CALCIUM TRANSPORT BY MITOCHONDRIA AND MICROSOMES IN DIFFERENT TYPES OF HEART FAILURE

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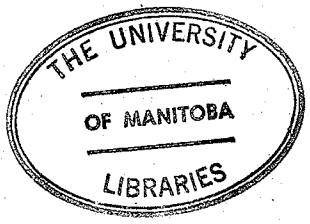

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ABSTRACT

In order to examine the role of changes in calcium transport by mitochondria and sarcoplasmic reticulum in the pathogenesis of heart failure, three types of failing hearts were employed in this study. Both mitochondrial and microsomal (fragments of sarcoplasmic reticulum) fractions were isolated from hearts failing due to oxygen or substrate lack, intracellular calcium deficiency or overload, and myopathy due to a genetic disorder. The energy dependent calcium binding and uptake abilities of the mitochondrial and microsomal fractions were determined by using the Millipore filtration technique and $^{45}CaCl_2$.

In rat hearts perfused for 10 minutes with hypoxic medium calcium binding and uptake by the microsomal fraction decreased significantly. However, mitochondrial calcium binding, but not uptake, decreased significantly on perfusing the hearts with hypoxic medium for 20 to 30 minutes when the microsomal calcium transport was markedly depressed. Reduction in calcium binding and uptake by the microsomal fraction as well as calcium binding by mitochondria of the hypoxic hearts recovered towards normal upon reperfusion with control medium. On the other hand, omitting glucose from the hypoxic medium accelerated the effects of hypoxia upon contractile force and mitochondria calcium binding as well as microsomal calcium binding and uptake. In contrast to the hypoxic hearts, the mitochondrial calcium uptake decreased significantly and the magnitude of depression in the microsomal calcium binding was appreciably greater in hearts made to fail to a comparable degree upon perfusion with substrate-free medium.

Heart failure due to intracellular calcium deficiency produced by

perfusing the isolated rat hearts with Ca^{++} -free medium resulted in a marked decline of calcium binding and uptake by mitochondrial fraction without any effect on the microsomal fraction. On the other hand, intracellular calcium overload, produced by reperfusing the rat hearts after 5 to 20 minutes of perfusion with Ca^{++} -free medium, decreased microsomal calcium binding and uptake, and increased mitochondrial calcium binding and uptake. When the hearts perfused with Ca^{++} -free medium in the presence of low sodium were reperfused with control medium appreciable augmentation in mitochondrial calcium transport and depression in microsomal calcium did not occur.

Intracellular calcium overload was also produced in rat hearts upon perfusion with Na^{+} -free or K^{+} -free medium. Perfusing the hearts with Na^{+} -free medium decreased microsomal calcium binding and uptake without any significant increase in the mitochondrial calcium transporting ability. Reperfusion of hearts following a 10 minute period of perfusion with Na^{+} -free medium resulted in a partial recovery of the microsomal calcium uptake. On the other hand, not only were microsomal calcium binding and uptake decreased, but mitochondrial calcium binding and uptake were also increased on perfusing the hearts with K^{+} -free medium. Both the augmented calcium uptake by mitochondria and the depressed calcium uptake by the microsomal fraction did not recover upon reperfusing the hearts following a 10 minute period of perfusion with K^{+} -free medium.

Both calcium binding and uptake by the microsomal fraction of hearts from myopathic hamsters (UM X 7.1) were decreased at early, moderate and severe stages of heart failure. Although calcium binding by the mitochondrial fraction of these hearts at early and moderate stages of failure was decreased,

mitochondrial calcium uptake was not significantly different from the control. Mitochondrial fractions of hearts from these animals at severe stage of failure had decreased calcium binding and uptake. On the other hand, mitochondrial calcium binding and uptake as well as microsomal calcium binding were decreased at early, moderate and late stages of heart failure in another strain of myopathic hamsters (BIO 14.6) whereas microsomal uptake decreased only in late stages of heart failure. In contrast, myocardial necrosis induced by oxidized isoprotenerol in the isolated rat hearts, decreased microsomal calcium uptake markedly without significant changes in mitochondrial calcium uptake as well as microsomal or mitochondrial calcium binding.

These results suggest that changes in the mitochondrial and microsomal calcium transporting abilities depend upon the degree and type of the heart failure. Depression in microsomal calcium transport seems to be a characteristic feature of failing hearts whereas mitochondrial calcium transport may be increased or decreased depending upon whether or not mitochondria serve as an adaptive mechanism for regulating intracellular calcium under the given conditions. In general, this study provides further support to the view that both mitochondria and sarcoplasmic reticulum by altering their abilities to regulate intracellular calcium play a crucial role in heart dysfunction.

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I. INTRODUCTION AND STATEMENT OF THE PROBLEM

It is now well recognized that calcium occupies a central position in the activation-contraction-relaxation cycle of heart muscle. Furthermore, different membrane systems, particularly sarcoplasmic reticulum and mitochondria, are believed to participate in raising and lowering the intracellular level of ionized calcium for initiating contraction and relaxation respectively. Although the relative contribution of different membrane systems in calcium release and uptake during the processes of heart contraction and relaxation is not clearly understood, both mitochondria and fragments of sarcoplasmic reticulum (heavy microsomes) have been demonstrated to accumulate large amounts of calcium by energy dependent mechanisms under *in vitro* conditions. Recent reports in the literature reveal defects in microsomal and mitochondrial calcium transport in certain types of failing hearts, however, the cause-effect relationship between changes in the membrane abilities to transport calcium and degree of heart failure has not been established. It is, therefore, the purpose of this investigation to provide some information concerning the role of mitochondrial and microsomal calcium transport in the pathogenesis of heart failure.

Since oxygen deficiency is known to cause a decrease in the ability of the heart to generate contractile force and thus heart failure, it was one of the objects of this research to test whether hypoxia has any influence upon calcium transporting ability of mitochondrial and microsomal fractions. The isolated rat heart perfused with hypoxic medium was used for this purpose because this model has been considered suitable by various investigators for studying biochemical changes

during the course of contractile failure. Hearts failing due to perfusion with substrate-free medium were used for comparison.

Recently intracellular calcium overload has been considered a crucial factor in the genesis of heart failure. Furthermore, the isolated rat heart perfused with control medium following a brief perfusion with Ca^{++} -free medium has been shown to be suitable model for studying the effects of intracellular calcium overload. In one series of experiments, therefore, we have investigated changes in calcium binding and uptake by mitochondrial and microsomal fractions in rat hearts perfused with Ca^{++} -free medium as well as following reperfusion. Since perfusion with Na^{+} -free or K^{+} -free medium is known to result in a marked increase in the intracellular concentration of calcium, rat hearts perfused with Na^{+} -free medium as K^{+} -free medium were used in this study for comparison.

Scarcity of good experimental models has been one of the major limiting factors for gaining insight into the pathophysiology of heart failure. A new strain of cardiomyopathic hamsters (UM-X7.1) provides us with an excellent opportunity to examine the abilities of subcellular fractions from hearts at different stages of failure to bind and accumulate calcium. These animals develop congestive heart failure with 100% incidence and can easily be grouped in terms of the disease on the basis of their age and clinical symptoms. In this study, therefore, we have employed these animals to examine the mitochondrial and microsomal calcium binding and uptake abilities under different experimental conditions. Another strain of hamsters (B 10 14.6) which has been extensively used for studying the defective

calcium transport during moderate and late stages of heart failure, was employed for comparison. Since myopathic hamster hearts are known to have focal necrosis, we have made some attempts to investigate changes in calcium transport by mitochondrial and microsomal fractions of hearts in which focal necrosis was induced experimentally by oxidized isoproterenol under in vitro conditions.

II. REVIEW OF LITERATURE

A. Present State of Knowledge Relative to Calcium Regulation in Heart:

The role of calcium as a mediator of the excitation-contraction coupling in muscle and the identification of "calcium pump" mechanism of the sarcoplasmic reticular system as the cause of relaxation appears to have been well documented (1,2). The release of calcium from this membranous structure to the myoplasm (3,4), in response to excitation of the muscle, is the way the sarcoplasmic reticulum is envisioned to function in excitation-contraction coupling. The calcium interacts with troponin (5) to unfasten the troponin-tropomyosin molecular lock thus enabling the contractile interaction of actin with myosin to occur. The sequestering of calcium by the sarcoplasmic reticulum depletes the myoplasm and troponin of calcium thus returning the muscle to the relaxed state. It should be recognized that most of the work concerning the excitation-contraction coupling and the relaxation processes has been done utilizing fragments sarcoplasmic reticulum of skeletal muscle. Some information concerning these events during the cardiac cycle of contraction and relaxation has also appeared in literature (6,7); however, the identity of the particulate factor in this regard is less certain for the cardiac muscle where sarcotubular system is comparatively sparse and mitochondria are abundant. In addition, calcium fluxes across sarcolemma have been considered to play a crucial role during the processes of myocardial contraction and relaxation (6).

It is pertinent to mention that mitochondria, like the fragments of

sarcoplasmic reticulum have been shown to accumulate calcium (8-11). From time to time, various investigators have expressed their concern that mitochondria, particularily in the case of heart, are involved in the regulation of intracellular calcium (12-18). Furthermore, mitochondria in the myocardial cell are claimed to play a major role in the in vivo regulation of calcium since these organelles were found to contain the highest specific activity upon exposure of the whole heart to radioactive calcium (19-21). On the other hand, the rate and extent of calcium transport by heart mitochondria are considered to be slower than those of the reticulum (12,20,22,23); however, none of the investigators have employed identical conditions for such studies with heart subcellular particles.

In spite of the fact that both sarcoplasmic reticulum and mitochondria can accumulate calcium in an energy dependent manner, a great many differences between these subcellular structures have been observed with respect to calcium transport. The uptake of calcium by heart mitochondria is inhibited by oligomycin, azide, dicumarol and dinitrophenol whereas these agents do not have a significant effect on the calcium uptake by the sarcoplasmic reticulum (1,20,24-28). Unlike the heart sarcoplasmic reticulum, the calcium binding by mitochondria is decreased in the presence of 5'-AMP, 3'-AMP or 5'-IMP. Furthermore, both 3'-AMP and adenosine have been found to inhibit the ATPase activity of mitochondria without affecting the reticular enzyme (25). Although the cardiac reticular fraction has been shown to contain more neutral lipids and phospholipids in comparison to the mitochondrial fraction (29,30), a satisfactory explanation for differences in the mechanisms of calcium transport by mitochondria and sarcoplasmic reticulum must

await further information.

Various investigators have attempted to improve the calcium pump activity of the cardiac sarcoplasmic reticulum by isolating and incubating these particles under different experimental conditions (16,23,31-39), however, no such effort has been made with respect to mitochondria. Scattered information on the influence of different ions on the calcium uptake in the reticular vesicles is also available. For example, Carsten (33) has reported that the rate of calcium uptake in the sarcoplasmic reticulum of the dog heart is neither affected by changes in K^+ or Na^+ concentrations nor by substitution of isosmolar amount of sucrose for 70% of the Na^+. On the other hand, Katz and Repke (34) have demonstrated that concentrations of KCl or NaCl below 0.1 M increased calcium uptake by the dog heart sarcoplasmic reticulum. Furthermore, replacement of KCl by equimolar amounts of NaCl decreased both the rate and extent of calcium uptake in the reticular vesicles. Palmer and Posey (40) have provided evidence that in heart sarcoplasmic reticulum, the reduction of calcium uptake by Na^+ is due to a rapid release of the bound calcium. Recently, Dransfeld et al (41) have reported that the calcium uptake in the cardiac mitochondria is enhanced by elevating the K^+/Na^+ ratio in the incubation medium. These studies have not been carried out in detail under identical experimental conditions nor do these reports provide enough experimental basis for the localization of the site for intracellular ionic competition which appears to be very critical for determining the regulation of myocardial contractility.

The work by different investigators concerning the effects of various

inotropic agents on calcium transport by heart microsomes has been reviewed recently (42-44). In spite of conflicting reports from various laboratories, it is believed that agents which increase calcium uptake by cardiac microsomes, increase myocardial contractility by making more calcium available for release during excitation. This hypothesis has been dealt with in detail by some investigators who have attempted to explain the positive inotropic effect of catecholamines through the participation of a cyclic AMP-protein kinase-microsomal calcium transport system (45-47). No information on this hypothesis with respect to mitochondrial or sarcolemmal calcium transport is available at present. It should be pointed out that various investigators have reported the isolation of heart sarcolemma (48-53); however, none of these workers have attempted to study the calcium binding property of this membrane. Although some reports concerning the release of calcium from the microsomal fraction under certain experimental conditions have been published (54-57), such information for mitochondria or sarcolemma is lacking. Therefore, further studies on the effects of various cardio-stimulants and cardio-depressants on calcium transporting properties of microsomal, mitochondrial and sarcolemmal fractions under identical conditions are needed to gain knowledge concerning the molecular mechanism of drug action on the heart.

From the foregoing discussion it is evident that various membranous systems, such as mitochondria, sarcoplasmic reticulum and sarcolemma, are intimately involved in the regulation of cardiac performance through the control of calcium movements. According to the current concept, the intracellular concentration of ionized calcium is increased as a result of an influx of extracellular calcium and release

from the intracellular calcium stores such as sarcoplasmic reticulum and possibly mitochondria on depolarization of the cardiac cell. It is generally believed that a wave of depolarization releases calcium directly from the sarcoplasmic reticulum however, it is also considered that influx of extracellular calcium and changes in the cytoplasmic concentrations of H^+, Na^+, and K^+ in the depolarized heart cell also release calcium from both sarcoplasmic reticulum and mitochondria. This increase in the intracellular free calcium ions relieves the inhibition of the troponin-tropomyosin system and activates myofibrillar ATPase, thus providing energy for contraction and sliding of the actin and myosin filaments. Relaxation of the heart, on the other hand, results from lowering the intracellular concentration of ionized calcium by different membranous systems such as sarcoplasmic reticulum, mitochondria and sarcolemma by energy-dependent processes. These movements of calcium during the activation contraction and relaxation phases of cardiac cycle are represented schematically in Figure 1. Although such a scheme is based on a great deal of indirect evidence, it is our belief that it helps us to readily appreciate the complex events which are occuring during cardiac contractions and relaxation cycle in normal and diseased states.

B. Present State of Knowledge Relative to Molecular Abnormalities in Heart Failure:

It is just over 20 years since Olson and Schwartz (58) discussed a hypothetical framework for the study of cardiac metabolism in relation to heart failure. It was proposed that cardiac metabolic processes could be divided into two general categories: those that led to ATP formation (which included substrate oxidation, electron transport and oxidative phosphorylation), and those that resulted in ATP

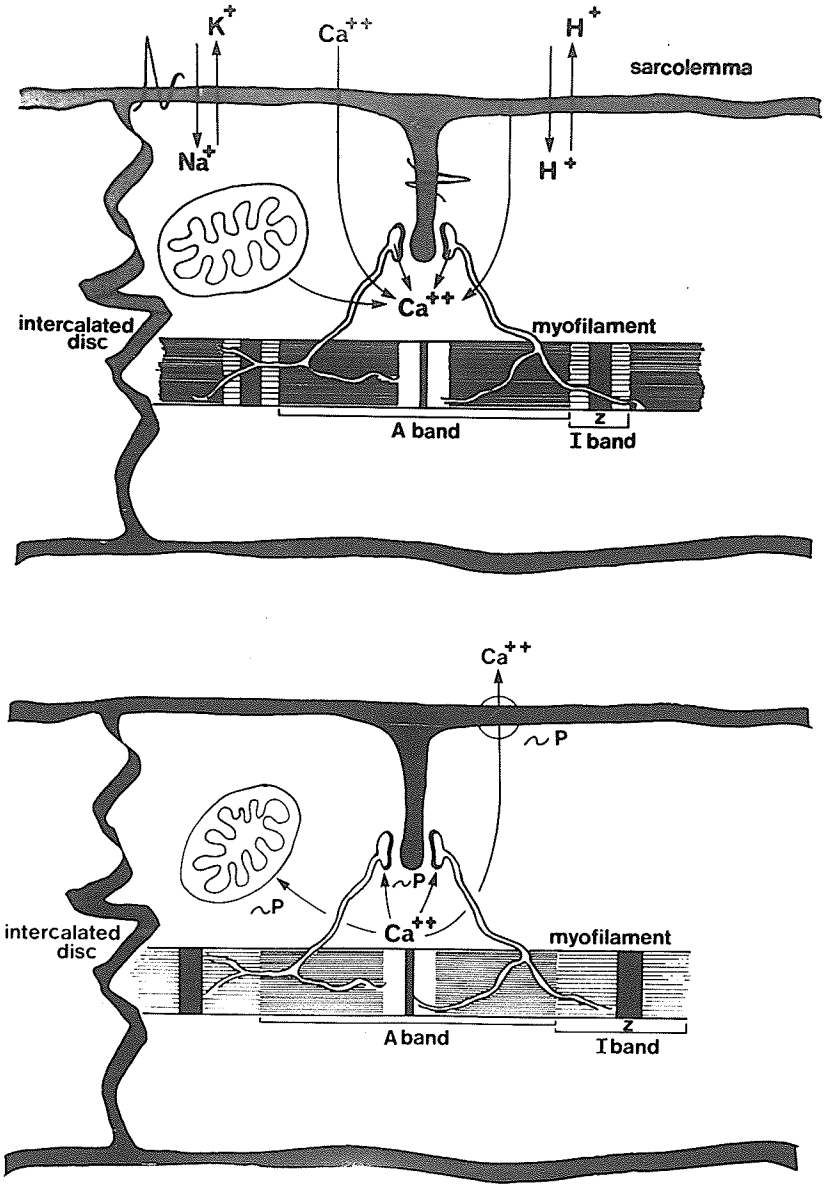

FIGURE 1. Schematic representation of heart cell during activation-contraction-relaxation cycle. Upper panel - contraction phase; lower panel - relaxation phase.

utilization (phosphorylations, general anabolic processes and myocardial cell function). It was also postulated that there exist two molecular classes of heart failure, i.e., those in which the defect lay in the generation of ATP and those in which the defect lay in the utilization of ATP (59). Abundant evidence has accumulated in the intervening years to justify this traditional formulation (60-63). Some investigators have demonstrated a defect in energy production (64-69) while others have claimed an impairment of the mechanisms for energy utilization (60,70, 71) in failing hearts. Reports are also available in the literature in which normal energy metabolism in the failing hearts has been indicated (72-77). The stores of norepinephrine, which are known to modulate myocardial function and metabolism, have also been shown to decrease in failing hearts (78-81); however, such a change has been reported to be secondary to heart failure (63,82,83). Thus, it appears that detailed biochemical mechanisms underlying the loss of contractility in heart failure remain obscure and the possibility of a disorder of regulation of cardiac metabolism in causing heart failure has not been ruled out at present.

The derangement of the excitation-contraction coupling mechanism in heart failure has also been reported (84-86). In order to provide experimental evidence in this regard numerous investigators have attempted to show an abnormality in the subcellular particles to accumulate calcium in different types of failing hearts (12,23,26,27,87-93). Some of the observations made by various investigators concerning biochemical changes in the failing heart can be interpreted to suggest an abnormality of cardiac sarcolemma in heart failure. For example, adenylate cyclase which is believed to be associated with the cell membrane, was markedly

altered in congestive heart failure induced by aortic constriction (94). It has recently been shown that changes in adenylate cyclase in heart failure induced by substrate-lack in the isolated hearts are dependent upon the degree of heart failure (95). Gold et al (96) failed to demonstrate any change in myocardial adenyl cyclase activity in the absence or presence of norepinephrine and fluoride in chronic heart failure produced by occluding the pulmonary artery in cats; however, adenyl cyclase acitvation by glucagon was lost in this preparation. Another line of evidence showing sarcolemmal abnormality in heart failure came from studies with membrane bound $Na^+ - K^+$ ATPase in different experimental models. Cardiac insufficiency induced by vitamin E or cobalt was shown to be associated with elevated levels of $Na^+ - K^+$ ATPase activity (68,97). Some investigators have shown a decrease in $Na^+ - K^+$ ATPase activity in failing hearts due to oxygen-lack (98), substrate-lack (99), aortic constriction (100) or myocardial ischemia (91). Other types of heart failure have been claimed to be associated with no change in $Na^+ - K^+$ ATPase activity (90,101). These results suggest abnormalities at the level of mitochondria, sarcoplasmic reticulum or sarcolemma in failing hearts; however, it is not known whether these changes are a cause or effect of heart failure.

A special strain of Syrian hamsters (BIO 14.6) which develop a hereditary cardiomyopathy has been regarded as an excellent model for studying the pathogenesis of congestive heart failure (102-106). The cardiac function in these animals has been shown to be markedly depressed (107-109). Some investigators (109-112) have reported an abnormality of oxidative phosphorylation in the hearts of myopathic hamsters while others have failed to observe such a lesion (113). Lochner et al

(109) found a small decrease in the level of creatine phosphate without significant changes in the concentration of other high energy phosphate stores of the intact myocardium of these animals; however, their results are difficult to interpret because the value of creatine phosphate for the control heart is far below the accepted level in the normal heart. A depression in the levels of creatine phosphate, ATP and AMP was reported by these workers (114) in the isolated perfused myopathic hearts without any change in the level of ADP. By using more specific fluorometric techniques, Fedelesova and Dhalla (115) demonstrated dramatic alterations in energy metabolism of the myopathic hamster heart. Some attempts have also been made to gain information concerning calcium transport in failing myopathic hamster hearts. For example, Gertz et al (116) reported a reduction in the ability of the myopathic sarcoplasmic reticulum to accumulate calcium in the presence of oxalate. Schwartz and his co-workers (111,112,117) showed a decrease in calcium uptake by mitochondria and sarcoplasmic reticulum of these animals. In addition to demonstrating changes in calcium binding and uptake by the subcellular particles of the myopathic hamster heart, it has been shown that the observed alteration in the calcium pump in the microsomal and mitochondrial fractions was not due to a reduction in the ATPase activities of these particles (28,118). Owens et al (119) have recently reported that the cholesterol: phospholipid molar ratio of the myopathic heart heavy microsomes was elevated and small differences were also apparent in the phospholipid composition of these membranes in comparison to the control. In extensive studies on calcium transport in the myopathic hamster hearts (28,118), animals with moderate and advanced degrees of heart failure were

employed, but detailed information in hearts before the onset of failure and at the initial stages of heart failure still remains to be obtained in order to determine whether such a lesion forms a primary factor in the pathogenesis of heart failure in this model. No change in adenylate cyclase activity of the myopathic hamster hearts with a moderate degree of failure was observed while at advanced stages of heart failure, the response to fluoride was decreased whereas that to norepinephrine was abolished (120). In myopathic hamster hearts with a moderate degree of heart failure, the $Na^+ - K^+$ ATPase activity was also increased (121) but no information in this regard is available in hearts before the onset of failure as well as at the initial stages of heart failure in these animals. Thus the BIO 14.6 strain of cardiomyopathic hamsters has served a useful purpose in providing valuable information concerning the pathogenesis of congestive heart failure.

Recently another strain of cardiomyopathic hamsters (UM-X7.1) has been developed with 100% incidence of congestive heart failure (122). These hamsters show an abnormal EKG pattern and the sarcolemma obtained from failing hearts at severe stages reveal dramatic changes in the activities of adenylate cyclase (in the presence of epinephrine and NaF), Ca^{++} ATPase, Mg^{++} ATPase, and $Na^+ - K^+$ ATPase (123). This, to the best of our knowledge, is the first demonstration of abnormality in sarcolemma in any model of heart failure. The results of Gelband and Bassett (124) concerning the depressed resting potential, action potential overshoot, and action potential maximum rate of rise in cardiac muscles from cats with right ventricular failure due to pulmonary artery obstruction can also be interpreted to reflect a defect at the level of sarcolemma. It is pointed out that these

cardiomyopathic animals (UM-X7.1) show generalized edema, pulmonary edema, liver congestion and cardiac hypertrophy. It is these animals which we used at different stages of heart failure to determine the significance of the observed defects of membranous systems.

C. Role of Calcium in Heart Failure:

According to a concept of Fleckenstein (125-127), depressed contractility of the failing cardiac muscle having normal energy supply can be restored by providing extra calcium whereas, calcium is not effective in failing hearts having depleted stores of the energy-rich phosphates. The restoration of contractility by calcium in certain types of failing hearts is believed to be due to an improvement of the excitation-contraction coupling process. Direct support to this hypothesis was provided by the work of Kaufmann et al (128) who employed papillary muscle from hypertrophied right ventricles of cats with artificial stenosis of the pulmonary artery. These investigators observed that depressed contractility in the absence of any alteration of the electrical activity of the failing muscles can be restored to the same level as with control preparations by adding extra calcium into the bathing medium. These observations can be interpreted to suggest that a sufficient amount of calcium was not released from the intracellular stores on depolarization in these failing heart muscles. On the other hand, Fleckenstein and co-workers (129) have recently suggested that a number of non-coronarogenic cardiomyopathies result from an intracellular overload of calcium, which is mainly due to a marked increase in the transmembrane calcium influx. This hypothesis is primarily based on the observations that factors such as corticosteroids, dihydrotachysterol and NaH_2PO_4,

which sensitize the myocardium to catecholamine-induced necrotization, act by potentiating calcium uptake; whereas agents such as verapamil, prenylamine and vascoril, which greatly reduce transmembrane calcium influx, show beneficial effects. From these studies one is tempted to conclude that sarcolemmal damage may result in an intracellular overload of calcium and subsequently heart failure.

Depressed abilities of mitochondria and microsomes to accumulate calcium can be conceived to elevate the intracellular concentration of ionized calcium. Such a change in the properties of mitochondria and sarcoplasmic reticulum like that of sarcolemma produce an overload of intracellular free calcium which may then result in derangement of myocardial metabolism, ultrastructure and function. Numerous investigators have attempted to show an abnormality of sub-cellular particles to accumulate calcium in different types of heart failure. For example, Gertz et al (87) have reported that the ability of heavy microsomes to accumulate calcium was markedly impaired in the spontaneously failing dog heart-lung preparation. Failure of isolated rat hearts to generate contractile force on perfusion with substrate-free medium was found to be associated with depression in calcium transport by both mitochondria and fragments of sarcoplasmic reticulum (12,26,93). The ability of the sarcoplasmic reticulum of the ischemic dog heart to bind and accumulate calcium has also been shown to decrease (88,89,130). Reduced rates of calcium binding and uptake by microsomal and mitochondrial fraction of the failing human hearts have also been reported (90,131) ligation of the right pulmonary artery was demonstrated to result in heart failure and depressed calcium uptake by the microsomal fraction (27). Myocardial necrosis induced by isoproterenol in vivo

has also been shown to be associated with reduction in calcium binding by the microsomal fraction (132). These results in addition to those reported above for the myopathic hamster hearts suggest that defects in the regulatory mechanisms for the movement of intracellular calcium have an important bearing on the pathogenesis of heart failure. It should be pointed out that none of the previous investigators have reported changes in calcium transport by subcellular particles before the onset or at the early phases of heart failure.

The dual role of calcium in maintaining, as well as in deteriorating, heart function and ultrastructure has been well recognized in isolated rat heart preparations (133-137). Recent studies in our laboratory have shown that reducing the amount of extracellular sodium during perfusion of the heart with calcium-free medium delays failure of contractile function, augments recovery and prevents ultrastructural damage (137). It has also been reported that perfusing the rat hearts with normal medium following pre-perfusion with calcium-free medium results in a marked increase in myocardial calcium content (138). Likewise, hearts perfused with Na^+ -free or K^+ -free medium failed to generate contractile force and were found to contain elevated levels of intracellular calcium (138). We believe these isolated perfused heart preparations form excellent models for studying the effects of intracellular calcium overload on different membranous systems. In contrast, failing hearts due to perfusion with hypoxic medium were demonstrated to contain lower levels of intracellular calcium and showed depressed calcium influx, whereas no change in the intracellular calcium levels was noted in failing hearts due to perfusion with substrate-free medium (138). Significant depression of mitochondrial

and microsomal levels of calcium was observed in failing hearts due to lack of substrate and oxygen (139). It should be mentioned that, unlike substrate-depleted hearts, no information concerning the abilities of mitochondrial and microsomal fractions of the hypoxic hearts to transport calcium is available in the literature.

III. MATERIALS AND METHODS

A. Animals:

In one series of experiments a new strain of myopathic hamsters (UM-X7.1) at different stages of heart failure was employed. These animals have recently been described as forming a useful model for studying the pathogenesis of heart failure (123,140,141). This line of hamsters has been established by cross-breeding homozygous diseased animals with healthy hamsters and by recovering the mutant gene in the F_2 generation. The disease in these animals is transmitted through an autosomal recessive gene and a prenecrotic stage has been found to occur within 20 to 30 days of age. A necrotizing stage has been observed between 30 to 120 days and depending upon its severity is followed by varying degrees of heart failure with 100% incidence. For the purpose of this study we have chosen to employ these hamsters at early (150 day old), moderate (175 day old) and severe (200 day old) stages of heart failure. The criteria for grouping these animals in different stages of heart failure were based upon the degrees of cardiac hypertrophy, liver congestion, hydrothorax, ascites and subcutaneous edema. Another well established strain of myopathic hamsters (BIO-14.6) at different stages of heart failure (102-106, 138,142) was used for comparison purposes. Normal healthy inbred Syrian hamsters of the respective age groups were employed as controls.

Male albino or hooded rats weighing 300 to 400 g. were also used in the present study. All animals were fed laboratory animal diet ad libitum and sacrificed by decapitation.

B. Chemicals and Reagents:

The chemicals used in the present study were of analytical grade and purchased from Fisher Scientific or Can-Lab. The enzyme grade sucrose was obtained from Mann Research Co. Di-sodium ATP and Tris base were purchased from Sigma Chemical Co., whereas $^{45}CaCl_2$ was obtained from New England Nuclear. Deionized distilled water was used in all experiments.

C. Rat Heart Perfusion:

The hearts were quickly dissected out and placed in cold oxygenated Krebs-Henseleit solution. Fat and connective tissues were removed and the hearts were arranged for coronary perfusion according to the method of Langendorff as described elsewhere (69,83,98,137). The perfusion medium was Krebs-Henseleit medium (control medium), containing (in mM): NaCl 120; $NaHCO_3$ 25.4; KCl 4.8; KH_2PO_4 1.2; $MgSO_4$ 0.86; $CaCl_2$ 1.25 and glucose 11. This solution was gassed with 95% O_2 and 5% CO_2 mixture and maintained at 37°C. The pH of the medium was 7.4. The perfusion pressure was approximately 80 cm H_2O. The spontaneously beating hearts were equilibrated for 10 minutes with control medium before they were employed in experiments carried out in the present study. All these hearts were perfused in an open system and were punctured with the tip of fine scissors at the beginning of the perfusion in order to avoid fluid accumulation in the ventricles.

In one set of experiments, hearts were made hypoxic by perfusion for different intervals with Krebs-Henseleit medium gassed with 95% N_2 and 5% CO_2.

In some experiments, hearts were also perfused with aerobic or hypoxic medium in which glucose was omitted. Hearts were also perfused with aerobic perfusion medium in which Ca^{++}, Na^{+} or K^{+} was omitted. In the perfusion medium in which any constituent was omitted, the osmolarity was maintained by adding an equimolar amount of sucrose or Tris-HCl. When the hearts were perfused with oxidized isoproterenol the perfusion medium containing the sympathomimetic amine was gassed with 95% O_2 and 5% CO_2 for 15 hours before starting the perfusion. The control hearts were perfused with control aerobic for comparable lengths of time for any given experimental condition. In some of the experiments, the values for control hearts under different conditions were grouped together since these were overlapping and were not statistically different from each other.

A resting tension of 1 g. was applied to all the hearts used in this study in starting the perfusion and the contractile force was monitored on a Grass polygraph by using a force displacement transducer. In some of the experiments coronary flow was maintained at 10 ml/min and the hearts were paced electrically by a square wave stimulator (Phipps and Bird Co.) with the pulses at 1.5X the threshold voltage and of 10 ms. duration, at a frequency of 5/sec.

D. Isolation of Subcellular Fractions:

Control and experimental rat hearts were placed in chilled 0.25 M sucrose solution containing 1 mM EDTA, pH 7.0. Two to three hearts receiving the same treatment were pooled together for preparing subcellular fractions by differential centrifugation. The hearts were diced and homogenized in 10% (w/v) of the sucrose -EDTA medium in a Waring blendor for 30 sec (2X15 sec) at a medium speed

setting. In some experiments, the heart homogenates were made by a glass homogenizer and Teflon pestle driven by an electric motor for a total of 8 to 10 passes. No difference was observed between the results obtained by these two methods of homogenization under our experimental conditions. The homogenate was filtered through four layers of gauze and centrifuged at 1,000X g for 10 minutes to remove cell debris, nuclei, myofibrills and other material. The supernatant was centrifuged at 10,000X g for 20 minutes to obtain a crude mitochondrial pellet. The supernatant was further centrifuged at 40,000X g for 45 minutes to obtain a crude heavy microsomal fraction containing fragments of sarcoplasmic reticulum.

The crude mitochondrial pellet obtained above was washed, gently suspended in the homogenizing medium using a glass homogenizer and centrifuged at 1,000X g for 10 minutes. The sediment was discarded and the supernatant centrifuged at 8,000X g for 10 minutes. The mitochondrial pellet thus obtained was washed and suspended in 0.25 M sucrose solution, pH 7.0, at a protein concentration of 2 to 4 mg/ml. The crude microsomal pellet obtained above was washed and gently suspended in 0.6 M KCl solution containing 20 mM Tris-HCl, pH 6.8 by using a glass homogenizer. This suspension was centrifuged at 100,000 x g for 10 minutes and the supernatant was further centrifuged at 40,000X g for 45 minutes. The pellet thus obtained was washed and suspended in 0.25 M sucrose solution, pH 7.0, at a protein concentration of about 1.0 mg/ml. This method of isolating the subcellular fractions is essentially similar to that described elsewhere (28). The procedure for isolating subcellular fractions was carried out at 1° to 4°C in the cold room and the

preparations were used immediately after isolation. In each set of experiments with rat hearts, other methods for isolating mitochondrial and microsomal fractions were also employed in order to examine if the observed changes were due to an artifact of the isolation procedure. For this purpose, mitochondria were prepared by homogenizing the heart in medium containing 0.18 M KCl, 10 mM EDTA and 0.5% albumin, pH 7.4, according to the method of Sordahl and Schwartz (143). On the other hand, microsomal fraction was isolated by making heart homogenate in 10 mM $NaHCO_3$, 5 mM NaN_3 and 15 mM Tris-HCl, pH 6.8 according to the procedure described by Harigaya and Schwartz (23). It is pointed out that most of the experiments with control and myopathic hamster hearts were carried out by the methods of Sordahl and Schwartz (143) and Harigaya and Schwartz (23). These methods did not yield results different from those obtained by the sucrose method outlined above.

E. Measurement of Calcium Binding and Uptake:

It is pointed out that the term calcium binding is applied in this study to indicate calcium accumulation by microsomal and mitochondrial fractions in the absence of oxalate and inorganic phosphate (Pi) respectively and it is understood that this usage employs an arbitrary meaning for binding. On the other hand, the term calcium uptake is applied to indicate calcium accumulation by microsomes and mitochondria in the presence of oxalate and Pi respectively. The methods employed for calcium binding and uptake are similar to those employed in this laboratory (25, 28,120).

1. Calcium Binding:

Both mitochondrial or microsomal fractions were incubated in 1 to 2 ml of

medium containing 100 mM KCl, 10 mM $MgCl_2$, 4 mM ATP, and 20 mM Tris-HCl pH 7.0. These membranes were incubated at 25°C for 2 minutes before starting the reaction with 0.1 mM $^{45}CaCl_2$. The protein concentration for these fractions in the incubation medium varied from 0.1 to 0.3 mg/ml. The reaction was terminated at the desired time intervals by Millipore filtration (pore size 0.45μ). The filtration was carried out by using Millipore filtration assembly attached to a 3 ml disposable syringe and was achieved within 2 seconds. The filtrate was tested to ensure it was free of protein. In some experiments calcium binding was determined by varying the pH or $^{45}CaCl_2$ concentration of the incubation medium. The radioactivity in the filtrate (0.1 ml) was measured in duplicate by a Packard liquid scintillation spectrophotometer by using 10 ml of the Bray's solution in disposable vials.

2. Calcium Uptake:

The microsomal fraction (0.02 to 0.04 mg/ml) was incubated for 2 minutes at 37°C in 1 to 2 ml of medium containing 100 mM KCl, 10 mM $MgCl_2$, 4 mM ATP, 5 mM potassium oxalate, and 20 mM Tris-HCl, pH 7.0. On the other hand, the mitochondrial fraction (0.1 to 0.3 mg/ml) was incubated for 2 minutes at 37°C in 1 to 2 ml medium containing 100 mM KCl, 10 mM $MgCl_2$, 4 mM KH_2PO_4, 4 mM succinate and 20 mM Tris-HCl, pH 7.0. The reaction in both cases was started by adding 0.1 mM $^{45}CaCl_2$ and terminated by the Millipore filtration as mentioned above. Some experiments, as indicated in the text, were carried out employing different concentrations of $^{45}CaCl_2$ or different pH of the incubation medium. The radioactivity in the filtrate (0.1 ml) was estimated in the Packard liquid scintillation spectrometer by using 15 ml of the Bray's solution in disposable

vials.

F. Determination of ATPase Activity:

The ATPase activity was assessed by determining the inorganic phosphate produced due to the hydrolysis of ATP during the incubation period. To study the total ATPase activity of either microsomes or mitochondria, the subcellular fraction was incubated in a medium containing 100 mM KCl, 10 mM $MgCl_2$, 4 mM potassium-oxalate, 0.1 mM $CaCl_2$, and 20 mM Tris-HCl, pH 7.0, at 37°C, in a total volume of 1.0 ml (final protein concentration was 0.1 to 0.2 mg/ml). The reaction was started by addition of 4 mM ATP and terminated by Millipore filtration. The basal ATPase activity of the microsomes was determined in the absence of $CaCl_2$ but in the presence of 0.2 mM EGTA. The difference between the total and basal ATPase activity represents the Ca^{++} -stimulated ATPase activity of the membrane fraction. The inorganic phosphate present in the filtrate was determined by the method of Fiske and Subbarow (144).

G. Determination of Protein Concentration:

Protein concentration of the subcellular fractions was determined according to the method of Lowry et al (145), using bovine serum albumin as standard. The results for calcium binding and uptake as well as for ATPase activity were expressed in terms of mg protein of the subcellular fraction.

H. Electron Microscopic Studies:

The final pellets of mitochondria and microsomes from the control and hypoxic hearts were fixed overnight in 5% gluteraldehyde in 0.1 M phosphate buffer, pH 7.4, diced into pieces of about 0.5 mm thick, washed overnight in 0.1 M

phosphate buffer, pH 7.4 and post-fixed for 1 hour in osmium tetraoxide. These specimens were dehydrated in a graded ethanol series (50 to 100%) and embedded in Araldite-502 epoxy resin. Thin sections were made on a Porter-Blum MT II ultramicrotome using glass knives, and stained with Reynold's lead citrate. The sections were examined and photographed using a Zeiss EM-9S electron microscope.

I. Marker Enzyme Activities:

The mitochondrial and microsomal fractions were isolated according to the procedures described above whereas the sarcolemmal fractions was isolated according to the method of McNamara et al (53). The activities of cytochrome C oxidase, glucose - 6 - phosphatase, ouabain-sensitive $Na^+ - K^+$ ATPase were determined according to the methods described elsewhere (28,53,146,147).

J. Analysis of Data:

Each observation was made with at least 3 to 4 different preparations. Both control and experimental preparations were made under identical conditions simultaneously. Mean $\pm$ S.E. for each point was calculated and the level of significance of the difference between control and experimental groups was determined by the students "t" test. The P values <0.05 were taken to reflect significant difference between control and experimental preparations.

IV. RESULTS

A. Calcium Transport by Subcellular Fractions of Hearts Made to Fail due to Oxygen or Substrate Lack:

When isolated rat hearts were perfused with hypoxic medium, the contractile force declined by about 40, 60 and 70% of the control within 5, 10 and 30 minutes respectively. On the other hand, the contractile force began to decline at 30 minutes and was about 50, 30 and 3% of the control within 60, 90 and 120 minutes respectively on perfusing the hearts with substrate-free medium. These results were similar to those already reported from our laboratory (98,99).

In the first series of experiments, both mitochondrial and heavy microsomal fractions were isolated from rat hearts perfused with hypoxic medium for 5, 10, 20 or 30 minutes and calcium binding and uptake were determined. The results shown in Figure 2 reveal that both calcium binding and uptake by microsomes were decreased significantly ($p < 0.05$) in hearts perfused for 10 or more minutes with hypoxic medium. Furthermore, calcium binding, but not calcium uptake, by mitochondria decreased significantly ($p < 0.05$) on perfusing the hearts with hypoxic medium for 20 or 30 minutes. The decrease in calcium uptake by microsomes, but not by mitochondria, in hearts perfused for 30 minutes with hypoxic medium was also apparent when determinations were made at different time intervals of incubation of these fractions (Figure 3).

Since calcium transport by subcellular fractions is considered to be an energy dependent process, the ATP hydrolyzing ability of mitochondrial and microsomal fractions from hearts perfused with control and hypoxic medium for 30 minutes

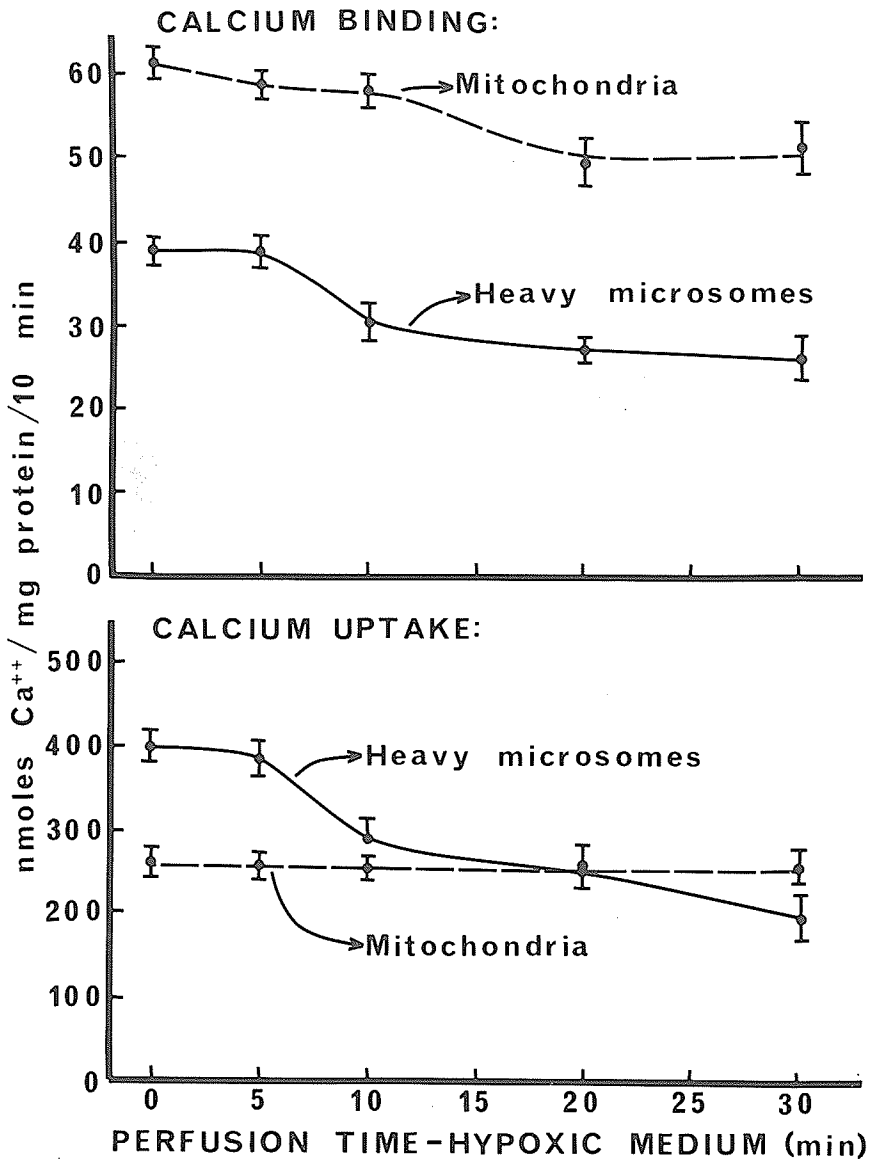

FIGURE 2. Calcium binding and uptake by subcellular fractions of hearts perfused with hypoxic medium for different intervals. Each value is a mean ± S.E. of 6 - 8 experiments.

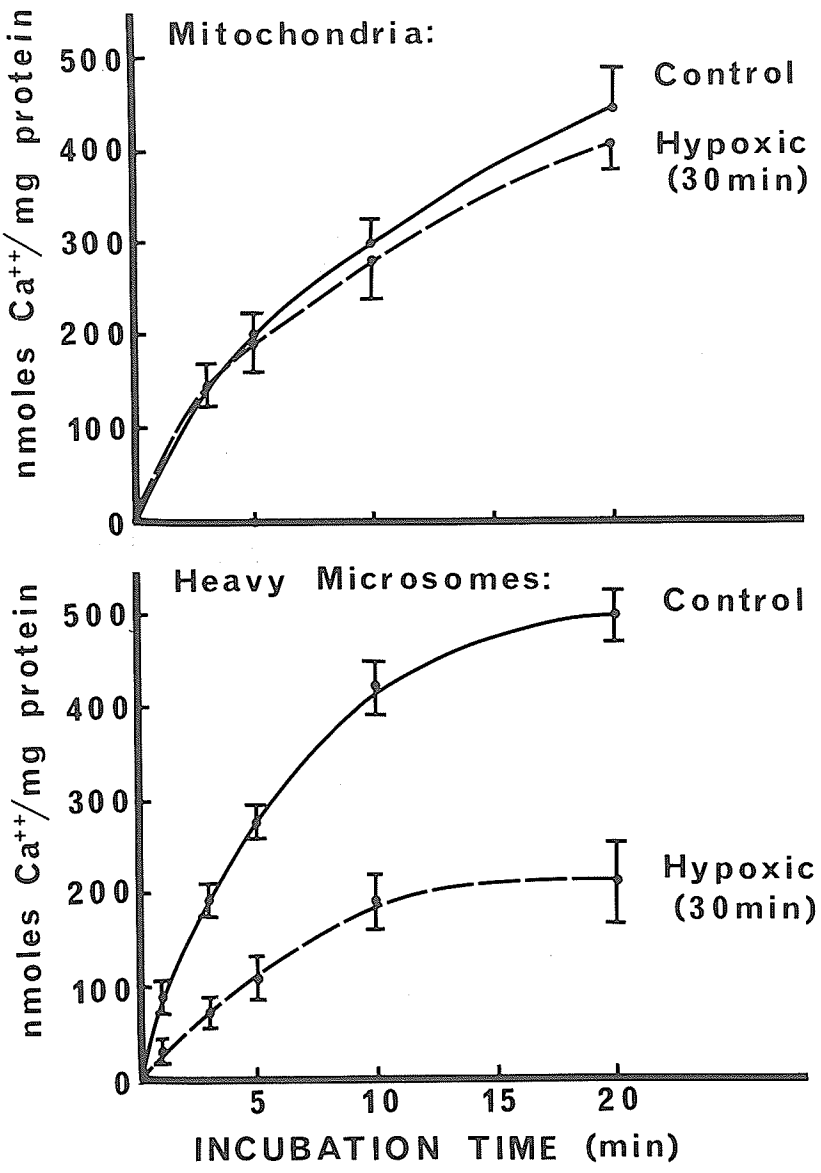

FIGURE 3. Time-course of calcium uptake by subcellular fractions of hearts perfused for 30 min with control or hypoxic medium. Each value is a mean ± S.E. of 6 experiments.

was determined. The results in Table I show that the total ATPase activity of mitochondria and the basal ATPase activity of microsomes from hypoxic hearts were not different ($p > 0.05$) from the control. However, Ca^{++} -stimulated ATPase activity of the microsomal fraction from the hypoxic hearts was significantly ($p < 0.05$) decreased.

It was observed that the yields of both mitochondrial and microsomal fractions of hearts perfused for 30 minutes were not different ($p > 0.05$) from the control (Table I). It can be seen from Table 2 that neither sodium-azide nor oligomycin exerted an appreciable effect on the microsomal fractions from the control and hypoxic hearts; however, these agents produced an equal degree of inhibition upon calcium binding by mitochondrial fractions from the control and hypoxic hearts.

The mitochondrial and microsomal fractions were also examined electronmicroscopically. The electron micrographs of the fractions from the hypoxic hearts are shown in Figure 4, whereas those from the control hearts are similar to those described by Dhalla (12); varying degrees of swelling and damage to the ultrastructure of mitochondria were seen in pellets from the hearts perfused with hypoxic medium for 30 minutes. On the other hand, the appearance of vesicles in the microsomal pellets from the hypoxic hearts was not different from the control.

The data shown in Table 3 indicate that depression in mitochondrial and microsomal calcium binding and microsomal calcium uptake in hearts perfused for 30 minutes with hypoxic medium was reversible upon reperfusing the hypoxic hearts with control medium for 15 minutes. The contractile force of these hypoxic hearts recovered to about 75% of the control under these conditions. Furthermore,

TABLE 1

ATP HYDROLYSIS BY THE SUBCELLULAR FRACTIONS AND THEIR YIELDS FROM CONTROL AND HYPOXIC HEARTS

	ATPase activity (μmoles Pi/mg protein/5 min)			Yield of subcellular fractions (mg protein/g heart)	
	Mitochondria	Heavy microsomes			
	Total	Basal	Ca^{++} - stimulated	Mitochondria	Microsomes
Control	3.26 ± 0.33	5.50 ± 0.42	0.87 ± 0.12	0.88 ± 0.11	0.22 ± 0.04
Hypoxic (30 min)	3.47 ± 0.41	5.58 ± 0.49	0.39 ± 0.08	0.80 ± 0.13	0.20 ± 0.04

Each value is a mean ± S.E. of 4 - 6 experiments.

TABLE 2

INFLUENCE OF AZIDE AND OLIGOMYCIN ON CALCIUM BINDING BY SUBCELLULAR FRACTIONS OF CONTROL AND HYPOXIC HEARTS

	Calcium binding (% of values without inhibitors)			
	Mitochondria		Heavy microsomes	
Additions	Control	Hypoxic (30 min)	Control	Hypoxic (30 min)
-	100	100	100	100
Sodium azide (5 mM)	46.8 ± 4.1	51.4 ± 3.2	92.8 ± 3.3	95.0 ± 2.8
Oligomycin (2.5 µg/ml)	51.1 ± 3.6	52.5 ± 3.8	92.9 ± 2.1	89.0 ± 3.0

The results are mean ± S.E. of 4 experiments.

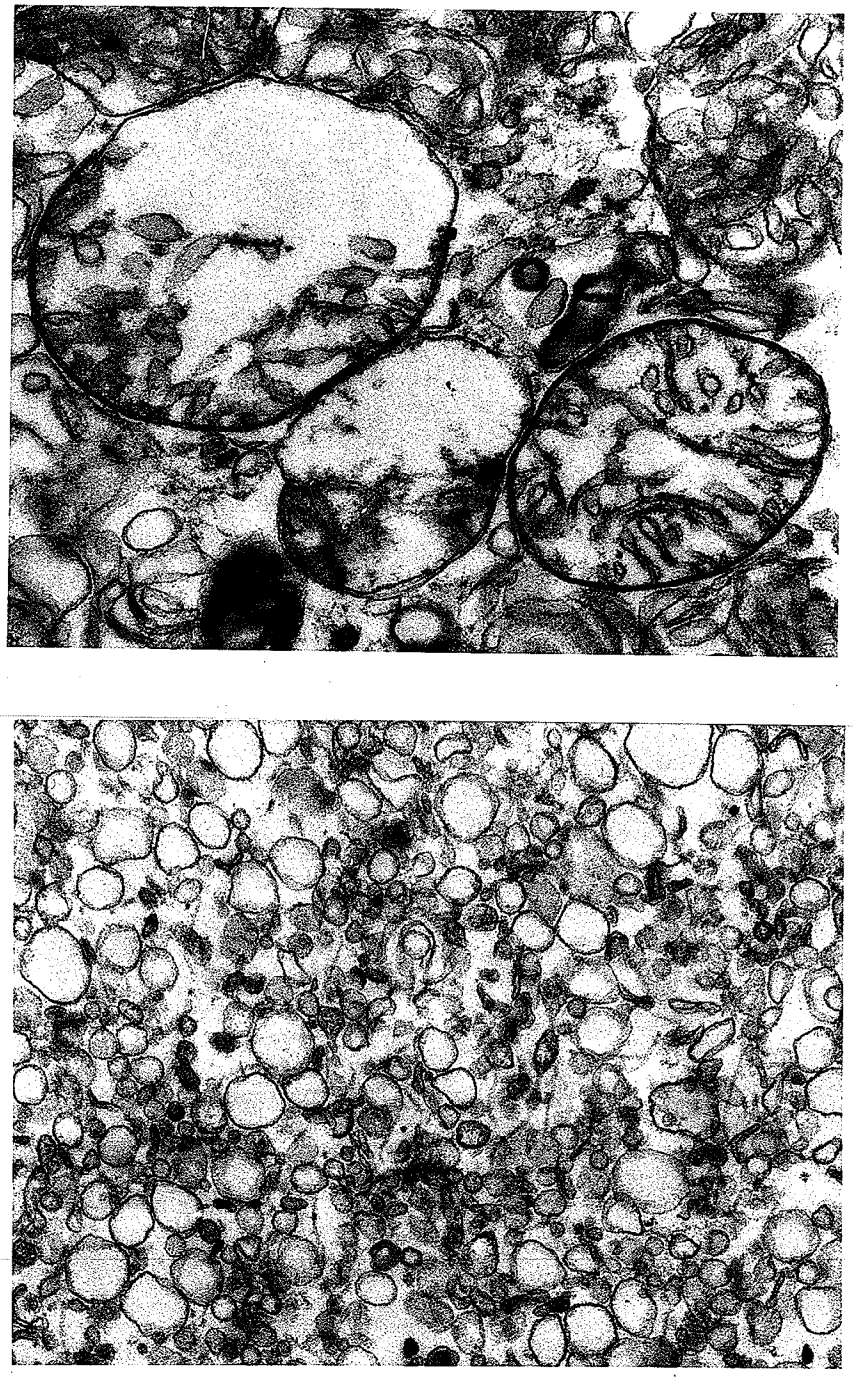

FIGURE 4. Electron micrographs of pellets of the subcellular fractions isolated from hearts perfused for 30 min with hypoxic medium. Upper panel - mitochondrial fraction; lower panel - heavy microsomal fraction (X - 27,000). The electron micrographs of pellets from control hearts were similar to those described by Dhalla (12).

TABLE 3

RECOVERY OF CALCIUM ACCUMULATING ABILITIES OF THE SUBCELLULAR FRACTIONS OF HYPOXIC HEARTS

	Calcium accumulation (% of the control values)			
	Mitochondria		Heavy microsomes	
	Binding	Uptake	Binding	Uptake
Control	100	100	100	100
Hypoxic (30 min)	85.8 ± 2.7	96.7 ± 3.2	79.1 ± 3.4	44.0 ± 5.8
Hypoxic (30 min) + Aerobic (15 min)	99.7 ± 1.6	95.1 ± 2.4	100.9 ± 2.2	95.6 ± 2.8

The results are mean ± S.E. of 6 experiments. The control calcium binding and uptake by mitochondria were 60 ± 4.9 and 255 ± 14.7 and by heavy microsomes were 36 ± 3.2 and 410 ± 18.6 nmoles Ca^{++}/mg protein/10 min respectively.

omission of glucose from the perfusion medium was found to potentiate the depressant effect of hypoxia on mitochondrial and microsomal calcium binding and microsomal calcium uptake (Table 4). It should be pointed out that hearts perfused with hypoxic and substrate-free medium failed to generate contractile force within 30 minutes.

In another series of experiments, the calcium uptake ability of the subcellular fractions from the hypoxic hearts was compared with that from hearts with substrate-free medium. For this purpose, the isolated rat hearts were paced electrically and perfused with hypoxic or substrate-free medium until their ability to generate contractile force decreased by about 66% of the control. From the results given in Table 5 it can be seen that calcium uptake by mitochondria from substrate-depleted hearts, unlike that from the hypoxic hearts, was significantly ($p < 0.05$) less than the control value. The calcium uptake by the microsomal fraction of the substrate-depleted hearts was depressed to an extent similar to that for the hypoxic hearts. On the other hand, calcium binding by the microsomal fraction from the substrate-depleted hearts was decreased to a greater extent ($p < 0.05$) than that from the hypoxic hearts (Table 6) when the determinations were performed at different pH or calcium concentrations of the incubation medium.

B. Calcium Transport by Subcellular Fractions of Hearts made to Fail due to Perfusion With Medium Deficient in Different Cations:

1. Hearts failing due to Ca^{++}-free perfusion:

The contractile force declined to zero within 30 seconds to one minute after perfusing the isolated rat hearts with calcium-free medium. The hearts perfused with calcium-free medium for 5 minutes failed to recover upon reperfusion with

TABLE 4

INFLUENCE OF SUBSTRATE-LACK ON THE CALCIUM ACCUMULATING ABILITIES OF THE SUBCELLULAR FRACTIONS OF THE HYPOXIC HEART

Conditions	Calcium accumulation (nmoles Ca^{++}/mg protein/10 min)			
	Mitochondria		Heavy microsomes	
	Binding	Uptake	Binding	Uptake
Control	61.9 ± 3.5	260 ± 21.3	38.7 ± 3.2	396 ± 13.6
Hypoxic (10 min)	57.0 ± 3.1	251 ± 23.0	30.3 ± 1.8	281 ± 20.8
Substrate-free (10 min)	58.1 ± 4.2	235 ± 14.9	36.8 ± 2.2	358 ± 19.5
Substrate-free hypoxic (10 min)	48.6 ± 2.7	240 ± 27.4	25.2 ± 2.1	159 ± 15.9

Each value is a mean ± S.E. of 6 experiments.

TABLE 5

CONTRACTILE FORCE AND CALCIUM TRANSPORT BY SUBCELLULAR FRACTIONS OF THE ELECTRICALLY PACED CONTROL, HYPOXIC AND SUBSTRATE-DEPLETED HEARTS

	Contractile force (g/g heart)	Calcium uptake ($nmoles\ Ca^{++}$/mg protein/10 min)	
		Mitochondria	Heavy microsomes
Control	1.92 ± 0.32	272 ± 18.3	385 ± 11.4
Hypoxic	0.60 ± 0.11	261 ± 9.2	182 ± 16.5
Substrate-depleted	0.65 ± 0.09	203 ± 13.2	231 ± 10.5

Each value is a mean ± S.E. of 4 - 6 experiments. A resting tension of 1 g was applied to the heart at the beginning of the experiment and the hearts were stimulated at a rate of 250 beats/min with a square pulse stimulii just above the threshold. Coronary flow was maintained at 10 ml/min. Perfusion with hypoxic or substrate-free medium was carried out till the contractile force declined by about 66% of the initial. Control hearts were perfused for comparable lengths of time and their results were grouped together.

TABLE 6

CALCIUM BINDING AT DIFFERENT pH OR CALCIUM CONCENTRATIONS BY MICROSOMAL FRACTION OF CONTROL, HYPOXIC OR SUBSTRATE-DEPLETED HEARTS

	Calcium binding (nmoles Ca^{++}/mg protein/2 min)		
Conditions	Control	Hypoxic	Substrate-depleted
A. pH values:			
6.0	37.1 ± 1.5	25.2 ± 0.7	13.5 ± 0.5
6.5	39.5 ± 2.3	25.9 ± 1.2	12.8 ± 0.7
7.0	36.5 ± 1.9	25.0 ± 1.4	12.6 ± 0.7
8.0	32.4 ± 1.4	23.1 ± 1.1	11.3 ± 0.8
B. Calcium concentrations:			
12.5 μM	19.0 ± 0.5	8.5 ± 0.2	2.9 ± 0.2
50 μM	31.1 ± 1.7	18.6 ± 0.8	7.5 ± 0.4
100 μM	38.5 ± 1.9	24.5 ± 0.7	12.0 ± 0.5
200 μM	36.4 ± 1.1	27.1 ± 1.3	15.2 ± 0.7

Each value is a mean ± S.E. of 4 - 6 experiments. Perfusion with hypoxic or substrate-free medium was carried out until the contractile force declined by about 66% of the initial. Control hearts were perfused for comparable lengths of time.

control perfusion medium containing 1.25 mM Ca^{++}. These results are in agreement with those reported from this laboratory (137).

In one series of experiments, calcium binding and uptake by subcellular fractions of hearts perfused with Ca^{++} -free medium for different intervals were investigated. Both calcium binding and uptake by mitochondrial fraction were significantly ($p < 0.05$) decreased as early as 5 minutes after Ca^{++} - free perfusion (Figure 5). On the other hand, microsomal calcium binding and uptake were not altered ($p > 0.05$) during a 40 minute period of perfusion with Ca^{++} -free medium (Figure 6). The depression in mitochondrial calcium transport, unlike that by microsomes, was also apparent when these fractions from hearts perfused for 20 minutes with Ca^{++} -free medium were incubated for different intervals under in vitro conditions (Figures 7 & 8). Using four to six hearts, we have observed that the abilities of both mitochondrial and microsomal fractions to transport calcium were not altered upon perfusion with Ca^{++} -free medium for 1 to 2 minutes.

In another series of experiments, the effect of reperfusion with control medium of Ca^{++} -deprived hearts was tested on calcium transport by subcellular fractions. It can be seen from Table 7 that calcium binding by the mitochondrial fraction from hearts perfused for 5 minutes with Ca^{++} -free medium was higher ($p < 0.05$) than the control values upon reperfusion for 10 minutes with control medium. On the other hand, microsomal calcium binding was markedly decreased under these conditions. Reperfusion for 10 minutes of hearts perfused with Ca^{++} -free medium for 5 or 10 minutes was also found to elevate mitochondrial calcium uptake and depress microsomal calcium uptake significantly ($p < 0.05$) at different intervals of

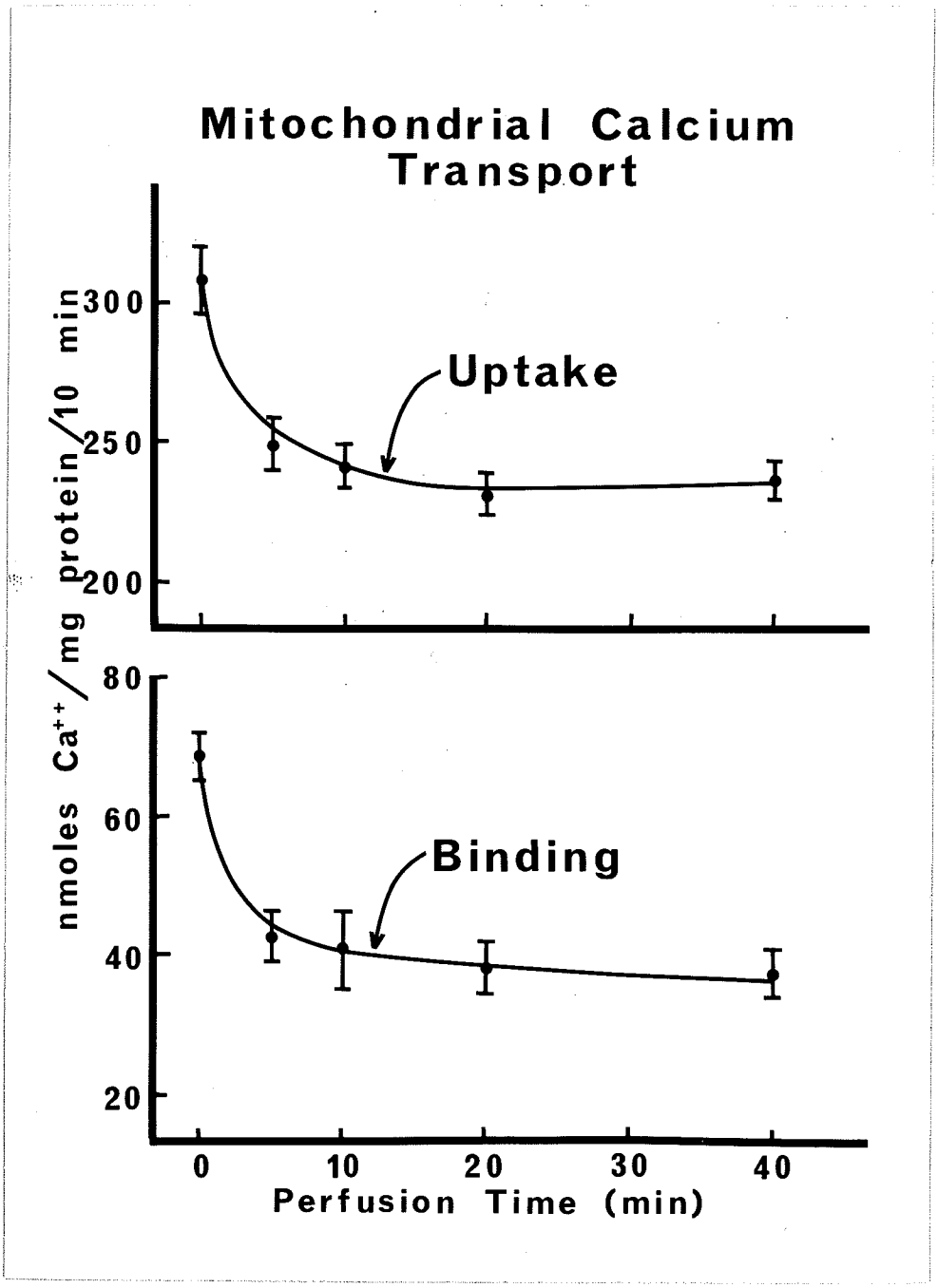

FIGURE 5. Calcium binding and uptake by mitochondrial fraction of hearts perfused with Ca^{++} - free medium for different intervals. Each value is a mean $\pm$ S.E. of 6 - 8 experiments.

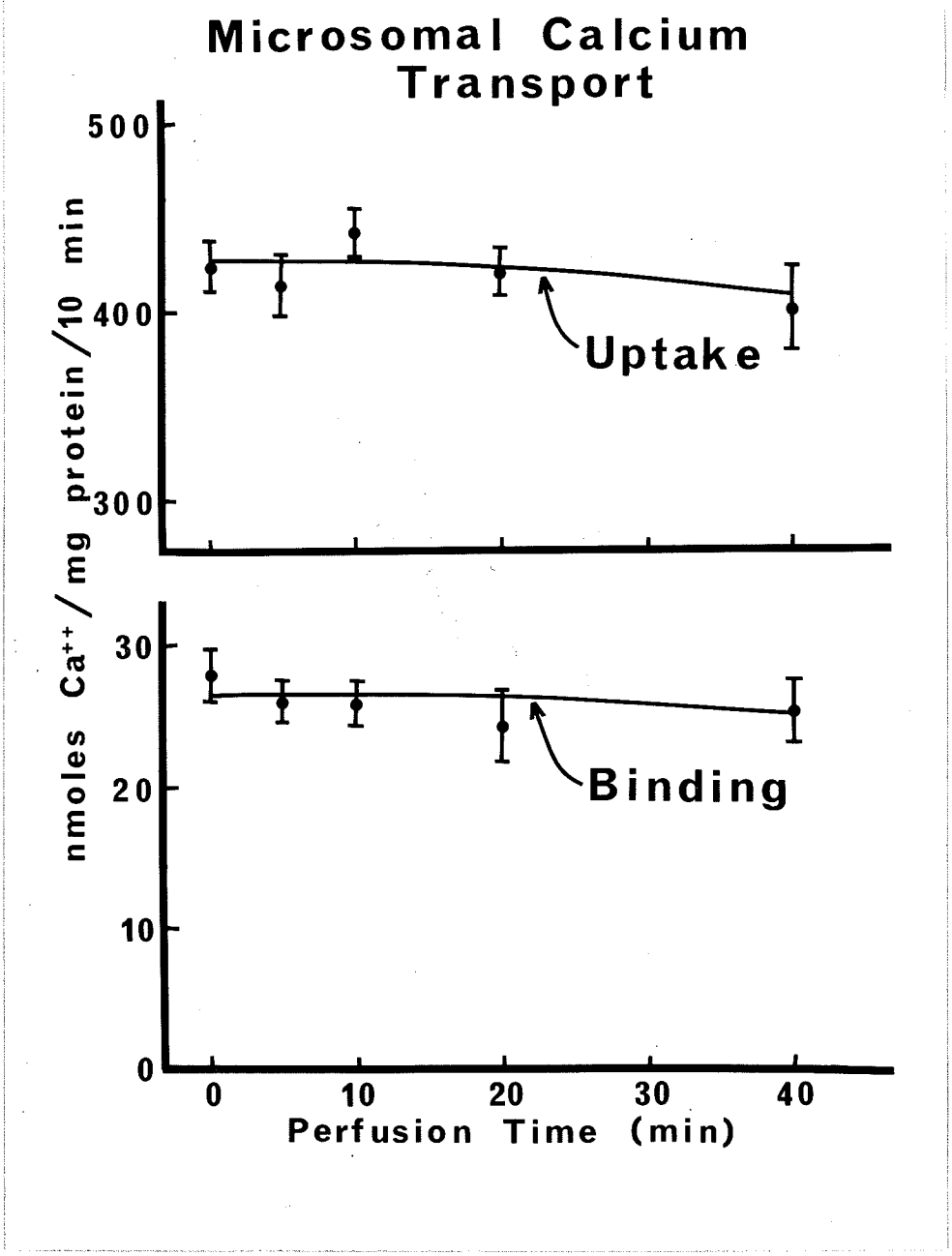

FIGURE 6. Calcium binding and uptake by heavy microsomal fraction of hearts perfused with Ca^{++} - free medium for different intervals. Each value is a mean $\pm$ S.E. of 6 - 8 experiments.

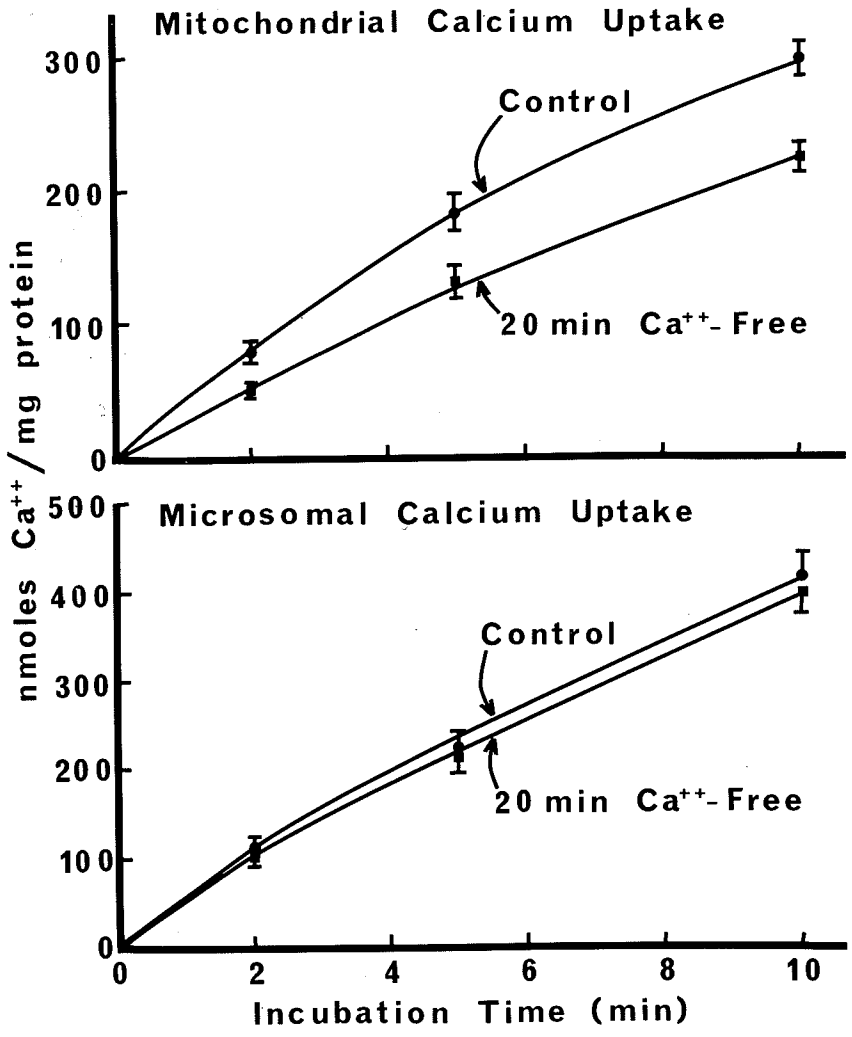

FIGURE 7. Time-course of calcium uptake by mitochondrial and heavy microsomal fractions of hearts perfused with control or Ca^{++}-free medium for 20 min. Each value is a mean $\pm$ S.E. of 6 experiments.

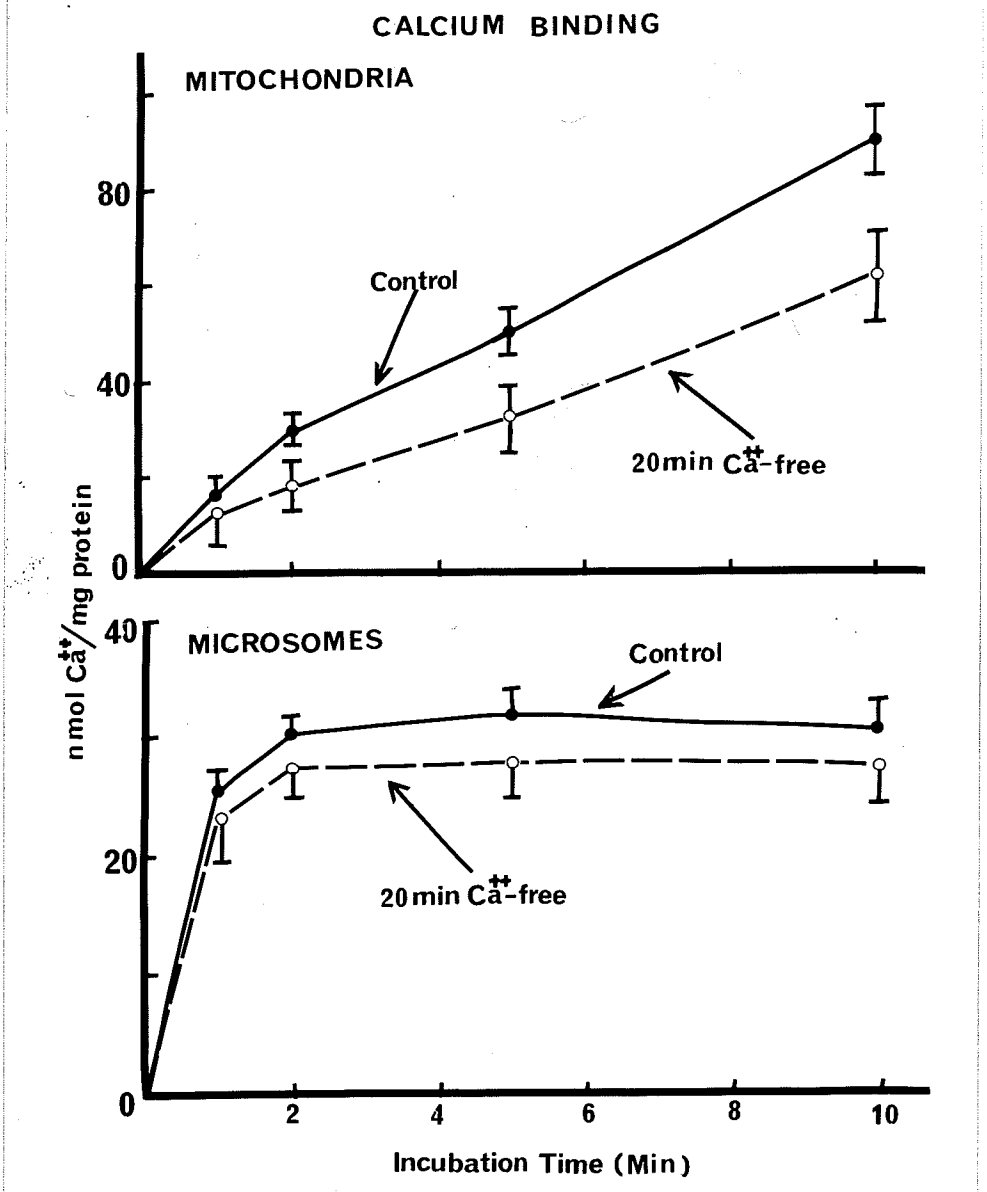

FIGURE 8. Time-course of calcium binding by mitochondrial and heavy microsomal fractions of hearts perfused with control or Ca^{++} - free medium for 20 min. Each value is a mean $\pm$ S.E. of 6 experiments.

TABLE 7

EFFECT OF PERFUSION WITH MEDIUM CONTAINING 1.25 mM Ca^{++} ON Ca^{++} BINDING BY SUBCELLULAR FRACTIONS OF HEARTS PERFUSED WITH Ca^{++} - FREE MEDIUM

	Ca^{++} Binding (nmoles Ca^{++} /mg protein)			
	Mitochondria		Microsomes	
	3 min	5 min	3 min	5 min
Control	24.7 ± 1.8	33.6 ± 2.6	31.9 ± 2.0	31.1 ± 2.2
5 min Ca^{++} - free medium	16.0 ± 0.6	22.8 ± 1.2	30.0 ± 2.7	29.4 ± 2.4
5 min Ca^{++} - free perfusion followed by 10 min control perfusion	31.5 ± 1.7	48.7 ± 3.4	19.8 ± 1.4	16.5 ± 1.3

Each value is a mean ± S.E. of 6 experiments. Control hearts were perfused for 15 min with normal medium containing 1.25 mM Ca^{++}.

incubation (Table 8).

Calcium binding by microsomal and mitochondrial fractions of hearts perfused with Ca^{++} -free medium for 20 minutes with or without subsequent reperfusion for 10 minutes with control medium was determined at different pH or calcium concentrations of the incubation medium. The data in Table 9 indicate that the calcium binding by the microsomal fraction from Ca^{++} -deprived hearts was not different ($p > 0.05$) from the control whereas that from reperfused hearts was decreased significantly ($p < 0.05$) at all pH or calcium concentrations employed. On the other hand, under similar conditions of incubation, calcium binding by the mitochondrial fraction was markedly decreased in calcium-deprived hearts and markedly increased in reperfused hearts in comparison to the control (Table 10). It should be pointed out that neither oligomycin (2.5 μg/ml) nor sodium azide (5 mM) inhibited calcium binding by microsomes obtained from control, calcium-deprived or reperfused hearts whereas these agents produced 50 to 60% inhibition of calcium binding by the mitochondrial fractions of these control and experimental hearts.

The ATP hydrolyzing ability of subcellular fractions from calcium deprived hearts and reperfused hearts was also studied. No significant ($p > 0.05$) change in mitochondrial or total microsomal ATPase activity was observed on perfusing the hearts for 20 or 40 minutes with Ca^{++} -free medium (Table 11). Although microsomal Ca^{++} -stimulated ATPase activity was decreased slightly under these conditions, this change was not significant ($p > 0.05$). On the other hand, both total and Ca^{++} -stimulated ATPase activities of the microsomal fraction, unlike mitochondrial ATPase activity, were significantly ($p < 0.05$) decreased upon reperfusing the Ca^{++} -deprived

TABLE 8

EFFECT OF PERFUSION WITH MEDIUM CONTAINING 1.25 mM Ca^{++} ON CALCIUM UPTAKE BY THE SUBCELLULAR FRACTIONS OF HEARTS PREPERFUSED WITH Ca^{++} - FREE MEDIUM FOR 5 OR 10 MINUTES

Time of incubation (min)	Ca^{++} Uptake (% of control)			
	After 5 min Ca^{++} - free preperfusion		After 10 min Ca^{++} - free preperfusion	
	Mitochondria	Microsomes	Mitochondria	Microsomes
2	217 ± 12.5	87 ± 5.0	237 ± 13.0	78 ± 6.2
5	177 ± 8.8	62 ± 7.5	187 ± 13.7	55 ± 7.5
10	190 ± 10.0	63 ± 8.7	205 ± 11.3	56 ± 9.0

Each value is a mean ± S.E. of 6 experiments. Control hearts were perfused for 15 to 20 min with normal medium containing 1.25 mM Ca^{++}.

TABLE 9

CALCIUM BINDING AT DIFFERENT pH OR CALCIUM CONCENTRATIONS BY MICROSOMAL FRACTION OF HEARTS PERFUSED WITH Ca^{++} - FREE MEDIUM

Conditions	Calcium binding (nmoles Ca^{++}/mg protein/2 min)		
	Control	Ca^{++} - free (20 min)	Ca^{++} - free (20 min) followed by 10 min control perfusion
A. pH values:			
6.5	37.2 ± 1.6	35.4 ± 1.9	12.2 ± 0.9
7.0	34.5 ± 0.8	36.2 ± 2.1	10.3 ± 0.2
8.0	30.7 ± 1.1	32.5 ± 1.7	10.6 ± 0.4
B. Calcium concentrations:			
12.5 μM	16.8 ± 1.2	16.7 ± 0.8	8.5 ± 0.3
50 μM	29.2 ± 1.2	26.3 ± 1.1	10.2 ± 1.0
100 μM	35.4 ± 0.8	31.2 ± 1.5	10.1 ± 0.5

Each value is a mean ± S.E. of 6 experiments. Control hearts were perfused for 20 - 30 min with normal medium containing 1.25 mM Ca^{++}.

TABLE 10

CALCIUM BINDING AT DIFFERENT pH OR CALCIUM CONCENTRATIONS BY MITOCHONDRIAL FRACTION OF HEARTS PERFUSED WITH Ca^{++} - FREE MEDIUM

Conditions	Calcium binding (nmoles Ca^{++}/mg protein/5 min) Control	Ca^{++} - free (20 min)	Ca^{++} - free (20 min) followed by 10 min control perfusion
A. pH values:			
6.5	28.2 ± 1.0	14.1 ± 0.5	56.9 ± 2.8
7.0	31.4 ± 1.5	16.5 ± 0.3	61.5 ± 4.2
8.0	27.9 ± 0.8	16.2 ± 0.8	57.0 ± 3.1
B. Calcium concentrations:			
12.5 μM	15.2 ± 0.5	7.7 ± 0.2	22.9 ± 0.6
50 μM	24.0 ± 1.7	16.5 ± 0.6	37.4 ± 1.9
100 μM	35.5 ± 2.1	25.2 ± 0.7	59.2 ± 3.3

Each value is a mean ± S.E. of 6 experiments. Control hearts were perfused for 20 - 30 min with normal medium containing 1.25 mM Ca^{++}.

TABLE 11

ATPase ACTIVITY OF SUBCELLULAR FRACTIONS OF HEARTS PERFUSED WITH Ca^{++} - FREE MEDIUM

	ATPase activity (μmoles Pi/mg protein/min)		
	Mitochondria	Microsomes	
	Total	Total	Ca^{++} - stimulated
Control	1.57 ± 0.11	3.10 ± 0.13	0.21 ± 0.02
20 min Ca^{++} - free	1.67 ± 0.12	3.00 ± 0.18	0.15 ± 0.03
40 min Ca^{++} - free	1.79 ± 0.15	2.67 ± 0.11	0.14 ± 0.03
20 min Ca^{++} - free followed by 10 min control perfusion	1.71 ± 0.11	2.38 ± 0.17	0.08 ± 0.01

Each value is a mean ± S.E. of 6 experiments. Control hearts were perfused for 20 - 40 min with normal medium containing 1.25 mM Ca^{++}.

hearts.

Earlier studies from this laboratory (137) have shown that the contractile force of rat hearts perfused with Ca^{++} -free medium in the presence of low sodium (35 mM) for 5 minutes recovered completely upon reperfusion with control medium. It was therefore considered of interest to investigate if the above mentioned effects of reperfusion on calcium transport by subcellular fractions could be modified in hearts perfused with Ca^{++} -free medium in the presence of low sodium. The results shown in Table 12 reveal that reperfusion of hearts prepurfused for 5 minutes with Ca^{++} -free medium in the presence of low sodium neither increased mitochondrial calcium binding and uptake nor decreased microsomal calcium binding and uptake significantly ($p > 0.05$).

2. Hearts failing due to Na^{+} -free perfusion:

The isolated rat hearts failed to generate contractile force within 30 seconds of perfusion with Na^{+} -free medium. Reperfusion with control medium for 15 minutes of hearts perfused with Na^{+} -free medium for 10 and 20 minutes resulted in the recovery of the contractile force to the extent of 60 and 40% of the initial values respectively. These results are in agreement with those already reported from this laboratory (148).

Both mitochondrial and microsomal fractions were isolated from rat hearts after perfusion with Na^{+} -free medium for 5, 10 and 20 minutes and calcium binding and uptake were studied. The results in Figure 9 indicate that both microsomal calcium binding and uptake decreased ($p < 0.05$) on perfusing the hearts for a period of 20 minutes. Although mitochondrial calcium uptake and binding were

TABLE 12

EFFECT OF PERFUSION WITH MEDIUM CONTAINING 1.25 mM Ca^{++} ON CALCIUM ACCUMULATION BY SUBCELLULAR FRACTIONS OF HEARTS PERFUSED WITH CALCIUM-FREE MEDIUM IN THE PRESENCE OF LOW SODIUM (35 mM)

	Calcium accumulation (nmoles/mg protein/5 min)			
	Mitochondria		Microsomes	
Conditions	Binding	Uptake	Binding	Uptake
5 min Ca^{++} - free, low Na^{+}	37 ± 2.7	108 ± 6.2	36 ± 2.5	159 ± 7.9
5 min Ca^{++} - free, low Na^{+} followed by 10 min control perfusion	48 ± 4.1	132 ± 8.7	28 ± 2.1	183 ± 9.4

Each value is a mean ± S.E. of 5 experiments.

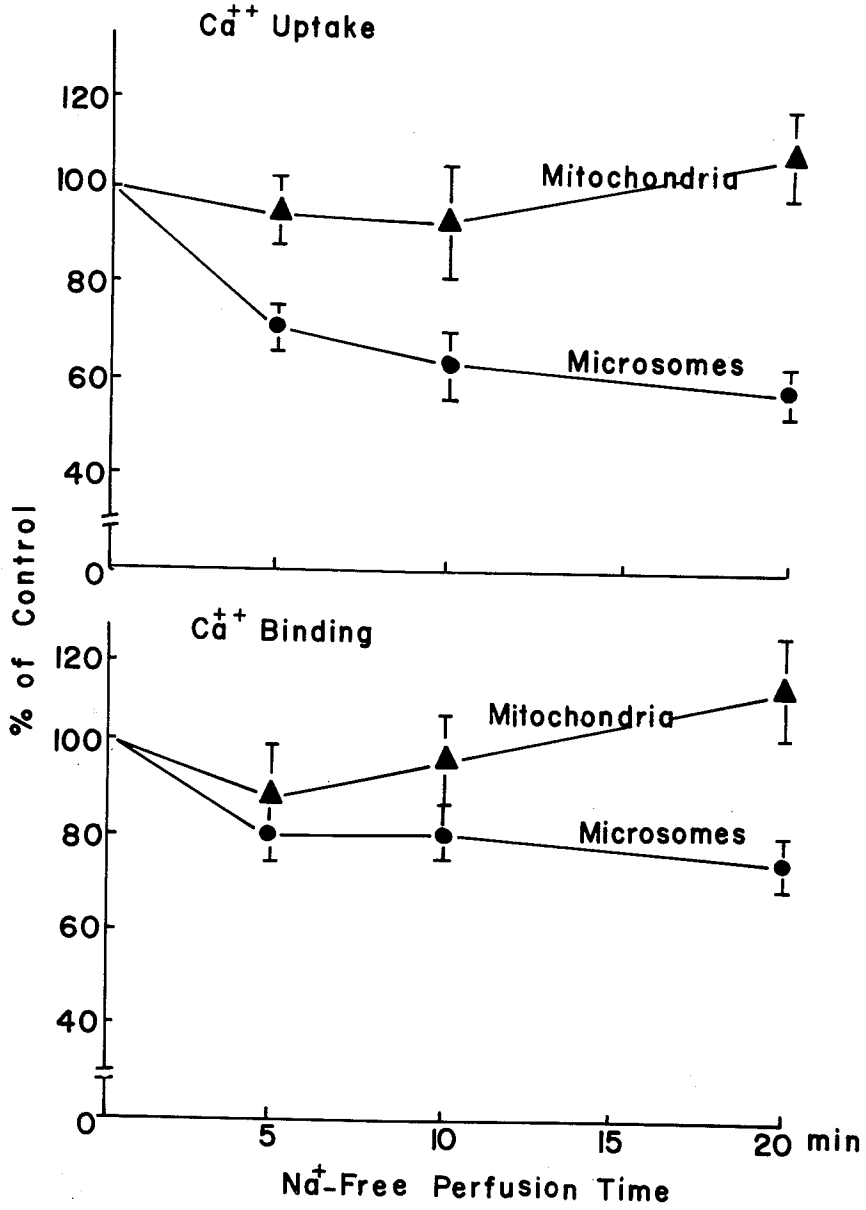

FIGURE 9. Calcium uptake and binding by subcellular fractions of hearts perfused with Na^+ - free medium for different intervals. Both calcium uptake and binding were measured for 10 min. Each value is a mean $\pm$ S.E. of 6 - 8 experiments.

higher than the control in hearts perfused for 20 minutes with Na^+ -free medium, this increase was not significant ($p > 0.05$). The depression in calcium uptake (Figure 10) and calcium binding (Figure 11) by the microsomal fraction, unlike that by the mitochondrial fraction, in hearts perfused for 20 minutes with Na^+ -free medium is further demonstrated when determinations were made at different intervals of incubation. Neither oligomycin (2.5 μg/ml) nor sodium azide (5 mM) which inhibited mitochondrial calcium transport by 50 to 60% influenced the calcium accumulation by the microsomal fraction of the control and experimental hearts.

Table 13 shows that a significant ($p < 0.05$) depression in calcium uptake by the microsomal fraction of hearts perfused for 10 minutes with Na^+ -free medium was observed when determinations were made at different pH or calcium concentrations of the incubation medium. Reperfusion with control medium of hearts preperfused with Na^+ -free medium resulted in partial recovery of microsomal ability to accumulate calcium (Table 13). Perfusion of the hearts with Na^+ -free medium for 5 minutes had no significant ($p > 0.05$) effect upon the mitochondrial or microsomal ATPase activities (Table 14). However, Ca^{++} stimulated ATPase activity of the microsomal fraction was decreased significantly ($p < 0.05$) upon perfusing the hearts with Na^+ -free medium for 20 minutes.

3. Hearts failing due to K^+ -free perfusion:

Although contractile force of the isolated rat hearts declined by about 25% of the initial value within 5 minutes of starting perfusion with K^+ -free medium, the contractions were irregular. The hearts failed to generate contractile force abruptly after about 10 minutes of perfusion with K^+ -free medium and did not recover upon reperfusion with control medium. These results are in agreement with

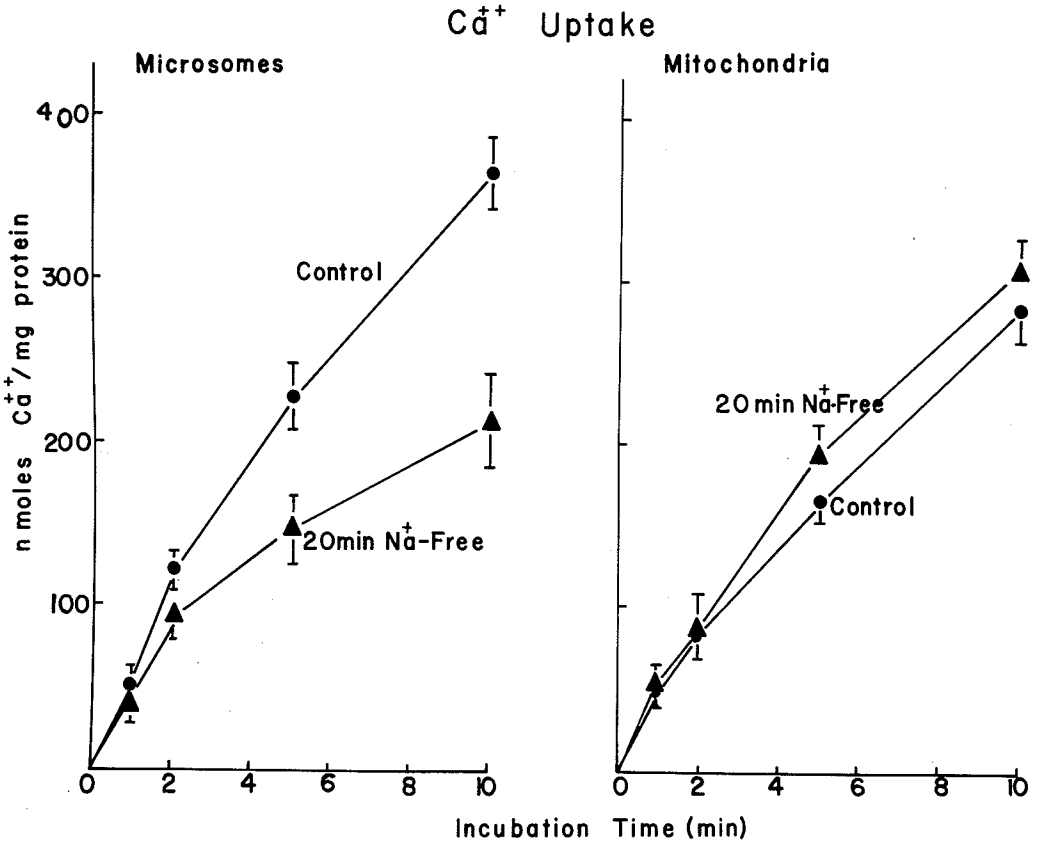

FIGURE 10. Time-course of calcium uptake by subcellular fractions of hearts perfused with control or Na^+ - free medium for 20 min. Each value is a mean $\pm$ S.E. of 6 experiments.

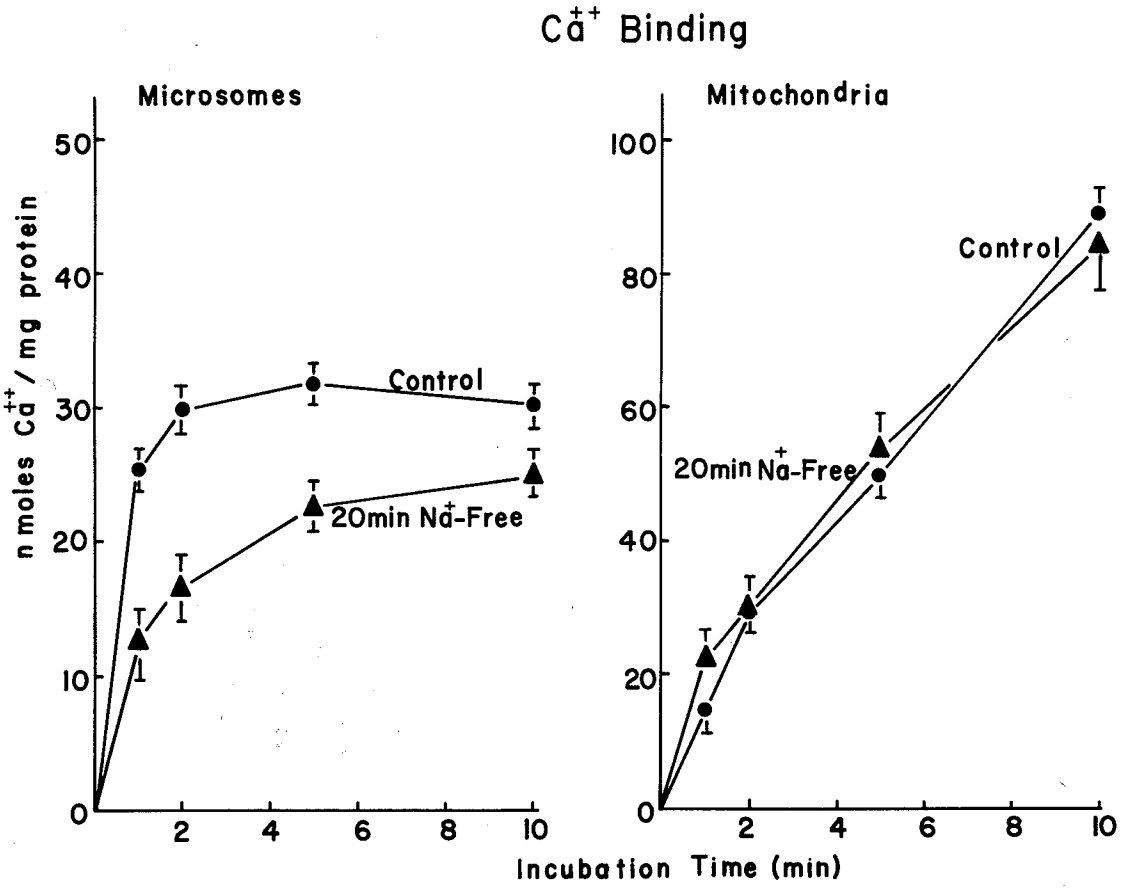

FIGURE 11. Time-course of calcium binding by subcellular fractions of hearts perfused with control or Na^+ - free medium for 20 min. Each value is a mean $\pm$ S.E. of 6 experiments.

TABLE 13

CALCIUM UPTAKE AT DIFFERENT pH OR CALCIUM CONCENTRATIONS BY HEAVY MICROSOMAL FRACTION OF HEARTS PERFUSED WITH CONTROL OR Na^+ - FREE MEDIUM FOR 10 MINUTES

Conditions	Calcium uptake (nmoles/mg protein/10 min)		
	Control	Na^+ - free	Na^+ - free followed by 15 min control perfusion
A. pH values:			
6.6	471 ± 40	364 ± 29	425 ± 13
7.0	437 ± 31	245 ± 30	375 ± 20
7.8	388 ± 22	172 ± 24	320 ± 25
B. Calcium concentrations:			
50 μM	383 ± 35	224 ± 18	315 ± 24
100 μM	416 ± 27	269 ± 21	362 ± 17
250 μM	280 ± 15	65 ± 8	248 ± 22

Each value is a mean ± S.E. of 6 - 8 experiments.

TABLE 14

ATPase ACTIVITY OF SUBCELLULAR FRACTIONS OF HEARTS PERFUSED WITH CONTROL OR Na^+ - FREE MEDIUM

	ATPase activity (μmole Pi/mg/min)		
	Mitochondria	Microsomes	
	Total	Total	Ca^{++} stimulated
Control	1.72 ± 0.18	3.27 ± 0.27	0.23 ± 0.03
5 min Na^+ - free	1.45 ± 0.21	2.73 ± 0.31	0.25 ± 0.03
20 min Na^+ - free	1.70 ± 0.13	2.57 ± 0.24	0.12 ± 0.02

Each value is a mean ± S.E. of 6 to 8 experiments.

those reported from this laboratory (148).

The isolated rat hearts were perfused with K^+ -free medium for 5, 10 or 20 minutes and mitochondrial and microsomal fractions were isolated for calcium transport determination. The results shown in Figure 12 indicate that calcium uptake and binding by the mitochondrial fraction were increased ($p < 0.05$) whereas calcium uptake and binding by the microsomal fraction were decreased ($p < 0.05$) in hearts perfused with K^+ -free medium. The decrease in calcium binding and uptake by the microsomal fraction (Figure 13) and the increase in calcium binding and uptake by the mitochondrial fraction (Figure 14) were also apparent at different intervals of the incubation period. Oligomycin (2.5 μg/ml) and sodium azide (5 mM), had no appreciable effect upon calcium binding by microsomal fractions of the control and experimental hearts, whereas these agents decreased mitochondrial calcium binding by about 50 to 60% in both control and experimental preparations.

Reperfusion of hearts perfused with K^+ -free medium for 10 minutes did not reverse the augmented calcium uptake by mitochondria or the depressed calcium uptake by microsomes (Table 15). These patterns of change in calcium uptake by mitochondrial and microsomal fractions upon K^+ -free perfusion or reperfusion of K^+ -deprived hearts were also apparent when determinations were made at different pH or calcium concentrations of the incubation medium (Table 16). No significant ($p > 0.05$) changes in ATPase activities of mitochondrial and microsomal fractions were found upon perfusing the hearts for 10 or 20 minutes with K^+ -free medium (Table 17).

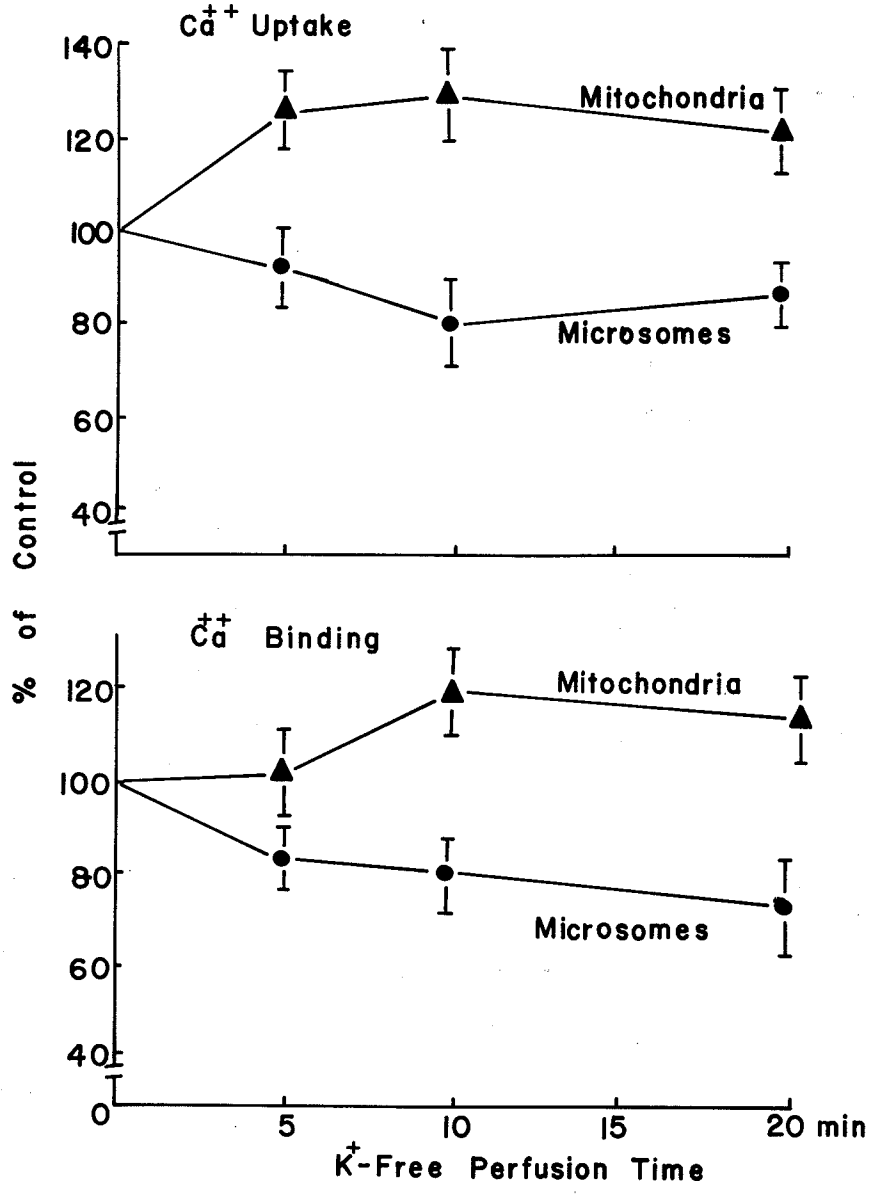

FIGURE 12. Calcium uptake and binding by subcellular fractions of hearts perfused with K^+ - free medium for different intervals. Both calcium uptake and binding were measured for 10 min. Each value is a mean $\pm$ S.E. of 8 experiments.

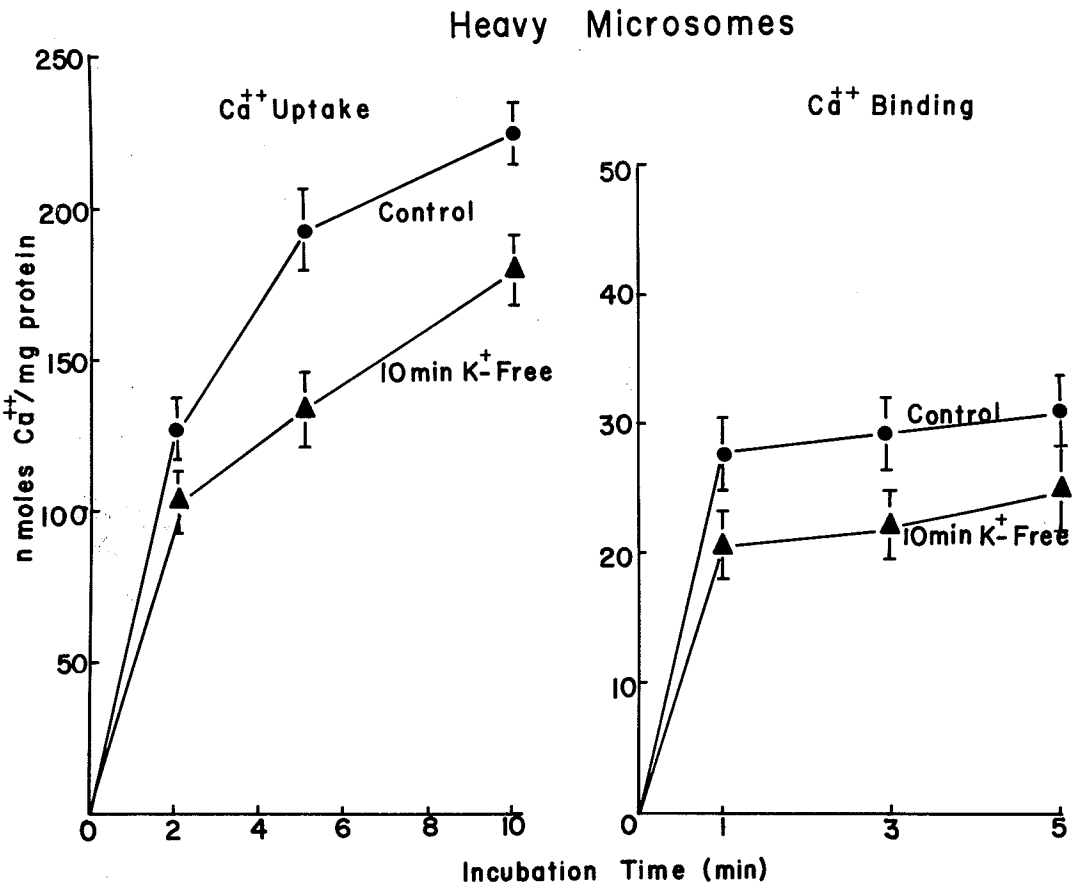

FIGURE 13. Time-course of calcium uptake and binding by heavy microsomal fraction of hearts perfused with K^+ - free medium for 10 min. Each value is a mean $\pm$ S.E. of 6 experiments.

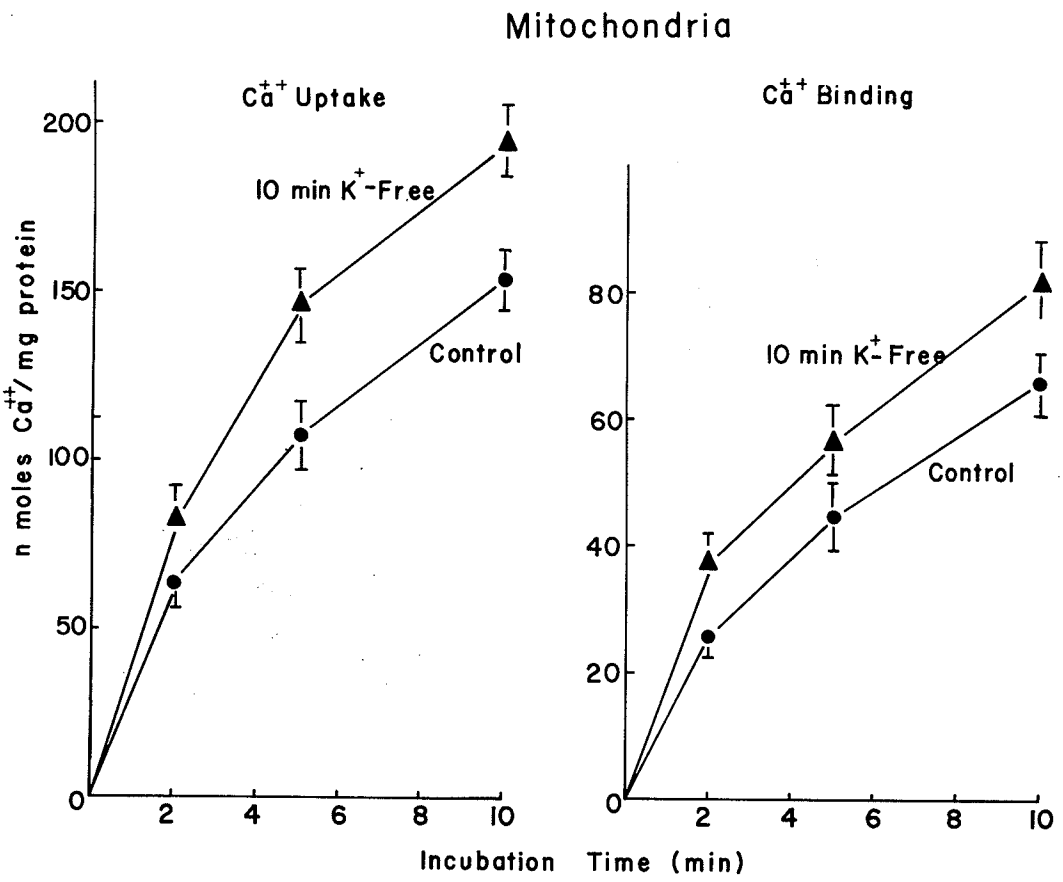

FIGURE 14. Time-course of calcium uptake and binding by mitochondrial fraction of hearts perfused with K^+ - free medium for 10 min. Each value is a mean $\pm$ S.E. of 6 experiments.

TABLE 15

CALCIUM UPTAKE BY SUBCELLULAR FRACTIONS OF HEARTS PERFUSED WITH CONTROL, K^+ - FREE OR K^+ - FREE FOLLOWED BY CONTROL MEDIUM

	Calcium uptake (nmoles/mg protein)			
	Mitochondria		Heavy microsomes	
	5 min	10 min	5 min	10 min
Control	104 ± 5	148 ± 8	197 ± 14	228 ± 17
10 min K^+ - free	142 ± 8	191 ± 11	139 ± 15	171 ± 16
10 min K^+ - free followed by 15 min control perfusion	137 ± 4	187 ± 9	145 ± 12	170 ± 19

Each value is a mean ± S.E. of 6 experiments.

TABLE 16

CALCIUM UPTAKE AT DIFFERENT pH OR CALCIUM CONCENTRATIONS BY SUBCELLULAR FRACTIONS OF HEARTS PERFUSED WITH CONTROL OR K^+ - FREE MEDIUM FOR 10 MINUTES

Conditions	Calcium uptake (nmoles/mg protein/10 min)					
	Mitochondria			Microsomes		
	Control	K^+ - free	K^+-free followed by 15 min control perfusion	Control	K^+ - free	K^+ - free followed by 15 min control perfusion
A. pH values:						
6.6	165 ± 11	218 ± 13	210 ± 18	249 ± 10	202 ± 7	210 ± 8
7.0	150 ± 8	197 ± 7	181 ± 12	225 ± 7	173 ± 5	171 ± 4
7.8	132 ± 7	171 ± 5	173 ± 10	198 ± 8	160 ± 7	158 ± 6
B. Calcium concentrations:						
50 μM	147 ± 10	188 ± 11	168 ± 8	212 ± 13	162 ± 6	160 ± 10
100 μM	152 ± 8	194 ± 13	187 ± 7	234 ± 7	176 ± 8	173 ± 11
250 μM	146 ± 7	190 ± 10	180 ± 4	219 ± 7	164 ± 3	169 ± 7

Each value is a mean ± S.E. of 4 - 6 experiments.

TABLE 17

ATPase ACTIVITY OF SUBCELLULAR FRACTIONS OF HEARTS PERFUSED WITH CONTROL OR K^+ - FREE MEDIUM

	ATPase activity (μmoles Pi/mg protein/min)		
	Mitochondria	Microsomes	
	Total	Total	Ca^{++} stimulated
Control	1.79 ± 0.12	2.98 ± 0.13	0.20 ± 0.02
10 min K^+ - free	1.98 ± 0.14	2.79 ± 0.12	0.23 ± 0.05
20 min K^+ - free	1.88 ± 0.10	2.73 ± 0.15	0.14 ± 0.02

Each value is a mean ± S.E. of 5 - 6 experiments.

C. Myopathic Failing Hearts:

In one series of experiments calcium binding by both mitochondrial and microsomal fractions of the hearts from myopathic hamster (UM-X7.1) at a severe stage of heart failure was examined. The calcium binding by these fractions of the myopathic hearts was significantly ($p < 0.05$) lower than the control values (Table 18). When the mitochondrial and microsomal fractions from the myopathic hamster heart homogenates were isolated in the presence of exogenously added sarcolemmal, microsomal or mitochondrial membranes prepared from the myopathic hearts, calcium binding by these fractions was not altered significantly ($p > 0.05$). Both sodium azide and oligomycin decreased calcium binding by mitochondrial fractions of the control and myopathic hearts to an equal extent without appreciably affecting calcium binding by the microsomal fractions (Table 19). The data on marker enzyme activities in the mitochondria and microsomes are given in Table 20 to indicate the degree of purity of the subcellular fractions employed in this study. Furthermore, the yields of mitochondrial and microsomal fractions of the myopathic hearts were not different ($p > 0.05$) from the control.

In another series of experiments, calcium binding by microsomal fractions of the control and myopathic hamster hearts at a severe stage of failure was monitored in the presence of different concentrations of calcium. The data was plotted according to the method of Lineweaver-Burk (149) and shown in Figure 15. The depression in calcium binding by the myopathic microsomal fractions appears to be associated with a decrease in the number of sites rather than changes in calcium binding constant. It was also found that calcium binding as well as calcium uptake by microsomal and

TABLE 18

CALCIUM BINDING BY SUBCELLULAR FRACTIONS ISOLATED FROM CONTROL AND MYOPATHIC HAMSTER (UM - X 7.1) HEART HOMOGENATES IN THE ABSENCE OR PRESENCE OF DIFFERENT EXOGENOUS CELLULAR MEMBRANES

		Calcium binding (n moles Ca^{++}/mg protein/2 min)	
	Additions	Mitochondria	Microsomes
Control	-	40 ± 3.1	49 ± 4.4
Myopathic	-	19 ± 2.8	22 ± 3.3
Myopathic	Sarcolemma	21 ± 3.2	25 ± 2.5
Myopathic	Microsomes	24 ± 2.3	23 ± 2.9
Myopathic	Mitochondria	20 ± 2.5	18 ± 3.6

Each value is a mean ± S.E. of 14 - 6 experiments. Sarcolemmal, Microsomal or mitochondrial membranes isolated from the myopathic hearts were added to the homogenates of the myopathic hearts where indicated before isolating the subcellular fraction for calcium binding determination. Myopathic hamsters at severe stage of heart failure and control healthy hamsters were 200 day old.

TABLE 19

EFFECT OF SODIUM AZIDE AND OLIGOMYCIN ON CALCIUM BINDING BY SUBCELLULAR FRACTIONS OF CONTROL AND MYOPATHIC HAMSTER (UM - X 7.1) HEARTS AT SEVERE STAGE OF HEART FAILURE

	Calcium binding (% of values without inhibitors)			
	Mitochondria		Microsomes	
Additions	Control	Myopathic	Control	Myopathic
-------	100	100	100	100
Sodium azide (5mM)	47 ± 2.9	56 ± 3.8	93 ± 3.3	97 ± 2.2
Oligomycin (2.5 µg/ml)	61 ± 3.4	59 ± 4.1	93 ± 2.9	96 ± 2.8

Each value is a mean ± S.E. of 4 experiments. Myopathic hamsters and control healthy hamsters were 200 day old.

TABLE 20

MARKER ENZYME ACTIVITIES AND YIELDS OF MITOCHONDRIAL AND MICROSOMAL FRACTIONS OF CONTROL AND MYOPATHIC HAMSTER (UM - X 7.1) HEARTS AT SEVERE STAGE OF HEART FAILURE

	Mitochondria		Microsomes	
	Control	Myopathic	Control	Myopathic
Cytochrome C oxidase	1103 ± 65	1070 ± 51	79 ± 7	91 ± 12
Glucose-6-phosphatase	0.08 ± 0.02	0.11 ± 0.03	1.59 ± 0.15	1.41 ± 0.17
Ouabain-sensitive $Na^+ - K^+$ stimulated ATPase	N.D.	N.D.	N.D.	N.D.
Yield (mg protein/g heart wt)	0.72 ± 0.08	0.65 ± 0.05	0.38 ± 03	0.42 ± 0.04

Each value is a mean ± S.E. of 4 experiments, N.D. - not detectable. Myopathic hamsters and control healthy hamsters were 200 day old. The cytochrome C oxidase activity is expressed as n moles cytochrome oxidized/mg protein/min whereas the activities of glucose-6-phosphatase and $Na^+ - K^+$ ATPase are expressed as μ moles Pi/mg protein/hr.

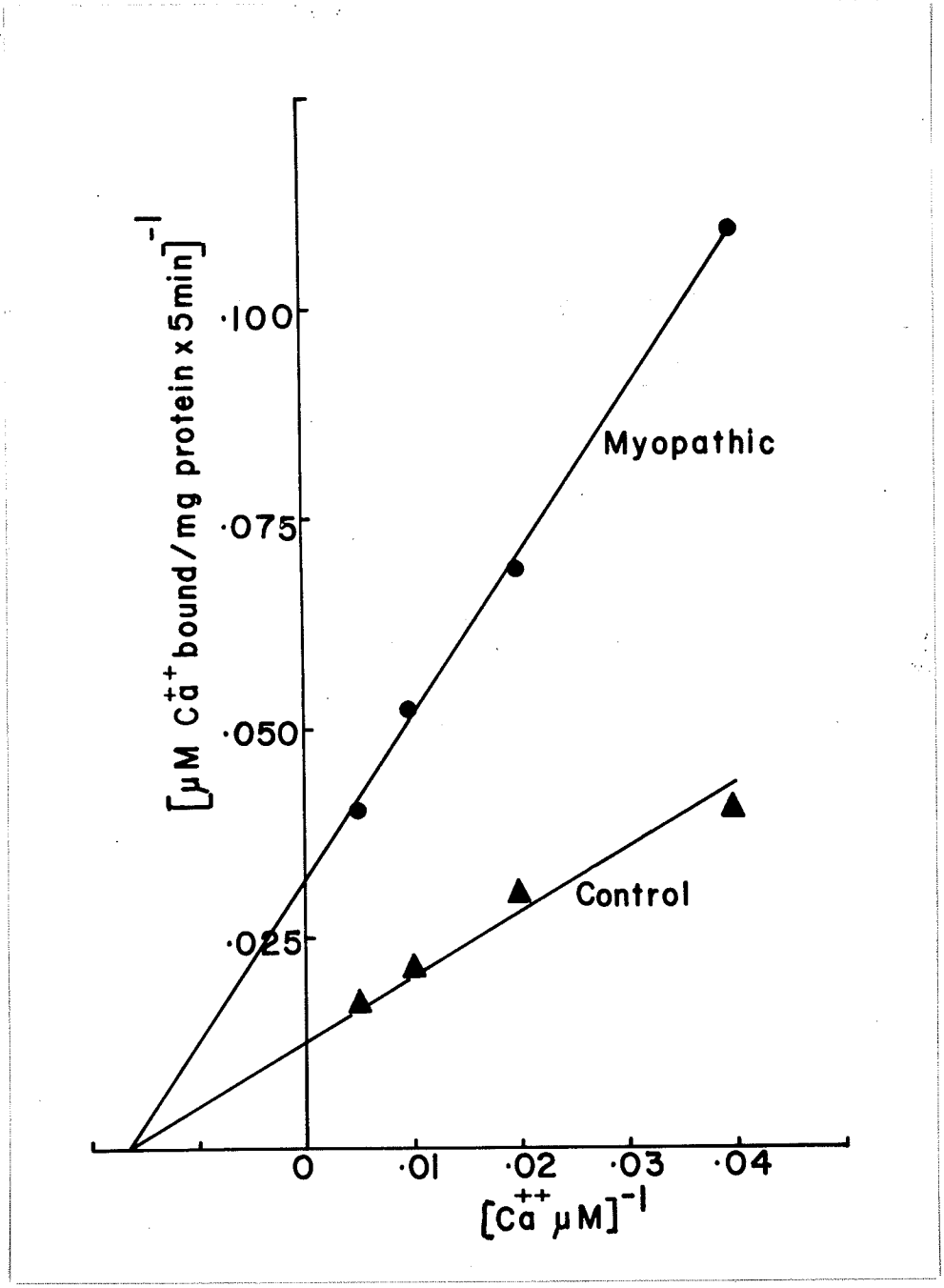

FIGURE 15. Lineweaver - Burk plot of data on calcium binding by microsomal fraction of control and myopathic hamster (UM -X7.1) hearts. Calcium binding was determined in the presence of different concentrations of total calcium in the incubation medium. Myopathic hamsters (200 day old) were at severe stage of failure whereas healthy hamsters (200 day old) served as controls. Each value is an average of 3 experiments.

mitochondrial fractions of the myopathic hamster hearts at severe stage of heart failure was less ($p < 0.05$) than the control values when determinations were made at different pH of the incubation medium (Table 21). The ATPase activities of the microsomal or mitochondrial fraction of the myopathic hearts were not different ($p > 0.05$) from the controls (Table 22).

The calcium accumulation abilities of both mitochondrial and microsomal fractions of the myopathic hamster (UM-X7.1) hearts at different stages of heart failure were examined. Although calcium binding by mitochondrial fractions from hearts at early, moderate and severe stages of failure was markedly decreased ($p < 0.05$), calcium uptake by the mitochondrial fraction decreased significantly ($p < 0.05$) only in hearts from severe stage of heart failure (Figure 16). Both calcium binding and uptake by the microsomal fractions of the myopathic hearts at early, moderate and severe stages of failure were significantly ($p < 0.05$) lower than the control (Figure 17).

For the purpose of comparison, calcium binding and uptake by mitochondrial and microsomal fractions of another strain of myopathic hamster (BIO 14.6) hearts at different stages of failure were tested. The data in Table 23 reveal that both calcium binding and uptake by mitochondrial fractions were decreased at all stages of failure in BIO 14.6 strain of myopathic hearts. Although calcium binding by microsomal fractions of the early, moderate and late stages of failure was significantly decreased ($p < 0.05$), the depression in calcium uptake by microsomal fraction was only significant ($p < 0.05$) in late stages of heart failure (Table 23).

In view of the presence of focal necrosis in myopathic hamster hearts, it

TABLE 21

CALCIUM ACCUMULATION AT DIFFERENT pH BY SUBCELLULAR FRACTIONS OF CONTROL OR MYOPATHIC HAMSTER (UM - X7.1) HEARTS AT LATE STAGES OF HEART FAILURE

	Calcium accumulation (nmoles/mg protein)							
	Mitochondria				Microsomes			
	Uptake/10 min		Binding/3 min		Uptake/10 min		Binding/3 min	
pH	Control	Myopathic	Control	Myopathic	Control	Myopathic	Control	Myopathic
6.6	257 ± 17	185 ± 21	35 ± 3	20 ± 2	745 ± 32	581 ± 29	36 ± 3	24 ± 2
7.0	270 ± 20	190 ± 16	43 ± 2	25 ± 3	805 ± 35	444 ± 31	50 ± 5	29 ± 3
7.8	243 ± 24	166 ± 19	25 ± 1	17 ± 2	670 ± 27	361 ± 29	44 ± 6	22 ± 4

Each value is a mean ± S.E. of 6 experiments.

TABLE 22

ATPase ACTIVITY OF SUBCELLULAR FRACTIONS OF CONTROL OR MYOPATHIC HAMSTER (UM-X7.1) HEARTS AT LATE STAGES OF HEART FAILURE

	ATPase activity (μmoles Pi/mg/min)		
	Mitochondria	Microsomes	
	Total	Total	Ca^{++} - stimulated
Control	2.10 ± 0.13	2.32 ± 0.19	0.96 ± 0.14
Myopathic	2.00 ± 0.22	2.42 ± 0.21	0.93 ± 0.18

Each value is a mean ± S.E. of 5 - 6 experiments.

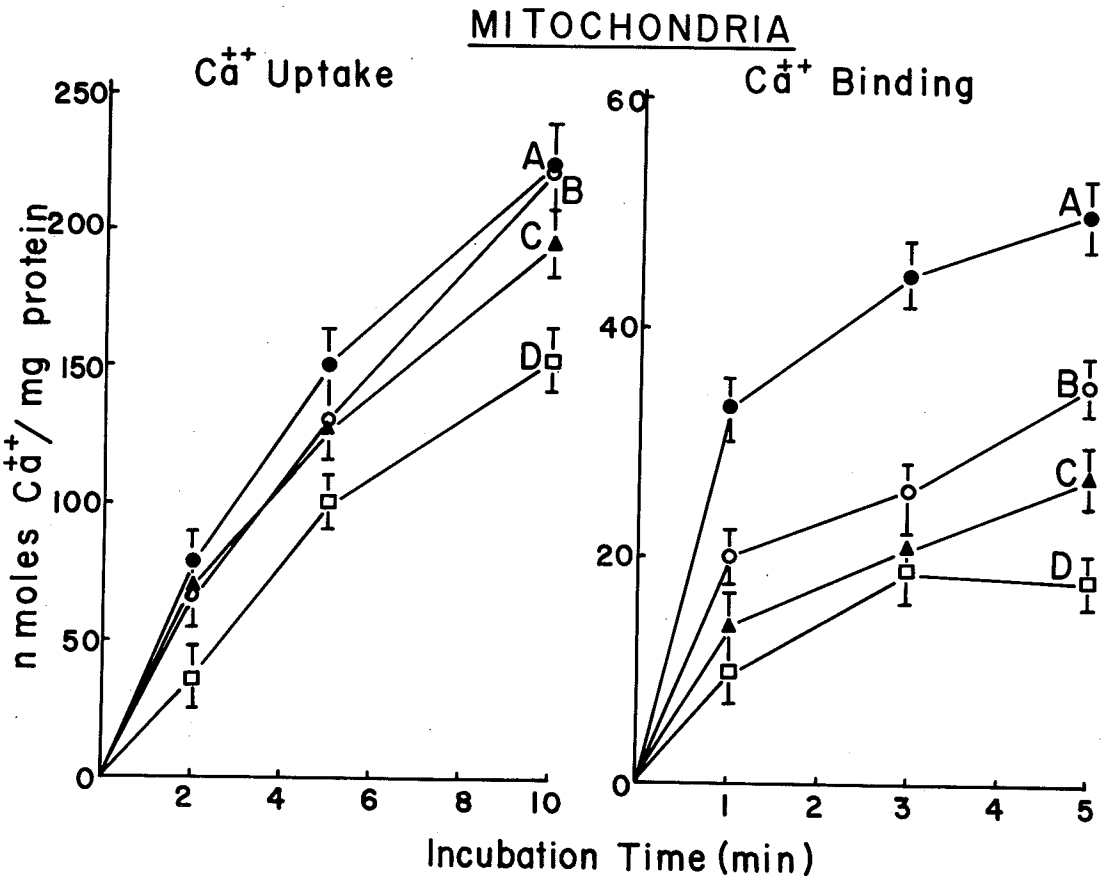

FIGURE 16. Time-course of calcium uptake and binding by mitochondrial fraction of control and myopathic hamster (UM -X7.1) hearts at different stages of heart failure. A - control healthy hamsters (150 - 200 day old); B - Myopathic hamsters at early stage of failure (150 day old); C - Myopathic hamsters at moderate stage of failure (175 day old); D - Myopathic hamsters at severe stage of failure (200 day old). Each value is a mean $\pm$ S.E. of 6 - 8 experiments.

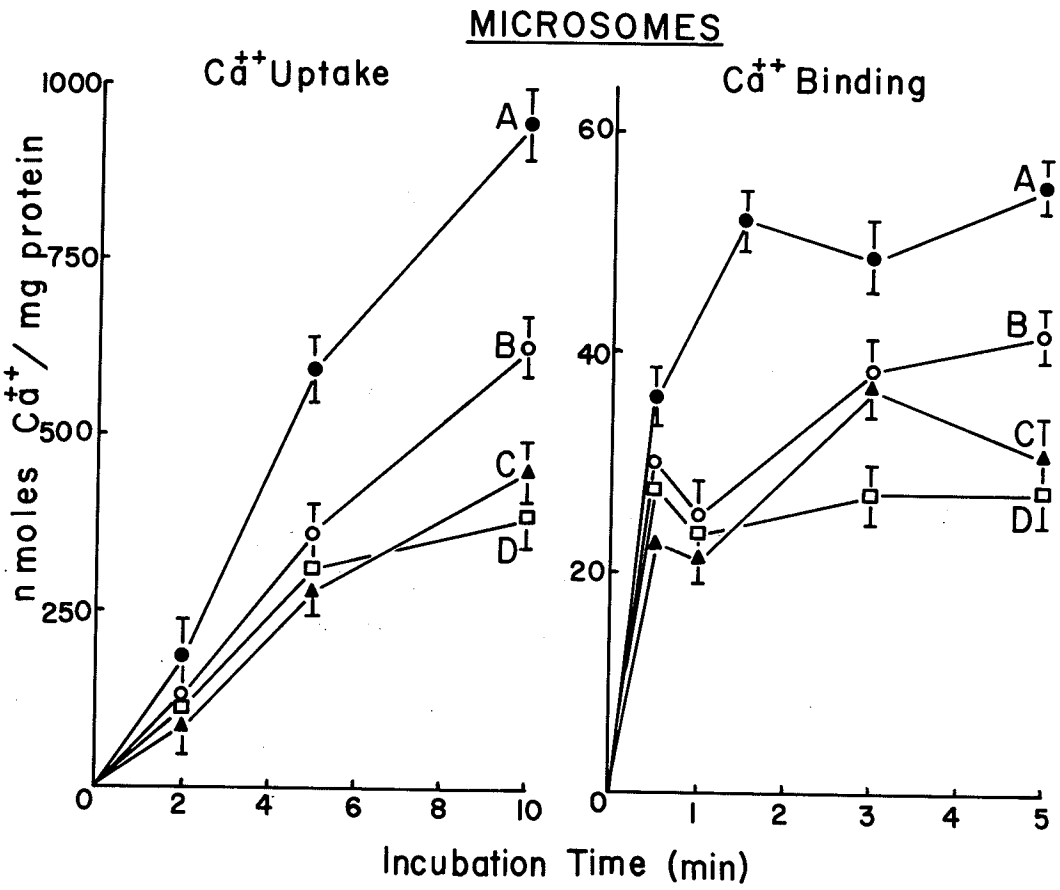

FIGURE 17. Time-course of calcium uptake and binding by microsomal fraction of control and myopathic hamster (UM -X7.1) hearts at different stages of heart failure. A - Control healthy hamsters (150 - 200 day old); B - Myopathic hamsters at early stage of failure (150 day old); C - Myopathic hamsters at moderate stage of failure (175 day old); D - Myopathic hamsters at severe stage of failure (200 day old). Each value is a mean ± S.E. of 6 - 8 experiments.

TABLE 23

CALCIUM ACCUMULATION BY SUBCELLULAR FRACTIONS OF HEARTS AT VARYING DEGREES OF CONGESTIVE HEART FAILURE IN GENETICALLY MYOPATHIC HAMSTERS (BIO 14.6)

Stages of failure	Calcium accumulation (% of control)			
	Mitochondria		Microsomes	
	Binding	Uptake	Binding	Uptake
Early stage (150 - 170 day old)	83 ± 4.9	80 ± 5.2	74 ± 4.2	97 ± 3.1
Moderate stage (200 - 230 day old)	50 ± 6.1	56 ± 4.7	50 ± 2.9	93 ± 4.2
Late stage (260 - 280 day old)	33 ± 3.4	30 ± 2.2	47 ± 1.8	67 ± 3.9

Each value is a mean ± S.E. of 6 - 8 experiments. Normal healthy hamsters of the respective age group served as controls.

was considered of interest to investigate the abilities of mitochondrial and microsomal fractions of hearts in which focal necrosis was induced experimentally. In an earlier study from this laboratory it has been demonstrated that oxidized isoproterenol, but not the fresh isoproterenol, produces focal necrosis in isolated perfused rat hearts (150). Perfusing the heart with oxidized isoproterenol did not decrease calcium uptake by mitochondria significantly ($p > 0.05$), whereas microsomal calcium uptake was markedly reduced ($p < 0.05$). No significant ($p > 0.05$) changes in mitochondrial or microsomal calcium binding were seen in hearts perfused with oxidized isoproterenol (Table 24). The results for calcium transport by subcellular fractions of hearts perfused with fresh isoproterenol were not different ($p > 0.05$) from the control. Oxidized isoproterenol was also found to decrease mitochondrial and microsomal calcium uptake when added in incubation medium without signficantly ($p > 0.05$) altering mitochondrial calcium binding (Table 25). Although microsomal calcium binding was increased when the incubation medium contained oxidized isoproterenol, the results were not significantly ($p > 0.05$) different from the control. The mitochondrial and microsomal ATPase activities were also not altered ($p > 0.05$) by the oxidized isoproterenol under both in vivo and in vitro conditions (Table 26).

TABLE 24

CALCIUM ACCUMULATION BY SUBCELLULAR FRACTIONS OF HEARTS PERFUSED WITH MEDIUM CONTAINING OXIDIZED ISOPROTERENOL (100 mg/l) FOR DIFFERENT INTERVALS

	Calcium accumulation (nmoles/mg protein)			
	Uptake/10 min		Binding/5 min	
Conditions	Mitochondria	Microsomes	Mitochondria	Microsomes
Control	222 ± 13	253 ± 16	104 ± 19	23 ± 5
Oxidized isoproterenol				
3 min	205 ± 15	169 ± 19	92 ± 9	19 ± 7
5 min	190 ± 9	155 ± 14	89 ± 11	28 ± 5
10 min	202 ± 10	123 ± 17	96 ± 12	24 ± 6

Each value is a mean ± S.E. of 6 experiments.

TABLE 25

IN VITRO EFFECT OF OXIDIZED ISOPROTERENOL ON CALCIUM ACCUMULATION BY SUBCELLULAR FRACTIONS OF RAT HEARTS

	Calcium accumulation (nmoles/mg protein)			
	Uptake /10 min		Binding /5 min	
Conditions	Mitochondria	Microsomes	Mitochondria	Microsomes
Control	238 ± 10	240 ± 16	103 ± 8	29 ± 5
Oxidized isoproterenol				
100 µg/ml	179 ± 12	159 ± 11	100 ± 11	40 ± 6
200 µg/ml	108 ± 13	122 ± 15	101 ± 10	41 ± 9

Each value is a mean ± S.E. of 6 experiments.

TABLE 26

IN VITRO AND IN VIVO EFFECTS OF OXIDIZED ISOPROTERENOL ON ATPase ACTIVITY OF SUBCELLULAR FRACTIONS OF HEARTS

	ATPase Activity (μmoles Pi/mg protein/min)		
	Mitochondria	Microsomes	
	Total	Total	Ca^{++} stimulated
A. In vivo (10 min perfusion)			
Control	2.80 ± 0.32	3.41 ± 0.26	0.29 ± 0.04
Oxidized Isoproterenol (100 mg/l)	2.78 ± 0.29	3.41 ± 0.21	0.27 ± 0.03
B. In vitro			
Control	2.93 ± 0.18	3.40 ± 0.19	0.31 ± 0.05
Oxidized Isoproterenol			
100 μg/ml	2.88 ± 0.20	3.35 ± 0.21	0.29 ± 0.05
200 μg/ml	2.90 ± 0.21	3.37 ± 0.15	0.30 ± 0.03

Each value is a mean ± S.E. of 5 - 6 experiments.

V. DISCUSSION

A. Hearts failing Due to Oxygen or Substrate Lack:

In the present study we have demonstrated that the ability of the microsomal fraction to bind and accumulate calcium was impaired upon perfusing the hearts with hypoxic medium for a period of 10 minutes or longer. On the other hand, mitochondrial calcium binding, but not the uptake, decreased in hearts perfused with hypoxic medium for 20 to 30 minutes. These changes in microsomal calcium binding and uptake as well as mitochondrial calcium binding in hypoxic hearts are unlikely to be due to the presence of some contaminants because both control and hypoxic preparations were made under identical conditions. Furthermore, both azide and oligomycin inhibited calcium binding by the control and hypoxic mitochondrial preparations to an equal extent without appreciably affecting the microsomal calcium binding. Electron microscopic examination of the microsomal pellets did not reveal the presence of myofibrils or intact mitochondria. In addition, the yields of the subcellular fractions from the hypoxic hearts were not different from those of the control.

In contrast to the hypoxic hearts, electrically paced hearts made to fail to an equal degree by perfusion with substrate-free medium showed a decline in mitochondrial calcium uptake and more dramatic decrease in the microsomal calcium binding. This depression in microsomal calcium binding by hypoxic and substrate-depleted heart preparations was also apparent at different pH and calcium concentrations of the incubation medium. It should be noted that calcium transport by subcellular fractions has been shown to be impaired in spontaneously beating hearts on

perfusion with substrate-depleted medium (12,26,93). Both mitochondrial and microsomal calcium stores in hearts failing due to substrate and oxygen lacks have also been shown to decrease (139). However, in this study it has been demonstrated that omission of glucose from the perfusion medium accelerated the inhibitory effects of hypoxia on calcium transport by subcellular fractions. In this regard, it is pointed out that microsomal calcium binding and mitochondrial calcium uptake have been reported to be reduced in chronically ischemic dog hearts (130). Furthermore, depression in calcium binding (89) or calcium uptake (88) has been observed in acute ischemic dog hearts after 60 to 90 minutes of coronary occlusion. Therefore, it is likely that, in conditions such as ischemic heart disease and myocardial infarction, changes in the calcium transporting abilities of the subcellular organelles are occurring due to limited supply of both oxygen and substrate.

Depression in the abilities of subcellular organelles of the hypoxic heart to bind and accumulate calcium would raise the intracellular level of ionized calcium. These events will be associated with an increase in the relaxation time as well as contracture of the hypoxic myocardium. This is exactly what has been shown in rat hearts upon perfusion with hypoxic medium (69,151). At any rate, the results of the present study demonstrate that changes in the abilities of the subcellular fractions are reversible upon reperfusing the hypoxic hearts with aerobic medium. Furthermore, reduction in the ability of the hypoxic microsomal fraction to accumulate calcium may be due to a decrease in the activity of Ca^{++} -stimulated ATPase, which has been considered to provide energy for the transport of calcium across the microsomal membranes. Although the mechanisms for depression in calcium binding by

hypoxic heart mitochondria are not clear, it is possible that this defect, like that in the microsomal vesicles, may be a reflection of changes in the membrane properties. This is consistent with an earlier suggestion that hypoxia may induce membrane alteration by changing the phospholipid-protein composition (98,152). Changes in in the phospholipid composition of the myocardium due to hypoxia has been reported recently (153).

Impaired abilities of the subcellular particles to accumulate calcium would result in decreased calcium available for release from the intracellular stores upon activation of the hypoxic heart. This mechanism may explain the depression in the ability of the hypoxic heart to generate contractile force. However, it was found that perfusing hearts with hypoxic medium for 5 minutes decreased contractile force by about 40% of the control without any changes in the calcium binding and uptake abilities of mitochondrial and microsomal fractions. This could be interpreted to suggest that changes in subcellular particles to transport calcium due to alterations in their membrane properties are secondary to depression in the contractile force of the hypoxic hearts. It could also be possible that the subcellular particles of the hypoxic heart during initial phases of failure may recover during the isolation and incubation periods. At any rate, hypoxia has also been shown to decrease sarcolemmal calcium binding and calcium uptake in addition to altering the sarcolemmal enzyme activities (98,138,152). Furthermore, abnormal concentrations of different cytoplasmic constituents, in addition to structural damage, have also been suggested to influence calcium transport in failing hearts due to hypoxia (25,93,131). In view of dramatic changes in energy metabolism due to hypoxia (69,151,154-157), the onset of failure

of the hypoxic heart to generate contractile force has been thought to be most likely due to an insufficiency in the processes of energy generation, whereas abnormalities in the processes of energy utilization subserving the mechanisms for maintaining the ionic gradients and excitation-contraction and relaxation are secondary in nature (69). Thus the present study demonstrating defects in the abilities of hypoxic heart mitochondrial and microsomal fractions to transport calcium can be considered to be only one of the various abnormalities associated with failing hearts due to hypoxia.

B. Hearts Failing Due to Intracellular Calcium Deficiency and Calcium Overload:

In the present study we have demonstrated a decrease in calcium binding and uptake by the mitochondrial fraction without any changes in the ability of the microsomal fraction to transport calcium on perfusing the hearts with Ca^{++} -free medium. However, hearts failed to generate contractile force within 30 seconds to 1 minute upon perfusion with Ca^{++} -free medium without any change in the abilities of the mitochondrial and microsomal fractions to bind and accumulate calcium. It has been shown that cessation of contractile activity of the heart on perfusion with Ca^{++} -free medium occurred in the absence of any change in the surface electrical activity (137,158,159). Recently, uncoupling of the electrical and mechanical events has been reported to be due to removal of a labile calcium component occurring within 30 seconds of Ca^{++} -free perfusion (136) and the decline in the contractile force can be readily understood in terms of membrane calcium fluxes (160,161). Thus it appears that the oberved reduction in the mitochondrial calcium binding and uptake is secondary to heart failure due to Ca^{++} -free perfusion. It is pointed out that

perfusion with Ca^{++} -free medium has been shown to produce intracellular calcium deficiency in the rat heart muscle (137,138).

Prolonged perfusion of hearts with Ca^{++} -free medium has been shown to produce dramatic changes in the ultrastructure of mitochondria, sarcoplasmic reticulum and cell membrane (136). Therefore, it is possible that the observed changes in mitochondrial calcium transport may reflect alterations in the mitochondrial membrane, whereas our inability to detect changes in microsomal calcium transport can be explained on the basis that sarcoplasmic reticular membrane is more resistant to changes occurring as a consequence of Ca^{++}-free perfusion. It should be noted that a decrease in sarcolemmal Na^{+} -K^{+} ATPase, Mg^{++} -ATPase and adenylate cyclase activities has also been reported on perfusing the hearts with Ca^{++}-free medium (138). These results are consistent with the view that calcium plays an important role in maintenance of membrane integrity.

It was interesting to find that the ability of the microsomal fraction to bind and accumulate calcium was markedly decreased upon reperfusing the hearts following perfusion with Ca^{++} -free medium for 5 minutes or longer. Furthermore, results of the present study show that mitochondrial calcium binding and uptake were markedly increased upon reperfusing the Ca^{++} -deprived hearts. Inability of the Ca^{++} -deprived hearts to recover contractile force has also been shown to be accompanied by extensive damage to the ultrastructure of the myocardium (133-135,137). Recent studies from this laboratory have revealed a marked decrease in sarcolemmal Na^{+} -K^{+}-ATPase, Ca^{++}-ATPase, Mg^{++} -ATPase and adenylate cyclase activities (138) upon repurfusing the Ca^{++} -deprived hearts. These results suggest that

reperfusion of Ca^{++} -deprived hearts causes alterations in various cardiac membrane systems.

Perfusion of Ca^{++} -deprived hearts with control medium has been reported to produce a marked increase in the intracellular calcium contents (138). Calcium overload in the cell has been suggested to result in disruption of the cellular structures by lowering the energy state of the myocardium (137). It can be conceived that the intracellular calcium overload will enhance the splitting of the high energy phosphate compunds as well as depress energy production by inhibiting mitochondrial functions. Both creatine phosphate and ATP contents of Ca^{++} -deprived hearts have been found to decrease upon reperfusion with control medium (Dhalla- unpublished observation). Thus it appears that intracellular calcium overload affects different biochemical events involved in the maintenance of cardiac structure and function and the inability of the Ca^{++} -deprived hearts to recover upon reperfusion may be a consequence of these complex metabolic changes.

The depression in microsomal calcium transport and augmentation in mitochondrial calcium transport due to intracellular calcium overload in Ca^{++} - deprived hearts was seen at different pH and calcium concentrations of the incubation medium. Although the observed decrease in microsomal calcium uptake may be explained on the basis of depression in the microsomal Ca^{++} -stimulated ATPase activity, the mechanisms for the observed increase in mitochondrial calcium transport due to intracellular calcium overload are not clear. It is possible that mitochondrial membranes are altered in such a manner that these organelles serve as an adaptive mechanism under conditions of intracellular calcium overload. However, it is of interest

to note that lowering the concentration of sodium in the Ca^{++} -free medium, not only prevented depression in microsomal calcium transport or augmentation in mitochondrial calcium transport, but also enabled the Ca^{++} -deprived heart to recover contractile force upon reperfusion with control medium. Lowering the concentration of sodium in the medium during Ca^{++} -free perfusion has been suggested to prevent intracellular calcium overload upon reperfusion (137). These results indicate that intracellular calcium overload may produce alterations in various cardiac membrane systems and lend further support to the hypothesis that intracellular calcium overload may play an important role in the pathogenesis of heart failure.

It has been shown in the present study that both microsomal calcium binding and uptake are depressed upon perfusing the hearts with Na^{+} -free or K^{+} -free medium. The microsomal Ca^{++} -ATPase activity was also found to decrease upon Na^{+} -free or K^{+} -free perfusion for 20 minutes. These changes in the microsomal calcium pump mechanisms in hearts perfused with Na^{+} -free or K^{+} -free medium may be due to intracellular calcium overload. This is borne out by a recent study from this laboratory that calcium contents of the hearts were increased upon perfusion with Na^{+} -free or K^{+} -free medium (138). If intracellular calcium overload is an important mechanism for depressing the microsomal "calcium pump" in hearts perfused with Na^{+} -free and K^{+} -free medium, then alterations similar to those observed in sarcoplasmic reticulum should be apparent in other membrane systems such as mitochondria and sarcolemma upon reperfusing the Ca^{++} -deprived hearts. In an earlier study changes in sarcolemma have been shown to occur in hearts on perfusion with Na^{+} -free or K^{+} -free medium (138). The results of this study

clearly indicate that mitochondrial calcium binding and uptake were increased in hearts perfused with K^+ -free medium. Feeding animals with low potassium diet has also been shown to decrease microsomal calcium transport and to increase mitochondrial calcium transport in the heart (162).

Although both mitochondrial calcium binding and uptake tended to increase in hearts upon perfusion with Na^+ -free medium, the results were not significant. It is possible that in the case of Na^+ -free perfusion, intracellular Na^+ -deficiency may be preventing mitochondrial membrane changes in response to intracellular calcium overload. It should be noted that while hearts perfused for 10 minutes with Na^+ -free medium regained contractile force by about 60% of the intitial value, the hearts perfused for 10 minutes with K^+ -free medium completely failed to recover contractile activity upon reperfusion. Such differences may be due to differences in the intracellular levels of sodium (138) since this cation has been suggested to play a deleterious role in heart failure due to intracellular calcium overload (137).

C. Myopathic Failing Hearts:

Previous studies from this laboratory have revealed that microsomal and mitochondrial calcium binding are decreased in myopathic hamster (BIO 14.6) hearts at moderate and late stages of heart failure (28,118,142). In addition, it has been reported that mitochondrial calcium uptake is decreased in these myopathic hearts at moderate and late stages of failure, whereas microsomal calcium uptake is reduced in late stages only. The present findings with BIO-14.6 myopathic hamsters confirm the earlier results and further demonstrate that microsomal calcium binding, but not

uptake, as well as mitochondrial calcium binding and uptake are impaired in BIO-14.6 myopathic hamster hearts at early stages of failure. Other investigators have also shown varying degrees of depression in calcium transport by the mitochondrial and microsomal fractions of failing hearts from these animals (111,112,116,117,163). The defective calcium transport by these subcellular fractions of myopathic hearts has been reported to be due to changes in their cholesterol-phospholipid compositions (119,163).

In the present study we have shown that microsomal calcium binding and uptake as well as mitochondrial calcium binding in a new strain of myopathic hamster (UM-X7.1) hearts were decreased in early, moderate and severe stages of heart failure. On the other hand, mitochondrial calcium uptake did not decrease in early and moderate stages of heart failure, although it was depressed in severe stages. The impairment in calcium transport by both mitochondrial and microsomal fractions of the myopathic hearts at severe stages of failure was seen at different pH of the incubation medium and was not associated with any alteration in ATPase activities of these subcellular fractions. Such a defect in calcium transport can be explained on the basis of uncoupling of "calcium pump" mechanisms due to disorganization of the intracellular membranes (26).

The study of kinetics of calcium binding by the microsomal fractions of control and myopathic hearts revealed that the number of binding sites, but not the binding constant, was reduced in the failing hearts. Furthermore, the data on marker enzyemes and yields of subcellular fractions indicate that the impairment of the myopathic hearts is not due to the presence of some contaminants. This is

strengthened by the experiments which showed no difference in mitochondrial or microsomal calcium binding when the fractions were isolated from the myopathic heart homogenates in the absence and presence of different exogenous membranes. Oligomycin and sodium azide, which inhibited mitochondrial calcium binding in control and myopathic preparations to an equal degree, had no appreciable effect on the microsomal calcium binding. Therefore, it seems clear that the defect in calcium transport by the myopathic subcellular fractions is real and may be of crucial importance in the pathogenesis of heart failure in UM-X7.1 strain of myopathic hamsters.

Although morphologic studies using BIO-14.6 and UM-X7.1 strains of myopathic hamsters did not reveal any difference in the expressions of myopathy due to genetic variation (140), the biochemical data indicate some differences in the failing hearts of these animals. For instance, it was found that mitochondrial calcium uptake in the UM-X7.1 myopathic hearts unlike the BIO-14.6 myopathic hearts, at early and moderate stages of failure was not different from the control. On the other hand, microsomal calcium uptake in the BIO-14.6 myopathic hearts, unlike the UM-X7.1 myopathic hearts, at early and moderate stages of failure was not different from the control. These observations are not surprising because studies from this laboratory have indicated that sarcolemmal Na^{+}-K^{+} ATPase, Mg^{++}-ATPase and Ca^{++}-ATPase are decreased in UM-X7.1 myopathic hearts but increased in BIO-14.6 myopathic hearts. Thus, it is apparent that there are differences in the biochemical functions of cardiac membrane systems in these two types of myopathic hamsters, which are probably due to genetic differences.

Since heart failure in myopathic hamsters is preceded by varying degrees of focal necrosis it is possible that changes in calcium transport by subcellular particles of the myopathic hearts are caused by the same process which causes these focal lesions. Although oxidized isoproterenol (150) has been shown to decrease mitochondrial and microsomal calcium uptake under in vitro conditions, this necrotizing agent had no affect on calcium binding by these membranes. Furthermore, perfusing the hearts with oxidized isoproterenol decreased microsomal calcium uptake without significantly affecting mitochondrial calcium uptake or mitochondrial and microsomal calcium binding. Microsomal calcium transport, but not mitochondrial calcium transport, has also been reported to decrease in isoproterenol-induced myocardial necrosis under in vivo conditions (132). Therefore it seems that the pattern of changes in the calcium transport system in the myopathic hamster hearts is quite different from that of the experimentally induced myocardial necrosis Clearly, further experiments are needed to establish the cause-effect relationship between myocardial necrosis and changes in calcium transport by subcellular fractions of the myopathic hearts.

In view of the preventive effects of a calcium antagonistic compound, isoptin, in the development of myocardial lesions and heart failure in myopathic hamsters (140), it is possible that the observed changes in calcium transport by subcellular fractions of the myopathic hearts are due to intracellular calcium overload. However, the pattern of changes in calcium transport by subcellular particles of the myopathic hamster heart is different from that obtained in calcium-overloaded hearts produced under an acute experimental condition by reperfusing the Ca^{++} -

deprived hearts as well as by perfusing with Na^+ -free or K^+ -free medium. Such a difference in the pattern of changes in calcium transport systems may be a reflection of the degree and type of heart failure.

VI. CONCLUSIONS

In this study calcium transporting abilities of the mitochondrial and microsomal fractions of different types of failing hearts were examined. From the results the following conclusions are drawn:

1. Although microsomal calcium binding and uptake were decreased upon perfusing rat hearts with hypoxic medium, these changes appear secondary to the decline in the ability of these hearts to generate contractile force.

2. Omission of glucose from the perfusion medium accelerated the inhibitory effects of hypoxia upon microsomal calcium binding and uptake as well as mitochondrial calcium binding. In contrast to the hypoxic hearts, hearts failing due to substrate-lack were found to show a decrease in mitochondrial calcium uptake.

3. Intracellular calcium deficiency produced by perfusing rat hearts with Ca^{++}-free medium was associated with a decline in mitochondrial calcium binding and uptake without any changes in microsomal calcium transport.

4. Intracellular calcium overload produced by reperfusing Ca^{++}-deprived hearts was shown to result in reducing microsomal calcium transport and augmenting mitochondrial calcium transport.

5. Although microsomal calcium binding and uptake also decreased on producing intracellular calcium overload upon perfusing hearts with Na^{+}-free or K^{+}-free medium, a significant increase in mitochondrial calcium binding and uptake was demonstrated only in hearts perfused with K^{+}-free medium.

6. Calcium binding by both mitochondrial and microsomal fractions was decreased in early, moderate and severe stages of heart failure in two types of myopathic

hamsters (UM-X7.1 and BIO 14.6); however, depression in mitochondrial calcium uptake in the UM-X7.1 strain of animals and depression in microsomal calcium uptake in the BIO 14.6 strain of animals occurred in late stages of heart failure only.

7. Myocardial necrosis due to oxidized isoproterenol in the isolated rat heart was found to be associated with a marked depression in microsomal calcium uptake without a significant decrease in mitochondrial calcium uptake.

8. It is suggested that depression in microsomal calcium transport, depending upon the degree and type of failing heart, plays an important role in the pathogenesis of heart failure, whereas mitochondrial calcium transport may increase or decrease depending upon whether or not mitochondria are serving as an adaptive mechanism for regulating intracellular calcium.

REFERENCES

1. Weber, A. 1966. Energized calcium transport and relaxing factors. In: Current Topics in Bioenergetics (D.R. Sanadi, ed.) Vol. I, pp. 203-254, Academic Press, New York.

2. Ebashi, S. and M. Endo. 1968. Calcium ion and muscle contraction. Prog. Biophys. Mol. Biol. 18:123-183.

3. Winegrad, S. 1968. Intracellular calcium movements of frog skeletal muscle during recovery from tetanus. J. Gen. Physiol. 51:65-83.

4. Winegrad, S. 1970. The intracellular site of calcium activation of contraction in frog skeletal muscle. J. Gen. Physiol. 55:77-88.

5. Fuchs, F. and F.N. Briggs. 1968. The site of calcium binding in relation to the activation of myofibrillar contraction. J. Gen. Physiol. 51:655-676.

6. Nayler, W.G. 1963. The significance of calcium ions in cardiac excitation and contraction. Am. Heart J. 65:404-411.

7. Sandow, A. 1965. Excitation-contraction coupling in skeletal muscle. Pharmacol. Rev. 17:265-320.

8. Slater, E.C. and K.W. Cleland. 1953. The effect of calcium on the respiratory and phosphorylative activities of heart muscle sarcosomes. Biochem. J. 55:566-580.

9. Lehninger, A.L. 1962. Water uptake and extrusion by mitochondria in relation to oxidative phosphorylation. Physiol. Rev. 43:467-517.

10. Sanadi, D.R. 1965. Energy-linked reactions in mitochondria. Ann. Rev. Biochem. 34:21-48.

11. Chance, B. 1965. The energy-linked reaction of calcium with mitochondria. J. Biol. Chem. 240:2729-2748.

12. Dhalla, N.S. 1969. Excitation-contraction coupling in heart I. Comparison of calcium uptake by the sarcoplasmic reticulum and mitochondria of the rat heart. Arch. Int. Physiol. Biochem. 77:916-934.

13. Haugaard, N., E.S. Haugaard, N.H. Lee and R.S. Horn. 1969. Possible role of mitochondria in regulating cardiac contractility. Fed. Proc. 28:1657-1662.

14. Sulakhe, P.V. and N.S. Dhalla. 1970. Excitation-contraction coupling in heart IV. Energy-dependent calcium transport in the myocardium of the developing rat. Life. Sci. 9:1363-1370.

15. Fanburg, B. 1964. Calcium in the regulation of heart muscle contraction and relaxation. Fed. Proc. 23:922-955.

16. Fanburg, B. and J. Gergely. 1965. Studies on adenosine triphosphate-supported calcium accumulation by cardiac subcellular particles. J. Biol. Chem. 240:2721-2728.

17. Brierley, G.P., E. Murer and E. Bachman. 1964. Studies on ion transport. III. The accumulation of calcium and inorganic phosphate by heart mitochondria. Arch. Biochem. Biophys. 105:89-102.

18. Ueba, Y., Y. Ito and C.A. Chidsey. 1971. Intracellular calcium and myocardial contractility. I. The influence of extracellular calcium. Am. J. Physiol. 220:1553-1557.

19. Dhalla, N.S., D.B. McNamara and P.V. Sulakhe. 1970. Excitation-contraction coupling in heart. V. Contribution of mitochondria and sarcoplasmic reticulum in the regulation of calcium concentration in the heart. Cardiol. 55:178-191.

21. Horn, R.S., A. Fyhn and N. Haugaard. 1971. Mitochondrial calcium uptake in the perfused contracting rat heart and the influence of epinephrine on calcium exchange. Biochem. Biophys. Acta. 226:459-466.

22. Nayler, W.G. and J.R. Hasker. 1966. Effect of caffeine on calcium in subcellular fractions of cardiac muscle. Am. J. Physiol. 211:950-954.

23. Harigaya, S. and A. Schwartz. 1969. Rate of calcium binding and uptake in normal animal and failing human cardiac muscle. Circ. Res. 25:781-794.

24. Martonosi, A. and R. Feretos. 1964. Sarcoplasmic reticulum. I. The Uptake of Calcium by sarcoplasmic reticulum fragments. J. Biol. Chem. 239:648-658.

25. Sulakhe, P.V., D.B. McNamara and N.S. Dhalla. 1971. Excitation-contraction coupling in heart. VIII. Influence of adenine nucleotides on calcium binding by subcellular fractions of rat heart. J. Biochem. 70:571-580.

26. Muir, J.R., N.S. Dhalla, J.M. Orteza and R.E. Olson. 1970. Energy linked calcium transport in subcellular fractions of the failing rat heart. Circ. Res. 26:429-438.

27. Suko, J., J.H.K. Vogel and C.A. Chidsey. 1970. Intracellular calcium and myocardial contractility. III. Reduced calcium uptake and ATPase of the sarcoplasmic reticular fraction prepared from chronically failing calf hearts. Circ. Res. 27:235-237.

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

29. Gloster, J. and P. Harris. 1969. The lipid composition of mitochondrial and microsomal fractions of human myocardial homogenates. Cardiovasc. Res. 3:45-51.

30. Gloster, J. and P. Harris. 1970. The lipid composition of mitochondrial and microsomal fractions from human ventricular myocardium. J. Mol. Cell Cardiol. 1:459-465.

31. Inesi, G., S. Ebashi and S. Watanabe. 1964. Preparation of vesicular relaxing factor from bovine heart tissue. Am. J. Physiol. 207:1339-1344.

32. Pretorius, P.J., W.G. Pohl, C.S. Smithen and G. Inesi. 1969. Structural and functional characterization of dog heart microsomes. Circ. Res. 25:487-499.

33. Carsten, M.E. 1967. Cardiac sarcotubular vesicles: Effects of ion, ouabain and acetyl strophanthidin. Circ. Res. 20:599-605.

34. Katz, A.M. and D.I. Repke. 1967. Na^{+} and K^{+} sensitivity of Ca^{++} uptake and Ca^{++} binding by dog cardiac microsomes. Circ. Res. 21:767-775.

35. Katz, A.M. and D.I. Repke. 1967. Quantitative aspects of dog cardiac microsomal calcium binding and calcium uptake. Circ. Res. 21:153-162.

36. Katz, A.M., D.I. Repke, J.E. Upshaw and M.A. Polascik. 1970. Characterization of dog cardiac microsomes: Use of zonal centrifugation to fractionate sarcoplasmic reticulum (Na^{+} -K^{+})-activated ATPase and mitochondrial fragments. Biochem. Biophys. Acta. 205:473-490.

37. Namm, D.H., E.L. Woods, and J.L. Zucker. 1972. Incorporation of the terminal phosphate of ATP into membranous protein of rabbit cardiac sarcoplasmic reticulum. Correlation with active calcium transport and study of the effect of cyclic AMP. Circ. Res. 31:308-316.

38. Streter, F., N. Ikemoto and J. Gergely. 1970. The Effect of lyophilization and dithiothreitol on vesicles of skeletal and cardiac muscle sarcoplasmic reticulum. Biochem. Biophys. Acta. 203:354-357.

39. McCollum, W.B., H.R. Besch, Jr., M.L. Entman and A. Schwartz. 1972. Apparent initial binding rate of calcium by canine cardiac relaxing system. Am. J. Physiol. 223:608-614.

40. Palmer, R.F. and V.A. Posey. 1967. Ion effects on calcium accumulation by cardiac sarcoplasmic reticulum. J. Gen. Physiol. 50:2085-2096.

41. Dransfeld, H., K. Greff, A. Schon and B.T. Ting. 1969. Calcium uptake in mitochondria and vesicles of heart and skeletal muscle in presence of potassium, sodium, k-strophanthin and pentobarbital. Biochem. Pharmacol. 18:1735-1345.

42. Lee, K.S. and W. Klaus. 1971. The subcellular basis for the mechanism of inotropic action of cardiac glycosides. Pharmacol. Rev. 23:193-261.

43. Kones, R.J. 1973. Molecular and ionic basis of altered myocardial contractility. Res. Comm. Chem. Path. Pharmacol. 5(suppl. I):1-84.

44. Martonosi, A. 1972. Biochemical and clinical aspects of sarcoplasmic reticulum function. In: Current Topics in Membranes and Transport. (C.F. Bonner and A. Kleinzeller, eds.) Vol. 3, pp. 83-197, Academic Press, New York.

45. Kirchberger, M.A., M. Tada, D.T. Repke and A.M. Katz. 1972. Cyclic adenosine 3',5'- monophosphate-dependent protein kinase stimulation of calcium uptake by canine cardiac microsomes. J. Mol. Cell Cardiol. 4:673-680.

46. Katz, A.M. and D.I. Repke. 1973. Calcium-membrane interaction in the myocardium: Effects of ouabain, epinephrine and 3', 5'-cyclic adenosine monophosphate. Am. J. Cardiol. 31:193-201.

47. Wray, H.L., R.R. Gray and R.A. Olson. 1973. Cyclic adenosine 3', 5'-monophosphate-stimulated protein kinase and a substrate associated with cardiac sarcoplasmic reticulum. J. Biol. Chem. 248:1496-1498.

48. Stam, A.C., Jr., W.B. Weglicki, Jr., D. Feldman, J.C. Shelburne and E.H. Sonnenblick. 1970. Canine myocardial sarcolemma -- Its preparation and enzymatic activity. J. Mol. Cell Cardiol. 1:117-130

49. Sulakhe, P.V. and N.S. Dhalla. 1971. Excitation-contraction coupling in heart. VI. Demonstration of calcium activated ATPase in the dog heart sarcolemma. Life. Sci. 10:185-191.

50. Kidwai, A.M., M.A. Radcliffe, G.Duchon and E.E. Daniel. 1971. Isolation of plasma membrane from cardiac muscle. Biochem. Biophys. Res. Comm. 45:901-910.

51. Dietz, G. and K.D. Hepp. 1971. Calcium stimulated ATPase of cardiac sarcolemma. Biochem. Biophys. Res. Comm. 44:1041-1049.

52. Tada, M., J.O. Finney, M.H. Swartz and A.M. Katz. 1972. Preparation and properties of plasma membranes from guinea pig hearts. J. Mol. Cell Cardiology. 4:417-426.

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

54. Nakamaru, Y. and A. Schwartz. 1970. Possible control of intracellular calcium metabolism by (H^+). Sarcoplasmic reticulum of skeletal and cardiac muscle. Biochem. Biophys. Res. Comm. 41:830-836.

55. Entman, M.L., P.C. Gillette, E.T. Wallick, B.C. Pressman and A. Schwartz. 1972. A study of calcium binding and uptake by isolated cardiac sarcoplasmic reticulum: The use of a new ionophore (X537A). Biochem. Biophys. Res. Comm. 48:847-857.

56. Entman, M.L., J.C. Allen, E.P. Bornet, P.C. Gillette, E.T. Wallick and A. Schwartz. 1972. Mechanisms of calcium accumulation and transport in cardiac relaxing system (sarcoplasmic reticulum membranes): Effects of verapamil, D-600, X537A and A23187. J. Mol. Cell Cardiol. 4:681-688.

57. Entman, M.L., E.P. Bornet and A. Schwartz. 1972. Phasic components of calcium binding and release by canine cardiac relaxing system (sarcoplasmic reticulum). J. Mol. Cell Cardiol. 4:155-169.

58. Olson, R.E. and W.B. Schwartz. 1951. Myocardiac metabolism in congestive heart failure. Medicine. 30:21-42.

59. Olson, R.E. 1956. Molecular events in cardiac failure. Am. J. Med. 20:159-162.

60. Fleckenstein, A., H.J. Doring and H. Kammermeier. 1967. Experimental heart failure due to inhibition of utilization of high energy phosphates. In: International Symposium on the Coronary Circulation and Energetics of the Myocardium, Milan, pp. 220-230, Karge, Basel and New York.

61. Opie, L.H. 1968. Metabolism of the heart in health and disease I. Am. Heart J. 76:685-698.

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

63. Opie, L.H. 1969. Metabolism of the heart in health and disease III. Am. Heart J. 77:383-410.

64. Argus, M.F., J.C. Arcos, V.M. Sardesai and J.L. Overby. 1964. Oxidative rates and phosphorylation in sarcosomes from experimentally induced failing rat heart. Proc. Soc. Exptl. Biol. Med. 117:380-383.

65. Lindenmayer, G.E., L.A. Sordahl and A. Schwartz. 1968. Reevaluation of oxidative phosphorylation in cardiac mitochondria from normal animals and animals in heart failure. Circ. Res. 23:439-450.

66. Schwartz, A. and K.S. Lee. 1962. Study of heart mitochondria in glycolytic metabolism in experimentally induced cardiac failure. Circ. Res. 10:321-332.

67. Wollenberger, A., B. Kleitke and G. Raabe. 1965. Some metabolic characteristics of mitochondria from chronically over-loaded hypertrophied hearts. Exptl. Mol. Path. 2:251-260.

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

69. Dhalla, N.S., J.C. Yates, D.A. Walz, V.A. McDonald and R.E. Olson. 1972. Correlation between changes in the endogenous energy stores and myocardial function due to hypoxia in the isolated perfused rat heart. Can. J. Physiol. Pharmacol. 50:333-345.

70. Furchgott, R.F. and J. De Gubareff. 1958. High energy phosphate content of cardiac muscle under various experimental conditions which alter contractile strength. J. Pharmacol. Exptl. Therap. 124:203-218.

71. Luchi, R.F., E.M. Kritcher and P.T. Thyrum. 1969. Reduced cardiac myosin adenosine-triphosphatase activity in dogs with spontaneously occurring heart failure. Circ. Res. 24:513-519.

72. Buckley, N.M. and K.K. Tsuobi. 1961. Cardiac nucleotides and derivatives in acute and chronic ventricular failure of the dog heart. Circ. Res. 9:618-625.

73. Chidsey, C.A., E.C. Weinbach, P.E. Pool and A.G. Morrow. 1966. Biochemical studies of energy production in the failing human heart. J. Clin. Invest. 45:40-50.

74. Olson, R.E. 1959. Myocardial metabolism in congestive heart failure. J. Chron. Dis. 9:442-464.

75. Olson, R.E. 1964. Abnormalities of myocardial metabolism. Circ. Res. 15(Suppl. II):109-117.

76. Plaut, G.W.E. and M.M. Gertler. 1959. Oxidative phosphorylation studies in normal and experimental congestive heart failure in guinea pig: A comparison. Ann. N.Y. Acad. Sci. 72:515-517.

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

78. Chidsey, C.A., E. Braunwald, A.G. Morrow and D.T. Mason. 1963. Myocardial norepinephrine concentrations in man: Effect of reserpine and congestive heart failure. New Eng. J. Med. 269:653-658.

79. Chidsey, C.A., G.A. Kaiser, E.H. Sonnenblick, J.F. Spann and E.J. Braunwald. 1964. Cardiac norepinephrine stores in experimental heart failure in the dog. J. Clin. Invest. 43:2386-2393.

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

81. Vogel, J.H.K., D. Jacobowitz and C.A. Chidsey. 1969. Distribution of norepinephrine in the failing bovine heart. Correlation of Chemical analysis and fluorescence microscopy. Circ. Res. 24:71-84.

82. Dhalla, N.S., K.J.R. Naidu, B. Bhagat and K. Cristensen. 1971. Biochemical basis of heart function I. Relation of catecholamine stores and contractile force in isolated rat heart. Cardiovasc. Res. 5:376-382.

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

84. Sandow, A. 1965. Excitation-contraction coupling in skeletal muscle. Pharmacol. Rev. 17:265-320.

85. Endman, K.A.P. 1965. Drugs and properties of heart muscle. Ann. Rev. Pharmacol. 5:99-118.

86. Briggs, F.N., E.W. Gertz and M.L. Hess. 1966. Calcium uptake by cardiac vesicles: Inhibition by amytal and reversal by ouabain. Biochem. J. 345:122-131.

87. Gertz, E.W., M.L. Hess, R.F. Lain and F.N. Briggs. 1967. Activity of the vesicular calcium pump in the spontaneously failing heart-lung preparation. Circ. Res. 20:477-484.

88. Lee, K.S., H. Ladinsky and J.H. Stuckey. 1967. Decreased Ca^{++}-uptake by sarcoplasmic reticulum after coronary artery occlusion for 60 and 90 minutes. Circ. Res. 21:439-444.

89. Nayler, W.G., J. Stone, V. Carson and D. Chipperfield. 1971. Effect of ischaemia on cardiac contractility and calcium exchange-ability. J. Mol. Cell Cardiol. 2:125-143.

90. Lindenmayer, G.E., L.A. Sordahl, S. Harrigaya, J.C. Allen, H.R. Besch and A. Schwartz. 1971. Some biochemical studies on subcellular systems isolated from fresh recipient human cardiac tissue obtained during transplantation. Am. J. Cardiol. 27:277-283.

91. Schwartz, A., J.M. Wood, J.C. Allen, E.P. Bornet, M.L. Entman, M.A. Goldstein, L.A. Sordahl and M. Suzuki. 1973. Biochemical and morphological correlates of cardiac ischemia I. Membrane systems. Am. J. Cardiol. 32: 46-61.

92. Sordahl, L.A., W.B. McCollum, W.G. Wood and A. Schwartz. 1973. Mitochondria and sarcoplasmic reticulum function in cardiac hypertrophy and failure. Am. J. Physiol. 224:497-502.

93. Dhalla, N.S. 1974. Defect in calcium regulatory mechanisms in heart failure. In: Myocardial Biology: Recent Advances in Studies on Cardiac Structure and Metabolism, (N.S. Dhalla, ed.) Vol. 4, pp. 331-345, University Park Press, Baltimore.

94. Sobel, B.E., P.D. Henry, A. Robinson, C. Bloor and J. Ross, Jr. 1969. Depressed adenyl cyclase activity in the failing guinea pig heart. Circ. Res. 24:507-512.

95. Dhalla, N.S., P.V. Sulakhe, R.L. Khandelwal and R.E. Olson. 1972. Adenyl cyclase activity in the perfused rat heart made to fail by substrate lack. Cardiovasc. Res. 6:344-352.

96. Gold, H.K., K.H. Prindle, G.S. Levey and S.E. Epstein. 1970. Effects of experimental heart failure in the capacity of glucagon to augment myocardial contractility and activate adenyl cyclase. J. Clin. Invest. 49:999-1006.

97. Dransfeld, H., J. Lipinski and E. Borsh-Galetke. 1971. Die Na^{+}-K^{+}-activierte transport-ATPase bei experimenteller Herzinsuffizienzy duich Kobaltchloride. N.S. Arch. Pharmakol. 270:335-342.

98. Balasubramanian, V., D.B. McNamara, J.N. Singh and N.S. Dhalla. 1973. Biochemical basis of heart function X. Reduction in the Na^+ -K^+ - stimulated ATPase activity in failing rat heart due to hypoxia. Can. J. Physiol. Pharmacol. 51:504-510.

99. Dhalla, N.S., J.N. Singh, M. Fedelesova, V. Balasubramanian and D.B. McNamara. 1974. Biochemical basis of heart function XIII. Sodium-potassium stimulated adenosine triphosphatase activity in the perfused rat heart made to fail by substrate lack. Cardiovas. Res. 8:227-237.

100. Yasaki, Y. and J. Fujii. 1972. Depressed Na^+ -K^+ -ATPase activity in the failing rabbit heart. Jap. Heart J. 13:73-83.

101. Mead, R.J., M.B. Peterson and J.D. Welty. 1971. Sarcolemmal and sarcoplasmic reticular ATPase activities in the failing canine heart. Circ. Res. 24:14-19.

102. Bajusz, E. 1969. Hereditary cardiomyopathy: A new disease model. Am. Heart J. 77:686-696.

103. Bajusz, E. 1969. Dystrophic calcification of the myocardium as a conditioning factor in the genesis of congestive heart failure. Am. Heart J. 78:202-210.

104. Bajusz, E., J.R. Baker, C.W. Nixon and F. Homburger. 1969. Spontaneous hereditary myocardial degeneration and congestive heart failure in a strain of Syrian hamsters. Ann. N.Y. Acad. Sci. 156:105-129.

105. Bajusz, E., F. Homburger, J.R. Baker and L.H. Opie. 1966. The heart muscle in muscular dystrophy with special reference to involvement of the cardiovascular system in the hereditary myopathy of the hamster. Ann. N.Y. Acad. Sci. 138:213-231.

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

107. Brink, A.J. and A. Lochner. 1969. Contractility and tension development of the myopathic hamster (B10-14.6) heart. Cardiovasc. Res. 3:453-458.

108. Brink, A.J. and A. Lochner. 1967. Work performance of the isolated perfused beating heart in the hereditary myocardiopathy of the Syrian hamster. Circ. Res. 21:391-401.

109. Lochner, A., L.H. Opie, A.J. Brink and A.R. Bosman. 1968. Defective oxidative phosphorylation in hereditary myocardiopathy in the Syrian hamster. Cadiovasc. Res. 3:297-307.

110. Opie, L.H., A. Lochner, A.J. Brink, F. Homburger and C.W. Nixon. 1964. Oxidative phosphorylation in hereditary myocardiopathy in the Syrian hamster. Lancet II:1213-1214.

111. Schwartz, A., G.E. Lindenmayer and S. Harigaya. 1968. Respiratory control and calcium transport in heart mitochondria from the cardiomyopathic Syrian hamster. Trans. N.Y. Acad. Sci. Ser. II. 30:951-954.

112. Lindenmayer, G.E., S. Harigaya, E. Bajusz and A. Schwartz. 1970. Oxidative phosphorylation and calcium transport of mitochondria isolated from cardiomyopathic hamster hearts. J. Mol. Cell Cardiol. 1:249-259.

113. Wrogemann, K., M.C. Blanchaer and B.E. Jacobson. 1972. Oxidative phosphorylation in cardiomyopathic hamsters. Am. J. Physiol. 222:1453-1457.

114. Lochner, A., A.J. Brink and J.J. Van Der Walt. 1970. The significance of biochemical and structural changes in the development of the myocardiopathy of the Syrian hamsters. J. Mol. Cell Cardiol I:47-64.

115. Fedelesova, M. and N.S. Dhalla. 1971. High energy phosphate stores in the hearts of genetically dystrophic hamsters. J. Mol. Cell Cardiol. 3: 93-102.

116. Gertz, E.W., A.C. Stam, Jr., and E.H. Sonnenblick. 1970. A quantitative and qualitative defect in the sarcoplasmic reticulum in the hereditary cardiac myopathy of the Syrian hamster. Biochem. Biophys. Res. Comm. 40:746-753.

117. McCallum, W.B., C. Crow, S. Harigaya, E. Bajusz and A. Schwartz. 1970. Calcium binding by cardiac relaxing system isolated from myopathic Syrian hamsters (Strain 14.6, 82.62 and 40.54). J. Mol. Cell Cardiol. 1:445-458.

118. Sulakhe, P.V. and N.S. Dhalla. 1973. Excitation-contraction coupling in heart X. Further studies on energy-linked calcium transport by subcellular particles in the failing heart of myopathic hamster. Biochem. Med. 8:18-27.

119. Owens, K., W.B. Weglicki, E.H. Sonnenblick and E.W. Gertz. 1972. Phospholipid and cholesterol content of ventricular tissue from the cardiomyopathic Syrian hamster. J. Mol. Cell Cardiol. 4:229-236.

120. Sulakhe, P.V. and N.S. Dhalla. 1972. Adenyl cyclase activity in failing hearts of genetically myopathic hamsters. Biochem. Med. 6:471-482.

121. Sulakhe, P.V. and N.S. Dhalla. 1973. Alterations in the activity of cardiac Na^+ -K^+ -stimulated ATPase in congestive heart failure. Exptl. Mol. Path. 18:100-111.

122. Bajusz, E. and G. Jasmin. 1972. Hereditary disease model of congestive cardiomyopathy: Studies on a new line of Syrian hamster. Fed. Proc. 31:621 (abs.).

123. Singh, J.N., N.S. Dhalla, D.B. McNamara, E. Bajusz and G. Jasmin. 1974. Membrane alteration in failing hearts of cardiomyopathic hamster. In: Recent Advances in Studies on Cardiac Structure and Metabolism, Vol. 5 (A. Fleckenstein, ed.) University Park Press, Baltimore. (in press)

124. Gelband, H. and A.L. Bassett. 1973. Depressed transmembrane potentials during experimentally induced ventricular failure in cats. Circ. Res. 32:625-634.

125. Fleckenstein, A. 1964. Die Bedeutung der energiereicheu phosphate fur Kontraktilitat und Tonus des Myokards. Verh. dtsch. Ges. inn. Med. 70:81.

126. Fleckenstein, A. 1967. Stoffwechselprobleme bei der myokardin-suffizienz. Verh. Deutsch. Ges. Path. 51:15-29.

127. Fleckenstein, A. 1968. Experimentelle Pathologie der akuten und chronishen Herzinsuffizienz. Ferh. Dtsch. Ges. Kreislforsch. 34:15.

128. Kaufmann, R.L., H. Homburger and H.Wirth. 1971. Disorder in excitation-contraction coupling of cardiac muscle from cats with experimentally produced right ventricular hypertrophy. Circ. Res. 28:346-357.

129. Fleckenstein, A., J. Janke, H.J. Doring, O. Leader. 1974. Myocardial fiber necrosis due to intracellular Ca^{++} overload -- A new principle in cardiac pathophysiology. In: Recent Advances in Studies on Cardiac Structure And Metabolism, (N.S. Dhalla, ed.) Vol. 4, pp.563-580, University Park Press, Baltimore.

130. Schwartz, A., J.M. Wood, J.C. Allen, E.P. Bornet, M.L. Entman, M.A. Goldstein, L.A. Sordahl and M. Suzuki. 1973. Biochemical and morphologic correlates of cardiac ischemia. Am. J. Cardiol. 32:46-61.

131. Dhalla, N.S., C.W. Tomlinson, J.C. Yates, S.L. Lee, K.G. Varley, I.F.M. Borowski and J. Barwinsky. 1974. Role of mitochondrial calcium transport in failing heart. In: Recent Advances in Studies on Cardiac Structure and Metabolism, (A. Fleckenstein and N.S. Dhalla, eds.) Vol. 5, University Park Press, Baltimore. (in press).

132. Varley, K.G. and N.S. Dhalla. 1973. Excitation-contraction coupling in heart XII. Subcellular calcium transport in isoproterenol induced myocardial necrosis. Exptl. Mol. Path. 19:94-105.

133. Muir, A.R. 1967. The effects of divalent cations on the ultrastructure of perfused rat heart. J. Anat. 101:239-261.

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

135. Zimmerman, A.N.E., W. Daems, W.C. Hulsmann J. Snijder, E. Wisse, and D. Durrer. 1967. Morphological changes of heart muscle caused by successive perfusion with calcium-free and calcium containing solutions (calcium paradox). Cardiovasc. Res. 1:201-209.

136. Tomlinson, C.W., J.C. Yates and N.S. Dhalla. 1974. Relationship among changes in intracellular calcium stores, ultrastructure and contractility of myocardium. In: Recent Advances in Studies on Cardiac Structure and Metabolism. (N.S. Dhalla, ed.), Vol. 4, pp. 331-345. University Park Press, Baltimore

137. Yates, J.C. and N.S. Dhalla. 1974. Structural and functional changes associated with failure and recovery of hearts after perfusion with Ca^{++} -free medium. J. Mol. Cell Cardiol. (in press).

138. Dhalla, N.S., C.W. Tomlinson, J.N. Singh, D.B. McNamara, J.A.C. Harrow and J.C. Yates. 1974. Role of sarcolemmal changes in cardiac pathophysiology. In: Recent Advances in Studies in Cardiac Structure and Metabolism, (P.E. Roy and N.S. Dhalla, eds.) Vol. 5, University Park Press, Baltimore (in press).

139. Tomlinson, C.W. and N.S. Dhalla. 1973. Excitation-contraction coupling in heart IX. Changes in the intracellular stores of calcium in failing hearts due to lack of substrate and oxygen. Cardiovasc. Res. 7:470-476.

140. Jasmin, G. and Bajusz. 1973. Polymyopathie et cardiomyopathie hereditaire chez le hemster de Syrie. Inhibition selective des lesions du myocarde. Ann. Anat. Path. 18:49-66.

141. Borowski, I.F.M., J.A.C. Harrow, E.T. Pritchard and N.S. Dhalla. 1974. Changes in electrolyte and lipid contents in the myopathic hamster (UM-X7.1) skeletal and cardiac muscles. Res. Commun. Chem. Path. Pharmacol. 7:443-451.

142. Dhalla, N.S., P.V. Sulakhe, M. Fedelesova and J.C. Yates. 1974. Molecular abnormalities in cardiomyopathy. Adv. Cardiol. 13:282-300.

143. Sordahl, L.A. and A. Schwartz. 1967. Effects of dipyridamole on heart muscle mitochondria. Mol. Pharmacol. 3:509-515.

144. Fiske, C.H. and Y. Subbarow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375-400.

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

146. Nordlie, R.C. and W.J. Arion. 1966. Glucose-6-phosphatase. In: Methods in Enzymology.(S.P. Colowick and N.O. Kaplan, eds.) Vol. 9, pp. 613-625, Academic Press, New York.

147. Smith, L. and P.W. Camerino. 1963. Comparison of polarographic and spectrophotometric assays for cytochrome oxidase activity. Biochemistry 2:1428-1432.

148. Yates, J.C. 1973. Electrical, mechanical and ultrastructural events in hearts perfused with media deficient in various cations. M.Sc. Thesis, Faculty of Medicine, University of Manitoba.

149. Lineweaver, M. and D. Burk. 1934. Determination of enzyme dissociation constants. J. Am. Chem. Soc. 56:658-666.

150. Yates, J.C. and N.S. Dhalla. 1974. Induction of myocardial necrosis in the isolated perfused rat hearts with oxidized isoproterenol. (submitted for publication).

151. Olson, R.E., N.S. Dhalla and C.N. Sun. 1972. Changes in energy stores in the hypoxic heart. Cardiology 56:114-124.

152. McNamara, D.B., P.V. Sulakhe, J.N. Singh and N.S. Dhalla. 1973. Adenyl cyclase activity in the hypoxic heart. In: Myocardial Metabolism: Recent Advances in Studies on Cardiac Structure and Metabolism, (N.S. Dhalla, ed.), Vol. 3, pp. 303-309. University Park Press, Baltimore.

153. Weglicki, W.B., K. Owens, C.W. Urschel, J.R. Serur and E.H. Sonnenblick. 1973. Hydrolysis of myocardial lipids during acidosis and ischemia. In: Myocardial Metabolism: Recent Advances in Studies on Cardiac Structure and Metabolism,(N.S. Dhalla, ed.), Vol. 3, pp. 781-793, University Park Press, Baltimore.

154. Regan, D.M., W.W. Davis, H.E. Morgan and C.R. Park. 1964. The regulation of hexokinase and phosphofructokinase activity in heart muscle. Effects of alloxan diabetes, growth hormone, cortisole and anoxia. J. Biol. Chem. 239:43-49.

155. Morgan, H.E. and A. Parmeggiani. 1964. Regulation of glycogenolysis in muscle II. Control of glycogen phosphorylase reaction in isolated perfused heart. J. Biol. Chem. 239:2435-2439.

156. Williamson, J.R. 1966. Glycolytic control mechanism II. Kinetics of intermediate changes during the aerobic, anoxia transition in perfused rat heart. J. Biol. Chem 241:5026-5036.

157. Sheuer, J., and S.W. Stezoski. 1970. Protective role of increased myocardial glycogen stores in cardia anoxia in the rat. Circ. Res. 27:835-849.

158. Locke, F.S. and O. Rosenheim. 1907. Contribution to the physiology of the isolated heart. The consumption of dextrose by mammalian cardiac muscle. J. Physiol. 36:205-220.

159. Mines, G.R. 1913. On functional analysis by the action of electrolytes. J. Physiol. 46:188-235.

160. Bailey, L.E. and H.A. Sures. 1971. The effect of ouabain on the washout and uptake of calcium in the isolated cat heart. J. Pharmacol. Exptl. Ther. 178:259-270.

161. Langer, A. 1968. Ion fluxes in cardiac excitation and contraction and their relation to myocardial contractility. Physiol. Rev. 48:708-757.

162. Sack, D.W., N.D. Kim and C.E. Harrison. 1974. Contractility and subcellular Ca^{++} metabolism in chronic potassium deficiency. Am. J. Physiol. 226:756-763.

163. Owens, K., R.C. Ruth, N.B. Weglicki, A.C. Stam and E.H. Sonnenblick. 1974. Fragmented sarcoplasmic reticulum of the cardiomyopathic Syrian hamster: Lipid composition, calcium transport, and Ca^{++} -stimulated ATPase. In: Myocardial Biology: Recent Advances in Studies on Cardiac Structure and Metabolism, (N.S. Dhalla, ed.), Vol. 4, pp. 541-550. University Park Press, Baltimore.