SARCOPLASMIC RETICULAR CALCIUM TRANSPORT IN CONGESTIVE HEART FAILURE SECONDARY TO MYOCARDIAL INFARCTION

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Presented to the University of Manitoba

In Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy

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BY

MUHAMMAD NASIR AFZAL

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

DOCTOR OF PHILOSOPHY

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I. ABSTRACT

Earlier studies have revealed that the sarcoplasmic reticulum (SR) by virtue of its ability to accumulate Ca^{2+}, plays an important role in the relaxation of the cardiac muscle. Although varying degrees of defects in the Ca^{2+}-transport activity of SR has been reported in different types of failing hearts, no information on the status of SR function during the development of congestive heart failure due to myocardial infarction is available in the literature. This study was, therefore, undertaken to examine the status of SR in congestive heart failure subsequent to myocardial infarction upon ligating the left coronary artery in rats for 4, 8, and 16 weeks. The left heart function was assessed with an intraventricular pressure transducer and SR membrane fractions from the right ventricle and the viable left ventricle were used for biochemical studies. The hemodynamic data revealed that the left ventricular end-diastolic pressure was increased, whereas the rates of contraction and relaxation were decreased significantly in experimental animals at 4, 8, and 16 weeks of coronary ligation as compared to controls. Although the presence of ascites was evident at 4 weeks, other signs of congestive heart failure, like lung congestion, were present at 8 and 16 weeks post-myocardial infarction in experimental rats . On the basis of these data, the experimental animals at 4, 8, and 16 weeks after the induction of myocardial infarction were considered to represent early, moderate and severe stages of congestive heart failure.

The SR Ca^{2+} uptake activities measured in the presence of oxalate were decreased progressively in the viable left ventricle of the experimental animals at 4, 8, and 16 weeks post-myocardial infarction. On the other hand, SR Ca^{2+} uptake activity in the right ventricle

was increased at 4 and 8 weeks but no change was apparent at 16 weeks after coronary occlusion. The decrease in SR Ca^{2+} uptake in the left ventricle and an increase in right ventricle were associated with corresponding changes in maximal velocity values (Vmax) without any alteration in the affinity for Ca^{2+}. The opposite changes in the right and left ventricles were dependent on the scar size as well as the time after inducing myocardial infarction and reflect difference in the functional status of the left and the right hearts in animals subsequent to myocardial infarction. The observed changes in SR Ca^{2+} uptake in both right and left ventricles were not due to any artifact associated with the isolation and purification of the SR vesicles or leakage of Ca^{2+} through the Ca^{2+}-release channels. These findings suggest differential remodelling of the SR membranes with respect to the Ca^{2+} pump mechanism in the left and the right ventricle during the development of congestive heart failure.

Since SR Ca^{2+} uptake is an energy dependent process, the SR Ca^{2+} ATPase in the left ventricles of experimental animals with severe stages of heart failure was characterized. The SR Ca^{2+}-stimulated ATPase activity, when examined in the presence of NaN_3 or different concentrations of KCl was decreased in the failing hearts. The rate of ATP hydrolysis at various concentrations of Ca^{2+} as well as ATP was found to be depressed in experimental heart preparations. Double reciprocal plots of the results showed a decrease in Vmax for ATP hydrolysis without any change in the affinity of experimental SR for Ca^{2+} or ATP. In contrast to the left ventricle, the SR Ca^{2+}-stimulated ATPase activity in the right ventricle from animals with congestive heart failure was higher than the control values. These results indicate that changes in SR Ca^{2+}-transport activities during the development

of congestive heart failure were due to alterations in the Ca^{2+}-pump mechanisms.

It is now well known that phosphorylation of SR membranes with cAMP-dependent protein kinase or calmodulin is associated with an increase in the Ca^{2+}-pump activity. In this study we examined the SR Ca^{2+}-ATPase activities in presence of various concentrations of cAMP-dependent protein kinase as well as calmodulin. The results indicated that addition of these phosphorylating agents produced a concentration dependent stimulation of the Ca^{2+}-ATPase in both control and experimental preparations; however, the enzyme activities in the failing hearts in presence of low concentrations of these agents were decreased in comparison to the control preparations. Similar changes were also observed when the measurements were made in the presence of different concentrations of Ca^{2+}. The phosphorylation activities of the experimental SR preparations due to cyclic AMP-protein kinase as well as calmodulin were lower than the control preparations. Although the stimulatory effects of both cyclic AMP dependent protein kinase and calmodulin on Ca^{2+}-ATPase and phosphorylation activities in experimental preparations were greater than those in control SR, such differences in sensitivities appear to be due to depressed base line activities in experimental preparations. These results indicate defects in regulatory mechanisms for SR Ca^{2+}-pump system in the failing heart.

The mechanisms of depressed Ca^{2+}-pump activities in experimental preparations were studied by examining the composition of SR with respect to SH groups as well as protein and phospholipid contents. No differences in the total and free SH groups as well as electrophoretic pattern of protein peaks in the polyacrylamide gel were seen between control and failing heart preparations. No changes in cholesterol contents or

cholesterol/phospholipids ratio in the experimental SR preparations were evident. On the other hand, marked changes in the phospholipid composition of the SR from failing hearts were seen; lysophosphatidylcholine, sphingomyelin and phosphatidylinositol contents were increased whereas phosphatidylcholine and phosphatidylserine contents were decreased. In view of the important role of phospholipids in modifying the SR Ca^{2+}-pump activities, it appears that the observed changes in SR Ca^{2+}-pump in failing hearts may partly be due to the altered phospholipid composition of the SR membrane.

In order to assess if the alterations in SR Ca^{2+}-pump system in the failing hearts were due to any defects at the genetic level, the levels of mRNA for Ca^{2+}-ATPase in the left ventricles of control and experimental animals at 4, 8, and 16 weeks were measured by using Northern and slot blot analysis. The levels of GAPDH mRNA and 28S rRNA were estimated as indication of the total mRNA and total rRNA, respectively. The relative amounts of mRNA for Ca^{2+}-ATPase with respect to mRNA levels of GAPDH and 28S rRNA were decreased in experimental hearts at 4, 8. and 16 week post-myocardial infarction. These results suggest that the depressed SR Ca^{2+}-pump activity in failing hearts may partly be due to abnormalities in the gene transcription during the development of CHF.

II. BACKGROUND AND REVIEW OF LITERATURE

1. Pathophysiology of Cardiac Dysfunction in Congestive Heart Failure

Congestive heart failure (CHF) is a pathophysiological state in which the heart is unable to supply sufficient oxygenated blood to meet the metabolic demands of the body and is thus associated with poor venous return as well as fluid accumulation in the lungs and peripheral tissues. The importance of examining the epidemiology of CHF may be appreciated by considering the magnitude of the problem (1) as the prevalence of CHF in the United States is estimated to be 2.3 million people. The increasing prevalence of CHF since 1968 is because of the aging population as well as due to improvements in medical therapy which has resulted in longer lives for patients with cardiovascular disease (1,2). The number of hospital discharges for CHF has more than doubled from 1973 to 1986 in the United States (3). A recent study has confirmed that CHF is the most common discharge diagnosis in the major diagnostic category of diseases and disorders of the circulatory system in elderly patients (4). Physicians now realize that the prognosis of patients with heart failure is far worse than originally anticipated; only 50 % of patients are still alive 5 years after the onset of signs and symptoms of CHF (5). Although precise data are not available, approximately 40 % of patients die suddenly when they are in stable condition, while others die primarily due to signs and symptoms of progressive cardiac dysfunction during the course of CHF (5).

The major pathophysiologic processes that contribute to the development of CHF

include loss of cardiac muscle, pressure overload, volume overload, decreased contractility and restricted filling (6). Etiological studies of congestive heart failure reveal that the most prevalent cause of CHF is the coronary artery disease followed by systemic hypertension, valvular heart disease, cardiomyopathy and congenital heart disease. Coronary artery disease comprises approximately two-thirds of patients with severe class IV heart failure (6). There are a number of factors that contribute to CHF in the presence of coronary artery disease. The most important of these is the loss of muscle due to myocardial infarction and subsequent scar formation. Depression in the pumping ability of the heart and ventricular reserve naturally follows from the reduction in the number of contractile elements and ventricular compliance. When a part of heart muscle is lost during infarction, there occurs an increase in the work load on the remaining viable muscle. The manner in which pressure or volume overload eventually leads to an irreversible reduction in the intrinsic contractility, it is likely that the same stress may be operative in the viable ventricular muscle with large myocardial infarcts (6).

In response to reduced cardiac output as a result of decreased contractility, a number of neurohormonal mechanisms are activated to preserve the circulatory homeostasis in the course of CHF (7). There is a good relation between the severity of CHF and the level of circulating catecholamines which presumably maintain the cardiovascular function in the face of decreased intrinsic contractility. Circulating catecholamine levels are increased in CHF due to an increase in the sympathetic tone and release of catecholamines from the adrenal medulla (8). Any change in cardiac performance that threatens renal blood flow is detected by sensory receptors in the renal arterioles and leads to the release of renin from

the kidney. Most patients with CHF demonstrate an excessive activation of the renin-angiotensin system at rest as well as during exercise. Once formed, angiotensin II may potentiate the effects of the other vasoconstrictive hormones in heart failure; this is particularly achieved by facilitating the central and peripheral effects of the sympathetic system as well as by enhancing the release of vasopressin from the pituitary. All these systems may then act in concert to greatly exacerbate conditions in patients with failing hearts. It has thus become evident over the past several years that the compensatory mechanisms which are helpful in maintaining cardiac function during initial stages, have the potential for overshooting and producing deleterious effects over a prolonged period (6).

As congestive heart failure has become one of the most serious health problems and common cause of death, different experimental models of heart failure have been developed for assessment of the failing myocardium. However, there are very few models of congestive heart failure secondary to myocardial infarction (9). In this regard, Johns and Olson (10) published the first comprehensive description of surgical ligation of the coronary artery in rats. These investigators preferred rats over dogs because the size of infarction and mortality due to infarction in dogs was unpredictable, presumably due to collateral blood supply and differences among various breeds. By establishing the arterial network of the heart and the incidence of mortality and infarction in rats, these investigators set the stage for further studies of experimentally produced myocardial infarction leading to congestive heart failure. Several years later with some modifications of the earlier techniques, Selye et al (11) were able to show larger myocardial infarct secondary to coronary occlusion and longer survival of the experimental animals. This rat model of myocardial infarction was used to study

morphological features of the evolving infarcts and the effect of various interventions on the infarct size (12-14) as well as the metabolic and mechanical adaptations in these hearts (15). Although the rat heart lacks collateral circulation and therefore is not similar to human heart, this model of infarction is useful by virtue of the reproducibility of the infarct size. Rat is also preferred over large animals because of the relative low mortality and cost, predictability of inducing a large size infarct and development of CHF in a large proportion of surviving animals.

Qualitative and quantitative changes during pathological evolution have been studied in the rat model of myocardial infarction during the past three decades. Histological study of 1-21 day old myocardial infarcted rats showed that following a mild and acute inflamatory response at the margins of the necrotic myocardium, there is chronic inflamation, vascular and collagenous proliferation and resorption of necrotic tissue which progresses until scar formation is complete usually by 21 days (16). In another study, the process of scar reabsorption has been carefully assessed by morphometric techniques in rat heart and it was shown that infarcts affecting an average 23 % of the left ventricle were characterized by a 27 % hypertrophic growth and infarcts with an average of 50 % loss of muscle, resulted in 83 % expansion of the spared myocardium (17). Cellular hypertrophy in both cases involved a significant increase in the myocyte transverse area and the myocyte length. These alterations combined with the deficient reconstitution of myocardial mass following large infarcts resulted in 25 %, 29 %, and 30 % deficits in the absolute amounts of capillary lumen, surface and length per ventricle, respectively (17).

Measurement of scar size is a valuable indicator of the extent of damage to the

myocardium. Left ventricular function in rats with large myocardial infarction was assessed in a study in which the relationship between infarct size and ventricular performance was defined in rats 3 weeks after the left coronary artery ligation (18). A small change in baseline hemodynamics or peak indices of cardiac pumping and pressure generating ability was observed in experimental animals with small left ventricular infarct (4-30 %) when compared to controls. Experimental animals with moderate myocardial infarction (31-45 %) had normal baseline hemodynamics but the cardiac pump function was reduced upon volume overload. Rats with large infarcts (45 %) had congestive heart failure characterized by elevated filling pressures, reduced cardiac output, and minimum capacity to respond to pre-load and after-load stress. Subsequent work indicated that coronary ligation in the rat provided an experimental model of graded left ventricular dysfunction, in which the impairment of left ventricular function was directly related to the extent of myocardial loss (19,20).

The presence and extent of left ventricular dilatation was a parameter used in the prognosis for survival of post-infarct patients (21). Left ventricular distention and dilatation were illustrated by upward and rightward movement on the pressure-volume relationship in experimental animals when compared to control (19,20). It was suggested that increased ventricular volume helped to maintain peak stroke volume despite the linear reduction of ejection fraction index with increasing infarct size in animals 3-4 weeks after the induction of myocardial infarction. A study of the time course (4, 7, 10, 20, and 35 weeks) of hemodynamic changes in rats with healed severe myocardial infarction (43 %) revealed that at 4 weeks, peak left ventricular blood pressure, left ventricular maximum rate of

contraction, mean blood pressure, and systemic vascular resistance were decreased when compared to control (22). Furthermore, the left ventricular end-diastolic pressure was increased by 313 % in these experimental animals. These investigators found no further significant deviation in blood pressure, left ventricular end-diastolic pressure, left ventricular blood pressure, and rate of left ventricular contraction after 4 weeks of infarction. However, cardiac output and systemic vascular resistance changed progressively. These changes were accompanied by decreased blood flow to stomach, small intestine and kidney by 35 weeks which reflects progressive cardiac decompensation and terminal heart failure in these animals (22). Recent use of this model for investigation of pharmacological tolerance to nitrate therapy has revealed that the rapid reduction of left ventricular end diastolic pressure caused by glyceryl trinitrate resembles that seen in man and therefore, could be used as a model for examining the mechanisms of action and tolerance of different pharmacological agents (23).

Under normal physiological conditions, heart works as a synchronized unit but under pathological conditions the response of each area of the heart may be different from other according to the pathological stress. In humans, the most common cause of right-sided heart failure and pulmonary hypertension is left-sided heart failure (6). When left ventricular filling pressure is elevated, right ventricular systolic pressure increases to maintain the pressure gradient across the pulmonary bed. Hence, the elevation of left ventricular end-diastolic pressure produced by extensive infarction appears to result in right ventricular hypertrophy and has been linked to the hemodynamic state of left ventricle (18,24). Since morphological adaptation of the right ventricle in animals with large left ventricle infarct is

characterized by concentric hypertrophy (25) whereas that of the viable nonischemic left ventricle is both concentric and accentric hypertrophy (17,26), it can be argued that changes in the left ventricle may differ from those in the right ventricle. This view is consistent with the observation that region-specific differences in contractile proteins are manifested in hearts subsequent to myocardial infarction (26). Thus, left ventricular dysfunction may eventually cause severe right ventricular overload and dysfunction such that the right ventricle becomes a limiting factor in heart failure.

To overcome the loss of a part of ventricle, the surviving myocardium undergoes hypertrophy to maintain the cardiac function. Myocardial hypertrophy after myocardial infarction has long been thought to be beneficial as a nonspecific compensatory mechanism for increasing ventricular mass and functional capacity of the infarcted hearts (27). Cardiac hypertrophy involves a sequence of events including initiating signals, coupling mechanisms and regulation of gene expression. The initiating signals include cell deformation, neurotransmitters and hormones, resulting in activation of membrane channels and enzymes. The mechanisms of decompensation of the hypertrophied heart leading to mechanical failure could involve impaired function of any of these steps in this complex pathway. While the validity of this concept remains to be established, the cellular processes associated with the incidence of hypertrophy in infarcted rat heart has yielded some useful information (17,25). An early report of cardiac hypertrophy in experimental rat hearts 12 weeks after the induction of myocardial infarction suggested that overall cardiac hypertrophy was about 170 % when compared to control (28). Recent work on rat heart with medium (>15-30 %) or large (>30 %) infarct size has shown that the myocytes had undergone significant

hypertrophy after 5 weeks of coronary ligation (29). In a study regarding growth response of the right ventricle in rats following ligation of the left coronary artery, the weight of the right ventricle was increased by 30 % and this hypertrophic change was characterized by 17 % ventricular wall thickening and 13 % greater diameter of myocytes (25). Inadequate growth of the microvasculature that supports tissue oxygenation was evidenced by relative decreases in capillary luminal volume density, capillary luminal surface density, and by an increase in the average maximum distance from the capillary wall to the mitochondria of myocytes. These authors suggested that inadequate compensation of the capillary bed results in alterations of the structural properties of the microvasculature implicated in oxygen avilablity, diffusion, and transport. The surviving ventricle may be vulnerable to additional ischemic episodes (25). In a study regarding biological basis of myocardial function in hypertension and coronary disease, it was concluded that myocardial hypertrophy is a biological adjustment to the environment and chronic heart failure is an adaptation indicating its limits (30).

In different animal models, the heart failure is commonly preceeded by cardiac hypertrophy to prevent acute cardiac insufficiency. Under in vivo conditions, the ventricular function in cardiac hypertrophy is normal or increased whereas in heart failure with clinical signs such as pulmonary congestion, liver congestion and ascites, ventricular function is markedly depressed (31,32). Cardiac muscle from nonfailing hypertrophied hearts, when examined under in vitro conditions, has been shown to generate normal, increased, or decreased contractile force. Increased and decreased velocities of muscle shortening in non failing hypertrophied cardiac muscles have been observed to be associated with high and low

myosin ATPase activities respectively (33). Such diffferences in the functional and biochemical properties of the nonfailing hypertrophied hearts are considered to be due to several factors such as the degree of ventricular wall stress, the duration of stress, the nature of inciting stimulus as well as the species, age and health of the animals (32,43). Accordingly, it has been proposed that nonfailing cardiac hypertrophy is physiological when the contractile function is normal or supernormal but it is pathological when the contractile function is depressed (32). It is therefore considered important to study the course of alterations in an animal model of congestive heart failure to gain detailed information regarding the mechanisms of these abnormalities in the surviving myocardium without any drug intervention.

The functional, biochemical and morphological alterations that occur in ischemic myocardium have been studied extensively, however, the adaptive process that occurs in surviving tissue of the well healed, infarcted heart is not understood. Several associated biochemical changes have been described but no precise cause-and-effect relationship has been determined. Studies regarding defective mitochondrial ATP production as a mechanism for reduced contractile force development have shown that heart failure can occur in the presence of normal myocardial perfusion and oxygen availability (34,35). From studies involving the measurement of oxidative phosphorylation and the high-energy phosphate content in failing heart, it is apparent that probably, the changes in mitochondrial function are not related to development of heart failure because the contractility of these hearts is impaired before the occurrence of any defect in mitochondrial function (36,37). Defects in myocardial energy utilization have also been postulated to play a role in the development

of heart failure because the efficiency of the heart, manifested as the ratio of work performed to oxygen utilized, is depressed in chronic myocardial failure. The possibility of a defect in the conversion of metabolic energy to contractile work has been implied by evidence which indicates that myosin heavy chains are differentially expressed and are associated with altered myofibrillar ATPase activity in heart failure (38-40). However, it has been suggested that this remodelling of the myocardium may increase the efficiency of the myocardium and thus represents a beneficial alteration rather than a cause leading to the development of heart failure (37,41).

The importance of calcium for the viability of the cardiac cell has been well recognized for almost a century. Recent advances in research involving calcium movements in the heart have been highly valuable for the formation of new concepts with respect to the physiological and pathological aspects of calcium metabolism in the myocardium. It is now well established that Ca^{2+} plays an important role in the excitation-contraction cycle of the cardiac cells. It has been suggested that abnormalities in intracellular Ca^{2+} metabolism may be the basis of depressed contractility in heart failure. Specifically, either intracellular Ca^{2+}-overload or intracellular Ca^{2+}-deficiency may be responsible for defective myocardial contractility as these events may initiate the disruption of energy-generating processes or abnormal activation of the contractile machinery (42). The striated muscle cell consists of two membrane systems, which are involved in the process of excitation-contraction coupling. The outer surface membrane, called the sarcolemma (SL), surrounds the entire muscle cell and is excited by the innervating nerve to propagate an action potential to initiate excitation-contraction coupling. The invaginations of sarcolemma into the fibre interior are

called transverse tubules or T-tubules. The T-tubule system is responsible for the spread of depolarization to the interior of the fibre and the accompanying membrane charge movement. The muscle cell has an extensive internal membrane system called the sarcoplasmic reticulum (SR), which is a membranous network surrounding myofibrils. The SR is responsible for sequestration of Ca^{2+} to allow relaxation, storage of Ca^{2+} during relaxation and release of Ca^{2+} to initiate contraction. Since cardiac sarcolemma and sarcoplasmic reticular membranes are known to participate in the beat-to-beat regulation of myoplasmic Ca^{2+} levels (43-46), a great deal of research has been focussed on abnormal sarcolemmal function in failing myocardium and some work has been carried out to identify sarcoplasmic reticular defects in heart failure. Evidence for rapid regulation of myoplasmic Ca^{2+} levels by mitochondria is lacking, but these organelles are capable of accumulation a large quantity of Ca^{2+} in vitro as well as under certain pathological conditions (45,47,48). Thus in light of the importance of Ca^{2+} ion in excitation-contraction coupling, it is intended to summarize the interaction of Ca^{2+} with myofibrils, sarcolemma, mitochondria, and sarcoplasmic reticulum in cardiac contraction and relaxation process in health and disease.

2. Role of contractile proteins in normal and failing hearts

The heart is composed of a syncytium of muscle cells with connective tissue matrix. Sarcomere, which forms 50 % of the cell mass, is the smallest functional unit of cardiac muscle and has the ability to shorten and to develop tension (49). The length of cardiac sarcomeres varies between 1.6 to 2.2 u and is dependent upon the state of contracture (50,51). Myofibrils are arranged end to end giving a striated appearance to the cardiac myocyte (51). The thin filaments surround the thick filaments and interact by overlapping ; two strands of actin form a double helix to comprise a thin filament (52). Although troponin and tropomyosin are known to play a regulatory role for the interaction of actin and myosin, an additional protein with a molecular weight of 150 KD (C-protein) has been identified in cardiac muscle (53). This C-protein has been shown to serve as a substrate for phosphorylation by cAMP-dependent protein kinase, however, the role of the interaction of this protein with other proteins in the contraction process is speculative (54). While actin and myosin form cross-bridges and shorten against mechanical load at the expense of the chemical energy contained in the high-energy phosphate bonds of ATP, the tropomyosin and troponin proteins confer Ca^{2+} sensitivity on the contractile machinery (55). Troponin C is the subunit which binds Ca^{2+} and troponin I is known to inhibit Mg^{2+}-stimulated actomyosin (55). Binding of Ca^{2+} to the troponin C subunit removes the inhibition on binding of troponin I to actin, and thus actin and myosin are allowed to interact for contraction. Cardiac muscle can respond to increases in preload with increased contractile force over the physiologic range which is observed clinically as graded improvements in cardiac

performance when diastolic volume of the heart is increased (56,57). A study concerning the effects of length on Ca^{2+} sensitivity during tension development on the isolated demembranated cardiac muscle was performed in which the cardiac troponin C was substituted with skeletal troponin C (58). The results suggest that intrinsic molecular properties of the cardiac troponin C subunit confer the length-induced autoregulation of the troponin complex which in turn regulates the activation of the actin filaments. In vitro evidence indicates that phosphorylation of serine, threonine, or tyrosine residues of the troponin I subunit by cAMP-dependent protein kinase occurs in the myocardium (59). Since the phosphorylation of troponin I in the isolated troponin complex was shown to reduce Ca^{2+} affinity, this catecholamine-specific phosphorylation process has been suggested to be associated with an increase in the rate of relaxation of the heart (60). The dark central A band of the sarcomere (thick filament), is about 1.5 u in length and is composed of myosin proteins (56). Myosin consists of a rodlike tail section each with a globular head made up of two heavy-chain subunits which are the site of ATPase activity (61).

Composition of the myosin head is related to the intrinsic ATPase activity of the myofibrils and the speed of contraction of the myocardium. Three myosin isozymes V_1, V_2, and V_3 have been described, to be composed of alpha-alpha, alpha-beta, and beta-beta subunits, respectively (40,41). Myosin ATPase activity is highest in the V_1 and lowest in the V_3 isoform. It is known that the presence of a given myosin isoform is not associated with any alteration in Ca^{2+} sensitivity of cardiac myofibrils (41). Remodelling of the failing heart as a shift in myocardial isozyme content from V_1 to V_3 has been demonstrated in many types of experimental heart disease (62-65). This isozyme shift is believed to occur at the

transcriptional level and has been demonstrated in a wide variety of mammalian hearts subjected to pressure overload (66). Support for the transcription regulation of these protein changes is evidenced in response to stimulation of alpha 1-adrenoceptors in neonatal rat cardiomyocytes and it has been suggested that hypertrophy in these cells is characterized by selective up-regulation of early developmental contractile protein isogenes, including those for skeletal alpha-actin and beta-myosin heavy chain (67). Alteration of contractile proteins is not confined to the myosin heavy-chains as changes in human light-chain myosin isoforms have also been demonstrated in human hearts subjected to increased mechanical stress (68). Increased synthesis of V_3 myosin isozyme could explain many of the functional changes such as the characteristic depression of the force-velocity curve in the failing heart. With regard to energy utilization, it has been suggested that the increase in mechanical energy derived from ATP used at the expense of depressed rate of myocardial contraction may actually be beneficial in heart failure (69). A shift of myosin isozyme contents is known to occur in the atria of failing human heart but has not been observed in human ventricle (70). Since the majority of myosin isozyme content of myocardium from ventricles of large mammals is V_3 (71), the shift in isozymes in response to hemodynamic overload may be precluded in these species. In this regard, autopsy samples from patients with hypertrophic cardiomyopathy assayed for myosin Ca^{2+}-stimulated ATPase and isoenzyme content, indicated that similar ATPase activities existed in control and cardiomyopathic heart and no evidence for the presence of different isozymes was found (72). Therefore, the mechanism involving changes in contractile proteins may not be held solely responsible for cardiac pump dysfunction observed in heart failure. The possibility of structural changes in actin, troponin, and

tropomyosin in the failing heart requires detailed investigation for any meaningful conclusion. Since it is possible to vary the maximum shortening velocity of failing hearts by pharmacologic intervention that enhances intracellular Ca^{2+} movements, it can be argued that altered sarcoplasmic reticular, mitochondrial, or sarcolemmal function rather than contractile proteins may play a role in pathogenesis of contractile failure.

3. Role of mitochondria in normal and failing hearts

The observation that isolated mitochondria could take up large amounts of Ca^{2+} is more than 25 years old (45) but recent experimental data have shown that the importance of mitochondria as cytosolic Ca^{2+} buffer has been overestimated. The precise definition of kinetic parameters of the energy-linked Ca^{2+} uptake reactions have shown the affinity of mitochondria for Ca^{2+} as 10 uM (Km) and the Vmax of 0.6 umol per mg of protein per min (45). In addition, mitochondria in situ are shown to contain between 1 and 2 nmoles of Ca^{2+} per mg of protein (73). At submicromolar free cytosolic Ca^{2+} levels, the rate of Ca^{2+} uptake by the low affinity system of mitochondria is marginal; however, this uptake is postulated to be continuous due to the presence of a transmembrane potential and this could eventually lead to calcification of the organelle (45). It should also be pointed out that the mitochondrial Ca^{2+}-uptake in normal myocardium is known to be balanced by electroneutral release of Ca^{2+} in exchange for transmembrane Na^+ so that a slow dynamic equilibrium of Ca^{2+} movement is achieved (74). It has been suggested that extramitochondrial Na^+ may play a role in the release of Ca^{2+} from the mitochondrial stores but a review of data from the studies of this nature is unable to support the theory that mitochondria can be an important means of delivery of Ca^{2+} in the normal myocardium (45). Nevertheless, increases in intracellular Na^+ by pharmacological means such as digitalis treatment may evoke release of intramitochondrial Ca^{2+} to the cytoplasm (45). Evidence from existence of mitochondrial H^+/Ca^{2+} exchange and Na^+/H^+ exchange mechanisms have been put forward by several investigators (75-78) but their significance in terms of

contractile function of the cardiac muscle is not clear at present.

The role of mitochondria in handling Ca^{2+} homeostasis in experimental heart disease has been studied in a number of animal models (43). In rabbits with heart failure due to aortic regurgitation, cellular Ca^{2+} contents were examined and were found to be normal, however, a redistribution of cellular Ca^{2+} to the mitochondria was noted (48). This redistribution of cellular Ca^{2+} from abnormal sarcoplasmic reticulum to the mitochondria may decrease the level of Ca^{2+} available to the contractile machinery and thus reduce the ability of cardiac muscle to generate contractile force. Conversely, the rate and capacity of mitochondrial Ca^{2+} uptake from the hearts of cardiomyopathic hamsters are reduced (79) and this may produce intracellular Ca^{2+} overload which may then impair contractile force development in these animals. Although cardiac mitochondria are not considered to participate in the delivery of Ca^{2+} for the genesis of the Ca^{2+} transients, these organelles may play an important role in the disruption of intracellular Ca^{2+} homeostasis during pathological conditions by storing away the excess cytosolic Ca^{2+}. Mitochondria may continue accumulating Ca^{2+} for a relatively long time without intolerable disturbance to their function and thus could serve as a safety device of great importance to the cell (45). Nonetheless, overloading mitochondria with Ca^{2+} can depress energy production and this would impair contractile force development.

4. Role of sarcolemma in normal and failing hearts

It is now well established that cardiac contraction is initiated when an action potential depolarizes the cell. Cardiac sarcolemma plays an important role in the generation and maintenance of transmembrane gradients of Na^+, K^+ and Ca^{2+} which are essential for membrane excitability. Operating together, cardiac sarcolemmal membrane -bound ion channels, ion-exchange systems and ATPase pumps contribute to the regulation of membrane potential and cardiac excitation-contraction coupling process. A rapid Ca^{2+} influx is achieved through the opening of voltage sensitive Ca^{2+} channels in the plasma membrane and the triggering of Ca^{2+} induced Ca^{2+} release depends critically on the rate of Ca^{2+} increase around the sarcoplasmic reticulum (80). In cardiac cells, two types of Ca^{2+} channels, namely L type and T type, have been identified (81,82). The L type channel, is the major pathway for voltage gated Ca^{2+} entry in mammalian cardiac cells leading to excitation-contraction coupling and intracellular Ca^{2+} transients (83). The experimental findings indicate that influx of Ca^{2+} via L type channels can provide an adequate source of Ca^{2+} to trigger the release of Ca^{2+} from the sarcoplasmic reticulum and are therefore compatible with a Ca^{2+}-induced Ca^{2+} release mechanism (84).

The functional status of Ca^{2+} channels in the cell can be monitored by determining the specific binding of Ca^{2+}-antagonists (85). Reports of increased density of Ca^{2+} channels/receptors in crude membrane preparations from hearts of genetically cardiomyopathic hamsters (86-88) raise the possibility that intracellular Ca^{2+} overload via augmented sarcolemmal Ca^{2+} influx may be the molecular basis of pathological alterations

seen in these hearts. However, no change in Ca^{2+} channel density was seen in older cardiomyopathic hamsters with extensive cardiac hypertrophy and congestive heart failure (89). In ischemic heart disease, it has been shown that [^{3}H]-nitrendipine and [^{3}H]-verapamil binding densities are reduced in hearts subjected to global ischemia or hypoxia-reoxygenation injury (90,91). Likewise the density of Ca^{2+} channels in the viable left ventricles in rats with congestive heart failure due to myocardial infarction was decreased (92). In a recent study, the level of mRNA encoding the Ca^{2+} channels was decreased by 47 % in the left ventricle from the patients with heart failure as compared to control (93). Furthermore, the number of Ca^{2+} channels in hypertrophied right ventricle of rats with congestive heart failure secondary to large left ventricular myocardial infarction were not changed as compared to control values (92). Thus the status of Ca^{2+} channels may depend on the type of heart failure.

Sympathetic nervous system plays an important role in the regulation of heart function and its influence on the heart is mediated primarily by the activation of ß-adrenoceptors and also by α-adrenoceptors to some extent (94,95). While the positive inotropic effect of catecholamines in the heart is mainly due to activation of ß-adrenoceptors, alpha 1-adrenoceptors have also been shown to exist in the heart. Depressed inotropic response of the myocardium to adrenergic stimulation has been demonstrated in both clinical heart failure and in a variety of experimental models of heart disease (96-99). The density of ß-adrenoceptors has been shown to be reduced without any change in their ligand binding affinity in congestive heart failure due to idiopathic cardiomyopathy as well as ischemic cardiomyopathy and congestive heart failure secondary to myocardial infarction

(100-102). On the other hand, no change was observed in congestive heart failure due to doxorubicin-induced cardiomyopathy (103), whereas the density of ß-adrenoceptors was increased in congestive heart failure due to aortic constriction in guinea pigs (104). Congestive heart failure due to aortic constriction in dogs was associated with an increase in the density but a decrease in the affinity of ß-adrenergic receptors (105). Furthermore, some investigators (105) have reported an increase while others (107) have observed a decrease in the density of ß-adrenoceptors in congestive heart failure due to a genetically linked cardiomyopathy in hamsters. While an increase in the density of α-adrenoceptors has been reported to occur in congestive heart failure due to aortic banding in guinea pigs (104), conflicting results showing both increase or no change in the density of α-adrenoceptors in congestive heart failure in cardiomyopathic hamsters (107,108) as well as in patients with heart failure of various etiologies (109,110) have appeared in the literature. A careful analysis of the results from these studies indicate that alterations in adrenergic receptors in the myocardium may depend upon the type and stage of congestive heart failure.

Efforts have been made by many investigators to elucidate the physiological and pathophysiologic role of sarcolemmal Na^+-Ca^{2+} exchange system in the heart since the discovery of Na^+-dependent Ca^{2+} transport twenty years ago (40,111,112). The kinetic parameters suggest that the sarcolemmal Na^+-Ca^{2+} exchange sytsem is capable of rapid movements of Ca^{2+} in and out of myocardial cell. In recent electrophysiological studies, sarcoplasmic reticulum Ca^{2+} release has demonstrated to occur in response to graded sarcolemmal Ca^{2+} influx (46) where the Na^+-Ca^{2+} exchange mechanism has been suggested to cause transarcolemmal Ca^{2+}-influx subsequent to a rapid influx of Na^+ through the fast

Na^+ channels (113). On the other hand, Bridge et al (114) have provided evidence to suggest that Ca^{2+} entering the heart cell during excitation is extruded by the Na^+-Ca^{2+} exchange system. Thus, sarcolemmal Na^+-Ca^{2+} exchange may be important in mediating both influx and efflux of Ca^{2+} and thus may be implicated in contractile function of the heart.

The Ca^{2+}-stimulated ATPase localized in the cardiac sarcolemma and described biochemically as Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase, is generally believed to be involved in transsarcolemmal Ca^{2+} extrusion (43,44,115). Sarcolemmal Ca^{2+}-pump is known to be under the control of phosphorylation/dephosphorylation reactions, mediated by Ca^{2+}-calmodulin dependent and cAMP-dependent protein kinases (116,117). This mechanism of regulation may be important in balancing the increased influx of Ca^{2+} due to beta adrenoceptor activation of the slow Ca^{2+}-channels. Alterations in sarcolemmal Na^+-Ca^{2+} exchange and Ca^{2+} pump activities have been assessed in several experimental animal models of heart failure. Recent studies have demonstrated defects varying in magnitude and direction in these sarcolemmal activities due to myocardial ischemia (118,119), hypoxia-reoxygenation injury (120), chronic diabetes (121,122) and hypertrophy resulting from either hypertension or aortic banding (29). These findings reiterate the possibility that the type and stage of heart failure is related to alterations in these sarcolemmal activities. The most extensively studied model of heart failure with respect to Na^+-Ca^{2+} exchange and Ca^{2+} pump activities is the genetic cardiomyopathic hamster. Decreased Na^+-Ca^{2+} exchange and Ca^{2+} pump activities in 120 and 280 days old (UM-X7.1 strain) as well as depressed Na^+-Ca^{2+} exchange activity in 360 days old (BIO 14.6) cardiomyopathic hamsters was observed

(89,123). ATP-dependent uptake has been observed to be decreased in early failure stage of the UM-X7.1 variety of hamsters. Depressed sarcolemmal Na^+-Ca^{2+} exchange without any changes in the sarcolemmal Ca^{2+}-pump activity has been reported in the viable left ventricle in rats with congestive heart failure due to myocardial infarction (124). The behaviour of these sarcolemmal systems in the pathogenesis of these disease states has led investigators to suggest that depression of their activity may lead to reduced Ca^{2+}-efflux from the myocardium and this may contribute to the occurrence of intracellular Ca^{2+}-overload.

The Na^+-K^+ pump, measured biochemically as Na^+-K^+ ATPase activity, is well known to be localized in the cardiac sarcolemma and carries out an uphill transport of sodium and potassium ions at the expense of ATP hydrolysis (125-127). Regulation of intracellular homeostasis of Na^+ and K^+ is vital to normal cardiac cell function as homeostasis of both of these ions is necessary in the maintenance of electrical properties of myocardium. The concept that a rise in intracellular Na^+ concentration via inhibition of the Na^+-K^+ pump enzyme contributes to sarcolemmal Ca^{2+} influx by activating sarcolemmal Na^+-Ca^{2+} exchange system was emphasized by Schwartz et al (126). Thus the inotropic effects of digitalis on the myocardium may be effected by this putative mechanism. Nevertheless, any change in the operation of sarcolemmal Na^+-K^+ ATPase activity could conceivably alter Ca^{2+} movements in the cell and thus may be seen to modify the cardiac contractile function. Many early studies were directed at the status of the sarcolemmal Na^+-K^+ ATPase enzyme in both human and experimental heart failure as it was the first cardiac enzyme to be localized at the cardiac sarcolemmal membrane. Decreased Na^+-K^+ ATPase

activity has been observed in isolated rat heart subjected to hypoxia-reoxygenation or ischemia-reperfusion injury (128), failing human heart (129), UM.X7.1 cardiomyopathic hamsters (130), rabbits with left ventricular hypertrophy and failure due to pressure overload (131) and viable left ventricle in rats with congestive heart failure due to myocardial infarction (124). However, increased Na^+-K^+ ATPase activity was observed in BIO 14.6 strain cardiomyopathic hamsters (132) and canine hearts with volume or pressure overload due to mitral valve insufficiency or aortic banding, respectively (133,134). The bidirectional changes observed in different types of failure once again support the hypothesis that these changes are dependent on etiology of the disease. Given the variation of results, it is unlikely that a change in function of this enzyme is a primary cause for contractile failure.

5. Role of sarcoplasmic reticulum in normal and failing hearts

a. SR function in normal hearts

Sarcoplasmic reticulum is a highly specialized network of membranes, which is believed to participate in cardiac contraction and relaxation by raising and lowering the cytoplasmic levels of Ca^{2+}. This membrane system is considered to represent a rapidly exchangeable calcium pool which plays an important role in heart function and metabolism (45). The main functions of SR are known to be Ca^{2+} sequestration, Ca^{2+} storage and Ca^{2+} release. The wave of excitation travelling along the transverse tubule is known to release Ca^{2+} from the sarcoplasmic reticular stores for initiating cardiac contraction. On the basis of its ability of accumulate Ca^{2+} by energy-dependent mechanisms, SR lowers the intracellular concentration of Ca^{2+} and initiates the relaxation phase of cardiac muscle. The sequestrated Ca^{2+} is stored in SR for release in the next contraction cycle. Thus any alteration in the function of SR can affect the cardiac contraction-relaxation cycle (43,45).

A role for SR in the cellular homeostasis of Ca^{2+} in muscle was first indicated about 30 years ago by experiments of Hasselbach and Makinose (135) in which a preparation of muscle microsomes, was shown to remove significant amounts of Ca^{2+} from the medium in a process that was dependent on ATP. Now it is evident that a spectrum in the relative contribution of SR to the Ca^{2+} transient exists with respect to the type of muscle and the species. For example, rat myocardium receives the largest contribution from the sarcoplasmic reticulum, and rabbit the least (136). Since SR regulate intracellular Ca^{2+} and hence the contractile state of the muscle, it is not unexpected that muscles of different

contractile properties contain correspondingly varying amounts of the SR membrane. The quantitative estimates of SR content (volume/surface area) have correlated well with the physiological profile of the muscle, the more the SR, the faster the muscle will contract and relax. Stereological data reveal considerable difference not only in total SR complement in different muscle types, but in distinct regions of the SR in each sarcomere. Regardless of species, atrial tissue SR is nearly double the volume of that in ventricle and regardless of muscle type, the volume of terminal cisternae is related inversely to the isometric contraction time. Depending on the type of muscle, the SR constitutes only 0.5 to 10 % of the cellular volume, yet it plays a major role in the regulation of muscle contractility. Such quantitative data are important when considering ultrastructural changes in certain muscle pathologies (137). For example, Anversa and colleagues found that in the left ventricle of renal hypertensive rats, the surface area and volume of SR are increased over control values and that the volume of SR in the epicardium of hypertensive animals is larger than that of the endocardium (137).

This intracellular membrane system has been the subject of numerous in situ and in vitro studies using structural, physiological and biochemical techniques. The literature is replete with morphological terms for various subregions or distinct features of SR, however, in general terms, the total SR complement may be broadly divided into two morphological categories: junctional SR and nonjunctional SR. The SR tubules which are closely opposed and attached to external membranes, either the sarcolemma or its derivative, transverse tubules (T-tubules) are known as junctional SR (138). In cardiac muscle this region of SR, is also termed terminal cisternae. This anatomical apposition is called dyad which is present

at the level of each Z band (139). The major content of this part of SR is a calcium binding glycoprotein called calsequestrin (140). Bridging the 12 to 14 nm gap between the junctional SR and T tubule membranes in cardiac muscle are regularly spaced, densely staining projections, termed as SR feet or junctional feet (141). The function of the SR feet seems to be the mechanical adhesion of the junctional SR to the transverse tubule membrane. The longitudinal reticulum which faces the myofibrils, is known as free SR because it does not participate in the formation of junctions with other membrane systems (142). Compared to the degree of specialization in junctional SR, the nonjunctional SR is considerably simpler, although not without morphological differentiation. The major function of the longitudinal reticulum appears to be the sequestration of Ca^{2+} during the relaxation phase (143). The Ca^{2+} pump is uniformly distributed in this part of the SR membrane. Unlike the junctional SR, the longitudinal network of SR does not contain calsequestrin (137).

In view of non-availability of suitable techniques for assessing the function of SR in vivo, various investigators have resorted to isolation of the fragments of SR (microsomal fraction) for studying its Ca^{2+} accumulating properties under in vitro conditions. The SR vesicles can be easily isolated and purified in considerable quantities from cardiac muscle by differential centrifugation of muscle homogenates. The major nonmembranous contaminant, actomyosin, can be removed by extracting the preparation of sarcoplasmic reticulum vesicles with 0.6 M KCl, which solubilizes actomyosin without disrupting the SR vesicles (144). The homogenization and isolation do not give rise to randomized inversion of the membrane vesicles and therefore they retain the proper orientation for Ca^{2+} transport (145). These membrane vesicles are composed of several proteins and lipids organized such

that the various proteins are situated within the basic matrix of a lipid bilayer (146). The SR vesicles are identified by their ability to accumulate Ca^{2+} from ATP containing solutions and by their Ca^{2+}dependent ATPase activity. ATP-dependent Ca^{2+} accumulation by these organelles in the presence of permeant ions, such as oxalate, is termed as Ca^{2+} uptake. Ca^{2+} uptake by the microsomal fraction is coupled with Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase, which serves as a calcium pump (43).

Ebashi (147) was first to show that isolated SR vesicles accumulated Ca^{2+} with high affinity in the presence of ATP. Hasselbach (148) was first to describe Ca^{2+} accumulation as active transport against Ca^{2+} gradient, a Ca^{2+} gradient which derives its energy from the hydrolysis of ATP through an ATPase enzyme incorporated in the SR membrane. Purified cardiac SR vesicles have been shown to consist of a heterogenous population of vesicles with respect to structure and function (149). It has been found that SR vesicles with different buoyant densities, termed light and heavy sarcoplasmic reticulum differed with respect to protein composition and electron-dense content. The data from biochemical and morphological studies has shown that heavy SR vesicles are derived from the terminal cisternae of the SR and the light SR vesicles are derived from the longitudinal SR (150). The heavy junctional SR vesicles have been identified by their high calsequestrin content and sensitivity to ryanodine which are not found in light vesicles or free SR (149).

The SR membranes from canine heart have been studied extensively and several of their protein constituents have been identified and characterized (151). Among these are 105 KD Ca^{2+} transporting ATPase (152,153), phospholamban, a 27-28 KD pentamer whose phosphorylation is associated with stimulation of Ca^{2+} uptake (153,154), calsequestrin, a 55

KD protein that binds Ca^{2+} with the SR with the low affinity and high capacity (155,156), two glycoproteins, 53 KD and 130 KD, whose function remains unknown but which may be involved in intraluminal Ca^{2+} binding and regulation of Ca^{2+} uptake (156) and a 400 KD ryanodine-sensitive Ca^{2+} channel (157,158). The Ca^{2+} transporting ATPase of cardiac SR like phospholamban is the product of a gene expression in cardiac and slow-twitch skeletal muscle (152,153) but not in fast-twitch skeletal muscle. Calsequestrin is also the product of two separate genes, one of which predominates in fast and slow-twitch skeletal muscle and the other of which predominates in cardiac muscle (155). Less is known regarding the genetics of other SR proteins, 53 and 130 KD glycoproteins. The genetics of ryanodine-sensitive Ca^{2+} channel, whose cardiac and skeletal muscle forms are similar in apparent molecular weight and chemical properties (157,158) remain undefined. Significant differences in the ryanodine-binding properties of cardiac and skeletal muscle SR have been noted, raising the possibility that the cardiac and skeletal muscle proteins are the products of separate genes or these are alternate-splice or post-translationally mediated products of the same gene. These differences might just as plausibly result from differences in the molecular environments of the ryanodine receptors in cardiac and skeletal muscle sarcoplasmic reticulum.

b. Calsequestrin in SR function

Campbell et al (156) were first to identify and purify calsequestrin, the second major protein constituent of SR, from the canine heart SR. Calsequestrin is a high capacity, moderate affinity, Ca^{2+} binding protein localized in the lumen of junctional sarcoplasmic reticulum in cardiac and skeletal muscles (159,160)). This acidic protein, which binds 300

nmol Ca^{2+} per milligram protein is therefore probably responsible for Ca^{2+} storage within the SR (151). The apparent molecular weight of cardiac calsequestrin is 45 KD when measured in neutral SDS gels and 55 KD when measured in alkaline SDS gels. Cardiac calsequestrin stains blue with Stains-All and has been identified as a component of ryanodine-sensitive cardiac SR (156). Recent anatomical evidence from rat myocardium indicates that the Ca^{2+} accumulated across the lumen of the sarcoplasmic reticulum is then bound to calsequestrin stored in the junctional SR (161), and it has been suggested that the junctional SR is a potential source of the releaseable Ca^{2+}. Although the exact role of calsequestrin in cardiac muscle is not clear, results from a study using skeletal muscle suggest that Ca^{2+}-dependent conformational changes in calsequestrin affect the junctional face of the membrane-bound proteins including the 400 KD Ca^{2+} release channel and that calsequestrin may then regulate Ca^{2+} release into the cytoplasm (162). It is known that cardiac and skeletal calsequestrin bind similar amounts of Ca^{2+}, even though structural differences exist between the cardiac and skeletal types (163,164), presumably by acting as a charged surface protein rather than by presenting multiple discrete Ca^{2+} binding sites (163). In recent years, the major SR proteins controlling Ca^{2+} release, reuptake and storage have been isolated and their primary structures have been determined (165). Calcium exchanges between the cytosol and SR are influenced by calsequestrin, which acts as a calcium buffer (166). Two isoforms of calsequestrin, a cardiac isoform and a fast-twitch muscle isoform have been identified (167,168). In a recent study involving the expression of calsequestrin mRNA, it was found that the expression of calsequestrin did not change in hyperthyroid and hypothyroid ventricular muscles (169) indicating that the storage capacity

of the SR was unaltered in these pathological states. More recently, it has been found that there is no difference in the level of cardiac calsequestrin mRNA between the patients with end-stage heart failure and their normal controls (93).

c. Ca^{2+}Release from SR

Excitation of the surface membrane of mammalian cardiac muscle releases Ca^{2+} from the SR into the cytoplasm which then activates the myofilaments and initiates contraction. During the last 10 years, three general hypotheses have been proposed to explain the nature of the excitation-contraction coupling (84) (a). Charge movement coupled Ca^{2+} release, in which voltage dependent movement of fixed charges in the sarcolemma mechanically gates the release of Ca^{2+} from the SR. (b). Ca^{2+} induced Ca^{2+} release, in which Ca^{2+} influx across the sarcolemma triggers the release of Ca^{2+} from the SR and (c). Inositol 1,4,5-triphosphate (IP3) induced Ca^{2+} release, in which IP3 acts as a diffusible second messenger to trigger release of Ca^{2+} from the sarcoplasmic reticulum.

The cisternae of SR in the proximity of transverse tubules, which inflect periodically from the sarcolemma at the level of the Z lines, are anchored to tubular system by feetlike projections (142) forming the so-called dyadic junctions in heart muscle. Somehow, the depolarization of the cell membrane is transmitted through the transverse tubules to the interior of the muscle cell, thus causing efflux of Ca^{2+} from the neighbouring cisternae of the reticulum. Evidently, a portion of the reticular membrane acts as a sensor for the excitation wave spreading from the plasma membrane enveloping the cell to the transverse tubules. It is believed that the feetlike projections that connect the transverse tubules with

the reticular cisternae are physically involved in the transmission of the excitation to the interior of the cell but the nature of the signal that will eventually direct the release of the Ca^{2+} from SR, whether mechanical, electrical, or chemical, is not known (45). Charge movement in cardiac muscle has many similarities to that in skeletal muscle thus raising the possibility that Ca^{2+} release is coupled to charge movements. However, several lines of evidence indicate that charge movement coupled Ca^{2+} release does not occur in cardiac muscle (84).

The hypothesis of Ca^{2+} induced Ca^{2+} release was originally proposed for the mechanism of excitation-contraction coupling in skeletal muscle (170,171) and later, largely through the work of Fabiato, it was extended to the cardiac muscle (172-174). According to this theory, the rapid influx of Ca^{2+} ions across the sarcolemma triggers the release of Ca^{2+} from SR via Ca^{2+} release channels, thereby providing a large quantity of Ca^{2+} required for initiating contraction. Experimental evidence from mechanically skinned cardiac cells (172-175), isolated SR (176-179) and intact cardiac myocytes (180) show that Ca^{2+} satisfies the first requirement of an excitation-contraction coupling messenger, in that it can release Ca^{2+} from the SR. Although the key role of sarcolemmal Na^{+}-Ca^{2+} exchange is to extrude Ca^{2+} from the cytoplasm, this system might also serve to drive Ca^{2+} into the cell. Leblanc and Hume (113) showed that Ca^{2+} release, in guinea pig ventricular myocytes which is small in amplitude, can occur in the virtual absence of an inward Ca^{2+} current. Since this release was associated with activation of tetrodotoxin sensitive Na^{+} channels and required extracellular Ca^{2+}, the authors concluded that Ca^{2+} release was triggered by the influx of Ca^{2+} via Na^{+}-Ca^{2+} exchange in response to Na^{+} influx through tetrodotoxin sensitive Na^{+} channels. Very

recently, Sham et al (181) disputed these findings which they believe are due to incomplete block of the Ca^{2+} channels or incomplete voltage control. Nonetheless the SR protein that mediates Ca^{2+} release has recently been isolated and purified (157,182,183) and is generally referred to as the SR Ca^{2+} release channel or ryanodine receptor. Subsequent studies have shown that in the presence of micromolar concentrations of Ca^{2+}, the SR Ca^{2+} channel is activated allowing Ca^{2+} release into the cytosol. (157,182-184). Experiments on intact cardiac cells provided evidence that Ca^{2+} release is specific for Ca^{2+} influx but not for Ba^{2+} or Na^{+} (185). Although the detailed mechanism of physiological SR Ca^{2+} release remains to be determined, recent rapid mixing-vesicle ion flux and planar lipid bilayer-single channel measurements have suggested that cardiac SR Ca^{2+} release is mediated by a high conductance Ca^{2+} channel that is activated by micromolar Ca^{2+} and millimolar ATP and inhibited by millimolar Mg^{2+} (186,187). The positive inotropic effect of caffeine, which is associated with a prolonged Ca^{2+} transient is known to interact directly with the Ca^{2+} release channel by increasing the open time and sensitivity of the channels to activation by Ca^{2+} (188,189). The presence of a similarly ligand-gated Ca^{2+} conducting channel has been previously observed in heavy junctional derived SR vesicle fractions from skeletal muscle (186-187).

The Ca^{2+} release behavior of heavy cardiac and skeletal SR vesicles is significantly affected by the neutral plant alkaloid, ryanodine, which binds specifically to the cardiac and skeletal Ca^{2+} release channels (178). At nanomolar concentrations, ryanodine locks the channel into a long-lived open subconductance state, while at concentrations above 10 μM, ryanodine completely closes the channel (190). Using [^{3}H]-ryanodine as a Ca^{2+} release

channel-specific ligand, the ryanodine receptor from skeletal muscle has been purified by sequential column chromatography and by density gradient centrifugation (157). Lai et al (158) showed that the cardiac ryanodine receptor, like the skeletal receptor, migrated through sucrose gradients as a large complex and displayed a major polypeptide band of molecular weight of 400 KD on SDS-polyacrylamide gels. Incorporation of the purified receptor into planar lipid bilayers induced single Ca^{2+} channel currents with conductance and gating kinetics similar to those of native cardiac Ca^{2+} release channels. It has been suggested that the purified cardiac ryanodine receptor complex contains the Ca^{2+} conducting pore of the cardiac Ca^{2+} release channel, although retention of the full range of regulatory sites of the native channel has not been demonstrated (158).

A further clue to the precise subcellular origin of ryanodine sensitive and ryanodine insensitive cardiac SR vesicles is given by measuring the responses of the isolated SR subfractions to the inorganic, polycationic staining agent, ruthenium red (191). It has been demonstrated that ruthenium red increases Ca^{2+} uptake by ryanodine sensitive (junctional, heavy) SR vesicles but has no significant effect on Ca^{2+} uptake by ryanodine insensitive (free, light) SR vesicles isolated from the heart (191,192). The effects of ruthenium red on Ca^{2+} uptake by cardiac SR vesicles support the idea that ryanodine sensitive cardiac SR vesicles originate from junctional SR in myocardium and that ryanodine insensitive cardiac SR vesicles originate from free SR (193). Ruthenium red stimulates net Ca^{2+} uptake by junctional cardiac SR vesicles at much lower concentrations than does ryanodine. Half-maximal stimulation by ruthenium red occurs at a concentration near 10^{-7} M whereas several hundred fold higher concentration is required for a similar effect by ryanodine (194,195).

The combination of ryanodine plus ruthenium red added to junctional cardiac SR vesicles gives no further stimulation of Ca^{2+} uptake than does either agent added alone, suggesting that both agents increase Ca^{2+} uptake by a similar mechanism (193-195). As is true for ryanodine, no increase in Ca^{2+}-ATPase activity is associated with ruthenium red stimulation of Ca^{2+} uptake by junctional cardiac SR vesicles. This observation suggests that ruthenium red increases net Ca^{2+} uptake into junctional cardiac SR vesicles by blocking Ca^{2+} efflux from the vesicles in a manner similar to that which has been proposed to explain the effect of ryanodine on net Ca^{2+} transport in the SR vesicles (193-195).

The role of IP_3 as a second messenger is well established in many cell types, where it functions by releasing Ca^{2+} from the intracellular stores (196). Recent studies have shown that in these cells IP_3 acts by directly opening Ca^{2+} release channels (197-198). The properties of this IP_3 gated Ca^{2+} release channel (IP_3 receptor) seem to be quite different from the Ca^{2+} gated Ca^{2+} release channel (ryanodine receptors) (197). Although hormone stimulated IP_3 synthesis has been clearly demonstrated in cardiac myocytes, the functional role of IP_3 in excitation-contraction coupling is as yet unclear and controversial (199). However, the limited information regarding IP_3 induced Ca^{2+} release in cardiac cells suggests that IP_3 should not be considered as a major physiological trigger mechanism for Ca^{2+} release. Recent data from skinned cardiac cells suggest that IP_3 only plays a pathological role in cardiac arrythmogenesis by enhancing spontaneous Ca^{2+} oscillations (200). The mechanism by which IP_3 enhances Ca^{2+} oscillation or induces Ca^{2+} release is not yet clear. It may either directly gate Ca^{2+} release channels (201) or modify the gating of ryanodine receptor (202).

Although some information concerning calcium release from SR in regional ischemia in dogs hearts is available, SR release activity has not been examined in a detailed manner in other types of heart failure. In a rat model of global ischemia, it was found that the decrease in Ca^{2+} uptake caused by ischemia is not due to any defect in Ca^{2+} pumping capabilities but is due to an increased efflux through the ryanodine sensitive calcium release channel of cardiac SR (203). When this activity was measured in the presence of SR calcium channel blocker (ryanodine), no significant difference was observed in Ca^{2+} uptake properties of SR in ischemic hearts as compared to control. In another study, low concentrations of ryanodine can prevent ventricular arrhythmias during acute myocardial ischemia and reperfusion in the rat heart (204). In another study, junctional SR membrane vesicles were isolated from chronically failing human hearts, incorporated into an artificial planar phospholipid bilayer and the activity of single Ca^{2+} release channels was investigated under voltage-clamp conditions. The properties of these channels were similar to those previously reported from normal animal tissue and this provided evidence that the function of individual Ca^{2+} release channels is not altered in failing hearts (205). The primary structure of the Ca^{2+} release channel has been determined by Otsu et al (206). Two distinct isoforms of the ryanodine receptor have been identified in cardiac and skeletal muscle (206). In one study regarding ryanodine receptor mRNA, the expression of mRNA coding the ryanodine receptor was upregulated in hyperthyroidism and downregulated in hypothyroidism; these results were in parallel with mRNA levels for Ca^{2+}-ATPase (93). In a very recent study, the molecular basis for the systolic and diastolic dysfunction, which are characteristics of end-stage heart failure in humans were explored. The mRNA levels for

ryanodine receptors were found to be decreased in ischemic cardiomyopathy but there was no change in idiopathic dilated cardiomyopathy as compared to control (207). These findings indicate that changes in Ca^{2+} release channels depend on the type of heart disease.

d. Ca^{2+}-stimulated ATPase activity in SR

The SR is comprised of about one third lipids and two thirds proteins. The Ca^{2+}-ATPase enzyme, accounting for about two thirds of the total proteins of the SR membrane system, carries out the enzymatic function of Ca^{2+} transport (208). The localization of Ca^{2+}-ATPase in skeletal and cardiac muscle has been determined by Jorgensen et al (209). Indirect immunofluorescent staining has shown that the Ca^{2+}-ATPase is concentrated in the longitudinal sarcoplasmic reticulum and in nonjunctional regions of the terminal cisternae. It has a highly asymmetric structure with respect to the membrane in which a major portion (60 %) of its mass protrudes into the cytoplasm as a pear-shaped head with a narrow stalk, while the rest is buried within the membrane. The Ca^{2+}-ATPase consists of a single polypeptide that is able to couple the hydrolysis of one molecule of ATP to the active transport of two Ca^{2+} ions across the SR membrane (210). The ATP-binding site is located in the cytoplasm portion of the enzyme, whereas the Ca^{2+} binding site is formed from its transmembrane segments. Because these two functional sites are presumably separated from each other, the coupling of ATP hydrolysis and Ca^{2+} movement probably occurs indirectly. In addition to one active site and two high affinity Ca^{2+} binding sites, this enzyme also contains one phosphorylation site per Ca^{2+}-ATPase molecule (210). The purified ATPase possesses all of the properties attributed to the Ca^{2+} transport system and when

reconstituted into phospholipid bilayer, it transports Ca^{2+} against a concentration gradient (211). MacLennan (140) was first to successfully purify the Ca^{2+}-ATPase with high ATPase activity from rabbit skeletal muscle SR.

The structure of the Ca^{2+}-ATPase molecule has been extensively studied and information has been obtained about the primary structure of the ATPase and various functional sites within the ATPase (146,210). The Ca^{2+}-ATPase has an apparent molecular weight of 100 to 120 KD based on SDS gel electrophoresis (154,212). The Ca^{2+}-ATPase has been cleaved at specific sites by limited digestion of intact SR vesicles with trypsin (212,213). The first cleavage produces two fragments of molecular weight 50 to 60 KD (fragment A) and 45 to 56 KD (fragment B). The second cleavage of the Ca^{2+}-ATPase produces further subfragments of A fragment, which have molecular weight of 30 (A_1) and 20 (A_2) KD (212,213). Localization of several major functional sites within the ATPase has been determined using limited digestion (212,214). The site for phosphoenzyme formation has been shown to reside in the intact ATPase and in the A fragment or A_1 subfragment after limited digestion (212). The high affinity Ca^{2+} binding sites of ATPase, directly involved in the transport mechanism, are defined as Ca^{2+} transport sites and have been localized to the A fragment or A_2 subfragment of Ca^{2+}-ATPase (215). It appears that A_1 subfragment contains the site involved in ATP hydrolysis and the A_2 subfragment contains the high affinity Ca^{2+} binding sites involved in Ca^{2+} transport (151).

Binding of two Ca^{2+} ions to the high-affinity Ca^{2+} site from the cytoplasmic side of the SR membrane induces phosphorylation of the enzyme by ATP to form the ADP-sensitive phosphoenzyme (E1P). Ca^{2+} ions in the E1P are occluded in that, they cannot be

exchanged with Ca^{2+} ions in the aqueous medium on either side of the SR membrane. E1P is then converted spontaneously to the ADP-insensitive phosphoenzyme (E2P) and at the same time, Ca^{2+} ions are exposed on the luminal surface and released. E2P is subsequently hydrolyzed to yield a dephosphoenzyme, which then starts another round of the reaction cycle. The Ca^{2+}-ATPase utilizes a magnesium-ATP complex as a physiological substrate. It is well known that Mg^{2+} is required for the rapid turnover of Ca^{2+}-ATPase. Mg^{2+} derived from the Mg^{2+}-ATP complex remains bound to the Ca^{2+}-ATPase at least until E2P is hydrolyzed and that binding of this Mg^{2+} is responsible for induction of the high rate of enzyme turnover. Binding of Ca^{2+} with unphosphorylated Ca^{2+}-ATPase as analyzed in equilibrium measurements revealed that the stoichiometry for high-affinity Ca^{2+} binding is 2 mole per mole of the phosphorylated site (208,210).

e. Modification of SR function

The SR membrane might be looked upon as a specialized lipid-protein environment which is sensitive to alterations in either constituent or in their ratio. There is considerable evidence for a highly specific association of certain phospholipids with a Ca^{2+}-ATPase molecule and any alteration in this lipid-protein interaction may have dramatic effects on calcium transport and Ca^{2+} ATPase (149,216). Isolated SR contains 90 to 110 phospholipid molecules per ATPase peptide together with minor amounts of neutral lipids and cholesterol. These phospholipids comprise mainly of phosphatidylcholine (PC) as the major component, together with phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI). Phosphatidylethanolamine has been reported to be distributed

primarily in the outer (cytoplasmic) monolayer of the membrane while phosphatidylcholine is evenly distributed and phosphatidylserine is primarily found in the inner monolayer. For the study of lipid-protein interaction, it is important that procedures are established by which it is possible to substitute the normal lipid environment with any other lipid species at will. The most utilized procedures employ addition of exogenous lipid to SR or purified ATPase with cholate or deoxycholate followed by sucrose density centrifugation to isolate lipid-substituted vesicles or lipid-protein complexes from the detergent containing samples. From these types of studies, it has been learned that the hydrophobic length of the bilayer probably is the decisive factor for the expression of enzyme activity (217).

There are a mumber of functionally important amino acid residues which regulate the ATPase enzyme activity in its denatured or native conformation stage (218). Most important of these are cysteinyl, lysine and histidine residues. Out of 24-25 cysteinyl residues, 17-19 SH groups are titrated with DTNB in denatured enzyme whereas 13-15 SH groups are reactive in the native conformation. More recently it was found that the number of crucial thiols is two or more and blocking of different thiols inhibits different elementary steps of the enzyme reaction (219). Chemical modification of lysine residues of Ca^{2+}-ATPase with fluorescamine results in the inhibition of Ca^{2+}-ATPase activity by blocking EP formation or EP decomposition (220). One or more histidine residues may also be involved in the function of Ca^{2+}-ATPase since photoinactivation of SR membrane in the presence of Rose Bengal or Methylene Blue results in a relative modification of histidine residues accompanied by inactivation by both Ca^{2+} uptake and ATPase activities (221). Chemical modification of these residues results in the inhibition of Ca^{2+}-ATPase activity (218).

Monovalent cations exert regulatory influences on the Ca^{2+}-ATPase of sarcoplasmic reticulum. In detailed studies, carried by Jones and Watanabe, the potency of various monovalent cations like K^+, Na^+, NH_4^+, Cs^+, and Li^+ for stimulating Ca^{2+}-ATPase activity was determined and K^+ was found to be the monovalent cation most effective (222). They have suggested that K^+ stimulates enzyme activity primarily by increasing the rate of decomposition of phosphoprotein. The increase in activity induced by K^+ is modulated by ATP concentration present in the incubation medium; both K^+ and ATP exert allosteric control over the rate-limiting step of the reaction (222).

f. Phosphorylation of SR membrane

The ß-adrenergic actions of catecholamines represent a key control mechanism that regulates the metabolic, electrical, and mechanical performance of the myocardium. Adenosine 3',5', monophosphate (cAMP) serves as the intracellular second messenger for these regulatory processes in which ß-adrenergic activation of a sarcolemmal adenyl cyclase causes intracellular cAMP levels to rise (223). This second messenger appears to exert most of its cellular effects by activating cAMP dependent protein kinases (cAMP-PKs). These enzymes catalyze phosphorylation of a number of proteins which alter several aspects of the excitation-contraction process in the myocardium including increases in the flux of ions across the sarcolemma and SR (223).

Cardiac SR can form two classes of phosphoprotein that differ in both molecular weight and chemical characteristics (224). The Ca^{2+} pump ATPase of the cardiac SR, which serves as an energy transducer that translocates Ca^{2+} across the membrane, forms a

phosphoprotein intermediate by incorporating the terminal phosphate of ATP into a hydoxylamine-labile acylphosphate (224). The cardiac SR is able to form a second type of phosphoprotein when reacted with cAMP-PK (225). In this phosphoprotein, the terminal phosphate of ATP is incorporated mainly into a serine residue of a 22KD protein that exhibits the stability characteristics of a phosphoester (225). This phosphorylatable protein, which represents one of the sites at which cAMP-PK exerts its action, appears to function as a modulator of the Ca^{2+}-ATPase and Ca^{2+} pump of cardiac SR. The formation of a phosphoester phosphoprotein when cardiac SR is incubated with cAMP in the absence and presence of exogenous cAMP-PK is independent of Ca^{2+} up to 0.1 mM but is markedly dependent on cAMP between 0.1 and 10 μM (225). Other lower molecular weight proteins are also phosphorylated by cAMP-PK and the chemical characteristics of these smaller proteins are distinct from those of phospholamban (226).

Phospholamban phosphorylation occurrs in cardiac SR preparations which are virtually freed from sarcolemmal vesicles by means of density gradient centrifugation. Phospholamban is intimately associated with the SR membrane and appears to be an acidic proteolipid (226). A portion of this molecule is exposed at the surface of SR vesicles as evidenced by the observation that it can be phosphorylated by exogenous cAMP-PK (226). The phospholamban content of the cardiac SR is estimated to be about 4-6 % of the total protein (223). During Ca^{2+} transport into the SR, the ATPase undergoes a complex series of reactions in which several phosphorylated intermediates (EP) are sequentially formed and degraded (224). Steady state EP levels are not altered by phospholamban phosphorylation at saturating concentrations of Ca^{2+} and ATP but the rate-limiting step of EP decomposition

is markedly enhanced (224). Major changes in the Ca^{2+} affinity occur in two of the rate determining steps during the turnover of the ATPase that are enhanced by phospholamban phosphorylation suggesting that phopholamban may exert its action by regulating Ca^{2+} mediated conformational changes of the ATPase. This interpretation is supported by the findings of Kirchberger et al (225) that phospholamban phosphorylation alters the sensitivity of the Ca^{2+} pump.

Phospholamban is a product of a single gene. It is expressed in SR of cardiac, slow-twitch skeletal and smooth muscle where the SERCA 2 type Ca^{2+} pump is expressed. Phospholamban consists of five monomers with molecular weight of 60 KD. The monomer is an amphipathic polypeptide with C-terminal transmembrane and N-terminal cytoplasmic domain. The cytoplasmic domain has phosphorylation sites catalyzed by cAMP and calmodulin-dependent PKs. One line of evidence indicates that phospholamban acts as a suppressor of Ca^{2+} pump ATPase and that its phosphorylation suppresses the inhibiting effects. Recent studies with synthetic peptides of phospholamban further revealed that phospholamban inhibits the Ca^{2+} pump ATPase at two sites in different manners, the cytoplasmic domains for Vmax and intramembrane domain for Kca; phosphorylation of phospholamban suppressed these two inhibiting effects (227). Thus, phospholamban may act as a regulator of Ca^{2+} pump ATPase in cardiac SR by a direct protein-protein interaction.

It has been reported that phosphate could be incorporated into phospholamban, in the presence of Ca^{2+} and calmodulin and formation of this phosphoprotein with stability characteristics of a phosphoester was dependent on calmodulin but independent of cAMP-PK (226). It is postulated that phospholamban could be phosphorylated by an endogenous

PK, associated with the SR, that was activated by calmodulin and Ca^{2+} (226). In this regard preincubation of cardiac SR with calmodulin was found to enhance the rate of calcium uptake and that calmodulin dependent phosphorylation of phospholamban was accompanied by an enhanced rate of Ca^{2+} transport (226). At physiological concentrations of Ca^{2+} (0.1-10 μM), stimulation of Ca^{2+} transport by either cAMP-PK or calmodulin-PK systems appears to occur independently. It has been reported that these effects on Ca^{2+} transport were additive in accord with the finding that phosphorylation by the two kinases occurs independently (223). Phosphorylation of cardiac SR by either cAMP or Ca^{2+}-calmodulin-dependent mechanisms results in approximately two fold increase in the steady-state rate of oxalate or phosphate-facilitated Ca^{2+} transport measured at optimal physiological Ca^{2+} concentration (223). The cAMP mediated acceleration of Ca^{2+} uptake by cardiac SR may explain two of the major mechanical effects of catecholamines on heart muscle, abbreviation of systole and increased contractility (228,229). Thus, the cAMP mediated increase in the rate of Ca^{2+} accumulation by SR could explain the faster rate of relaxation because of the increased rate at which Ca^{2+} would be removed from troponin in catecholamine-stimulated myocardium. Enhanced accumulation of SR Ca^{2+} could increase the amount of Ca^{2+} stored within the cell as some of the Ca^{2+} that would otherwise be lost during diastole (228). This could increase the amount of Ca^{2+} available for delivery to the contractile proteins in subsequent contraction, thereby producing the positive inotropic effect of catecholamines. The abbreviation of systole seen in the catecholamine-stimulated heart might be explained by an action of phosphorylated phospholamban to accelerate spontaneous transition from Ca^{2+} uptake to Ca^{2+} release in the SR (223).

It is postulated that the effects of drugs, fatty acids and ethanol on the SR membrane are mediated either directly by interaction with the calcium pump protein of the SR membrane or indirectly by an alteration of the physico-chemical properties of the bulk lipid phase and lipid-protein interface of the SR lipid bilayer (231). Local anesthetics such as lidocaine and tetracaine, have been shown to inhibit calcium transport and calcium ATPase activity of both cardiac and skeletal SR (231,232). General anesthetics such as halothane and enfluane also effect the functional properties of the SR with many of these effects exhibiting a complex concentration-dependence similar to local anesthetics (231). Calcium channel blockers and ß-adrenergic receptors blocking agents appear to act on the SR nonspecifically rather than through mechanisms related to their calcium channel or ß-adrenergic blocking actions. At concentrations $< 10^{-5}$ M, where these agents act as calcium channel blockers in whole heart, verapamil and D-600 were found to have no effect on active calcium transport and passive Ca^{2+} release of cardiac SR (233), whereas, inhibition of Ca^{2+} transport was observed at concentrations in the range of 10^{-5} to 10^{-3} M (234). ß-adrenergic receptor blocking drugs such as propranolol and timolol have been shown by several investigators to inhibit calcium transport and total ATPase activity of both cardiac and skeletal SR (235). Fatty acids and fatty acid derivatives such as palmitylcarnitine have complex effects on SR membrane ATPase systems, ranging from complete inhibition to two-fold increase in ATPase activity and other coupled biological reactions (231). Alcohols, especially, ethanol have been shown to effect the functional and presumably structural states of several biological membranes including SR. Solvation of ethanol into and partial solubilization of the lipid bilayer may be cause of the increase in ATPase activity whereas inhibition of

calcium transport may be caused by a direct interaction with the calcium pump protein (236,237).

g. Role of SR Ca^{2+}-transport in heart failure

Varying degrees of defects in SR Ca^{2+} transport have been identified in various experimental models of heart failure as well as in failing human hearts in the last three decades (43). One of the first experiments designed to assess SR function was carried out in microsomes prepared from spontaneously failing canine heart-lung preparations which showed depressed Ca^{2+} uptake and Ca^{2+}-ATPase activities (230). Depressed activity of the sarcoplasmic reticulum Ca^{2+}-ATPase could lead to a reduced amount of Ca^{2+} available for contraction and ultimately could alter the function of the cardiac pump by slowing the normal relaxation phase (44,238). Observations of a depressed rate of SR Ca^{2+} uptake have been made in failing heart muscle obtained from humans (239), and in a number of experimental models employing rabbits (240) and hamsters (79,241). Alterations in SR Ca^{2+} uptake were obvious with the development of left ventricular failure in rabbits with hypertrophied hearts secondary to aortic insufficiency (242). Depressed calcium uptake activities of the microsomal fraction were noted without any change in the SR Ca^{2+}-ATPase activity indicating uncoupling of Ca^{2+} pump in cardiomyopathic hamsters (79) and bacterial infected cardiomyopathy in rabbits (243). The SR vesicles isolated from left ventricles of human hearts with idiopathic dilated cardiomyopathy did not show any change in Ca^{2+} uptake properties and accordingly it was suggested that abnormal Ca^{2+} handling in the myocardium of these patients was not a result of any intrinsic alteration of Ca^{2+} uptake by

the SR (244).

Attempts to unravel the rational basis for the pathogenesis of heart failure are always accelerated by new ideas and the application of new technologies. Only recently, molecular biology provided new insight into the mechanisms responsible for the alterations of contraction and relaxation observed during cardiac hypertrophy and heart failure. Determination of the expression of genes encoding various SR proteins are under investigation in various models of heart failure. Recent reports have indicated that alterations observed in SR function in heart failure are at the transcriptional level as hypertrophy of rat and rabbit hearts was associated with decreased myocardial level of SR Ca^{2+}-ATPase mRNA and protein concentration (245,246). Altered SR Ca^{2+}-ATPase gene expression as compared to controls has been reported in a group of patients with idiopathic dilated cardiomyopathy, coronary artery disease with myocardial infarction, or valvular insufficiency (247). Accordingly, it was concluded that a decrease in Ca^{2+}-ATPase mRNA in the myocardium plays an important role in the alterations of Ca^{2+} movements and myocardial relaxation reported during end-stage heart failure (247). Molecular mechanisms of hypertrophied failing heart and abnormalities of the diastolic properties and contractility were studied and it was reported that the mRNA levels of Ca^{2+}-ATPase were decreased significantly by pressure overload and were correlated with corresponding protein levels (248).

Many membrane proteins are difficult to purify and once purified are difficult to reconstitute into their native environments. Monoclonal antibodies may be useful probes for the evaluation of the function and the mechanism of action of such membrane proteins

(249). Antibodies are exquisitely selective proteins that can bind to a single target among millions of irrelevant sites. Monoclonal and polyclonal antibodies to the major SR proteins of rabbit skeletal and canine cardiac muscle were used to identify and characterize the corresponding components of human cardiac SR (250). In this study, immunoblots of SR from the normal left ventricles and excised failing left ventricles of patients with idiopathic dilated cardiomyopathy were compared in search of qualitative differences in the protein patterns of the failing hearts. No such differences were found with respect to the Ca^{2+}-ATPase, 53 KD glycoprotein, the ryanodine-sensitive Ca^{2+}-channel, calsequestrin or phospholamban (250).

Since phospholamban plays a regulatory role in Ca^{2+}-ATPase activity, a defect in the phospholamban protein may lead to abnormal Ca^{2+}-ATPase activity. This possibility was studied with the use of phospholamban-specific monoclonal antibodies, where binding of this antibody to isolated SR prepared from normal and failing hearts was taken as simulation of phospholamban phosphorylation by cAMP-dependent protein kinase (251). Stimulation of SR Ca^{2+}-ATPase was found to be similar among failing and normal hearts and these investigators suggested that the pathogenesis of heart failure in idiopathic dilated cardiomyopathy is independent of intrinsic alteration of this mechanism. Furthermore, oxalate-supported Ca^{2+} uptake by cardiac SR was examined in microsomes prepared from left ventricle free wall with idiopathic dilated cardiomyopathy and no significant difference in Ca^{2+} uptake was found as compared to control. On the basis of these observations it was concluded that abnormalities in the regulation of Ca^{2+} in failing human myocardium cannot be ascribed to changes in the level or function of the Ca^{2+} transporting ATPase or

phospholamban in the SR (252).

It is important to consider the particular etiology of heart failure when studies on the function of subcellular organelles are carried out in humans because changes in intracellular Ca^{2+} handling are not identical in every type or stage of heart failure (43). Furthermore, consideration of specific abnormalities of cellular membrane function in the pathogenesis of any given type of heart failure is necessary (43). With regard to animal models of heart failure, intracellular Ca^{2+} overload is believed to exist in experimental models of genetic cardiomyopathy and isoproterenol induced cardiomyopathy (43,44), hypertrophy due to pressure overload (253), diabetic cardiomyopathy (254,255), and acute ischemia-reperfusion injury (256). In another study intracellular Ca^{2+} transients were measured with aequorin from patients with idiopathic dilated cardiomyopathy, ischemic heart disease, valvular insufficiency and hypertrophic cardiomyopathy and were found to be markedly diminished and prolonged in comparison to the control (257). During different pathological conditions, the sensitivity of myocardium to extracellular Ca^{2+} has been shown to be changed (253,258-260). In one study, the response of infarcted heart to extracellular Ca^{2+} was monitored and it was observed that the inotropic response to a decrease in extracellular Ca^{2+} was greater in infarcted hearts than in sham controls (261). Although changes in myofibrillar sensitivity to Ca^{2+} may be the cause of abnormalities in cardiac function in patients, the sensitivity of the myofibrils to Ca^{2+} is known to be unaffected by the different structural elements of the thick filaments that occur as myosin isozymes (262).

Relaxation abnormalities are often more important than contraction abnormalities in the pathogenesis of the clinical syndrome of heart failure (263). Experimental studies

have shown that impaired relaxation is among the initial abnormalities to appear in failing hearts. In fact, diastolic heart failure is characterized by increased resistance to diastolic filling of cardiac ventricles (238). Impaired Ca^{2+} handling has been reported to occur in a variety of conditions like hypertrophic cardiomyopathy, myocardial ischemia and congestive heart failure, characterized by diastolic dysfunction and might play a unifying role as a basic mechanism for diastolic heart failure (238). Ca^{2+} transients of muscles from failing hearts have been reported to be markedly prolonged and these muscles have shown a diminished capacity to restore a low resting Ca^{2+} levels during diastole, which is consistent with reduced rate of sequestration of Ca^{2+} (253,264). Accordingly, it can be concluded that impaired uptake of Ca^{2+} by SR result in a rise in resting tension, leading to diastolic heart failure. The exact role of SR in terms of Ca^{2+} uptake and Ca^{2+} release at different stages of congestive heart failure has not yet been cleary defined. Conflicting results regarding SR Ca^{2+} transport in heart failure has been reported as recently as 1989 (244). It also remains to be determined whether changes in SR Ca^{2+} transport activity in failing heart arise from abnormalities in the Ca^{2+} pump protein or alterations in the phospholipid composition of these membranes. Thus in view of real gaps concerning the nature of SR Ca^{2+} transport defects in failing hearts and the mechanisms of these abnormalities, the present study is an attempt to gain a detailed information to extend our existing knowledge in this field. Furthermore, it is designed to understand the alterations in SR Ca^{2+} transport in hemodynamically assessed preparations for relating changes at both a biochemical and molecular level with functionl abnormalities in congestive heart failure secondary to myocardial infarction as a result of coronary ligation.

III. METHODS

1. Experimental model

Myocardial infarction was induced in the male Sprague Dawley rats (175-200 g) by occlusion of the left coronary artery as described by Selye et al (11). The animals were anaesthetized with ether and the heart was exposed through the left thoracotomy. The anterior descending branch of the left coronary artery was ligated 2 mm from the origin of the aorta with 6-0 silk suture, the heart was repositioned in the chest and the incision was closed with a purse-string suture. A mixture of 95% oxygen and 5% CO_2 was supplied to the animal under the positive pressure during the operative procedure. Sham operated animals were treated in the same way except that the artery was not ligated. All these animals were fed rat chow and water ad libitum untill used at 4, 8 and 16 weeks after the operation, for hemodynamic and biochemical studies. The mortality rate in this experimental model was about 40 % within 48 hours of the operation. Rats were sacrificed by decapitation, hearts were removed and washed in cold normal saline. The atria and the connective tissue around the heart were removed and right and left ventricles were separated by careful dissection. Lungs were also taken out, weighed, dried in an oven for 48 hours and wet/dry lung weight ratio was calculated.

Quantification of infarct size was carried out where hearts from control and experimental animals were fixed in 10 % formalin and processed for histological examination. A planimeter technique (18) was used to measure the size of the entire endocardial circumference and the infarcted portion of the left ventricle. The percentage

of the infarcted left ventricle was calculated from these measurements and this percentage was extrapolated to the respective experimental groups. Unless otherwise indicated in the text, experimental animals with >30 % scar size of the left ventricle were used in this study.

2. Hemodynamic assessment

Control and experimental animals were anaesthetized with an injection of pentobarbital sodium (50 mg/kg; Nembutal, I.P.). After intubation of the trachea to maintain adequate ventilation, the right carotid artery was exposed and a microtip pressure transducer (Model SPR-249, Miller Instruments, Houston, Tx.) was introduced into the left ventricle through a proximal arteriotomy. The catheter was advanced carefully through the lumen of the carotid artery untill the tip of the transducer entered into the left ventricle. The catheter was secured in place with a silk suture, tied around the artery and connected to a dynograph recorder (Model RSHA, Beckman, Fullerton, CA). Left ventricular pressures, rate of contraction (+dP/dt), rate of relaxation (-dP/dt) and heart rate were recorded after 10-15 min of stablization of the heart function (254,265). The changes in these hemodynamic parameters were used to characterize the mild, moderate and severe stages of CHF at 4, 8, and 16 weaks after coronary ligation.

3. Isolation of sarcoplasmic reticulum vesicles

Membrane fractions enriched with SR were isolated according to the procedure described by Harigaya Schawartz (239). This preparation has been commonly used in our laboratory as well as by other investigators for studying Ca^{2+} uptake and Ca^{2+}-ATPase

activities in SR membrane vesicles (63,266-268). In some experiments, only crude membrane (homogenate) was used to study Ca^{2+} transport. Viable non-ischemic left ventricle and right ventricular tissue were homogenized separately in a Waring blender in a medium containing 10 mM $NaHCO_3$, 5 mM NaN_3, and 15 mM Tris-HCl (pH 6.8) at a medium speed for 45 sec. The homogenate was centrifuged at 10,000 g for 20 min, the pellet was discarded and the supernatant was again centrifuged at 40,000 g for 30 min. The 10,000 to 40,000 g sediment was suspended in 0.6 M KCl and 20 mM Tris-HCl(pH 6.8) to solubilize the contractile proteins and centrifuged at 40,000 g for 45 min. The final pellet was washed and suspended in 0.25 M sucrose and 20 mM Tris-HCl (pH 6.8) and frozen immediately in liquid N_2 unless used in next one hour. All isolation steps were carried out at 0-4 °C. To test if the observed changes in SR Ca^{2+} uptake are not due to an artifact of the method of the isolation, SR preparations from control and experimental tissues were isolated by another method (269), different from that employed mainly in this study. Protein estimation of all membranes was carried out by the method of Lowry et al (270).

4. Determination of sarcoplasmic reticulum Ca^{2+} uptake

The SR Ca^{2+} uptake activity was determined using the Millipore filtration technique (63,266). The membranes (0.03-0.07 mg/ml) were incubated in the presence of 100 mM KCl, 20 mM Tris-HCl (pH 6.8), 5 mM $MgCl_2$, 5 mM K-oxalate, 5 mM NaN_3 and the desired concentration of ^{45}Ca (0.1-10 uM). The concentration of free calcium in solutions was buffered by ethylene-glycol-bis (ß-aminoethyl ether)-N,N,N,N-tetra-acetic acid (EGTA) to obtain activities between 0.1-10 uM. For this purpose, dissociation constants for H^+, Na^+,

K^+, Ca^{2+} and Mg^{2+} binding to EGTA were taken from Fabiato (271). The reaction was started with 5 mM ATP and 0.2 ml sample was filtered at the desired time through a Millipore filter (size 0.45 um) and immediately washed two times with 3 ml ice cold buffer. The filters were dried and then counted for radioactivity by using the standard liquid scintillation counting technique (266,267). Appropriate blanks without ATP were included in all experiments to calculate the ATP dependent Ca^{2+} uptake. All experiments were done in triplicate. Since Ca^{2+} uptake in both control and experimental SR preparations declined by 15 % within 2 hr of completing the isolation procedure, freshly prepared (within 60 min) SR vesicles or frozen preparations were used for biochemical determinationn. Freshly prepared membranes frozen in liquid N_2 and kept at -80 °C for 2 to 3 days did not show any significant loss in the Ca^{2+} uptake and Ca^{2+}-stimulated ATPase activities.

5. Determination of Ca^{2+}-stimulated ATPase activities

Total (Mg^{2+} and Ca^{2+}) and basal (Mg^{2+}) ATPase activities were determined in a reaction medium containing 100 mM KCl, 20 mM Tris HCl, 5 mM $MgCl_2$ and 5 mM NaN_3. Reaction was started with addition of 5mM Tris ATP in presence of 0.05-0.08 mg/ml of SR protein and was terminated with 1 ml of 12 % wt/vol cold trichloroacetic acid (TCA). Inorganic phosphate liberated during reaction was estimated in protein free filtrate by spectrophotometric method (63,266,267). The Ca^{2+}-stimulated Mg^{2+}-dependent ATPase activity is reported as the difference between the total and basal ATPase activities. The sensitivity of Ca^{2+}-stimulated ATPase to potassium was examined at different concentrations of K^+ (222). Ca^{2+}-stimulated ATPase activities were also studied in presence of different

concentrations of ATP (0.01-10 mM) for determination of Km and Vmax values for control and experimental SR preparations. In these experiments an ATP regenerating system was used to maintain a constant amount of ATP available during the reaction procedure. For this purpose, 0.05 mg/ml of pyruvate kinase and 2 mM phosphoenolpyruvate (PEP) was included in the reaction medium to maintain constant concentrations of ATP (222).

To study the regulatory role of phosphorylating interventions on Ca^{2+}-ATPase activity, SR membrane vesicles were incubated in presence of cAMP dependent protein kinase (PK) and calmodulin at 37° for 5 min (229,272). The Ca^{2+}-ATPase pump activities of phosphorylated preparations in control and experimental SR were measured in presence of different concentrations of these agents at 10 uM free calcium in the medium. The Ca^{2+}-stimulated ATPase activities were also studied in the presence of different concentrations of calcium in the same assay medium where 30 ug of PK or 5uM calmodulin was used for membrane phosphorylation.

6. Phosphorylation of SR membranes

SR preparations (50-60 ug) were phosphorylated in 200 ul of a solution containing 50 mM Tris HCl buffer (pH 6.8), 100 mM KCl, 5mM $MgCl_2$, 10 uM free Ca^{2+} and 1 mM [^{32}P]-ATP (28.5 Ci/mmol) in the absence and presence of 5 uM calmodulin or 5uM cAMP together with 30 ug/ml protein kinase (268). Following incubation for 2 min. at 25°, the reaction was terminated by addition of 2 ml of ice cold 10 % trichloroacetic acid. The pellet was washed 3 times at 0° by resuspension in 0.1 ml of 1 N NaOH and precipitation with 2 ml of 5 % TCA. The pellet was then dissolved at room temperature in 0.1 ml of 1

N NaOH and the radioactivity was determined in 10 ml of scintillation fluid.

7. Determination of sulfhydryl group contents

Sulfhydryl (SH groups) contents of SR membranes were determined at 25° with 5,5-dithiobis (2 nitrobenzoic acid) (DTNB), according to the procedure of Ellman (273). For total SH group quantification, the reaction medium contained 2% SDS, 80 mM sodium Phosphate buffer (pH 8) and 0.5 mg/ml EDTA. An aliquot of 200 ul SR protein (0.5 mg/ml) was added in 1.0 ml of buffer mixture and 30 ul of 6 mM DTNB (in 100 mM Na Phosphate buffer) and the absorbance at 412 nm was measured after 30 min of incubation. Then 6 mM of N-ethylmaleimide (NEM) were added and the reading was taken after 3 min. For free SH group determination SR membrane (200 ul) was incubated with 100 mM sodium phosphate buffer and 6 mM DTNB. The first reading was taken after 3 min of incubation and the second after 3 min of addition of NEM. Calculation of the SH groups was based on a molar extinction coefficient of 13,600 M/cm at 412 nm for the thiophenol reaction product and is expressed per 1.5×10^5 g of protein.

8. Determination of membrane phospholipids and cholesterol contents

Phospholipids from cardiac SR (2 mg protein) were extracted in 2:1 chloroform, methanol solution (274). The lipid extracts were dried in the presence of N_2 and the individual phospholipids were separated by two dimentional thin layer chromatography. Plates were dried, sprayed with 5 % sulphuric acid, heated at 150 °C for 15 min and then visualized under ultra violet light. The identified phospholipid spots were estimated by

measuring phospholipid phosphate liberated during reaction. Membrane cholesterol was estimated by cholesterol esterase method using cholesterol kit reagents of Sigma Chemicals (St. Louis MO).

9. Malondialdehyde (MDA) assay

Lipid peroxidation was determined by the thiobarbituric acid method for the estimation of malondialdehyde (MDA) contents as described (275). Heart homogenate 10 % wt/vol was prepared in 0.2 M Tris, 0.16 M KCl buffer pH 7.4 and incubated for one hour at 37 °C. An aliquot of 1.0 ml of 40 % trichloroacetic acid (TCA) was added in it. After mixing, 1 ml of 2 % sodium thiobarbituric acid was added. The tubes were boiled for 15 min and cooled on ice. Then 2 ml of 70 % TCA was added and tubes were allowed to stand for 20 min and centrifuged at 800 g for 15 min. The color was read at 532 nm; commercially available MDA was used as standard.

10. Catecholamines determination

For catecholamines determination, ventricular tissue was homogenized by a polytron homogenizer with 1 % NaS_2 in 0.4 HCl (1:10 wt/vol), the homogenate was carefully aspirated, volume measured and the pH adjusted to 8.6 with 1 M Tris-HCl. Catecholamines in this supernatant were extracted by using activated aluminium oxide and measured by employing high performance liquid chromatography with electrochemical detection as described by Hallman et al (276).

11. Characterization of SR vesicles

The purity of the preparation was assessed by standard enzymatic markers for sarcolemma, myofibrils and mitochondria. Ouabain sensitive Na^+-K^+ ATPase activities of SR were determined as described elsewhere (277). The K^+-EDTA stimulated myosin ATPase activity was measured by the procedure as described by Martin et al (278). Cytochrome C-oxidase activity was determined by previously published methods (279). Rotenone-insensitive NADPH cytochrome C-reductase was measured as described (280). Electrophoresis of proteins in sodium dodecyl sulfate (SDS) polyacrylamide gel (14%) was performed according to the method of Laemmli (281). Molecular weights of the protein peaks were estimated by running known molecular weight standard under the identical conditions.

12. Extraction of RNA

Total RNA was isolated from the viable left ventricles using the guanidine thiocyanate-phenol-chloroform extraction procedure described by Chomczynski et al (282). The final pellet was suspended in 0.5 ml of double distilled sterile water. The absorbance at 260 nm and 280 nm was used to calculate the total RNA concentration for all samples. These values were divided by viable left ventricle weight to calculate total RNA levels present in control and experimental hearts. These samples were stored at -80 °C until used for Northern and slot blot analysis.

13. Northern blot analysis

The mRNA levels of Ca^{2+}-ATPase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and ribosomal RNA (28S rRNA) were quantitated using Northern blot analysis (93). 20 ug of total RNA were fractionated on 1% agarose gel containing 1 M formaldehyde and blotted onto a nitrocellulose membrane (Schleicher & Schuell Inc. Keene, N.Y). These filters were baked for one hr at 80 °C and UV crosslinked to nitrocellulose in an UV Stratalinker (Stratagene, CA).

14. Slot blot analysis

The RNA slot blot method was used to analyse the expression levels of Ca^{2+}-ATPase, GAPDH, and 28S rRNA. Total RNA was denatured in 50 % formamide, 7 % formaldehyde and 1 X SSC by heating at 65° for 15 min followed by cooling on ice (93). Serial amounts (3 and 6 μg) of total RNA from each sample of the left ventricles was loaded onto the nitrocellulose membranes (Schleicher & Schuell) of a slot blot apparatus . The same amount of tRNA was also loaded to detect non-specific binding of probes. Cross linking in an UV Stratalinker was done after these filters were baked in an oven at 80 °C for one hour.

15. cDNA probes

For Ca^{2+}-ATPase, a 1.7-kb cDNA fragment containing the protein coding region and the 3' untranslated region of the rabbit cardiac/slow-twitch Ca^{2+}-ATPase (SERCA 2a) was used (246). For glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 1.63-kb chicken GAPDH cDNA was used (93). Mouse 28S rRNA fragment was used to verify that a

constant amount of RNA was loaded onto each lane during Northern and slot blot analysis.

16. Filter hybridization

The nitrocellulose membranes prepared under the conditions described for Northern and slot blot analysis were pre-hybridized for 4-6 hr and then hybridized with ^{32}P-labelled cDNA fragments for Ca^{2+}-ATPase, GAPDH and 28S rRNA as probes. After overnight hybridization, these filters were washed twice with standard saline citrate (SSC) and 0.1 % sodium dodecyl sulphate (SDS) solution for 15 min at 50°C and dried for 3 hours at room temperature. The filters were then exposed to Kodak X-Omat-AR film. After autoradiography, the radiolabelled mRNA bands were quantitated by densitometer scans using Photofinish and Scanplot computer programs. The optical density of mRNA for each of the Ca^{2+}-ATPase bands was divided by that for GAPDH and 28S band optical density. The relative level of the mRNA for Ca^{2+}-ATPase corrected against that for GAPDH and 28S rRNA in each sample were calculated as percentage of mean values of the corresponding message level in the control groups (93).

17. Statistical analysis

The results are presented as mean ± SE and the statistical difference between mean values for two groups was evaluated by the Student's t-test for biochemical data; a P value <0.05 was taken to reflect a significant difference. Since values for sham operated control at 4,8 and 16 weeks of operation were not different from each other, the results from these groups were combined. The statistical difference between two groups was

evaluated by Non-parametric unpaired Kroskil test using Macintosh Statview computer program.

IV. RESULTS

1. General characteristics and hemodynamic status of experimental animals

The occlusion of coronary artery in rats produced a wide spectrum of scar formation (15 to 55 %) in the left ventricle. Histology of a heart, 16 weeks after coronary artery ligation as compared to control is shown in Figure 1. Complete replacement of necrotic myocardium by connective tissue 16 weeks after coronary ligation indicates the success of operative procedure. Thickening of the left and right ventricular walls as well as of the interventricular septum is obvious as compared to control heart. Furthermore, dilatation of the left and right ventricles can be appreciated in the experimental hearts. The results regarding the general characteristics of experimental animals showing > 30 % scar size are given in Table 1. Although the weight of viable left ventricle increased markedly ($P<0.05$) at 16 weeks, it should be noted that the apparent weight of the viable left ventricle was significantly less at 4 weeks and was not different at 8 weeks of myocardial infarction. However, as reported earlier (22,92,266), the normalized left ventricular weight, at 4 and 8 weeks, when the infarcted tissue was taken into account increased by about 30 and 90 %, respectively, when the infarcted tissue was taken into account. The right ventricles showed varying degrees of hypertrophy at 4, 8 and 16 weeks of inducing myocardial infarction. No difference in the scar weights of the left ventricular free wall was seen among the 4, 8, and 16 weeks experimental groups. Although a progressive increase in ascites was noted in experimental animals, the lung wet/dry wt ratio, an indicator of lung congestion, increased at 8 and 16 weeks only.

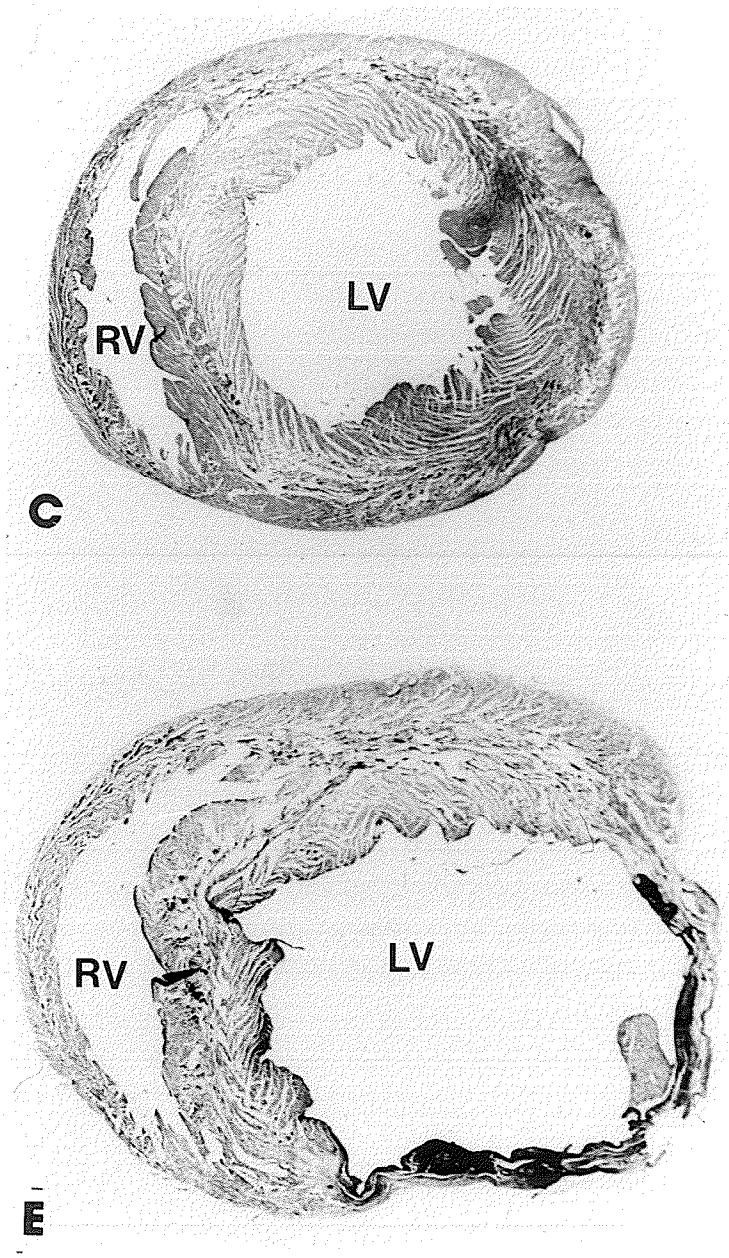


Figure 1. Cross section of rat heart at 16 weeks post-ligation of the left coronary artery or sham operation. Dilatation and hypertrophy of the left and right ventricles (LV, RV) and extensive replacement of LV free wall with scar tissue is evident. C-control; E-experimental.

Table 1. General characteristics of experimental rats at 4, 8 and 16 weeks after coronary artery ligation

Parameter	4 Weeks		8 Weeks		16 Weeks	
	Control	Experimental	Control	Experimental	Control	Experimental
Body wt (g)	440 ± 15	450 ± 18	521 ± 10	503 ± 14	588 ± 16	550 ± 20
Scar wt (mg)	-	300 ± 8	-	330 ± 10	-	350 ± 12
Viable LV wt (mg)	780 ± 40	590 ± 40*	920 ± 70	970 ± 50	1000 ± 60	1280 ± 60*
RV wt (mg)	220 ± 20	300 ± 20*	250 ± 30	390 ± 40*	280 ± 30	430 ± 40*
LV/Body wt (mg/g)	1.76 ± 0.1	1.31 ± 0.2	1.76 ± 0.1	1.92 ± 0.2	1.70 ± 1.0	2.32 ± 0.1*
RNA/LV wt (μg/mg)	1.80 ± 0.3	1.76 ± 0.4	1.69 ± 0.3	1.70 ± 0.2	1.87 ± 0.4	1.45 ± 0.4
Lung wet/dry wt ratio	3.77 ± 0.3	4.08 ± 0.3	3.88 ± 0.3	5.09 ± 0.3*	3.89 ± 2.0	5.46 ± 0.2*
Ascities (ml)	-	1.20 ± 0.1*	-	3.00 ± 01*	-	6.0 ± 0.1*

Results are mean ± SE of 8-10 experiments for each group. *$P < 0.05$ vs. sham-operated controls.

The results described in Table 2 indicate that the left ventricular end-diastolic pressure (LVEDP) was increased whereas the rates of contraction (+dP/dt) and relaxation (-dP/dt) decreased significantly ($P < 0.05$) at 4, 8 and 16 weeks of coronary ligation. The left ventricular systolic pressure (LVSP) and heart rate (HR) were decreased in 16 weeks experimental animals only. These changes are consistent with those reported earlier with this experimental model indicating mild, moderate and severe stages of congestive heart failure at 4,8 and 16 weeks of coronary occlusion, respectively (92,266). It should be mentioned that the division of experimental animals into mild, moderate and severe stages of CHF with respect to time after coronary occlusion is arbitrary and is used for the purpose of comparison of hemodynamic and clinical changes with alterations in biochemical parameters. Since 80 to 90 % experimental animals died within 20 to 24 weeks of inducing myocardial infarction, no hemodynamic assessment was carried out at this late stage of congestive heart failure.

2. SR Ca^{2+} uptake activities in failing hearts

Ca^{2+} uptake activities of SR from right ventricle and the viable left ventricle were studied in 4 weeks experimental animals and the results are shown in Figures 2 and 3. A time course study of Ca^{2+} uptake revealed a marked depression in SR preparations from the left ventricle whereas a significant increase was evident in the right ventricular preparations (Figure 2). An opposite pattern of changes in SR Ca^{2+} uptake activities, when measured at different concentrations of Ca^{2+}, was also seen in the left and right ventricles (Fig 3). A Lineweaver-Burk plot analysis of the data showed that the depression

Table 2. Hemodynamic parameters of experimental rats at 4, 8, and 16 weeks after coronary artery ligation.

Parameter	4 Weeks		8 Weeks		16 Weeks	
	Control	Experimental	Control	Experimental	Control	Experimental
LVSP (mmHg)	132 ± 4	124 ± 8	125 ± 6	109 ± 8	123 ± 7	85 ± 7*
LVEDP (mmHg)	2.3 ± 1.0	10.2 ± 2.1*	2.2 ± 1.1	12.5 ± 1.3*	3.1 ± 1.3	15.0 ± 1.5*
+dP/dt (mmHg/s)	5408 ± 101	4330 ± 400*	5402 ± 380	4240 ± 129*	5650 ± 501	3316 ± 312*
-dP/dt (mmHg/s)	4880 ± 215	3117 ± 400*	5000 ± 301	3440 ± 201*	5210 ± 414	2590 ± 301*
HR (beats/min)	401 ± 6	398 ± 30	391 ± 16	340 ± 11	382 ± 15	285 ± 12*

Results are mean ± SE of 8-10 experiments for each group. LVSP, left ventricular systolicpressure; LVEDP, left ventricular end-diastolic pressure; +dP/dt, rate of contraction; -dP/dt, rate of relaxation; HR, heart rate. *$P<0.05$ vs. sham-operated controls.

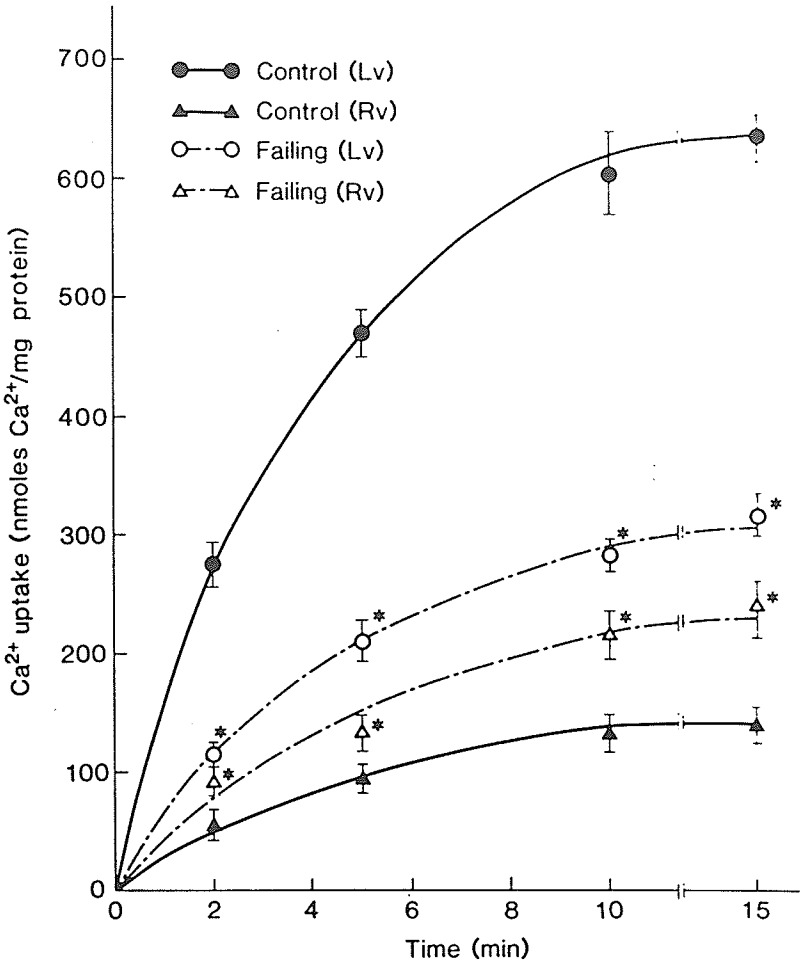


Figure 2. SR Ca^{2+} uptake activity at various times of incubation in the right and left ventricles from control and 4 weeks experimental hearts. The concentration of Ca^{2+} used was 10 uM. Values presented are mean ± SE of 8 control and 4 experimental hearts. *P <0.05 vs respective control.

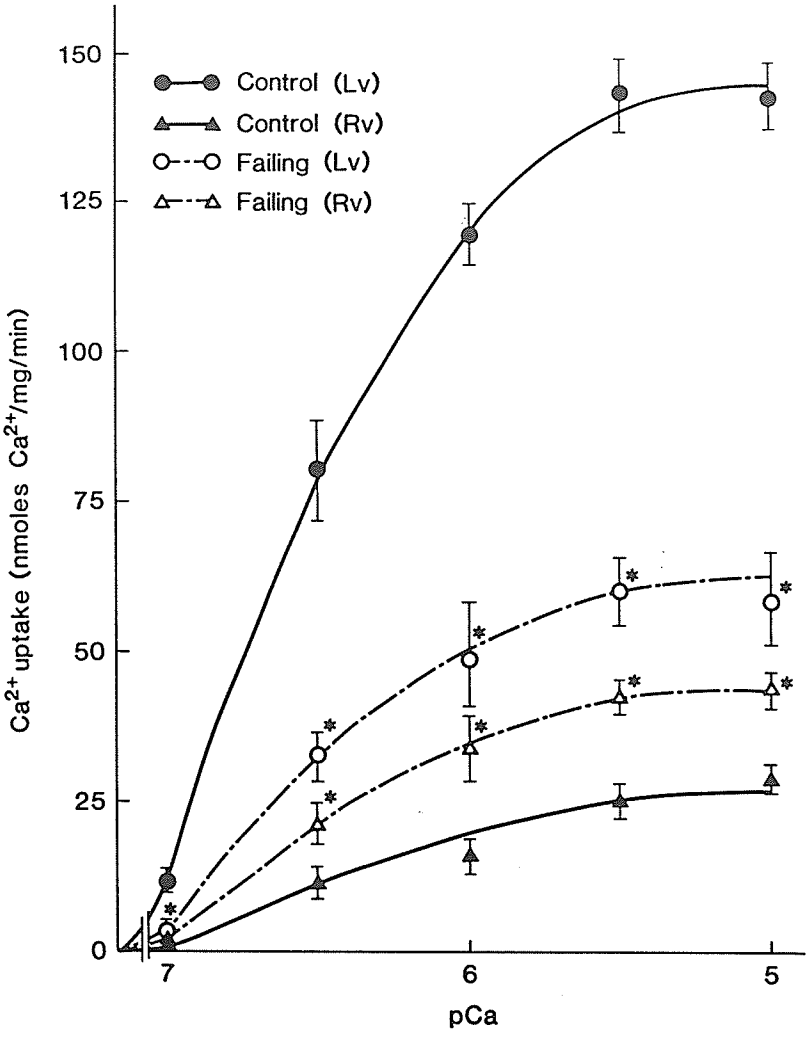


Figure 3. SR Ca^{2+} uptake at different concentrations of Ca^{2+} in the right and left ventricles from control and 4 weeks experimental hearts. Ca^{2+} uptake was measured during the first 2 min period as the reaction was linear under the assay conditions employed in this study. Values are mean ± SE of 6 controls and 5 experimental hearts. *P <0.05 vs respective control.

of SR Ca^{2+} uptake in the left ventricle was associated with a decrease in the maximal velocity (Vmax) in 4 weeks experimental animals (Table 3). On the other hand, the Vmax value for Ca^{2+} uptake in SR from the right ventricle was increased at 4 weeks after the induction of myocardial infarction. The results in Table 3 also indicate that the values for Vmax in left ventricles were decreased at 8 and 16 weeks whereas those for the right ventricles were increased at 8 weeks and unaltered at 16 weeks of coronary occlusion. The dissociation constants (Ka) for the SR Ca^{2+} uptake in left and right ventricles were not altered at 4, 8 and 16 weeks of myocardial infarction. It should be pointed out that the Vmax and Ka values in right ventricle SR Ca^{2+} uptake were lower and higher than those in the left ventricles, respectively. Other investigators (283,284) have also reported lower values for SR Ca^{2+} uptake in the right ventricle in comparison to those in the left ventricle and these differences in the SR Ca^{2+} uptake activities were suggested to reflect functional differences in both ventricles (283).

Earlier studies have indicated an excellent relationship between scar size and impairment of cardiac performance in rats upon occluding the coronary artery (18). Since 16 weeks experimental animals with less than 15 % scar size had no ascites whereas those with 15 to 30 % scar size showed ascites comparable to what was seen at 4 weeks experimental animals with 30 to 55 % scar size, it was considered interesting to study changes in SR Ca^{2+} uptake with respect to scar formation in the left ventricle due to coronary occlusion. The results in Table 4 show no changes in SR Ca^{2+} uptake in both left and right ventricles from animals with less than 15 % scar size. On the other hand, a scar size-dependent progressive decrease in SR Ca^{2+} uptake was seen in the viable left

Table 3. Kinetic parameters for sarcoplasmic reticulum Ca^{2+} uptake in control and experimental hearts (4 weeks).

	Left Ventricle		Right Ventricle	
	K_a (μM)	V_{max} (nmol/mg/min)	K_a (μM)	V_{max} (nmol/mg/min)
Control	0.42 ± 0.06	150 ± 4.0	0.61 ± 0.04	30 ± 1.5
4 Week	0.43 ± 0.08	78 ± 3.7*	0.52 ± 0.06	45 ± 0.9*
8 Weeks	0.41 ± 0.06	54 ± 3.1*	0.54 ± 0.09	37 ± 1.3*
16 Weeks	0.39 ± 0.07	32 ± 2.9*	0.58 ± 0.05	25 ± 1.2

Each value is mean ± SE of 12 control and 4 experimental animals at 4, 8, and 16 weeks after inducing myocardial infarction. Ca^{2+} uptake was measured in the presence of different concentrations of calcium and dissociation constant (K_a) as well as maximum velocity (V_{max}) values were calculated from the Lineweaver-Burk plots. Because the data for 4,8 and 16 weeks sham controls was not different from each other, the results were grouped together. * $P < 0.05$ vs control.

Table 4. Sarcoplasmic reticulum Ca^{2+} uptake in experimental hearts with different scar size at 4, 8, and 16 weeks after ligation of the left coronary artery.

	Scar Size			
	Control	<15 %	15-30 %	>30 %
	Ca^{2+} uptake (nmol Ca^{2+}/mg/min)			
Left ventricle				
4 Weeks	132 ± 4.7	127 ± 5.1	90 ± 3.2*	56 ± 2.6*
8 Weeks	140 ± 4.4	132 ± 4.8	76 ± 2.2*	46 ± 2.1*
16 Weeks	149 ± 5.6	140 ± 3.8	65 ± 2.1*	37 ± 1.3*
Right ventricle				
4 Weeks	29 ± 1.4	26 ± 1.8	37 ± 1.6*	42 ± 1.0*
8 Weeks	25 ± 1.2	23 ± 3.0	32 ± 1.2*	35 ± 1.3*
16 Weeks	24 ± 2.1	26 ± 2.2	22 ± 1.3	23 ± 0.9

Results are mean ± SE of 5-6 experiments for each group at 4, 8, and 16 weeks. The concentration of Ca^{2+} used here was 10 μM. *$P<0.05$ vs. control.

ventricle during the development of congestive heart failure in rats with scar size from 15-30 % and greater than 30 %. The activities of SR Ca^{2+} uptake in right ventricle were increased at 4 and 8 weeks with scar size of 15-30 % and 30-55 % but were unaltered in 16 weeks experimental animals. In 20 weeks experimental animals (n=3) with large scar size (40-50 %), the SR Ca^{2+} uptake in the left ventricle was decreased by almost 80 % whereas that in the right ventricle was decreased by almost 35 % of the respective control values. Thus it appears that there is a progressive decrease in SR Ca^{2+} uptake in the left ventricle whereas SR Ca^{2+} uptake in the right ventricle showed a biphasic change during the development of congestive heart failure.

3. Methodological consideration for SR Ca^{2+} uptake

Since Ca^{2+} uptake measured by the Millipore filtration technique represents the net Ca^{2+} accumulation in SR vesicles as Ca^{2+} influx and Ca^{2+} efflux occur simultaneously during the incubation period, it can be argued that the observed changes in SR Ca^{2+} uptake are due to differences in Ca^{2+} efflux activities in the control and experimental preparations. This possibility was ruled out by including ruthenium red, an inhibitor of SR Ca^{2+} efflux (285), in the incubation medium. The data in Table 5 indicates that the pattern of changes in Ca^{2+} uptake activities of the experimental preparations in the presence of ruthenium red were similar to those seen in its absence (Table 3). Similar changes in the SR Ca^{2+} uptake activities of the left and right ventricles from the 4 weeks experimental animals (n=2) were seen when 100 to 500 uM ryanodine, another inhibitor of Ca^{2+} release, was included in the incubation medium (data not shown).

Table 5. Ca^{2+} uptake in cardiac SR of control and experimental hearts (4 and 16 weeks) in presence of ruthenium red.

	Calcium uptake (nmoles Ca^{2+}/mg/min)		
	Time after infarction	Control	Experimental
Left ventricle			
	4 weeks	137 ± 2.2	75.6 ± 1.3*
	16 weeks	148 ± 1.2	50.3 ± 1.7*
Right ventricle			
	4 weeks	32.7 ± 1.3	50.4 ± 1.3*
	16 weeks	28.3 ± 1.1	29.7 ± 0.4

Values are expressed as mean ± SE of 3-4 experiments for each group. The concentration of calcium used was 10 uM whereas the concentration of ruthenium red used was 2.5 uM.
*$P < 0.05$ vs respective control.

In order to see if the observed changes in SR Ca^{2+} uptake were not due to an artifact of the method of isolation, SR preparations from the control and experimental tissues were isolated by a method (269) different from that employed in this study. The results shown in Figure 4 indicate a progressive depression in Ca^{2+} uptake in the left ventricles at 4,8 and 16 weeks and an increase in Ca^{2+} uptake in the right ventricles at 4 and 8 weeks of myocardial infarction. Since varying degrees of proteolysis can be seen to occur upon homogenizing the diseased hearts, it is possible that the observed changes in the SR preparations are due to differential effects of the proteolytic enzymes. Accordingly, hearts from both control and experimental (4 weeks) animals were homogenized in the presence of 0.1 mM phenylmethylsulfonyl fluoride (PMSF), an inhibitor of the proteolytic enzymes (286), and SR vesicles were isolated for Ca^{2+} uptake determination. The results for the Ca^{2+} uptake activities for the right and left ventricular SR preparations from control and 4 weeks experimental animals (n=2) were similar to those shown in Table 3. Furthermore, the pattern of changes in Ca^{2+} uptake activities in the 4 weeks experimental (n=2) right and left ventricles did not change when SR preparations were obtained from tissue homogenates by employing a glass homogenizer or a polytron. In another set of experiments, oxalate supported Ca^{2+} uptake was determined in tissue homogenates of the left and right ventricles from both control and 4 weeks experimental animals, according to the methods described by Solaro and Briggs (287). The data in Table 6 indicate that oxalate supported activity was decreased in the left ventricle and increased in the right ventricle. The absence or presence of NaN_3, a mitochondrial Ca^{2+} transport inhibitor, in the homogenate did not alter the pattern of results.

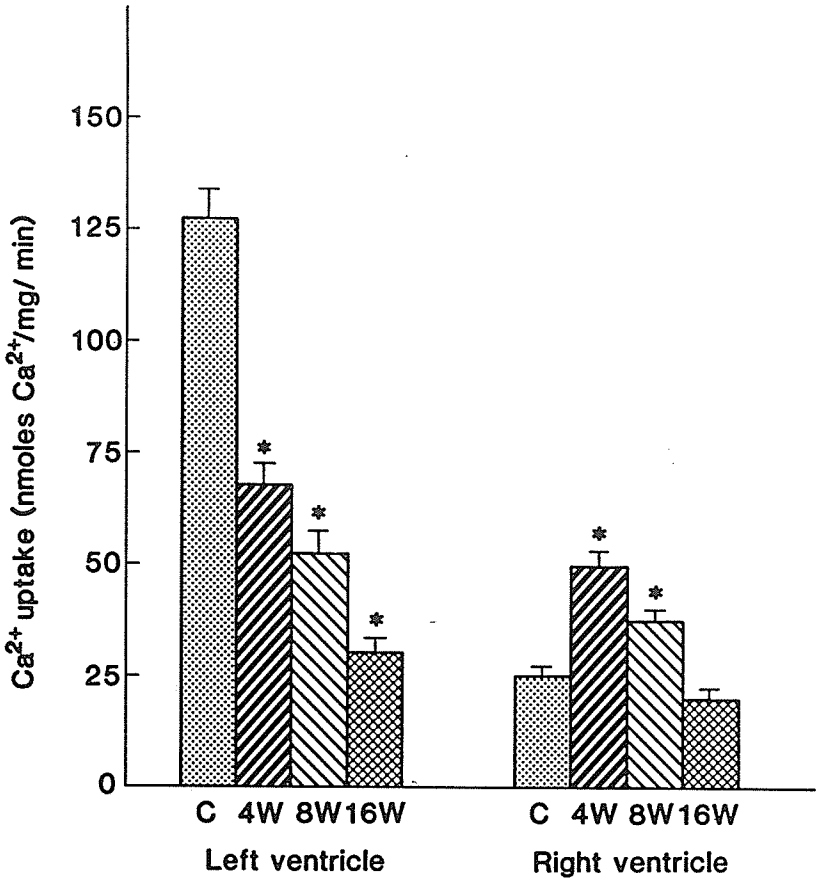


Figure 4. Ca^{2+} uptake activity measured in cardiac SR at different stages of cardiac failure 4,8, and 16 weeks in the left and right ventricles as compared to control. Ca^{2+} uptake was measured in presence of 10 uM calcium. Each point represents mean ± SE of 8 controls and 6 experimental hearts in each group. *P <0.05 vs respective control.

Table 6. Oxalate supported Ca^{2+} uptake in homogenate from control and 4 weeks experimental hearts.

	Ca^{2+} uptake (nmoles Ca^{2+} /mg/min)			
	Left ventricle		Right ventricle	
	Control	Experimental	Control	Experimental
In absence of NaN_3				
	50 ± 2.1	30 ± 1.3*	12 ± 0.8	19 ± 0.9*
In presence of NaN_3				
	46 ± 1.0	28 ± 0.9*	11 ± 0.5	18 ± 0.9*

Values are mean ± SE of 4 experiments. The concentration of Ca^{2+} was 10 uM whereas that of NaN_3 was 5 mM *P <0.05 vs control.

From these experiments it is apparent that the observed changes in the sarcoplasmic reticular Ca^{2+} uptake in both right and left ventricle were not due to any artifact associated with the isolation and purification of the SR vesicles. In addition, these results also rule out the possibility that we are dealing with a select population of SR vesicles which is isolated from the experimental heart by the methods employed in this study. Along with that it was concluded that SR Ca^{2+} uptake activities are regional-specific and time dependent in right and left ventricles and these changes are directly related to the extent of myocardial infarction in these hearts. Because Ca^{2+} uptake in SR is dependent on ATP hydrolysis, the Ca^{2+}-stimulated ATPase activities of SR preparations were studied in control and experimental animals. To characterize the changes in SR Ca^{2+}-stimulated ATPase, animals at severe stages of congestive heart failure were used and these studies were limited to the left ventricular tissue only.

4. SR Ca^{2+}-stimulated ATPase activities in failing hearts

In one set of experiments, the Mg^{2+}-ATPase and Ca^{2+}-stimulated ATPase activities of control and failing SR preparations were studied in presence and absence of 5 mM sodium azide, an inhibitor of mitochondrial ATPase. The Ca^{2+}-stimulated ATPase activities was decreased in failing heart as compared to control without any significant change in the Mg^{2+}-ATPase activity (Table 7). It is also obvious from the results that the contribution of mitochondrial ATPase activity was minimal (Table 7). Since the Ca^{2+}-stimulated ATPase activity is known to be affected by monovalent cations (222), the sensitivity of

Table 7. Mg^{2+}-ATPase and Ca^{2+}-stimulated ATPase activities in presence and absence of sodium azide (5mM) in the left ventricles of control and experimental hearts (16 weeks).

	ATPase activity (nmoles Pi/mg/min)	
	Control	Experimental
A. In the absence of sodium azide		
Mg^{2+} ATPase	4482 ± 85	4624 ± 78
Ca^{2+}-stimulated ATPase	115 ± 2.1	35 ± 2.3*
B. In the presence of sodium azide		
Mg^{2+} ATPase	4596 ± 96	4645 ± 85
Ca^{2+}-stimulated ATPase	109 ± 5.0	31 ± *1.2

Results are mean ± SE of 4 experiments for each group. Significantly different $^{*}P<0.05$ vs control.

Ca^{2+}-stimulated ATPase to potassium was examined in control and experimental left ventricle. The enzyme activity was stimulated by potassium and the maximal activity was seen at 100 to 150 mM concentrations of potassium in both control and failing heart preparations; however, the Ca^{2+}-stimulated ATPase activity in the experimental SR was depressed at all concentrations of K^+ (Figure 5).

The Ca^{2+}-stimulated Mg^{2+}-dependent ATPase activities were also examined in SR preparations at different concentrations of calcium to test the Ca^{2+} sensitivity of SR in the left ventricles of animals in severe stages of heart failure. The enzyme activity was depressed at all concentrations of calcium in experimental heart preparations as compared to control (Figure 6). The Lineweaver-Burk plot of the data revealed no change in Ka values for Ca^{2+} but the V_{max} was depressed in experimental hearts as compared to control (Table 8). Since increasing the ATP concentration is known to stimulate the enzyme turnover and increase the net ATP hydrolysis, a study was undertaken to examine SR Ca^{2+}-stimulated ATPase activity in presence of different concentrations of ATP (0.01-10 mM) in control and experimental preparations. The rate of ATP hydrolysis was depressed in experimental heart preparations as compared to control at all concentrations of ATP tested (Figure 7). The results revealed a marked depression in Vmax value without any changes in the Km values in the failing heart preparations (Table 8). From these results it is evident that the affinities of Ca^{2+} pump ATPase for both Ca^{2+} and ATP were unchanged but the capacity of the enzyme to hydrolyse ATP was markedly decreased in experimental heart SR as compared to control.

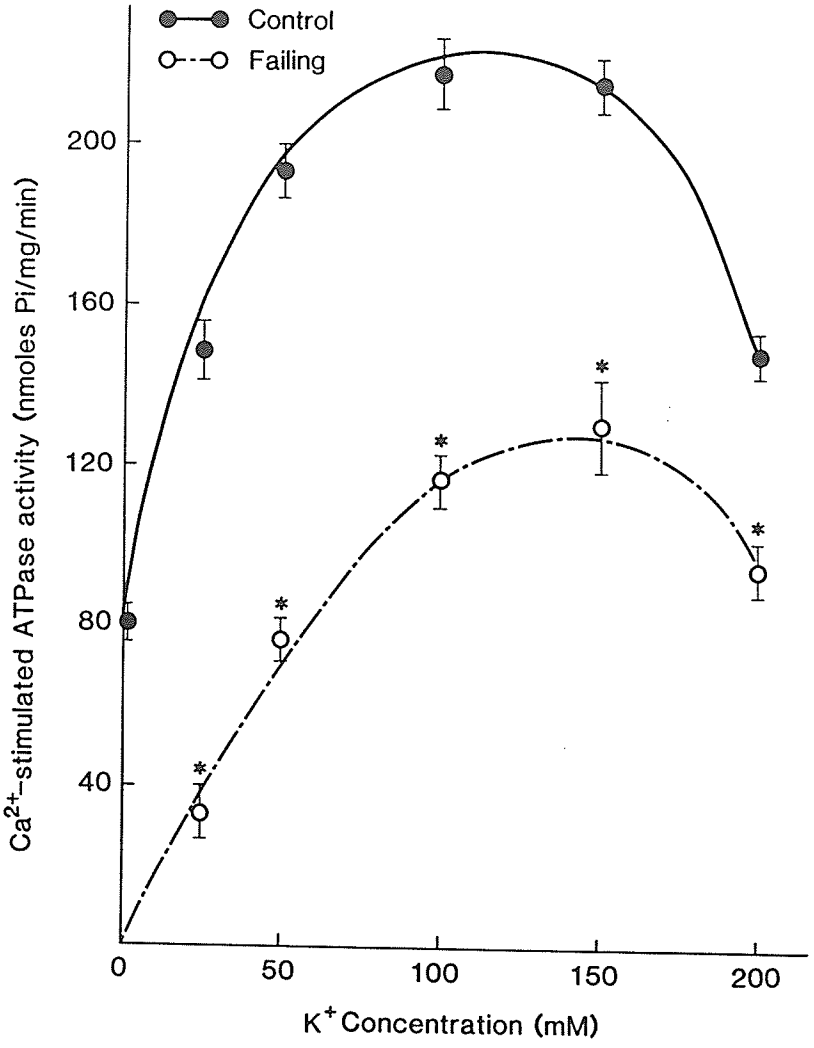


Figure. 5. Effect of different concentrations of KCl on SR Ca^{2+}-stimulated ATPase activity in control and experimental hearts (16 weeks). Results are mean ± SE of 4 experiments. *Significantly different from control $P<0.05$.

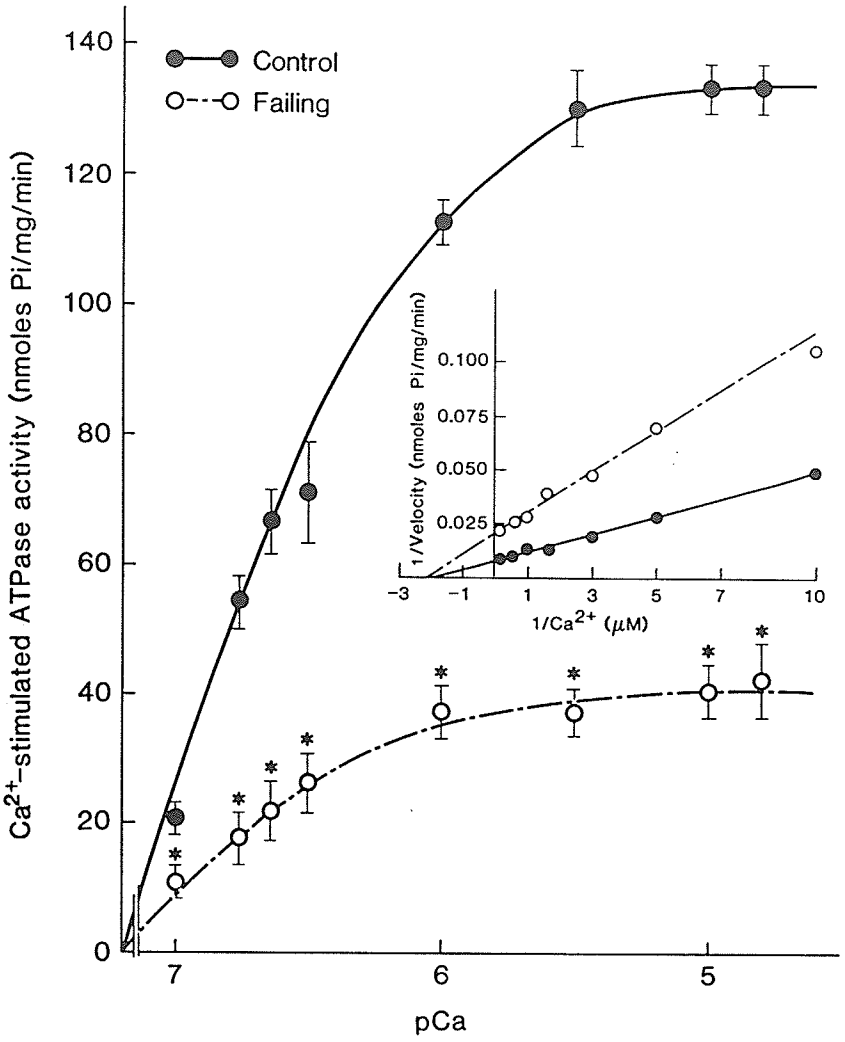


Figure 6. Cardiac SR Ca^{2+}-stimulated ATPase activity in control and experimental (16 weeks) hearts at various concentrations of Ca^{2+}. *$P<0.05$ vs control. Each value represents mean ± SE of 4 experiments. Inset shows double reciprocal plots of data from a typical experiment.

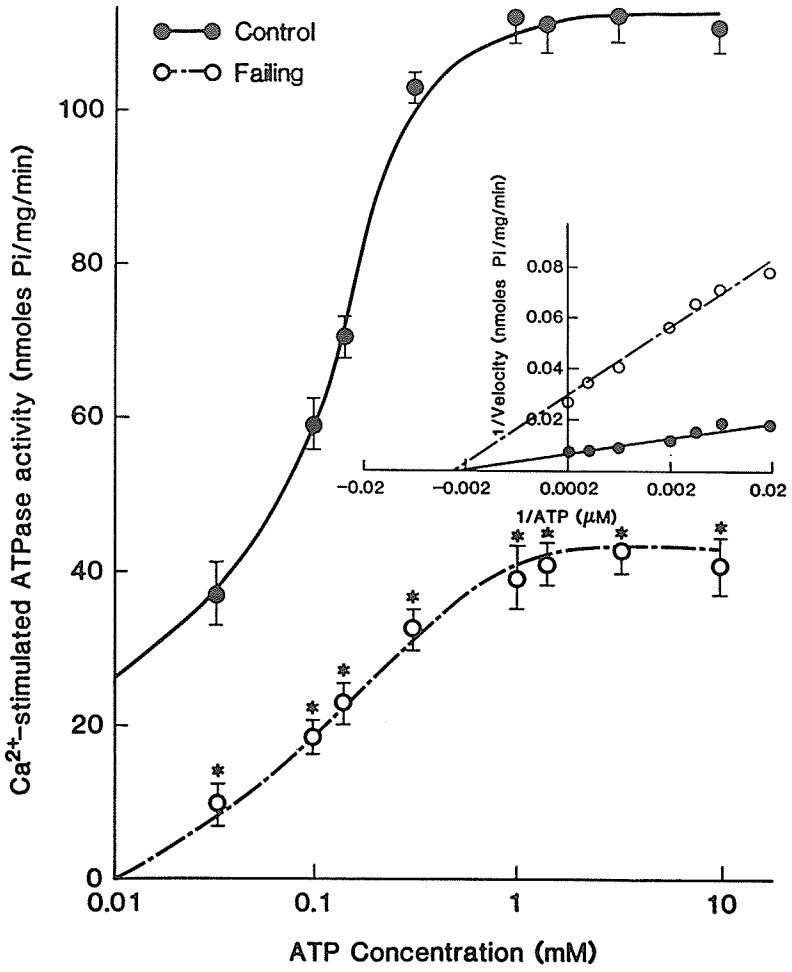


Figure 7. SR Ca^{2+}-stimulated ATPase activities in presence of different concentrations of ATP in control and experimental animals (16 weeks). Results are mean ± SE of 4 experiments. *$P < 0.05$ vs control. Inset shows double reciprocal plot of data from a typical experiment.

Table 8. Kinetic parameters for cardiac SR Ca^{2+}-stimulated ATPase in presence of different concentrations of Ca^{2+} and ATP in the left ventricles of control and experimental hearts (16 weeks).

	Control	Experimental
A. Different concentrations of Ca^{2+}		
Ka	0.42 ± 0.3	0.38 ± 0.2
Vmax	139 ± 9	42 ± 4*
B. Different concentrations of ATP		
Km	0.18 ± 0.2	0.21 ± 0.2
Vmax	147 ± 15	47 ± 8*

Values are mean ± SE of 5 experiments. Significantly different from controls $^{*}P<0.05$.

5. Regulation of SR Ca^{2+}-stimulated ATPase in failing hearts

Since phosphorylation of SR membrane by cAMP-dependent protein kinase or calmodulin is known to increase Ca^{2+}-ATPase activity (223-225), we studied the enzyme activities of SR preparations upon treatment with cAMP-dependent PK or calmodulin. The addition of cAMP-dependent PK in the medium produced a concentration-dependent stimulation of enzyme activity in both control and experimental preparations. The Ca^{2+}-stimulated ATPase activity in the experimental SR was lower than the control values at low concentrations of cAMP-dependent protein kinase; however at higher conentrations the Ca^{2+}-ATPase activities in experimental heart preparations was near control values (Table 9). Effect of calmodulin on Ca^{2+}-ATPase activity was also concentration dependent; increasing concentrations of calmodulin increased the enzyme activity in both control and experimental SR preparations (Table 10). The Ca^{2+}-ATPase activities in the experimental preparations was lower in comparison to the control values at low concentrations, but not at higher concentrations of calmodulin. It can be seen from the Table 9 and 10 that the stimulatory effects of both cAMP-protein kinase and calmodulin, when expressed as fold activation from the basal values were higher in the experimental SR preparations. The effects of low concentrations of cAMP dependent PK and calmodulin were also tested in presence of different concentrations of calcium (0.1-10 uM). The increase in Ca^{2+}-stimulated ATPase activity was calcium dependent after addition of PK (30 ug) and calmodulin (5 uM) in the medium ; however, the enzyme activities in the experimental preparations were lower than the control values (Table 11). K_a calculated from the data did not show any change in the affinity of Ca^{2+} whereas the Vmax values were decreased in

Table 9. Effect of cAMP-dependent protein kinase on SR Ca^{2+}-stimulated ATPase activity in control and experimental hearts (16 weeks).

	Ca^{2+}-stimulated ATPase activity (nmoles Pi/mg/min)	
	Control	Experimental
Protein kinase (ug)		
0	112 ± 4	37 ± 2*
5	155 ± 5 (1.4)	94 ± 4* (2.6)
15	231 ± 6 (2.0)	165 ± 4* (4.5)
30	296 ± 7 (2.6)	235 ± 6* (6.3)
45	340 ± 5 (3.0)	290 ± 8* (7.8)
60	380 ± 9 (3.4)	341 ± 11 (9.2)

Results are mean ± SE of 4-5 experiments. The concentration of cAMP in protein kinase experiments was 1 uM. The concentration of free Ca^{2+} used here was 10 uM. *P <0.05 vs control is significantly different. The figures in parenthesis indicate fold activation with respect to the basal values in the absence of cAMP-protein kinase.

Table 10. Effect of calmodulin on SR Ca^{2+}-stimulated ATPase activity in control and experimental hearts (16 weeks).

	Ca^{2+}-stimulated ATPase activity (nmoles Pi/mg/min)	
	Control	Experimental
Calmodulin (uM)		
0	116 ± 4	40 ± 4*
1	135 ± 3 (1.1)	80 ± 4* (2.0)
3	290 ± 5 (1.6)	145 ± 5* (3.6)
5	252 ± 5 (2.2)	206 ± 7* (5.0)
10	296 ± 10 (2.6)	285 ± 11 (7.0)
20	352 ± 11 (3.0)	320 ± 10 (8.0)

Results are mean ± SE of 4-5 experiments. The concentration of free Ca^{2+} used here was 10 uM. *P <0.05 vs control is significantly different. The figures in parenthesis indicate fold activation with respect to the basal values in the absence of calmodulin.

Table 11. Ca^{2+}-stimulated ATPase activity in presence of different concentrations of calcium in phosphorylated SR membrane in control and experimental hearts (16 weeks).

	Ca^{2+}-stimulated ATPase activity (nmoles Pi/mg/min)			
	Conc. of Ca^{2+}	Without	Calmodulin	cAMP
Control	0.1	22 ± 1	60 ± 2*	65 ± 4*
	0.5	51 ± 2	101 ± 3*	140 ± 6*
	1.0	85 ± 3	130 ± 4*	220 ± 7*
	5.0	115 ± 5	190 ± 6*	293 ± 8*
	10.0	114 ± 5	204 ± 7*	301 ± 9*
	20.0	100 ± 4	207 ± 7*	306 ± 8*
Experimental	0.1	7 ± 1	27 ± 2*	63 ± 3*
	0.5	19 ± 3	70 ± 5*	101 ± 6*
	1.0	27 ± 2	108 ± 4*	180 ± 8*
	5.0	37 ± 2	130 ± 3*	240 ± 9*
	10.0	39 ± 2	153 ± 6*	246 ± 11*
	20.0	36 ± 3	150 ± 6*	251 ± 10*

Results are mean ± SE of 4-5 experiments. Calmodulin 5 uM and Protein kinase 30 ug/ml was used in the incubation medium. *P <0.05 vs control (without addition of calmodulin or cAMP dependent PK) is significantly different.

experimental SR preparations in comparison to the control values (Table 12).

To gain some insight into the phosphorylation mechanisms, SR from control and experimental animals were incubated in presence of [^{32}P]-ATP. As shown in Table 13, ^{32}P incorporation was decreased in experimental hearts as compared to controls. When these membranes were incubated in the presence of protein kinase or calmodulin, ^{32}P incorporation was increased both in control and experimental hearts but the activity in the experimental heart preparations was lower in comparison to the control values. However, it should be noted that stimulatory effects of both cyclic AMP-dependent protein kinase and calmodulin, when expressed in terms of fold stimulation from the basal values, were higher in the experimental preparations. This table also shows the effects of protein kinase inhibitor and calmodulin inhibitor (compound 48/80) on SR phosphorylation. These inhibitors decreased the ^{32}P incorporation in control and experimental SR preparations close to their respective basal values.

6. Mechanisms of SR Ca^{2+}-pump defect

The results in Table 14 indicate that the yields of SR membranes in control and experimental hearts were not different from each other. Since sulfhydryl groups of proteins are important for SR function, any pathological condition can effect the SR Ca^{2+}-stimulated ATPase activity by changing the sulfhydryl groups of the membrane. However, both total and free SH group contents in experimental preparations were not different from those in the control SR (Table 14) indicating that the integrity of SH groups may have been maintained during severe stages of congestive heart failure. One mechanism for the

Table 12. Kinetic parameters of Ca^{2+}-stimulated ATPase in in phosphorylated SR membrane in the left ventricle of control and experimental hearts (16 weeks).

	Ka		Vmax	
	(uM Ca^{2+})		(nmol Pi/mg/min)	
	Control	Experimental	Control	Experimental
No addition	0.46 ± 0.2	0.48 ± 0.2	115 ± 6	39 ± 2
Protein kinase	0.51 ± 0.2	0.54 ± 0.3	317 ± 8*	260 ± 12*
Calmodulin	0.48 ± 0.4	0.52 ± 0.5	209 ± 9*	153 ± 5*

Results are mean ± SE of 5 experiments. Protein kinase 30 ug/ml and Calmodulin 5 uM was used in the incubation medium. *P <0.05 vs control (without addition of cAMP-dependent PK or calmodulin).

Table 13. ^{32}P incorporation in presence of cAMP-dependent PK, calmodulin and their inhibitors in the left ventricles of control and experimental hearts (16 weeks).

	Control	Experimental
without addition	60 ± 3.8	28 ± 2.1*
+ cAMP and protein kinase	160 ± 4.2 (2.7)	104 ± 3.6* (3.7)
+ cAMP, protein kinase and protein kinase inhibitor	55 ± 2.6 (0.9)	25 ± 1.3* (0.9)
+ Ca^{2+}-calmodulin	148 ± 3.5 (2.5)	98 ± 1.8* (3.5)
+ Ca^{2+}-calmodulin and compound 48/80	58 ± 1.3 (1.0)	27 ± 1.7* (1.0)

Values are mean ± SE of 3-4 experiments and are expressed in pmol ^{32}P/min/mg protein. The concentration of different substrates were 5 uM cAMP, 30 ug/ml protein kinase, 100 ug/ml protein kinase inhibitor, 5 uM calmodulin and 30 ug/ml compound 48/80. The figures in parenthesis indicate fold stimulation with respect to the basal values in the absence of cAMP-protein kinase or calmodulin.

Table 14. Cardiac norepinephrine and malondialdehyde contents as well as SR sulfhydryl (SH groups) content in control and experimental hearts (16 weeks).

	Control	Experimenral
SR Yield (mg protein/g heart)	1.82 ± 0.10	1.97 ± 0.15
SR total SH groups (mol/10^5g SR protein)	5.41 ± 0.82	6.27 ± 0.75
SR free SH groups (mol/10^5g SR protein)	2.30 ± 0.21	2.86 ± 0.24
Cardiac malondialdehyde content (nmol/g heart)	30.75 ± 0.90	25.90 ± 0.90
Cardiac norepinephrine content (ng/g heart)	223.00 ± 8.30	152.00 ± 9.70*

Results are mean ± SE of 4-5 experiments. *P < 0.05 vs. control is taken as significant.

observed changes the SR Ca^{2+} stimulated ATPase activity in the experimental hearts may be through the O_2 free radical lipid peroxidation. But this does not seem to be the case since the level of MDA in experimental hearts did not change significantly in comparison to the control value (Table 14). In this regard, the failing hearts used in this study seem to be different from those employed by others because increased MDA levels in ischemic dog myocardium during acute heart failure (288) and decreased MDA levels in stable phase of hypertrophy due to pressure overload has been shown in rats (289). Nonetheless, it can be seen from Table 14 that norepinephrine contents in failing hearts were decreased similar to those observed elsewhere (290). Because alterations in membrane lipid composition are known to affect the Ca^{2+}-stimulated ATPase activity, phospholipid and cholesterol contents were determined in control and experimental SR preparations (Table 15). Concentrations of phosphatidylcholine (PC) and phosphatidylserine (PS) were decreased but concentrations of lysophosphatidylcholine (LPC), sphingomyelin (Sph) and phosphatidylinositol (PI) were increased in experimental hearts SR as compared to control. These changes in the membrane phospholipid composition of the experimental heart SR may be an indication of changes in the membrane composition during this pathological condition. However, the results also indicate that the cholesterol contents as well as cholesterol/phospholipid ratio in experimental SR preparations were not altered.

To determine whether the depression in SR function in failing hearts may be a consequence of a differential purity of this fraction, activities of various selected marker enzymes for different subcellular organelles were examined (Table 16). The enzyme activities as well as purity factors for glucose 6-phosphatase and rotenone insensitive

Table 15. Phospholipid composition and cholesterol contents from cardiac SR of control and experimental hearts (16 weeks).

	Control	Experimental
Phospholipids (nmol/mg)		
Phosphatidylcholine	130 ± 3.90	107 ± 3.06*
Lysophosphatidylcholine	3.91 ± 0.31	7.43 ± 1.06*
Phosphatidylethanolamine	97 ± 7.2	102 ± 9.94
Sphingomyelin	4.95 ± 0.47	6.97 ± 0.44*
Phosphatidylserine	19.0 ± 1.36	10.5 ± 0.36*
Phosphatidylinositol	4.68 ± 0.76	10.1 ± 0.86*
Diphosphatidylglycerol	35.0 ± 1.30	27.2 ± 2.84
Phosphatidic acid	2.17 ± 1.31	1.87 ± 0.4
Total phospholipids	346 ± 27.5	312 ± 26.8
Cholesterol (nmol/mg)	112 ± 5.8	124 ± 7.17
Cholesterol/phospholipids ratio	0.322 ± 0.021	0.397 ± 0.025

Values are mean ± SE of 3-4 experiments. Significantly different from control (*P <0.05).

Table 16. Specific activities of marker enzymes in the left ventricular SR from control and experimental hearts (16 weeks).

	Control	Experimental
Glucose-6-Phosphatase (umoles Pi/mg/hr)	2.98 ± 0.24 (9.8)	2.83 ± 0.21 (10.2)
Rotenone-insensitive NADPH cytochrome C reductase (umoles/mg/min)	38.52 ± 2.55 (10.2)	36.46 ± 2.71 (10.6)
Ouabain-sensitive Na^+-K^+ ATPase (umoles Pi/mg/hr)	1.65 ± 0.12 (0.8)	1.60 ± 0.15 (0.9)
Cytochrome C oxidase (nmoles/mg/min)	42.12 ± 3.24 (0.5)	40.18 ± 3.11 (0.4)

Results are mean ± SE of 5-6 experiments. The values in parenthesis represent the purity factors for each marker and are calculated as the ratio of activities for marker enzymes in SR/homogenate.

NADPH cytochrome Creductase, well known markers for SR membranes in experimental preparations were not different from control values. Ouabain sensitive Na^+-K^+ ATPase activity in SR was similar in control and experimental hearts and exhibited no increase in purity over homogenate values. These preparations did not show any Na^+- Ca^{2+} exchange activity because no calcium release from the Ca^{2+}-loaded SR preparations was seen upon adding 80 mM Na^+. Furthermore 2 uM vanadate, which is known to depress calcium uptake in sarcolemmal vesicles by 60-70 %, inhibited the SR calcium uptake in both control and experimental hearts by about 4 %. This would suggest minor sarcolemmal contamination of these vesicles. It should also be pointed out that the K^+-EDTA-stimulated ATPase activity was undetectable in control and experimental heart preparations. Further extraction of SR vesicles with 0.6 M KCl, which is believed to remove myofibrils, did not affect the calcium uptake activities , ruling out the presence of substantial myofibrillar contamination in these vesicles. Furthermore, we found similar levels of cytochrome C-oxidase activity and no appreciable azide sensitive Mg^{2+}-ATPase activity in both fractions, indicating that mitochondrial contamination in these preparations was minimal and comparable in control and experimental heart samples. These findings suggest that the results observed in this study were not due to a difference in the relative purity of these membranes. The protein gel profile for SR preparations indicated no new peak in the experimental heart SR as compared to control. Furthermore, no change in the magnitude of the major protein peaks in experimental hearts was evident (data not shown). This indicates that there was not any change in the protein composition of the experimental heart SR as compared to control.

7. Expression of mRNA for SR Ca^{2+}-ATPase

In view of the relationship between mRNA content and Ca^{2+}-ATPase protein concentration in SR (291), we examined the expression level of mRNA for cardiac Ca^{2+}-ATPase in the left ventricles of control and experimental animals at 4, 8 and 16 weeks using Nothern blot analysis (Figure 8). For normalization of the Ca^{2+}-ATPase mRNA values, GAPDH mRNA and 28S rRNA values were used as an indication of the total mRNA and total rRNA respectively (93). The relative amounts of Ca^{2+}-ATPase mRNA present in the left ventricle versus GAPDH mRNA in different groups of experimental animals are presented in Figure 9. The level of Ca^{2+}-ATPase mRNA relative to GAPDH mRNA was significantly decreased in the left ventricles at 4, 8 and 16 weeks post-myocardial infarction hearts as compared to sham operated controls (Figure 9A). To reconfirm these observations, and to see that equal amount of RNA was loaded for each sample, blotted filters were also hybridized with cDNA for 28S rRNA. When the ratio of the mRNA for Ca^{2+}-ATPase was calculated relative to the 28S rRNA, the same trend of changes was apparent (Fig. 9B) indicating that in the left ventricles of the experimental hearts, mRNA levels for Ca^{2+}-ATPase are not only decreased relative to GAPDH, but also to ribosomal RNA as compared to controls.

To further quantitate the variation in the level of expression of gene, we performed slot blot analysis (Figure 10). GAPDH mRNA and 28S rRNA values were used for normalization of SR Ca^{2+}-ATPase mRNA. The relative amounts of Ca^{2+}-ATPase mRNA present in the left ventricles versus GAPDH mRNA in different groups of animals was decreased as compared to control (Figure 11A). The same trend of changes was also

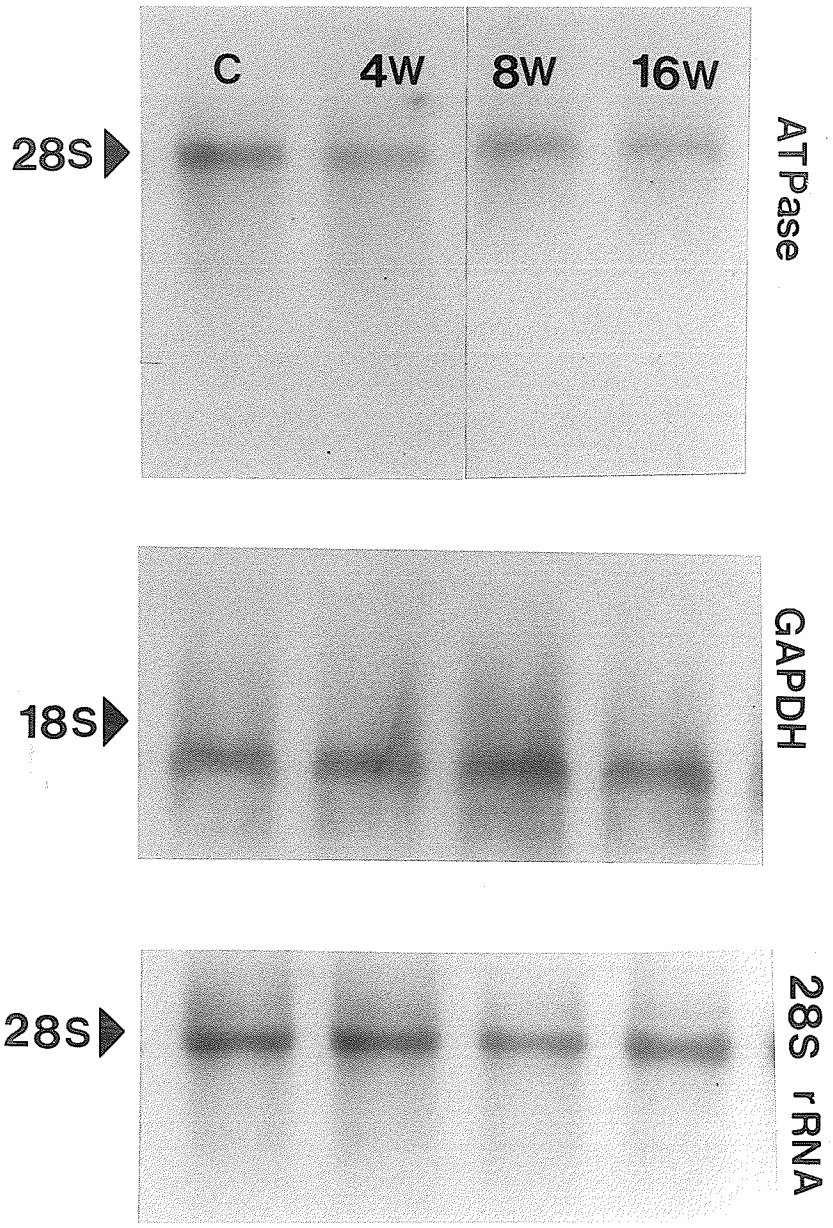


Figure 8. Northern blots of cardiac Ca^{2+}-ATPase mRNA, GAPDH mRNA and 28S rRNA in the left ventricle from control and experimental animals (4, 8, and 16 weeks post-myocardial infarction).

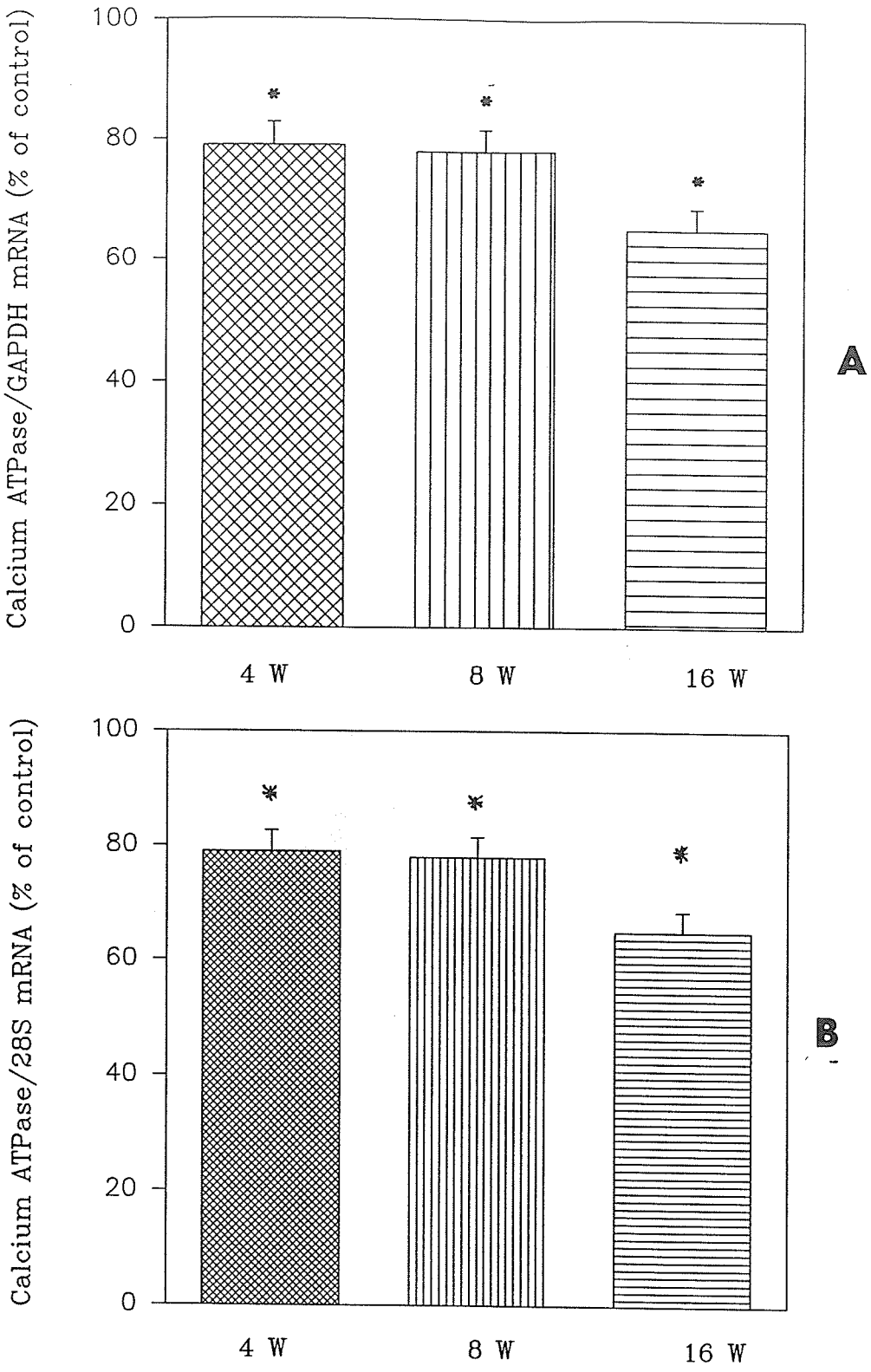


Figure 9. Northern blot analysis: Bar graphs showing quantitation of mRNA for Ca^{2+}-ATPase in control and experimental hearts at 4, 8, and 16 weeks post myocardial infarction.The values are corrected with mRNA of GAPDH and 28S r RNA levels and are shown as the percentage of the control. Each value is a mean ± SE of 6-8 experiments.
* $P < 0.05$.

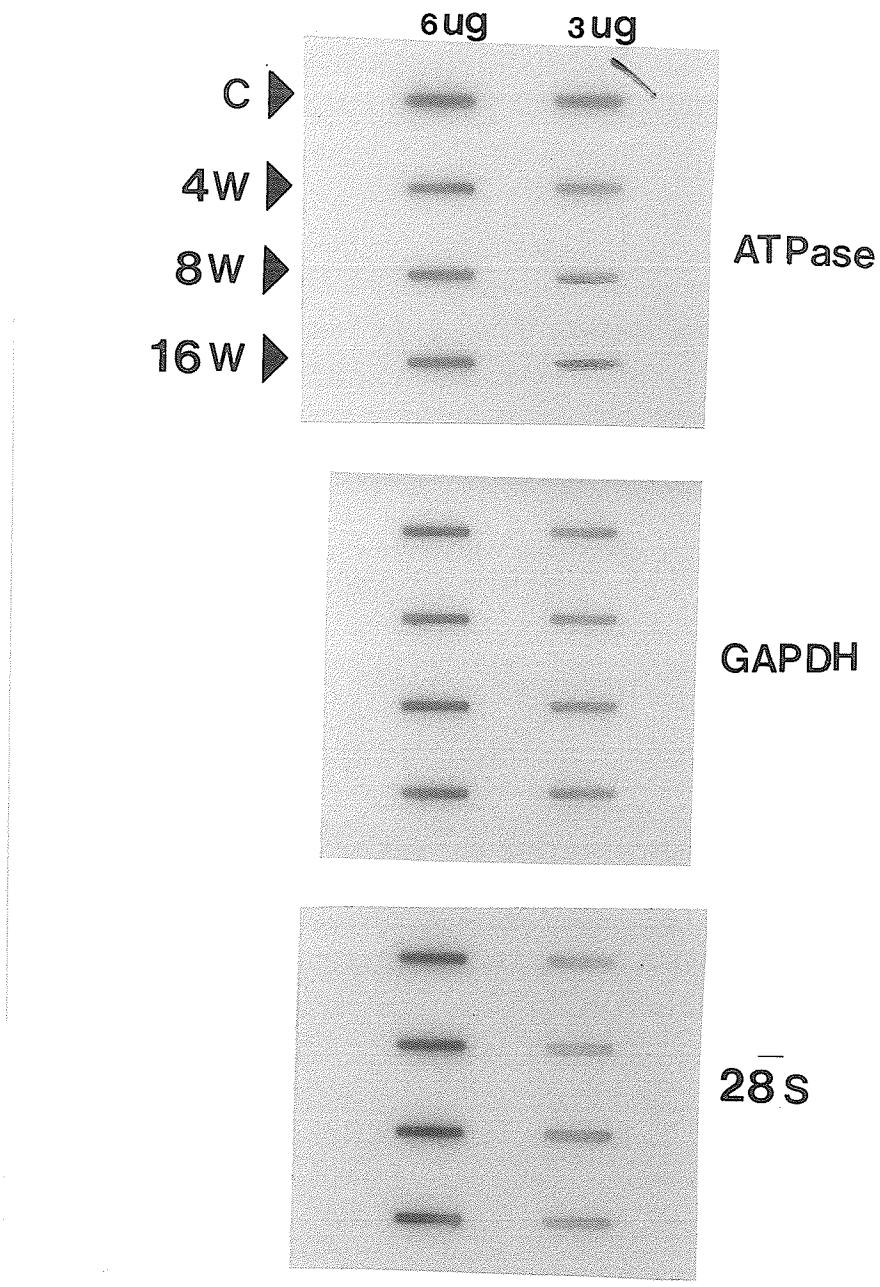


Figure 10. RNA slot blots of the cardiac Ca^{2+}-ATPase, GAPDH and 28S rRNA in control and experimental hearts at 4, 8, and 16 weeks after ligation of the left coronary atery. Total RNA of 3 and 6 μg was loaded onto each slot.

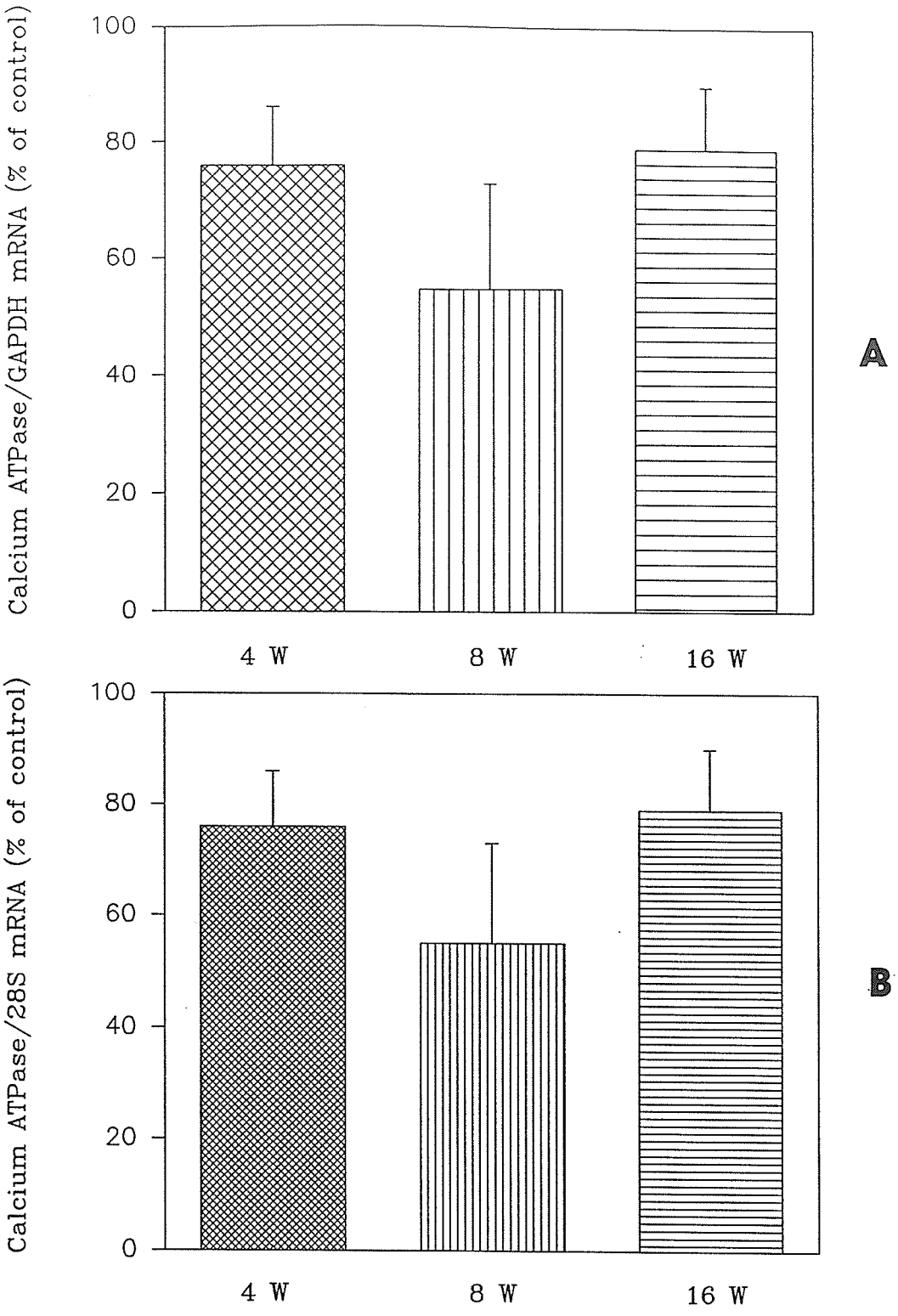


Figure 11. Slot blot analysis: Bar graph showing quantitation of mRNA for Ca^{2+}-ATPase in control and experimental hearts at 4, 8, and 16 weeks post myocardial infarction.The values are corrected with mRNA of GAPDH and 28S r RNA levels and are shown as the percentage of the control.

obvious when the calculation was made relative to 28S mRNA (Figure 11B). These results indicate that the mRNA levels of SR Ca^{2+}-ATPase responsible for calcium uptake are decreased in mild, moderate and severe stages of heart failure as compared to control animals.

V. DISCUSSION

The rat model of congestive heart failure has been used by many investigators to study the structural, functional and morphological characteristics of surviving myocardium (17,92,261,266). The left coronary artery ligation resulted in 5-50 % infarction of the left ventricles in experimental animals after 16 weeks of induction of myocardial infarction. Heart failure in these animals was associated with hypertrophy of the remaining myocardium and decreased rate of contraction and relaxation. Increased LVEDP, congested lungs and presence of ascites were the confirmation of circulatory failure in this condition. It is the current view that cellular growth in hypertrophy is proportional to the amount of stress imposed on the heart. On such a basis, it can be assumed that following myocardial infarction, the extent of cellular enlargement in the surviving tissue would be proportional to the magnitude of the myocardial cell loss. During the development of congestive heart failure, heart not only loses a part of its contractile mass but the remaining myocardium develops hypertrophy. Anversa et al (17) have shown that after large infarcts of the left ventricle, the hypertrophied surviving myocardium was characterized by an 18 % reduction in the capillary surface and 16 % increase in the diffusion distance for O_2. Therefore a deficit in capillary adaptation can be seen to exist following myocardial infarction. Since the magnitude of the deficit depends on the extent of hypertrophy which is related to infarct size (17), it appears that the surviving myocardium in the left ventricle may be relatively ischemic. However, unlike the ischemic myocardium, the malondialdehyde content, an index of increased lipid peroxidation due to oxidative stress, was not increased

in the failing myocardium due to myocardial infarction. Accordingly, it is apparent that viable non-ischemic left ventricle tissue is suitable for biochemical studies of the failing heart in the experimental model. Furthermore, it should be noted that norepinephrine content in the failing hearts due to myocardial infarction are decreased and this observation is consistent with the results obtained from other types of failing hearts (290).

A novel finding in this study was that Ca^{2+} uptake in SR preparations from the viable left ventricle decreased whereas that from the right ventricle increased upon inducing myocardial infarction. Depressed SR Ca^{2+} uptake in the left ventricle and increased SR Ca^{2+} uptake in the right ventricle were associated with corresponding alterations in the Vmax values without any change in the affinity of Ca^{2+} transport system for Ca^{2+}. Since Ca^{2+}-stimulated ATPase activity in the left ventricular SR was decreased while that in the right ventricular SR was increased in the experimental animals, it is evident that the observed alterations in SR Ca^{2+} uptake activities are due to corresponding changes of the Ca^{2+}-pump mechanisms in the SR membrane. Whether or not these changes are due to alterations in density of the Ca^{2+}-pumping units in the SR membranes from right and left ventricles cannot be decided on the basis of information available at this time. However, from the results presented in this study it appears that alterations in the SR Ca^{2+}-pump activities are not due to any cross contamination with other subcellular organelles or artifacts associated with the isolation of SR vesicles.

A decrease in SR Ca^{2+} uptake activity is usually interpreted to reflect an impairment in the rate of relaxation of the cardiac muscle (43,44). The depressed SR Ca^{2+} uptake would also decrease the intracellular Ca^{2+} stores, which are available for release upon excitation

of the cardiac cell and are considered to be responsible for the contractile force development (43,44). Although a progressive decrease in SR Ca^{2+} uptake in the viable left ventricle was seen to occur during the development of congestive heart failure, such changes did not exhibit a definite relationship with depressed left heart function because the contractile activity of the left ventricle in 4 and 8 weeks experimental animals was not different from each other. This may be due to different types of adaptive changes which may occur to maintain heart function during the development of congestive heart failure. Furthermore, alterations in myofibrillar ATPase activities need to be examined during the development of congestive heart failure to fully understand the relationship of biochemical defects with contractile failure. Nevertheless, it is noteworthy that the observed decrease in SR Ca^{2+} uptake in the left ventricle was dependent upon scar size, which has been demonstrated to show an excellent relationship with the degree of impairment in the left heart function due to myocardial infarction in rats (18). Accordingly, it is suggested that a defect in the SR Ca^{2+}-pump mechanism in the left ventricle may contribute towards contractile abnormalities in the left heart function subsequent of myocardial infarction.

Opposite changes in SR Ca^{2+} uptake in the right and left ventricles at 4 and 8 weeks of myocardial infarction may reflect differences in the functional status of the right and left hearts in animals subsequent to myocardial infarction. In fact the right and left ventricles were found to exhibit hyperfunction and hypofunction 4 weeks after myocardial infarction in rats, respectively (292). Thus it appears that while the left ventricle is in a failing stage, the right ventricle is in a compensatory stage. Such a view is consistent with previous observations where SR Ca^{2+} uptake was increased at an early stage of myocardial

hypertrophy exhibiting hyperfunction but was decreased at a late stage of hypertrophy showing hypofunction due to pressure overload (293,294). Since the right ventricular SR Ca^{2+} uptake was depressed at terminal stages of heart failure (20 weeks after occluding the coronary artery), it appears that changes in the right ventricular SR Ca^{2+} uptake were biphasic in nature. However, due to a lack of functional data for the right ventricle during the development of congestive heart failure, it is difficult to draw any conclusion regarding the significance of the Ca^{2+} uptake results. Nevertheless it should be noted that an increase in both cardiac function and SR Ca^{2+} uptake has been observed in animals upon exercise (295) whereas a depression in these parameters has been reported in a wide variety of experimental models of heart disease (43,44,63,267). In view of the concept that changes in different membrane systems in hypertrophied and failing hearts are dependent upon the stage and type of the heart disease (43,44), some caution should be exercised in comparing data from one experimental model to another. At any rate, the observed differential alterations in the right and left ventricular SR Ca^{2+}uptake at 4 weeks of myocardial infarction may be specific because no changes in Ca^{2+} channels at the sarcolemmal level were seen in this experimental model of congestive heart failure (92). Although both biochemical and molecular mechanisms responsible for the observed differential changes in SR preparations from right and left ventricles in animals with congestive heart failure remain to be investigated, the results in this study provide evidence for differential remodelling of the SR membranes in the right and left ventricles.

The results of this study indicate that the depressed Ca^{2+}-stimulated ATPase activity in SR from the failing ventricle was associated with a depression in the maximal velocity of

the enzymatic reaction. Since the Ka value for Ca^{2+} and Km value for ATP were not altered in the failing heart preparations, it is evident that the observed depression in the SR Ca^{2+}-stimulated ATPase was not related to any changes in the affinity of the enzyme for Ca^{2+} or ATP. Because neither the SR protein yield nor the marker enzyme activities in the failing heart preparations were different from their respective control values, it is unlikely that the decreased Ca^{2+}-stimulted ATPase activity is due to any cross contamination by other subcellular organelles. This is further evident from the fact that no new peak was seen in the gel electrophoretic protein pattern of the experimental preparations. Although inactivation of SH groups in the SR Ca^{2+}-stimulated ATPase is known to depress the enzyme activity (218,219), this does not seem to be the case for failing heart preparations as no changes in the total or free SH groups contents were apparent. Since the cholesterol/phospholipid ratio, an important index of membrane fluidity (297) did not change in the experimental SR, it is improbable that the decreased Ca^{2+}-stimulated ATPase activity is due to any alteration in the membrane fluidity. On the other hand, phosphatidylcholine and phosphatidylserine contents were decreased while lysophsphatidylcholine, sphingomyelin and phosphatidylinositol contents were increased in the failing heart SR preparations. Since changes in the phospholipid composition of the SR membrane are known to exert a profound effect on the Ca^{2+}-stimulated ATPase activity (217,296,297), the observed alterations in the phospholipid contents of the failing heart SR can be seen to explain the depressed enzyme activity in these preparations.

In addition to changes in the composition of membrane phospholipids, other factors such as alterations in membrane phosphorylation by cyclic AMP dependent kinase as well

as calcium-calmodulin system are known to regulate the SR Ca^{2+}-stimulated ATPase activity (223). In this regard, it should be noted that cyclic AMP protein kinase dependent and calcium-calmodulin dependent phosphorylation have been reported to augment the SR Ca^{2+}-stimulated ATPase activity (224,272) and thus any decrease in one or both types of membrane phosphorylations could be associated with a depression in the enzyme activity. The results of this study have indicated depressed phosphorylation activities by both cyclic AMP-protein kinase and calcium-calmodulin systems in the failing heart SR preparations. Furthermore, the activation of Ca^{2+}-ATPase in the presence of low concentrations of exogenous cyclic AMP-dependent protein kinase or calmodulin in the failing heart SR preparations was lower than the control values. Although the fold activation of Ca^{2+}-stimulated ATPase activity in the experimental SR by low concentrations of cyclic AMP-protein kinase or calmodulin was higher than that in the control preparations, this apparent increase in the sensitivity of failing heart SR was due to depressed basal values of the enzyme activity. This view is consistent with similar observations in the failing heart preparations with respect to membrane phosphorylations in the presence of both cyclic AMP-protein kinase as well as calmodulin. Since the Ca^{2+}-stimulated ATPase activities in the experimental SR preparations in the presence of high concentrations of exogenous cyclic AMP-dependent protein kinase as well as calmodulin were not different from the respective control values, it can be argued that the depressed enzyme activity in the failing heart SR may be due to decreased cyclic AMP-dependent protein kinase and calmodulin contents. This view is further substantiated by the fact that the endogenous phosphorylation activity in the failing heart SR was less than the control preparations. On the basis of these

observations, it is suggested that the depressed SR Ca^{2+}-stimulated ATPase activity in the failing heart may partly be due to abnormalities in both cyclic AMP-protein kinase and calmodulin-dependent mechanisms for membrane phophorylation.

While the depressed SR Ca^{2+}-stimulated ATPase activity can be considered to account for the observed decrease in the Ca^{2+}-uptake in the experimental preparations, the data in this study does not provide any information concerning changes in the amount of Ca^{2+}-pump protein in the SR membrane. Nonetheless we examined the mechanisms for the observed altertions in the SR function during different stages of congestive heart failure by determining the status of mRNA encoding the SR Ca^{2+}-ATPase. It was demonstrated that the expression of the mRNA for cardiac Ca^{2+}-ATPase was down regulated at 4, 8 and 16 weeks-post myocardial infarction. The depression in the levels of mRNA for Ca^{2+}-ATPase was seen by employing two techniques, Northern blot analysis and slot blot analysis. Furthermore, similar results with respect to changes in the relative amounts of mRNA for Ca^{2+}ATPase were obtained by using two internal standards namely mRNA for GAPDH and mRNA for 28S. These results are in agreement with reports that the mRNA levels for SR Ca^{2+}-ATPase were decreased in hypothyroid as well as failing hearts of different etiologies (169,246,247). Furthermore, the expression of calcium pump (Ca^{2+}-ATPase) was co-ordinately upregulated in hyperthyroidism and downregulated in hypothyroidism (246). It should be pointed out that the changes in mRNA expression for Ca^{2+}-ATPase were parallel to alterations in the enzyme activity at mild, moderate and severe stages of congestive heart failure. Although these data support the hypothesis that changes are occuring at the mRNA level, it remains to be determined whether the regulation of the SR Ca^{2+}-pump ATP

expression is occuring at the transcriptional or post transcriptional level.

VI. SUMMARY AND CONCLUSIONS

1. The clinical signs and hemodynamic assessment of rats revealed mild, moderate and severe stages of congestive heart failure at 4, 8 and 16 weeks after the occlusion of the left coronary artery, respectively.
2. During the development of congestive heart failure, the SR Ca^{2+} uptake activities in the viable left ventricle were decreased progressively at mild moderate and severe stages of heart failure.
3. The SR Ca^{2+} uptake activities in the right ventricle were increased at 4 and 8 weeks but no change was evident at 16 week post-infarction.
4. Opposite changes in SR Ca^{2+} uptake activities in left and right ventricles from the experimental animals were also seen when the release of Ca^{2+} from the SR vesicles was blocked by a Ca^{2+}-release channel blocker, ruthenium red.
5. The observed changes in SR Ca^{2+} uptake activities in the both the left and the right ventricles from experimental animals were associated with corresponding alterations in the Vmax values without any changes in the affinity of SR to Ca^{2+}.
6. The changes in Ca^{2+} uptake activities in the left and right ventricles were time dependent and were related to the scar size in the left ventricle of experimental animals.
7. Opposite changes were observed in the SR Ca^{2+}-stimulated ATPase activities in the left and right ventricles reflecting alterations of the Ca^{2+} pump

mechanisms in SR membranes in the experimental hearts.

8. The depressed activity of the SR Ca^{2+}-stimulated ATPase in the left ventricle at severe stages of heart failure was associated with a decrease in the Vmax values for Ca^{2+}-ATPase without any change in the affinity for Ca^{2+} and ATP.

9. When the SR Ca^{2+}-ATPase activities was measured in the presence of cAMP-dependent PK or calmodulin, the experimental left hearts showed more sensitivity but less enzyme activities at low concentrations of these phosphorylating agents as compared to control values; however in the presence of high concentrations of these agents, Ca^{2+}-ATPase activities in experimental left heart SR were not different from the control values.

10. The phosphorylation activities due to cAMP-dependent PK or calmodulin were decreased in the failing heart SR preparations indicating abnormalities of the regulatory mechanisms for the SR Ca^{2+}-pump system.

11. No changes in the total or free SH group contents of the left ventricle SR was seen in experimental animals. Marker enzyme studies revealed that the observed changes in the experimental hearts were not due to cross contamination or artefact of the method for isolation of membranes.

12. Marked changes were found in the lipid composition of the left ventricular SR in failing hearts and these may partly explain the observed changes in the SR Ca^{2+}-pump system.

13. The levels of mRNA for SR Ca^{2+}-ATPase were decreased in the left ventricle at mild, moderate and severe stages of congestive heart failure suggesting that

the observed changes in SR Ca^{2+}-pump may partly be due to down regulation of the genetic process.

14. The results of this study suggest that the contractile abnormalities in the failing heart as a consequence of myocardial infarction may be due to defects in the SR Ca^{2+}-pump system.

15. The observed changes in SR Ca^{2+}-transport in failing heart are suggested be due to alterations in the lipid composition of the SR membrane, defects in the cyclic AMP and calmodulin mediated regulation of the SR, as well as down regulation of the gene transcription for the SR Ca^{2+}-stimulated ATPase.

16. The SR Ca^{2+} transport changes in the left ventricle were opposite to those observed in the right ventricles from animals with congestive heart failure. This suggests differential remodelling of the SR membranes in the right and left ventricles during the development of congestive heart failure due to myocardial infarction.

VII. REFERENCES

1. BROPHY, J.M. Epidemiology of congestive heart failure: Canadian data from 1970 to 1989. Can. J. Cardiol. 8: 495-498, 1992.
2. SMITH, W.M. Epidemiology of congestive heart failure. Am J. Cardiol. 55: 3A-8A, 1985.
3. GHALI, J.K., R.E. COOPER and E. FORD. Trends in hospitalization rates for heart failure in the United States, 1973-1986. Arch. Intern. Med. 150: 769-773, 1990.
4. ANDERSON, G.M., J.P. NEWHOUSE and L.L. ROSS. Hospital care for elderly patients with diseases of the circulatory system. N. Eng. J. Med. 321: 1443-1448, 1989.
5. FRANCIS, G.S. Development of arrythmias in the patient with congestive heart failure: Pathophysiology, prevalence and prognosis. Am. J. Cardiol. 57: 3B-7B, 1987.
6. PARMLEY, W.W. Pathophysiology of congestive heart failure. Am. J. Cardiol. 55: 9A-14A, 1985.
7. PACKER, M. Neurohormonal interactions and adaptations in congestive heart failure. Circulation 77: 721-730, 1988.
8. FRANCIS, G.S., S.R. GOLDSMITH and J.N. COHEN. Relationship of exercise capacity to resting left ventricular performance and basal plasma nor-epinephrine levels in patients with congestive heart failure. Am. Heart J. 104:

725-731, 1982.

9. PFEFFER, J.M., M.A. PFEFFER, P.J. FLETCHER and E. BRAUNWALD. Ventricular performance in rats with myocardial infarction and failure. Am. J. Med. 76: 99-103, 1984.

10. JOHNS, T.N.P., and B.J. OLSON. Experimental myocardial infarction. A method of coronary occlusion in small animals. Ann. Surg. 140: 675-682, 1954.

11. SELYE, H., E. BAJUSZ, S. GRASSO, AND P. MENDELL. Simple techniques for the surgical occlusion of coronary vessels in the rat. Angiology 11: 398-407, 1060.

12. KAUFMAN, N., T.L. GAVAN, AND R.W. HILL. Experimental myocardial infarction in the rat. Arch. Pathol. 67: 482-488, 1959.

13. BAJUSZ, E., AND G. JASMIN. Histochemical studies on the myocardium following experimental interference with coronary circulation after occlusion of the coronary artery. Acta Histochem. 18: 222-237, 1964.

14. DUSEK, J., G. RONA, AND D.S. KAHER. Healing process in the marginal zone of an experimental myocardial infarct: Findings in the surviving cardiac muscle cells. Am. J. Pathol. 62: 321-338, 1971.

15. BESTER, A.J., E. BAJUSZ, AND A.L. LOCHNER. Effect of ischaemia and infarction on the metabolism and function of the isolated perfused rat heart. Cardiovasc. Res. 6: 284-294, 1972.

16. FISHBEIN, M.C., D. MACLEAN, AND P.R. MAROKO. Experimental myocardial infarction in the rat. Qualitative and quantitative changes during

pathologic evolution. Am. J. Pathol. 90: 57-70, 1978.

17. ANVERSA, P., C. BEGHI, Y. KIKKAWA, AND G. OLIVETTI. Myocardial infarction in rats. Infarct size, myocyte hypertrophy, and capillary growth. Circ. Res. 58: 26-37, 1986.

18. PFEFFER, M.A., J.M. PFEFFER, M.C. FISHBEIN, P.J. FLETCHER, J. SPADARO, R.A. KLONER, AND E. BRAUNWALD. Myocardial infarct size and ventricular function in rats. Circ. Res. 44: 503-512, 1979.

19. FLETCHER, P.J., J.M. PFEFFER, M.A. PFEFFER, AND E. BRAUNWALD. Left ventricular diastolic pressure-volume relations in rats with healed myocardial infarction: Effects on systolic function. Cir. Res. 49: 618-626, 1981.

20. FLETCHER, P.J., J.M. PFEFFER, M.A. PFEFFER, AND E. BRAUNWALD. Effects of hypertension on cardiac performance in rats with myocardial infarction. Am. J. Cardiol. 50: 488-496, 1982.

21. FEILD, B.J., R.O. RUSSEL JR., AND R.E. MORASKI. Left ventricular size and function and heart size in the year following myocardial infarction. Circulation 50: 331-339, 1974.

22. DEFELICE, A., R. FRERING, AND P. HORAN. Time course of hemodynamic changes in rats with healed severe myocardial infarction. Am. J. Physiol. 257: H289-H296, 1989.

23. BAUER, J.A., AND H.L. FUNG. Effects of chronic glyceryl trinitrate on left ventricular haemodynamics in a rat model of congestive heart failure:

Demonstration of a simple animal model for the study of in vivo tolerance. Cardiovasc. Res. 24: 198-203, 1990.

24. PFEFFER, J.M., M.A. PFEFFER, AND E. BRAUNWALD. Influence of chronic captopril therapy on the infarcted left ventricle of the rat. Circ. Res. 57: 84-95, 1965.

25. ANVERSA, P., C. BEGHI, S.L. MCDONALD, V. LEVICKY, Y. KIKKAWA, AND G. OLIVETTI. Morphometry of right ventricular hypertrophy induced by myocardial infarction in rat. Am. J. Pathol. 116: 504-513, 1984.

26. GREENEN, D.L., A. MALHOTRA, AND J. SCHEUER. Regional variation in rat cardiac myosin isoenzymes and ATPase activity after infarction. Am. J. Physiol. 256: H745-H750, 1989.

27. RUBIN, S.A., M.C. FISHBEIN, AND H.J.C. SWAN. Compensatory hypertrophy in the heart after myocardial infarction in the rat. J. A. Coll. Cardiol. 1: 1435-1441, 1983.

28. NORMAN, T., AND C.R. COERS. Cardiac hypertrophy after coronary artery ligation in rats. Arch. Pathol. 69: 181-184, 1960.

29. NAKANISHI, H., N. MAKINO, T. HATA, H. MATSUI, K. YANO, AND T. YANAGA. Sarcolemmal Ca^{2+} transport activities in cardiac hypertrophy caused by pressure overload. Am. J. Physiol. 257: H349- H356, 1989.

30. SWYNGHEDAUW, B., S. RABENOU, F. CARRE. Chronic heart failure: biological bases of myocardial function. Presse. Med. 21: 475-481, 1992.

31. GROSSMAN, W. Cardiac hypertrophy: Useful adaptation or pathological

process. A. J. Med. 69: 576-584, 1980.

32. WILKMAN-COFFELT, J., W. W. PARMLEY, AND D. T. MASON. The cardiac hypertrophy process: Analysis of factors determining pathological vs. physiological development. Circ. Res. 45: 697-707, 1979.

33. SCHEUER, J., AND A. K. BHAN. Cardiac contractile proteins, adenosine triphosphatase avtivity and physiological function. Circ. Res. 45: 1-12, 1979.

34. BING, R.L. The biochemical basis of myocardial failure. Hosp. Pract. 18: 93-112, 1983.

35. COOPER, G.I.V., R.J. TOMANEK, J.D. EHRHARDT, AND M.L. MARCUS. Chronic progressive pressure overload of the cat right ventricle. Circ. Res. 48: 488-497, 1981.

36. POOL, P.E., J.F. SPANN JR., R.A. BUCCINO, E.J. SONNENBLICK, AND E. BRAUNWALD. Myocardial high energy phosphate stores in cardiac hypertrophy and heart failure. Circ. Res. 21: 365-373, 1967.

37. KATZ, A.M. Biochemical defect in the hypertrophied and failing heart. Circulation 47: 1076-1079, 1973.

38. ALPERT, N.R., AND M.S. GORDON. Myofibrillar adenosine triphosphate activity in congestive heart failure. Am. J. Physiol. 202: 940-946, 1962.

39. GORDON, M.S., AND A.L. BROWN. Myofibrillar adenosine triphosphate activity of human heart tissue and congestive failure: Effects of ouabain and calcium. Circ. Res. 18: 534-542, 1966.

40. SCHWARTZ, K., Y. LECARPENTIER, J.L. MARTIN, A.M. LOMPRE, J.J.

MERCADIER, AND B. SWYNGHEDAUW. Myosin isoenzymic distribution correlates with speed of myocardial contraction J. Mol. Cell. Cardiol. 13: 1071-1075, 1981.

41. RUPP, H., AND R. JACOBS. Myocardial transition between fast and slow type muscle as monitored by population of myosin isozymes. In: The Regulation of Heart Function. Rupp, H. (ed.), Thieme, Inc., New York. pp. 271, 1986.

42. DHALLA, N.S. Involvement of membrane systems in heart failure due to intracellular calcium overload and deficiency. J. Mol. Cell. Cardiol. 9: 661-667, 1976.

43. DHALLA, N.S., P.K. DAS, AND G.P. SHARMA. Subcellular basis of cardiac contractile failure. J. Mol. Cell. Cardiol. 10: 363-385, 1978.

44. DHALLA, N.S., G.N. PIERCE, V. PANAGIA, P.K. SINGAL, AND R.E. BEAMISH. Calcium movements in relation to heart function. Basic Res. Cardiol. 77: 117-139, 1982.

45. CARAFOLI, E. Intracellular Ca^{2+} homeostasis. Annu. Rev. Biochem. 56: 395-433, 1987.

46. CALLEWAERT, G.L., L. CLEEMANN, AND M. MORAD. Epinephrine enhances Ca^{2+}-current-regulated Ca^{2+} release and Ca^{2+} reuptake in rat ventricular myocytes. Proc. Natl. Acad. Sci. USA 85: 2009-2013, 1988.

47. SORDAHL, L.A., W.B. MCCOLUM, W.G. WOOD, AND A. SCHWARTZ. Mitochondria and sarcoplasmic reticulum function in cardiac hypertrophy and

failure. Am. J. Physiol. 224: 497-502, 1973.

48. ITO, Y., AND C.A. CHIDSEY. Intracellular calcium and myocardial contractility. IV. Distribution of calcium in the failing heart. J. Mol. Cell. Cardiol. 4: 507-517, 1972.

49. SCHAPER, J., E. MEISER, AND G. STAMMLER. Ultrastructural morphometric analysis of myocardium from dogs, rats, hamsters, mice and from human hearts. Circ. Res. 56: 377-391, 1985.

50. HAMRELL, B.B., AND N.R. ALPERT. Cellular basis of mechanical properties of hypertrophied myocardium. In The Heart and Cardiovascular System. Fozzard, H.A., E. Haber, R.B. Jennings, A.M. Katz, and H.E. Morgan (eds), Raven Press, New York, pp 1507-1524, 1986.

51. SOMMER, J.R., AND R.B. JENNINGS. Ultrastructure of cardiac muscle. In: THe Heart and Cardiovascular System, Fozzard, H.A., E. Haber, R.B. Jennings, A.M. Katz, and H.E. Morgan (eds), Raven Press, New York, pp 61-100, 1986.

52. MALHOTRA, A., S. PENPARGKUL, F.S. FEIN, E.H. SONNENBLICK, AND J. SCHEUER. The effect of streptozotocin-induced diabetes in rats on cardiac contractile proteins. Circ. Res. 49: 1243-1250, 1981.

53. JEACOCKE, S.A., AND P.J. ENGLAND. Phosphorylation of a myofibrillar protein of Mr 150,000 in perfused rat heart, and the tentative identification of this as C-protein. FEBS Lett. 122: 129-132, 1980.

54. ENGLAND, P.J. The phosphorylation of cardiac contractile proteins. In: The

Regulation of Heart Function. Rupp. H. (ed) Thieme Inc., New York, pp 223-233, 1986.

55. POTTER, J.D., AND J. GERGELY. Troponin, tropomyosin, and actin interactions in the Ca^{2+} regulation of muscle contraction. Biochemistry 13: 2697-2703, 1974.

56. STROBECK, J.E., AND E.H. SONNENBLICK. Myocardial contractile properties and ventricular performance. In: The Heart and Cardiovascular System, Fozzard, H.A., E. Haber, R.B. Jennings, A.M. Katz, and H.E. Morgan (eds), Raven Press, New York, pp 31-49, 1986.

57. GUZ, A. Chairman's introduction. In: The Physiological Basis of Starling's Law of the Heart. Porter, R., and D.W. Fitzsimmons (eds), Associated Scientific, Amstermad, pp 1-6, 1974.

58. BABU, A., E.H. SONNENBLICK, AND J. GULATI. Molecular basis for the influence of muscle length on myocardial performance. Science 240: 74-76, 1988.

59. COLE, H.A. AND S.V. PERRY. The phosphorylation of troponin-I from cardiac muscle. Biochem. J. 149: 525-533, 1975.

60. ROBERTSON, S.P., J.D. JOHNSON, M.J. HOLROYDE, E.G. KRANIAS, J.D. POTTER, AND R.J. SOLARO. The effect of troponin-I phosphorylation on the Ca^{2+}-binding properties of the Ca^{2+}-regulatory site of bovine cardiac troponin. J. Biol. Chem. 257: 260-263, 1982.

61. LOWEY, S., H.S. SLAYTER, A.G. WEEDS, AND H. BAKER. Substructure

of the myosin molecule. 1. Subfragments of myosin by enzymatic degradation. J. Mol. Biol. 42: 1-29, 1969.

62. RUPP, H., V. ELIMBAN, AND N.S. DHALLA. Diabetes-like action of intermittent fasting on sarcoplasmic reticulum Ca^{2+}-pump ATPase and myosin isoenzymes can be prevented by sucrose. Biochem. Biophys. Res. Commun. 164: 319-325, 1989.

63. AFZAL, N., G.N. PIERCE, V. ELIMBAN, R.E. BEAMISH, AND N.S. DHALLA. Influence of verapamil on some subcellular defects in diabetic cardiomyopathy. Am. J. Physiol. 256: E453-E458, 1989.

64. MUSCH, T.I., R.L. MOORE, P.G. SMALDONE, M. RIEDY, AND R. ZELIS. Cardiac adaptations to endurance training in rats with a chronic myocardial infarction. J. Appl. Physiol. 66: 712-719, 1989.

65. MERCADIER, J.J., A. M. LOMPRE, C. WISNEWSKY, J. L. SAMUEL, J. BERCOVICI, B. SWYNGHEDAUW, AND K. SCHWARTZ. Myosin isoenzymic changes in several models of rat cardiac hypertrophy. Circ. Res. 49: 525-532, 1981.

66. CHEVALIER, B., F. CALLENS, D. CHARLEMAGNE, C. DELCAYRE, A.M. LOMPRE, L. LELIEVINE, J.J. MERCADIER, J.M. MOLIC, P. MANSIER, L. RAPPAPORT, J.L. SAMUEL, K. SCHWARTZ, AND B. SWYGHENDAUW. Signal and adaptational changes in gene expression during cardiac overload. J. Mol. Cell. Cardiol. 21 (S5): 71-77, 1989.

67. SIMPSON, P.C., C.S. LONG, L.E. WASPE, C.J. HENRICH, AND C.P.

ORDAHL. Transcription of early developmental isogenes in cardiac myocyte hypertrophy. J. Mol. Cell. Cardiol. 21 (S5): 79-89, 1989.

68. HIRZEL, H.O., C.R. TUCHSCHMID, J. SCHNEIDER, H.P. KRAYENBUEHL, AND M. SCHAUB. Relationship between myosin isoenzyme composition, hemodynamics and myocardial structure in various forms of human cardiac hypertrophy. Circ. Res. 57: 729-740, 1985.

69. ALPERT, N.R., AND L.A. MULIERI. Increased myothermal economy of isometric force generation in compensated cardiac hypertrophy induced by pulmonary artery constriction in the rabbit. Circ. Res. 50: 491-500, 1982.

70. MERCADIER, J.J., P. BOUVERET, L. GORZA, S. SCHIAFFINO, W.A. CLARK, R. ZAK, B. SWYNGHEDAUW, AND K. SCHWARTZ. Myosin isoenzymes in normal and hypertrophied ventricular myocardium. Circ. Res. 53: 52-62, 1983.

71. CLARK, W.A., R.A. CHIZZONITE, A.W. EVERETT, M. RABINOWITZ, AND R. ZAK. Species correlations between cardiac isozymosins. J. Biol. Chem. 257: 5449-5454, 1982.

72. PAGANI, E.D., A.A. ALOUSI, A.M. GRANT, T.M. OLDER, S.W. DZIUVBAN, AND P.D. ALLEN. Changes in myofibillar content and Mg^{2+}ATPase activity in ventricular tissues from patients with heart failure caused by coronary artery disease, cardiomyopathy, or mitral valve insufficiency. Circ. Res. 63: 380-385, 1988.

73. SOMLYO, A.P., A.V. SOMLYO, AND H. SCHUMAN. Electron probe

analysis of vascular smooth muscle. Composition of mitochondria, nuclei and cytoplasm. J. Cell. Biol. 81: 316-335, 1979.

74. CARAFOLI, E., R. TIOZZO, G. LUIGI, F. CROVETTI, AND C. KARATZING. The release of calcium from heart mitochondria by sodium. J. Mol. Cell. Cardiol. 6: 361-371, 1974.

75. VERCOSI, A., B. REYNAFORJE, AND A.L. LEHNINGER. Stoichiometry of H^+ ejection and Ca^{2+} uptake coupled to electron transport in rat heart mitochondria. J. Biol. Chem. 253: 6379-6385, 1978.

76. AKERMAN, K.E.O. Effect of pH and Ca^{2+} on the retention of Ca^{2+} by rat liver mitochondria. Arch. Biochem. Biophys. 189: 256-262, 1978.

77. FISKUM, G., AND R.S. COCKRELL. Ruthenium red sensitive and insensitive calcium transport in rat liver and ehrlich ascities tumor cell mitochondria. FEBS Lett. 92: 125-128, 1978.

78. MITCHELL, P., AND J. MOYLE. Respiration-driven proton translocation in rat liver mitochondria. Biochem. J. 105: 1147-1162, 1967.

79. SULAKHE, P.V., AND N. S. DHALLA. Excitation-contraction coupling in heart. V11. Calcium accumulation in subcellular particles in congestive heart failure. J. Clin. Invest. 50: 1019-1027, 1971.

80. FABIATO, A. Time and calcium dependence of activation and inactivation of calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac purkinje cell. J. Gen. Physiol. 85: 247-289, 1985.

81. NILIUS, B., P. HESS, J.B. LANSMAN, AND R.W. TSIEN. A novel type of

cardiac calcium channel in ventricular cells. Nature 316: 443-446, 1985.

82. BEAN, B.P. Classes of calcium channels in vertebrate cells. Annu. Rev. Physiol. 51: 367-384, 1989.

83. CLEEMANN, L., AND M. MORAD. Role of Ca^{2+} channel in cardiac excitation contraction in the rat: evidence from Ca^{2+} transients and contraction. J. Physiol. (London) 432: 283-312, 1991.

84. CALLEWAERT, G. Excitation-contraction coupling in mammalian cardiac cells. Cardiovas. Res. 26: 923-932, 1992.

85. NILIUS, B., P. HESS, J. B. LANSMAN, AND R.W. TSIEN. A novel type of cardiac calcium channel in ventricular cells. Nature 316: 443-446, 1985.

86. WAGNER, J.A., I.J. REYNOLDS, H.F. WEISMAN, P. DUDECK, M.L. WEISFELDT, AND S.H. SNYDER. Calcium antagonist receptors in cardiomyopathic hamster: Selective increases in heart, muscle, brain. Science 232: 515-518, 1987.

87. FINKEL, M.S., E.S. MARKS, R.E. PATTERSON, E.H. SPEIR, K.A. STADMAN, AND H.R. KEISER. Correlation of changes in cardiac calcium channels with hemodynamics in Syrian hamster cardiomyopathy and heart failure. Life Sci. 41: 153-159, 1987.

88. KOBAYASHI, A., T. YAMASHITA, M. KANEKO, T. NISHIYAMA, H. HAYASHI, AND N. YAMAZAKI. Effects of verapamil on experimental cardiomyopathy in the BIO 14.6 Syrian hamster. J. A. Coll. Cardiol. 10: 1128-1134, 1987.

89. WAGNER, J.A., H.F. WEISMAN, A.M. SNOWMAN, I.J. REYNOLDS, K.L. WEISFELDT, AND S.H. SNYDER. Alterations in calcium antagonist receptors and sodium-calcium exchange in cardiomyopathic hamsters tissues. Circ. Res. 65: 205-214, 1989.

90 MATUCCI, R., F. BENNARDINI, M.L. SCIAMARELLA, C. BACCARO, I. STENARDI, F. FRANCONI, AND A. GIOTTI. [^{3}H]-nitrendipine binding in membrane obtained from hypoxic and reoxygenated heart. Biochem. Pharmacol. 36: 1059-1062, 1987.

91. NAYLER, W.G., J.S. DILLON, J.S. ELZ, AND M. MCKELVIE. An effect of ischemia on myocardial dihydropyridine binding sites. Eur. J. Pharmacol. 115: 81-89, 1985.

92. DIXON, I.M.C., S.L. LEE, AND N.S. DHALLA. Nitrendipine binding in congestive heart failure due to myocardial infarction. Circ. Res. 66: 782-788, 1990.

93. TAKAHASHI, T., P.D. ALLEN, R.V. LACRO, A.R. MARKS, A.R. DENNIS, F.J. SCHOEN, W. GROSSMAN, J.D. MARSH, AND S. IZUMO. Expression of dihydropyridine receptor (Ca^{2+} channel) and calsequestrin genes in the myocardium of patients with end-stage heart failure. J. Clin. Invest. 90: 927-935, 1992.

94. DHALLA, N.S., I.M.C. DIXON, AND R.E. BEAMISH. Biochemical basis of heart function and contractile failure. J. Appl. Cardiol. 6: 7-30, 1991.

95. LEE, H.R. α-adrenergic receptors in heart failure. Heart failure 5: 62-70,

1989.

96. HOMCY, C.J., S.F. VETNER, AND D.E. VATNER. ß-adrenergic receptor regulation in the heart in pathophysiological states: abnormal adrenergic responsiveness in cardiac disease. Annu. Rev. Physiol. 53: 137-159, 1991.

97. BOHM, M., DIET, G. FEILER, B. KEMKES, AND E. ERDMANN. α-adrenoceptors and ß-adrenoceptor-mediated positive inotropic effects in failing human myocardium. J. Cardiovas. Pharmacol. 12: 357-364, 1988.

98. NEWMAN, W.H. A depressed response of left ventricular contractile force to isoproterenol and norepinephrine in dogs with congestive heart failure. Am. Heart J. 93: 216-221, 1977.

99. FELDMAN, M.D., L. COPELAS, J.K. GWATHMEY, P. PHILLIPS, S.E. WARREN, F.J. SCHOEN, W. GROSSMAN, AND J.P. MORGAN. Deficient production of cyclic AMP: pharmacologic evidence of an important cause of contractile dysfunction in patients with end-stage heart failure. Circulation 75: 331-339, 1987.

100. BRISTOW, M.R., R. GINSBURG, W. MINOBE, R.S. CUBICCIOTTI, W.S. SAGEMAN, K. LURIE, M.E. BILLINGHAM, D.C. HARRISON, AND E.B. STINSON. Decreased catecholamine sensitivity and ß-adrenergic-receptor density in failing human hearts. N. Engl. J. Med. 307: 205-211, 1982.

101. DENNIS, A.R., W.S. COLUCCI, P.D. ALLEN, AND J.D. MARSH. Distribution and function of human ventricular ß-adrenergic receptors in congestive heart failure. J. Mol. Cell. Cardiol. 21: 651-660, 1989.

102. DIXON, I.M.C., AND N.S. DHALLA. Alterations in cardiac adrenoceptors in congestive heart failure secondary to myocardial infarction. Coronary Artery Disease 2: 805-814, 1991.

103. FU, l.X., K.G. SJOGREN, Q.M. LIANG, F. WAAGSTEIN, J. HOEBEKE, AND A. HJALMARSON. Activity of receptors coupled to guanine nucleotide binding regulatory protein in doxorubicin induced cardiomyopathy. Cardiovas. Res. 25: 145-150, 1991.

104. KARLINER, J.S., P. BARNES, M. BROWN, AND C. DOLLERY. Chronic heart failure in the guinea pig increases cardiac α- and ß-adrenoceptors. Eur. J. Pharmacol. 67: 115-118, 1980.

105. VATNER D.E., S.F. VATNER, A.M. FUJII, AND C.J. HOMCY. Loss of high affinity cardiac ß-adrenergic receptors in dogs with heart failure. J. Clin. Invest. 76: 2259-2264, 1985.

106. KARLINER, J.S., C. ALABASTER, H. STEPHENS, P. BARNES, AND C. DOLLERY. Enhanced noradrenaline response in cardiomyopathic hamsters: possible relation to changes in adrenoceptors studied by radioligand binding. Cardiovas. Res. 15: 296-303, 1981.

107 KAGIYA, T., M. HORI, K. IWAKURA, K. IWAI, Y. WATANABE, S. UCHIDA, H. YOSHIDA, A. KITABATAKE, M. INOVE, AND T. KAMADA. Role of increased α-adrenergic activity in cardiomyopathic Syrian hamster. Am. J. Physiol. 260: H80-H88, 1991.

108. SEN, L., B.T. LIANG, W.S. COLUCCI, T.W. SMITH. Enhanced α_1-

adrenergic responsiveness in cardiomyopathic hamster cardiac myocytes: relation to the expression of pertussis to toxin-sensitive G protein and α_1-adrenergic receptors. Circ. Res. 67: 1182-1192, 1990.

109. BRISTOW, M.R., W. MINOBE, R. RASMUSSEN, R.E. HERSHBERGER, AND B.B. HOFFMAN. α_1-Adrenergic receptors in non-failing and failing heart. J. Pharmacol. Exp. Ther. 247: 1039-1045, 1988.

110. VAGO, T., M. BEVILACQUA, G. NORBIATO, G. BALDI, E. CHEBAT, P. BERTORA, G. BAROLDI, AND R. ACCINNI. Identification of α-adrenergic receptors on sarcolemma from normal subjects and patients with idiopathic dilated cardiomyopathy: characteristics and linkage to GTP-binding protein. Circ. Res. 64: 474-481, 1989.

111. REUTER, H. Calcium measurements through cardiac cell membranes. Med. Res. Reviews 5: 427-440, 1985.

112. REUTER, H. Exchange of calcium ions in the mammalian myocardium: Mechanisms and physiological significance. Circ. Res. 34: 599-605, 1974.

113. LEBLANC, N. AND J.R. HUME. Sodium current-induced release of calcium from cardiac sarcoplasmic reticulum. Science 248: 372-376, 1990.

114. BRIDGE, J.H.B., J.R. SMOLEY, AND K.W. SPITZER. The relationship between charge movements associated with I_{Ca} and $I_{Na\text{-}Ca}$ in cardiac myocytes. Science 248: 376-378, 1990.

115. ST. LOUIS, P.J., AND P.V. SULAKHE. Adenosine triphosphate-dependent calcium binding and accumulation by guinea-pig cardiac sarcolemma. Can. J.

Biochem. 54: 946-956, 1976.

116. CARONI, P., AND E. CARAFOLI. Regulation of Ca^{2+}-pumping ATPase of heart sarcolemma by a phosphorylation-dephosphorylation process. J. Biol. Chem. 256: 9371-9373, 1981.

117. VETTER, R., H. HASSE, AND H. WILL. Potentiation effect of calmodulin and catalytic subunit of cyclic AMP-dependent protein kinase on ATP-dependent Ca^{2+} transport by sarcolemma. FEBS Lett. 148: 326, 1982.

118. BERSOHN, M.M., K.D. PHILIPSON, AND J.Y. FUKUSHIMA. Sodium-calcium exchange and sarcolemmal enzymes in ischemic rabbit hearts. Am. J. Physiol. 242: C288-C295, 1982.

119. CHEMNITIUS, J.M., Y. SASAKI, W. BURGER, AND R.J. BING. The effect of ischemia and reperfusion on sarcolemmal function in perfused canine hearts. J. Mol. Cell. Cardiol. 17: 1139-1150, 1985.

120. DIXON, I.M.C., D.A. EYOLFSON, AND N.S. DHALLA. Sarcolemmal Na^{+}-Ca^{2+} exchange activity in hearts subjected to hypoxia reoxygenation. Am. J. Physiol. 253: H1026-H1034, 1987.

121. HEYLIGER, C.E., A. PRAKASH, AND J.H. MCNEILL. Alterations in cardiac sarcolemmal Ca^{2+} pump activity during diabetes mellitus. Am. J. Physiol. 252: H540-H544, 1987.

122. MAKINO, N., K.S. DHALLA, V. ELIMBAN, AND N.S. DHALLA. Sarcolemmal Ca^{2+} transport in streptozotocin-induced diabetic cardiomyopathy in rats. Am. J. Physiol. 253: E202-E207, 1987.

123. MAKINO, N., G. JASMIN, R.E. BEAMISH, AND N.S. DHALLA. Sarcolemmal Na^+-Ca^{2+} exchange during the development of genetically determined cardiomyopathy. Biochem. Biophys. Res. Commun.133: 491-497, 1985.

124. DIXON, I.M.C, T. HATA, AND N.S. DHALLA. Sarcolemmaal calcium transport in congestive heart failure to myocardial infarction in rats. Am. J. Physiol. 262: H1387-1394, 1992.

125. SCHUURMANS-STEKHOVEN, F., AND S.L. BONTING. Transport adenosine triphosphatases: Properties and functions. Physiol. Rev. 61: 2-76, 1981.

126. SCHWARTZ, A., G.E. LINDENMAYER, AND J.C. ALLEN. The sodium-potassium adenosine triphosphatase: Pharmacological, physiological and biochemical aspects. Pharmacol. Rev. 27: 3-134, 1975.

127. GADSBY, D.C. The Na/K pump of cardiac cells. Annu. Rev. Biophys. Bioeng. 13: 373-398, 1984.

128. BALASUBRAMANIAN, V., D.B. McNAMARA, J.N. SINGH, AND N.S. DHALLA. Biochemical basis of heart function. X. Reduction in Na^+-K^+ stimulated ATPase activity in rat heart due to hypoxia. Can. J. Physiol. Pharmacol. 51: 504-410, 1973.

129. LINDENMAYER, G.E., L.A. SORDAHL, S. HARIGAYA, J.C. ALLEN, H.R. BESCH, JR., AND A. SCHWARTZ. Some biochemical studies on subcellular systems isolated from fresh recipient human cardiac tissue obtained during transplantation. Am. J. Cardiol. 27: 227-283, 1971.

130. PANAGIA, V., J.N. SINGH, M.B. ANAND-SRIVASTAVA, G.N. PIERCE, G. JASMIN, AND N.S. DHALLA. Sarcolemmal alterations during the development of genetically determined cardiomyopathy. Cardiovas. Res. 18: 567-572, 1984.

131. YAZAKI, Y., AND J. FUJII. Depressed Na^+-K^+ ATPase activity in the failing rabbit heart. Jap. Heart J. 13: 73-83, 1972.

132. SULAKHE, V.P., AND N.S. DHALLA. Alterations in the activity of cardiac Na^+-K^+-Stimulated ATPase in congestive heart failure. Exp. Mol. Pathol. 18: 100-111, 1973.

133. KHATTER, J.C., AND K. PRASAD. Myocardial sarcolemmal ATPase in dogs with induced mitral insufficiency. Cardiovas. Res. 10: 637-641, 1976.

134. PRASAD, K., J.C. KHATTER, AND B. BHARADWAJ. Intra- and extracellular electrolytes and sarcolemmal ATPase in the failing heart due to pressure overload in dogs. Cardiovas. Res. 13: 95-104, 1979.

135. HASSELBACH, W., AND M. MAKINOSE. Die Calciumpumpe der Erschlaffungsgrana des Muskels und ikre Abhangigkeit von der ATP-Spaltung. Biochem. Z. 333:518-528,1961.

136. FABIATO, A. Calcium release in skinned cardiac cells: Variations with species, tissues, and development. Fed. Proc. 41: 2238-2244, 1982.

137. ANVERSA, P., A. LOUD, F. GIACOMELLI, AND J. WIENER. Absolute morphometric study of myocardial hypertrophy in experimental hypertension. 11. Ultrastructure of myocytes and interstitium. Lab. Invest. 38: 597, 1978.

138. JOHNSON, E., AND J.R. SOMMER. A strand of cardiac muscle: its ultrastructure and the electrophysiologic implications of its geometry. J. Cell. Biol. 33: 103, 1967.

139. SCALES, D. J. Aspects of the mammalian sarcotubular system revealed by electron microscopy. J. Mol. Cell. Cardiol. 13: 373, 1981.

140. MACLENNAN, D.H., AND P.T.S. WONG. Isolation of a calcium-sequestring protein from sarcoplasmic reticulum. Proc. Natl. Acad. Sci. U.S.A. 68: 1231, 1971.

141. FRANZINI-ARMSTRONG, C., AND G. NUNZI. Junctional feet and particles in the triads of a fast twitch fibre. J. Muscle Res. Cell Motil. 4: 233, 1983.

142. FRANZINI-ARMSTRONG, C. Structure of sarcoplasmic reticulum. Fed. Proc. Fed. Am. Soc. Exp. Biol. 39: 2403, 1980.

143. SOMLYO, A.V., H. GONZALEZ-SERRATOS, H. SHUMAN, G. MCCLELLAN, AND A.P. SOMLYO. Calcium release and ionic changes in the sarcoplasmic reticulum of tetanized muscle: an electron-probe study. J. Cell Biol. 90: 577, 1981.

144. MARTONOSI, A. Sarcoplasmic reticulum. 1V. Solubilization of microsomal adenosine triphosphatase. J. Biol. Chem. 243: 71, 1968.

145. HASSELBACH, W. The reversibility of sarcoplasmic calcium pump. Biochem. Biophys. Acta. 515: 23, 1978.

146. TADA, M., T. YAMAMOTO, Y. TONOMURA. Molecular mechanism of

active calcium transport by sarcoplasmic reticulum. Physiol. Rev. 58: 1-79, 1978.

147. EBASHI, S. A granule-bound relaxation factor in skeletal muscle. Arch. Biochem. Biophys. 76: 410, 1958.

148. HASSELBACH, W. Relaxing factor and the relaxation of muscle. Prog. Biophys. Mol. Biol. 14: 169, 1964.

149. JONES, L.R., AND S.E. CALA. Biochemical evidence for functional heterogeneity of cardiac sarcoplasmic reticulum vesicles. J. Biol. Chem. 256: 11809, 1981.

150. MEISSNER, G. Isolation and characterization of two types of sarcoplasmic reticulum vesicles. Biochim. Biophys. Acta 389: 51, 1975.

151. CAMPBELL, K.P. Protein components and their roles in sarcoplasmic reticulum function. In Sarcoplasmic Reticulum in Muscle Physiology, Entman, M.L., and W.B.V. Winkle (eds), CRC Press, Florida, vol.1: 65-99, 1986.

152. BRANDL, C.J., N.M. GREEN, B. KORCZAK AND D.H. MACLENNAN. Two Ca^{2+} ATPase genes: homologies and mechanistics implications of deduced amino acid sequences. Cell 44: 597-607, 1986.

153. BRANDL, C.J., S. DELEON, D.R. MARTIN, D.H. MACLENNAN. Adult forms of Ca^{2+} ATPase of SR. J. Biol. Chem. 262: 3767-3774, 1987.

154. TADA, M., M.A. KIRCHBERGER, D.I. REPKE AND A.M. KATZ. The stimulation of Ca^{2+} transport in cardiac sarcoplasmic reticulum by adenosine

3,5,-monophosphate-dependent protein kinase. J. Biol. Chem. 249: 6174-6180, 1974.

155. FLIEGEL, L., E. LEBERER, N. GREEN, AND D.H. MACLENNAN. The fast-twitch muscle calsequestrin isoform predominates in rabbit slow-twitch soleus muscle. FEBS Lett. 242: 297-300, 1989.

156. CAMPBELL, K.P., D.H. MACLENNAN, A.O. JORGENSEN, AND M.C. MINTZER. Purification and characterization of cardiac calsequestrin from canine sarcoplasmic reticulum and identification of the 53,000 dalton glycoprotein. J. Biol. Chem. 58: 1238, 1983.

157. INUI, M., A. SAITO, AND S. FLEISCHER. Isolation of the ryanodine receptor from cardiac sarcoplasmic reticulum and identity with the feet structures. J. Biol. Chem. 262: 15637-15642, 1987.

158. LAI, F.A., K. ANDERSON, E. ROUSSEAU, Q-Y. LIU, AND G. MEISSNER. Evidence for a Ca^{2+} channel within the ryanodine receptor complex from cardiac sarcoplasmic reticulum. Biochem. Biophys. Res. Commun. 151: 441-449, 1988.

159. JORGENSEN, A.O., A.C-Y. SHEN, AND K.P. CAMPBELL. Ultrastructural localization of calsequestrin in adult rat atrial and ventricular muscle cells. J. Cell. Biol. 101: 257-268, 1985.

160. JORGENSEN, A.O., A.C-Y. SHEN, K.P. CAMPBELL, AND D.H. MACLENNAN. Ultrastructural localization of calsequestrin in rat skeletal muscle by immunoferritin labelling of ultrathin frozen sections. J. Cell. Biol.

97: 1573-1581, 1983.

161. JORGENSEN, A.O., R. BRODERICK, A.P. SOMLYO, AND A.V. SOMLYO. Two structurally distinct calcium storage sites in rat cardiac sarcoplasmic reticulum: An electron microprobe analysis study. Circ. Res. 63: 1060-1069, 1988.

162. IKEMOTO, N., M. RONJAT, L.G. MESZAROS, AND M. KOSHITA. Postulated role of calsequestrin in the regulation of calcium release from sarcoplasmic reticulum. Biochem. 28: 6764-6771, 1989.

163. SCOTT, B.T., H.K.B. SIMMERMAN, J.H. COLLINS, B. NADAL-GINARD, AND L.R. JONES. Complete amino acid sequence of canine cardiac calsequestrin deduced by cDNA cloning. J. Biol. Chem. 263: 8958-8964, 1988.

164. MITCHELL, R.D., H.K.B. SIMMERMAN, AND L.R. JONES. Ca^{2+} binding effects on protein confirmation and protein interactions of canine cardiac calsequestrin. J. Biol. Chem. 264: 1376-1381, 1988.

165. LYTTON, J., D.H. MACLENNAN. Sarcoplasmic reticulum. In: The Heart and Cardiovascular System, Fozzard, H.A, E. Haber, R.B. Jenning, A.M. Katz, and H.E. Morgan (eds), Raven Press Publishers, New York. Vol.2: 1992.

166. MACLENNAN, D.H., K.P. CAMPBELL, AND R.A.F. REITHMEIER. In: Calsequestrin, Calcium and cell function. Cheung, W.Y. (ed), Academic Press, Inc. New York, Vol 4: 151-173, 1983.

167. FLIEGEL, L., M. OHNISHI, M.C. CARPENTER, V.K. KHANNA, R.A.F. REITHMEIER, AND D.H. MACLENNAN. Amino acid sequence of rabbit

skeletal muscle calsequestrin deduced from cDNA and peptide sequencing. Proc. Natl. Acad. Sci. U.S.A. 84: 1167-1171, 1987.

168. ZARAIN-HERZBERG, A., L. FLIEGEL, AND D.H. MACLENNAN. structure of the rabbit fast-twitch skeletal muscle calsequestrin gene. J. Biol. Chem. 263: 4807-4812, 1988.

169. ARAI, M., K. OTSU, D.H. MACLENNAN, N.R. ALPERT, AND M. PERIASAMY. Effect of thyroid hormone on the expression of mRNA encoding sarcoplasmic reticulum proteins. Circ. Res. 69: 266-276, 1991.

170. FORD, L.E., AND R.J. PODOLSKY. Regenerative calcium release within muscle cells. Science 167: 58-59, 1970.

171. ENDO, M., M. TANAKA, AND Y. OGAWA. Calcium induced release of calcium from sarcoplasmic reticulum of skinned skeletal muscle fibres. Nature 228: 34-36, 1970.

172. FABIATO, A. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. Am. J. Physiol.245: C1-C14, 1983.

173. FABIATO, A. Time and calcium dependence of activation and inactivation of calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. J. Gen. Physiol. 85: 247-289, 1985.

174. FABIATO, A. Simulated calcium current can both cause calcium loading in and trigger calcium release from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. J. Gen. Physiol. 85: 291-320, 1985.

175. KENTISH, J.C., R.J. BARSOTTI, T.J. LEA, I.P. MULLIGAN, J.R. PATEL,

AND M.A. FERENZI. Calcium release from cardiac sarcoplasmic reticulum induced by photorelease of calcium or Ins $(1,4,5)P_3$. Am. J. Physiol. 258: H610-H615, 1990.

176. CHAMBERLAIN, B.K., P. VOLPE. AND S. FLEISCHER. Calcium-induced calcium release from purified cardiac sarcoplasmic reticulum vesicles. General characteristics. J. Biol. Chem. 258: 7540-7546, 1984.

177. ROUSSEAU, E., J.S. SMITH, J.S. HENDERSON, AND G. MEISSNER. Single channel and $^{45}Ca^{2+}$ flux measurements of the cardiac sarcoplasmic reticulum calcium channel. Biophys. J. 50: 1009-1014, 1986.

178. MEISSNER, G., AND J.S. HENDERSON. Rapid calcium release from cardiac sarcoplasmic reticulum vesicles is dependent on Ca^{2+} and is modulated by Mg^{2+}, adenine nucleotide, and calmodulin. J. Biol. Chem. 262: 3065-3073, 1987.

179. BENEVOLENSKY, D.S., E.V MENSHIKOVA, J. WATRAS, D.O. LEVITSKY, AND V.B. RITOV. Characterization of Ca^{2+} release from the sarcoplasmic reticulum of myocardium and vascular smooth muscle. Biomed. Biochem. Acta 46: S393-S398, 1987.

180. NABAUER, M., G.C.R. ELLIS-DAVIES, J.H. KAPLAN, AND M. MORAD. Modulation of Ca^{2+} channel selectivity and cardiac contraction by photorelease of Ca^{2+}. Am. J. Physiol.256: H916-H920, 1989.

181. SHAM, J.S.K., L. CLEEMAN, AND M. MORAD. Gating of the cardiac Ca^{2+} release channel,the role of Na^+ current and Na^+-Ca^{2+} exchange. Science 255:

850-853, 1992.

182. RADON, D.P., D.C. CEFALI, R.D. MITCHELL, S.M. SEILER, AND L.R. JONES. High molecular weight proteins purified from cardiac junctional sarcoplasmic reticulum vesicles are ryanodine-sensitive calcium channels. Circ. Res. 64: 779-789, 1989.

183. LINDSAY, A.R., AND A.J. WILLIAMS. Functional characterization of the ryanodine receptor purified from sheep cardiac sarcoplasmic reticulum. Biochem. Biophys. Acta 1064: 89-102, 1991.

184. ASHLEY, R.H., AND A.J. WILLIAMS. Divalent cation activation and inhibition of single calcium release channels from sheep cardiac sarcoplasmic reticulum. J. Gen. Physiol. 95: 981-1005, 1990.

185. NABAUER, M., G. CALLEWAERT, L. CLEEMANN, AND M. MORAD. Regulation of calcium release is gated by calcium current, not gating charge, in cardiac myocytes. Science 244: 800-803, 1989.

186. ANDERSON, K., F. ANTHONY, Q-Y. LIU, E. ROUSSEAU, H.P. ERICKSON, AND G. MEISSNER. Structural and functional characterization of the purified cardiac ryanodine receptor-Ca^{2+} release channel complex. J. Biol. Chem. 264: 1329-1335, 1989.

187. SMITH, J.S., R. CORONADO, AND G. MEISSNER. Single channel measurements of the calcium release channel from skeletal muscle sarcoplasmic reticulum. Activation by Ca^{2+} and ATP and modulation by Mg^{2+}. J.Gen. Physiol. 88: 573-588, 1986.

188. MORGAN, J.P., AND J.R. BLINKS. Intracellular Ca^{2+} transients in the cat papillary muscle. Can. J. Physiol. 60: 524-528, 1982.

189. ROUSSEAU, E., J. LA DINE, Q-Y. LIU, AND G. MEISSNER. Activation of the Ca^{2+} release channel of skeletal muscle sarcoplasmic reticulum by caffeine and related compounds. Arch. Biochem. Biophys. 267: 75-86, 1988.

190. IMAGAWA, T., J.S. SMITH, R. CORONADO, AND K.P. CAMPBELL. Purified ryanodine receptor from skeletal muscle sarcoplasmic reticulum is the Ca^{2+}-permeable pore of the calcium release channel. J. Biol. Chem. 262: 16636-16643, 1987.

191. JONES, L.R., AND S.D. BURROWS. Ruthenium red-induced stimulation of Ca^{2+} uptake by junctional sarcoplasmic reticulum vesicles isolated from heart. Fed. Proc. Am. Soc. Exp. Bio. 42: 1932, 1983.

192. MIYAMOTO, H., AND E. RACKER. Mechanism of calcium release from skeletal sarcoplasmic reticulum. J. Memb. Biol. 66: 193, 1982.

193. JONES, L.R., S.M. SEILER, AND B.V. WINKLE. Regional differences in sarcoplasmic reticulum function.In Entman, M.L., and W.B.V.Winkle (eds), Sarcoplasmic Reticulum in Muscle Physiology, CRC Press, Florida, Vol.1: 65-99, 1986.

194. JONES, L.R., AND S.E. CALA. Biochemical evidence for functional heterogeneity of cardiac sarcoplasmic reticulum vesicles. J. Biol. Chem. 256: 11809, 1981.

195. JONES. L.R., H.R. BESCH, JR., J.C. SUTKO, AND J.T. WILLERSON.

Ryanodine-induced stimulation of net Ca^{2+} uptake by cardiac sarcoplasmic reticulum vesicles. J. Pharmacol. Exp. Ther. 21: 1, 1979.

196. BERRIDGE, M.J. Inositol triphosphate and diacylglycerol: two interacting second messengers. Annu. Rev. Biochem. 56: 159-193, 1987.

197. BEZPROZVANNY, I., J. WATRAS, AND B.E. EHRLICH. Bell-shaped calcium-response curves of Ins (1,4,5) P_3-gated and calcium gated channels from endoplasmic reticulum of cerebellum. Nature 351: 751-754, 1991.

198. EHRLICH, B.E., AND J. WATRAS. Inositol 1,4,5-triphosphate activates a channel from smooth muscle sarcoplasmic reticulum. Nature 336: 583-586, 1988.

199. DE CHAFFOY, D., AND D.E. COURECELLES. Is there evidence of a role of the phosphoinositol-cycle in the myocardium ? Mol. Cell. Biochem. 88: 65-72, 1989.

200. ZHU, Y., AND T.M. NOSEK. Inositol triphosphate enhances Ca^{2+} oscillations but not Ca^{2+}-induced Ca^{2+} release from cardiac sarcoplasmic reticulum. Pflugers Arch. 418: 1-6, 1991.

201. BORGATTA, L., J. WATRAS, A.M. KATZ, AND B.E. EHRLICH. Regional difference in calcium-release channels from heart. Proc. Natl. Acad. Sci. U.S.A. 88: 2486-2490, 1991.

202. NOSEK, T.M., M.F. WILLIAMS, S.T. ZEIGLER, AND R.E. GODT. Inositol triphosphate enhances calcium release in skinned cardiac and skeletal muscle. Am. J. Physiol. 250: C807-C811, 1986.

203. FEHER, J.J., W.R. LEBOLT, AND N.H. MANSON. Differential effect of global ischemia on the ryanodine-sensitive and ryanodine-insensitive calcium uptake of cardiac sarcoplasmic reticulum. Circ. Res. 65: 1400-1408, 1989.

204. THANDROYEN, F.T., J. McCARTHY, K.P. BURTON, AND L.H. OPIE. Ryanodine and caffeine prevent ventricular arrythmias during acute myocardial ischemia and reperfusion in rat heart. Circ. Res. 62: 306-314, 1988.

205. HOLMBERG, S.R., AND A.J. WILLIAMS. The calcium-release channel from cardiac sarcoplasmic reticulum: function in the failing and acutely ischemic heart. Basic Res. Cardiol. 87: 255-268, 1992.

206. OTSU, K., H.F. WILLARD, V.K. KHANNA, F. ZORZATO, N.M. GREEN, AND D.H. MACLENNAN. Molecular cloning of cDNA encoding the Ca^{2+} release channel (ryanodine receptor) of rabbit cardiac muscle sarcoplasmic reticulum. J. Biol. Chem. 265: 13472-13483, 1990.

207. BRILLANTES, A.M., P.ALLEN, T. TAKAHASHI, S. IZUMO, AND A.R. MARKS. Difference in cardiac calcium release channel (ryanodine receptor) expression in myocardium from patients with end stage heart failure caused by ischemic verses dilated cardiomyopathy. Circ. Res. 71: 18-26, 1992.

208. DE MEIS, L., AND A.L. VIANNA. Energy interconversion by the Ca^{2+}-dependent ATPase of the sarcoplasmic reticulum. Annu. Rev. Biochem. 48: 275, 1979.

209. JORGENSEN, A.O., A.C. SHEN, P. DALY. AND D.H. MACLENNAN. Ultrastructural localization of the $Ca^{2+}+Mg^{2+}$-dependent ATPase of

sarcoplasmic reticulum in rat skeletal muscle by immunoferritin labelling of ultrathin frozen sections. J. Cell. Biol. 92: 409, 1982.

210. IKEMOTO, N., Structure and function of the calcium pump protein of sarcoplasmic reticulum. Annu. Rev. Physiol. 44: 297, 1982.

211. RACKER, E. Reconstitution of a calcium pump with phospholipids and a purified Ca^{2+}-adenosine triphosphatase from sarcoplasmic reticulum. J. Biol. Chem. 247: 8198, 1972.

212. THORLEY-LAWSON, D.A., AND N.M. GREEN. Studies on the location and orientation of proteins in the sarcoplasmic reticulum. Eur. J. Biochem. 40: 403, 1973.

213. INESI, G., AND D. SCALES. Tryptic cleavage of sarcoplasmic reticulum protein. Biochem. 13: 3298, 1974.

214. KLIP, A., R.A.F. REITHMEIER, AND D.H. MACLENNAN. Alignment of the major tryptic fragments of the adenosine triphosphatase from sarcoplasmic reticulum. J. Biol. Chem. 255: 6562, 1980.

215. PICK, U., AND E. RACKER. Inhibition of the (Ca^{2+}) ATPase from sarcoplasmic reticulum by dicyclohexyl carbodiimide. Evidence for localization of the Ca^{2+} binding site in the hydrophobic region. Biochemistry. 18: 108, 1979.

216. LENTZ, B.R., K.W. CLUBB, D.A. BARROW, AND G. MEISSNER. Ordered and disordered phospholipid domains coexist in membranes containing calcium pump protein of sarcoplasmic reticulum. Proc. Natl. Acad.

Sci. U.S.A. 80: 2917, 1983.

217. MAIRE, M.I., AND J.V. MOLLER. Protein-protein and protein-lipid interactions of the sarcoplasmic reticulum Ca^{2+}-ATPase. In: Sarcoplasmic Reticulum in Muscle Physiology. Entman, M.L., and W.B.W. Winkle (eds), CRC Press, Florida, Vol 1, 101-126, 1986.

218. MURPHY, A. Sulfhydryl group modification of sarcoplasmic reticulum membrane. Biochemistry 15: 4492-4496, 1976.

219. KAWAKITA, M., K. YASUOKA, AND Y. KAZIRO. Selective modification of functionally distinct sulfhydryl groups of sarcoplasmic reticulum Ca^{2+}, Mg^{2+} adenosine triphosphatase with N-ethylmaleimide. J. Biochem. Tokyo 87: 609-617, 1980.

220. HIDALGO, C., D.A. PETRUCCI, AND C. VERGARA. Uncoupling of Ca^{2+} transport in sarcoplasmic reticulum as a result of labelling lipid amino groups and inhibition of Ca^{2+} ATPase activity by modification of lysine residues of the Ca^{2+} ATPase polypeptide. J. Biol. Chem. 1981.

221. YU, B.P., E.J. MASORO, AND H.A. BERTRAND. The functioning of histidine residues of sarcoplasmic reticulum in Ca^{2+} transport and related activities. Biochemistry 13: 5083-5087, 1974.

222. JONES, L.R., H.R. BESCH, AND A.M. WATANABE. Regulation of the calcium pump of cardiac sarcoplasmic reticulum. Interactive roles of potassium and ATP on the phosphoprotein intermediate of the (K^+, Ca^{2+}) ATPase. J. Biol. Chem. 253: 1643-1653, 1978.

223. TADA, M., AND A.M. KATZ. Phosphorylation of the sarcoplasmic reticulum and sarcolemma. Annu. Rev. Physiol. 44: 401-423, 1982.

224. TADA, M., F. OHMORI, M. YAMADA, AND H. ABE. Mechanism of the stimulation of Ca^{2+}-dependent ATPase of cardiac sarcoplasmic reticulum by adenosine 3',5'-monophosphate-dependent protein kinase. Role of the 22,000-dalton protein. J. Biol. Chem. 254: 319-326, 1979.

225. KIRCHBERGER, M.A., M. TADA, A.M. KATZ. Adenosine 3,5, monophosphate-dependent protein kinase-catalyzed phosphorylation reaction and its relationship to calcium transport in cardiac sarcoplasmic reticulum. J. Biol. Chem. 249: 6166-6173, 1974.

226. BIDLACK, J.M., AND A.E. SHAMOO. Adenosine 3,5,-monophosphate-dependent phosphorylation of a 6000 and a 22,000 dalton protein from cardiac sarcoplasmic reticulum. Biochem. Biophys. Acta 632: 310-325, 1980.

227. SASAKI, T., M. INUI, Y. KIMURA AND M. TADA. Molecular mechanism of regulation of Ca^{2+} pump ATPase by phospholamban in cardiac sarcoplasmic reticulum. J. Biol. Chem. 267: 1674-1679, 1992.

228. LAMERS, J.M., AND H. T. STINIS. Phosphorylation of low molecular weight proteins in purified preparations of rat heart sarcolemma and sarcoplasmic reticulum. Biochem. Biophys. Acta 624: 443-459, 1980.

229. TADA, M., M.A. KIRCHBERGER, AND A.M. KATZ. Phosphorylation of a 22,000-dalton component of the cardiac sarcoplasmic reticulum by adenosine 3,5,-monophosphate-dependent protein kinase. J. Biol. Chem. 250: 2640-2647,

1975.

230. GERTZ, E.W., M.L. HESS, R.F. LAIN, AND F.N. BRIGGS. Activity of vesicular calcium pump in the spontaneously failing heart-lung preparation. Circ. Res. 20: 477-484, 1967.

231. HERBETTE, L., F.C. MESSINEO, AND A.M. KATZ. The interaction of drugs with the sarcoplasmic reticulum. Annu. Rev. Pharmacol. Toxicol. 22: 413-434, 1982.

232. KATZ, A.M., A.M. REPKE, D.I. CORKEDALE, AND S. SCHWARTZ. Effects of local anestheticts on calcium transport by canine cardiac microsomes (fragmented sarcoplasmic reticulum). Cardiovas. Res. 9: 764-769, 1975.

233. WATANABE, A.M., AND H.R. BESCH. Subcellular myocardial effects of verapamil and D600: Comparison with propranolol. J. Pharmacol. Exp. Ther. 191: 241-251, 1974.

234. ENTMAN, M.L., J.C. ALLEN, E. P. BORNET, P. C. GILLETTE, E. WALLICK, AND A. SCHWARTZ. Mechanisms of calcium accumulation and transport in cardiac relaxing system (sarcoplasmic reticulum membranes): Effects of verapamil, D600, X537A and A23187. J. Mol. Cell. Cardiol. 4: 681-687, 1972.

235. MESSINEO, F.C., AND A.M. KATZ. Effects of propranolol and timolol on calcium uptake by sarcoplasmic reticulum vesicles. J. Cardio. Pharmacol. 1: 449-459, 1979.

236. LITTLETON, J.M., S.J. GRIEVE, P.J. GRIFFITHS, AND G.R. JOHN. Ethanol-induced alterations in membrane phospholipid composition: possible relationship to development of cellular tolerance to ethanol. Adv. Exp. Med. Biol. 126: 7-19, 1980.

237. HARA, K., AND M. KASAI. The mechanism of increase in ATPase activity of SR vesicles treated with n-alcohols. J. Biochem. 82: 1005-1017, 1977.

238. GROSSMAN, W. Diastolic dysfunction and congestive heart failure. Circulation 81: III 1-III 7, 1990

239. HARIGAYA, S., AND A. SCHWARTZ. Rate of calcium binding and uptake in normal animal and failing human cardiac muscle. Circ. Res. 25: 781-794. 1971.

240. SORDAHL, L.A., W.G. WOOD, AND A. SCHWARTZ. Production of cardiac hypertrophy and failure in rabbits with ameroid clips. J. Mol. Cell. Cardiol. 1: 341-344, 1970.

241. McCOLLUM, W.B., C. CROW, S. HARIGAYA, E. BAJUSZ, AND A. SCHWARTZ. Calcium binding by cardiac relaxaing system isolated from myopathic Syrian hamsters. J. Mol. Cell. Cardiol. 1: 445-457, 1970.

242. ITO, Y., J. SUKO, AND C.A. CHIDSEY. Intracellular calcium binding and myocardial contractility. V. Calcium uptake of sarcoplasmic reticulum fractions in hypertrophied and failing rabbit hearts. J. Mol. Cell. Cardiol. 6: 237-247, 1974.

243. TOMLINSON, C.W., S.L. LEE, AND N.S. DHALLA. Abnormalities in heart

membranes and myofibrils during bacterial cardiomyopathy in the rabbit. Circ. Res. 39: 82-92, 1976.

244. MOVSESIAN, M.A., M.R. BRISTOW, AND J. KRALL. Ca^{2+} uptake by cardiac sarcoplasmic reticulum from patients with idiopathic dilated cardiomyopathy. Circ. Res. 65: 1141-1144, 1989.

245. KOMURO, I., M. KURABAYASHI, Y. SHIBAZAKI, F. TAKAKU, AND Y. YAZAKI. Molecular cloning and characterization of a Ca^{2+} and Mg^{2+}-dependent adenosine triphosphatase from rat cardiac sarcoplasmic reticulum. J. Clin. Invest. 83: 1102-1108, 1989.

246. NAGAI, R., A. ZARAIN-HERZBERG, C.J. BRANDL, J. FUJII, M. TADA, D.H. MACLENNAN, N, ALPERT, AND P. PERIASMY. Regulation of myocardial Ca^{2+}-ATPase and phospholamban mRNA expression in response to pressure overload and thyroid hormone. Proc. Natl. Acad. Sci. U.S.A. 86: 2966-2970, 1989.

247. MERCADIER, J.J., A.M. LOMPRE, P. DUC, K.R. BOHELER, J.B. FRAYSSE, C. WISNEWSKY, P.D. ALLEN, M. KOMAJDA, AND K. SCHWARTZ. Altered sarcoplasmic reticulum Ca^{2+}-ATPase gene expression in human ventricle during end-stage heart failure. J. Clin. Invest. 85: 305-309, 1990.

248. KATOH, Y., I. KOMURO, H. YAMAGUCHI, AND Y. YAZAKI. Molecular mechanism of hypertrophied failing heart--abnormalities of the diastolic properties and contractility. Jpn. Circ. J. 56: 694-700, 1992.

249. SUZUKI, T., AND J.H. WANG. Stimulation of bovine cardiac sarcoplasmic reticulum Ca^{2+} pump and blocking of phospholamban phosphorylation and dephosphorylation by a phospholamban monoclonal antibody. J. Biol. Chem. 261: 7018-7023, 1986.

250. MOVSESIAN, M.A., C. LEVEILLE, J. KRALL, J. COLYER, J.H. WANG AND K.P. CAMPBELL. Identification and characterization of proteins in sarcoplasmic reticulum from normal and failing human left ventricles. J. Mol. Cell. Cardiol. 22: 1477-1485, 1990.

251. MOVSESIAN, M.A., J. COLYER, J.H. WANG, AND J. KRALL. Phospholamban-mediated stimulation of Ca^{2+} uptake in sarcoplasmic reticulum from normal and failing hearts. J. Clin. Invest. 85: 1698-1702, 1990.

252. MOVSESIAN, M.A. Calcium uptake by sarcoplasmic reticulum and its modulation by cAMP-dependent phosphorylation in normal and failing human myocardium. Basic Res. Cardiol. 87: 277-284, 1992.

253. GWATHMEY, J.K., AND J.P. MORGAN. Altered calcium handling in experimental pressure-overload hypertrophy in the ferret. Circ. Res. 57: 836-843, 1985.

254. AFZAL, N., P.K. GANGULY, K.S. DHALLA, G.N. PIERCE, P.K. SINGAL, AND N.S. DHALLA. Beneficial effects of verapamil in diabetic cardiomyopathy. Diabetes. 37: 936-942, 1988.

255. DHALLA, N.S., G.N. PIERCE, I.R. INNES, AND R.E. BEAMISH. Pathogenesis of cardiac dysfunction in diabetes mellitus. Can. J. Cardiol. 1:

263-281, 1985.

256. TANI, M. Mechanisms of Ca^{2+} overload in perfused ischemic myocardium. Annu. Rev. Physiol. 52: 543-559, 1990.

257. GWATHMEY, J.K., L. COPELAS, R. MACKINNON, F.J. SCHOEN, M.D. FELDMAN, W. GROSSMAN, AND J.P. MORGAN. Abnormal intracellular calcium handling in myocardium from patients with end-stage heart failure. Circ. Res. 61: 70-76, 1987.

258. GWATHMEY, J.K., M.T. SLAWSKY, R.J. HAJJAR, G.M. BRIGGS, AND J.P. MORGAN. Role of intracellular calcium handling in force-interval relationships of human ventricular myocardium. J. Clin. Invest. 85: 1599-1613, 1990.

259. KAUFMAN, R.L., H. HOMBURGER, AND H. WIRTH. Disorder in excitation-contraction coupling of cardiac muscle from cats with experimentally produced right ventricular hypertrophy. Circ. Res. 28: 346-357, 1971.

260. GINSBERG, R., L.J. ESSERMAN, AND M.R. BRISTOW. Myocardial performance and extracellular ionized calcium in severely failing human heart. Ann. Intern. Med. 98: 603-606, 1983.

261. FELLENIUS, E., C.A. HANSEN, O. MJOS, AND J.R. NEELY. Chronic infarction decreases maximum cardiac work and sensitivity of heart to extracellular calcium. Am. J. Physiol. 249: H80-H87, 1985.

262. RUPP, H. The Ca^{2+} responsiveness of myofilaments in terms of ATPase activity, shortening velocity and tension generation. In: The Regulation of Heart Function. H. Rupp, (ed), Thieme, Inc., New York, 234-248, 1986.

263. KATZ, A.M. Cellular mechanisms in congestive heart failure. Am. J. Cardio. 62: 3A-8A, 1988.

264. MORGAN, J.P., R. ERNY, P.D. ALLEN, W. GROSSMAN AND J.K. GWATHMEY. Abnormal intracellular handling, a major cause of systolic and diastolic dysfunction in ventricular myocardium from patients with heart failure. Circulation 81 (Supppl. 111): 21-32, 1990.

265. ZIMMER, H.G. Measurement of left ventricular hemodynamic parameters in closed chest rats under control and various pathological conditions. Basic Res. Cardiol. 78: 77-84, 1983.

266. AFZAL, N., AND N.S. DHALLA. Differential changes in the left and right ventricular calcium transport in congestive heart failure. Am. J. Physiol. 262: H868-H874, 1992.

267. GANGULY, P.K., G.N. PIERCE, K.S. DHALLA, AND NS. DHALLA. Defective sarcoplasmic reticular calcium transport in diabetic cardiomyopathy. Am. J. Physiol. 244 : E528-E535, 1983.

268. LIMAS, C.J. Enhanced phosphorylation of myocardial sarcoplasmic reticulum in experimental hyperthyroidism. A. J. Physiol. 234: H426-H4321, 1978.

269. SULAKHE, P.V. AND N.S. DHALLA. Adenylate cyclase of heart sarcotubular membranes. Biochem. Biophys. Acta. 293: 379-396, 1973.

270. LOWRY, O.H., N.J. ROSENBROUGH, A.L. FARR, AND R.J. RANDALL. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275, 1951.

271. FABIATO, A., AND F. FABIATO. Calculator programme for computing the composition of solutions containing multiple metals and ligands used for experiments of skinned muscle cells. J. Physiol. 75: 463-505, 1979.

272. KIRCHBERGER, M.A., AND T. ANTONETZ. Calmodulin-mediated regulation of calcium transport and (Ca^{2+} + Mg^{2+}) activated ATPase activity in isolated cardiac sarcoplasmic reticulum. J. Biol. Chem. 257: 5685-5691, 1982.

273. BOYNE, A.F., AND G.L. ELLMAN. A methodology for analysis of tissue sulfhydryl components. Analytical Biochem. 46: 639-653, 1972.

274. PUMPHREY, A.M. Incorporation of ^{32}P orthophosphate into brain-slice phospholipids and their precursors. Biochem J. 112: 61-120, 1969.

275. SINGAL, P.K., AND G.N. PIERCE. Adriamycin stimulates low affinity Ca^{2+} binding and lipid peroxidation but depresses myocardial function.Am. J. Physiol. 250: H419-H425, 1986.

276. HALLMAN, H., L.O. FARNEBO, B. HAMBERGER, AND G. JOHNSON. A sensitive method for the determination of plasma catecholamines using liquid chromatography with electrochemical detection. Life Sci. 23: 1049-1052, 1978.

277. BESCH, H.R., L.R. JONES, AND A.M. WATANABE. Intact vesicles of

cardiac sarcolemma. Evidence from vectorial properties of Na^+- K^+ ATPase. Circ. Res. 586-595, 1976.

278. MARTIN, A.F., E.D. PAGANI, AND R.J. SOLARO. Thyroxine induced redistribution of isoenzymes of rabbit ventricular myocardium. CirC. Res. 50: 117-124, 1982.

279. WATSON, D.C., AND A. TZAGOLOFF. Cytochrome oxidase from beef heart mitochondria. Methods Enzymol. 10: 245-250, 1967.

280. PAGOTTI, G., G.R. LAWFORD, AND P.N. CAMPBELL. Biosynthesis of microsomal nicotinamide-adenine dinucleotide phosphate cytochrome c. reductase by membrane bound and free polysomes from rat liver. Biochem. J. 112: 139-147, 1969.

281. LAEMMLI, U.K., AND M. FAVRE. Maturation of the head of bacteriophage T_4. I. DNA packaging events. J. Biol. Chem. 80: 575-599, 1973.

282. CHOMCZYNSKI, P., AND N. SACCHI. Single-step method of RNA isolation by acid guanidine thiocynate-phenol-chloroform extraction. Analytical Biochem. 162: 156-159, 1987.

283. DHALLA. N.S., P.V. SULAKHE, S.L. LEE, P.K. SINGAL, K.G. VARLEY, J.C. YATES. Subcellular Ca^{2+} transport in different areas of dog heart. Can. J. Physiol. Pharmacol. 58: 360-367, 1979.

284. SHLAFER, M., H. GELBAND, R.J. SUNG, R.F. PALMER, AND A.L. BASSETT. Time dependent alterations of myocardial microsomal yield and calcium accumulation in experimentally induced right ventricular hypertrophy

and failure. J. Mol.Cell. Cardiol. 10: 395-407, 1978.

285. GUPTA, M.P., I.M.C. DIXON, D. ZHAO, AND N.S. DHALLA. Influence of ruthenium red on heart subcellular calcium transport. Can. J. Cardiol. 5: 55-63, 1989.

286. TURINI, P., S. KUROOKA, M. STEER, A.N. CORBASCIO, AND T.P. SINGER. The action of phenylmethylsulfonyl fluoride on human acetylcholinestrase, chymotrypsin and trypsin. J. Pharmacol. Exp. Therap. 167: 98-104, 1969.

287. SOLARO, R.J. AND F.N. BRIGGS. Estimating the functional capabilities of sarcoplasmic reticulum in cardiac muscle. Cir. Res. 34: 531-540, 1974.

288. CHEN, L., Y. ZANG, M. ZHU, B. BARR, J. MO, AND D. WANG. Direct measurement of oxygen free radicals following cardiac failure of ischemic dog myocardium with electron spin resonance technique. Can. J. Cardiol. 8: 1066-1070, 1992.

289. GUPTA, M., AND P.K. SINGAL. Higher antioxidative capacity during a chronic stable hypertrophy. Circ. Res. 64: 398-406, 1989.

290. PIERPONT, G.L., G.S. FRANCIS, E.G. DEMASTER, M.J. OLIVARI, W.S. RING, I.F. GOLDENBERG, S. REYNOLDS, AND J.N. COHN. Heterogenous myocardial catecholamine concentrations in patients with congestive heart failure. Am. J. Cardiol. 60: 316-321, 1987.

291. KOMURO, I., M. KORABAYASHI, Y. SHIBAZAKI, F. TAKAKU, AND Y. YAZAKI. Molecular cloning and characterization of $Ca^{2+} + Mg^{2+}$-dependent

adenosine triphosphatase from rat cardiac sarcoplasmic reticulum. J. Clin. Invest. 83: 1102-1108, 1989.

292. ZIMMER, H.G., A.M. GERDES, S. LORTEL, AND G. MELL. Changes in heart function and cardiac cell size in rats with chronic myocardial infarction. J. Mol. Cell. Cardiol. 22: 1231-1243, 1990.

293. DHALLA, N.S., L.E. ALTO, C.E. HEYLIGER, G.N. PIERCE, V. PANAGIA, AND P.K. SINGAL. Sarcoplasmic reticular Ca^{2+} pump adaptation in cardiac hypertrophy due to pressure overload in pigs. Europ. Heart. J. 5 (Suppl F): 323-328, 1984.

294. LIMAS, C.J., S.S. SPIER, AND J. KAHLON. Enhanced calcium transport by sarcoplasmic reticulum in mild cardiac hypertrophy. J. Mol. Cell. Cardiol. 12: 1103-1116, 1980.

295. MALHOTRA, A.A., PENPARGKUL, T. SCHAIBLE, AND J. SCHEUER. Contractile protein and sarcoplasmic reticulum in physiologic cardiac hypertrophy. Am. J. Physiol. 241: H263-H267, 1981.

296. MEERSON, F.Z., V.E. KAGAN, Y.P. KOZLOV, L.M. BELKINA, AND Y.V. ARKHIPENKO. The role of lipid peroxidation in pathogenesis of ischemic damage and the antioxident protection of the heart. Basic Res. Cardiol. 77: 465-485, 1982.

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