholamine administration were not appreciably different with both diet groups developing a variety of rhythm disturbances and survival rates did not differ between them. However, sudden death (within the first 5 min) was observed only in the cholesterol fed group.

D. Biochemical Data

It has been reported previously that the membrane fraction isolated by the hypotonic shock-LiBr method originates from the myocardial cell membrane and is substantially free of other cellular contaminants such as myofilaments, mitochondria and

nuclei (Takeo et al,1979). Marker enzyme studies of this fraction indicated minimal contamination (3-5%) with other organelles (Anand et al,1977). The preparation consists of vesicles of variable shape and size with a well defined glycocalyx present on the external face of the plasma membrane (Takeo et al,1979; Matsukubo et al,1981). The preparation possesses a high specific activity of plasma membrane marker enzymes (McNamara et al,1974) as well as ATP independent calcium binding activity (Matsukubo et al,1981). In addition, neuraminidase sensitive sialic acid is found associated with the membrane fraction (Takeo et al,1980; Matsukubo et al,1981).

Sarcolemma isolated from rats fed either control or cholesterol diets for 6,12 or 24 weeks was compared with respect to protein yield, sialic acid, phospholipid and cholesterol content and the results are shown in Table 5. Sarcolemmal protein yield did not differ regardless of diet nor did the yield change throughout the feeding period in either group. Similarly, sarcolemmal cholesterol content did not differ significantly between groups at any time. Phospholipid content of membranes decreased over the feeding period in both control and cholesterol fed animals, and this depression was found to be 32% in the treated group at 24 weeks compared to 21% in control. Nevertheless, the moderately elevated (15%) cholesterol levels at 12 weeks in the treated group resulted in a significant increase ($p<0.05$) in the cholesterol/phospholipid molar ratio at this time. It should be noted this ratio was higher in the cholesterol

Table 5. Protein Yield, Phospholipid and Cholesterol Content of Sarcolemma Isolated from Rats Fed Control or 2% Cholesterol Supplemented Diets.

	6 weeks		12 weeks		24 weeks	
	C	CF	C	CF	C	CF
Yield (mg/g)	4.82 ± 0.58	4.41 ± 0.62	3.92 ± 0.38	3.24 ± 0.42	4.30 ± 0.33	4.54 ± 0.49
Sialic Acid (nmoles/mg)	34.6 ± 3.4	46.4 ± 5.1*	36.4 ± 4.9	35.1 ± 2.7	26.2 ± 2.4	26.9 ± 2.2
Sarcolemmal Cholesterol (nmoles/mg)	138.3 ± 4.0	146.0 ± 1.2	118.9 ± 18.8	136.7 ± 18.5	148.2 ± 9.7	136.9 ± 12.0
Sarcolemmal Phospholipid (nmoles/mg)	298.5 ± 8.5	303.8 ± 10.8	241.4 ± 29.0	244.6 ± 13.4	235.6 ± 10.4	206.8 ± 15.1
Cholesterol Phospholipid (molar ratio)	0.463 ± 0.002	0.482 ± 0.014	0.495 ± 0.055	0.555 ± 0.054*	0.629 ± 0.027	0.660 ± 0.017

Each value is a mean ± S.E. of 5-10 experiments. C = Control rats, CF = Cholesterol fed rats. * $P < 0.05$.

fed group at all times, but this was not significant at 6 or 24 weeks. By 24 weeks both diet groups experienced a significant increase in this ratio when compared to values obtained at 6 and 12 weeks. This probably is a result of the fall in phospholipid content of the sarcolemma with time as cholesterol values did not change appreciably.

Sialic acid content of sarcolemma was significantly greater ($p<0.05$) in the cholesterol group at 6 weeks. This parameter did not differ between groups at either 12 or 24 weeks. Decreased content of the amino sugar was observed in both groups by 24 weeks and in this regard resembles the decrease in phospholipid content with age described earlier.

Phospholipid composition of the sarcolemma was determined in all groups and these data are presented in Table 6. No significant changes were observed either within or between diet groups at any time.

The effect of diet on the calcium binding capacity of the sarcolemma is shown in Figure 20. After six weeks there were no differences between diet goups in binding in the presence of 0.05 mM $CaCl_2$ (Figure 20). However, binding was significantly greater ($p<0.01$) in the presence of 1.25 mM $CaCl_2$ in the cholesterol group, when compared to control. After 12 weeks on respective diets, sarcolemma isolated from hearts of cholesterol fed rats showed a significantly greater calcium binding capacity at 0.05 mM $CaCl_2$ than in control. Binding in the presence of 1.25 mM

Table 6. Phospholipid Composition of Rat Heart Sarcolemma from Animals Fed Control and 2% Cholesterol Rich Diets.

Phospholipid	6 weeks		12 weeks		24 weeks	
	C	CF	C	CF	C	CF
Phosphatidylcholine	36.6	31.8	34.2	31.6	32.6	35.9
Lysophosphatidycholine	2.5	3.6	1.6	3.2	3.4	3.4
Phosphatidylethanolamine	31.4	31.6	31.0	26.8	29.7	33.1
Phosphatidyserine	5.8	6.6	6.0	7.5	7.6	6.1
Phosphatidyinositol	3.1	4.3	3.2	4.8	3.7	3.1
diPhosphatidylglycerol	10.5	10.1	7.5	10.6	10.3	8.3
Phosphatidic acid	1.5	2.0	2.2	2.5	1.7	1.6
Sphingomyelin	5.5	6.0	11.2	8.9	7.6	6.5
Other Lipids	3.2	4.1	3.1	4.3	3.4	2.0

The results are expressed as percentage of total phospholipids and are average of 4 experiments. C = Control rats, CF = Cholesterol fed rats.

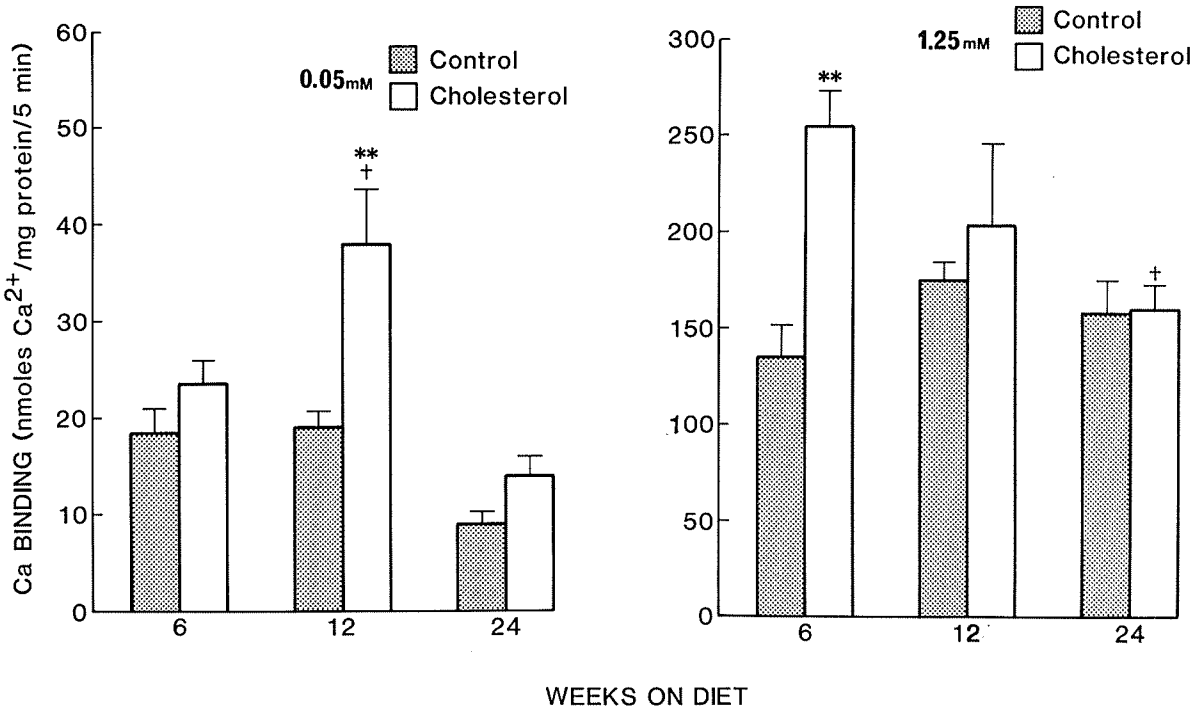


FIGURE 20: Calcium binding of the isolated rat heart sarcolemma from rats fed either control or 2% cholesterol diets for 6, 12 or 24 weeks.

$CaCl_2$ did not differ between the two groups at this time. By 24 weeks all differences in calcium binding between diet groups had been abolished. At both concentrations of calcium, binding was found to be independent of length of feeding in the control group. In the cholesterol group, in contrast, binding was significantly greater at 12 weeks in the presence of 0.05 mM $CaCl_2$ than at any other time. As well, there was a significant decrease in this activity, in the presence of 1.25 mM $CaCl_2$, by 24 weeks.

The effects of diet on the activity of Mg^{2+}ATPase, Na^+-K^+ATPase and ouabain sensitive ATPase were studied in rats fed control or cholesterol diets for 6,12 or 24 weeks. The results are shown in Table 7. Sarcolemma isolated from hearts of animals fed control diets exhibited no differences in Mg^{2+}ATPase activity regardless of the time of feeding. In contrast, the enzyme activity in sarcolemmal membranes isolated from hearts of animals fed cholesterol diets exhibited a significant dependence on the length of the feeding period. Activity at 12 weeks was found to be significantly greater than that observed at either 6 or 24 weeks. Mg^{2+}ATPase activity did not differ between diet groups at 6 weeks, however, by 12 weeks on the respective diets the activity in membranes from the cholesterol group was significantly greater than control. By 24 weeks, Mg^{2+}ATPase activity of sarcolemma from cholesterol fed animals was significantly less than in matched controls and had decreased 50% from values observed after 12 weeks. Na^+-K^+ATPase was stimulated

Table 7. Effect of 2% Cholesterol Diet on Rat Sarcolemmal Mg^{2+} ATPase and Na^{+}-K^{+} ATPase.

Time on Diet (weeks)	Enzyme Activity (μmoles Pi/mg protein/hr)					
	Mg^{2+} ATPase		Na^{+}-K^{+} ATPase		Ouabain Sensitivity (%)	
	C	CF	C	CF	C	CF
6	36.21 ± 2.40	33.47 ± 3.30	16.04 ± 1.48	23.37 ± 1.76**	74.3 ± 12.2	49.0 ± 8.2
12	39.44 ± 3.42	51.10 ± 4.23*	17.29 ± 1.44	20.81 ± 1.43*	76.2 ± 7.4	65.2 ± 1.6
24	40.10 ± 2.81	25.00 ± 2.88*	14.91 ± 1.24	12.75 ± 0.63	67.7 ± 4.1	53.0 ± 6.5

Each value is the mean ± S.E.M. of 5-10 experiments. C = Control rats, CF = Cholesterol fed rats.
** $P < 0.01$, * $P < 0.05$.

in sarcolemmal membranes from cholesterol fed rats of the 6 week group when compared to matched controls; the ouabain sensitivity of Na^+-K^+ATPase activity was 74% in control as compared to 49% in sarcolemma from cholesterol fed animals. At 12 weeks on the two diets Na^+-K^+ATPase remained elevated in membranes of the cholesterol group in comparison to controls; the ouabain sensitivity was 76% in control as compared to 65% in the cholesterol group. By 24 weeks, the activity of Na^+-K^+ ATPase in membranes of the cholesterol fed animals had fallen below that observed in controls and was 45% less than that recorded at 6 weeks and 39% less than the same activity at 12 weeks. Similarly, the ouabain sensitivity at 24 weeks was found to be 67% in controls and 53% in membranes from the cholesterol group.

The effects of diet on the activity of Ca^{2+}ATPase are shown in Figure 21. The activity of this enzyme in the presence of 1.25 mM $CaCl_2$ was dependent upon the length of the time on diet in both the control and cholesterol fed groups. In controls there was a significant increase in enzyme activity between 6 and 12 weeks. The activity did not differ at 24 weeks in this group. Sarcolemma isolated from cholesterol fed rats displayed a significantly greater Ca^{2+}ATPase activity at 12 weeks than that observed at either 6 or 24 weeks on diet and this activity also differed significantly from that observed in control at this time. In the presence of 4.00 mM $CaCl_2$, Ca^{2+}ATPase activity did not vary with time of feeding in the control group. In the sarcolemma from the cholesterol fed animals, however, this

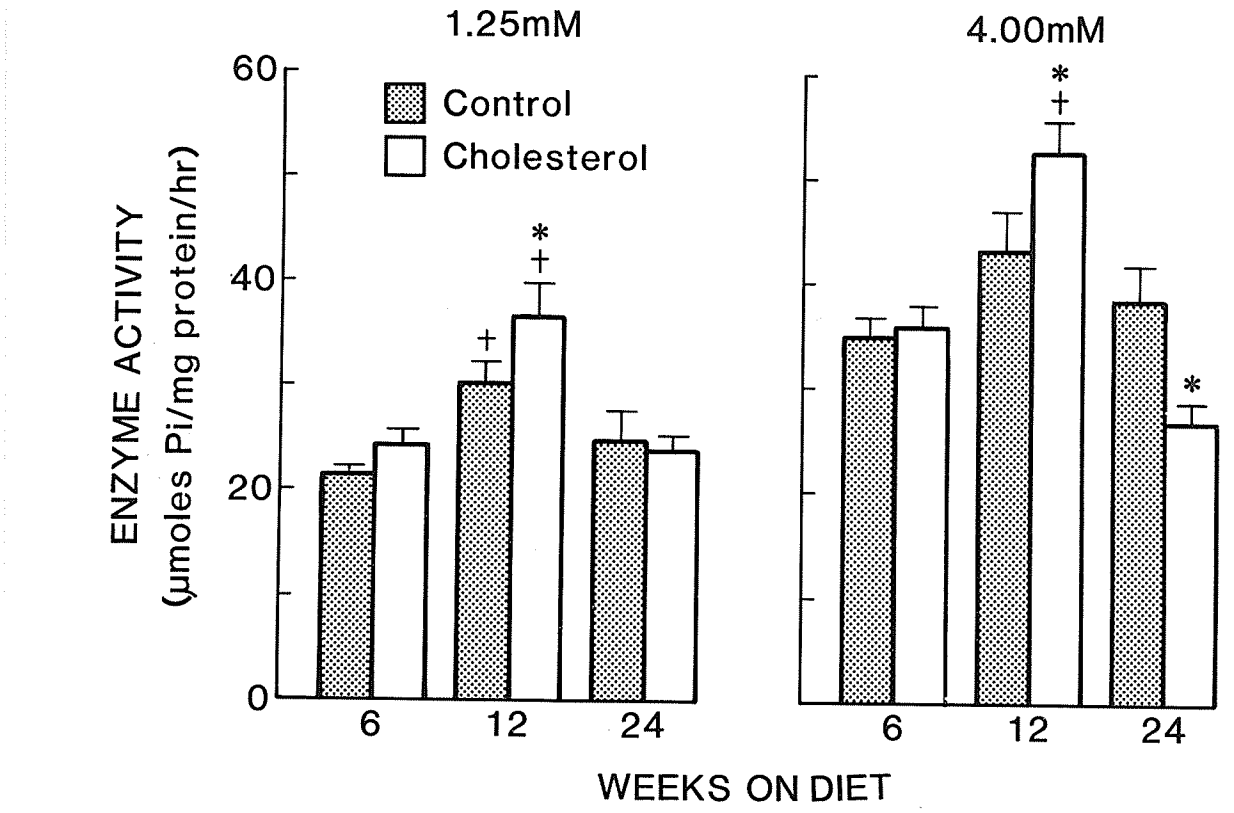


FIGURE 21: Effect of a 2% cholesterol diet on the Ca^{2+} ATPase activity of isolated rat heart sarcolemma.

Table 8. Effect of Concanavalin A on the Activity of Isolated Rat Heart Sarcolemmal ATPase.

Concanavalin A Conc (µg/mg protein)	Enzyme Activity (% of control)			
	Mg^{2+} ATPase	$Na^{+} + K^{+}$ ATPase	Ca^{2+} ATPase (1.25 mM)	Ca^{2+} ATPase (4.00 mM)
10	101 ± 3	95 ± 7	100	101
100	112 ± 4*	106 ± 10	121 ± 4**	116 ± 4**
1000	124 ± 4**	112 ± 10	123 ± 5**	124 ± 4**
α-mm[a] + 1000	99 ± 3	102 ± 1	98 ± 2	100 ± 3

a - α-mm - α methyl-d-mannoside (50 mM). Each value is a mean ± S.E.M. of 5 experiments. * $P < 0.05$, **$P < 0.01$.

activity was significantly greater at 12 weeks than at 6 or 24 weeks on diet. In addition, Ca^{2+}ATPase activity was significantly stimulated in sarcolemma of the 12 week group when compared to control and this activity declined by 50% at 24 weeks at which time it was significantly less than the control value.

To determine if changes in fluidity as suggested by the increase in the cholesterol/phospholipid ratio, might underlie the changes observed for sarcolemmal enzymes, the effect of concanavalin A on the activity of these proteins was studied. The results are presented in Table 8. At concentrations of concanavalin A of 100 ug/mg protein or greater, the activities of Mg^{2+}ATPase and Ca^{2+}ATPase were significantly stimulated and this stimulation was of the same magnitude as that observed for these enzymes in sarcolemma from cholesterol fed rats at 12 weeks. In contrast, the Na^{+} -K^{+}ATPase was not changed by treatment of membranes with concanavalin A. Inclusion of alpha-methyl-d-mannoside in the medium completely abolished stimulation and it was concluded the observed stimulation was attributable to binding of the lectin to the sarcolemma.

E. Heart Function Data

Figure 22 shows the spontaneous rate of isolated hearts from the 12 week control and cholesterol groups in response to glucose deprivation and hypoxia. During normoxia, heart rates were stable in both control and cholesterol groups, irrespective of the presence or absence of substrate. Similarly, rate did not differ

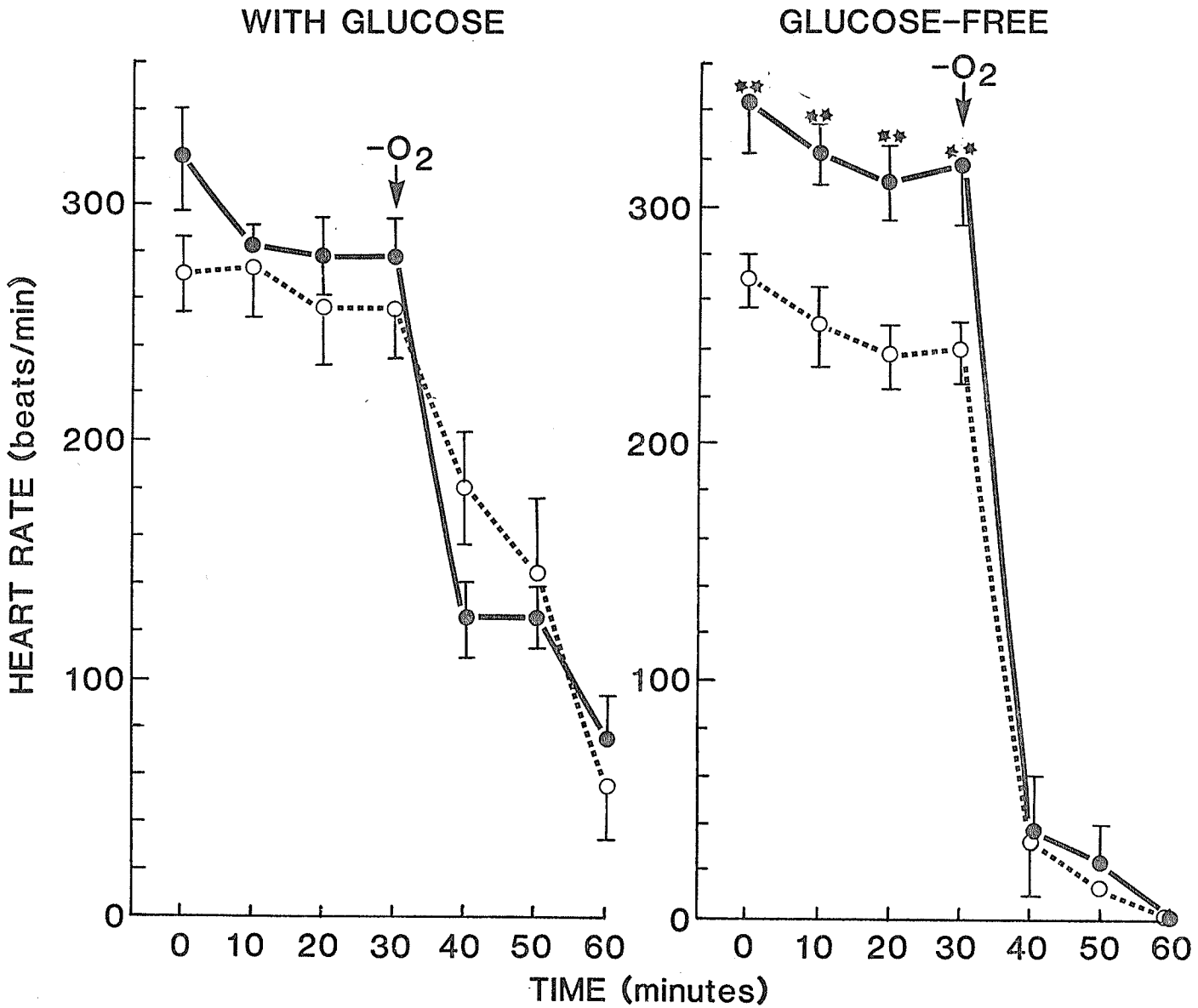


FIGURE:22 Spontaneous rate of hearts of rats fed either a control or 2% cholesterol diet in response to substrate deprivation and hypoxia. Points represent the mean $\pm$ S.E.M. of 5 experiments. ** $p < 0.01$
Closed circles-control, open circles-cholesterol fed

between the two diet groups in the presence of glucose. In glucose-free buffer, however, heart rates of the control group were significantly higher than in the cholesterol group and this difference was maintained throughout the normoxic period. Hypoxia caused a rapid decline in heart rate in all groups studied and absence of substrate significantly exacerbated this effect throughout hypoxia in controls and following 10 and 20 min in the cholesterol group. There was no significant effect of substrate at 30 min of hypoxia in this group.

Figure 23 depicts the effect of diet on tension development by isolated rat hearts. During normoxia there was a significant increase in developed tension at 20 and 30 min in the control group and at all times in the cholesterol group in the presence of substrate when compared to their glucose-free cohorts. While there was no difference in force development between the two groups in the absence of glucose, in the presence of substrate, the cholesterol group exhibited significantly higher values. Induction of hypoxia resulted in a comparable rapid decline in contractile force in the control group irrespective of the presence or absence of glucose. A similar pattern was observed for the cholesterol group, however, force development was significantly greater during the initial phase of hypoxia (10 min) in the presence of substrate. Force development during hypoxia did not differ between the diet groups in the presence or absence of glucose.

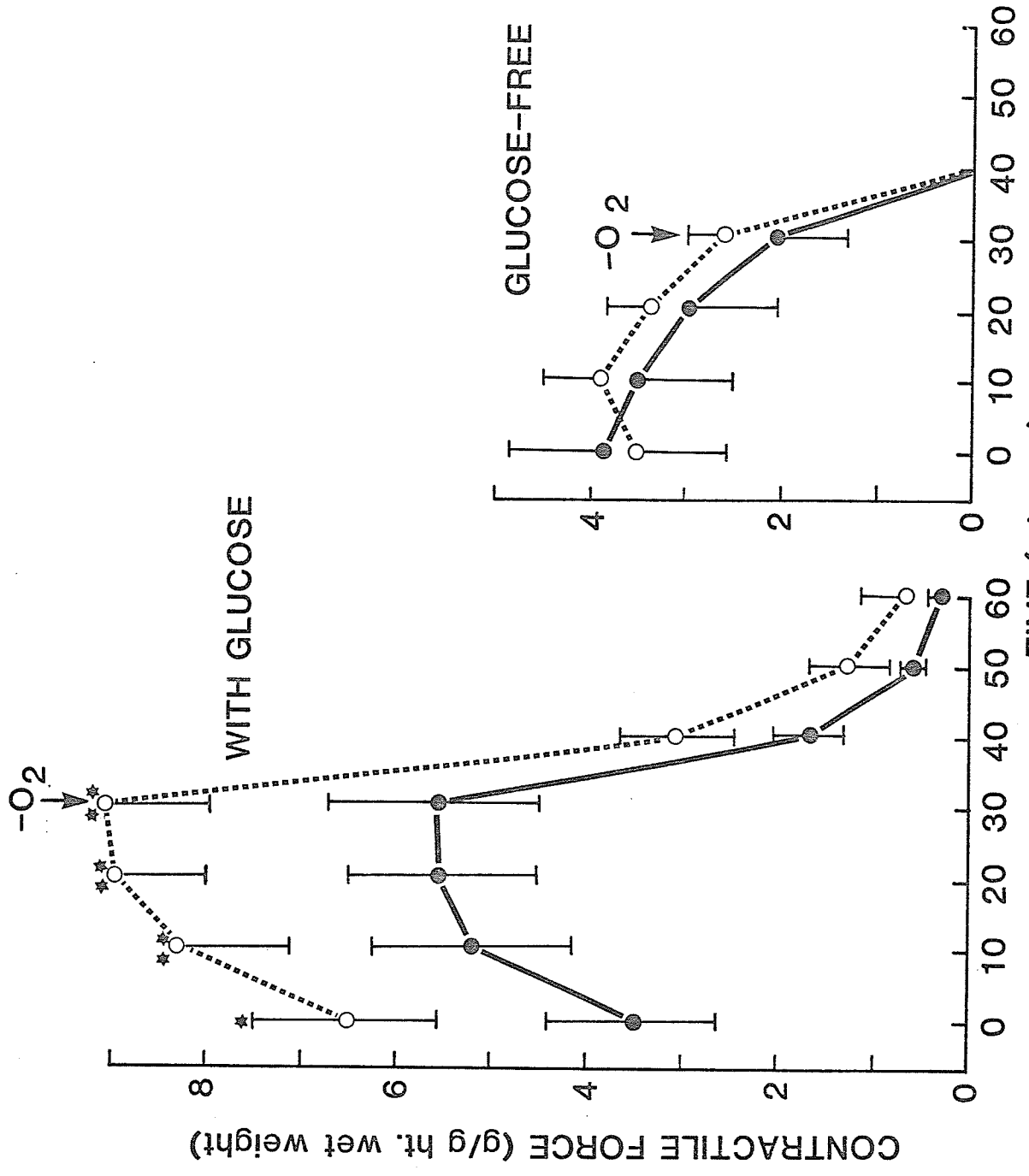


FIGURE 23: Contractile force of isolated hearts. Description as for Figure 22.

Figure 24 represents the influence of diet on time to maximal force generation in response to glucose deprivation and hypoxia. Glucose-free normoxic perfusion caused a significant decrease in the time to peak tension in the control group. In contrast, there was no effect of substrate on this parameter in the cholesterol group and time to peak tension was significantly prolonged when compared to controls. This difference was not observed in the presence of glucose. Induction of hypoxia in the presence of glucose decreased the time to peak tension in the controls, however, this effect was significant only at 30 min of hypoxia. Hypoxia resulted in a significant decrease in the time to peak tension at all times in the cholesterol group under these conditions. Time to peak tension did not differ between the two groups during hypoxia, however.

Figure 25 depicts the resting tension of isolated rat hearts from control and cholesterol groups. During normoxia there was no effect of substrate on this parameter in either diet group nor did responses differ between the two groups. Induction of hypoxia in the absence of glucose resulted in a significant increase in resting tension in both groups and these increases did not differ between them at 10 or 20 min of hypoxia. Following 30 min of hypoxia, however, the increase in resting tension was significantly greater in the cholesterol group. In the presence of substrate there was no significant change in resting tension in either group in response to hypoxic challenge.

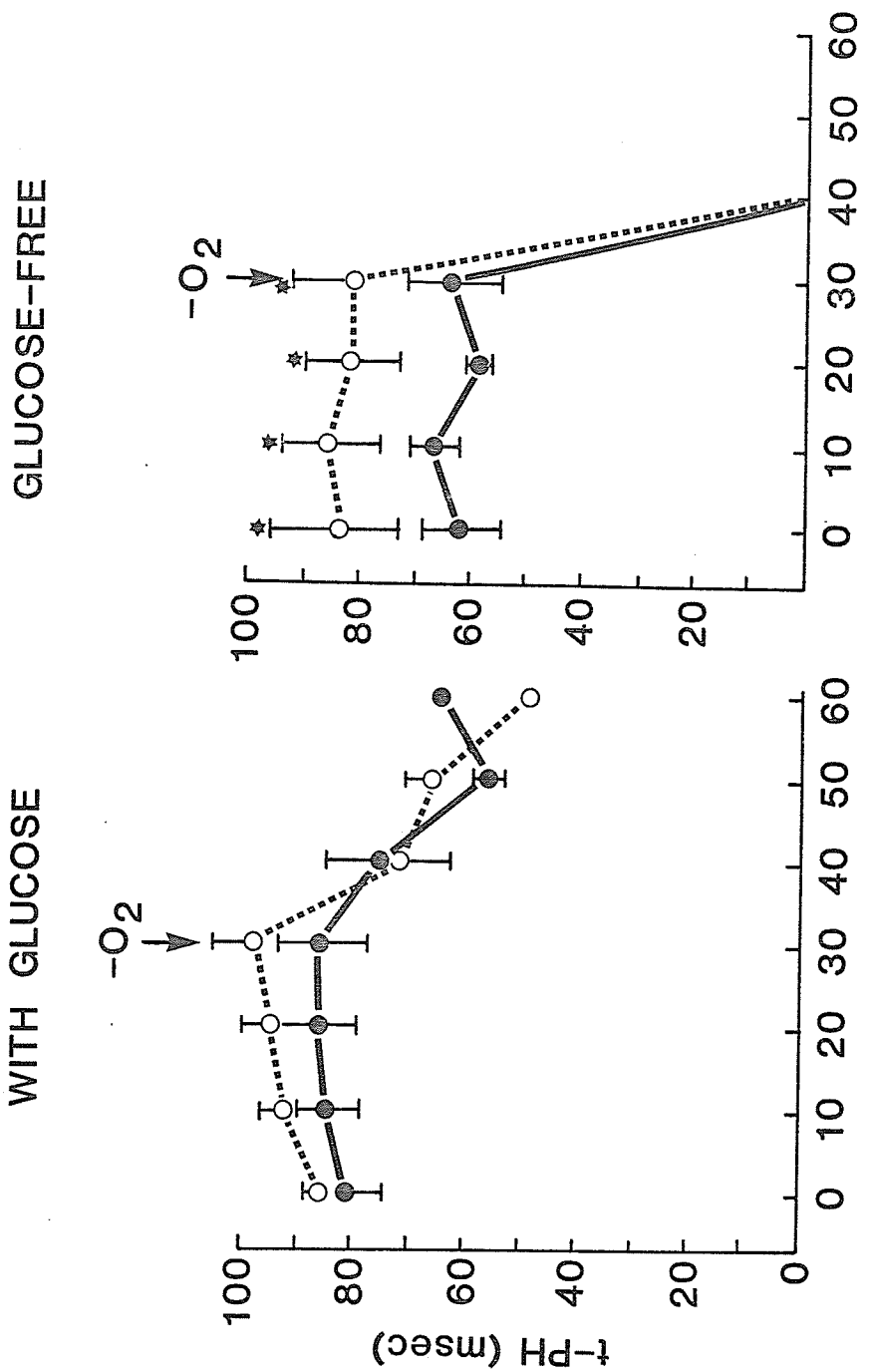


FIGURE 24: Time to peak height of developed tension of isolated hearts. Description as for Figure 22.

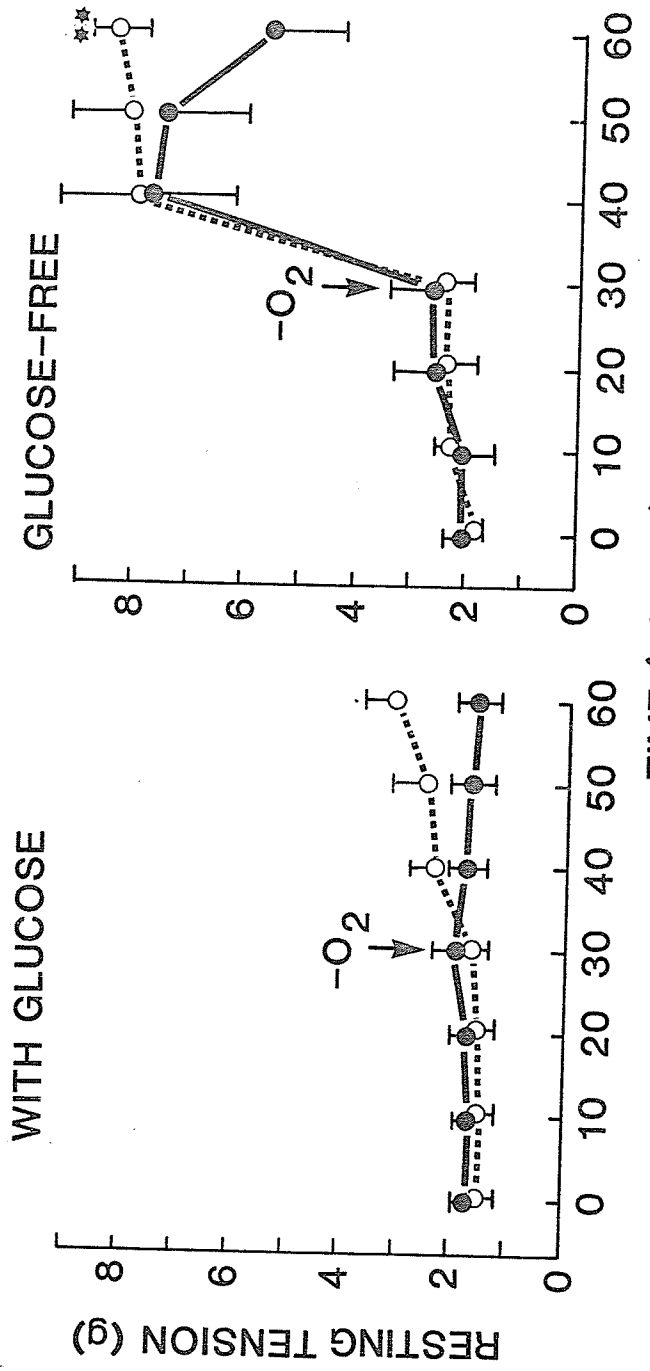


FIGURE 25: Resting tension of isolated hearts. Description as for Figure 22.

Figure 26 shows the coronary perfusion pressure of isolated rat hearts from control and cholesterol groups. During normoxia there were no significant effects of substrate in either diet group nor did values for coronary pressure differ between them. Induction of hypoxia in the control group produced a significant increase in coronary pressure at 20 and 30 min in the absence of glucose, however, this effect was not seen in its presence. The cholesterol group displayed no change in coronary perfusion pressure during hypoxia regardless of the presence or absence of substrate. Therefore there was a significant difference in the hypoxic response in glucose free perfusions between the two diet groups following 20 and 30 min of hypoxia.

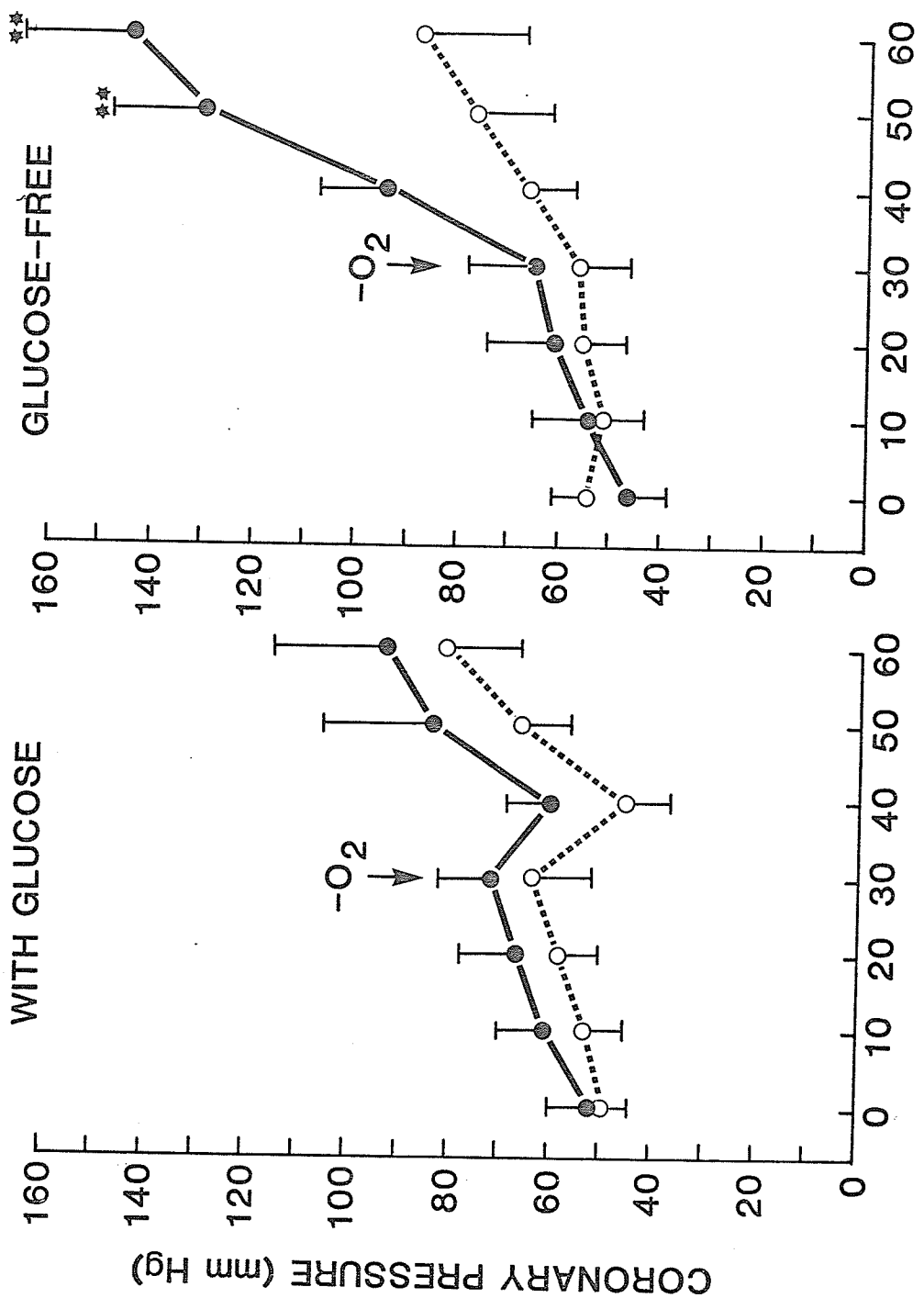


FIGURE 26: Coronary perfusion pressure of isolated hearts. Description as for Figure 22.

V. DISCUSSION

Cholesterol metabolism has been studied extensively in a number of species. Initially much of the research concerned itself with regulation by dietary intake and the role of modification of this process in the development of atherosclerosis and coronary artery disease. More recently, investigation of the role of cholesterol in membranes has extended the possible functions of this neutral sterol to a large number of membrane related phenomena. The direct effects of cholesterol on the myocardium have not been the subject of many studies, however, pathological changes in myocardial structure and function have been reported. Even less understood is the role of cholesterol in myocardial cell membranes, although one can assume as in other cell types, it most probably is involved with regulation of fluidity and permeability. While it is accepted that excessive dietary intake of cholesterol significantly increases the risk of developing coronary artery disease, the effects of diet on the structure and function of myocardial membrane systerms are not known. What is known, however, is that some forms of hereditary cardiomyopathy in animals are found associated with increased membrane cholesterol (Slack et al, 1980) and alteration in function, particularly in those processes involved in calcium homeostasis (Owens et al,1974). Calcium blockers were effective in preventing or delaying the necrotic process in these disorders (Jasmin and Solymoss,1975; Bhattacharya et al,1982) indicating the increase in membrane

cholesterol may alter permeability under these conditions. The possibility that storage of excess cholesterol in myocardial membranes in response to increased dietary intake might initiate a qualitatively similar process served as a basis for the present investigation.

Myocardial lipid storage is found in rabbits fed atherogenic diets (Ho and Taylor,1968; Ho et al,1974). The cholesterol content of the myocardium doubled in 2-3 months under these conditions and while other studies have demonstrated the presence of intracellular lipid droplets (David et al,1978) it is likely some of the sterol is sequestered within membranes (Sabine,1977). The effects of lipid storage on enzyme activities and ionic permeability of sarcolemma, sarcoplasmic reticulum and mitochondria are of considerable interest if there is a cardiomyopathic process occurring under these circumstances. The effects of diet on structural and functional properties of the myocardial cell are also pertinent to determine if membrane changes correlate with alterations in any of these parameters. Lastly, as many observations could be attributable to concomitant atherosclerosis or cardiac hypertrophy, it would seem desirable to eliminate these additional factors in order to determine which effects, if any, might be related directly to cholesterol.

The initial phase of the present study concerned itself with characterization of a model which would be consistent with these

objectives. Selection of the rat as the experimental model of choice was made to eliminate the influence of atherosclerosis. It has been shown by others this species is highly resistant to diet induced hypercholesterolemia and atherosclerosis. Nevertheless, the rat, like many other species including man, shares a common metabolic response to cholesterol supplementation and is therefore a legitimate model in which to study diet related myocardial damage (Mahley,1978).

The results of the present study confirmed the rat does not develop marked hypercholesterolemia, atherosclerosis or cardiac hypertrophy when fed a 2% cholesterol enriched diet for 6,12 or 24 weeks. Light microscopic analysis revealed no evidence of plaque development and the overall appearance indicated that coronary patency was maintained. The observation of a considerable number of leukocytes in the vessels and myocardial tissue of cholesterol fed animals did suggest that some type of inflammatory process might be occurring. These observation were confirmed at the electron microscopic level with leukocyte infiltration particularly evident in the 12 week group and a substantial increase in lysosomal activity present at 24 weeks. Idiopathic cholesterol pericarditis has been reported in man, in the absence of hypercholesterolemia and significant atherosclerosis (Stanley et al,1981). This disorder was associated with cholesterol precipitates and fibrotic degeneration and calcific necrosis of the epicardium. Whether the inflammatory reaction observed in the present study is a result

of a similar process is not known. Data on plasma cholesterol levels indicate that no marked hypercholesterolemia is present at any feeding period, although, a significant elevation in these values was observed in the treated group at 6 weeks. This was principally a result of a smaller degree of variability in values obtained in these animals as the actual percentage increase in cholesterol levels was uniform throughout the entire range of feeding periods. Ventricular weight/body weight ratio did not differ between diet groups indicating absence of cardiac hypertrophy. These findings in rats are in contrast to those observed in rabbits which have been shown to develop significant hypercholesterolemia and hypertrophy following feeding of atherogenic diets (Lee et al,1978; Peterson et al,1979). Our results do indicate, however, that cholesterol may be stored in the liver and hypertrophy of this tissue is significant following 24 weeks on the diet. This finding is consistent with the observations of others which indicate, in the rat, the liver is a major storage site for cholesterol (Mathe and Chevallier,1979).

Ultrastructural findings in this investigation were similar in some respects to those reported in cholesterol fed rats by Melax and Leeson (1975). The onset of changes however, was somewhat slower and may be related to the fact the rats were younger when placed on the diet in the present study. The alterations noted by Melax and Leeson (1975) at 4 weeks were not observed following 6 weeks on diet in this investigation. However, the findings after 12 weeks were similar to those

reported to occur at 10 weeks in the former study. Vacuolation, mitochondrial swelling and tortuosities of the intercalated disc region were all noted at this time. However, we did not observe widespread myelin figure formation nor separation of the intercalated disc, both observations having been made in the previous report. The mitochondrial swelling observed is significant as lesions of this organelle could lead to impairment of energy production in myocardial cells and thus impair contractile performance. This conclusion is supported by the results of the Langendorff study which indicated a greater reliance of the hearts from the cholesterol fed rats for exogenous glucose. This may suggest the myocardium of these animals may be utilizing anaerobic glycolysis to support failing oxidative metabolism. As well, the stimulation of sarcolemmal ATPases at 12 weeks, suggests energy ultilization could be increased. This combination of impaired production and increased utilization can be seen to lead to an imbalance in energy stores which has been suggested to underlie some forms of contractile failure (Dhalla et al,1978). Considerable lipid deposition was present and in this regard was similar to that reported previously (Melax and Leeson,1975; David et al,1978). It has been suggested this might impair movement of substrates and products in and out of the mitochondria (Melax and Leeson,1975). As well, loss of the proper geometric arrangement of the contractile elements might also result in impaired contractile performance. Nevertheless, force development was enhanced in hearts from

cholesterol fed animals provided exogenous glucose was provided. Therefore it would seem that the impaired performance under some experimental conditions was not due to mechanical interference by lipid inclusions and vacuoles. Changes in the contour of the nucleus were similar to those reported previously (Melax and Leeson, 1975). The consequences of lobulation of the nuclear membrane are not known but it might result in some impairment in protein synthesis. In this regard, David et al (1978) reported a decrease in mitochondrial protein yield in hypercholesterolemic rabbits and the present study found a decrease in total protein of ventricular homogenates of cholesterol fed rats, although yield when normalized for ventricular weights was not statistically different. The presence of a large number of leukocytes at this time (12 weeks) has not been reported previously but was a consistent finding in this study. While Melax and Leeson (1975) attributed ultrastructural changes in the myocardium of cholesterol fed rats to microvascular disease, no evidence of this disorder was observed in the present study. This is in good agreement with many other studies which report the absence of atherosclerosis in cholesterol fed rats.

Further evidence of damage was observed at 24 weeks when large numbers of lysosomes were found closely associated with all myocardial membrane systems but particularly with mitochondria. At this time, formation of myelin figures within mitochondria and encapsulation of the organelles by membrane bound structures was

also noted. It is of interest that exposure of cultured heart muscle cells to moderate levels of cholesterol or cholesterol esters has been found associated with increased permeability of lysosomal and mitochondrial membranes (Wenzel et al,1975). Our results also are suggestive of loss of myocardial membrane integrity. Lysosomes were observed to contain large numbers of electron dense bodies. It is possible these may be iron containing lysosomal enzymes. Whether increased fragility of lysosomal membranes due to cholesterol is causing enzyme leakage and damage or whether damage as a result of some other process stimulates greater lysosomal activity is not clear. Nevertheless, the close association of lysosomes with or within mitochondrial and sarcoplasmic reticular membranes is indicative that some autolytic process is occurring. In view of the relationship of cell injury in atherosclerosis to alterations in lysosomal and mitochondrial function (Curreri and Muller,1968; Murray et al,1968) the presence of a similar process in myocardial cells in this study is intriguing. It is not clear why some of the degenerative changes occurring at 12 weeks had resolved themselves at 24 weeks. The disappearance of the lipid inclusions may have been the result of lysosomal activity as these structures contain enzymes capable of catabolizing lipid substrates. Nonetheless, evidence of impaired mitochondrial structure persisted. As well, biochemical studies indicated a depression of some sarcolemmal enzyme activities. Whether this depression resulted from compromised ATP production or loss of

sarcolemmal integrity as a result of lysosomal activation is not known, but the widespread involvement of all membrane systems would seem to argue that both process may be occurring simultaeously.

Electrocardiographic studies on the two diet groups indicated substantial differences existed between them. In control animals, interval durations were comparable to those reported previously for rat by Fraser et al (1967) although the PR interval in this investigation more closely resembled that found by Sambhi and White (1960). Amplitude of the P and T waves was not different than that reported previously (Fraser et al,1967). The R wave amplitude was considerably lower and may be related either to the depth of anesthesia or to strain differences. In this regard, Fraser and associates (1967) reported the presence of an S wave in lead II which was not present in control animals in this study. Prolongation of the PR interval seen in cholesterol fed animals at 24 weeks may be indicative of an atrioventricular or intraventricular conduction disturbance. The tendency to prolongation of the QT interval noted in the cholesterol fed animals also would be in agreement with this conclusion. The most prominent difference in resting ECG pattern between control and cholesterol fed groups was the appearance of a prominent S wave in the treated animals at 12 weeks. This combined with a sharp positive deflection in lead aVR is indicative of conduction disturbance and left axis deviation (Lipman et al, 1973). While cholesterol fed animals of all three

groups appeared to have changes in the ST pattern, this finding was difficult to interpret. Apparent ST elevation and ST depression were both seen, the particular pattern being dependent upon the presence (12 weeks) or absence (6 or 24 weeks) of a high amplitude S wave. It has been argued by Sambhi and White (1960) that no true ST segment exists in rat ECG and therefore no elevation or depression of this component is possible. Nevertheless, patterns of the contiguous S and T waves differed between diet groups. The cholesterol fed animals of the 12 and 24 week groups were refractory to the positive chonotropic action of isoproterenol. It is not clear whether this too was the result of degenerative changes or if some alterations either in the beta receptor density or sensitivity had occurred. Alternatively, cholesterol has been reported to block extraneuronal reuptake of catecholamines and such an action might be expected to increase circulating levels (Salt and Iverson,1972). Subacute administration of catecholamines has been shown to reduce heart rate and prolong the PR interval in rats (Singal et al, 1981).

It was interesting that some of the alterations in ECG pattern seen in the cholesterol group prior to isoproterenol administration could be reproduced in the control group after injection of a dose of the drug known to produce massive myocardial infarction and 50-60% mortality (Wexler et al,1968). It seems likely that conduction disturbances occur in response to this treatment and this might account for the similarities in pattern. While the prominent S wave was a normal feature only of

the 12 week cholesterol fed animals, administration of isoproterenol resulted in the appearance of this wave in all groups studied. This would indicate the pattern could be produced as a result of conduction disturbance. Arrhythmias in response to isoproterenol were a prominent feature in all groups studied, however, sudden death occurred only in animals from the 12 and 24 weeks cholesterol fed groups. This may indicate these animals are more sensitive to the conduction disturbances caused by acute isoproterenol administration.

Electrocardiograms from the atherosclerotic rabbit share similarities in pattern to those described here. Lee et al (1978) reported ST depression and ST elevation which was potentiated by isoproterenol. Similar prominent S waves were also present but that study did not indicate which lead was used to make ECG recordings. In contrast to the findings reported here, Lee et al (1978) noted increased heart rate in 50% of rabbits subjected to isoproterenol infusion. However, this group did report that isoproterenol exacerbated ECG abnormalities and in this respect agree with the data presented here. Non-specific ST changes were also reported in cholesterol pericarditis by Stanley et al (1980) and ST depression has been noted in asymptomatic hyperlipoproteinemia in response to exercise (Olsson,1977).

The results presented here indicate that changes in the normal electrical conduction properties occur in cholesterol supplemented states. As tissue data and ultrastructural

examination indicate that neither hypertrophy nor atherosclerotic related ischemia are present, the observed changes may be related to degeneration of conducting tissues, membrane changes or possibly to a form of myocarditis. The latter possibility is suggested by microscopic evidence of inflammatory reaction discussed earlier and by resolution of some abnormalities observed at 12 weeks in the 24 week group. However, membrane mediated changes were suggested by the study of Alivisatos et al (1977) who demonstrated hyperpolarization of Purkinje fibres exposed to cholesterol. As well, increases in sodium permeability and possibly calcium permeability have been suggested to occur in hypercholesterolemic states (Pfeiffer et al,1978; Peterson et al,1979). Increases in intracellular sodium have also been demonstrated in failing myopathic hearts (Bajusz and Lossnitzer,1968). Lastly, destruction of some components of the conduction system has been reported in rabbits (Senges et al,1981) and rats (Melax and Leeson,1975) fed cholesterol rich diets. This study also demonstrated changes in the low resistance pathways in cholesterol fed animals of the 12 and 24 week groups. It is possible that any combination of these factors may be present in this study and contributing to the changes observed.

Biochemical studies on sarcolemmal membranes indicate that a gradual change in the adenosine triphosphatase activities as well as in calcium binding capacity occur in response to cholesterol. The earliest change in this regard was stimulation of the sodium pump activity. Such an increase was predicted by Pfeiffer et al

(1978) on the basis of the observation of increased myocardial sodium in hypercholesterolemic rabbits. As well, Alivisatos et al (1977) found a concentration dependent increase in ATPase activity of rat and dog heart homogenates in response to cholesterol. Hyperpolarization of puppy Purkinje fibre which could have resulted from pump stimulation was also noted in that study although no connection between the two phenomena was demonstrated. Stimulation of Na^+-K^+ATPase by cholesterol in the present study is not in agreement with the findings of Kimelberg (1975) on phosphatidylserine activated sheep kidney ATPase where incorporation of cholesterol was associated with inhibition of the enzyme. Red cell membrane Na^+-K^+ATPase has been reported to be unchanged by increased cholesterol (Cooper et al, 1975). As well, activity of this enzyme in rat liver plasmalemma was unaffected by in vitro incorporation of the sterol (Leoni et al,1982). Increased Na^+-K^+ATPase activity has been reported in sarcolemma from genetically myopathic hamsters (Sulakhe and Dhalla,1973). These authors concluded the increase might represent an adaptive change, perhaps related to increases in intracellular sodium. As well, increases in this enzyme activity, accompanied by decreases in high energy phosphate levels were reported in vitamin E deficient rats (Fedelesova et al,1971). Because of the influence of cholesterol on fluidity it seemed possible that at least the initial stimulation of pump activity observed here might be associated with alterations in this membrane characteristic. However, the failure of concanavalin A

to stimulate Na^+-K^+ATPase activity would seem to indicate in myocardial sarcolemma that this activity is not influenced primarily by membrane fluidity. This may explain why no changes in enzyme activity are noted in liver membranes exposed to cholesterol in vitro (Leoni et al,1982). As well, at 24 weeks the cholesterol/phospholipid molar ratio was elevated in both control and treated membranes while the enzyme activity was either unchanged (control) or depressed (cholesterol) when compared to that observed at 6 and 12 weeks when the ratio was smaller. The most potent stimulation of the enzyme in the cholesterol group occurred at 6 weeks when the C/P ratio did not differ appreciably from control. The conclusion of Kimelberg (1975) that cholesterol mediates its influence on the enzyme by modulating the physical state of membrane phospholipids does not appear to be justified in this study.

It is possible the early stimulation of the pump is secondary to increases in intracellular calcium due to cholesterol related permeability changes at the sarcolemma as suggested by others (Pfeiffer et al,1978; Peterson et al,1979). As well, it is possible inhibition of the sarcoplasmic reticulum Ca^{2+}ATPase activity may lead to a calcium overload in this state (Madden et al,1979;1981). An increase in intracellular calcium concentration can be seen to increase sodium pump activity by stimulation of the sodium-calcium exchange antiporter system. The energy for calcium movements by this mechanism is believed to be derived from the ATP hydrolysis by the sodium pump. If this is

the case, stimulation of Na^+-K^+ATPase probably reflects an early compensatory mechanism to restore correct ionic balance in the myocardial cell. This would be in agreement with observations in myopathic hamsters which display increased membrane cholesterol (Slack et al,1980), increased Na^+-K^+ATPase activity (Sulahke and Dhalla,1973) and an increase in intracellular sodium (Bajusz and Lossnitzer,1968). It should be noted the necrosis is blocked by calcium channel blockers (Jasmin and Solymoss,1975) indicating permeability characteristics for this cation are probably also altered in this type of cardiomyopathy. The depression of enzyme activity at 24 weeks would then represent a later, pathological development.

The effects of the diet on divalent cation ATPases were strongly dependent on time. Stimulation of Mg^{2+}ATPase by cholesterol has been reported in sheep kidney (Kimelberg,1975) and rat liver membranes (Riordan, 1980). Some investigators have found that cholesterol was without effect on this activity (Leoni et al,1982). In those studies reporting cholesterol related stimulation it was concluded that changes were related to increased membrane order (Kimelberg,1975; Riordan,1980). Stimulation of Mg^{2+}ATPase by concanavalin A has also been demonstrated (Carraway,1980; Riordan,1980) which would lend credence to this conclusion. In addition, membrane fluidizing agents, such as cis-vaccenic acid have been reported to inhibit enzyme activity (Riordan,1980; Leoni et al,1982) which also would be indicative that membrane fluidity exerts an influence on this

system. This study demonstrated comparable increases in Mg^{2+}ATPase activity by cholesterol feeding and by concanavalin A treatment. However, stimulation of the enzyme was observed only following 12 weeks in the cholesterol fed animals, suggesting either the increase was not mediated through cholesterol related changes in fluidity or that these changes were offset by some other occurrence at 24 weeks. The possibility of either impairment of ATP production by mitochondria or some loss of sarcolemmal membrane function must be considered in view of the lysosomal activity occurring at this time. As was the case with the Na^{+}-K^{+}ATPase, it is possible the late depression of Mg^{2+}ATPase activity may be a manifestation of advancing pathology under these conditions.

Ca^{2+}ATPase has also been reported to be stimulated by increasing membrane order, both in lymphocytes (Averdunk and Gunther,1980) and in mammary gland tumour cells (Carraway et al,1980). A similar result was obtained in this investigation in membranes from rats fed cholesterol rich diets for 12 weeks or by treatment of normal sarcolemma with concanavalin A. In this regard, the sarcolemmal enzyme differs from that of the sarcoplasmic reticulum which is inhibited by cholesterol (Madden et al,1979;1981). If the sarcolemmal enzyme is involved in calcium entry, as has been suggested (Dhalla et al,1981) then combination of stimulation of this activity with inhibition of the sarcoplasmic reticular pump might very well lead to an increase in intracellular calcium. This would be in agreement

with the conclusions of othersin hypercholesterolemic rabbits (Pfeiffer et al,1978; Peterson et al,1979). It would also provide a reasonable explanation for the calcium overload occurring in cardiomyopathic hamsters. Increases in mitochondrial membrane cholesterol are reported to depress oxidative phosphorylation in dogs (Rouslin et al,1981). Thus, the depressed activity of the enzyme at later stages may be due to depressed ATP production by mitochondria. At present, it is not possible to distinguish between the possible mechanisms for the effects which cholesterol feeding exerts on sarcolemmal enzyme activities. In cholesterol fed animals it seems likely at least two cholesterol related membrane changes are occurring, namely, alterations in fluidity and permeability. In addition, alterations in energy production by mitochondria are possible in view of the ultrastructural evidence of damage to these organelles. The interaction between these phenomena and possibly others probably determines the overall effect on sarcolemmal function. It is evident that differences exist in the response of the control and treated groups to increases in sarcolemmal cholesterol/phospholipid molar ratio. Increases in this parameter in the 24 week control group failed to elicit any change in sarcolemmal enzyme activities although there was a non-significant decrease in divalent cation ATPases at this time. Increasing age is associated with increased cholesterol levels in the rat (Yamamoto and Yamamura,1971) and therefore it is possible aging may initiate a process similar to that observed in the cholesterol fed rats. As enzyme activities

tend to fall with age, it is possible the increase in the control animals may initially offset some of this depression in membrane function by stimulation. This process might eventually lead to the deleterious changes observed in the treated groups suggesting that aging may be related to the cells ability to regulate lipid metabolism as suggested by others (Melax and Leeson,1975). However, it may be that other factors which are capable of modulating fluidity, such as phospholipid fatty acid composition, may offset fluidity changes brought about by increased cholesterol. It is apparent that cholesterol does alter sarcolemmal function, however, the mechanism by which it exerts its effects is far from clear and in the intact animal probably includes a wide spectrum of adjustments by the organism.

Calcium binding to the sarcolemma of the myocardial cell is necessary for maintaining contractility. This is amply demonstrated by the rapid decline in force which occurs in the absence of calcium (Shine et al,1971) or when this cation is displaced by lanthanum (Sanborn and Langer,1970). Studies have shown that proteins (Limas,1977), phospholipids (Philipson et all980) and sialic acid (Langer,1976;1978) all bind calcium at the sarcolemma. Which moiety represents the contractile dependent calcium binding pool, however, has not been satisfactorily resolved. The existence of at least two calcium binding pools on the sarcolemma was reported by Limas (1977). He found high and low affinity binding sites with affinity constant values for calcium of 2×10^{-5} M and 5.6×10^{-2} M respectively. Selective

treatment of sarcolemmal membranes with trypsin, phospholipase C and neuraminidase has indicated that proteins probably represent the high affinity site while phospholipids, proteins and sialic acid all contribute to the low affinity binding pool (Matsukubo et al,1981). Thus both low and high affinity sites were studied in an attempt to correlate changes in binding capacity with alterations in protein, phospholipid or sialic acid content in membranes from the two diet groups. In this regard, the increased activity of Ca^{2+}ATPase at 12 weeks in the cholesterol group may be reflected in the increase in binding in the presence of 0.05 mM $CaCl_2$ seen at this time. Whether this would be a result of an increased number of functional enzyme molecules or increased rate of ATP hydrolysis is uncertain. It is of interest that concanavalin A in addition to stimulating Ca^{2+}ATPase has been reported to increase both calcium binding and calcium uptake of lymphocyte plasma membranes (Averdunk and Gunther,1980). Whether the increases in ATP hydrolysis and binding in the presence of calcium in rats of the 12 week cholesterol fed group is a manifestation of the same type of phenomenon is uncertain. It is tempting to speculate that the increased binding at the low affinity site (1.25 mM $CaCl_2$) in the 6 week cholesterol fed animals might be due to the increase in sialic acid content as well as a tendency to an increase in acidic phospholipids. In this regard, increases in sialic acid have been reported for red cells from rabbits fed cholesterol rich diets (Pessina et al,1981).

In conclusion, the biochemical evidence presented here is consistent with the hypothesis that cholesterol can initiate alterations in sarcolemmal structure and function. In view of these observation, the effects of diet on contractile performance of the heart were seen as being especially significant in view of the cause and effect relationship between membrane integrity and cardiac function which has been proposed (Dhalla et al,1978).

Examination of a number of indices of contractility as well as the response to substrate deprivation and hypoxia was carried out in both groups at 12 weeks. The effects of removal of glucose, oxygen or both in the present study suggest that diet does play a direct role in the response of the isolated heart to these interventions. During aerobic glucose-free perfusion significant differences were observed between the diet groups with respect to heart rate and time to peak tension development. The hearts from the cholesterol fed group displayed a lower rate as well as a prolonged time to peak tension under these conditions. Perfusion of the isolated rat heart with substrate-free medium has been shown to cause release of endogenous catecholamines (Dhalla et al,1971). This can be seen to bring about an increase in heart rate as well as a decrease in time to peak tension development by activation of the adenylate cyclase system. The failure of hearts from the cholesterol fed group to exhibit this response may be due to the fact that they may be in a depleted state with respect to their endogenous

catecholamine supplies. Inhibition of extraneuronal reuptake of catecholamines in the heart by cholesterol has been reported (Salt and Iverson,1972). Conversely, as suggested by that study, sensitivity of the system may be reduced due to higher levels of circulating catecholamines during the feeding period. Attenuation of beta receptor mediated increases in heart rate have been reported for female rats chronically administered steroid oral contraceptives (Fregly and Thrasher,1977). As well, beta receptor density decline accompanied by a decrease in heart rate has been reported in diabetic rats (Savarese and Berkowitz,1979). Similarly, studies have shown attenuation of the inotropic response to norepinephrine in catecholamine cardiomyopathy (Fripp et al,1981) and a decrease in isoproterenol stimulated adenylate cyclase activity and cAMP production (Marsh et al,1980). This explanation of the current findings would be consistent with the observation that perfusion with glucose abolishes the observed differences in these two parameters. However, other studies have shown degenerative changes in nodal tissue and cardiac nerves (Senges et al,1981) as well as pathological changes in the intercalated disc (Melax and Leeson,1975) in response to cholesterol enriched diets. It is possible a similar process is occurring in the present study and that the defect is unmasked by substrate deprivation. The presence of a conduction defect in hearts from cholesterol fed animals was suggested by the ECG findings discussed earlier. The force generation by hearts from both diet groups was sensitive to substrate availability. This is

in contrast to the observations of Dhalla et al (1980) who found no significant effect of substrate deprivation on contractile force during the first 30 min of perfusion. This difference is probably a reflection of the fact that in the present study the initial equilibration period was also in the absence of substrate while in the former study equilibration was performed with substrate containing buffer. That study demonstrated a rapid decline in contractile force between 30-60 min of substrate-free perfusion, therefore the time course of contractile failure in control animals is not different in this investigation if the equilibration period is teken into account. The earlier onset of the decrease in contractile force in the cholesterol group suggests this group is more sensitive to the effects of glucose deprivation. It has been suggested the decline in contractile force in response to glucose-free perfusion may result from a decreased ability of hearts to utilize endogenous fuel supplies or some defect in calcium transporting systems (Dhalla et al,1980). These results do not allow us to distinguish between the two mechanisms but it is evident that a cholesterol rich diet accelerates the onset of contractile failure in the absence of glucose. The observation that in the presence of substrate hearts from the cholesterol group had significantly greater force development while no such differences were present in glucose-free medium may suggest cholesterol diet augments calcium delivery to the contractile apparatus when an exogenous fuel supply is available.

Hypoxia in the presence or absence of substrate elicited similar responses with respect to rate, contractile force and rate of force development in both diet groups. The hypoxic stimulus in the presence of glucose did cause a more rapid onset in reduction of the time to peak tension in the cholesterol group. This may be a reflection of the fact that these times were in fact prolonged during normoxia in this group when compared to controls. Therefore while the response to hypoxia was more rapid in hearts from treated rats, the actual values during hypoxia did not differ between diet groups.

Substrate availability had no appreciable effect on either resting tension or coronary pressure in either diet group during normoxia. Hypoxia, however, did unmask several differences. With respect to resting tension, the cholesterol group developed a greater degree of contracture after prolonged hypoxia in the absence of substrate which was abolished by its presence. These results seem to indicate a greater reliance of this group on exogenous fuel supply. This result would also indicate an greater increase in myocardial calcium content or alternatively a greater decrease in ATP availability in the cholesterol group relative to control under these conditions.

Only the control group showed any differences in coronary perfusion pressure in response to hypoxic challenge in the absence of substrate. Some studies have indicated increased vascular reactivity in response to both acute (Yokoyama and

Henry,1979) and chronic (Rosendorff et al, 1981) exposure to cholesterol. Others, however, have found no such differences (Johannson et al,1981). The reason for the apparent decrease in the reactivity of the cholesterol group in the present study is not known.

VI. PHYSIOLOGICAL CONSIDERATIONS AND CONCLUSIONS

The results of this investigation indicate that a cardiomyopathic process independent of atherosclerosis, may occur in response to a high cholesterol diet. The evidence of changes in structural, electrical, biochemical and functional parameters suggests the process is widespread and probably involves both compensatory and pathological alterations. Because of the importance of subcellular membranes in the regulation of myocardial function (Dhalla et al,1977) it is likely that changes in membrane structure may have initiated subsequent events. Early adjustments in membrane Na^{+}-K^{+}ATPase activity are in agreement with this contention. This stimulation did not appear to correlate well with changes in membrane fluidity and it maybe that it represents an adjustment to alterations in permeability, possibly involving sodium, calcium or both. Subsequent stimulation of the Ca^{2+}ATPase, on the other hand, most likely is a result of decreased membrane fluidity and would exacerbate any calcium related permeability defect. As well, cholesterol would be expected to distribute not only in sarcolemma but in other membrane systems (Sabine,1977). The consequences of increases in

membrane cholesterol in sarcoplasmic reticulum have been reported to involve depression of calcium uptake by this membrane system (Drabikowski et al,1972) and increased cholesterol in mitochondrial membranes depresses oxidative phosphorylation (Rouslin et al,1981). Whether this is mediated by changes in enzyme function or by conversion of ATP production capacity to calcium pumping by these organelles is not known. In this regard, data presented here did demonstrate evidence of damage to mitochondrial ultrastructure. It is possible the altered and increased calcium entry via stimulation of Ca^{2+}ATPase in the presence of defective calcium handling by the sarcoplasmic reticulum and impaired ATP production may have resulted in a calcium overload. As this state is associated with degenerative changes in myocardial structure and function (Yates and Dhalla,1975; Singal et al,1978;1979) it may underlie the damage observed in the present studies.

Another possible source of the observed myocardial damage is related to alterations in lipid metabolism. Increased lipolysis as a result of catecholamine stimulation may have led to lipid deposition in the heart of cholesterol fed animals. An increase in circulating catecholamines in hypercholesterolemic states has been suggested by Salt and Iverson (1972). It is recognised that fatty acids uncouple mitochondrial oxidative phosphorylation (Challoner and Steinberg,1966). Therefore, the presence of fat droplets in myocardial cells may reflect a decrease in the ability of the mitochondria to oxidize fatty acids in the

presence of an increased supply of these substrates. This would explain the protective effects of glucose on myocardial performance observed in isolated hearts from rats fed cholesterol rich diets in the present study. Another observation which is consistent with catecholamin mediated damage is the presence of conduction defects in cholesterol fed rats. Fat deposition has been found associated with conduction abnormalities (Balsaver et al, 1967) and may have contributed to the defects observed here. It is interesting that disappearance of some ECG abnormalities and the appearance of lysosomal activity coincided with the regression of lipid deposition. These organelles contain enzymes capable of breaking down lipids and the increase in their number may represent the cells response to lipid overload. Alternatively, constant exposure to catecholamines is known to decrease the responsiveness of the adenylate cyclase system. This could have resulted in an eventual decrease in lipolytic activity and resolution of increased delivery of lipid substrates to the myocardium. It should be noted that some evidence of refractoriness of the myocardium to catecholamines was seen in this study.

Diet related myocardial damage therefore, may reflect alterations in calcium homeostasis as a result of the interaction of cholesterol with subcellular membrane systems or catecholamine stimulated alterations in lipid metabolism. In this regard catecholamines are also believed to enhance calcium entry. It seems likely that some combination of both reactions occur and

result in cell damage. The late depression of sarcolemmal activities could be due either to direct membrane damage as a result of lysosomal action or to depressed mitochondrial function. Destruction of mitochondria by lysosomes would be expected to decrease ATP production. As well, these organelles may not be functioning optimally as a result of cholesterol or catecholamine related actions discussed earlier. Regardless of the mechanism, it is evident from the structural, biochemical and electrocardiographic findings that a pathological process is initiated by high cholesterol diets. It is difficult to differentiate between compensatory mechanisms and direct diet related membrane effects. However, the evidence would suggest that leucocyte and lysosomal activity and possibly enhanced Na^{+}-K^{+}ATPase activity represent the former. Lipid deposition and stimulation of Mg^{2+} and Ca^{2+}ATPases on the other hand would seem to mediate cell damage and therefore could be classified as pathological. By 24 weeks compensatory mechanisms appear to be no longer adequate and destruction of cellular elements and depression of membrane function have ensued.

In conclusion, this study has demonstrated a cholesterol mediated cardiomyopathy. The ability of cholesterol to modify cell structure and function directly suggests in susceptible individuals, diet related alteration of the myocardium may enhance their risk of developing heart disease when exposed to other causitive factors. This may explain why on an individual basis serum cholesterol levels are a poor indicator of risk while

the correlation holds true for the population as a whole. In view of the possible involvement of catecholamines in mediating some of the observed damage, the effects of stress superimposed on diet would be of particular interest. Finally, the observations reported here could be used as the basis for future studies on possible methods of prevention and treatment of lifestyle related heart disease.

VII. BIBLIOGRAPHY

Adel,H.N., Deming,Q.B., Brun,L. Genetic hypercholesterolemia in rats. Circulation 39:Suppl.III:1,1969..

Akera,T., Brody,T.M. The role of Na^+-K^+-ATPase in the inotropic actions of digitalis. Pharmacol. Rev. 29:187-220,1978.

Alivisatos,S.G.A., Papastavrou,C., Drouka-Liapati,E., Molyvdas,A.P., Nikitopoulou,G. Enzymatic and electrophysiological changes of the function of membrane proteins by cholesterol. Biochem. Biophys. Res. Comm. 79:677-683,1977.

Albrink,M.J., Meigs,J.W., Man,E.B. Serum lipids, hypertension and coronary artery disease. Am. J. Med. 31:4-11,1961.

Anand,M.B., Chauhan,M.S., Dhalla,N.S. Ca^{2+}/Mg^{2+} ATPase activities of heart sarcolemma, microsomes and mitochondria. J. Biochem. 82:1731- 1739,1977.

Anderson,O.S. In Membrane Transport in Biology. (G. Giebisch, C.C. Tostenson, H.H. Ussing,eds.) Springer-Verlag, Berlin, 1978. Vol.1,369-446.

Ashworth,L.A.E. and Green,C. Plasma membranes: Phospholipid and sterol content. Science 151:210-211,1966.

Averdunk,R. and Gunther,T. Effect of concanavalin A on Ca^{2+} binding, Ca^{2+} uptake and the Ca^{2+}ATPase of lymphocyte plasma membranes. Biochem. Biophys. Res. Comm. 97:1146-1153,1980.

Balasubramaniam,S., Mitropoulos,K.A., Venkatesan,S. Rat-liver acyl-coA: cholesterol acyltransferase. Eur. J. Biochem. 90:377-383,1978.

Balsaver,A.M., Morales,A.R., Whitehouse,F.W. Fat infiltration of myocardium as a cause of cardiac conduction defect. Am. J. Cardiol. 19: 261-265,1967.

Bajusz,E., Lossnitzer,K. A new disease model of chronic congestive heart failure. Studies on its pathogenesis. Trans. N.Y. Acad. Sci. 30:939-948,1968.

Bartlett,G.R. Phosphorus assay in column chromatography. J. Biol. Chem. 234:446-471,1959.

Bhattacharya,S.K., Palmieri,G.M.A., Bertorini,T.E., Nutting,D.F. The effects of diltiazem in dystrophic hamsters. Muscle and Nerve 5:73-78, 1982.

Bloj,B., Galo,M.G., Morero,R.D., Farias,R.N. Kinetic modifications of the acetylcholinesterase and (Ca^{2+}+Mg^{2+})-ATPase in rat erythrocytes by cholesterol feeding. J. Nutr. 106:1827-1839,1976.

Bloj,B., Galo,M.G., Morero,R.D., Farias,R.N. Heterogeneous effect of dietary cholesterol on acetylcholinesterase and ATPases of rat erythrocytes: Arrhenius plots. J. Nutr. 109:63-69,1979.

Bloj,B., Morero,R.D., Farias,R.N. Membrane fluidity, cholesterol

and allosteric transitions of membrane bound Mg^{2+}-ATPase, (Na^{+}+K^{+}) ATPase and acetylcholinesterase from rat erythrocytes. FEBS Lett. 38: 101-105,1973a.

Bloj,B., Morero,R.D., Farias,R.N., Trucco,R.E. Membrane lipid fatty acids and regulation of erythrocyte (Mg^{2+})-ATPase, (Na^{+}-K^{+})-ATPase and acetylcholinesterase from rats fed different fat supplemented diets. Biochim. Biophys. Acta 311:67-69,1973b.

Boggs,J.M. Intermolecular hydrogen bonding between lipids: influence on organization and function of lipids in membranes. Can. J. Biochem. 58:755-770,1980.

Boissel,J.P., Crouzet,B., Bourdillon,M.C., Blaes,N. Selection of a strain of rats with spontaneous high cholesterolemia. Atherosclerosis 39:11-18,1981.

Brown,M.S., Goldstein,J.L. Receptor-mediated endocytosis:Insights from the lipoprotein receptor system. Proc. Natl. Acac. Sci. USA 76:3330-3337,1979.

Camejo,G., Bosch,V., Arreaza,C., Mendez,H.C. Early changes in plasma lipoprotein structure and biosynthesis in cholesterol-fed rabbits. J. Lipid Res. 14:61-68,1973.

Carlson,L.A., Bottiger,L.E. Ischemic heart disease in relation to fasting levels of plasma triglycerides and cholesterol. Lancet 1: 865-867,1972.

Caroni,P., Carafoli,E. The (Ca^{2+}+Mg^{2+})ATPase of dog heart

sarcolemma. Nature 283:765-767,1980.

Caroni,P., Carafoli,E. The Ca^{2+} pumping ATPase of heart sarcolemma. Characterization, calmodulin dependence and partial purification. J. Biol. Chem. 256:3263-3270,1981.

Carafoli,E., Niggli,V. Purification and reconstruction of the calcium magnesium ATPase of the erythrocyte membrane. Ann. N.Y. Acad. Sci. 358: 159-168,1980.

Carraway,C.A.C., Corrado,F.J., Fogle,D.D., Carraway,K.L. Ecto-enzyme of mammary gland and its tumours. Ca^{2+} or Mg^{2+}-stimulated adenosine triphosphatase and its perturbation by concanavalin A. Biochem. J. 191:45-51,1980.

Challoner,D.R., Steinberg,D. Oxidative metabolism of myocardium as influenced by fatty acids and epinephrine. Am. J. Physiol. 211:897-902,1966.

Chapman,D. Some recent studies of lipids, lipid-cholesterol and membrane systems. In Biological Membranes, Vol.2 (D.Chapman and D.F.A. Wallach eds.) Academic Press,London. 91-144,1973.

Claret,M., Garay,R., Giraud,F. The effect of membrane cholesterol on the sodium pump in red blood cells. J. Physiol. 274:247-263,1978.

Coleman, R. Membrane-bound enzymes and membrane ultrastructure. Biochim. Biophys. Acta 300:1-30,1973.

Cook,G.M.W. Techniques for the analysis of membrane carbohydrates. In Biochemical Analysis of Membranes, (E.H. Maddy,ed.) Wiley and Sons,New York.1976.

Cooper,R.A. Abnormalities of cell-membrane fluidity in the pathogenesis of disease. N. Engl. J. Med. 297:371-377,1977.

Cooper,R.A., Arner,E.C., Wiley,J.S., Shattil,S.J. Modification of red cell membrane structure by cholesterol-rich lipid dispersions: a model for the primary spur cell defect. J. Clin. Invest. 55:115-126, 1975.

Cooper,R.A., Kimball,D.B., Durocher,J.R. Role of the spleen in membrane conditioning and hemolysis of spur cells in liver disease. N. Engl. J. Med. 290:1279-1284,1974.

Cooper,R.A., Shattil,S.J. Membrane cholesterol-is enough too much. N. Engl. J. Med. 302:49-51,1980.

Coraboeuf,E. Ionic basis of electrical activity in cardiac tissue. Am. J. Physiol. 234:H101-H116,1978.

Coronary Drug Project Research Group. Clofibrate and niacin in coronary heart disease. J. Am. Med. Assoc. 231:360-381,1975.

Curreri,P.W., Muller,B.F. Lysosomal enzyme activity in atherosclerosis. Rev. Surg. 25:371-377,1968.

David,H., Wilfert,K., Gunther,J., Pfeiffer,C., Gross,V., Behrisch,D. Ultrastrukurelle und funktionell veranderungen des

kaninchenmyokards nach Kholesterol futterung. Acta Biol. Med. Germ. 37:577-591,1978.

Dayton,S.,Pearce,M.L., Hasimoto,S., Dixon,W.J., Tomiyasu,U. A controlled clinical trial of a diet high in unsaturated fat in preventing complications of atherosclerosis. Circulation, Suppl.II,40:1-63,1969.

Dean,W.L., Tanford,C.J. Reactivation of lipid-depleted Ca^{2+}-ATPase by a nonionic detergent. J. Biol. Chem. 252:3551-3553,1977.

DeGier,J., Mandersloot,J.G., VanDeenen,L.L.M. Lipid composition and permeability of liposomes. Biochim. Biophys. Acta 150:666-675,1968.

DeGier,J., Mandersloot,J.G., VanDeenen,L.L.M. The role of cholesterol in lipid membranes. Biochim. Biophys. Acta 173:143-145,1969.

Demel,R.A., DeKruyff,B. The function of sterols in membranes. Biochim. Biophys. Acta 457:109-132,1976.

Demel,R.A., Kinsky,S.C., VanDeenen,L.L.M. Effects of temperature and cholesterol on the glucose permeability of liposomes prepared with natural and synthetic lecithins. Biochim. Biophys. Acta 150:655-665,

DePierre,J.W., Karnovsky,M.L. Plasma membranes of mammalian cells. A review of methods for their characterization and

isolation. J. Cell Biol. 56:275-303,1973.

Deuticke,B. Properties and structural basis of simple diffusion pathways in the erythrocyte membrane. Rev. Physiol. Biochem. Pharmacol. 78:1-97,1977. 1968.

Deuticke,B., Ruska,C. Changes of nonelectrolyte permeability in cholesterol loaded erythrocytes. Biochim. Biophys. Acta 433:638-653,1976.

Dhalla,N.S., Anand,M.B., Harrow,J.A.C. Calcium binding and ATPase activity of heart sarcolemma. J. Biochem. 79:1345-1350,1976.

Dhalla,N.S., Anand-Srivastava,M.B., Tuana,B.S., Khandelwal,R.L. Solubilization of a calcium dependent adenosine triphosphatase from rat heart sarcolemma. J. Mole. Cell. Cardiol. 13:413-423,1981.

Dhalla,N.S., Bhagat,B.D., Sulakhe,P.V., Olson,R.E. Catecholamine stores of the isolated rat heart perfused with substrate-free medium. J. Pharmacol. Exp. Ther. 177:96-101,1971.

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

Dhalla,N.S., Matoushek,R.F., Olson,R.E. Changes in contractile function and endogenous fuel supply in rat heart upon perfusion with substrate-free medium. In Advances in Myocardiology, Vol.2 (M. Tajuddin, B.Bhatia, H.H. Siddiqui and G. Rona,eds.) University Park Press, Baltimore.17-29,1980.

Dhalla,N.S., Ziegelhoffer,A., Harrow,J.A.C. Regulatory role of membrane systems in heart function. Can. J. Physiol. Pharmacol. 6: 1211-1234,1977.

Dietchy,J.M., Siperstein,M.D. Effect of cholesterol feeding and fasting on sterol synthesis in seventeen tissues of the rat. J. Lipid Res. 8:97-104,1967.

Drabikowski,W., Sazala,M.G., Wroniszewsku,A., Lagwinska,E., Drzewiecka, B. Role of cholesterol in the Ca^{2+} uptake and ATPase activity of fragmented sarcoplasmic reticulum. Biochim. Biophys. Acta 274:158-170, 1972.

Farias,R.N., Bloj,B., Morero,R.D., Sinerez,F., Trucco,R. Regulation of allosteric membrane-bound enzymes through changes in membrane lipid composition. Biochim. Biophys. Acta 415:231-251,1975.

Fedelesova,M., Sulakhe,P.V., Yates,J.C., Dhalla,N.S. Biochemical basis of heart function IV. Energy metabolism and calcium transport of vitamin E deficient rats. Can. J. Physiol. Pharmacol. 49:909-918,1971.

Fenster,L.J., Copenhaver,J.H. Phosphatidylserine requirement of (Na^+-K^+) -activated adenosine triphosphatase from rat kidney and brain. Biochim Biophys. Acta 137:406-408,1967.

Fiehn,W., Seiler,D. Alteration of erythrocyte (Na^+-K^+)-ATPase by replacement of cholesterol by desmosterol in the membrane.

Experientia 31:773-775,1975.

Fisher,W.R., Truitt,D.H. The common hyperlipoproteinemias. An understanding of disease mechanisms and their control. Ann. Intern. Med. 85:497-508,1976.

Folch,J., Lees,M., Sloane-Stanley,G.H. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-501,1957.

Frantz,I.D.Jr., Schneider, H.S., Hinkelman,B.T. Suppression of hepatic cholesterol synthesis in the rat by cholesterol feeding. J. Biol. Chem. 206:465-469,1954.

Fraser,R.S., Harley,C., Wiley,T. Electrocardiogram in the normal rat. J. Appl. Physiol. 3:401-402,1967.

Fregly,M.J. and Thrasher,T.N. Response of heart rate to acute administration of isoproterenol in rats treated chronically with norethynodrel,ethinylestradiol and both combined. Endocrinology 100: 148-154,1977.

Fripp,R.R., Lee,J.C., Downing,S.E. Inotropic responsiveness of the heart in catecholamine cardiomyopathy. Am. Heart J. 101:17-21,1981.

Frnka,J., Reiser,R. The effects of diet cholesterol on the synthesis of rat serum apolipoproteins. Biochim. Biophys. Acta 360:322-338,1974.

Gadsby,D.C., Cranefield,P.F. Direct measurement of changes in sodium pump current in canine cardiac Purkinje fibers. Proc. Natl. Acad. Sci. USA 76:1783-1787,1979.

Galo,M.G., Bloj,B., Farias,R.N. Kinetic changes of the erythrocyte (Mg^{2+}+Ca^{2+})-adenosine triphosphatase of rats fed different fat supplemented diets. J. Biol. Chem. 250:6204-6207,1975.

Geer,J.C., Crago,C.A., Little,W.C., Gardner,L.L., Bishop,S.P. Subendocardial ischemic myocardial lesions associated with severe coronary atherosclerosis. Am. J. Path. 98:663-680.1980.

Giraud,F., Claret,M., Bruckdorfer,K.R. The effect of membrane-fluidizing agents on sodium-pump activity in erythrocytes. Biochem. Soc. Trans. 8:132,1980.

Giraud,F., Claret,M., Bruckdorfer,K.R., Chailley,B. The effects of membrane lipid order and cholesterol on the internal and external cationic sites of the Na^{+}-K^{+} pump in erythrocytes. Biochim. Biophys. Acta 647:249-258,1981.

Glitch,H.G., Reuter,H., Scholz,H. The effect of internal sodium concentration on calcium fluxes in isolated guinea pig auricles. J. Physiol. (Lond.) 209:25-43,1970.

Gofman,J.W., Young,W., Tandy,R. Ischemic heart disease, atherosclerosis and longevity. Circulation 34:679-685,1966.

Gottleib,M.H. Roles of cholesterol exchange between human

erythrocytes. Biochim. Biophys. Acta 600:530-541,1980.

Grunze,M., Deuticke,B. Changes of membrane permeability due to extensive cholesterol depletion in mammalian erythrocytes. Biochim. Biophys. Acta 356:125-130,1974.

Grunze,M., Forst,B., Deuticke,B. Dual effect of membrane cholesterol on simple and mediated processes in human erythrocytes. Biochim. Biophys. Acta 600:800-869,1980.

Hageman,J.A.S., Gould,R.G. The in vitro interchange of cholesterol between plasma and red cells. Proc. Soc. Exp. Biol. Med. 78:329-332, 1951.

Hamby,R.I., Gupta,M.D., Young,M.W. Clinical and hemodynamic aspects of single vessel coronary artery disease. Am. Heart J. 85:458-466,1973.

Hanai,T., Haydon,D.A., Taylor,J. The influence of lipid composition and of some adsorbed proteins on the capacitance of black hydrocarbon membranes. J. Theor. Biol. 9:422-432,1965.

Hasin,Y., Shimoni,Y., Stein,O., Stein,Y. Effect of cholesterol depletion on the electrical activity of rat heart myocytes in culture. J. Mole. Cell. Cardiol. 12:675-683,1980.

Hjermann,I., Helgeland,A., Holme,I., Lund-Larsen,P.G., Leren,P. A randomised intervention trial in the primary prevention of coronary heart disease. Proc. of the Eigth Congress on Cardiology. Basel:Karger:164,1980.

Ho,K.J., Pang,L.D., Taylor,C.R. Mode of cholesterol accumulation in various tissues of rabbits after prolonged exposure to various serum cholesterol levels. Atherosclerosis 19:561-566,1974.

Ho,K.J., Taylor,C.B. Comparative studies on tissue cholesterol. Arch. Path. 86:585-596,1968.

Horrobin,D.F. A new concept of lifestyle related cardiovascular disease: the importance of interactions between cholesterol, essential fatty acids prostaglandin E and thromboxane A_2. Med. Hypotheses 6:785-800,1980.

Hughes,B.P. Lipid changes in Duchenne muscular dystrophy. J. Neurosurg. Psychiatry 35:658-663,1972.

Imai,Y., Matsumara,H. Genetic studies on induced and spontaneous hypercholesterolemia in rats. Atherosclerosis 18:59,1973.

Jackson,R.L., Gotto,A.M.Jr. Hypothesis concerning membrane structure, cholesterol and atherosclerosis. Atherosclerosis Rev. 1:1-21,1976.

Jain,M.K. Role of cholesterol in biomembranes and related systems. Curr. Top. Membr. Transp.5:1-57,1975.

Jarnefelt,J. Lipid requirements of functional membrane structures as indicated by the reversible inactivation of (Na^+-K^+)-ATPase. Biochim. Biophys. Acta 266:91-96,1972.

Jasmin,G., Sólymoss,B. Prevention of hereditary cardiomyopathy in

the hamster by verapamil and other agents. Proc. Soc. Exp. Biol. Med. 149: 193-198,1975.

Johannsen,V.J., Mark,A.L., Marcus,M.L., Armstrong,M.L. Effects of dietary hypercholesterolemia on coronary vascular responsiveness in vivo. Circulation 64,Suppl.4:267,1981.

Kannel,W.B., Castelli,W.P., Gordon,T., MacNamara,P.M. Serum cholesterol, lipoproteins and risk of coronary heart diseases. The Framingham Study. Ann. Intern. Med. 24:1-17,1971.

Katz,A.M., Messineo,F.C. Lipid-membrane interactions and the pathogenesis of ischemic damage in the myocardium. Circ. Res. 48:1-16,1981.

Katz,A.M., Messineo,F.C., Herbette,L. Ion channels in membranes. Circulation 65,Suppl.1:2-10,1982.

Kimelberg,H.K. Alterations in phospholipid-dependent $(Na^{+}+K^{+})$-ATPase activity due to lipid fluidity. Effects of cholesterol and Mg^{2+}. Biochim. Biophys. Acta 413:143-156,1975.

Kimelberg,H.K., Papahadjopoulos,D. Phospholipid requirements for $(Na^{+}+K^{+})$-ATPase activity:Head-group specificity and fatty acid fluidity. Biochim. Biophys. Acta 282:277-292,1972.

Kimelberg,H.K., Papahadjopoulos,D. Effects of phospholipid acyl chain fluidity, phase transitions and cholesterol on $(Na^{+}+K^{+})$-stimulated adenosine triphosphatase. J. Biol. Chem. 249:1071-1080,1974.

Kroes,J., Ostwald,R., Keith,A. Erythrocyte membranes-compression of lipid phases by increased cholesterol content. Biochim. Biophys. Acta 274:71-74,1972.

Kushwaha,R.S., Hazzard,W.R., Englbom,J. High density lipoprotein metabolism in normolipemic and cholesterol-fed rabbits. Biochim. Biophys. Acta 530:132-143,1978a.

Kushwaha,R.S., Hazzard,W.R., Harker,L.A., Englbom,J. Lipoprotein metabolism in baboons. Effect of feeding cholesterol-rich diet. Atherosclerosis 31:65-76,1978b.

Langer,G.A. Events at the cardiac sarcolemma:localization and movement of contractile dependent calcium. Fed. Proc. 35:1274-1278,1976.

Langer,G.A. The structure and function of the myocardial cell surface. Am. J. Physiol. 235:H461-H468,1978.

Lasser,N.L., Roheim,P.S., Edelstein,D., Eder,H.A. Serum lipoproteins of normal and cholesterol fed rats. J. Lipid Res. 14:1-8,1973.

Lee,R.J., Zaidi,I.H., Baky,S.H. Pathophysiology of the atherosclerotic rabbit. Envir. Health Prospec. 26:225-231,1978.

Lenaz,G. The role of lipids in membranes. In Subcellular Biochemistry (D.B. Roodyn,ed.) Plenum, New York. 223-343,1979.

Leoni,S., Leely,P., Mangiantini,M.R., Spagnuolo,S. Effect of free

fatty acids and cholesterol in vitro on liver plasma membrane-bound enzymes. Experientia 38:102-104,1982.

Limas,C.J. Calcium binding sites in rat myocardial sarcolemma. Arch. Biochem. Biophys. 179:302-309,1977.

Lipman,B.S., Massie,E., Kleiger,R.E. Clinical scalar electrocardiography. Year Book Medical Publishers, Chicago,1973.

Lowry,O.D., Rosebrough,N.J., Farr,A.L., Randall,R.J. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275,1951.

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

Madden,T.D., Chapman,D., Quinn,P.J. Cholesterol modulates activity of calcium dependent ATPase of the sarcoplasmic reticulum. Nature 279: 538-541,1979.

Madden,T.D., King,M.D., Quinn,P.J. The modulation of Ca^{2+}-ATPase activity of sarcoplasmic reticulum by membrane cholesterol. The effect of enzyme coupling. Biochim. Biophys. Acta 641:265-269,1981.

Mahley,R.W. Alterations in plasma lipoproteins induced by cholesterol feeding in animals including man. In Disturbances in Lipid and Lipoprotein Metabolism (J.M. Dietschy, A.M.Gotto Jr., J.A. Ontko,eds.) American Physiological Society,Bethesda MD. 181-197,1978.

Mahley,R.W., Holcombe,K.S. Alterations of plasma lipoproteins and apoproteins following cholesterol feeding in the rat. J. Lipid Res. 18:314-324,1977.

Mahley,R.W., Innerarity,T.L., Brown,M.S., Ho,Y.K., Goldstein,J.L. Cholesteryl ester synthesis in macrophages: stimulation by B-very low density lipoproteins from cholesterol fed animals of several species. J. Lipid Res. 21:970-980,1980.

Mahley,R.W., Weisgraber,K.H., Innerarity,T. Canine lipoproteins and atherosclerosis II. Characterization of the plasma lipoproteins associated with athergenic and non-atherogenic hyperlipidemia. Circ. Res. 35:722-733,1974.

Mahley,R.W., Weisgraber,K.H., Innerarity,T. Atherogenic hyperlipoproteinemia induced by cholesterol feeding in the Palas monkey. Biochemistry 15:2979-2985,1976.

Mahley,R.W., Weisgraber,K.H., Innerarity,T., Brewer,H.B., Assman,G. Swine lipoproteins and atherosclerosis. Changes in plasma lipoproteins and apoproteins induced by cholesterol feeding. Biochemistry 14:2817-2823, 1975.

Mak,W.W-N., Wong,J.T-F. Relationship between membrane fluidity and capping of receptors for concanavalin A. Can. J. Biochem. 58:1421-1429,1980.

Mann,G.V. Diet-Heart: End of an era. N. Engl. J. Med. 297:644-650,1977.

Marsh,J.D., Barry,W.H., Neer,E.J., Alexander,W., Smith,T.W. Desensitization of chick embryo ventricle to the physiological and biochemical effects of isoproterenol. Evidence for uncoupling of the B-receptor-adenylate cyclase complex. Circ. Res. 47:493-501,1980.

Mathe,D., Chevallier,F. Effects of dietary cholesterol on the dynamic equilibrium of cholesterol in rats. J.Nutr. 109:2076-2084,1979.

Matsukubo,M.P., Singal,P.K., Dhalla,N.S. Negatively charged sites and calcium binding in the isolated rat heart sarcolemma. Basic Res. Cardiol. 76:16-28,1981.

McElhaney,R.N., DeGier,J., Van Der Naut-kok,E.C.M. The effect of alterations in fatty acid composition and cholesterol content on the permeability of Mycoplasma laidlawii B cells and derived liposomes. Biochim. Biophys. Acta 298:500-512,1973.

McNamara,D.B., Sulakhe,P.V., Singh,J.N., Dhalla,N.S. Properties of the heart sarcolemmal Na^+-K^+ATPase. J. Biochem. 75:795-803,1974.

Melax,H., Leeson,T.S. Comparative electron microscope studies of the myocardium in adult rats fed on normal and cholesterol diets. J. Mole. Cell. Cardiol. 7:195-202,1975.

Miettinen,M., Turpeinen,O., Karvonen,M.K., Elosuo,R., Paavilainen,E. Effect of cholesterol-lowering diet on mortality

from coronary heart disease and other causes. A twelve year clinical trial in men and women. Lancet 2:835-838,1972.

Mitropoulos,K.A., Venkatesan,S., Balasubramaniam,S. On the mechanism of regulation of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase and acyl coenzyme A acyltransferase by dietary fat. Biochim. Biophys. Acta 619:247-257,1980.

Miyamoto,H., Racker,E. Solubilization and partial purification of the Ca^{2+}/Na^{+} antiporter from the plasma membrane of bovine heart. J. Biol. Chem. 255:2656-2658,1980.

Moraski,R.E., Russel,R.O.Jr., Smith,M., Rackley,C.E. Left ventricular function in patients with and without myocardial infarction and one, two or three vessel coronary artery disease. Am. J. Cardiol. 35:1-10, 1975.

Morcos,N.G., Drummond,G.I. $(Ca^{2+}+Mg^{2+})$-ATPase in enriched sarcolemma from dog heart. Biochim. Biophys. Acta 598:27-39,1980.

Murray,M., Schrodt,G.R., Berg,H.F. Mitochondrial alterations in human and experimental atherosclerosis. Biochem. Med. 2:118-135,1968.

Nayler,W.G. Some factors involved in the maintainance and regulation of cardiac contractility. Circ. Res. 20,21,Suppl. 3:213-217,1967.

Nelson,G.J. Composition of neutral lipids from erythrocytes of common mammals. J. Lipid Res. 8:374-379,1967.

Noguchi,T., Freed,S. Dissociation of lipid components and reconstitution at −75 C of Mg^{2+} dependent, Na^{+} and K^{+} stimulated, adenosine triphosphatase in rat brain. Nature (New Biol.) 230:148-150,1971.

Ohki,E. The electrical capacitance of phospholipid membranes. Biophys. J. 9:1195-1205,1969.

Okano,G., Matsuzuka,H., Shimojo,T. A comparative study of the lipid composition of white, intermediate, red and heart muscle in rats. Biochim. Biophys. Acta 619:167-175,1980.

Oliver,M.F. Trials using drugs to control hyperlipidaemia. In Lipoproteins, Atherosclerosis and Coronary Heart Disease (Miller,N.E., Lewis,B.,eds.) Elsevier,Amsterdam.165-195,1981a.

Oliver,M.F. Serum cholesterol-the knave of hearts and the joker. Lancet: 1090-1095,1981b.

Olsson,A.G. Exercise ST segment depressions in asymptomatic hyperlipoproteinaemia and its relation to various lipoprotein classes. Adv. Exp. Med. Biol. 82:118-120,1977.

Owens,K., Ruth,R.C., Weglicki,W.B., Stam,A.C., Sonnenblick,E.H. Fragmented sarcoplasmic reticulum of the cardiomyopathic Syrian hamster:lipid composition, Ca^{++} transport and Ca^{++} stimulated ATPase. In Recent Advances in Studies on Cardiac Structure and Metabolism, Vol.4 (A. Fleckenstein, G. Rona,eds.) University Park Press,Baltimore. 540-550,1974.

Palatini,P., Dabbeni-Sala,F., Bruni,A. Reactivition of phospholipid-depleted sodium, potassium-stimulated ATPase. Biochim. Biophys. Acta 288:413-422,1972.

Papahadjopoulos,D., Cowden,M., Kimelberg,H. Role of cholesterol in membranes. Membrane permeability and enzyme activity. Biochim. Biophys. Acta 330: 8-26,1973.

Papahadjopoulos,D., Nir,S., Ohki,S. Permeability properties of phospholipid membranes:effct of temperature and cholesterol. Biochim. Biophys. Acta 266:561-583,1971.

Papahadjopoulos,D., Watkins,J.C. Phospholipid model membranes II. Permeability properties of hydrated liquid crystals. Biochim. Biophys. Acta 135:639-652,1967.

Patton,N.M., Brown,R.V., Middleton,C.C. Familial cholesterolemia in pigeons. Atherosclerosis 19:307-311,1974.

Pessina,G.P., Paulesu,L., Bocci,V. Red cell modifications in cholesterol fed rabbits. Int. J. Biochem. 13:803-810,1981.

Peter,J.B., Fiehn,W. Diazacholesterol myotonia: Accumulation of desmosterol and increased adenosine triphosphatase activity of sarcolemma. Science 179:910-912,1973.

Peters,W.H.M., Fleuren-Jacobs,A.M.M., DePont,J.J.H.H.M., Bonting,S.L. Studies on $(Na^{+}+K^{+})$-Activated ATPase. Content and role of cholesterol and other neutral lipids in highly purified

rabbit kidney enzyme preparation. Biochim. Biophys. Acta 649:541-549,1981.

Peterson,D.W., Griffith,D.W., Napolitano,C.A. Decreased myocardial contractility in papillary muscles from atherosclerotic rabbits. Circ. Res. 45:338-346,1979.

Pfeiffer,C., Flemming,B., Gunther,J., Vetter,R., Goos,H.,Pfeifer,K. Veranderungen der electromechanischen ankopplung beim Kaninchenmyokard nach Vertutterung von Kholesterol. Acta Biol. Med. Germ. 37:1037-1047,1978.

Portman,O.W., Alexander,M., O´Malley,J.P. Metabolism of free and esterfied cholesterol and apolipoproteins of plasma low and high density lipoproteins. Biochim. Biophys. Acta 619:545-558,1980.

Poznansky,M., Kirkwood,D., Solomon,A.K. Modulation of red cell K^+ transport by membrane lipids. Biochim. Biophys. Acta 330:351-355,1973.

Pumphrey,A.M. Incorporation of [P^{32}] orthophosphate into brain slice phospholipids and their precursors. Biochem. J. 112:61-70,1969.

Quinn,P.J., Chapman,D. The dynamics of membrane structure. CRC Critical Reviews in Biochemistry. 1-117,1980.

Rackley,G.E., Russel,R.O.Jr. Left ventricular function in acute and chronic coronary artery disease. Ann. Rev. Med. 26:105-120,1975.

Reuter,H. Properties of two inward membrane currents in heart. Ann. Rev. Physiol. 41:413-424,1979.

Reynolds,E.S. The use of lead citrate of high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17:208-210,1963.

Riley,J.W., Glickman,R.M., Green,P.H.R., Tall,A.T. The effect of chonic cholesterol feeding on intestinal lipoproteins in the rat. J. Lipid Res. 21:942-952,1980.

Riordan,J.R. Ordering of bulk membrane lipid or protein promotes activity of plasma membrane Mg^{2+}ATPase. Can. J. Biochem. 58:928-934,1980.

Roelofsen,B. The (non) specificity in the lipid-requirement of calcium and (sodium plus potassium)-transporting adenosine triphosphatases. Life Sci. 29:2235-2247,1981.

Rosendorff,C., Hoffman,J.I.E., Verrier,E.D., Rouleau,J., Boerboom,L.E. Cholesterol potentiates the coronary artery response to norepinephrine in anesthetized and concious dogs. Circ. Res. 48:320-329,1981.

Rosenhammer,G., Carlson,L.A. Effect of combined clofibrate, nicotinic acid treatment in ischaemic heart disease. Atherosclerosis 37:129-138,1980.

Ross,A.C., Minick,C.R., Zilversmit,D.B., Equal atherosclerosis in

rabbits fed cholesterol-free, low fat diet or cholesterol supplemented diet. Atherosclerosis 29:201-315,1978.

Ross,A.C., Zilversmit,D.B. Chylomicron remnant cholesteryl esters as the major constituent of very low density lipoproteins in plasma of cholesterol-fed rabbits. J. Lipid Res. 8:169-181,1977.

Roth,R.I., Patsch,J.R. Metabolism of the "arginine rich" protein in the rabbit. Fed. Proc. 37:1322,1974.

Rouser,G., Nelson,G.J., Fleishcer,S., Simon,G. Lipid composition of animal cell membranes, organelles and organs. In Biological Membranes (D. Chapman,ed.) Academic Press, London. 5-70,1968.

Rouslin,W., MacGee,J., Wesselman,A.R., Adams,R.J., Gupta,S. Canine myocardial ischemia: Increased mitochondrial cholesterol, a marker of mitochondrial membrane injury. J. Mole. Cell. Cardiol. 12:1475-1482, 1980.

Sabine,J.R. Cholesterol. M.Dekker, New York. 1977.

Salt,P.J., Iverson,L.L. Inhibition of the extraneuronal uptake of catecholamine in the isolated rat heart by cholesterol. Nature (New Biol.) 238:91-92,1972.

Sambhi,M.P., White,F.D. The electrocardiogram of the normal and hypertensive rat. Circ. Res. 8:129-134,1960.

Sanborn,W.D., Langer,G.A. Specific uncoupling of excitation and contraction in mammalian cardiac tissue by lanthanum. J. Gen.

Physiol. 56:191-217,1970.

Savarese,J.J., Berkowitz,B.A. B-Adrenergic receptor decrease in diabetic rat heart. Life Sci. 25:2075-2078,1979.

Schwartz,A., Lindenmayer,G.E., Allen,J.C. The sodium-potassium adenosine triphosphatase. Pharmacological, physiological and biochemical aspect. Pharmacol. Rev. 27:1-134,1975.

Seiler,D., Fiehn,W. Effect of cholesterol oxidation on (Na^+,K^+)ATPase activity of erythrocyte membranes. Experientia 32:849-850,1976.

Senges,J., Weihe,E., Brachmann,J., Pelzer,D., Nimmerich,H., Kubler,W. Effects of hypercholesterolaemia without ischemia on some electrophysiological and ultrastructural properties of the rabbit heart. J. Mole. Cell. Cardiol. 13:253-264,1981.

Shine,K.I., Serena,S.D., Langer,G.A. Kinetic localization of contractile calcium in rabbit myocardium. Am. J. Physiol. 221:1408-1417,1971.

Shinitzky,M., Borochov,H., Wilbrandt,W. Lipid fluidity as a physiological regulator of membrane transport and enzyme activities. In Membrane Transport in Erythrocytes (U.V. Lassen, H.H. Ussing, J.O. Weith,eds.) Munksgaard, Copenhagen. 91-102,1980.

Shinitzky,M., Henkart,P. Fluidity of cell membranes-Current concepts and trends. Int. Rev. Cytol. 60:121-147,1979.

Shurtliff,D. Some characteristics related to the incidence of cardiovascular disease and death. Framingham Study: 18-year follow-up. The Framingham Study, Section 30 (W.B. Kannel, T. Gordon,eds.) Washington,D.C. US Government Printing Office 1974. (DHEW publication (NIH) No. 74-599).

Singal,P.K., Dhillon,K.S., Beamish,R.E., Dhalla,N.S. Protective effect of zinc against catecholamine-induced myocardial changes. Electrocardiographic and ultrastructural studies. Lab. Invest. 44:426-433,1981.

Singal,P.K., Matsukubo,M.P., Dhalla,N.S. Calcium-related changes in the ultrastructure of mammalian myocardium. Br. J. Exp. Path. 60:96-106, 1979.

Singal,P.K., Yates,J.C., Lee,S.L., Dhalla,N.S. Effects of Na^{+} and K^{+}- free perfusion on the heart ultrastructure and subcellular Ca^{2+} transport. J. Mole. Cell. Cardiol. 10,Suppl.1:105,1978.

Skipski,V.P. Lipid composition of lipoproteins in normal and diseased states. In Blood Lipids and Lipoproteins:Quantitation, Composition and Metabolism (G.J. Nelson,ed.) Wiley-Interscience, New York. Chap.11,1972.

Slack,B.E., Boegman,R.J., Downie,J.W., Jasmin,G. Cardiac membrane cholesterol in dystrophic and verapamil-treated hamsters. J. Mole. Cell. Cardiol. 12:179-185,1980.

Stanley,R.J., Subramanian,R., Lie,J.T. Cholesterol pericarditis

terminating as contrictive calcific pericarditis. Am. J. Cardiol. 46:511-514,1981.

Stuart,M.J., Gerrard,J.M., White,J.G. Effect of cholesterol on production of thromboxane B_2 by platelets in vitro. N. Engl. J. Med. 302:6-10,1980.

Sulakhe,P.V., Dhalla,N.S. Excitation-contraction coupling in heart VII. Calcium accumulation in subcellular particles in congestive heart failure. J. Clin. Invest. 50:1019-1027,1971.

Sulakhe,P.V., Dhalla,N.S. Alterations in the activity of cardiac Na^+-K^+- stimulated ATPase in congestive heart failure. Exp. Mole. Pathol. 18:100-111, 1973.

Swift,L.L., Manowitz,N.R., Dunn,G.D., LeQuire,V.S. Isolation and characterization of hepatic Golgi lipoproteins from hypercholesterolemic rats. J. Clin. Invest. 66:415-425,1980.

Szabo,B. Dual methanism for the action of cholesterol on membrane permeability. Nature 252:47-49,1974.

Tada,M., Yamamoto,T., Tonomura,Y. Molecular mechanism of active calcium transport by sarcoplasmic reticulum. Physiol. Rev. 58:1-79,1977.

Takeo,S., Daly,M.J., Anand-Srivastava,M.B., Dhalla,N.S. Influence of neuraminidase treatment on rat heart sarcolemma. J. Mole. Cell. Cardiol. 12:211-217,1980.

Takeo,S., Duke,P., Taam,G.M.L., Singal,P.K., Dhalla,N.S. Effects of lanthanum on heart sarcolemmal ATPase and calcium binding activities. Can. J. Physiol. Pharmacol. 57:496-503,1979.

Tall,A.R., Atkinson,D., Small,D.M., Mahley,R.W. Characterization of the lipoproteins of atherosclerotic swine. J. Biol. Chem. 252:728-729,1977.

Tanaka,R., Sakamoto,T. Molecular structure in phospholipid essential to activiate (Na^+;K^+-Mg^{2+})-dependent ATPase and (K^+-Mg^{2+})-dependent phosphatase of bovine cerebral cortex. Biochim. Biophys. Acta 193: 384-393,1969.

Tanaka,R. Strickland,K.P. Role of phospholipids in the activation of Na^++K^+ activated adenosine triphosphatase of beef brain. Arch. Biochem. Biophys. 111:583-592,1965.

Taussky,H.H., Shorr,E. A microcolorimetric method for the determination of inorganic phosphorous. J. Biol. Chem. 202:675-685,1953.

Thomas,R.C. Electrogenic sodium pump in nerve and muscle cells. Physiol. Rev. 52:563-594,1972.

Vahouny,G.V., Tamboli,A., Vander Malen,M., Jensen,H., Turi,J-S, Schotz, M.C. Lipoprotein lipase activity of adult rat cardiocytes. Biochim. Biophys. Acta 620:63-69,1980.

Van Zupphen,L.F.M., Den Bieman,M.G.C. Cholesterol response in inbred strains of rats, Rattus norvegicus. J. Nutr.

111:1833-1838,1981.

Walker,J.G., Wheeler,K.P. Polar head-groups and acyl side-chain requirements for phospholipid dependent (Na^++K^+)-ATPase. Biochim. Biophys. Acta 394:135-144,1975.

Warren,G.B., Houslay,M.D., Metcalfe,J.C., Birdsall,N.J.M. Cholesterol is excluded from the phospholipid annulus surrounding an active calcium transport protein. Nature 255:684-687,1975.

Warren,L. The thiobarbituric acid assay of sialic acids. J. Biol. Chem. 234:1971-1975,1959.

Wenzel,D.G., Acosta,D., Kretsinger,W.B. Cholesterol and beta-lipoprotein on lipid inclusions and lysosomal and mitochondrial permeability of cultured heart muscle and endothelial cells. Res. Commun. Chem. Pathol. Pharmacol. 12:789-792,1975.

Wexler,B.C., Judd,J.R., Kittinger,G.W. Myocardial necrosis induced by isoproterenol in rats - Changes in serum protein, lipoprotein, lipids and glucose during active necrosis and repair in arteriosclerotic and non-arteriosclerotic animals. Angiology 19:665-676.1968.

Wheeler,K.P., Walker,J.A., Barker,D.M. Lipid requirement of the membrane sodium plus potassium - dependent adenosine triphosphatase system. Biochem. J. 146:713-722,1975.

Wheeler,K.P., Whittam,R. ATPase activity of the sodium pump needs phosphatidylserine. Nature 225:449-450,1970.

Wiley,J.S., Cooper,R.A. Inhibition of cation co-transport by cholesterol enrichment of human red cell membranes. Biochim. Biophys. Acta 413:425-431, 1975.

Wilson,J.D. Relation between dietary cholesterol and bile acid excretion in the rat. Am. J. Physiol. 203:1029-1032,1962.

Yamori,Y, Kitamura,Y., Nara,Y., Iritani,N. Mechanism of hypercholesterolemia in arteriolipidosis-prone rats (ALR). Jap. Circ. J. 45:1068-1073,1981.

Yates,J.C., Dhalla,N.S. Structural and functional changes associated with failure and recovery of hearts after perfusion with Ca^{++}-free medium. J. Mole. Cell. Cardiol. 7:91-103,1975.

Yokoyama,M., Henry,P.D. Sensitization of isolated canine coronary arteries to calcium ions after exposure to cholesterol. Circ. Res. 45: 479-486,1979.