STRUCTURAL AND FUNCTIONAL CHANGES IN THE ISOLATED RAT HEART FOLLOWING PERFUSION WITH CATECHOLAMINES, THEIR METABOLITES AND OXIDATION PRODUCTS

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JOHN CHARLES YATES
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ABSTRACT

The effects of fresh and oxidized isoproterenol, epinephrine, metanephrine, dihydroxymandelic acid, vanilmandelic acid and adrenochrome on the mechanical function and ultrastructure of the isolated perfused rat heart were studied. In contrast to fresh isoproterenol, isoproterenol oxidized for 6 to 8 hours decreased contractile force, maximum rate of force development and maximum rate of relaxation. Hearts perfused with oxidized isoproterenol (100 mg/l) were unable to generate contractile force in about 35 minutes, and showed a marked increase in resting tension. Although fresh isoproterenol at high concentrations employed in this study did not show an increase in contractile force, the maximum rates of force development and relaxation were increased in comparison to control. Both fresh and oxidized isoproterenol decreased times for peak tension and for 1/2 relaxation. The hearts perfused with oxidized isoproterenol, but not with fresh isoproterenol, showed other ultrastructural damage which is commonly seen in myocardial necrosis. The absorption spectrum of the oxidized isoproterenol solution resembled that of adrenochrome. The ability of the solution of isoproterenol oxidized for 16 to 24 hours to depress cardiac function and induce necrosis was markedly less than that of the one oxidized for 6 to 8 hours. Perfusion of isolated hearts with epinephrine or metanephrine significantly increased contractile force. Vanilmandelic acid and dihydroxymandelic acid did not alter contractile force development, whereas adrenochrome (50 mg/l) caused a decline in contractile force with complete disappearance of contractile activity by 30 minutes. The increase in contractile force with epinephrine (50 mg/l) was associated with an increase in resting tension and maximum rates of force development and relaxation, and decreases of times for peak tension development and 1/2 relaxation. Hearts perfused with adrenochrome showed an early decline followed by a steady increase in resting tension. Maximum rates of force development and relaxation were reduced and times for peak tension development and 1/2 relaxation were increased. Hearts perfused with adrenochrome (50 mg/l) but not epinephrine, metanephrine, dehydroxy-mandelic acid and vanilmandelic acid, showed ultrastructural damage characteristic of myocardial necrosis which was evident following 10 minutes or more of perfusion. Adrenochrome concentrations of 10 or 25 mg/l altered the appearance of mitochondria

after 30 minutes of perfusion. These results are consistent with the view that oxidation products of catecholamines, but not catecholamines per se, are responsible for inducing myocardial necrosis and failure.

Infusion of epinephrine (1 mg/l) during perfusion with adrenochrome partially maintained contractile force during the first 15 minutes of perfusion but did not alter the severity of ultrastructural changes due to adrenochrome. Reduction of adrenochrome in solution by ascorbic acid, dithiothriotol or cysteine resulted in an increased severity of ultrastructural damage. Increasing the Ca^{++} or K^{+} concentration or decreasing the Na^{+} concentration of the adrenochrome containing perfusion medium partially maintained contractile force but increased the severity of ultrastructural damage. Reducing the K^{+} concentration of the medium did not alter the failure of contractile force development but increased the severity of necrosis due to adrenochrome. Reducing the Ca^{++} or increasing the Mg^{++} concentration of the perfusion medium completely prevented myocardial necrosis due to adrenochrome. Omission of Mg^{++} from the perfusion medium did not alter either the time course of contractile failure or the severity of necrosis due to adrenochrome. The calcium antagonist D-600 reduced the severity of adrenochrome - induced ultrastructural damage, but normal structure of the sarcomeres was not maintained. The α - receptor blocking drugs, tolazoline and dibenamine, and the adrenergic neuron blocking agents, quanethidine and bretylium, did not alter the development of contractile failure and necrosis due to adrenochrome. The β - receptor blocking compounds, propranolol and practolol, effectively protected the heart from adrenochrome-induced necrotic damage, and partially prevented contractile failure. The hydrazine - type monoamine oxidase inhibitor, iproniazide, completely prevented ultrastructural damage and partially maintained contractile force development in adrenochrome perfused hearts. The non-hydrazine type monoamine oxidase inhibitor, tranylcypromine, partially protected the isolated heart against adrenochrome necrosis, but disruption of mitochondrial structure was still seen. Tranylcypromine did not significantly improve contractile force development during adrenochrome perfusion. These results for the most part parallel the influence of similar ionic and pharmacological interventions on the severity of necrosis produced by injection or infusion of large quantities of catecholamines,

and further support the opinion that these catecholamine - induced lesions are the result of an accumulation of toxic quantities of catecholamine oxidation products in the myocardium.

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I. INTRODUCTION AND STATEMENT OF THE PROBLEM

It has been known for many years that epinephrine (1, 2) and norepinephrine (3) can cause cardiac lesions when administered in large doses. Subsequently Rona et. al. (4) discovered that the synthetic catecholamine, isoproterenol, is capable of producing consistent myocardial lesions when injected at dose levels far below the lethal dosage for this drug. This has provided a challenging experimental model for studying mechanisms by which catecholamines induce myocardial lesions. Injection of catecholamines in animals also produces a number of dramatic pharmacological effects including changes in hemodynamic factors such as peripheral resistance, arterial blood pressure, cardiac output, venous return and coronary flow; increases heart rate and cardiac work, thereby causing increased myocardial oxygen demand; releases further amounts of catecholamines from the adrenergic nerve endings; produces alterations in lipid and carbohydrate metabolism; and results in the accumulation of exogenous lipids in the heart. As a consequence of these factors it has been difficult to determine whether catecholamines do in fact exert a direct toxic influence on the myocardium, or whether necrosis is in some way secondary to other actions of catecholamines as has been proposed by some investigators (4 - 10). On the other hand, evidence and arguments to the effect that cardiovascular, hemodynamic and other changes following administration of catecholamines cannot completely account for the occurrence of myocardial necrosis in different experimental models have been presented by various investigators (11 - 15). A further complicating factor in this problem is the fact that catecholamines readily undergo oxidation and it has been suggested that the oxidation products of catecholamines rather than catecholamines per se are responsible for the cardiotoxicity observed following admisinstration of these agents (16, 17). Furthermore, other workers have claimed that the products of catecholamine metabolism, especially those produced during the monoamine oxidase reaction, may be involved in the induction of myocardial necrosis (18). Thus, in spite of a considerable research effort by many investigators devoted to elucidating these mechanisms, there is as yet no clear cut explanation of the sequence of events by which cardiac cell necrosis results from an injection of catecholamines.

The present study is therefore primarily directed to the problem of whether or not catecholamines, their oxidation products or metabolites are indeed capable of a direct toxic influence on the heart. The isolated perfused rat heart preparation which has been empolyed for this study appears to be an ideal model for this purpose for several reasons. These include elimination of hemodynamic factors, neural mechanisms, availability of exogenous lipids, and other physiological parameters which tend to complicate the production of heart lesions in intact animals. Furthermore, changes in contractile events can be monitored concomitantly and the heart can be readily fixed at a desired time for ultrastructural examination. Fresh isoproterenol, oxidized isoproterenol, fresh epinephrine, adrenochrome and various metabolites of epinephrine will be used for investigating changes in ultrastructure and contractile functions of the isolated perfused rat hearts. In addition to studying the time-course and dose-responses to the cardiotoxic agents, attempts will be made to elucidate the mechanisms subserving the functional and morphological alterations. For this purpose, hearts will be perfused in the absence or presence of the cardiotoxic substance with media of different cationic compositions or containing various pharmacologic agents which are known to influence the catecholamine-induced myocardial necrosis in vivo. It is hoped that this study will extend our existing knowledge concerning the modes of catecholamine - induced contractile failure and myocardial cell damage.

II. REVIEW OF THE LITERATURE

A. Cardiotoxicity of Epinephrine, Norepinephrine and Isoproterenol:

The cardiotoxic properties of catecholamines have been known at least since 1905 when the occurrence of cardiac lesions after epinephrine administration was reported by Ziegler (2), and these lesions were subsequently described in detail by Pearce (1) and Josue (19). Fleisher and Loeb (20 - 23) reported that injection of sparteine sulphate or caffeine sodium benzoate followed by epinephrine provided an easy and certain method of producing myocardial lesions in rabbits. Lesions visible to the naked eye occurred in 60% of the animals, and microscopic examination revealed further lesions in a still larger percentage. These investigators suggested that excessive mechanical strain was at least one of the factors responsible in producing the lesions. Christian et . al . (24) confirmed these findings and further demonstrated that sparteine sulphate alone did not produce myocardial lesions. In 1913 a full histological account of these epinephrine induced myocardial changes was provided by Anitschkow (25) and epinephrine was thereafter shown to produce cardiac lesions in man as well (26, 27). While most of these reports dealt with relatively high dose levels of epinephrine, small endocardial lesions were also observed in the left ventricle of dog hearts following continuous infusion for 120 to 289 hours of epinephrine at rates considered to be well below the maximum physiological rate of secretion by the adrenal glands (28).

Within a few years after norepinephrine came into widespread use as a primary pressor agent, reports of norepinephrine induced myocardial lesions began to appear. In the course of a study on the effects of prolonged infusion of norepinephrine on arterial pressure of rabbits, Blacket et . al . (29) found that the hypertension resulting from norepinephrine infusion could not be maintained, even with increasing dose levels, and that blood pressure fell drastically when infusion was discontinued. Although the possible release of vasodilator substances was discussed in connection with these findings, these investigators apparently did not consider the possibility of impaired cardiac function. Norepinephrine did not increase the survival rate of dogs in hemorrhagic shock when compared with controls (30) and in fact this agent was found to show a higher incidence of cardiac lesions

in this condition (31). The observation of an increased frequency of nonspecific myocarditis and an apparent common denominator of norepinephrine therapy in humans prompted Szakacs and Cannon (3) to study the effects on the heart of continuous intravenous infusions of therapeutic doses of norepinephrine in dogs. Focal myocarditis was found to be uniformly present in the hearts of these animals in association with subendocardial and subepicardial hemorrhages. Being aware of the epinephrine induced myocardial lesions previously reported (32), Nahas et. al. conducted a series of experiments with both epinephrine and norepinephrine using a heart lung preparation in which the heart was isolated from all secondary hormonal or nervous influences (33). All hearts which had been perfused with epinephrine, norepinephrine, or both proved to have extensive lesions of the myocardium. In a quantitative study on the pathological effects of norepinephrine, Szakacs and Mehlman (34) found that dosages considered physiologic and indeed harmless, if administered for short periods of time, might become lethal after prolonged infusion. Thus duration of infusion is an important factor in determining whether a particular dose level of norepinephrine is likely to produce myocardial lesions.

In addition to myocardial cell damage, norepinephrine was also demonstrated to produce derangements of metabolic processes in the heart. For example Maling and Highman (35) reported a fatty degeneration of the myocardium under the influence of high doses of norepinephrine. In subsequent studies (36, 37) remarkable similarities were found in heart triglyceride content and serum enzyme levels after doses of epinephrine and norepinephrine and following infarction produced by coronary artery occlusion. However, these studies did not attempt to examine the relationship between changes in lipid metabolism and the occurrence of myocardial necrosis due to catecholamines.

The discovery that severe myocardial necrosis could be consistently produced in rats with doses of isoproterenol which constitute an amazingly small fraction of the median lethal dose (4, 38) opened the way for a systematic examination of catecholamine - induced myocardial lesions. Although the LD_{50} of isoproterenol in rats was reported to be 680 mg/kg, doses as low as 0.02 mg/kg produced microscopic focal necrotic lesions. The severity of myocardial damage was closely related to the dosage of isoproterenol used, and varied from focal lesions affecting single cells to

massive infarcts involving large portions of the left ventricle. Lesions were generally found to be localized in the apex and left ventricular subendocardium, being observed less frequently in the papillary muscle and right ventricle. A comparison of the cardiotoxic effects of epinephrine, norepinephrine, and isoproterenol in rats (39) showed the lesions produced to be qualitatively similar, but the lesions which were seen after isoproterenol treatment were more severe than those produced by epinephrine or norepinephrine. Whereas the median lethal doses of isoproterenol, epinephrine, and norepinephrine were found in this study to be 581 mg/kg, 6.1 mg/kg, and 8.6 mg/kg respectively, isoproterenol was found to be 29 to 72 times more potent in producing myocardial lesions of equal severity than epinephrine or norepinephrine. Isoproterenol was also found to produce apical lesions and disseminated focal necrosis in dogs (40), however, these lesions were frequently fatal to these animals and the median lethal dosage was much lower. Myocardial lesions similar to those produced by catecholamine injections have also been reported in patients with pheochromocytomas (3, 41 - 43), subarachnoid hemorrhage and various other intracranial lesions (44 - 47), and following electrical stimulation of the stellate ganglion (48, 49) or hypothalamus (50) in experimental animals. These studies not only demonstrate that catecholamines are capable of producing myocardial necrosis but also suggest that myocardial cell damage seen in patients may be the result of high levels of circulating catecholamines.

B. Morphological and Biochemical Changes Associated with Catecholamine - induced Myocardial Necrosis:

Although a number of long term studies have been conducted on the development and healing of catecholamine - induced infarcts, the present discussion will be limited to early events (i.e., less than 48 hours after injection) in the production of necrosis.

1. Ultrastructural changes: The time course of ultrastructural changes following isoproterenol injections in rats has been studied extensively. The earliest changes, evident within 4 to 6 minutes, are disorientation of myofilaments, irregular sarcomere length, occasional regions of contracture or rupture of myofilaments, and some slight dilatation of the sarcoplasmic reticulum (51 - 53). By ten minutes mitochondrial swelling is evident, with the occasional occurrence of electron - dense bodies (53).

By this time disorganization and fragmentation of myofibrils is prominent. It has been reported (52, 54) that there is no correlation between mitochondrial damage and disruption of myofilaments; normal appearing mitochondria being found among fragmented filaments, and swollen mitochondria with ruptured cristae and electron dense deposits among apparently undamaged sarcomeres. Within 30 minutes to an hour, electron dense granular deposits are frequently noted within mitochondria, numerous lipid droplets are seen, margination of nuclear chromatin is observed, a disappearance of glycogen granules is noted, various degrees of swelling and disruption of transverse and longitudinal tubules and mitochondria are seen, and there is a spectrum of damage to the contractile filaments, ranging from irregular bands of greater or less than normal density in sarcomeres of irregular length, to fusion of sarcomeres into confluent masses and granular disintegration of the myofilaments (51 - 53, 55). Over the course of the next few hours all of these changes become progressively more widespread throughout the myocardium, and more severe (55, 56). Interstitial and intracellular edema as well as extensive inflammation also becomes evident (46, 55). Herniation of intercalated discs and extensive vacuolization was also seen within several hours of isoproterenol injection (52). Comparison of the effects of norepinephrine and epinephrine with isoproterenol has shown the effects of these three drugs to be qualitatively identical at the cellular level (54 - 59), with the exception that glycogen depletion (55, 57) and fat deposition (59) were much more extensive with epinephrine than with isoproterenol or norepinephrine. Maruffo (11) has also reported similar alterations following isoproterenol administration in monkeys.

From the foregoing evidence it appears that alterations of the contractile filaments begin with irregularities in length and disalignment of the sarcomeres which is usually associated with an increased thickness and density of the Z-band. Contracture ensues, with the Z-bands becoming indistinct, and actin and myosin filaments can no longer be distinguished. Granular disintegration of the sarcomeres follows with the appearance of large empty spaces within the muscle cells, and this fragmentation likely contributes to swelling of the cell. The tubular elements and mitochondria commence swelling very soon after catecholamine injection, and the mitochondrial matrix is subsequently decreased in electron density. Swelling

of the transverse tubules and sarcoplasmic reticulum is not as consistent a finding as the mitochondrial swelling, and may not be evident with certain fixatives. Following norepinephrine injection the T-system is dilated and much more extensively branched than in normal animals. Rupture of the cristae and deposition of electron dense material in the mitochondria represent the final stages in the disruption of these organelles. Accumulation of lipid droplets and disappearance of glycogen granules is not usually evident until these other changes have occurred to some degree and are probably due to well known metabolic effects of catecholamines. Herniation of intercalated discs and vacuolization are probably secondary to the swelling and disruption of subcellular organelles and the disintegration of myofilaments.

2. Histological changes: The earliest change which can be visualized under the light microscope is the appearance of darkly stained contraction bands at 10 minutes after isoproterenol injection in thin sections from Araldite embedded ventricle pieces (11). Focal myocardial degeneration, characterized by loss of striations with sarcoplasmic smudging, lipid accumulation, margination of nuclear chromatin, and increased cytoplasmic eosinophilia is evident in 2 - 6 hours following injection of isoproterenol (10, 11) or 4 - 5 hours after beginning infusion of norepinephrine (60). Capillary dilatation and interstitial edema are evident by this time in isoproterenol treated hearts (11) and have been observed much earlier with norepinephrine (54).

Interstitial edema is usually associated with subendocradial and subepicardial congestion and hemorrhages following administration of catecholamines and is characteristically present in damaged areas of the myocardium even after 72 hours (4, 14, 40, 54, 55, 60). Rosenblum et. al. (14) and Ferrans et. al. (55) have made comparative studies which indicate that interstitial edema and inflammation are much more prominent following epinephrine or norepinephrine injections even though isoproterenol is more potent in producing cellular damage, suggesting that edema and inflammation result from mechanisms different from those causing necrotic tissue damage.

Within 12 to 24 hours, myocardial tissue damage is readily apparent and characterized by certain histological staining properties. Fibers with highly eosinophilic cytoplasm alternate with normal fibers giving the myocardium a mottled

appearance (4). Swelling, segmentation and fragmentation, and hyalinization of fibers is evident. Fibers become homogenous, strongly eosinophilic, Periodic - Acid - Schiff's (PAS) positive, and stain pink or deep red with Cason's trichrome (4, 10, 40, 55, 58). Fat deposition is usually evident as well (58).

3. Histochemical changes: Histochemical alterations subsequent to administration of lesion producing doses of catecholamines have been reported in detail (55 - 57, 60). A marked loss of glycogen, as visualized by PAS reaction, is seen within 30 minutes, and is most marked following epinephrine administration. Accumulation of PAS positive material is seen at one hour and in an increasing number of fibers over the next 24 hours. This is associated with loss of normal striations and appearance of clear vacuoles. A metachromatic substance is usually observed in areas of interstitial edema and inflammation.

All three catecholamines produce a biphasic change in the activity of the oxidative enzymes succinic dehydrogenase, NAD diaphorase, lactic dehydrogenase, isocitric dehydrogenase, malic dehydrogenase, β - hydroxbutyric dehydrogenase, α - glycerophosphate dehydrogenase, glutamic dehydrogenase, and ethanol dehydrogenase. There is a rapid increase in the activity of the enzymes evident within 5 minutes in various individual fibers, followed by a gradual decline in activity until by 6 to 12 hours certain areas of the myocardium having markedly diminished oxidative enzyme activity are interspersed with fibers of normal activity. Decline in oxidative enzyme activity of certain fibers progresses until frank necrosis is evident and there is complete loss of activity. In each case the degree of change of activity is proportional to the normal level of activity of the enzyme involved. Cytochrome oxidase activity is unchanged until evidence of early necrosis is seen after 6 to 12 hours, at which time activity of this enzyme decreases as well.

An increased number of lipid droplets is observed at 30 minutes. Fatty change is more evident in the endocardial region than elsewhere, and has been reported by Ferrans et. al. (55) to be least apparent with epinephrine, which is in direct contradiction of the findings of Lehr et. al. (59). The reason for this discrepancy in these results is not clear; however, fibers which contain large lipid droplets show decreased activity of oxidative enzymes and cytochrome oxidase. Furthermore, all of the three agents cause a slight irregular increase in the staining

of cytoplasm for lysosomal esterase activity (59).

4. Biochemical Changes: Following catecholamine administration, coronary blood flow, myocardial oxygen uptake, and cardiac respiratory quotient were increased (61). Blood content of glucose, triglycerides, and nonesterified fatty acids increased markedly without any change in blood cholesterol levels whereas total serum protein content was decreased (15, 62 - 64). Glycogen and lactate content of the heart decreased rapidly after injection of isoproterenol. Glycogen levels then rose above control levels after two hours, whereas lactate content slowly returned to control levels over about 5 hours (65). Within 24 hours after isoproterenol injection aldosterone production increased whereas glucocorticoids and total steroids were decreased (63, 64). Serum levels of glutamate-pyruvate transaminase, glutamate-oxaloacetate transaminase (aspartate amino-transferase), lactate dehydrogenase, and creatine phosphokinase were all greatly elevated during the acute phase of necrotization following catecholamine administration (61, 63, 64, 66).

The extraction of nonesterified fatty acids by the heart has been reported to be decreased in comparison to controls following epinephrine infusion, whereas the extraction of triglycerides was increased (61). Likewise, there was no significant increase in the contents of free fatty acids or phospholipids in the left ventricle while there was a significant increase in the triglyceride content of every layer of the left ventricular wall, following epinephrine infusion, the greatest increase in triglyceride content occurred in the endocardium (13). The uptake of C^{14} - labeled triglycerides has also been found to increase during norepinephrine infusion and was associated with a small but significant increase in $C^{14}O_2$ production, whereas synthesis of triglycerides from C^{14} - acetate or palmitate was unchanged (67). These findings are consistent with the appearance of numerous lipid droplets reported in histological and ultrastructural studies.

The myocardial content of hexosamines became greatly elevated within 24 hours after an injection of isoproterenol and this increase in mucopolysaccharide could not be attributed to fibroblasts or other infiltrating cells (68). Cardiac aspartate amino-transferase (glutamate-oxaloacetate transaminase) activity decreased in a time and dose dependent manner following isoproterenol injection (69). This decrease in

activity correlates well with the occurrence and severity of macroscopic lesions. Total cardiac lactate dehydrogenase activity fell as well following isoproterenol injection (70). This depression of activity is long term, lasting several days and appears to be due to a decrease in the ratio of H to M isoenzymes. These findings are consistent with the loss of these enzymes from the heart as evidenced by the increase in plasma concentrations of transaminases and lactate dehydrogenase.

Sobel et. al. (17) have reported that a single, large subcutaneous dose of epinephrine, norepinephrine, or isoproterenol produced uncoupling of oxidative phosphorylation in rat heart mitochondria, although these catecholamines in vitro did not affect normal rat heart mitochondria. A reduced respiratory control index of heart mitochondria 24 hours after isoproterenol injection has also been reported by Stanton and Schwartz (71) and more recently by Vorbeck et. al. (72). The results of several studies on cardiac adenine nucleotides following isoproterenol injection in rats are somewhat contradictory. Hattori et. al. (73) reported a decrease in the level of all adenine nucleotides, but no change in relative amounts of ATP, ADP and AMP. Kako (74), on the other hand, found a 26 to 34 % decrease in ATP, and 18 - 30% decrease in creatine phosphate levels, a decrease in both the ATP/ADP and the ATP/AMP ratios, but no significant difference in the levels of ADP, AMP, CP, glycogen, lactate, pyruvate, lactate/pyruvate ratio, triglycerides, cholesterol or phospholipids. Studies of this sort are difficult to evaluate, firstly because the results are expressed in terms of gram wet heart weight, which increases following catecholamine administration due to a large increase in extracellular fluid volume (74, 75), and secondly because the scattered portions of the myocardium undergoing necrotic change are "diluted" within a very large mass of cardiac tissue which has been affected only slightly or not at all. The work of Fleckenstein (76, 77), in which he showed a very large decrease in both ATP and creatine phosphate stores of the myocardium, appears to be the best representation of changes in high energy phosphates presently available. A large increase in the orthophosphate content of the myocardium was also found. These results suggest lowering of the energy state of myocardium due to high doses of catecholamines and this change appears to be at least partly due to an impairment in the process of energy production.

Pelouch et . al . (78, 79) have found that myosin extracted from rat hearts following isoproterenol injections contained a large component consisting of a stable aggregated form of myosin, whereas only the monomeric form of myosin was extracted from control animals. The first phase of aggregation involved a low polymer, probably a dimer, and there was no evidence of proteolytic damage of the myosin. The aggregated form of myosin did not possess any ATPase activity. On the other hand, Fedelesova et . al . (80) have reported that injection of a lesion producing dose of isoproterenol caused an elevation of cardiac myofibrillar ATPase activity and an increased accumulation of Ca^{++} by heart mitochondria and microsomes as well as decreased high energy phosphate stores. ATP - dependent calcium binding by microsomes but not by mitochondria was found to decrease in rat and rabbit hearts after isoproterenol (81). Thus changes in the subcellular components involved in the process of energy utilization in the catecholamine - induced myocardial necrosis are controversial and require further studies.

5. Electrolyte changes: The earliest and most significant changes in tissue ion content following catecholamine administration were found to be a decrease in both magnesium and phosphate, which were evident by 3 hours and persisted beyond 24 hours (58, 59, 82, 83). An increase in myocardial calcium content was observed as well, but was not as pronounced in the earlier stages of necrosis, prior to about 6 hours after catecholamine injection. More recent studies have shown the uptake of extracellular Ca^{45} to increase 6 to 7 fold with isoproterenol (76,77) although the absolute increase in myocardial content was only of the order of 50 to 100%. The sodium content of the myocardium did not change until about 24 hours, at which time it was increased, which may be a reflection of an increased interstitial fluid volume (58, 59, 82, 83).

Alterations of myocardial potassium content are less certain. Although a transient loss of myocardial potassium has been frequently reported (75, 84 - 86), this is a short term phenomenon not lasting more than 1 to 5 minutes, and may be a result of the increased frequency of contraction (87). In this regard, Daggett et . al . (88) have reported that norepinephrine caused a dose dependent uptake of potassium by the isolated heart. In studies concerned with the cardiotoxicity of epinephrine both an increase (59) and a decrease (13) in the potassium content of

the myocardium have been reported. Stanton and Schwartz (71) have also reported a decrease of myocardial potassium content following isoproterenol injection. As mentioned previously, myocardial content determinations are complicated by both the increase of interstitial fluid volume which accompanies necrosis, and by the admixture of necrotic and normal fibers which characterizes "multifocal disseminated" necrosis.

Measurements of serum levels of electrolytes three hours after isoproterenol injection have revealed an increase of serum magnesium and a decrease of calcium and sodium levels (83). By 7 hours, magnesium returned to control levels and serum potassium was increased, while calcium and sodium levels remained significantly depressed. By 24 hours all serum electrolyte levels returned to normal with the exception of calcium, which remained slightly low. Regan et. al. (61) have reported that, after an initial period of uptake of potassium and phosphate, loss of these ions from the left ventricle was evident. Thus serum electrolyte measurements appear to confirm the loss of magnesium and phosphate from the heart and the uptake of calcium as early, important events in the etiology of catecholamine induced necrosis. Since both net increases and decreases of myocardial and serum potassium have been found at different times, it is possible that potassium may be taken up by more or less undamaged myocardial cells while it is being released from fibers undergoing necrotic changes.

C. Factors Influencing Catecholamine Cardiotoxicity:

Monoamine oxidase inhibitors (MAOI) of the hydrazine type have been found to decrease the incidence and severity of myocardial lesions following catecholamine administration (65, 71, 89 - 93) and to antagonize increases in myocardial water, sodium, and chloride as well as loss of potassium (71). The hydrazine type inhibitors investigated include isocarboxazide, iproniazide, pivaloylbenzhydrazine, and phenylzine. On the other hand, the non-hydrazine type MAO inhibitors such as tranylcypromine, pargyline, and RO5-7071 have been reported by some workers to be ineffective (65, 92, 93), although Leszkovsky and Gal (94) reported a reduction of the severity of isoproterenol induced lesions with pargyline and another nonhydrazine MAOI identified as E-250. Muller (18) also

found that hydrazine type MAOI protected the heart whereas non-hydrazine type MAOI did not, but pointed out that hydrazine type inhibitors are long lasting in their effects whereas tranylcypromine is a competitive blocker with an intensive but transient effect and thus the inhibition produced by this drug may be of insufficient duration to afford protection.

The β - receptor blocking compounds, propranolol, pronethalol and dichloroisoproterenol were found to reduce the incidence and severity of myocardial lesions induced by isoproterenol (82, 83, 89, 95 - 97), although Wenzel and Lyon (70) found pronethalol to be ineffective. In another study, Wenzel and Chau (69) have reported that pronethalol afforded some protection against the loss of myocardial aspartate aminotransferase (AAT) activity caused by epinephrine, norepinephrine, and high doses of isoproterenol but potentiated the loss of AAT activity with moderate lesion producing doses of isoproterenol. Propranolol has also been found to ameliorate or completely prevent electrolyte shifts (increased myocardial Ca; decreased Mg and P) associated with isoproterenol induced necrosis (83, 95), thus producing an apparent dichotomy between the occurrence of lesions and electrolyte shifts since lesions are still seen, although less severe (95). In view of the proportion of the ventricle not undergoing necrotic damage in these experiments, it is difficult to know whether the alterations of electrolytes are truly and completely prevented. Kako (98) has reported that propranolol reduced the amount by which myocardial ATP declined following isoproterenol - induced damage. One can thus conclude that the β-adrenergic blocking agents are capable of modifying certain cardiotoxic effects of catecholamines.

The α - adrenergic blocking compounds, such as azapetine, phentolamine, dibenamine, dihydroergocryptin, phenoxybenzamine and tolazoline were ineffective against isoproterenol (65, 70, 96, 97, 99), but reduced somewhat the incidence and severity of lesions caused by α - receptor agonists, such as phenylephrine (82, 99), epinephrine (70, 82, 99, 100) and norepinephrine (70, 99). The α - blockers also ameliorated the loss of myocardial AAT and lactic dehydrogenase activity, and shifts of electrolytes caused by epinephrine and norepinephrine (59, 70, 101) and were usually more effective against epinephrine lesions when used in combination with a β - blocker (59, 82, 99). It should be pointed out that isoproterenol has been shown

to reduce the endogenous norepinephrine stores from the nerve endings (102) and it is possible that the endogenously released norepinephrine may also be participating in producing the cardiotoxic effects upon injecting the animals with isoproterenol.

The post-ganglionic blocker guanethidine has been reported to be ineffective (65, 97) or to increase the severity (94) of necrosis caused by isoproterenol. Another post-ganglionic blocker, isocaramidine, has also been found to have no effect on isoproterenol - induced necrosis (65). Reserpine, which is known to decrease catecholamine stores, has been reported to be without effect (65, 93) or to increase the severity (18, 94) of isoproterenol-induced lesions. Pyrogallol, a catechol - O - methyl transferase inhibitor, also increased the severity of lesions (18). Serotonin and nialimide administered together reduced the severity and incidence of myocardial lesions (103). Other drugs found not to influence the production of lesions by isoproterenol are the vasodilators sodium nitrite, aminophylline, dipyridamole, and hexobendine, and the psychosedatives chlorpromazine, chlordiazepoxide, meprobamate, and amitriptyline (65). In this regard it is worth mentioning that most of these drugs are not specific with respect to their site of action and conclusions drawn from such studies should be interpreted with some caution.

Inhibition of lipolysis by nicotinic acid or beta pyridyl carbinol decreased the amount by which isoproterenol infusion increased the myocardial oxygen consumption (104). Chronic administration of nicotine in high doses tended to increase the severity and incidence of lesions produced by isoproterenol (105). The calcium blockers, Verapamil, D-600, prenylamine, and vascoril reduced the severity of lesions and prevented the decrease in high energy phosphate stores and accumulation of calcium by the myocardium caused by isoproterenol injections (76, 77, 106). Thus it is apparent that agents which affect lipid and calcium metabolism are also capable of modifying the cardiotoxic effects of high doses of catecholamines.

The mineralocorticoids such as deoxycorticosterone, and 9 α - fluorocortisol increased the severity of myocardial lesions (38, 77, 89, 90, 107), the level of Ca^{45} accumulation by the heart (76, 77), and the severity of high energy phosphate depletion (77) caused by isoproterenol injections. Among the other steroids, estrone and testosterone also increased the severity of necrotic lesions (89, 90), whereas

estrogen, progesterone, glucocorticoids, cortisone and 9 α - fluoro - 16 hydrocortisol are without effect as were ACTH or adrenalectomy (89, 90, 108). High sodium or low potassium diets were similar to mineralocorticoid therapy in increasing the severity of lesions, whereas low sodium or high potassium diets reduced the incidence and severity of lesions (89, 90, 107). Administration of KCl, $MgCl_2$ or NH_4Cl_2 reduced the severity of lesions (65, 77, 109) and protected against the electrolyte shifts and reduction of high energy phosphate stores (67,77, 109). On the other hand, if plasma Mg^{++}, K^+, of H^+ concentrations were low, isoproterenol-induced lesions were potentiated (110). Administration of K^+, Mg^{++} - aspartate administered together with isoproterenol has also been found to prevent or reduce the changes in myofibrillar ATPase activity, Ca^{++} accumulation by mitochondria and microsomes, and high energy phosphate stores (80), and to decrease the severity of ultrastructural damage to the myocardium (111).

Thyroxin and hyperthyroidisim increased the severity of lesions (39, 89, 90) whereas thyroidectomy, thiouracil, or propylthiouracil decreased the extent of necrosis with isoproterenol (39, 89, 90, 93). Calciferol and another antirachitic agent, dihydrotachysterol, increased the severity of necrotic lesions, as did $NaHPO_4$ and increased blood pH (76, 77, 89). The increased severity of the lesions was associated with a further increase in the uptake of Ca^{45} and a greater fall of high energy phosphate stores of the heart. Likewise, thyrocalcitonin, reduction of plasma calcium with EDTA, and a decrease in blood pH decreased the extent of both lesions and electrolyte shifts (77).

Administration of glucose, lactate, or pyruvate had no effect on the extent and severity of catecholamine - induced lesions (65). Sex and breed in rats did not influence the severity of the myocardial damage, although the severity was increased with increased body weight and excess body fat (89, 90, 112, 113). The severity of lesions did increase with age, but this is probably an indirect effect related to the increase in body weight with age (114). Starvation or restricted food intake likewise tended to reduce the severity of lesions (89, 90, 115).

Previous myocardial damage markedly reduced the severity of lesions produced by high doses of isoproterenol (116 - 118). This protective effect disappeared with time, was independent of the part of the heart previously damaged,

and did not result from necrosis of extracardiac tissues. Similarly, previous isoproterenol injections (112, 119, 120) and coronary arteriosclerosis (64, 121) increased the resistance of the heart to isoproterenol - induced damage. Cardiac hypertrophy (116) or a simultaneous hypoxia (92) increased the extent and severity of lesions. A higher ambient temperature also potentiated the necrotic effect of isoproterenol, possibly due to the increased work load of the heart during thermoregulatory vasodilation (122) as well as changes in the calcium transport mechanisms (81). On the other hand, high altitude acclimation or hyperbaric oxygen tended to protect the heart against necrotic damage (123 - 125). Isolation stress or cold exposure both increased the severity of isoproterenol - induced lesions and electrolyte shifts (89, 90, 112, 126, 127), although this may be an indirect result of increased mineralocorticoid production which occurs under these conditions (126 - 128).

Thus, it would appear that factors tending to increase the work load of the heart, increase the metabolic rate of the heart, interfere with oxygen supply to myocardial cells, favor the electrolyte changes, or favor mobilization of lipids aggravate the necrotic influence of catecholamine administration. On the other hand, factors which block the stimulatory effects of catecholamines, thereby reducing cardiac work, or otherwise reduce myocardial metabolic rate, aid in the supply of oxygen to the myocardium, limit the mobilization of lipids, or counteract the ionic shifts can at least reduce the severity of necrotic changes. Although the protective effect of MAOI compounds is still enigmatic, it is clear from the above that an imbalance between oxygen availability and work, the metabolism of lipids and the alteration of electrolyte balance are all crucial factors contributing to the etiology of catecholamine lesions. The evidence available in the existing literature does not permit the identification of a single molecular lesion which can be considered responsible for the pathogenesis of myocardial necrosis due to catecholamines.

D. Hypotheses Concerning the Mechanisms by which Catecholamines Elicit Cardiac Lesions:

It has been for the most part assumed that the cardiac cell necrosis that occurs following administration of catecholamines in large amounts is due to a defect in the supply of energy for the maintenance of essential cellular processes. Various

theories have been proposed with regard to the cause of the energy deficiency and the nature of the irreversible step following decreased energy availability. The major hypotheses include: a relative cardiac hypoxemia due to increased cardiac work and myocardial oxygen demands, aggravated by hypotension in the case of isoproterenol (10, 40); coronary arterial vasoconstriction (spasm) causing endocardial ischemia (7, 8); inadequate perfusion of the endocardium due to impaired venous drainage of the heart (129); hypoxia due to direct oxygen wasting effects of catecholamines or their oxidation products (16); interference with mitochondrial oxidative phosphorylation by free fatty acids (104, 130), massive calcium influx (77), or formation of adrenochromes (17). Potassium depletion (131), altered permeability of the myocardial cell membrane through elevation of plasma non-esterified free fatty acids (132) and depletion of intracellular magnesium required for many ATP-dependent enzymatic processes (82, 109) have also been suggested to explain myocardial necrosis due to catecholamines.

The concept of a relative cardiac hypoxemia due to increased cardiac work and myocardial oxygen demands was advanced by Rona et. al. (10, 40) following their discovery of the very great cardiotoxicity of isoproterenol. It was found that both high and low doses of isoproterenol produced similar increases in heart rate in dogs, but a much more profound fall in blood pressure was seen with higher lesion producing doses of isoproterenol, and it was suggested that the fall in aortic blood pressure was of such a degree that a reduced coronary flow could be inferred. It was further postulated that the necrotic lesions are an ischemic infarct due to a decreased coronary flow during a time when both amplitude and frequency of cardiac contractions are increased. Thus the greater cardiotoxicity of isoproterenol as compared to epinephrine or norepinephrine is attributed to the dramatic hypotension produced by this drug, and various factors, such as previous myocardial damage or previous isoproterenol injections, activate metabolic processes which provide cardiac muscle cells with an enhanced adaptation to withstand the increased demand and relative hypoxia produced by isoproterenol (133).

On the other hand, Rosenblum et. al. (14) found that mephenteramine, dl-ephedrine and d-amphetamine produced lesions in less than 50% of animals, although these agents increase blood pressure while ephedrine and amphetamine have

a positive inotropic effect. Accordingly, drugs with both positive inotropic and chronotropic actions may not produce cardiac lesions. Also, methoxamine which has no positive inotropic effect was found to produce cardiac lesions. In another study (15), the hemodynamic effects of "pharmacologic" and "lesion - producing" doses of sympathomimetics were compared in cats. It was found that lesion-producing doses of isoproterenol caused a decrease in aortic flow and heart rate as compared to pharmacologic doses, but these were still above control values. Stroke work was greater with lesion-producing doses as compared to pharmacologic doses, but mean aortic pressure, which determines the coronary perfusion pressure, was not reduced by lesion-producing doses of isoproterenol. Thus there is evidence of impaired function of the myocardium, but the hemodynamic changes do not appear adequate to produce insufficient myocardial perfusion. As a result of these findings it was suggested that the effects of isoproterenol were due to some direct action on the myocardial cell and not solely to the hemodynamic effects. Maruffo (11) also considered it unlikely that in experimental monkeys, the coronary flow could have been greatly reduced by isoproterenol, since blood pressure remained above that found in shock and was associated with increased cardiac output. Strubelt and Siegers (134) also concluded that hypotension is non-essential for production of cardiac necrosis by isoproterenol after finding that verapamil was effective in protecting the heart from isoproterenol-induced necrosis even though blood pressure fell almost twice as much when verapamil was administered together with isoproterenol as it did following administration of isoproterenol alone.

Another hypothesis closely related to that of coronary insufficiency of hemodynamic origin is that of a relative ischemia resulting from coronary vascular changes. Handforth (7) injected India ink at a pressure of 50 mm Hg into the aorta of hearts excised from hamsters 5 - 30 minutes after isoproterenol injection. The dye failed to penetrate large areas of the endocardium in 6 out of 14 hearts from isoproterenol injected hamsters, although uniform filling of capillaries was seen in control hearts. It was suggested that dilatation of arteriovenous shunts might be responsible for the endocardial ischemia, since coronary flow is usually found to be increased with isoproterenol. In a subsequent study, however (8), it was noted that

the superficial veins draining these areas were also not filled with ink. A similar experiment was performed with bismuth oxychloride in which these larger particles did not reach the veins. This evidence was interpreted to indicate a true endocardial ischemia, postulated to be due to arterial constriction rather than arteriovenous shunts. More consistent evidence of endocardial ischemia was seen at one hour than had been observed at 5 - 30 minutes, therefore coronary artery constriction was considered to be more important than peripheral vasodilation since the drop in peripheral resistance occurred within minutes of injection. A coronary arteritis was also found 1 - 3 days after isoproterenol injection (8) which was considered to support the concept of a vascular component in the etiology of necrosis. Ostadol and Poupa (135) repeated these experiments and reported a marked occlusion of coronary vessels in 69% of animals at 30 minutes, 33% at 60 minutes, and practically no occlusion at 24 hours after isoproterenol injection. These workers assumed this to be due to spasm of the coronary vessels, but the importance of peripheral resistance changes is emphasized by their observation that, whereas occlusion was evident with injection of India ink at 50 mm Hg, uniform filling of capillaries was seen even in isoproterenol treated animals if an injection pressure of 100 mm Hg was used. Jasmin (129) found that it was possible to reproduce essentially similar pathological changes by surgical occlusion of efferent vessels, and on this basis suggested that impairment of venous drainage via venospasm largely accounts for the adverse effects of sympathomimetic amines.

The occurrence of coronary arterial or venous spasms is of course only conjectural, and a somewhat simpler explanation of the impaired perfusion of the endocardium can be inferred from certain other studies of the circulation in the heart. Blood flow to left ventricular subendocardial muscle has been suggested to be compromised during systole and to occur mainly during diastole because intramyocardial compressive forces are greatest in this region (136, 137). Furthermore, it has recently been shown (138) that when aortic diastolic pressure was lowered or diastole shortened (by pacing) and myocardial oxygen demands simultaneously raised, myocardial performance was found to be impaired. In a subsequent study (139), coronary flow distribution was determined by scintillation counting of the distribution of ^{141}Ce, ^{85}Sr, ^{46}Sc labelled microspheres (12-15 μ diameter) injected into the left

atrium during isoproterenol infusion. When isoproterenol was infused at a rate which failed to maintain an increase in contractile force, it was found that subendocardial flow fell by 35% while subepicardial flow increased by 19%. Thus, although spasm of coronary arteries and /or veins may well occur, it is possible that increased cardiac activity, reduced aortic pressure, and greatly decreased diastole could also be responsible for an underperfusion of the endocardium.

A serious challenge to the concept of impaired ventricular perfusion as the primary cause of necrosis was presented by the findings of Regan et. al. (13) who used ^{85}Kr clearance methods to study perfusion of the ventricle during epinephrine infusion in dogs. Evidence of cardiac necrosis was obtained by 75 minutes after the start of epinephrine infusion, but ^{85}Kr clearance studies showed no difference in the rate of clearance from inner, middle, and outer layers of the left ventricle in either the control or epinephrine treated hearts. Thus, there was no evidence for ischemia of sub-endocardial tissue as a causal factor in the epinephrine-induced necrosis. On the other hand, a more recent study by Boutet et. al. (5) in which isoproterenol was infused in rats at a low concentration, a decreased passage of the trace substance horseradish peroxidase from the capillaries to the myocardial interstitium was observed. Thus this controversy still remains to be resolved.

The hypothesis that the previously described vascular factors are the primary cause of necrotic lesions has also been tested by Ostadal et. al. (12) using the turtle heart as a unique model in which perfusion of the endocardium is avascular. In the turtle heart, the internal spongy masculature is supplied by diffusion from the ventricular lumen via intertrabecular spaces, while the outer compact layer is supplied by the coronary artery branching off the aorta. Isoproterenol injections were found to produce necrotic lesions in the spongy layer of the turtle heart, which does not support the opinion that isoproterenol-induced cardiac necroses are due to a vascular mechanism and are in fact ischemic infarcts.

As Bajusz (140) has pointed out in his careful survey of data on various types of cardiac necroses, catecholamine - induced necrosis must be considered to be of a mixed pathogenesis involving both direct metabolic actions on the fibers as well as factors secondary to vascular and hemodynamic effects. Although cardiac lesions induced by epinephrine, methoxamine and isoproterenol are indistinguishable

as regards both distribution and histologic characteristics, it would appear that the methoxamine-induced lesion is a typical secondary cardiomyopathy, while that due to epinephrine and isoproterenol is of a mixed type in which both hypoxia secondary to vascular and hemodynamic effects as well as direct metabolic effects on the heart muscle have a role. It is not, then, unreasonable to regard vascular and hemodynamic effects as complicating factors which greatly aggravate some more direct toxic influence of catecholamines on myocardial cells, in view of which it is readily understood how a reduction in the extent and severity of catecholamine-induced lesions is brought about by interventions which specifically block the peripheral vascular changes, prevent the positive inotropic and chronotropic effects of these drugs on the heart, or which improve the delivery of oxygen to the myocardium.

Many years ago Raab (16) attributed the cardiotoxic actions of catecholamines to their oxygen wasting effect. According to him:

> "The most conspicuous reaction of myocardial metabolism to the administration of adrenaline is an intense enhancement of local oxygen consumption which, in certain dosages, by far exceeds the demand of simultaneously increased myocardial muscular work, and which is only partially compensated by a simultaneous increase of coronary blood flow. In these respects adrenaline is able, so to speak, to mimic the anoxiating effects of coronary insufficiency in the absence of any real coronary anomaly. It should be emphasized, however, that the tissue anoxia resulting from the administration of adrenaline is probably not caused by adrenaline itself but by an oxidation product of adrenaline (Bruno Kisch's omega) which acts as an oxidation catalyst even in very high dilutions."

In a later study (141) it was found that identical electrocardiographic changes occurred during cardiac sympathetic nerve stimulation, electrically induced muscular exercise, or intravenous injection of norepinephrine or epinephrine, when coronary artery dilatability is impaired and during exogenous anoxia or partial occlusion of the coronary arteries. This was taken as evidence that the increased

O_2 consumption caused by catecholamines produced a relative hypoxia if coronary flow could not be sufficiently increased. A crucial point in this concept concerns the origin of the increased O_2 consumption with catecholamines - whether it is due to a decreased efficiency of oxygen utilization, or to increased demand. Lee and Yu (142) found the oxygen consumption of resting papillary muscles not to be increased by catecholamines even in concentrations ten times higher than those effective in stimulating oxygen consumption of contracting papillary muscles, and concluded that the increased oxygen consumption of the intact heart following administration of epinephrine or norepinephrine is secondary to the increased contractility. On the other hand, Weisfeldt and Gilmore (143) reported that low doses of norepinephrine exerted a maximal inotropic effect with little or no increase of O_2 consumption, while larger doses had no further inotropic effect but did increase O_2 consumption, indicating that it is excessive catecholamine concentrations which cause "oxygen-wasting." Klocke et. al. (144) studied the increase in oxygen consumption produced by catecholamines in the isolated dog heart. It was found that the increase of oxygen consumption of the potassium-arrested heart caused by catecholamines was 5 - 20% of that found in the beating heart, and thus concluded that most, but not all, of the increased oxygen consumption was secondary to hemodynamic alterations and increased cardiac work. In a similar comparison of the effects of epinephrine on oxygen consumption in beating and arrested hearts, Challoner and Steinberg (145) found that about one third of the increment in oxygen consumption in beating hearts was accounted for by a metabolic effect dissociable from increased work. Haug and Øye (146) confirmed an increased oxygen consumption in arrested hearts with epinephrine, and reported that this effect could be blocked by dichloroisoproterenol but not by phentolamine. From these studies it is evident that catecholamines can cause an increase in oxygen consumption that is not related to increased cardiac work or activity and the concept of decreased efficiency or "oxygen-wasting" is therefore justified. Furthermore, Raab (16) has suggested that this oxygen-wasting effect was actually due to an oxidation product of epinephrine, and one such oxidation product, adrenochrome, has been shown to uncouple mitochondria (147). The uncoupling of mitochondria by adrenochrome was antagonized by glutathione in high concentration, probably due to a direct reduction of

adrenochrome since the characteristic red color of adrenochrome was lost when glutathione was added in the presence or absence of mitochondria, whereas oxidized glutathione did not effect uncoupling by adrenochrome.

Sobel et . al . (17) found the P/O ratio of heart mitochondria from animals given norepinephrine, epinephrine or isoproterenol to be significantly lower than controls. RCI and QO_2 were similar to control, but unfortunately the control RCI values in these experiments were very low. A good relationship between elevation of myocardial catecholamine content and depression of P/O ratio in mitochondria was observed. Whereas propranolol pretreatment enhanced the increase in myocardial catecholamines and caused a more marked depression of mitochondrial P/O ratios, dibenzyline inhibited both the increase in catecholamine contents and the decrease in the mitochondrial P/O ratio. Reserpine pretreatment caused a depletion of myocardial catecholamines and prevented the depression of the mitochondrial P/O ratio. Since the three catecholamines used did not effect the P/O ratio of normal rat heart mitochondria in vitro at a concentration of 10^{-3}M, it was concluded that this was not a direct action of the catecholamine on the mitochondria, and it was suggested that adrenochrome or one of its metabolites might be responsible for the observed effects (17). Stanton and Schwartz (71) also studied the oxidative phosphorylation of heart mitochondria from isoproterenol treated rats, but found the RCI was reduced without affecting the P/O ratio. Unfortunately, no data on QO_2 was given. Since these measurements were made 17 hours after the administration of isoproterenol they may not reflect important changes occurring in the development of myocardial cell necrosis. It is not possible to draw any definite conclusions from these studies as regards the effects of catecholamines on mitochondrial respiration, although uncoupling of oxidative phosphorylation is certainly indicated and would explain both the "oxygen-wasting" effect and the depletion of myocardial high energy phosphate stores caused by large doses of catecholamines. Likewise, the possibility that the agent producing this uncoupling might be adrenochrome or some one of its metabolities is intriguing.

Having found that heart mitochondria from catecholamine treated rats were uncoupled, Sobel et . al . (17) determined the free fatty acid levels of the mitochondria since free fatty acids are known to uncouple mitochondria. No

differences in mitochondrial free fatty acid content or composition was found and it was thus concluded that the observed uncoupling was not due to accumulation of fatty acids. Furthermore, ephedrine, which produced no significant changes in plasma non-esterified free fatty acids, can cause cardiac lesions (132). Nevertheless, Mjos (104) found that inhibition of lipolysis by nicotinic acid, beta pyridyl carbinol, or high plasma glucose concentrations during infusion of isoproterenol could substantially reduce the increase in myocardial oxygen consumption, possibly by preventing an uncoupling action of high intracelluldr concentrations of free fatty acid in the heart following catecholamine administration. Hoak et. al. (130) have also postulated a causal relationship between the increases in plasma free fatty acids following norepinephrine administration and the occurrence of cardiac lesions. Although the evidence fails to implicate elevated levels of free fatty acids as primary agents in mitochondrial uncoupling following administration of catecholamines, from the findings of Mjos (104) as well as the previous correlation of severity of lesions with the amount of body fat (89), it is evident that metabolism of free fatty acids in some way aggravates the cardiotoxic effects of catecholamines.

In view of the close relationship between electrolyte shifts and the occurrence of necrotic lesions, Lehr (82) has suggested that changes in myocardial electrolyte content initiated by altered ionic transfer ability of myocardial cells at the plasma membrane and subcellular membrane sites contribute to irreversible failure of cell function. It is the loss of cellular magnesium, in particular, he suggested (109) that is most critical in the pathogenesis of irreversible damage. In this regard, it was pointed out that Mg^{++} is an important prosthetic or activator ion participating in the function of many enzymes involved in phosphate transfer reactions, including utilization of ATP. Unfortunately, this mechanism is not adequate to explain the reduction in high energy phosphate content in the myocardium (77) since interference with utilization would have the opposite effect. On the other hand, magnesium is reported to cause a decrease in the respiration supported uptake of calcium by isolated heart mitochondria (148) and could thus be important in regulating mitochondrial function in terms of oxidative phosphorylation versus calcium uptake.

Raab (131) has similarly argued that it is the derangement of myocardial

electrolyte balance, most specifically the loss of K^+ and Mg^{++} ions from the myocardium, which is the central mechanism in a variety of cardiomyopathies. But this derangement of electrolyte balance is considered to be secondary to an inadequate supply of energy for transmembrane ion pumps required for maintenance of electrolyte equilibrium which occurs with oxygen deficiency or impaired energy production. Bajusz (140) has also suggested that electrolyte shifts are an important component in the development of irreversible damage produced by both direct and indirect pathogenic mechanisms, and that myocardial resistance is related to the ability of the heart to maintain a normal electrolyte balance in the face of potentially cardiotoxic episodes.

Fleckenstein et. al. (77) found that the isoproterenol - induced necroses and decline in high energy phosphates were associated with a 6 - 7 fold increase in the rate of radioactive calcium uptake and a doubling of net myocardial calcium content, and suggested that isoproterenol causes a greatly increased influx of calcium which overloads the fiber. It was postulated that the intracellular calcium overload initiates a high energy phosphate deficiency by excessive activation of Ca-dependent intracellular ATPases and by impairing mitochondrial oxidative phosphorylation. When high energy phosphate exhaustion reaches a critical level, fiber necrosis results. This hypothesis attempts to explain why myocardium can be sensitized to isoproterenol-induced necrosis by factors, such as 9 α - fluorocortisol acetate, dihydrotachysterol, NaH_2PO_4, high extracellular calcium, or increased blood pH, which favor calcium overload. Consistent with this hypothesis, K and Mg salts, low extracellular calcium, thyrocalcitonin, low blood pH, or specific blockers of transmembrane calcium fluxes protect the heart against isoproterenol, presumably by preventing calcium overload. In support of this concept of a central role for Ca^{++} in the pathogenesis of necrosis is the finding that spontaneous necrotization of cardiac tissues of myopathic hamsters is prevented by treatment with the calcium blocker verapamil (149, 150). Also, necrosis of skeletal muscle fibers can be induced through mechanical injury of the cell membrane permitting Ca^{++} - influx, and can be prevented by elimination of Ca^{++} from the Ringer solution or by an outward electric current which blocks Ca^{++} influx (151).

Unfortunately, there is no direct evidence that it is in fact calcium which

produces the decline of high energy phosphates in the hearts of animals given isoproterenol, and therefore a causal relationship has not yet been established. Furthermore, Bloom and Davis (95) have found that myocardial calcium content increased in a manner well correlated to isoproterenol dose in the range from 0.1 10 μg/Kg, but did not further increase with higher dose levels required to produce myocardial lesions. Thus, it was suggested that the inotropic response is related to calcium entry, but that necrosis is due to some other factor, possibly including the intracellular metabolism of calcium. It was also shown by these workers that propranolol could completely block the increase of calcium content of the myocardium but would only reduce the incidence of lesions rather than preventing them. Bloom and Davis (95) have interpreted their results to indicate a mechanism other than calcium influx in isoproterenol-induced necrosis, but the dramatic modification of necrosis by factors influencing transmembrane calcium fluxes clearly suggests the involvement of calcium at some level in the etiology of necrosis caused by catecholamines.

Muller (18) has suggested that, on the basis of coincidence of localization of isoproterenol induced myocardial lesions and the highest myocardial MAO activity, the accumulation of amines metabolically formed during deamination of catecholamines may be the cause of necrosis in the heart. It is further pointed out that in young rats, heart MAO activity is less than in older rats, and that isoproterenol sensitivity is also less. None of the hypotheses discussed so far account for these findings as well as the protective effect of MAOI. Likewise, no specific explanation has been offerred for the changes in contractile proteins which are seen to occur in catecholamine-induced necrotic lesions except for the suggestion by Pelouch et. al. (79) that a direct interaction of catecholamine or some metabolite with the heavy meromyosin region of the myosin molecule is involved. It may very well be that lysosomes are activated due to calcium overload and decreased energy state of the myocardium after catecholamine injection and this may produce cellular damage. Furthermore, catecholamine are known to markedly increase the concentration of cyclic AMP in the heart, and it is likely that this agent in high concentrations may represent an important factor for causing catecholamine - induced myocardial necrosis.

In summary, the majority of the factors found to influence the severity of

catecholamine - induced lesions can be understood in terms of their effects on hemodynamic factors, delivery of oxygen to the myocardium, electrolyte balance, or the metabolism of calcium and lipids. It would thus appear that hemodynamic and coronary vascular factors contribute significantly to the severity of myocardial damage following catecholamine administration, but that some primary pathogenic mechanism acting directly on the myocardial cell is probably involved as well. Furthermore, it is clear that exhaustion of high energy phosphate stores and disruption of electrolyte balance are crucial events in the etiology of irreversible cell damage, and although the metabolism of lipids and calcium are clearly involved, the nature of the direct pathogenic influence following injection of catecholamines is yet unknown.

E. The Physiological Significance of Adrenochrome and Related Oxidation Products.

The oxidation products of epinephrine, adrenochrome and adrenolutin, have been identified in the heart, skeletal muscle, liver brain and kidney of rabbits (152 - 154) by paper chromatography and by their fluorescence properties. In addition to the spontaneous oxidation of epinephrine to adrenochrome by an autocatalytic process (155), adrenochrome is enzymatically formed in mammalian tissues. These enzymes include tyrosinases (156 - 161), and polyphenol oxidases from various sources (161 - 165), particularly in guinea pig and rat muscles (165). Other enzyme systems shown to actively convert epinephrine to adrenochrome are xanthine oxidase (166), leukocyte myeloperoxidase (167, 168), heart muscle cytochrome oxidase (169 - 171), a cyanide insensitive system present in heart and skeletal muscle (172), the cytochrome-indophenol oxidase system present in all tissues (172, 173), and an unidentified enzyme in cat salivary gland (174). The oxidation of epinephrine has also been reported to be catalyzed by cytochrome C and by methaemoglobin (175), to stabilize with the formation of adrenochrome in the presence of bicarbonate buffer, regardless of the oxidizing system used (176), and to occur in blood (177, 178).

Much of the work in the literature dealing with the physiological and pharmacological effects of epinephrine oxidation products was carried out before the structure of adrenochrome was known, and still more before a relatively pure preparation

could be obtained, as has been discussed by Hoffer in his review on this subject (179). Furthermore, due to the inherent instability of adrenochrome in solution, one can not be certain whether reports attributing either specific effects or the absence of certain effects can truly be considered as an accurate assessment of the adrenochrome activity. Nevertheless, it is well worth reviewing the broad spectrum of physiological activities which have been found to result from adrenochrome or at least from some closely related epinephrine oxidation product to which adrenochrome is converted.

Oxidized epinephrine solutions have been found to powerfully inhibit cardiac inotropism and chronotropism (157, 158, 162, 180 - 182), and to increase rather than decrease the tone of rabbit intestinal strips (183). Adrenochrome has been reported to increase blood pressure (184), 185), to be effective as a vasoconstrictor (186), to be a powerful hemostatic agent, and to greatly diminish capillary permeability (187, 188). Adrenochrome has frequently been reported to reduce blood sugar levels and potentiate the effects of insulin and has been described as an anti-insulinase (179, 189), although this claim has been disputed (190).

Administration of adrenochrome either by injection or by feeding can result in an increased oxygen consumption in humans and guinea pigs (191, 192), and has been reported to increase tissue oxygen consumption in vitro (193, 194). On the other hand, other workers have found that adrenochrome may stimulate, inhibit, or have no effect on tissue oxygen consumption depending upon the metabolic substrate utilized (195) or the adrenochrome concentration (196), which may explain reports that adrenochrome did not effect the oxygen uptake of rat muscle (197) and inhibited oxygen uptake and lactic acid production in dog heart slices (198).

Adrenochrome has also been reported to uncouple mitochondrial oxidative phosphorylation, depressing P:O ratios (147, 199, 200), and it has been suggested that adrenochrome may act as a hydrogen carrier between substrate and molecular oxygen with the formation of water and regeneration of adrenochrome after each cycle (199). It was also reported to be much more effective in inhibiting pyruvate oxidation than succinate oxidation (200). Adrenoxyl, a closely related epinephrine oxidation product, has been found to lower mitochondrial potassium content, decrease ATPase activity, and to alter mitochondrial P:O ratios (201). Adrenochrome also inhibited hexokinase and phosphofructokinase, thus inhibiting glucose phosphorylation

and glycolysis, while stimulating glycogen synthesis (172, 202 - 205) and the hexose monophosphate shunt (206, 207). Adrenochrome and adrenoxyl have both been found to be inhibitors of myosin ATPase activity in heart and smooth muscle (209 - 211). Adrenochrome and oxidized epinephrine solutions not only inhibited mono-amine oxidase in a variety of tissues (212-214) but the enzyme, alkaline phosphatase, also (215). It was thought that inhibition of all of the above enzymes is due at least partly to the reversible oxidation of sulfhydryl groups on the enzymes (210, 212). Other effects attributed to adrenochrome and adrenoxyl include antimitotic activity (216), reduction of coenzyme A levels in heart, kidneys and brain (217), anti-histaminic properties (179), and an increase in mitochondrial material of cultured cells (218). Thus it can be appreciated that adrenochrome is a highly reactive molecule chemically, and is not only capable of oxidizing protein sulfhydryl groups, but is also a dynamic catalyst for the deamination of a variety of amines and amino acids (219 - 223). Furthermore, it may very likely be capable of functioning as an oxidative hydrogen carrier acting upon either metabolic substrates (199) or $NADP^+$ (207) and thus altering or disrupting essential metabolic pathways.

The metabolism of adrenochrome and related epinephrine oxidation products has been studied in rabbits, cats, and dogs (224 - 226). Adrenochrome injected into rabbits rapidly disappeared from the blood and was transformed in the liver to adrenolutin which was removed from the system via the kidney (224). Most of it was excreted in the urine as adrenolutin (both free and conjugated), or in the form of a flourescent brown pigment, while a small amount was excreted unchanged. In cats and dogs, approximately 70 per cent was excreted in the form of a variety of adrenochrome reduction products and other indoles (225). An unstable yellow pigment has been observed in the urine of rats after injection of C^{14} labelled adrenochrome (227).

To summarize, there is evidence both for the presence of and the formation of adrenochrome in mammalian tissues, and adrenochrome has been shown to be capable of producing a wide variety of metabolic changes by interfering with numerous enzyme systems. Thus adrenochrome and / or other catecholamine oxidation products may be regarded as possible candidates for agents involved in

toxic manifestations occurring in conjunction with catecholamine excess or altered catecholamine metabolism. The injection of catecholamine into animals can be conceived to result in the formation of oxidation products such as adrenochrome in the circulating blood as well as in the myocardial cell. The accumulation of these oxidation products in myocardium could then directly or indirectly, acting by themselves or in conjunction with other effects of catecholamines, initiate processes leading to myocardial necrosis. The present study is therefore intended to provide some information on these aspects.

III. METHODS

Male Sprague-Dawley rats having a body weight of 300 - 350 g were decapitated, the hearts quickly removed and arranged for perfusion by the conventional Langendorff technique. The perfusion medium was Krebs-Henseleit solution containing NaCl, 120 mM; $NaHCO_3$, 25 mM; KCl, 4.8 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.2 mM; $CaCl_2$, 1.25 mM; and glucose, 8 mM. The perfusion solution, pH 7.4, was continually gassed with a mixture of 95% O_2 and 5% CO_2, and the perfusion temperature was maintained at 38°C. The perfusion rate (7.8 ml/min) was controlled by using a Harvard peristaltic pump in all experiments.

For certain experiments the composition of the perfusion medium was modified by varying the individual components. When sodium chloride concentration was reduced, an equivalent amount of sucrose was added to the solution. Corrections were not made for changes in the concentration of calcium chloride, potassium chloride or magnesium chloride. The pharmacologic agents which were utilized in this study were dissolved directly in the perfusion medium. These were: iproniazide (25 mg/l), tranylcypromine (25 mg/l) propranolol (1 mg/l), practolol (1 mg/l), dibenamine (25 mg/l), tolazoline (25 mg/l), D-600 (0.5 mg/l), guanethidine (2 mg/l) and bretylium (2 mg/l). Also employed were: ascorbic acid (1 mM), cysteine (0.5 mM), and dithiothriatol (0.5 mM).

Contractile force development and resting tension were monitered throughout each experiment by means of a steel hook in the apex of the heart connected via a short length of silk thread to a Grass force displacement transducer. Time to peak tension and time for 1/2 relaxation were calculated from the contractile force recordings obtained at the maximum speed of the recorder (100 mm/sec). The hearts were electrically driven at 360 beats/min throughout all experiments, except those involving altered K^+ concentration, using a square wave stimulator (Phipps & Bird) to apply pulses of 1-3 volts and 2 msec duration between two platinum electrodes located in the apex of the heart and in the intraventricular septum at the base of the heart. In experiments where the K^+ content of the perfusion medium was altered, hearts were driven at 180 beats/min to avoid arrythmias and fibrillation. The atria were removed and the atrio-ventricular node crushed to facilitate external

control of the heart rate.

The hearts were permitted a 20 minute period for equilibration following commencement of perfusion prior to the administration of isoproterenol, oxidized isoproterenol, epinephrine, dihydroxymandelic acid, metanephrine, vanilmandelic acid, or adrenochrome. In experiments designed to study the influence of altered ionic composition of the perfusion medium, or the presence of various pharmacologic agents, these modifications were present from the beginning of the perfusion in order that the hearts would be equilibrated to these factors prior to the administration of adrenochrome.

To minimize oxidation, isoproterenol was dissolved in the perfusion medium at a concentration of 4 mg/ml and infused into the perfusion cannula at a constant rate of 0.2 ml/min by means of a syringe pump (Sage, model 341). The final concentration of the drug reaching the heart was thus approximately 100 mg/liter. The infusion solution used for the study of the effects of unoxidized isoproterenol was freshly made, replaced at 5 - 10 minute intervals during the course of each experiment, and discarded if there was any evidence of colouration. The infusion solution used for the study of the effects of oxidized isoproterenol was made 6 to 8 hours prior to use, during which interval it was continuously gassed with 95% O_2 - 5% CO_2, and at the time of use was of a very dark reddish brown colour. When such solutions were permitted to stand for very long periods of time (16 to 24 hours) they became more transparent, having a yellow-brown colouration, and were found to be greatly reduced in potency. Spectral scans of these infusion solutions were carried out following a 40 fold dilution with Krebs-Henseleit solution (the solvent) using an Aminco DW-2 Spectrophotometer.

It was subsequently found that more potent solutions of spontaneously oxidized isoproterenol were obtained if solutions of perfusion medium containing the desired final concentration of isoproterenol were gassed with 95% O_2 - 5% CO_2 at room temperature for 12 hours prior to use. Solutions prepared in this manner have a pink to bright red colouration, depending upon the concentration of isoproterenol used, and were used to study the effects of concentration on contractile force.

For the experiments using adrenochrome, epinephrine, metanephrine, dihydroxymandelic acid, and vanilmandelic acid, small volumes of appropriate

concentration in perfusion medium previously gassed with 95% O_2 - 5% CO_2 were prepared just prior to use. In order to investigate the possible synergistic effect of epinephrine and adrenochrome, hearts were perfused with solution containing adrenochrome (50 or 25 mg/l) at a rate of 7.8 ml/min while a fresh epinephrine solution of a concentration of 40 mg/l was infused at a rate of 0.2 ml/min togive a final concentration of 1 mg/l of epinephrine in the perfusion medium reaching the heart.

At the end of each experiment the hearts were abruptly switched to perfusion with a solution of 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 5 minutes as a preliminary fixation for electron microscopic studies. Small portions of the left ventricle of each heart were subsequently dissected out, washed for 4 - 6 hours in 0.1 M phosphate buffer, and post-fixed for 1 hour in a solution of 1% OsO_4 in 0.1 M phosphate buffer. These specimens were then dehydrated in a graded ethanol series and embedded in Epon. Sections were made using a diamond or glass knives on a Sorvall MT - 2 ultramicrotome, stained with uranyl acetate and / or lead citrate, and examined using a Zeiss EM9S electron microscope.

At least four different hearts were used for each observation. The drugs used in this study were hydrochloride salts. The results were analyzed by the Student "t" test.

IV. RESULTS

A. Effect of Perfusion with Isoproterenol and Oxidized Isoproterenol on Contractile Force Development and Ultrastructure.

A systematic comparison of perfusion with unoxidized and oxidized isoproterenol (100 mg/liter) revealed a dramatic contrast in terms of their effects on force development parameters of the isolated rat hearts. Although the developed contractile tension was not significantly ($P>0.05$) greater than the control level with the infusion of freshly made isoproterenol solutions, the maximum rate of tension development was increased by 40%, and the time to peak tension was greatly reduced (Figure 1). Infusion of solution in which isoproterenol has undergone spontaneous oxidation at a concentration of 4 mg/ml for 6 to 8 hours, on the other hand, resulted in a steady decline in both developed tension and maximum rate of tension development, with failure of the heart to generate contractile force occurring at an average of 35 min (Figure 1). Although time to peak tension declined with fresh and oxidized isoproterenol, this change with oxidized drug was significantly ($P<0.05$) greater than that seen with fresh isoproterenol.

Resting tension was not significantly altered during infusion of fresh isoproterenol, while the maximum rate of relaxation was noticeably increased and the time for 1/2 relaxation reduced (Figure 2). By contrast, a dramatic increase in the resting tension of hearts perfused with oxidized isoproterenol was seen to coincide with failure of contractility (Figure 2). Furthermore, with oxidized isoproterenol the maximum rate of relaxation decreased significantly ($P<0.05$) below control levels, and fell steadily with continued perfusion until contractility failed. The time for 1/2 relaxation was decreased with oxidized isoproterenol to a similar degree as with fresh isoproterenol except in the few minutes just prior to complete loss of contractile function (Figure 2).

Electron microscopic examination of numerous sections from three hearts perfused with fresh isoproterenol (100 mg/liter) for one hour revealed no alteration of mitochondria, sarcoplasmic reticulum, sarcomeres, sarcolemma, or transverse tubules as compared to control hearts perfused for a similar period without isoproterenol (Figure 3, upper panel). Examination of 50 to 100 sections each from three hearts

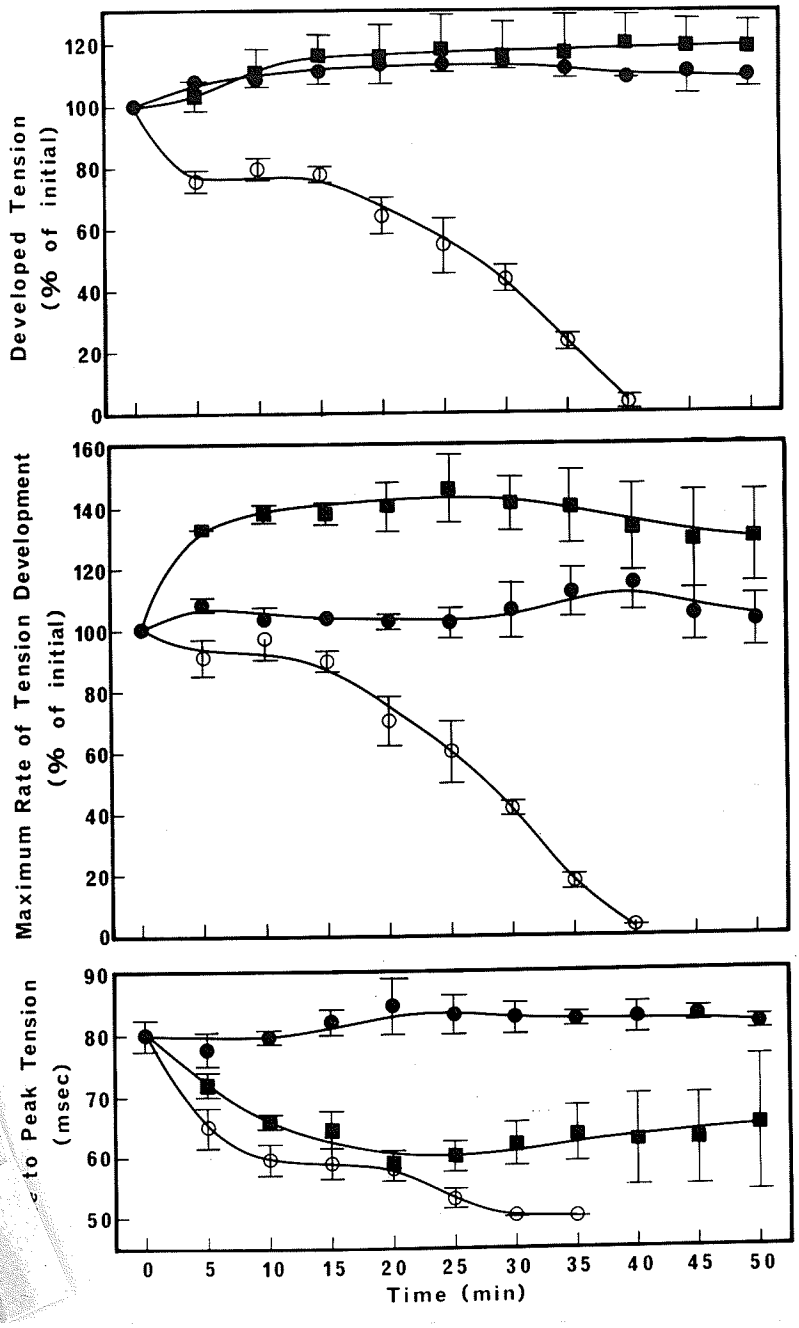

Figure 1: Effect of infusion of fresh and oxidized isoproterenol solution (100 mg/liter) on the developed tension, maximum rate of tension development, and time to peak tension of isolated perfused rat hearts. Control ●—●, infusion of fresh isoproterenol solution ■—■; infusion of oxidized isoproterenol solution ⊖—⊖. Each point is the mean ± standard error of 4 experiments. Mean initial contractile force was 8.2 ± 0.6 gm at a resting tension of 2.5 gm.

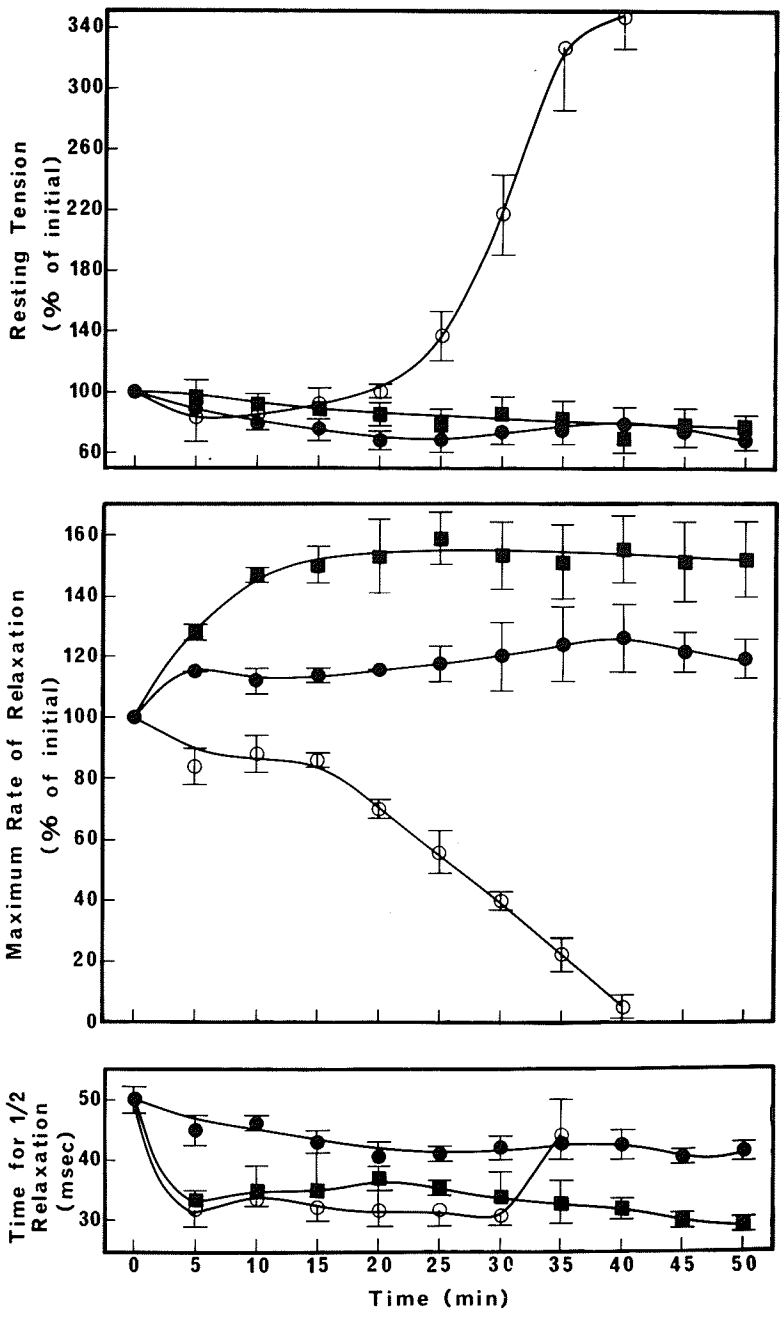

Figure 2: Effect of infusion of fresh and oxidized isoproterenol solutions (100 mg/liter) on the resting tension, maximum rate of relaxation, and time for 1/2 relaxation of isolated perfused rat hearts. Control ●—●; infusion of fresh isoproterenol solution ■—■; infusion of oxidized isoproterenol solution ○—○ . Each point is the mean ± standard error of 4 experiments.

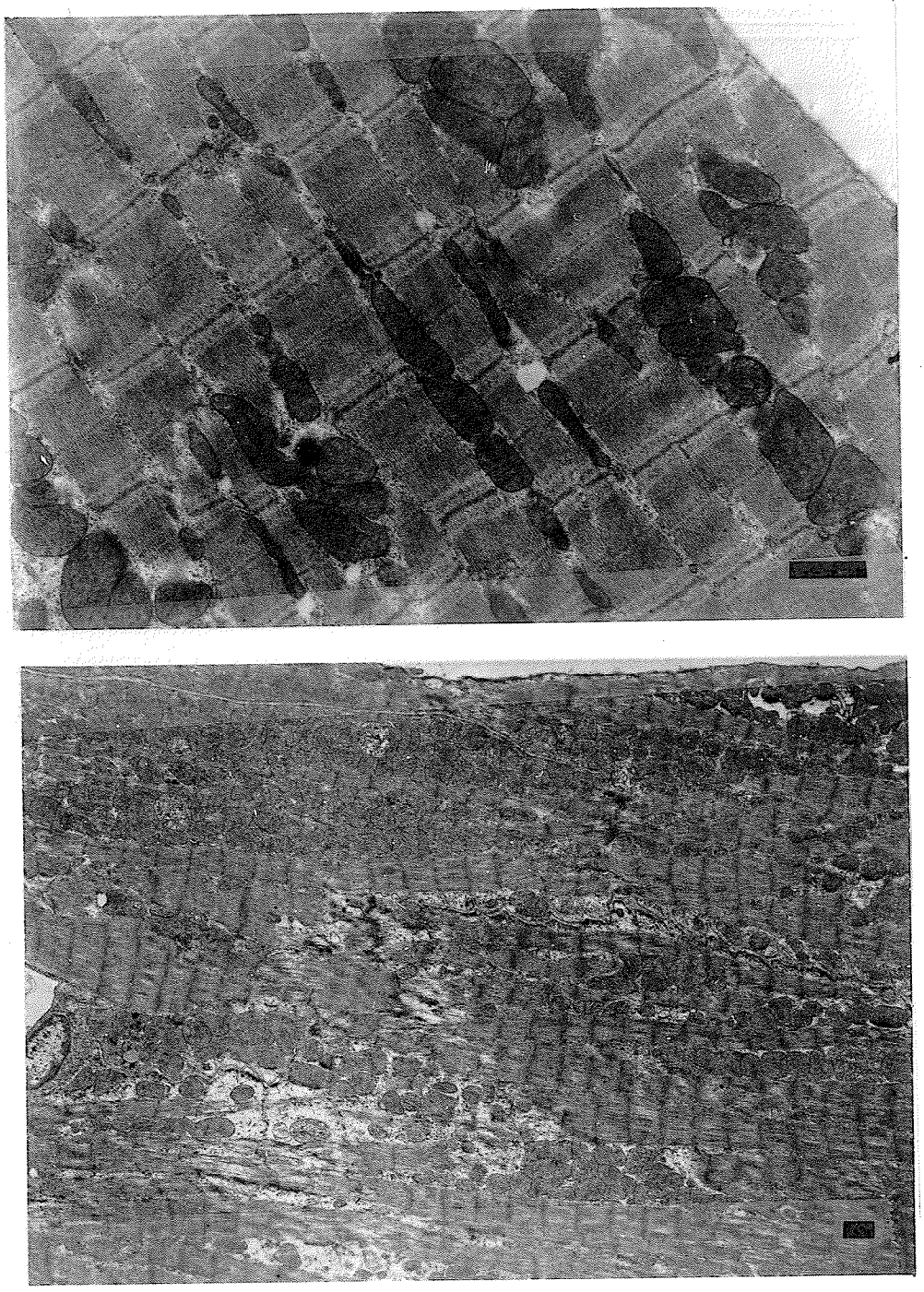

Figure 3: Upper panel - Electron micrograph of a section from an isolated rat heart perfused for one hour with fresh isoproterenol (100 mg/l). This micrograph is typical of all sections examined. Lower panel - electron micrograph of a section from an isolated rat heart perfused with oxidized isoproterenol (100 mg/l) until failure of contractile function. Black bar represents one micron.

perfused with oxidized isoproterenol until contractility disappeared (about 35 min perfusion), on the other hand, revealed frequent evidence of necrotic damage, including contracture of sarcomeres and regions of vacant cytoplasm (Figure 3, lower panel). Distinct evidence of disruption of contractile filaments was seen to occur in many regions (Figure 4, upper panel), as was swelling of mitochondria (Figure 4, lower panel). Changes such as those shown in these figures appeared to involve 1/3 to 1/2 more of all the cells seen in the sections examined.

Figure 5 shows spectral scans obtained from a 400 fold dilution (initial concentration 4 mg/ml) of freshly made isoproterenol solution and oxidized isoproterenol solution, as used in this study, and also of older solution, which had undergone a further colour change from reddish-brown to a more transparent yellow - brown. The latter solution was found to be of greatly reduced potency, in terms of cardiotoxic effects, with developed tension decreasing by less than 50% during 35 min of perfusion and not diminishing further even after more than one hour (results not shown). Fresh isoproterenol solutions show a major absorption peak at approximately 205 nm with a smaller maximum at 280 nm (Figure 5). The oxidized isoproterenol solution exhibiting a reddish-brown colour and having a high degree of cardiotoxicity exhibited absorption maxima at approximately 225 and 308 nm with a very broad peak in the visible region at 490 - 500 nm. These maxima agree closely with the absorption spectra reported for adrenochrome, a primary oxidation product of epinephrine (214). Older solutions exhibiting a yellow-brown colouration and having a greatly reduced cardiotoxic potency show broad maxima at 225 and 340 nm (Figure 5). The ultrastructural damage in hearts perfused with isoproterenol oxidized for 16 to 24 hours was not even half as extensive as seen in hearts perfused with isoproterenol oxidized for 6 to 8 hours. Perfusion of hearts with concentrations of fresh isoproterenol as high as 200 mg/liter for periods in excess of 2 hours resulted in no diminution of mechanical activity and no ultrastructural damage.

Solutions of isoproterenol which had undergone spontaneous oxidation for a longer period of time (12 hrs.) at a lower concentration (100 μg/ml) were found to be much more potent in terms of their effects on contractile failure of the isolated perfused heart (Figure 6). Perfusion with oxidized isoproterenol prepared in this manner at a concentration of 100 mg/l resulted in a rapid and dramatic decline of

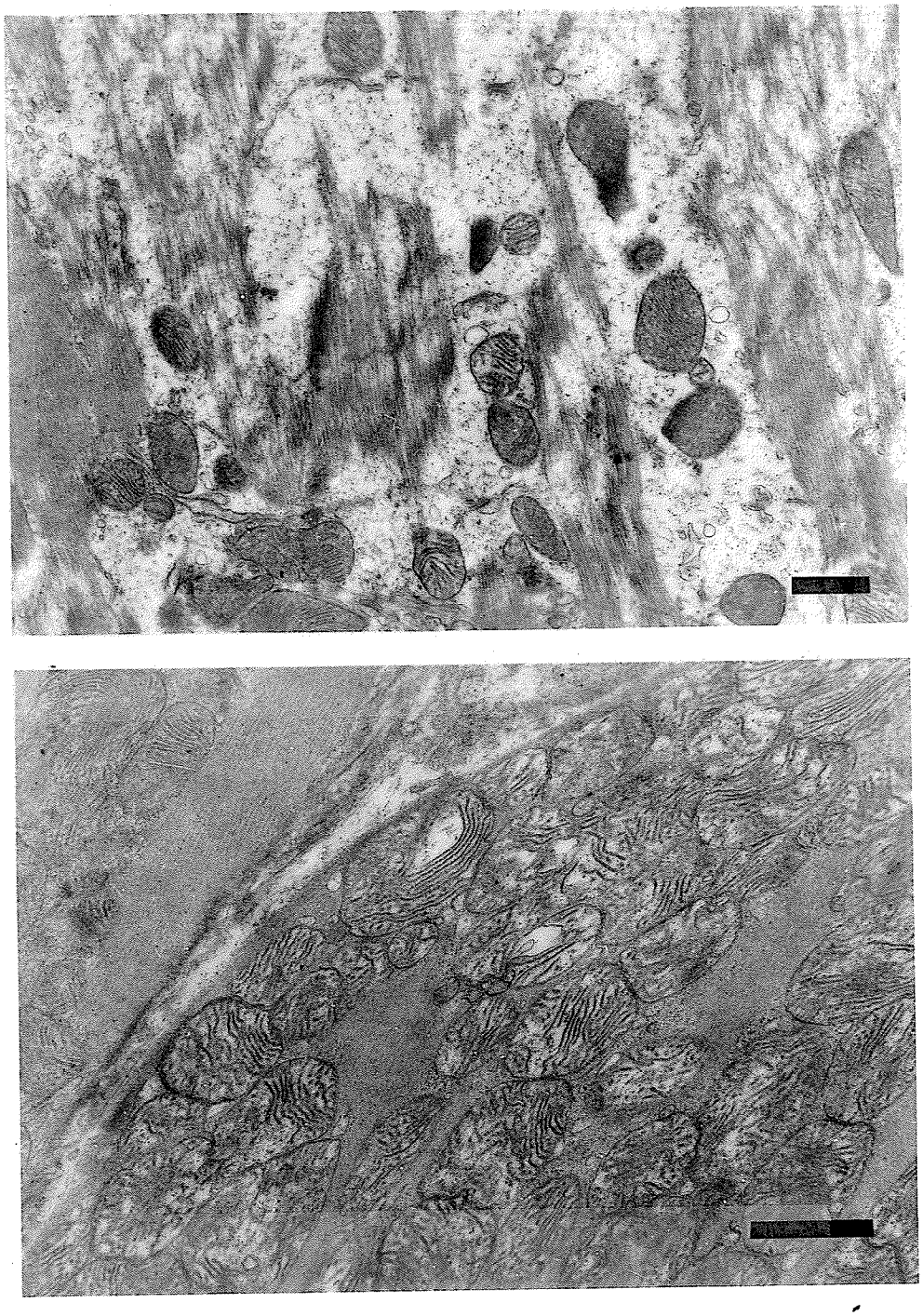

Figure 4: Electron micrographs of sections from isolated rat hearts perfused with oxidized isoproterenol (100 mg/l) until failure of contractile function. Upper panel indicating disrupted contractile filaments and the lower panel showing swollen mitochondria. Black bar represents one micron.

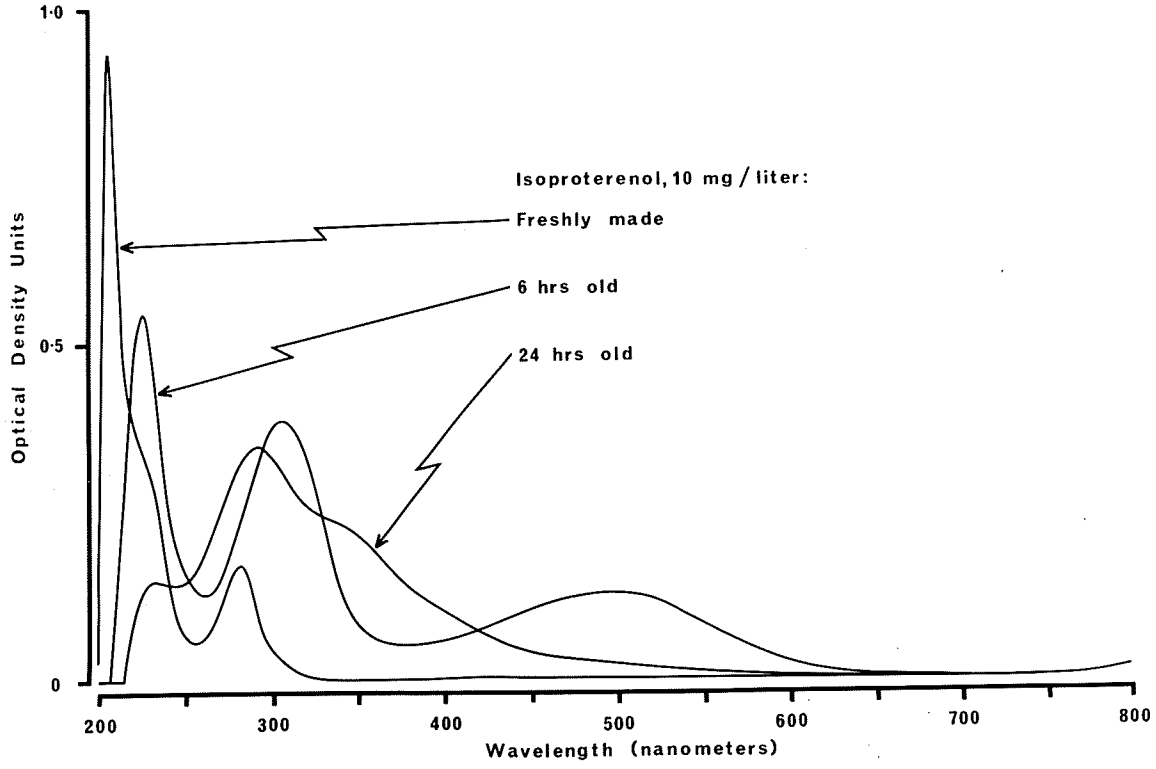

Figure 5: Absorption spectrum of a solution of isoproterenol in Kreb-Henseleit perfusion medium when freshly made, after sitting for several hours, at which time it is highly cardiotoxic, and after sitting overnight, at which time it's cardiotoxicity is greatly diminished.

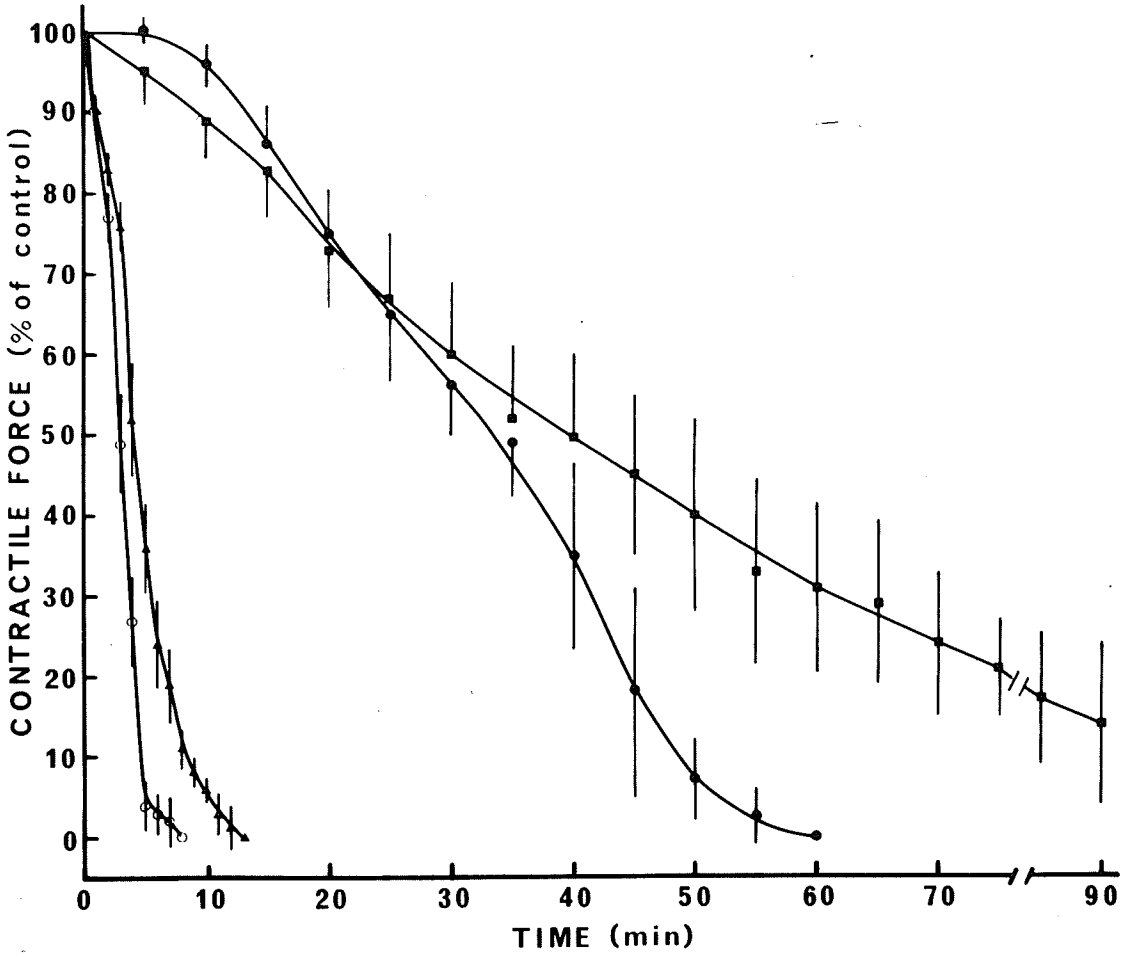

Figure 6: Effect of perfusion with various concentrations of oxidized isoproterenol on the contractile force of isolated perfused rat hearts. 10 mg/l ■—■ , 20 mg/l ●—● , 50 mg/l ▲—▲ , 100 mg/l ○—○ . Each point is the mean ± standard error of 4 experiments. Mean initial contractile force was 8.5 ± 0.3 gm at a resting tension of 2.5 gm.

contractile force to 8% of the initial level within 5 minutes, and complete failure of contractile activity by about 8 minutes. This pattern of contractile failure was found to vary according to the concentration of oxidized isoproterenol, with failure occurring at approximately 13 minutes at 50 mg/l, 60 minutes at 25 mg/l, and contractile force reduced to approximately 15% of control by 90 minutes at a concentration of 10 mg/l (Figure 6).

Electron microscopic examination of sections from hearts perfused for 15 minutes with 50 mg/l of isoproterenol oxidized at that concentration for 12 hours revealed similar necrotic damage to that found previously. Figure 7 illustrates the disruption of contractile filaments (upper panel) and swelling of mitochondria and sarcoplasmic reticulum (lower panel) typical of that found in these hearts.

B. Effect on Contractile Force Development and Ultrastructure of Perfusion with Epinephrine, its Metabolites, and Adrenochrome.

Due to the similarity of the absorption spectrum of oxidized isoproterenol solution to that of adrenochrome a comparison was made of the effects of epinephrine and commercially prepared adrenochrome (Sigma Chemicals) in terms of their effects on contractile function and ultrastructure in the isolated perfused rat heart. In addition, a series of experiments was carried out to determine whether any of the normally occurring metabolites of epinephrine, such as metanephrine, dihydroxymandelic acid, and vanilmandelic acid were capable of inducing necrotic damage to the myocardium.

Perfusion of isolated rat hearts with medium containing epinephrine (50 mg/l) resulted in a rapid increase in contractile force development reaching a peak mean value of just over 150% of control at 3 - 5 minutes and remaining well above the control throughout the experiment (Figure 8, upper panel). Metanephrine (50 mg/l) likewise produced a significant ($P<0.05$) but much smaller increase in contractile force development which was also maintained and increased gradually throughout the experiment. Neither dihydroxymandelic acid (50 mg/l) or vanilmandelic acid (50 mg/l) significantly ($P>0.05$) altered the development of contractile force as compared to control (Figure 8, upper panel). Adrenochrome (50 mg/l) on the other hand caused a steady decline in contractile force development until a complete disappearance of contractile activity had occurred by 30 minutes (Figure 8, upper panel). The increase in contractile

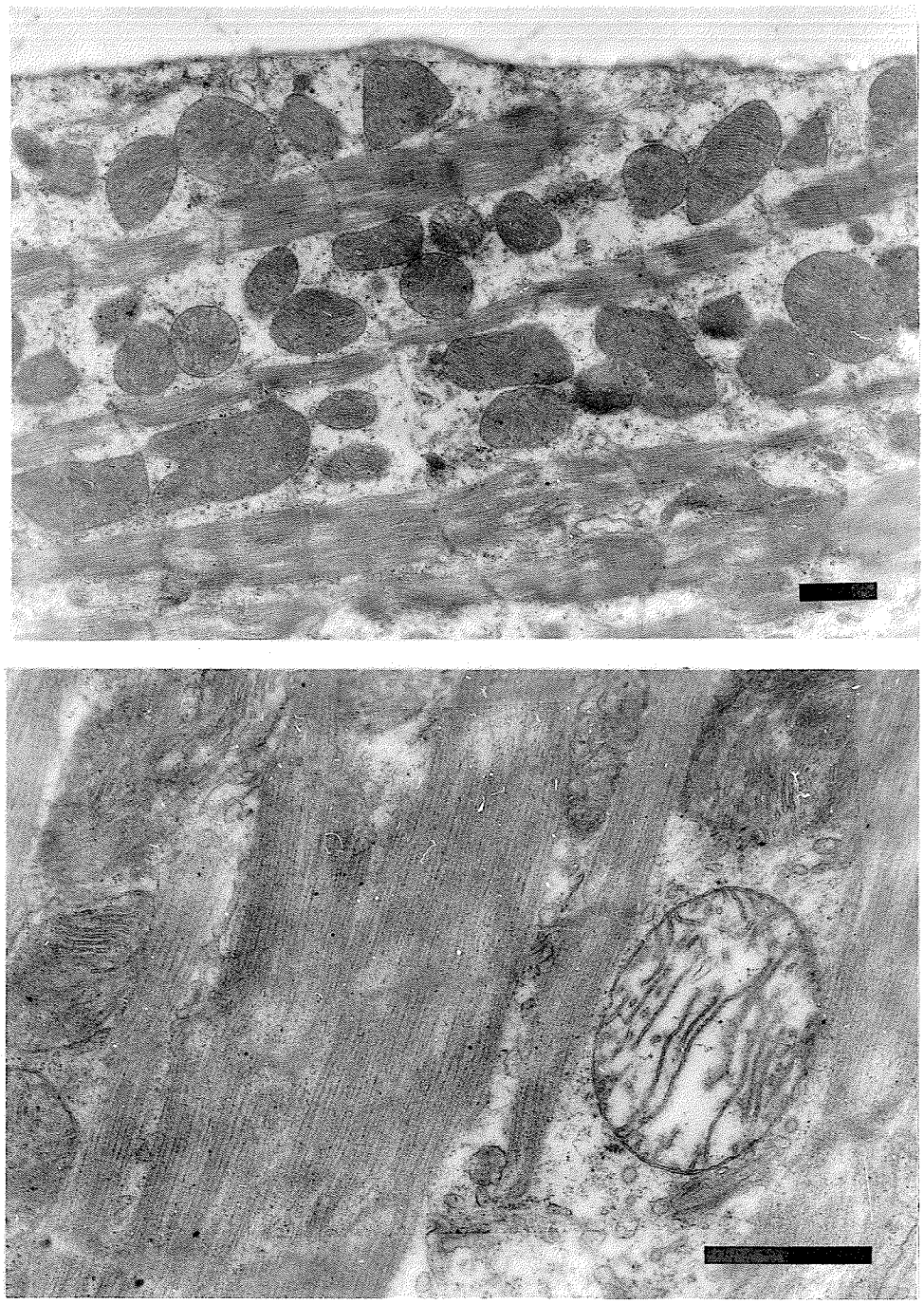

Figure 7: Electron micrographs of sections from isolated rat hearts perfused for 15 minutes with oxidized isoproterenol (50 mg/l). Upper panel demonstrates disruption of contractile filaments and the lower panel shows swelling of mitochondria. Black bar represents one micron.

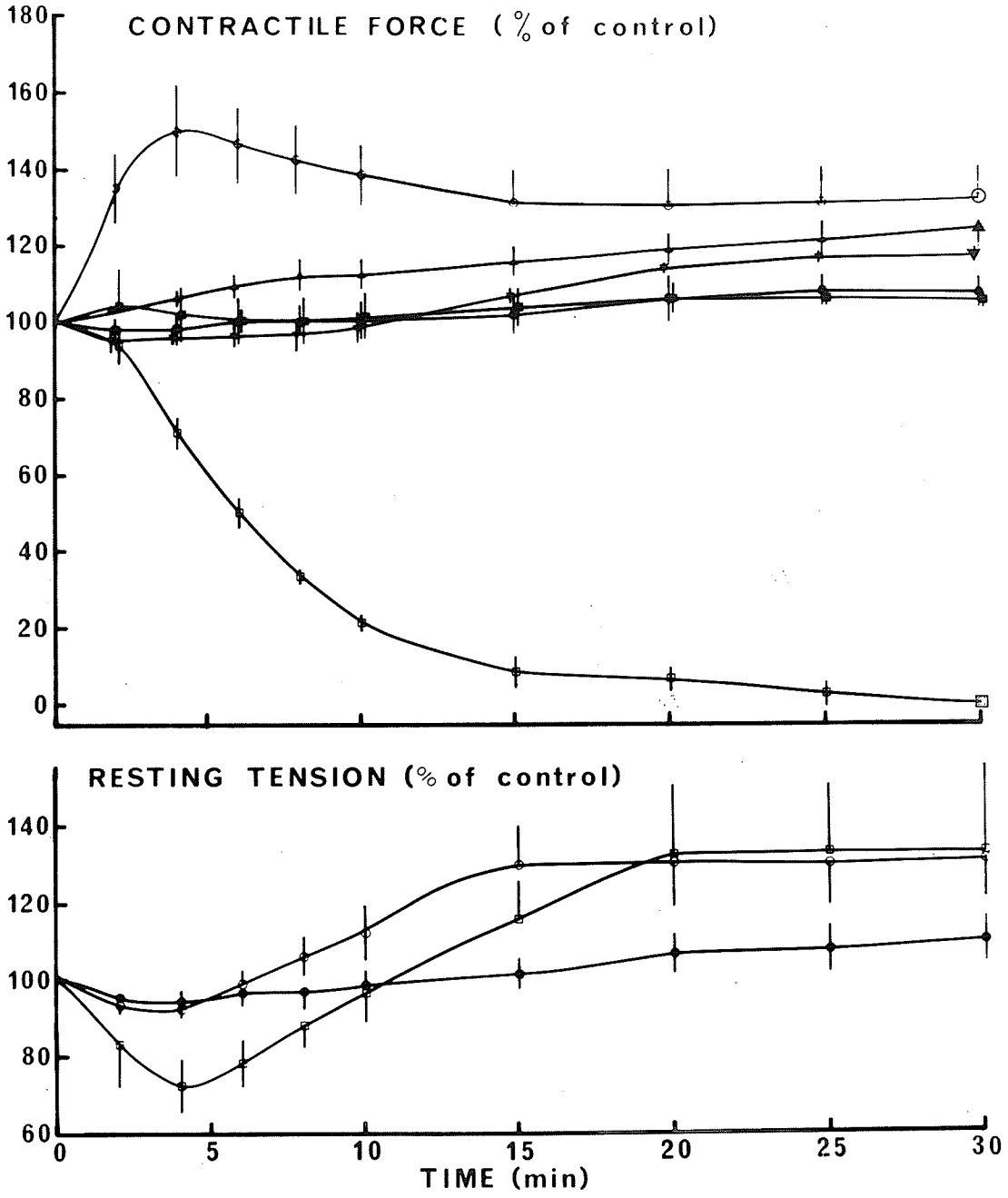

Figure 8: Upper panel - Effect of perfusion with epinephrine, metanephrine, dihydroxymandelic acid, vanilmandelic acid, or adrenochrome on the contractile force of isolated perfused rat hearts. Mean initial contractile force was 8.4 ± 0.4 gm at a resting tension of 2.5 gm. Lower panel - Effect of perfusion with epinephrine or adrenochrome on the resting tension of isolated perfused rat hearts. The initial resting tension was 2.5 gm. Epinephrine (50 mg/l) ○—○ , metanephrine (50 mg/l) ▲—▲ , vanilmandelic acid (50 mg/l) ▼—▼ , dihydroxymandelic acid (50 mg/l) ■—■ , adrenochrome (50 mg/l) □—□ , control ●—●. Each point is the mean ± standard error of 4 experiments.

force caused by perfusion with epinephrine was accompanied by a gradual increase in resting tension (Figure 8, lower panel) which then became stable at a level about 30 % above the initial resting tension. The effect of perfusion with adrenochrome on the resting tension of the heart was to produce an early decline to less than 75% of control during the first five minutes, followed by a steady increase in resting tension to a level not significantly ($P>0.05$) different from that seen upon perfusion with adrenaline (Figure 8, lower panel).

Epinephrine and adrenochrome produced dramatically different effects on both the rate of contractile force development and the time to peak tension (Figure 9). The powerful positive inotropic response to epinephrine is reflected by a large increase in rate of force development and by a decrease in the time to peak tension. By contrast, adrenochrome caused a steady and rapid decline in the rate of force development and a prolonged time to peak tension. Time to peak tension could not be accurately measured in hearts perfused with adrenochrome for longer than 10 minutes due to the greatly reduced amplitude of contractile force development (Figure 9, lower panel).

The effects of epinephrine and adrenochrome on the maximum rate of relaxation and the time for 1/2 relaxation were quite similar to their effects on the maximum rate of force development and time to peak tension. Epinephrine caused a large increase in the maximum rate of relaxation and a shortened time for 1/2 relaxation, whereas perfusion with adrenochrome resulted in a dramatic and steady decrease in the maximum rate of relaxation and a prolonged time for 1/2 relaxation (Figure 10).

Electron microscopic examination of sections from four hearts each perfused with epinephrine (Figure 11, upper panel), vanilmandelic acid (Figure 11, lower panel), metanephrine acid (Figure 12, upper panel), and dihydroxymandelic acid (Figure 12, lower panel), failed to reveal any ultrastructural evidence of myocardial necrosis. Preservation was good and the appearance of the mitochondria, sarcoplasmic reticulum, transverse tubules, sarcolemma, and sarcomeres was not different from that of control perfused rat hearts.

In contrast to these observations, examination of numerous sections from three of four hearts perfused with 50 mg/l adrenochrome revealed extensive evidence of necrotic damage including apparent dissolution of contractile filaments as well as

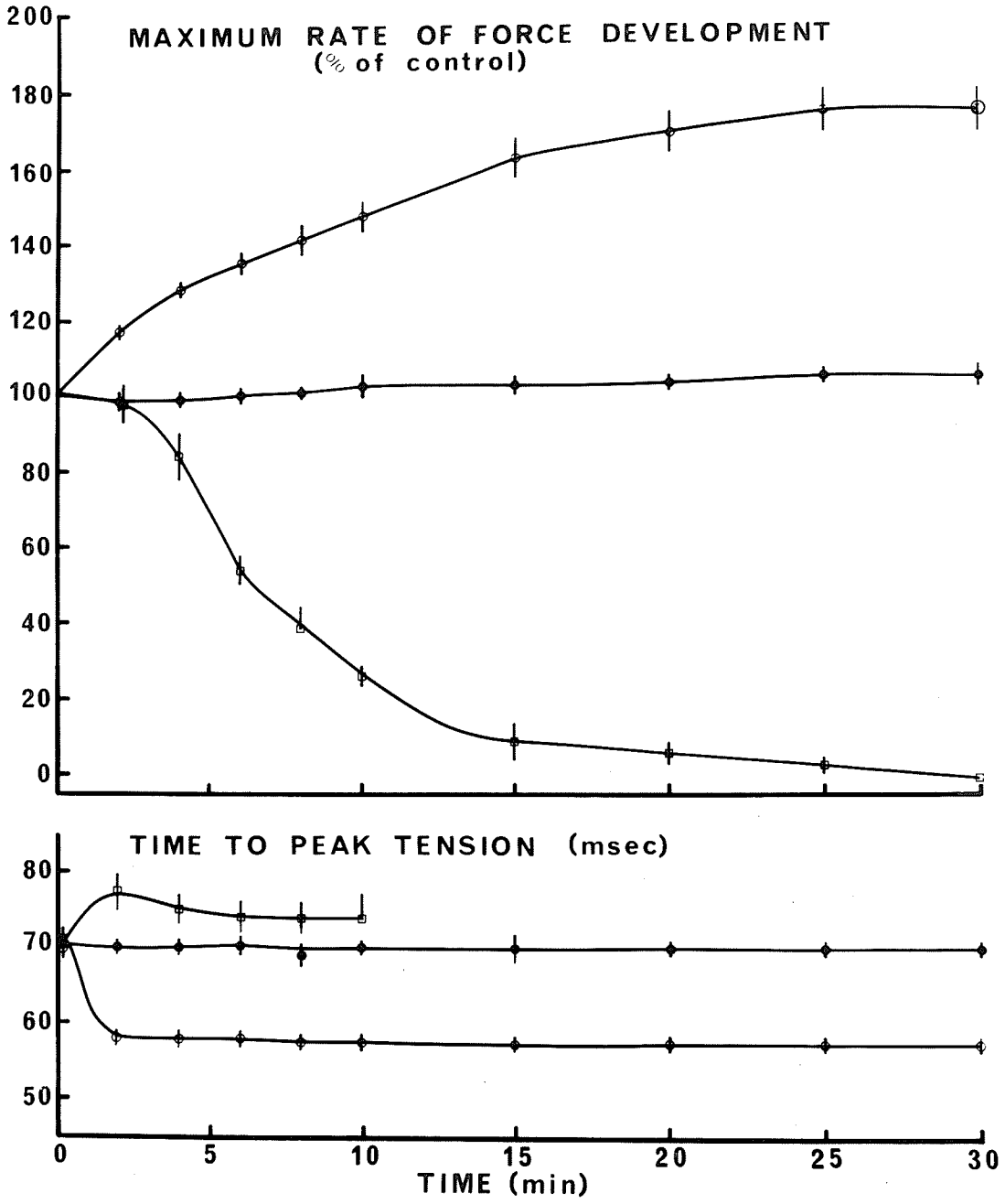

Figure 9: Effect of perfusion with epinephrine or adrenochrome on the maximum rate of force development and the time to peak tension of isolated perfused rat hearts. Epinephrine (50 mg/l) ⊖—⊖, adrenochrome (50 mg/l) ⊡—⊡ , control ●—● . Each point is the mean ± standard error of 4 experiments.

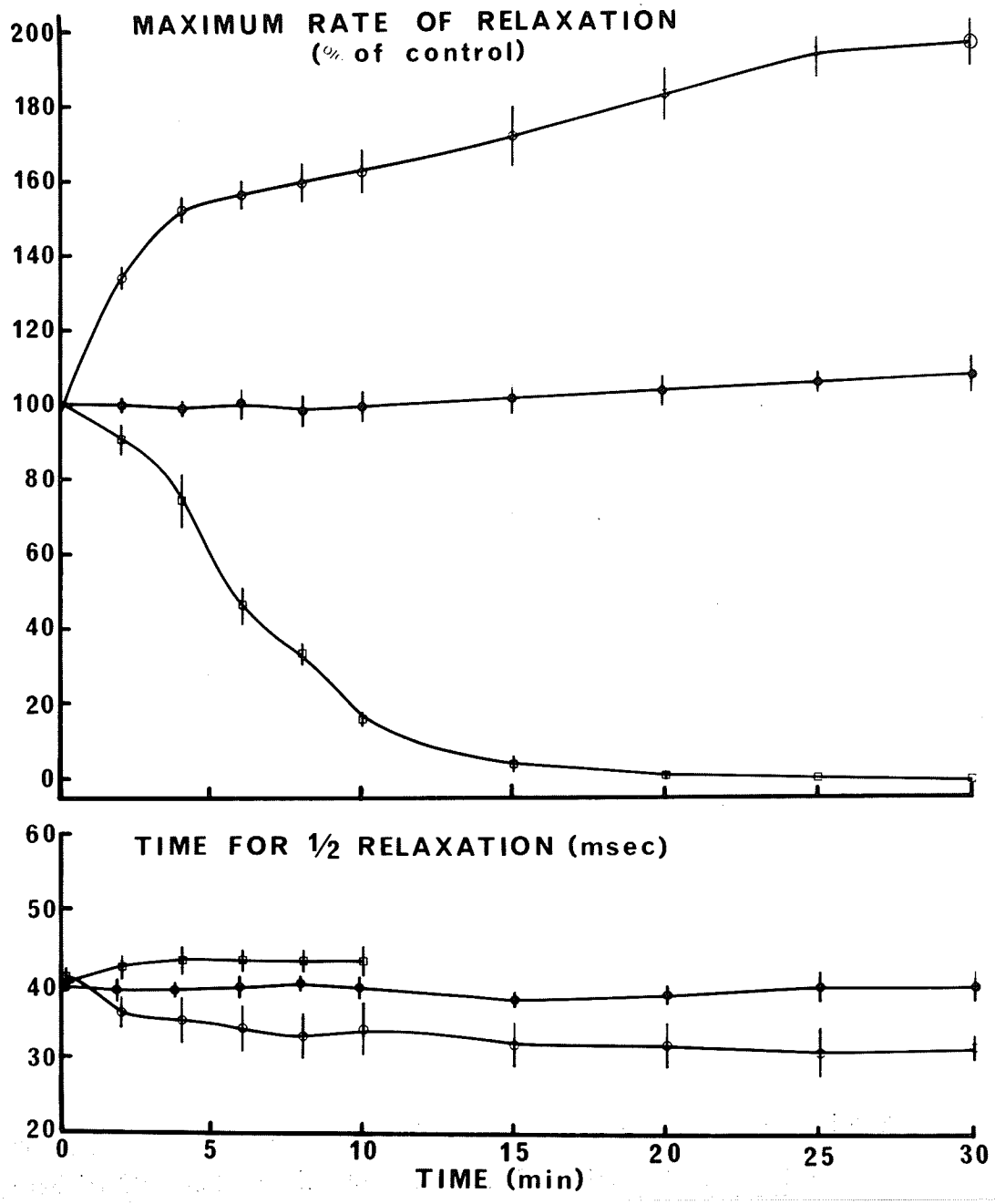

Figure 10: Effect of perfusion with epinephrine or adrenochrome on the maximum rate of relaxation and the time for 1/2 relaxation of isolated perfused rat hearts. Epinephrine (50 mg/l) ⊖—⊖, adrenochrome (50 mg/l) ⊡—⊡ , control ●—● . Each value is a mean ± standard error of 4 experiments.

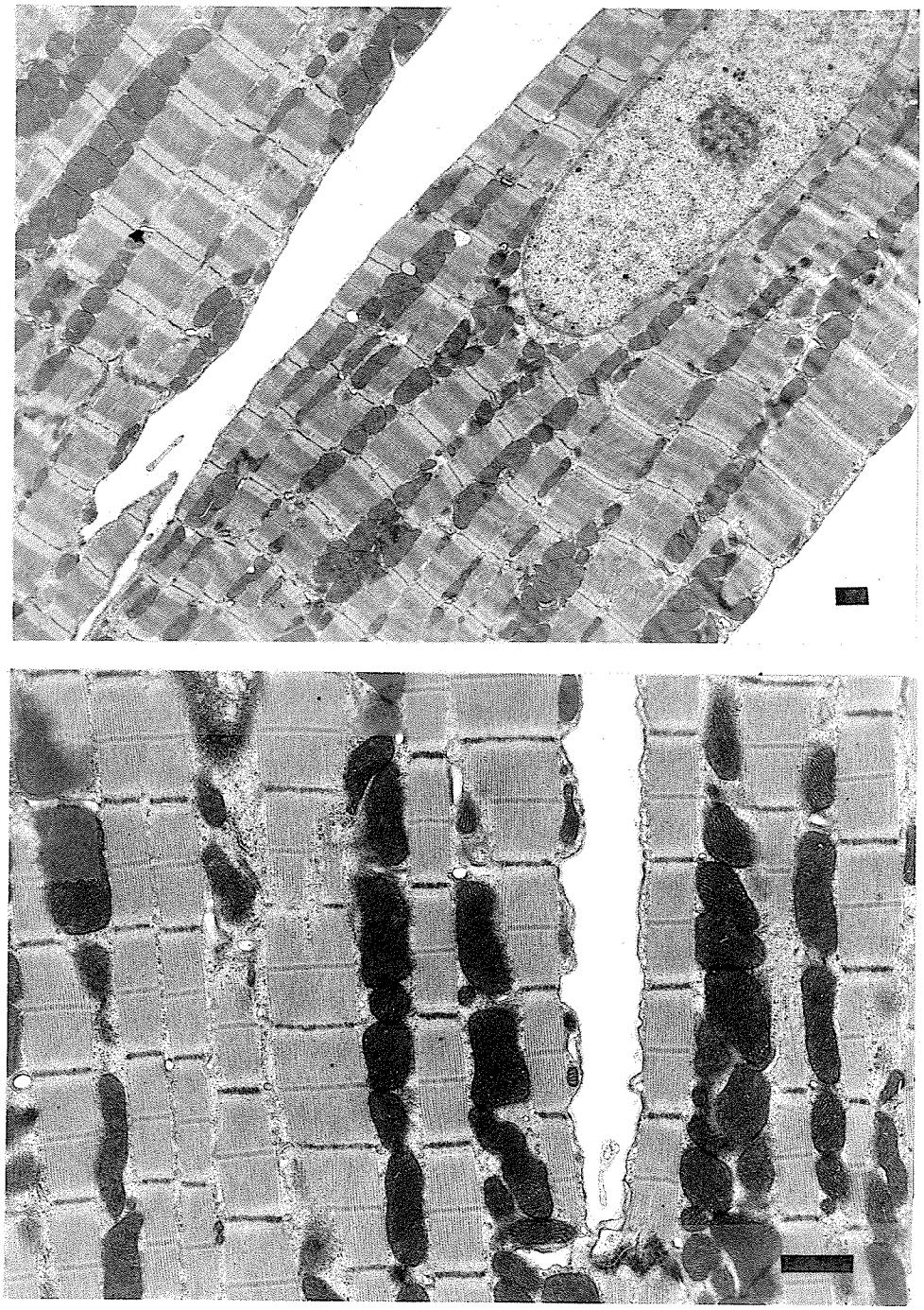

Figure 11: Upper panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with epinephrine (50 mg/l). Lower panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with vanilmandelic acid (50 mg/l). These micrographs are typical of sections examined from four hearts each perfused with epinephrine and vanilmandelic acid for 30 minutes. Black bar indicates one micron.

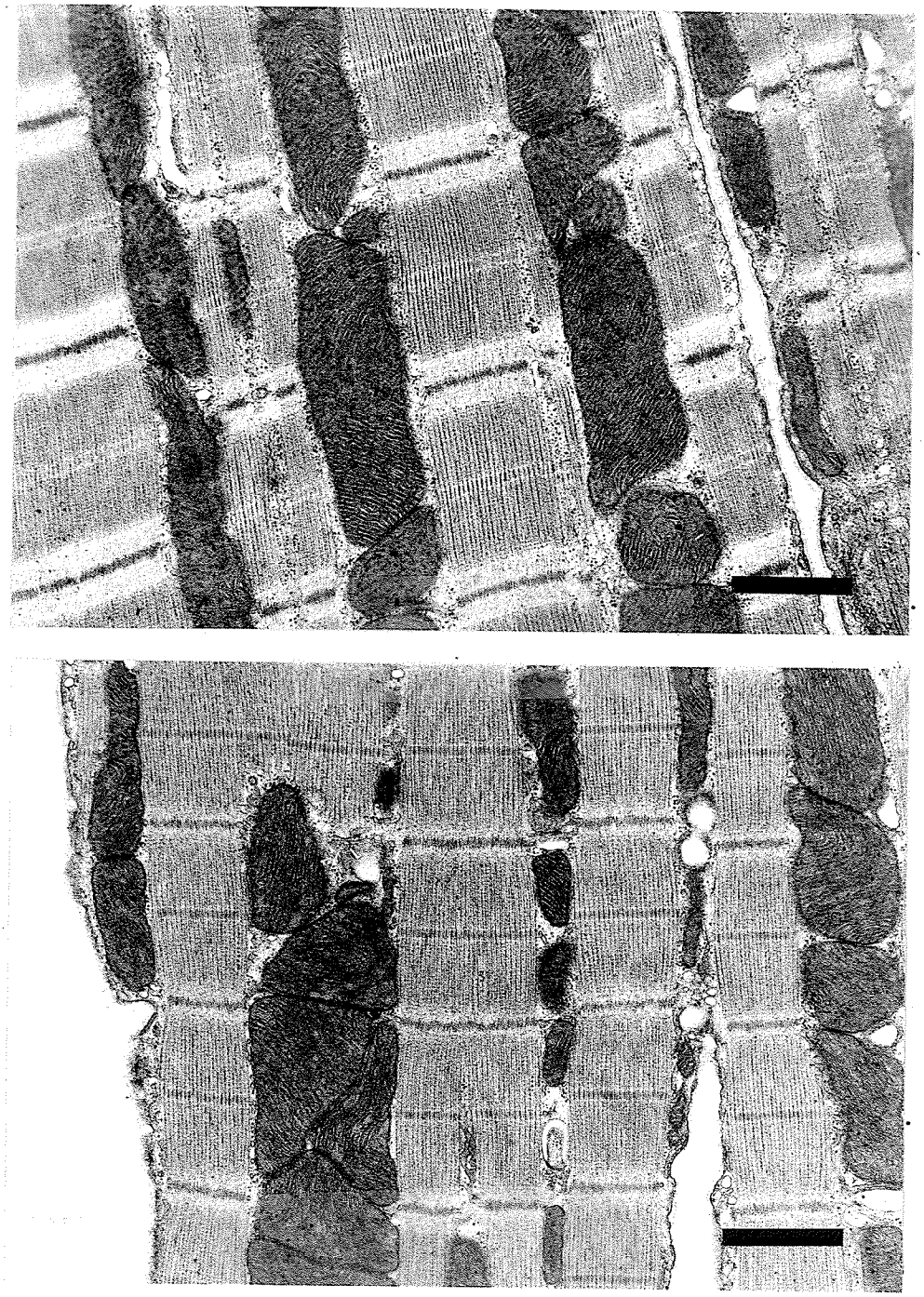

Figure 12: Upper panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with metanephrine (50 mg/l). Lower panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with dihydroxymandelic acid (50 mg/l). These micrographs are typical of all sections examined from four hearts each perfused with metanephrine and dihydroxymandelic acid for 30 minutes. Black bar represents one micron.

contracture in other areas (Figure 13, upper panel) and swelling of mitochondria and sarcoplasmic reticulum (Figure 13, lower panel). Ultrastructural changes such as these were estimated to involve from 1/2 to 2/3 or more of all ventricular tissue examined from these hearts. In the fourth heart perfused with 50 mg/l adrenochrome several areas showing damage similar to that found in the other three hearts were occassionally observed (Figure 14, upper panel), but by and large the appearance of most of the ventricular tissue examined in over a hundred sections from this heart was not different from that of the control (Figure 14, lower panel).

The effect on the time course of changes in contractile force development of perfusion with various concentrations of adrenochrome for 30 minutes is illustrated in Figure 15. The rate of decline of contractile force was increased and the mean contractile force at the end of 30 minutes was decreased over a range of concentrations from 1 mg/l to 50 mg/l of adrenochrome. Whereas 1 mg/l of adrenochrome produced virtually no change in contractile activity within the 30 minute period, 50 mg/l of adrenochrome resulted in complete failure of contractile activity by 30 minutes.

Electron microscopic examination of rat hearts perfused for 30 minutes with 1 mg/l of adrenochrome revealed no evidence of ultrastructural damage, nor did sections from hearts perfused with 5 mg/l of adrenochrome appear unusual except for occasional vacuolizations possibly representing the degeneration of individual mitochondria (Figure 16, upper panel). Although there was no overt disruption of subcellular organelles in hearts perfused with 10 mg/l of adrenochrome for 30 minutes, subtle alterations in the appearance of the mitochondria were evident (Figure 16, lower panel). In addition to being more electrondense, the cristae of these mitochondria were more tightly packed and were seen more often in a spiral rather than transverse configuration. These alterations in mitochondrial structure were found to be more pronounced following perfusion with an adrenochrome concentration of 25 mg/l (Figure 17, upper panel), and the mitochondrial cristae were more convoluted in appearance while the space between cristae was increased. Evidence of disruption of the contractile elements was observed as well (Figure 17, upper panel), although this was not as uniform a phenomenon as the mitochondrial changes and was not at all evident in many areas.

Four hearts each were fixed for electron microscopy following perfusion with

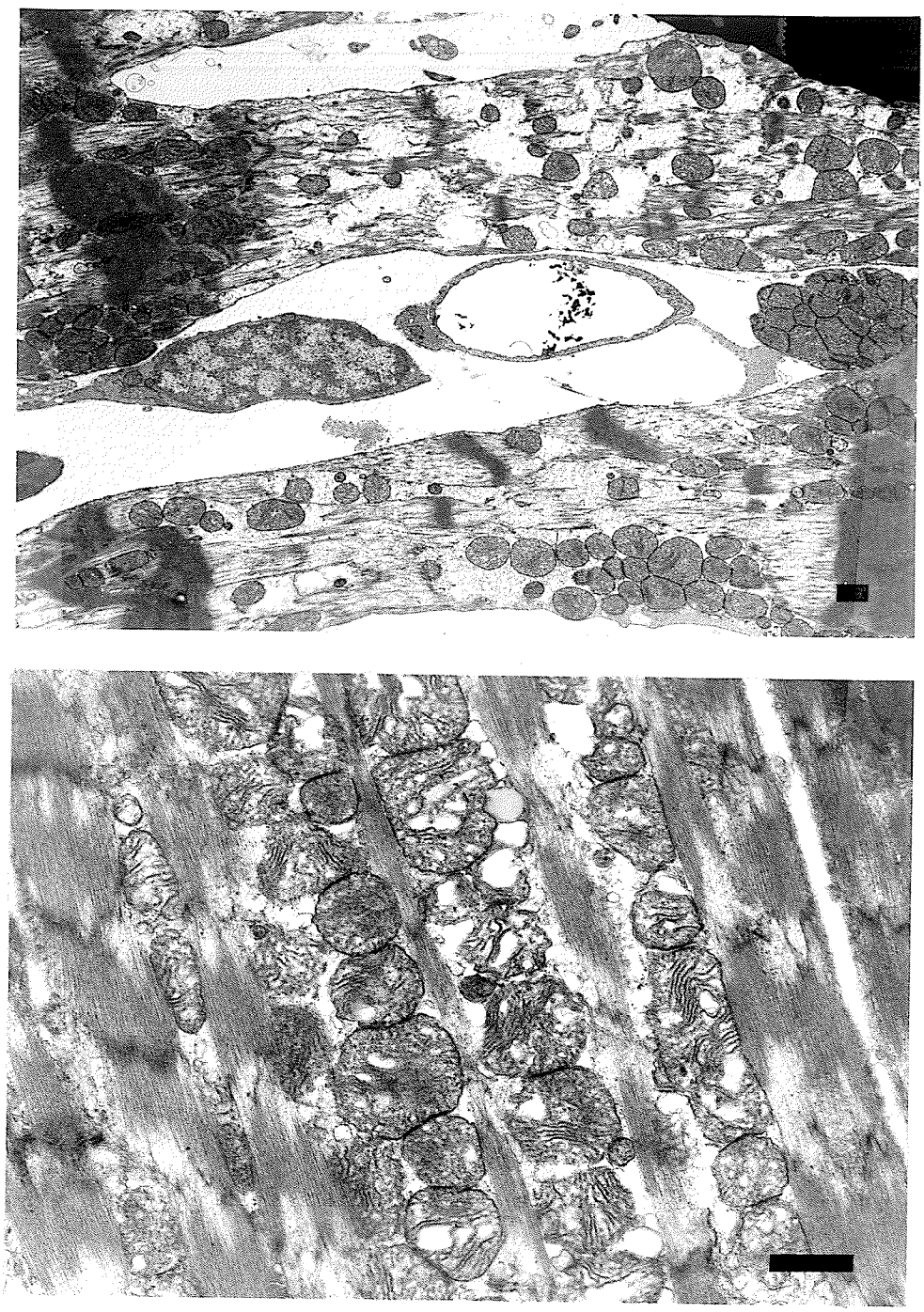

Figure 13: Electron micrographs of typical sections from isolated rat hearts perfused for 30 minutes with adrenochrome (50 mg/l). Upper panel illustrates contracture and dissolution of contractile filaments. Lower panel illustrates swelling and disruption of mitochondria and swelling of sarcoplasmic reticulum. These micrographs are typical of extensive areas of damage observed in three of four hearts perfused with adrenochrome for 30 minutes. Black bar represents one micron.

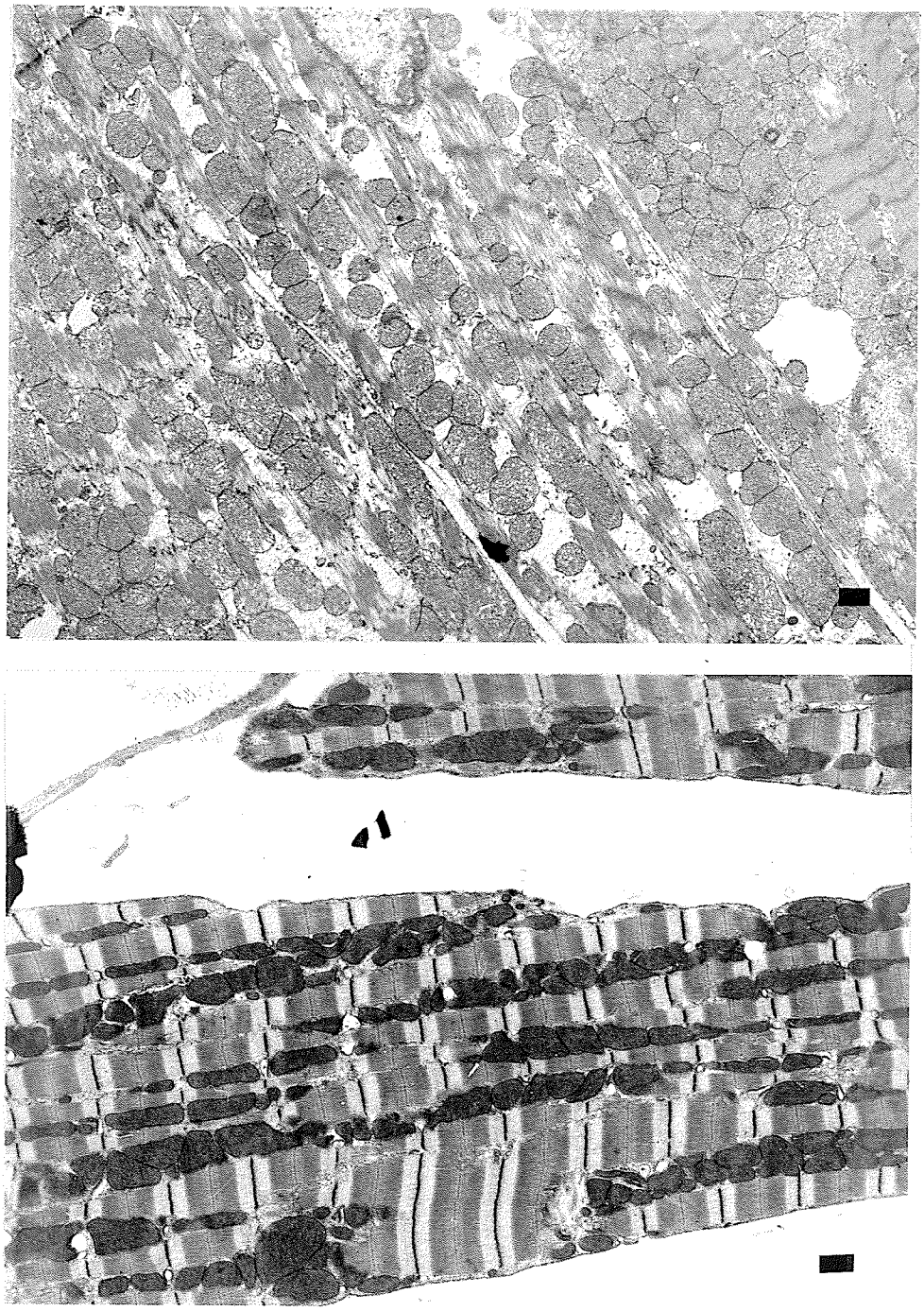

Figure 14: Upper panel - Electron micrograph of a section from an isolated rat heart perfused for 30 minutes with adrenochrome (50 mg/l), indicating necrotic damage similar to that shown in the previous figure. Lower panel - Electron micrograph of another region of the same heart shown in the upper panel. Ultrastructure is apparently normal and is typical of the majority of sections examined from this particular heart. Black bar indicates one micron.

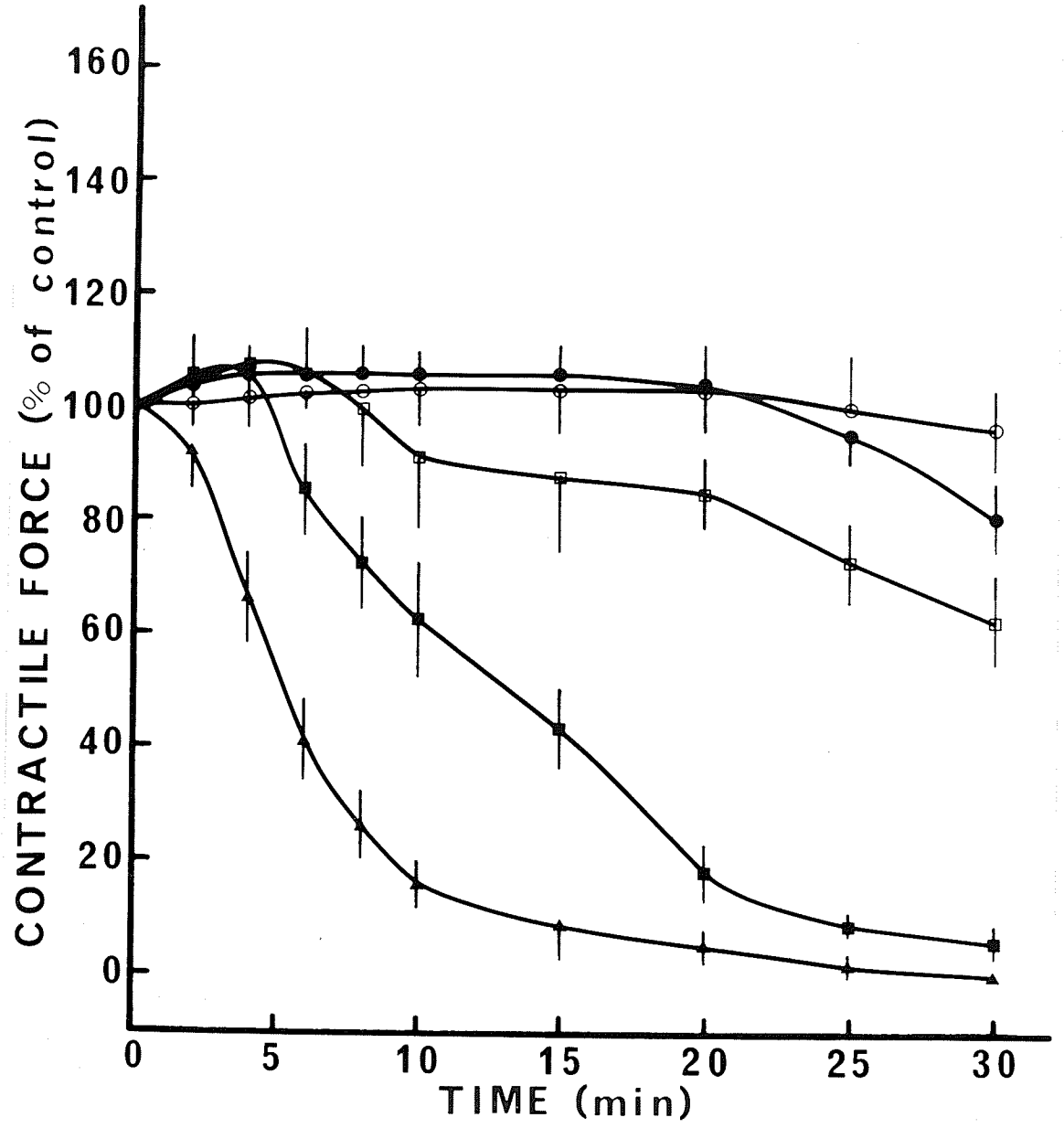

Figure 15: Effect of perfusion with various concentrations of adrenochrome on the contractile force of isolated rat hearts. 1 mg/l ○—○, 5 mg/l ●—● , 10 mg/l □—□ , 25 mg/l ■—■ , 50 mg/l ▲—▲ . Each point is the mean ± standard error of 4 experiments. Mean initial contractile force was 8.7 ± 0.4 gm at a resting tension of 2.5 gm.

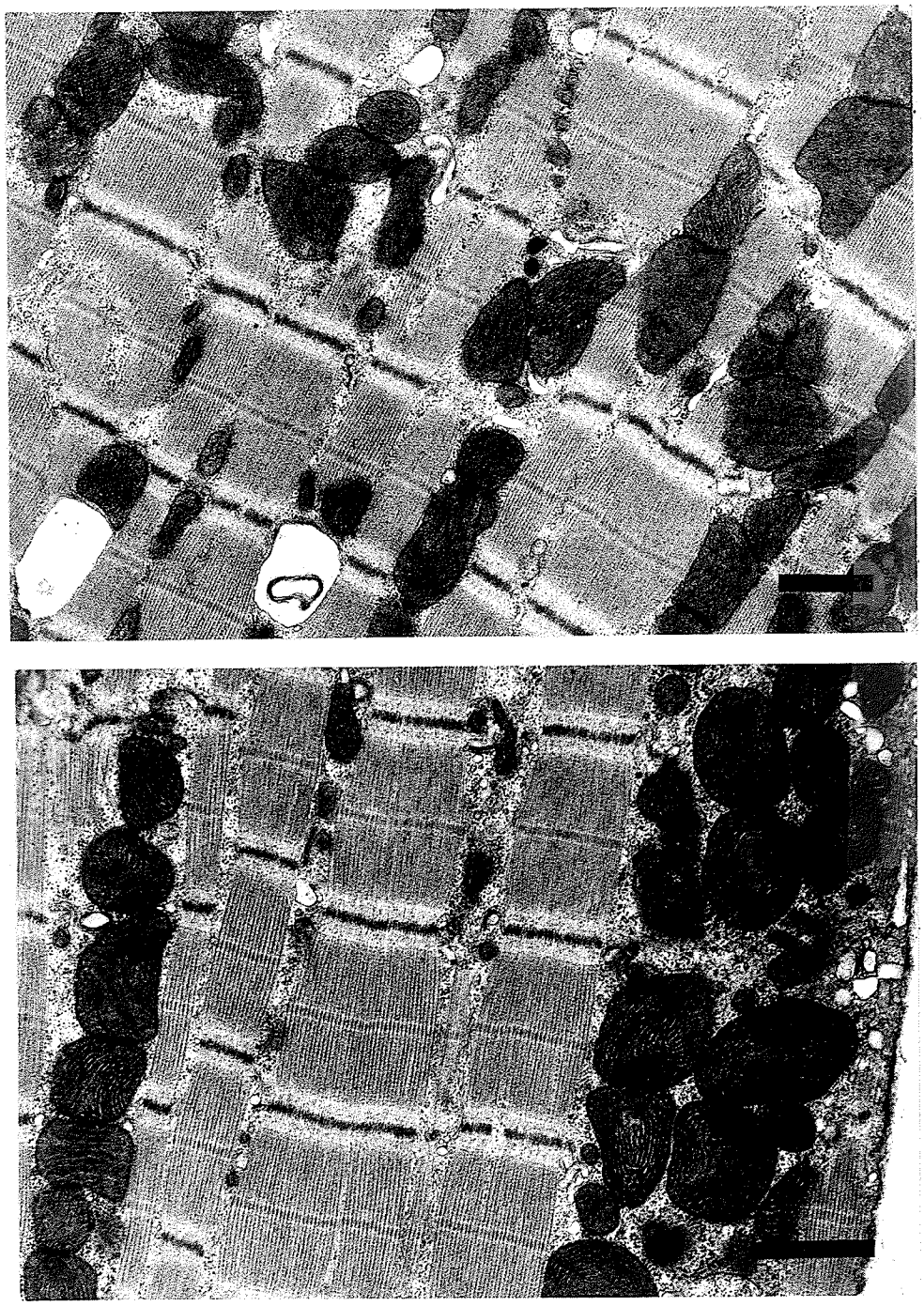

Figure 16: Upper panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (5 mg/l). Lower panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (10 mg/l). These micrographs are representative of sections examined from four hearts perfused at each concentration of adrenochrome. Black bar indicates one micron.

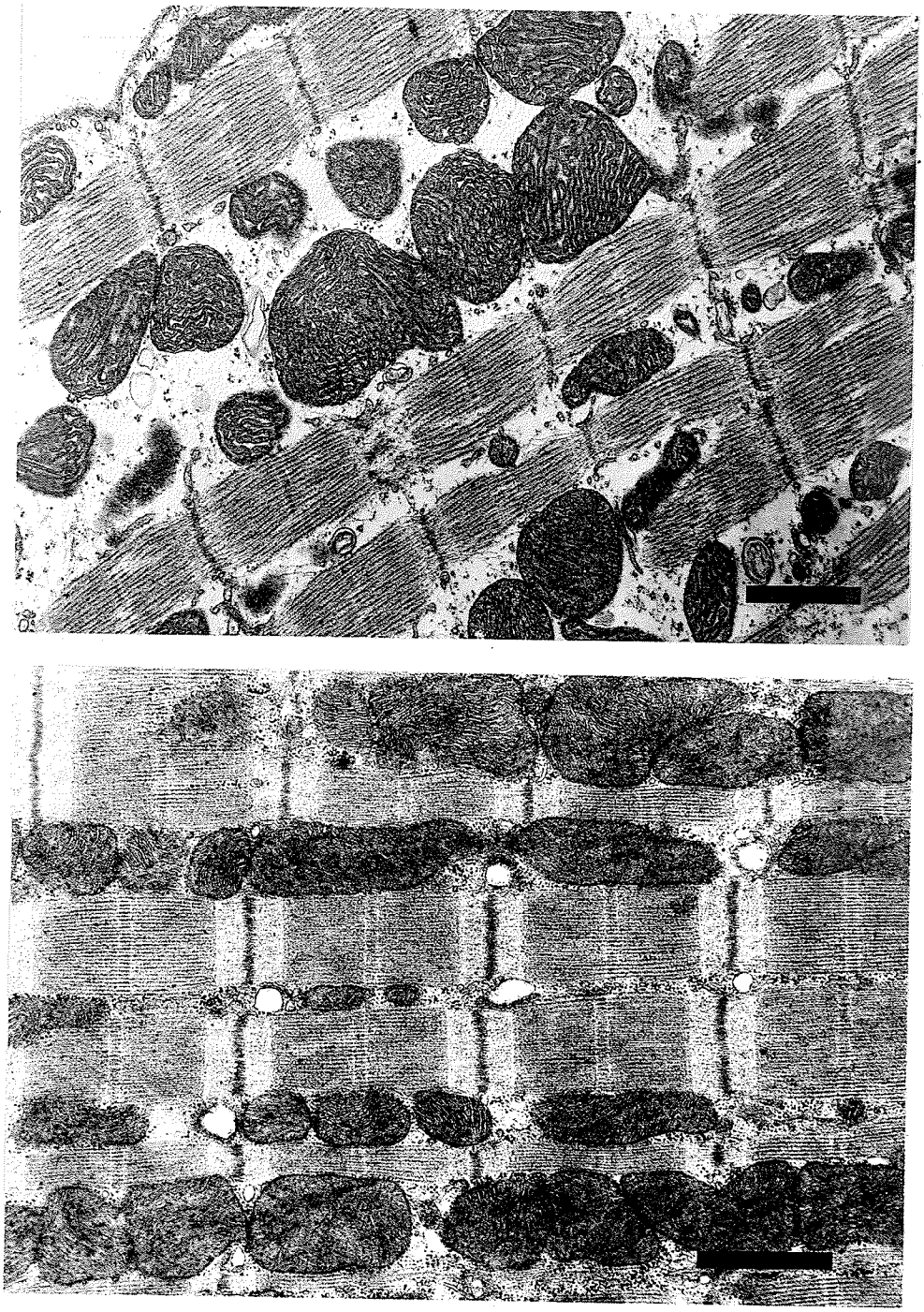

Figure 17: Upper panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (25 mg/l). Lower panel - Electron micrograph of a typical section from an isolated rat heart perfused for 5 minutes with adrenochrome (50 mg/l). These micrographs are representative of sections examined from four hearts each perfused under the conditions described. Black bar indicates one micron.

50 mg/l of adrenochrome for 5 minutes, 10 minutes, and 20 minutes in order to study the time course of development of ultrastructural damage. Electron microscopic examination of sections from these hearts revealed a dramatic difference in hearts perfused for 10 minutes with this concentration of adrenochrome as compared to those perfused for 5 minutes. At 5 minutes of perfusion with adrenochrome ultrastructure was not apparently different from control (Figure 17, lower panel) whereas by 10 minutes areas in which sarcomeres were both over-stretched and in contracture, in which contractile elements appeared to be undergoing dissolution, and in which disruption of mitochondria was evident were frequently observed (Figure 18). The severity of the damage observed in these hearts was similar to that seen at 30 minutes of perfusion with 50 mg/l of adrenochrome, although not as extensive. By 20 minutes of perfusion with 50 mg/l the extent of the damaged areas approached that seen at 30 minutes.

C. Influence of Epinephrine and Various Reducing Agents on Adrenochrome Induced Failure and Necrosis.

The effects of epinephrine infusion on necrosis and failure resulting from perfusion of isolated rat hearts with adrenochrome was studied for the following reasons. Firstly, a small increase in contractile force, although not statistically significant, was consistantly noted during the first few minutes of perfusion with 5, 10 and 25 mg/l of adrenochrome (Figure 15). This observation suggests the possibility of contamination of the adrenochrome preparation by traces of unoxidized epinephrine, though the product assay information provided by Sigma indicated a high degree of purity. Attempts to further purify the preparation were considered impractical due to the instability of adrenochrome. It was therefore considered essential to determine whether the presence of epinephrine was a significant factor influencing the effects of adrenochrome on the isolated perfused rat heart. Furthermore, adrenochrome formation in vivo must necessarily occur in the presence of catecholamines and possible synergistic effects must be considered.

The infusion of epinephrine at a rate calculated to produce a concentration of 1 mg/l in the medium during perfusion with 50 mg/l of adrenochrome resulted in maintenance of a significantly ($P<0.05$) greater contractile force during the first 15 minutes as compared to adrenochrome alone (Figure 19). Contractile force declined

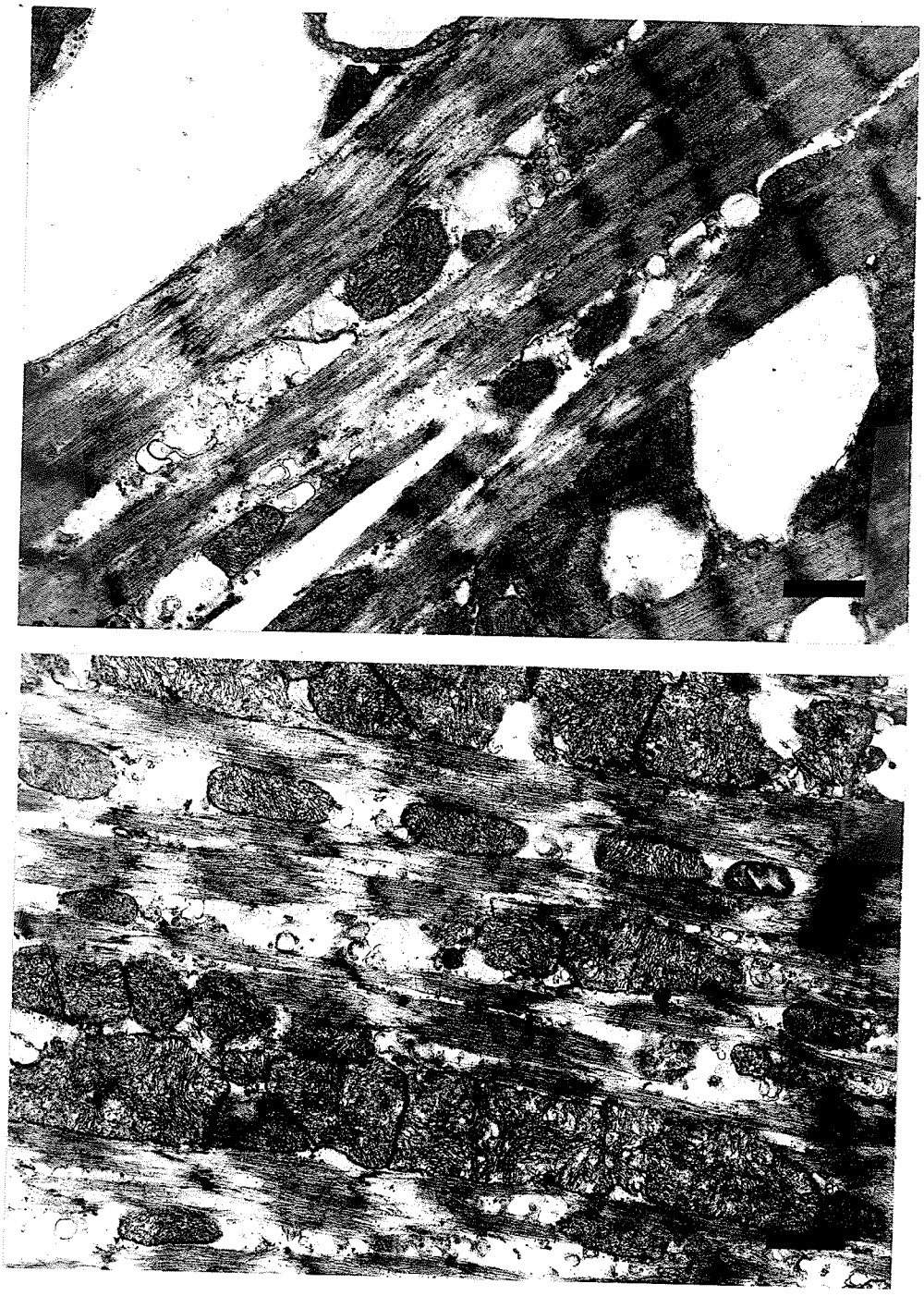

Figure 18: Electron micrographs of sections from isolated rat hearts perfused for 10 minutes with adrenochrome (50 mg/l). Upper panel illustrates both contracture and over-stretching of sarcomeres and the lower panel shows mitochondrial swelling with disruption of the cristae. Black bar indicates one micron.

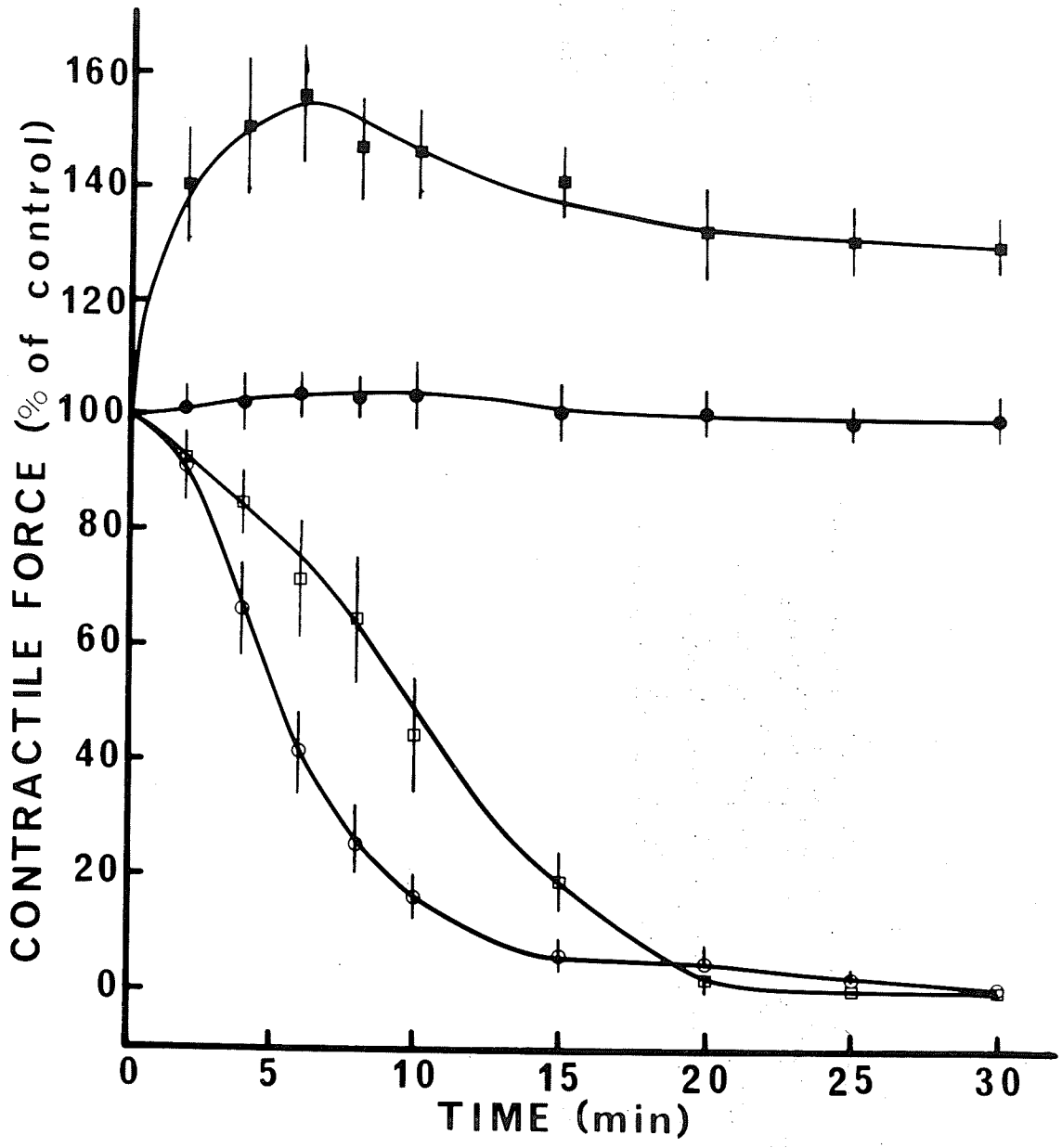

Figure 19: Effect of infusion of epinephrine (1 mg/l) in the absence and presence of adrenochrome (50 mg/l) on the contractile force of isolated rat hearts. Epinephrine (1 mg/l) ■—■, adrenochrome (50 mg/l) ○—○, adrenochrome (50 mg/l) plus epinephrine (1 mg/l) □—□, control ●—●. Each point is a mean ± standard error of 4 experiments. Mean initial contractile force was 8.4 ± 0.2 gm at a resting tension of 2.5 gm.

rapidly thereafter. Comparison of the ultrastructure of hearts after 30 minutes of perfusion with adrenochrome in the presence and absence of epinephrine (1 mg/l) failed to revealed any differences to suggest that epinephrine infusion potentiates the action of adrenochrome (Figure 20, upper panel). Similarly, electron microscopic examination of sections from hearts perfused for 30 minutes with 50 mg/l of adrenochrome during infusion of epinephrine (1 mg/l) revealed ultrastructural damage comparable in extent and severity to that of hearts perfused with 50 mg/l of adrenochrome alone. Thus, although epinephrine was moderately effective in maintaining contractile force during early stages of adrenochrome perfusion, it did not either potentiate or protect the heart against disruption of subcellular structures due to adrenochrome.

In another group of experiments 1 mM ascorbic acid was added to the adrenochrome solution in an attempt to prevent further oxidation of the adrenochrome. Colour changes of the solution upon addition of ascorbic acid indicated that some chemical alteration of the adrenochrome, probably a reduction, had occurred. Perfusion of isolated rat hearts with this solution containing 25 mg/l of adrenochrome together with 1 mM ascorbic acid was found to result in a more rapid decline of contractile force than was observed with 25 mg/l of adrenochrome alone (Figure 21). Two other reducing agents, dithiothriotol and cysteine, were subsequently found to produce even more marked colour changes of the adrenochrome solution. Perfusion of hearts with solutions to which 0.5 mM dithiothriotol was present in addition to 25 mg/l of adrenochrome produced a still more dramatic decline of contractile force than that seen with the ascorbic acid - adrenochrome experiments (Figure 21). Addition of 0.5 mM cysteine to perfusion medium containing 25 mg/l of adrenochrome did not significantly ($P>0.05$) alter the time course of decline of contractile force (Figure 21).

Electron microscopic examination of hearts perfused for 30 minutes with 25 mg/l of adrenochrome to which 1 mM ascorbic acid was added disclosed frequent areas of disruption of myocardial ultrastructure including contracture and disruption of sarcomeres (Figure 22, upper panel). Likewise, hearts perfused for 30 minutes with 25 mg/l of adrenochrome after addition of 0.5 mM dithiothriotol were found to have

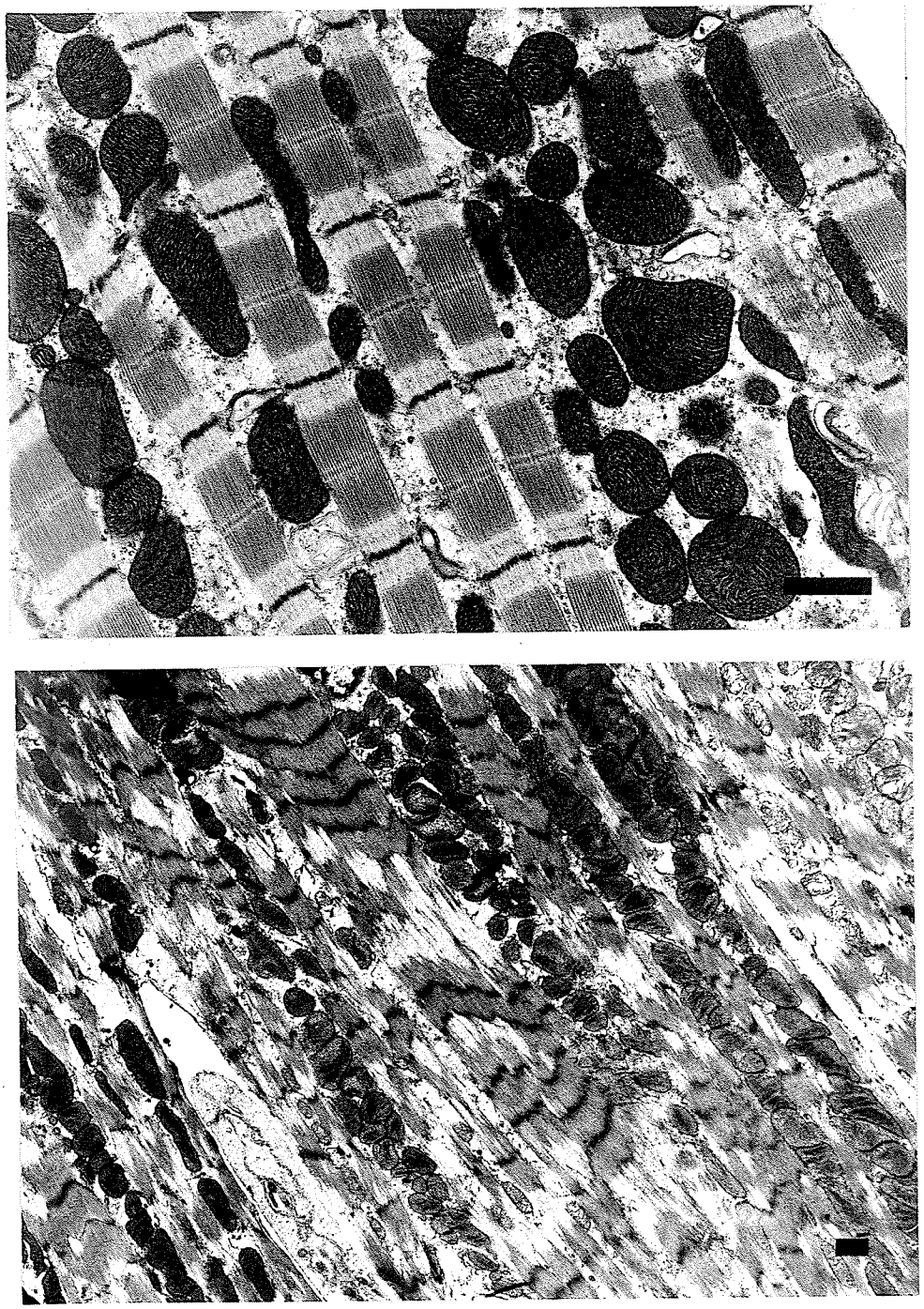

Figure 20: Upper panel - Electron micrograph of a typical section from an isolated rat heart perfused 30 minutes with adrenochrome (25 mg/l) plus epinephrine (1 mg/l). Lower panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (50 mg/l) plus epinephrine (1 mg/l). These micrographs are representative of sections from four hearts each perfused under the conditions described. Black bar indicates one micron.

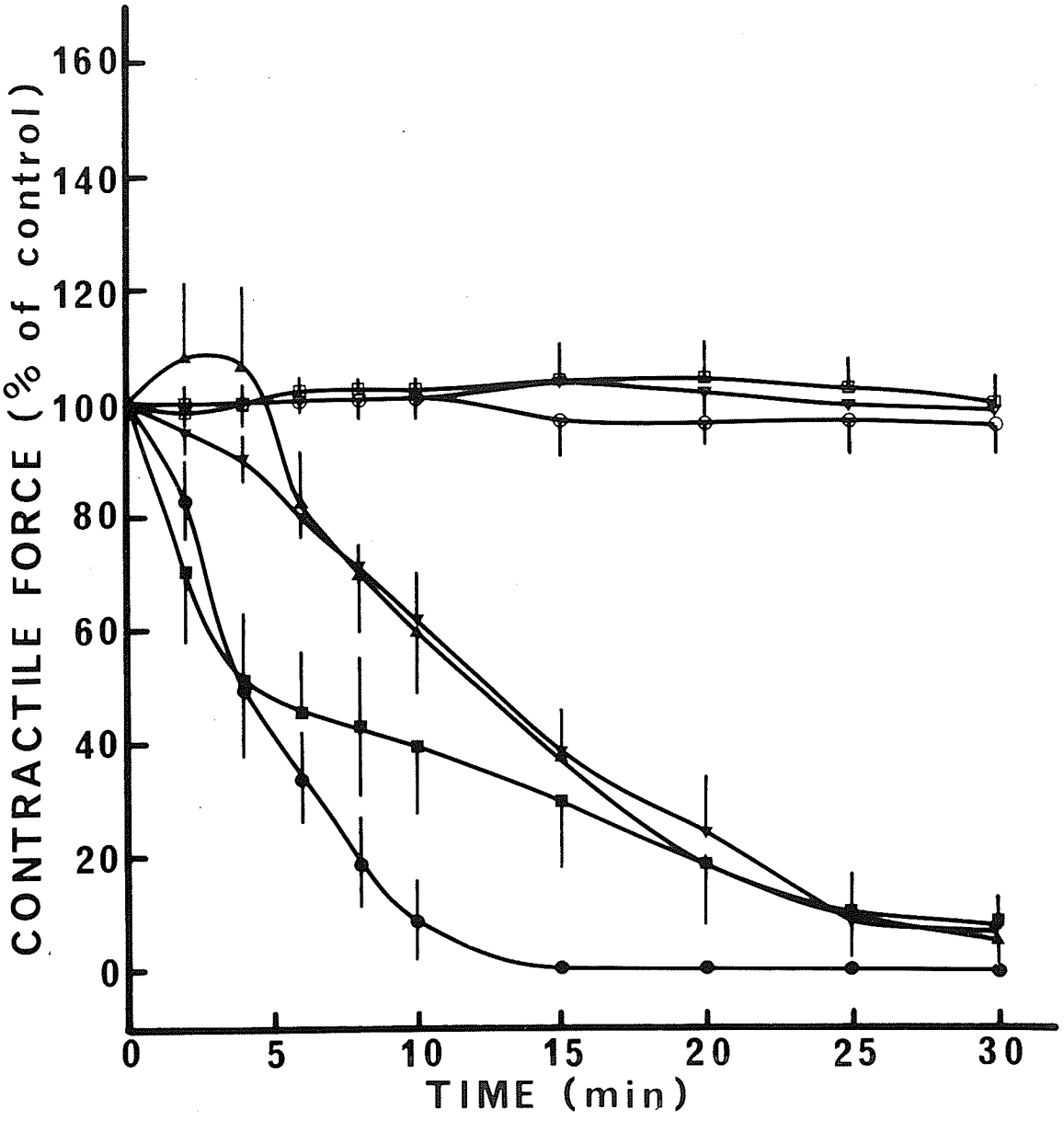

Figure 21: Effect of ascorbic acid (1 mM), cysteine (0.5 mM) and dithiothriotol (0.5 mM) on the time course of failure of contractile force development of isolated rat hearts due to perfusion with adrenochrome (25 mg/l). Ascorbic acid ⊡—⊡, cysteine ▽—▽, dithiothriotol ⊖—⊖, adrenochrome ▲—▲, adrenochrome plus ascorbic acid ■—■, adrenochrome plus cysteine ▼—▼, adrenochrome plus dithiothriotol ●—●. Each point is the mean ± standard error of 4 experiments. Mean initial contractile force values in grams were ascorbic acid - 7.1 ± 0.5; cysteine - 8.1 ± 0.6; dithiothriotol - 8.4 ± 0.5; and control - 8.7 ± 0.5 at a resting tension of 2.5 gm.

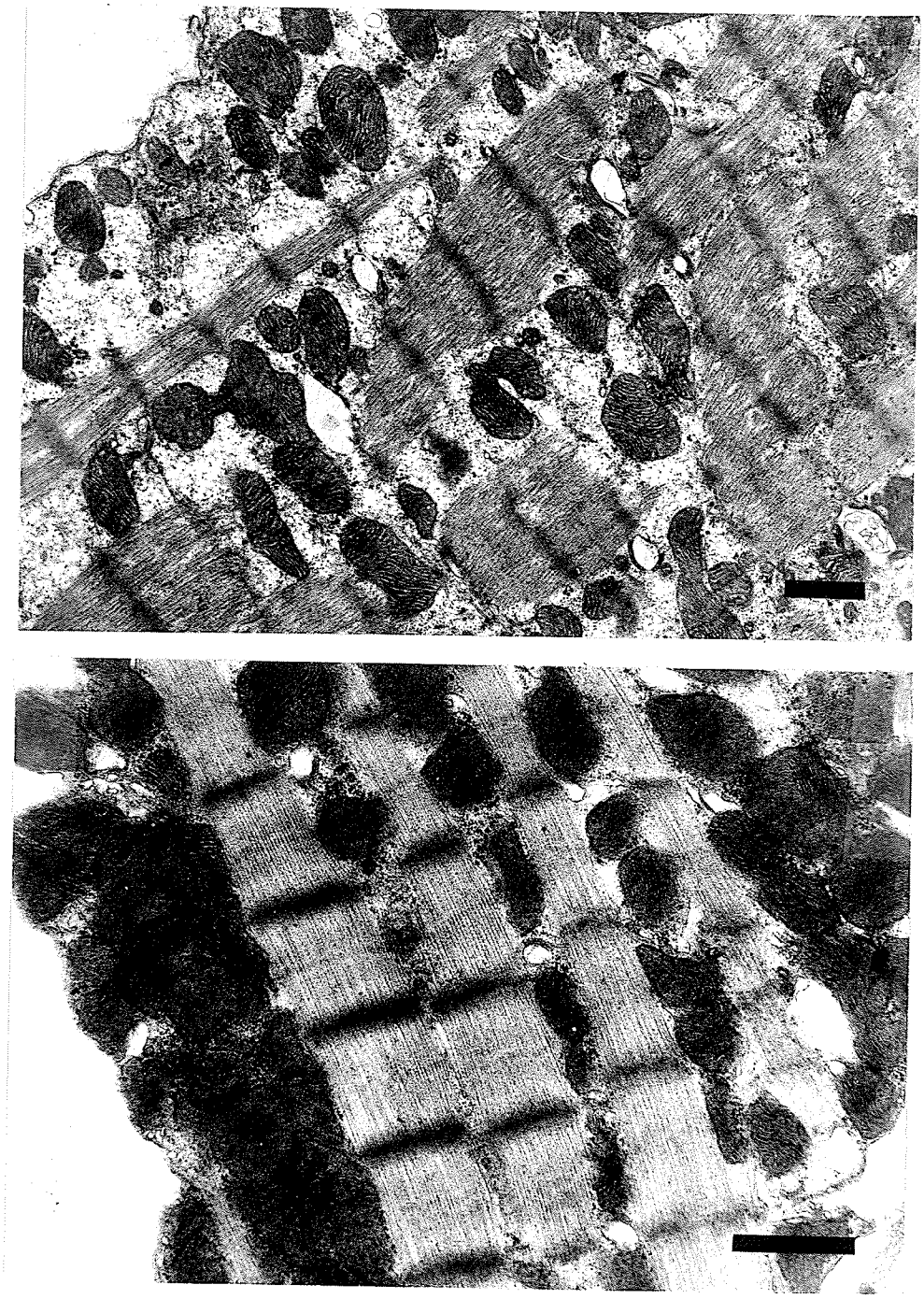

Figure 22: Upper panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (25 mg/l) in the presence of ascorbic acid (1 mM). Lower panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (25 mg/l) in the presence of dithiothriotol (0.5 mM). These micrographs are representative of sections examined from four hearts each perfused under the conditions described. Black bar represents one micron.

large areas in which contracture of sarcomeres was evident, and to be much more severely and extensively damaged throughout than hearts perfused with 25 mg/l of adrenochrome alone (Figure 22, lower panel). Although 0.5 mM cysteine also increased the severity of damage produced by 25 mg/l of adrenochrome (Figure 23, upper panel), widespread contracture was not observed, and much of the tissue examined was relatively normal except for a distinctive swelling of the transverse tubules (Figure 23, lower panel). Sections from isolated rat hearts perfused for similar periods of time with 1 mM ascorbic acid, 0.5 mM dithiothriotol or 0.5 mM cysteine in the absence of adrenochrome were not different from controls.

D. Influence of Altered Cation Concentrations of the Perfusion Medium on Adrenochrome Induced Failure and Necrosis.

Perfusion of isolated rat hearts with 25 mg/l of adrenochrome in perfusing medium containing 0.31 mM Ca^{++} did not significantly alter the time course of failure of contractile force as compared to hearts perfused with 25 mg/l of adrenochrome in the presence of 1.25 mM Ca^{++} (Figure 24). On the other hand, an increase in the Ca^{++} concentration of the medium to 2.5 mM prevented any decline of contractile force below control levels during the first 15 minutes of perfusion with 25 mg/l of adrenochrome (Figure 24). By thirty minutes, contractile force was decreased by only about 55% in hearts perfused with 25 mg/l of adrenochrome in medium containing 2.5 mM Ca^{++}, whereas contractile activity had ceased in hearts perfused with the same concentration of adrenochrome in the presence of 1.25 or 0.31 mM Ca^{++}.

Four hearts each were fixed following 30 minutes of perfusion with 25 or 50 mg/l of adrenochrome in medium containing 0.31 mM Ca^{++} to determine the influence of reduced extracellular Ca^{++} levels on the severity of ultrastructural damage due to adrenochrome. Examination of sections from these hearts showed that reduction of the Ca^{++} concentration of the medium to 0.31 mM had completely protected the myocardium from structural damage due to 50 mg/l of adrenochrome (Figure 25). Likewise, increasing the Ca^{++} concentration of the perfusion medium to 2.5 mM resulted in a considerable increase in both the extent and severity of ultrastructural damage produced by perfusion with 25 mg/l of adrenochrome (Figure 26). The structural integrity of adjacent myocardial cells separated by an intercalated

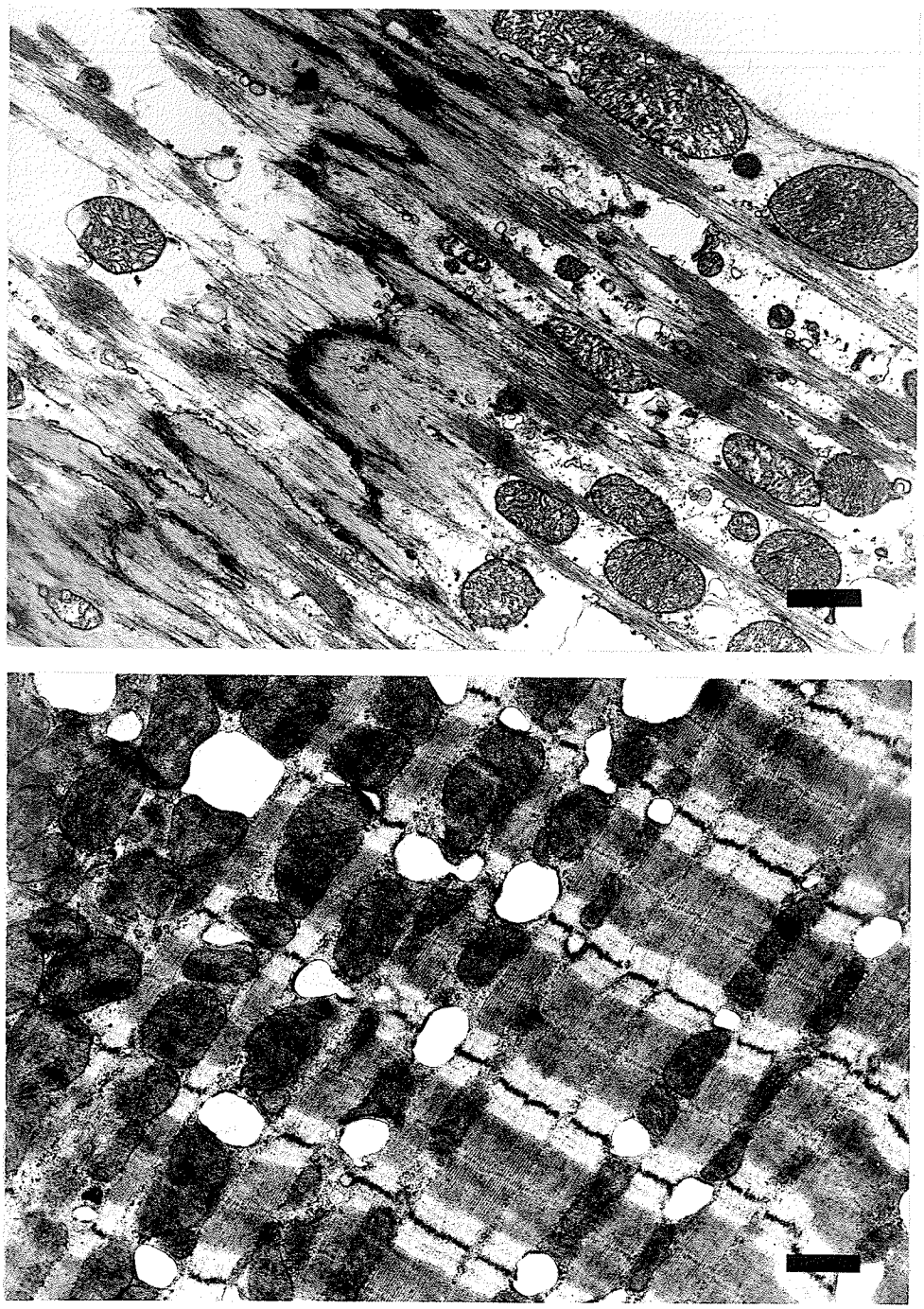

Figure 23: Electron micrographs of typical sections from isolated rat hearts perfused for 30 minutes with adrenochrome (25 mg/l) in the presence of cysteine (0.5 mM). Upper panel indicates areas of extensive damage whereas lower panel represents areas of minimal changes. These micrographs are representative of sections from four hearts perfused under the conditions described. Black bar represents one micron.

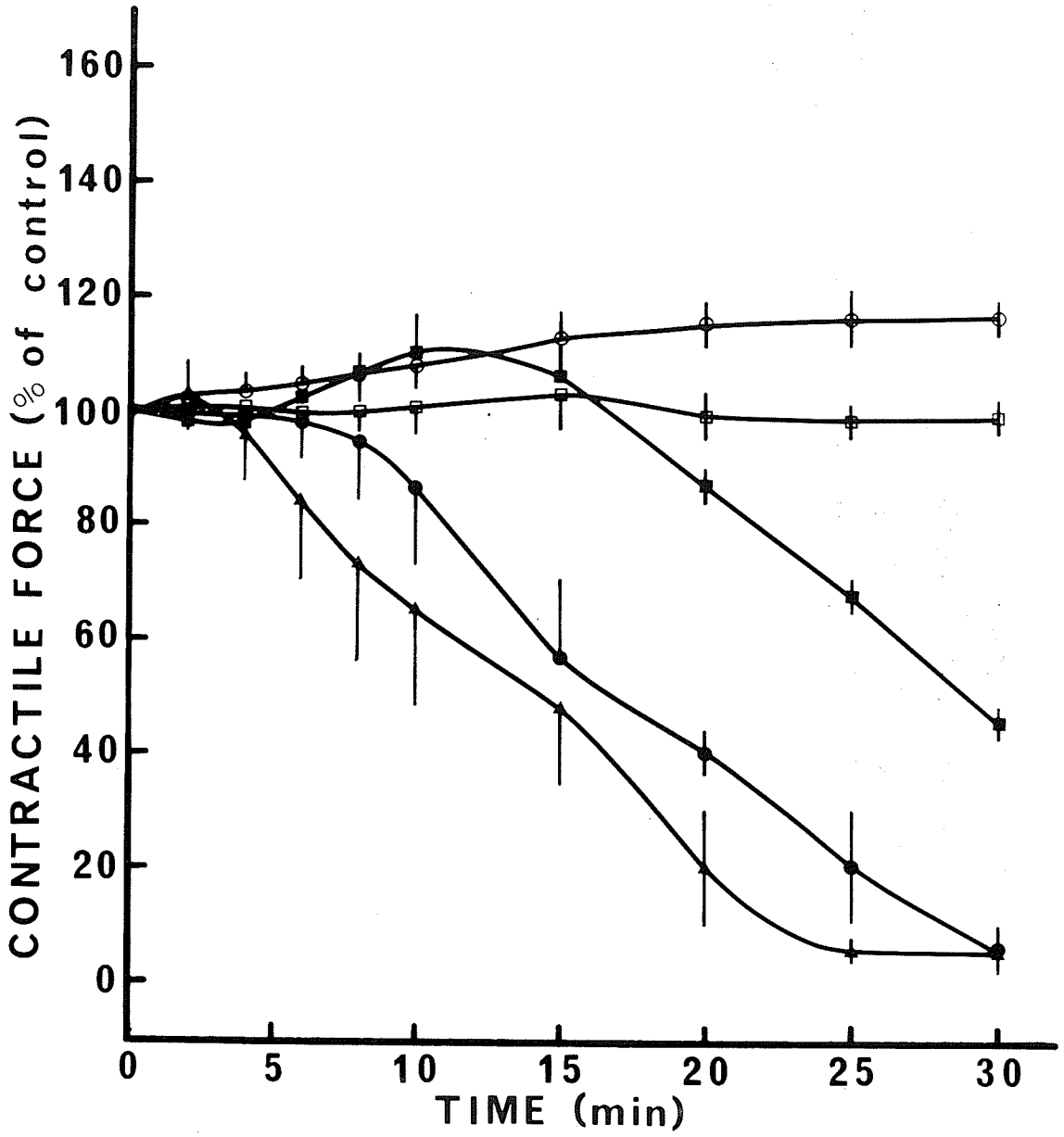

Figure 24: Effect of varying the Ca^{++} concentration of the perfusion medium on the time course of failure of contractile force development of isolated rat hearts due to adrenochrome (25 mg/l). 0.31 mM Ca^{++} ⊙—⊙, 0.31 mM Ca^{++} plus adrenochrome ●—●, 2.5 mM Ca^{++} □—□, 2.5 mM Ca^{++} plus adrenochrome ■—■, 1.25 mM Ca^{++} plus adrenochrome ▲—▲. Each point is the mean ± standard error of 4 experiments. Mean initial contractile force values in grams were : 0.31 mM Ca^{++} - 2.4 ± 0.6; 2.5 mM Ca^{++} - 12.2 ± 0.6; 1.25 mM Ca^{++} - 8.5 ± 0.8 at a resting tension of 2.5 gm.

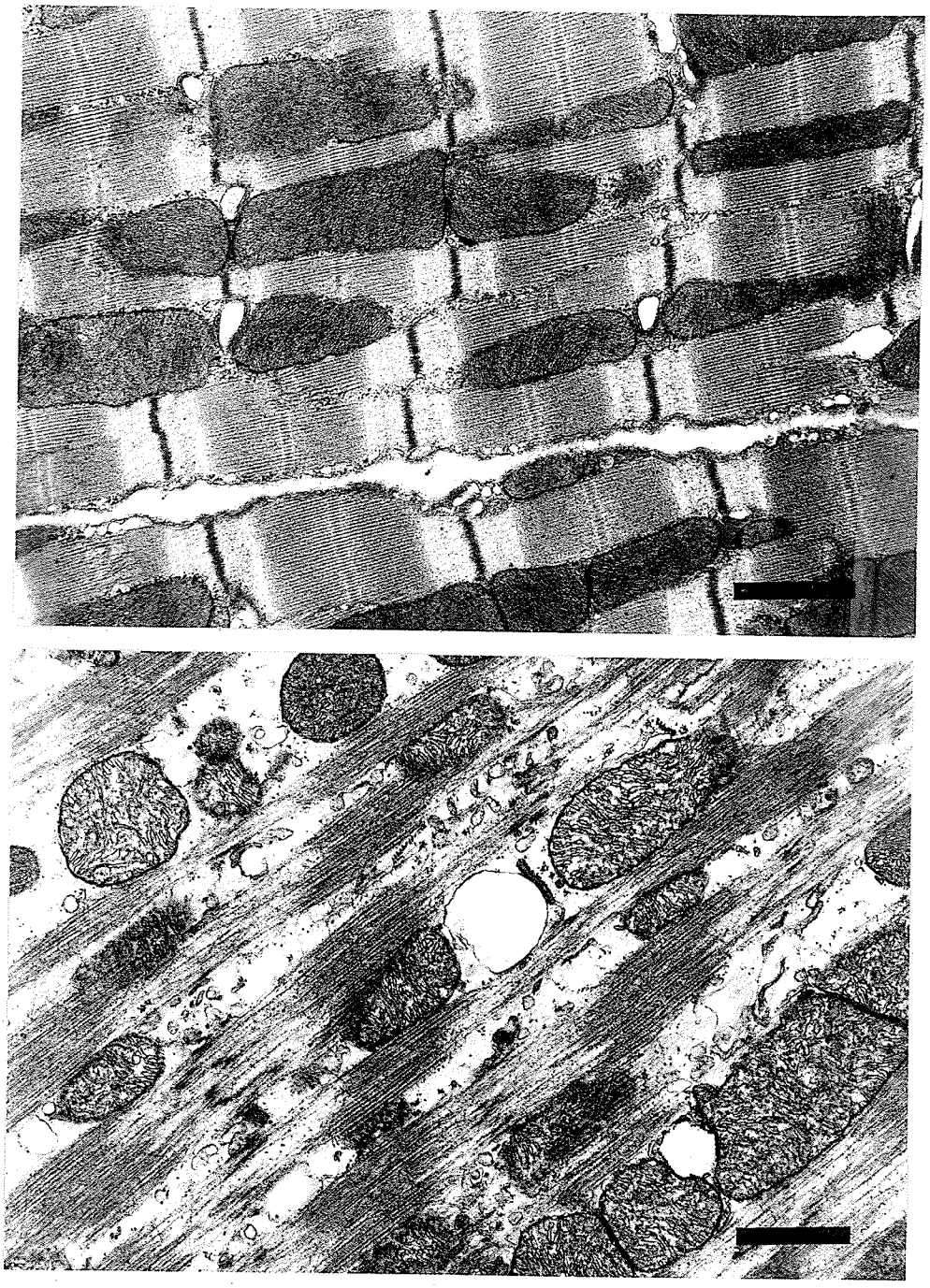

Figure 25: Upper panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (50 mg/l) in the presence of 0.31 mM Ca^{++}. Lower panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (50 mg/l) in the presence of 1.25 mM Ca^{++}. These micrographs are representative of sections examined from four hearts each perfused under the conditions described. Black bar indicates one micron.

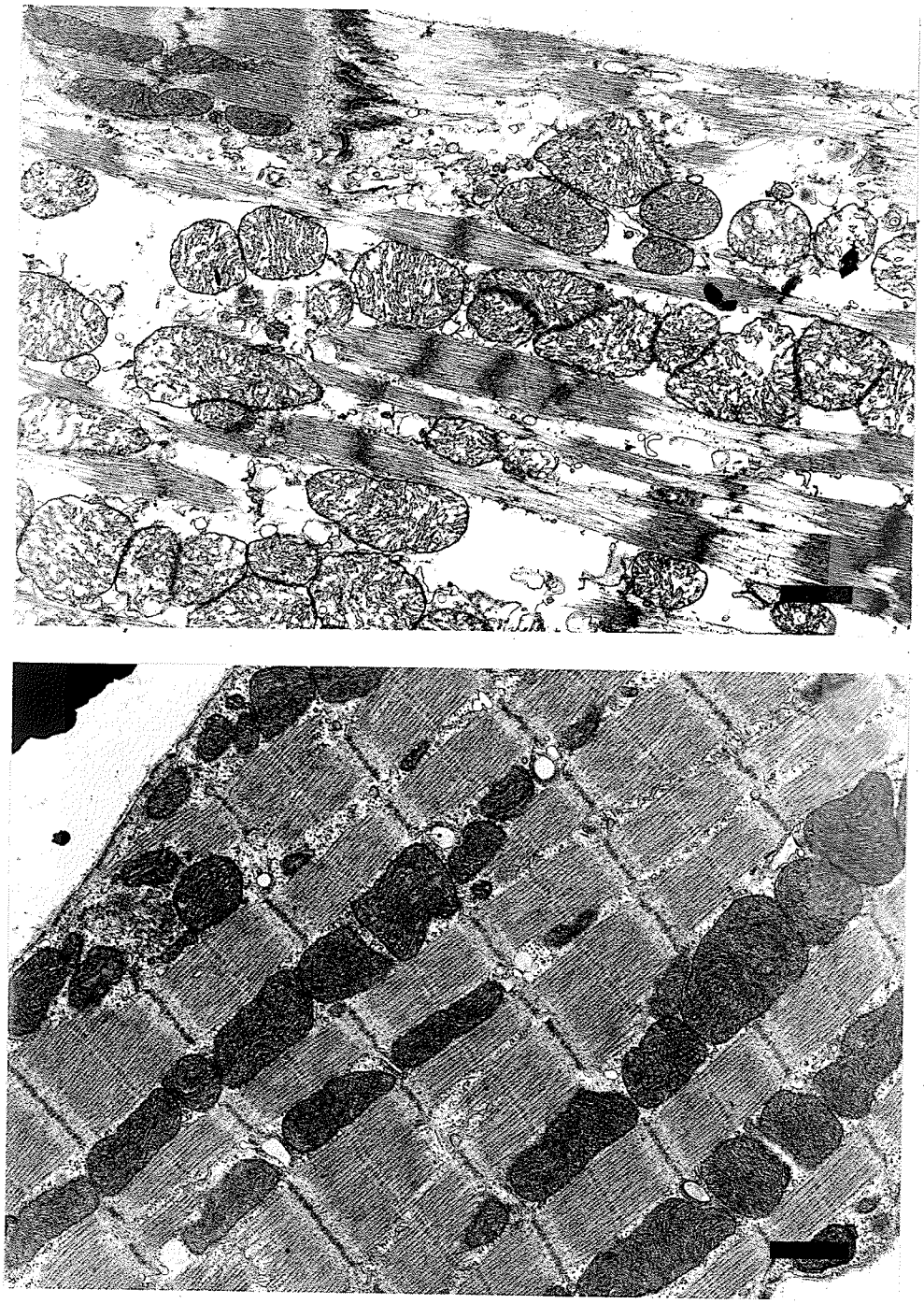

Figure 26: Upper panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (25 mg/l) in the presence of 2.5 mM Ca^{++}. Lower panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (25 mg/l) in the presence of 1.25 mM Ca^{++}. These micrographs are representative of sections examined from four hearts each perfused under the conditions described.

disc was often seen to be quite different in hearts perfused with 25 mg/l of adrenochrome in the presence of 2.5 mM Ca^{++}. An advanced degree of disruption of contractile elements and membranous organelles was frequently observed in one cell while its neighbor was affected only slightly or not at all (Figure 26, upper panel). Ultrastructure of hearts perfused with 2.5 mM Ca^{++} in the absence of adrenochrome was not different from controls.

The effect of reducing the Na^{+} concentration of the perfusion medium was found to be similar to increased Ca^{++} concentration in maintaining contractile force of isolated hearts during perfusion with 25 mg/l of adrenochrome. Contractile force was not significantly ($P>0.05$) less than control during the first ten minutes of perfusion with 25 mg/l of adrenochrome in medium containing 35 mM Na^{+} and had fallen by only 30% at 30 minutes (Figure 27). In contrast to this, the contractile force of hearts perfused with 25 mg/l of adrenochrome in the presence of 145 mM Na^{+} declined steadily after 5 minutes of perfusion and had been reduced to about 5% of the control level by 30 minutes (Figure 27).

Examination of the ultrastructure of hearts fixed after perfusion with 35 mM Na^{+} in the absence of adrenochrome disclosed a swelling of the sarcoplasmic reticulum due to this modification of the perfusing medium, but the structure of the sarcomeres, mitochondria, intercalated discs, and sarcolemma were not affected (Figure 28, upper panel). In hearts fixed after 30 minutes of perfusion with 25 mg/l of adrenochrome in the presence of 35 mM Na^{+} contracture of sarcomeres and mitochondrial swelling and disruption were evident (Figure 28, lower panel). A dramatic contrast was observed in the severity of ultrastructural changes of adjacent cells, similar to that described in hearts perfused with 25 mg/l of adrenochrome in medium containing 2.5 mM Ca^{++}. These changes represent a considerable increase in the severity of necrosis as compared to that produced by perfusion with 25 mg/l of adrenochrome in the presence of 145 mM Na^{+}.

Figure 29 shows the effect of increasing and decreasing the Mg^{++} concentration of the perfusion medium on the time course of failure of contractile force of hearts perfused with 25 mg/l of adrenochrome. Elimination of Mg^{++} from the perfusion medium did not significantly ($P>0.05$) effect the contractile force changes caused by 25 mg/l of adrenochrome over a period of 30 minutes (Figure 29). On the

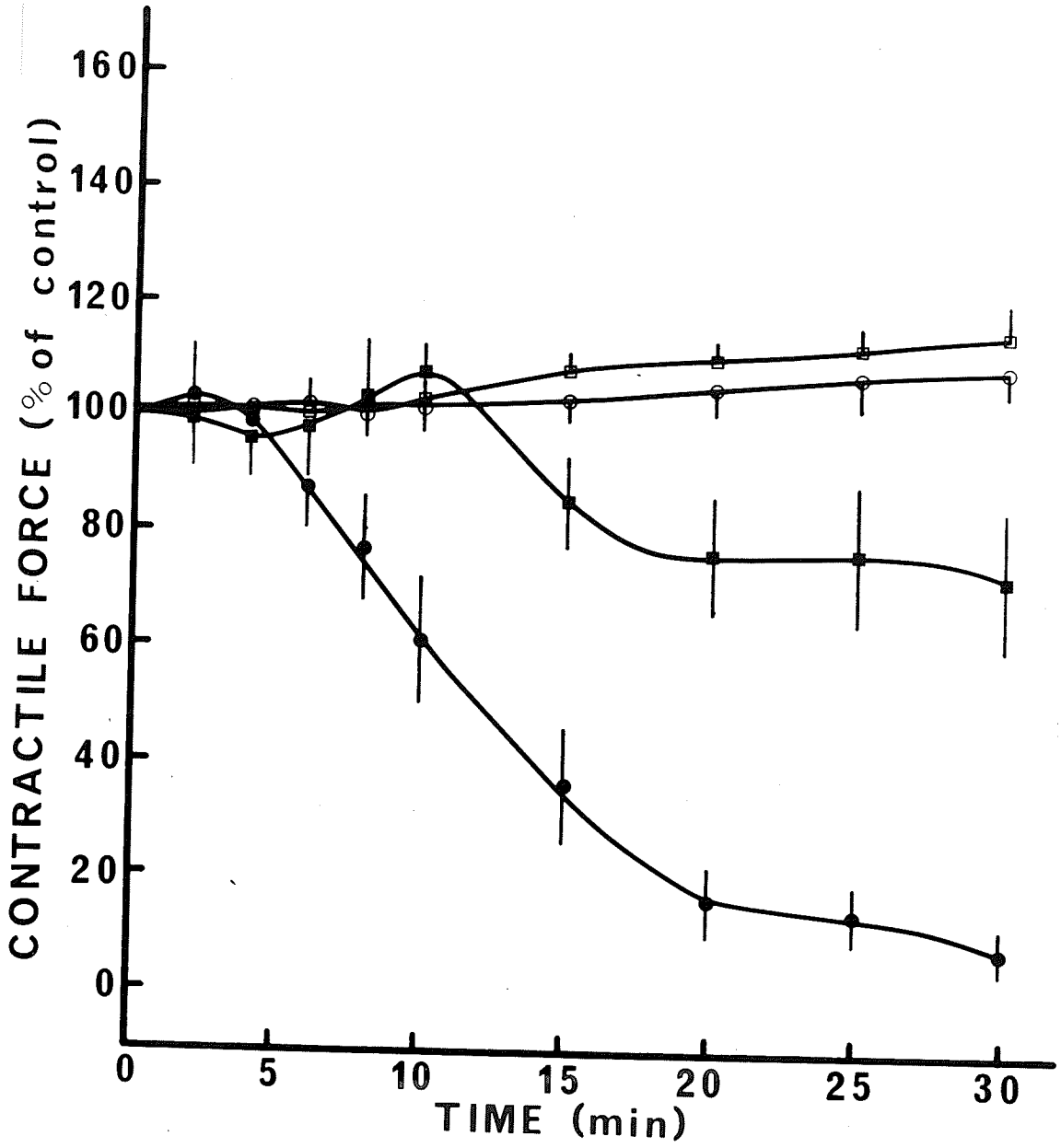

Figure 27: Effect of varying the Na^+ concentration of the perfusion medium on the time course of failure of contractile force development of isolated rat hearts due to adrenochrome (25 mg/l). 35 mM Na^+ ⊡—⊡, 35 mM Na^+ plus adrenochrome ■—■, 145 mM Na^+ ○—○, 145 mM Na^+ plus adrenochrome ●—●. Each point is the mean ± standard error of 4 experiments. Mean initial contractile force values in grams were: 35 mM Na^+ - 11.5 ± 0.6; 145 mM Na^+ - 8.4 ± 0.5 at a resting tension of 2.5 gm.

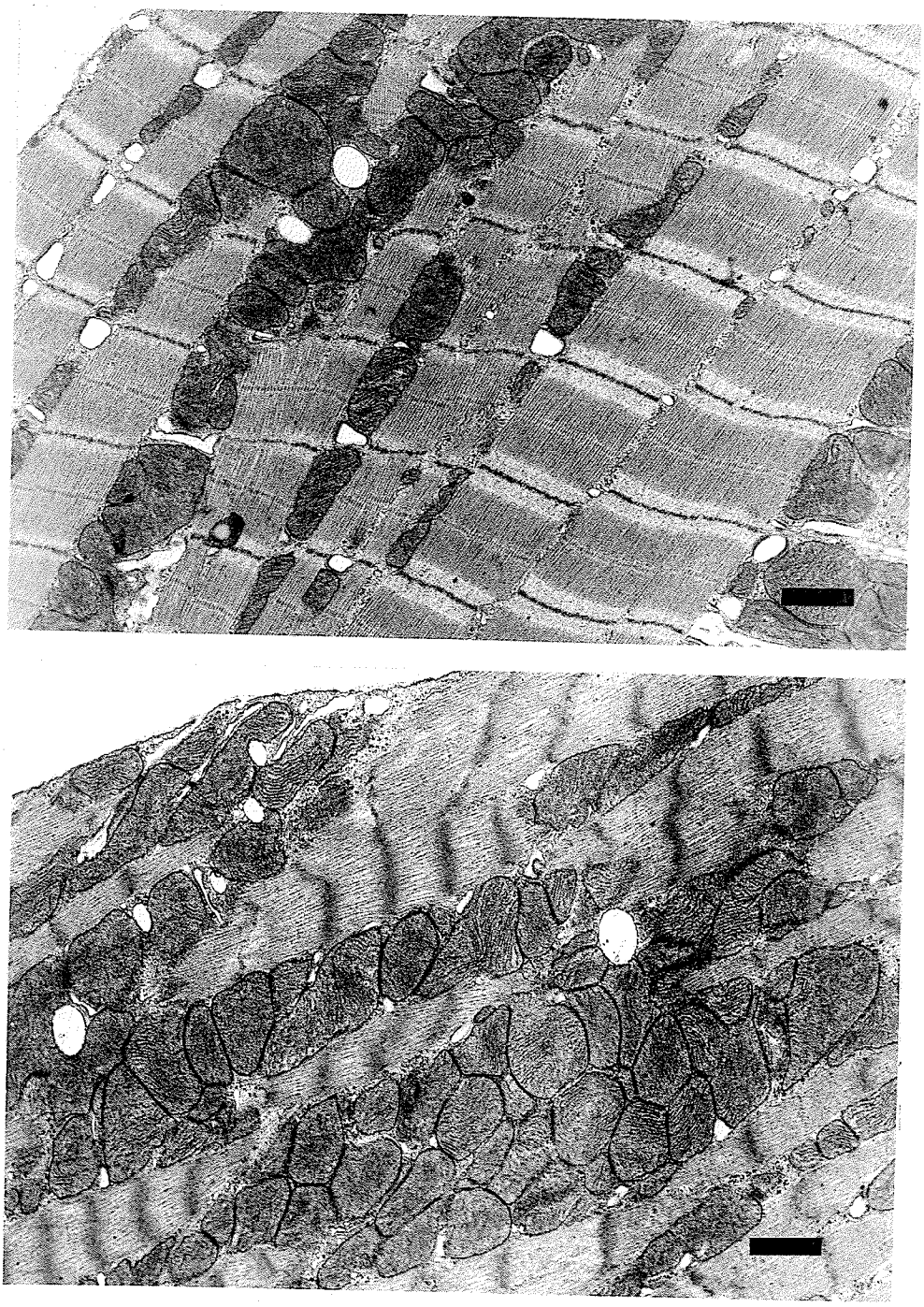

Figure 28: Upper panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with medium containing 35 mM Na^+. Lower panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (25 mg/l) in the presence of 35 mM Na^+. These micrographs are representative of sections examined from four hearts each perfused under the conditions described. Black bar indicates one micron.

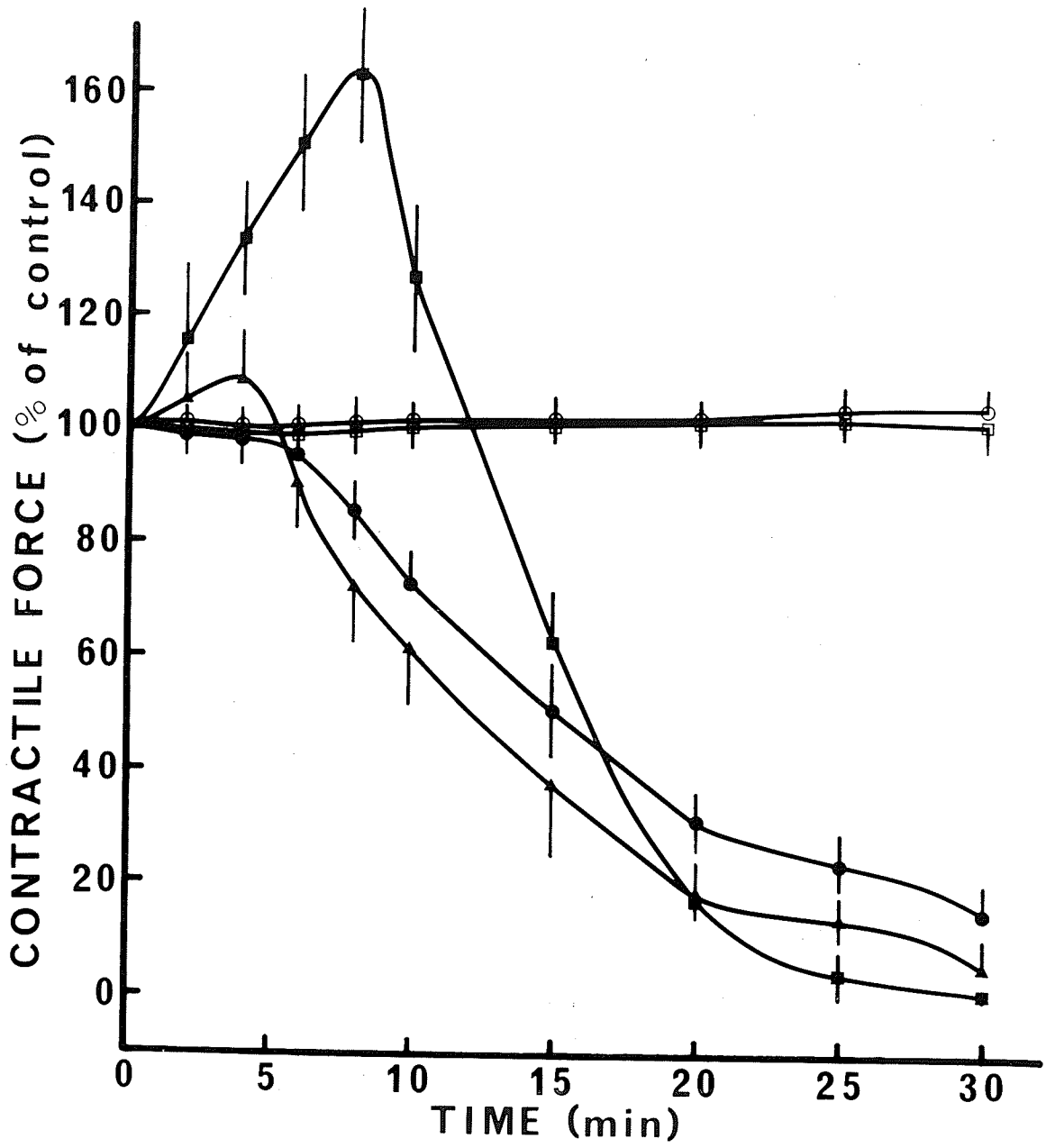

Figure 29: Effect of varying the Mg^{++} concentration of the perfusion medium on the time course of failure of contractile force development of isolated rat hearts due to adrenochrome (25 mg/l). 0 mM Mg^{++} ○—○, 0 mM Mg^{++} plus adrenochrome ●—●, 12 mM Mg^{++} □—□, 12 mM Mg^{++} plus adrenochrome ■—■, 1.2 mM Mg^{++} plus adrenochrome ▲—▲. Each point is a mean ± standard error of four experiments. Mean initial contractile force values in grams were: 0 mM Mg^{++} - 9.9 ± 0.7; 12 mM Mg^{++} - 1.9 ± 0.5; 1.2 mM Mg^{++} - 8.2 ± 0.7 at a resting tension of 2.5 gm.

other hand 12 mM Mg^{++}, which by itself supported a level of contractile force development of only 1.9 ± 0.5 gm at a resting tension of 2.5 gm, resulted in a steady increase in contractile force following initiation of perfusion with 25 mg/l of adrenochrome to a level of over 160% of control by eight minutes (Figure 29). Contractile force declined rapidly after the first eight minutes and during the last 10 minutes of the perfusion period was comparable to that of hearts perfused with 25 mg/l of adrenochrome in the presence of 1.2 mM Mg^{++}.

Omission of Mg^{++} from the perfusion medium did not alter the ultrastructural appearance of hearts fixed after perfusion for 30 minutes with either 25 mg/l of adrenochrome (Figure 30, upper panel), or 50 mg/l of adrenochrome as compared to hearts perfused with the same concentration of adrenochrome in the presence of 1.2 mM Mg^{++}, whereas 12 mM Mg^{++} effectively prevented necrotic changes at both adrenochrome concentrations. Figure 30 (lower panel) shows a typical speciman from a heart perfused for 30 minutes with 50 mg/l of adrenochrome at a Mg^{++} concentration of 12 mM in which mitochondria and sarcomeres are intact. The ultrastructure of these hearts was normal except for an occasional moderate degree of swelling of the transverse tubules.

Although in all other experiments reported in this study isolated rat hearts were paced at 360 beats/min, in the series of experiments designed to investigate the influence of altered K^+ concentration of the perfusion medium on the effect of adrenochrome the stimulation rate had to be reduced to 180/min in order to avoid irregular contractions and fibrillation. As can be seen in Figure 31, initiation of perfusion with 25 mg/l of adrenochrome resulted in an increased contractile force development at every K^+ concentration with this rate of stimulation, and this initial increase was most pronounced with a K^+ concentration of 18 mM. Furthermore, contractile force was maintained at a high level throughout the entire period of perfusion with 25 mg/l of adrenochrome in the presence of 18 mM K^+, and at 30 minutes was nearly 50% of control (Figure 31). The time course of failure of contractile force due to perfusion with 25 mg/l of adrenochrome was not significantly different at a K^+ concentration of 1.5 mM as compared to 6.0 mM (Figure 31).

Subcellular structures were found to be intact following perfusion for 30 minutes with medium containing 1.5 mM K^+ in the absence of adrenochrome (Figure 32, upper panel), while contracture of sarcomeres and swelling and disruption of

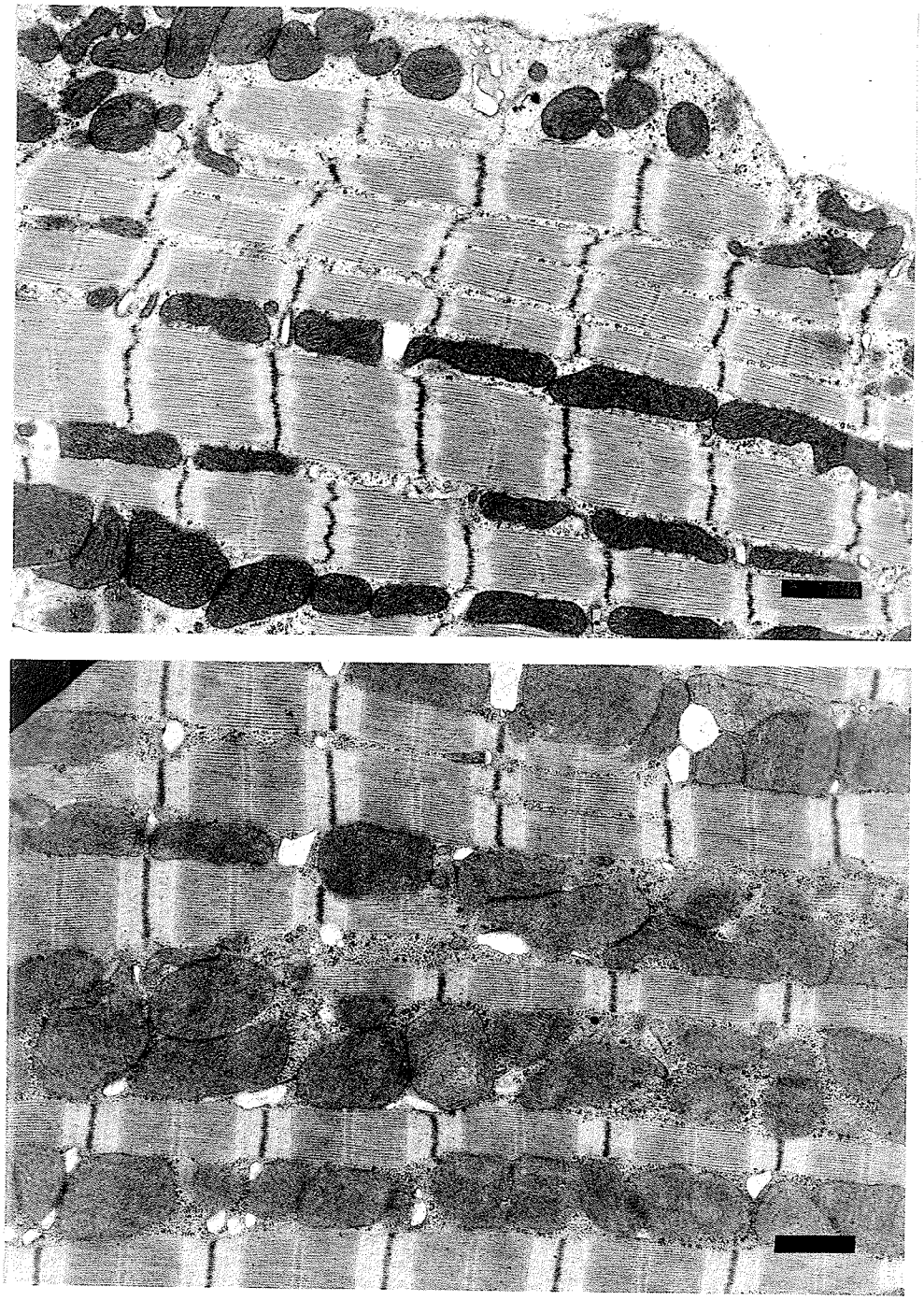

Figure 30: Upper panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (25 mg/l) in the absence of Mg^{++}. Lower panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (50 mg/l) in the presence of 12 mM Mg^{++}. These micrographs are representative of sections examined from four hearts each perfused under the conditions described.

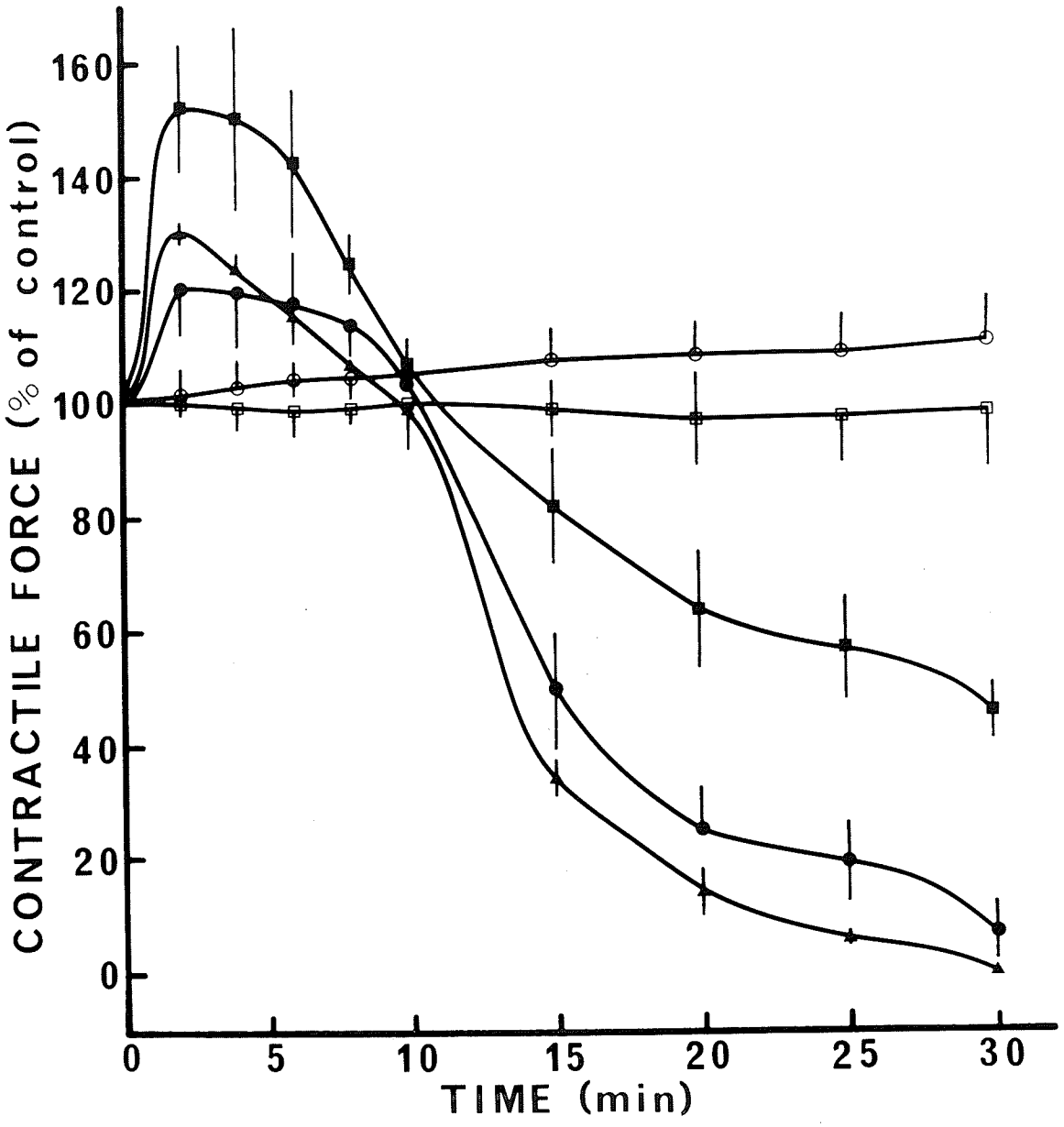

Figure 31: Effect of varying the K^+ concentration of the perfusion medium on the time course of failure of contractile force development due to adrenochrome (25 mg/l). 1.5 mM K^+ ○—○ , 1.5 mM K^+ plus adrenochrome ●—● , 18 mM K^+ □—□ , 18 mM K^+ plus adrenochrome ■—■ , 6 mM K^+ plus adrenochrome ▲—▲. Each point is the mean ± standard error of four experiments. Mean initial contractile force values in grams were: 1.5 mM K^+ - 9.9 ± 0.9; 18 mM K^+ - 5.2 ± 0.8; 6.0 mM K^+ - 8.7 ± 0.4 at a resting tension of 2.5 gm.

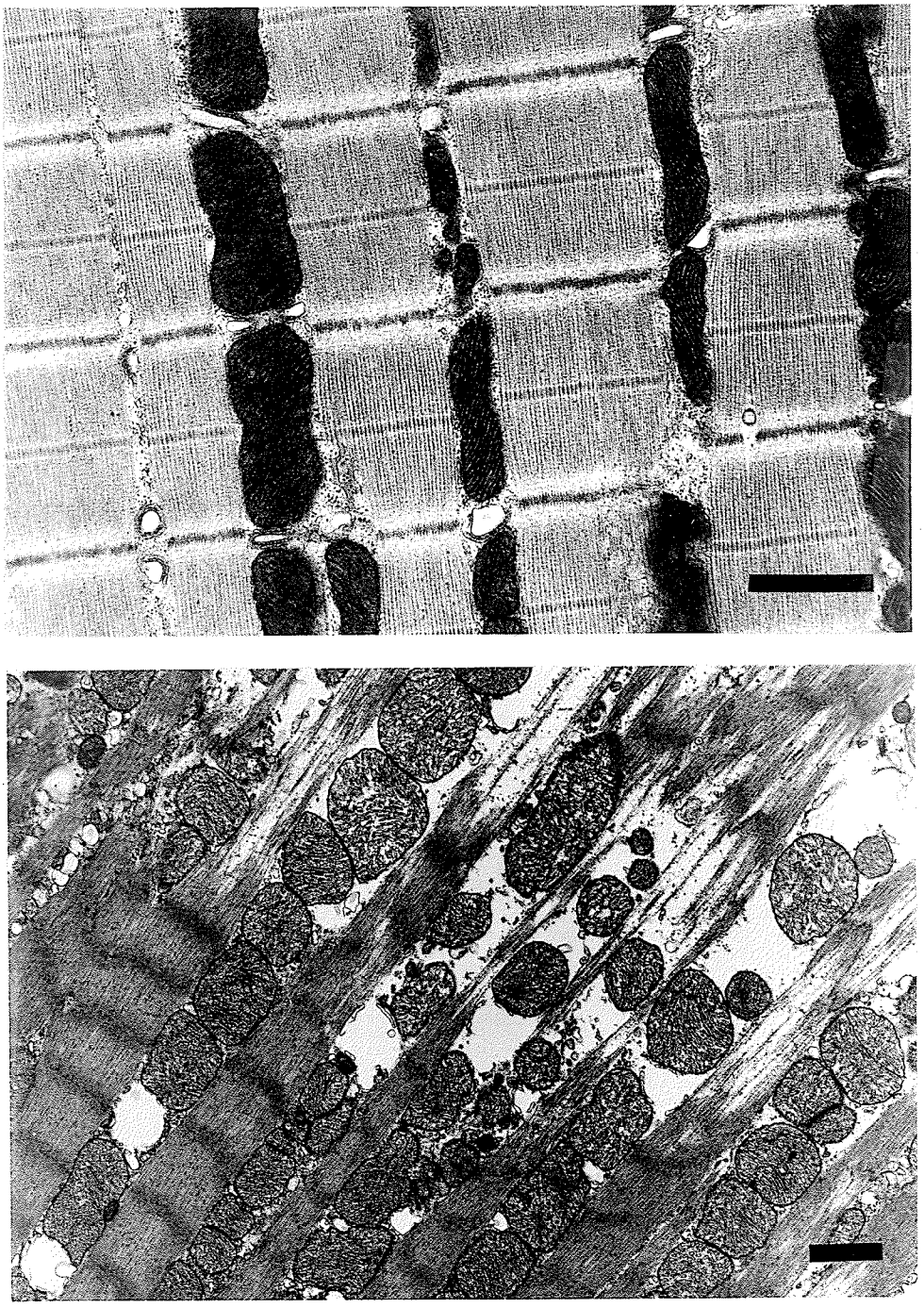

Figure 32: Upper panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with medium containing 1.5 mM K^+. Lower panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (25 mg/l) in the presence of 1.5 mM K^+. These micrographs are representative of sections examined from four hearts each perfused under the conditions described. Black bar indicates one micron.

mitochondria were frequently observed in hearts perfused in the presence of 25 mg/l of adrenochrome at this K^+ concentration (Figure 32, lower panel). Similarly, 18 mM K^+ did not itself produce any contracture, disruption, or mitochondrial swelling (Figure 33, upper panel), whereas perfusion for 30 minutes with 25 mg/l of adrenochrome in the presence of 18 mM K^+ did result in such damage to large portions of the left ventricular myocardium (Figure 33, lower panel).

E. Influence of Various Pharmacological Interventions on Adrenochrome Induced Failure and Necrosis.

The presence of the calcium antagonist D-600 at a concentration of 0.5 mg/l in the perfusing medium resulted in a decline and disappearance of contractile activity of the isolated rat heart within less than 10 minutes of perfusion. This fact made investigation of the effect of this agent on the time course of failure of contractile activity caused by adrenochrome impossible. The influence of 0.5 mg/l of D-600 on the morphological changes induced by adrenochrome was studied following 30 minutes of perfusion of these non-contracting hearts with 50 mg/l of adrenochrome. Electrical stimulation at 360 pulses/minute was continued and a resting tension of 2.5 gm was applied throughout each experiment. Electron microscopic examination of sections from the left ventricles of these hearts revealed ultrastructural changes which were quite different from those usually produced by 50 mg/l of adrenochrome. The most striking feature was a desalignment of the contractile filaments within the sarcomeres (Figure 34, upper panel). This alteration was seen in most, but not all, of the sections examined. Mitochondria, sarcoplasmic reticulum, and transverse tubules were in general, well preserved, although large vacuoles of uncertain origin were frequently observed. Nonetheless, the contracture and dissolution of contractile elements and swelling and disruption of mitochondria characteristic of perfusion with 50 mg/l of adrenochrome (Figure 34, lower panel) were completely absent in hearts perfused with 50 mg/l of adrenochrome in the presence of 0.5 mg/l of D-600. The ultrastructure of hearts perfused for 30 minutes with 0.5 mg/l of D-600 in the absence of adrenochrome was not different from controls.

Addition of either tolazoline (25 mg/l) or dibenamine (25 mg/l) which act as α - adrenergic receptor blocking agents to the perfusion medium was found not to

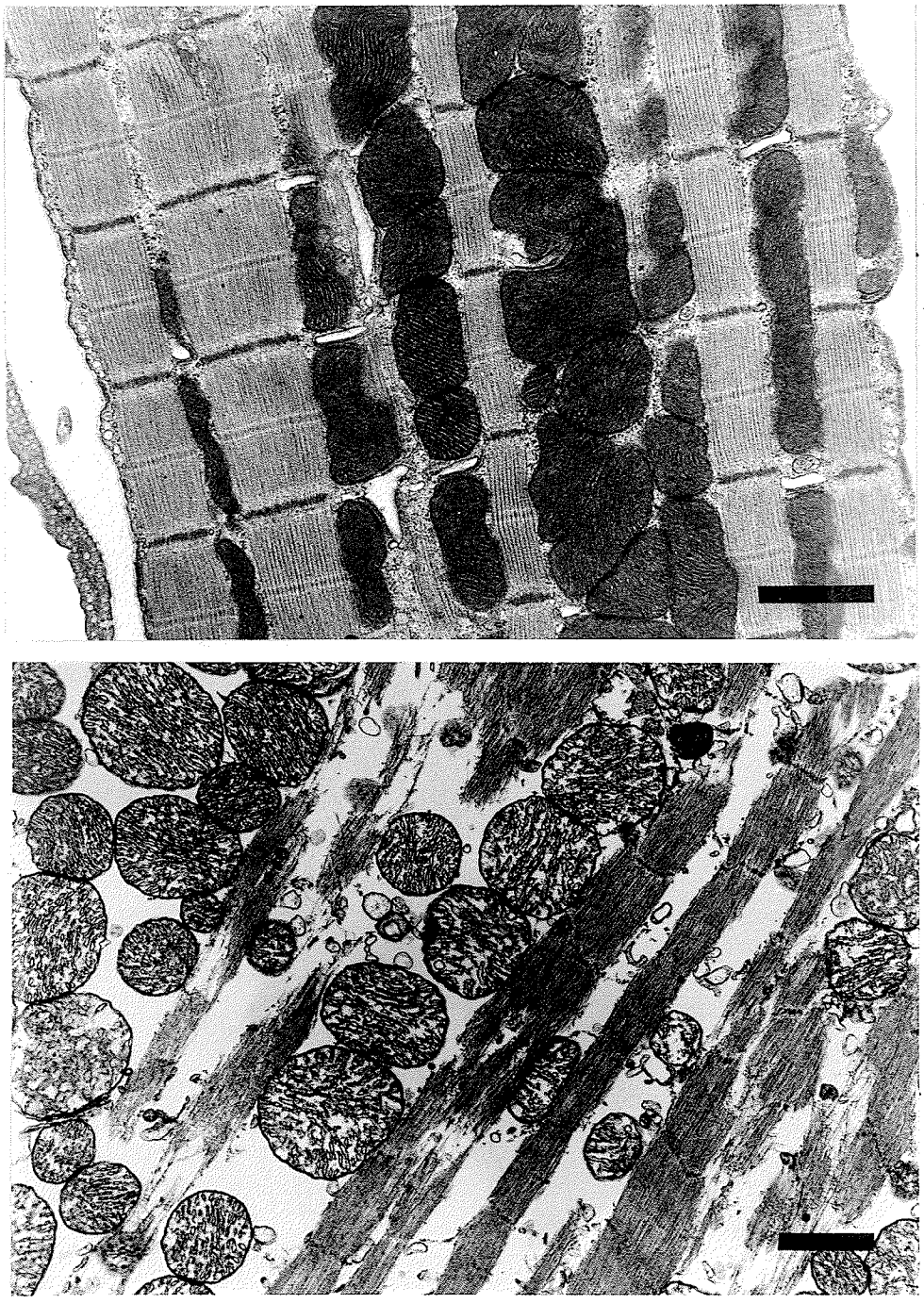

Figure 33: Upper panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with medium containing 18 mM K^+. Lower panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (25 mg/l) in the presence of 18 mM K^+. These micrographs are representative of sections examined from four hearts each perfused under the conditions described. Black bar indicates one micron.

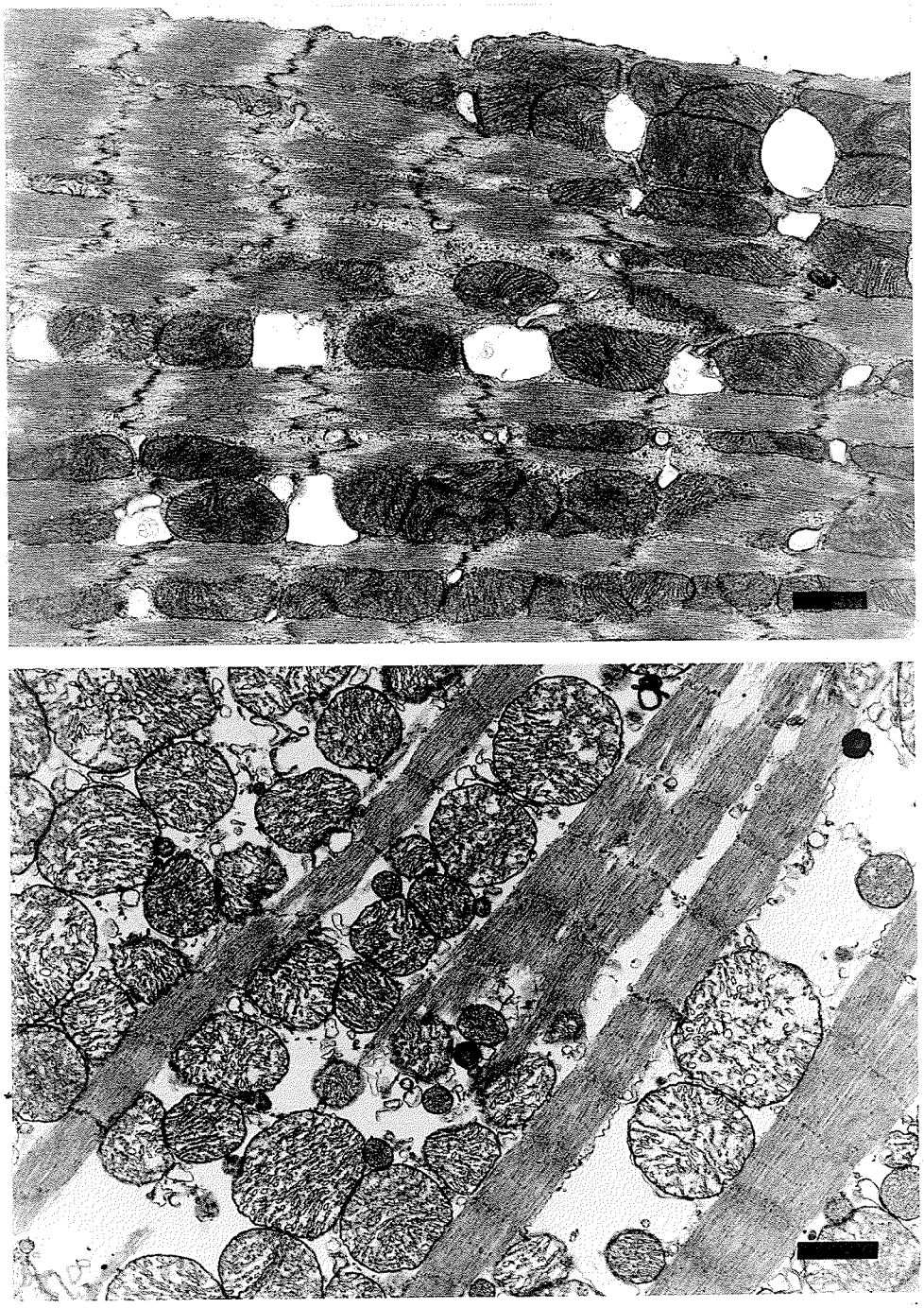

Figure 34: Upper panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (50 mg/l) in the presence of D-600 (0.5 mg/l). Lower panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (50 mg/l). These micrographs are representative of sections examined from four hearts each perfused under the conditions described. Black bar indicates one micron.

significantly ($P>0.05$) alter the time course of failure of contractile force development due to perfusion with 25 mg/l of adrenochrome (Figure 35). The presence of these agents in the perfusion medium was also ineffective in protecting isolated rat hearts from necrotic changes due to 30 minutes of perfusion with 50 mg/l of adrenochrome (Figure 36).

The presence of 1 mg/l of practolol, a β-adrenergic receptor blocking agent, in the medium was found to augment the contractile force of isolated rat hearts upon commencement of perfusion with 25 mg/l of adrenochrome, and force of contraction remained significantly ($P<0.05$) above that of hearts perfused with 25 mg/l of adrenochrome alone throughout the 30 minute perfusion period (Figure 37). Another β - receptor blocking agent, propranolol (1 mg/l), did not significantly ($P>0.05$) alter the course of failure of contractile force during the first 15 minutes of perfusion with 25 mg/l of adrenochrome, but significantly ($P<0.05$) reduced the rate of decline of contractile force during the remainder of the perfusion period (Figure 37). Upon electron microscopic examination of hearts fixed after perfusion for 30 minutes with 50 mg/l of adrenochrome in the presence of either propranolol (1 mg/l) or practolol (1 mg/l) myocardial ultrastructure was found to be well preserved, and no evidence of necrotic damage was observed (Figure 38).

The time course of failure of contractile force development of isolated rat hearts was not different ($P>0.05$) in the presence or absence of either of two adrenergic neuron blocking agents, quanethidine (2 mg/l) and bretylium (2 mg/l) (Figure 39). These agents also failed to protect the isolated heart from necrotic changes due to 30 minutes of perfusion with 50 mg/l of adrenochrome (Figure 40).

The hydrazine type mono-amine oxidase inhibitor iproniazide, at a concentration of 25 mg/l, was found to significantly ($P<0.05$) improve contractile force development from 6 to 30 minutes of perfusion with 25 mg/l of adrenochrome (Figure 41). At 30 minutes mean contractile force was over 40% of control level in the presence of iproniazide, whereas with 25 mg/l of adrenochrome alone the contractile force was only 5% of control at 30 minutes (Figure 41). On the other hand, tranylcypromine (25 mg/l) which is a non-hydrazine mono-amine oxidase inhibitor, was not effective ($P>0.05$) in altering the time course of contractile force changes due to adrenochrome (25 mg/l).

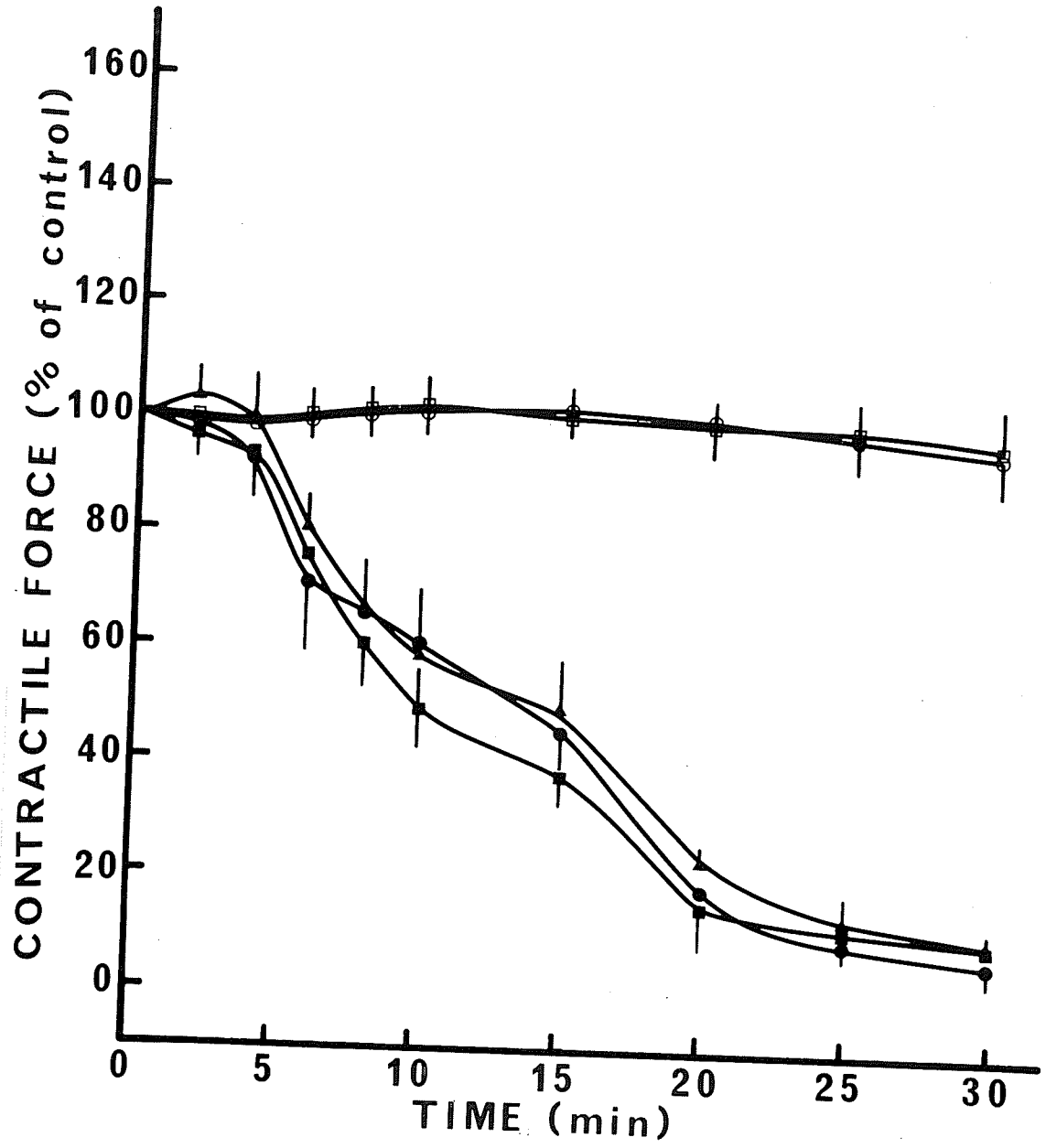

Figure 35: Effect of tolazoline (25 mg/l) and dibenamine (25 mg/l) on the time course of failure of contractile force development of the isolated rat heart perfused with adrenochrome (25 mg/l). Tolazoline ⊡—⊡, tolazoline plus adrenochrome ■—■, dibenamine ⊙—⊙, dibenamine plus adrenochrome ●—●, adrenochrome ▲—▲. Each point is the mean ± standard error of four experiments. Mean initial contractile force values in grams were: tolazoline - 8.8 ± 0.5; dibenamine - 8.5 ± 0.1; and control 9.6 ± 0.9 at a resting tension of 2.5 gm.

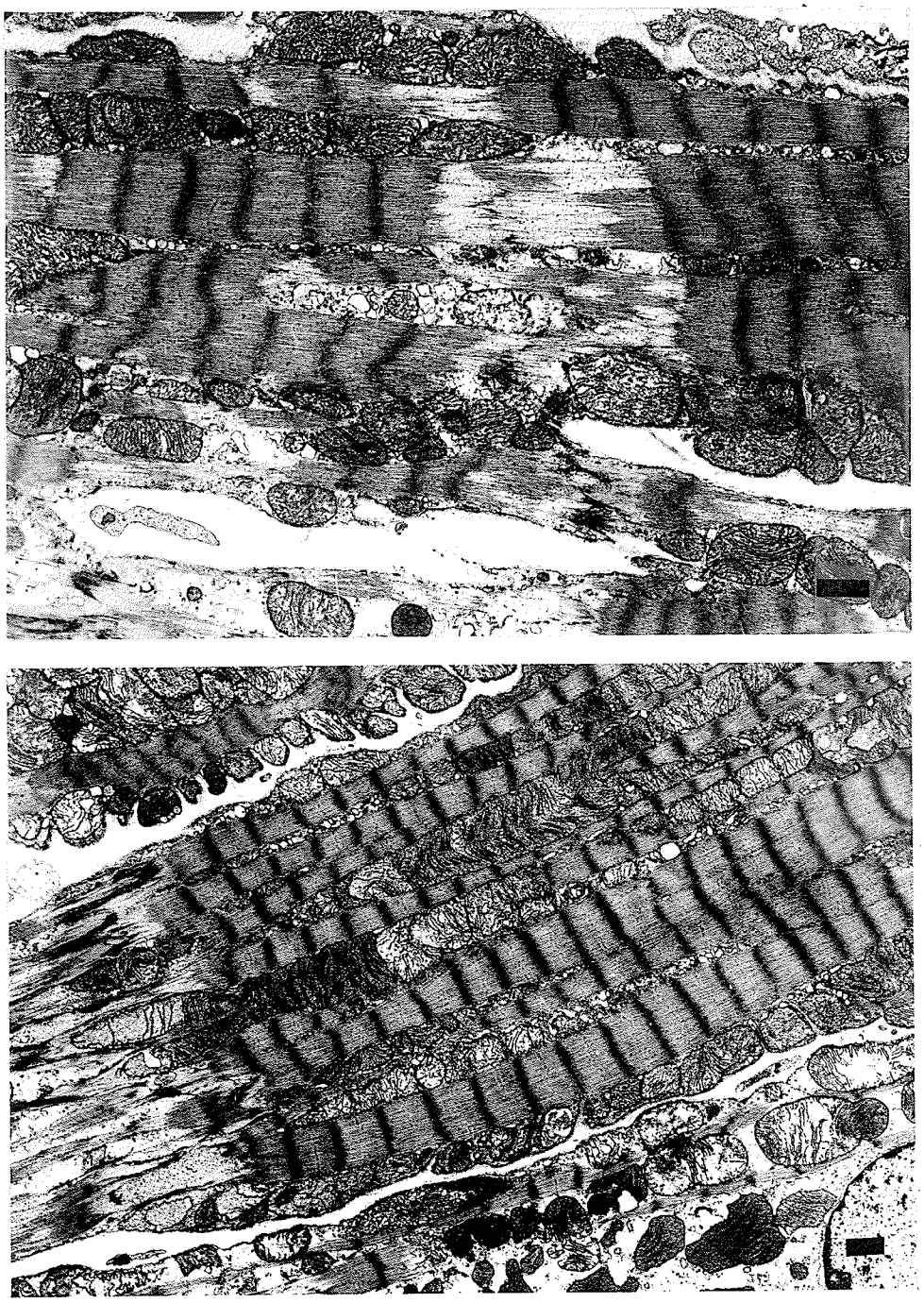

Figure 36: Upper panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (50 mg/l) in the presence of tolazoline (25 mg/l). Lower panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (50 mg/l) in the presence of dibenamine (25 mg/l). These micrographs are representative of sections examined from four hearts each perfused under the conditions described. Black bar indicates one micron.

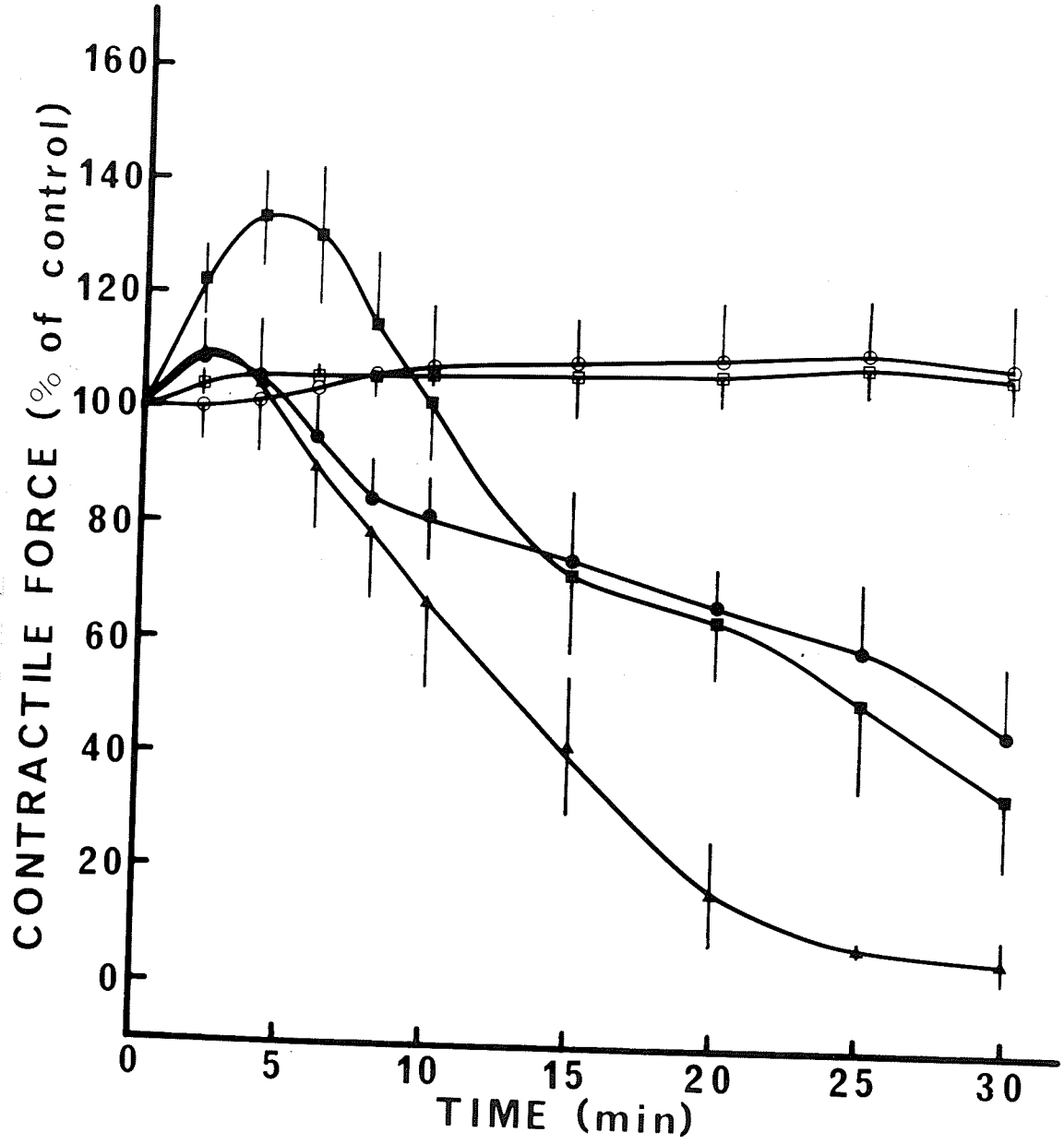

Figure 37: Effect of propranolol (1 mg/l) and practolol (1 mg/l) on the time course of failure of contractile force development of isolated rat hearts due to perfusion with adrenochrome (25 mg/l). Propranolol ⊙—⊙ , propranolol plus adrenochrome ●—●, practolol ⊡—⊡, practolol plus adrenochrome ■—■, adrenochrome ▲—▲. Each point is the mean ± standard error of four experiments. Mean initial contractile force values in grams were: propranolol - 7.5 ± 0.6; practolol - 6.5 ± 0.7; control 8.6 ± 0.8 at a resting tension of 2.5 gm.

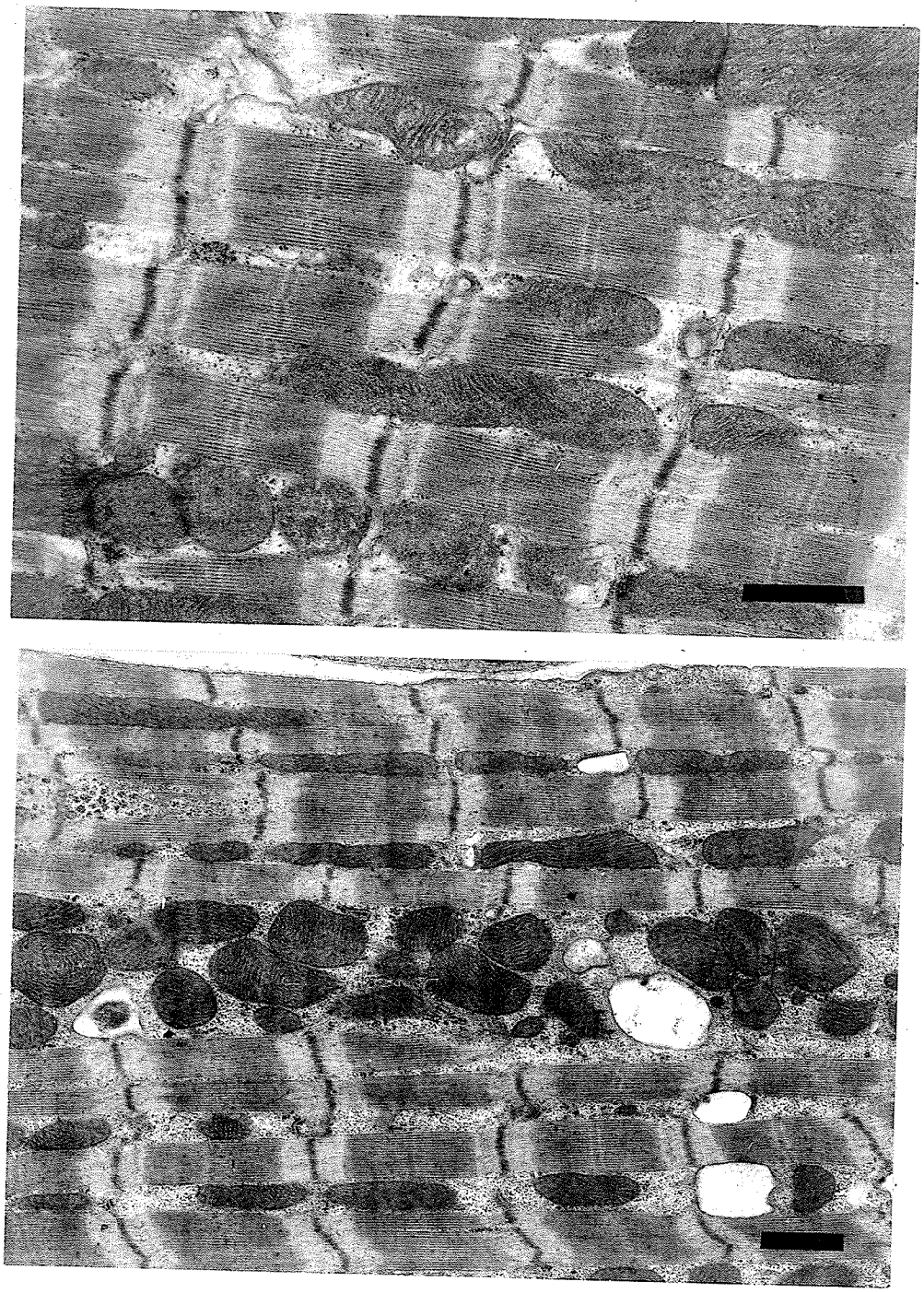

Figure 38: Upper panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (50 mg/l) in the presence of propranolol (1 mg/l). Lower panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (50 mg/l) in the presence of practolol (1 mg/l). These micrographs are representative of sections examined from four hearts each perfused under the conditions described. Black bar indicates one micron.

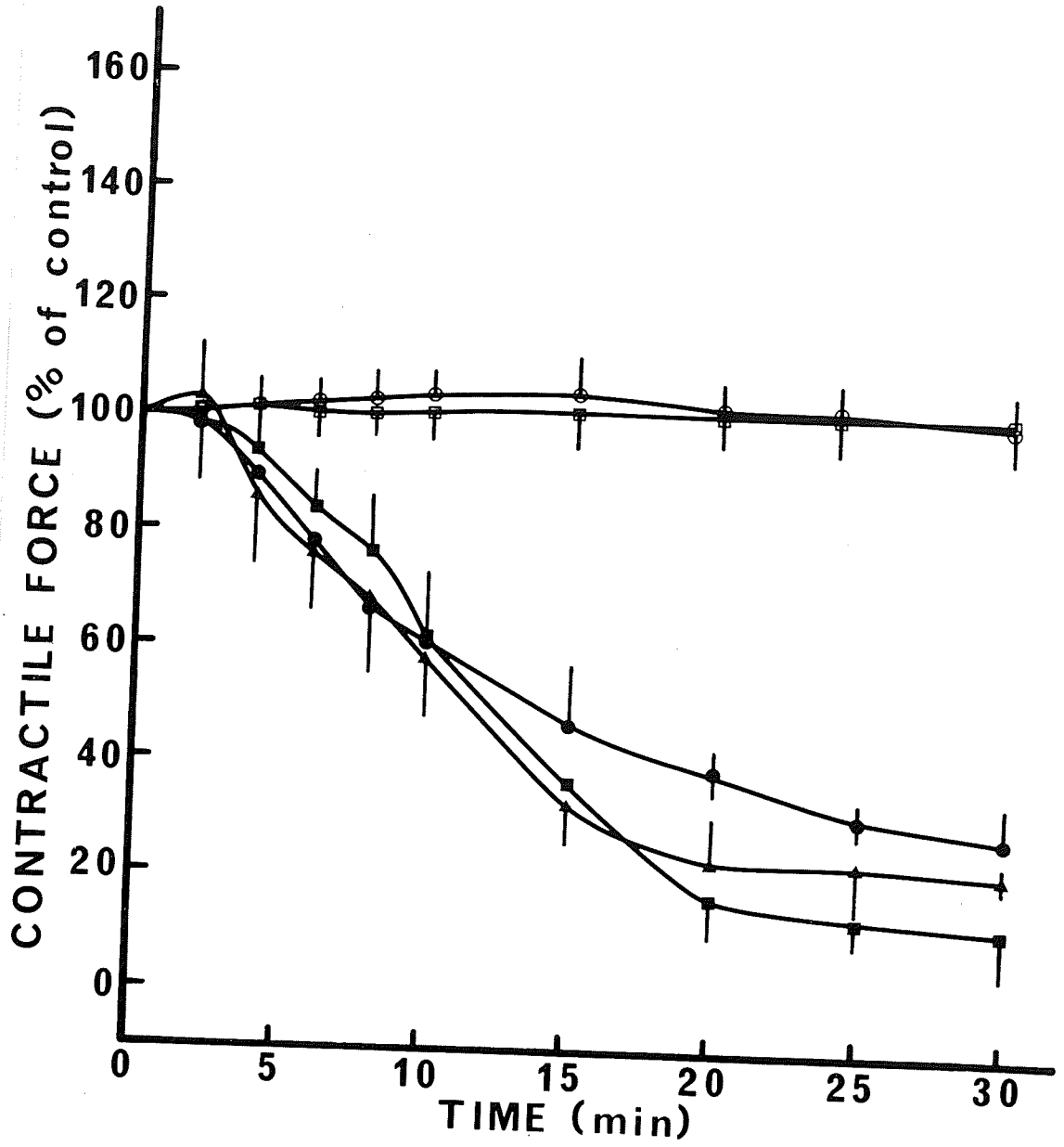

Figure 39: Effect of guanethidine (2 mg/l) and bretylium (2 mg/l) on the time course of failure of contractile force development of isolated rat hearts due to perfusion with adrenochrome (25 mg/l). Guanethidine ⊡—⊡, guanethidine plus adrenochrome ■—■, bretylium ⊙—⊙, bretylium plus adrenochrome ●—●, adrenochrome ▲—▲. Each point is the mean ± standard error of four experiments. Mean initial contractile force values in grams were: guanethidine - 8.5 ± 0.6; bretylium - 8.5 ± 0.5; and control - 7.8 ± 0.6 at a resting tension of 2.5 gm.

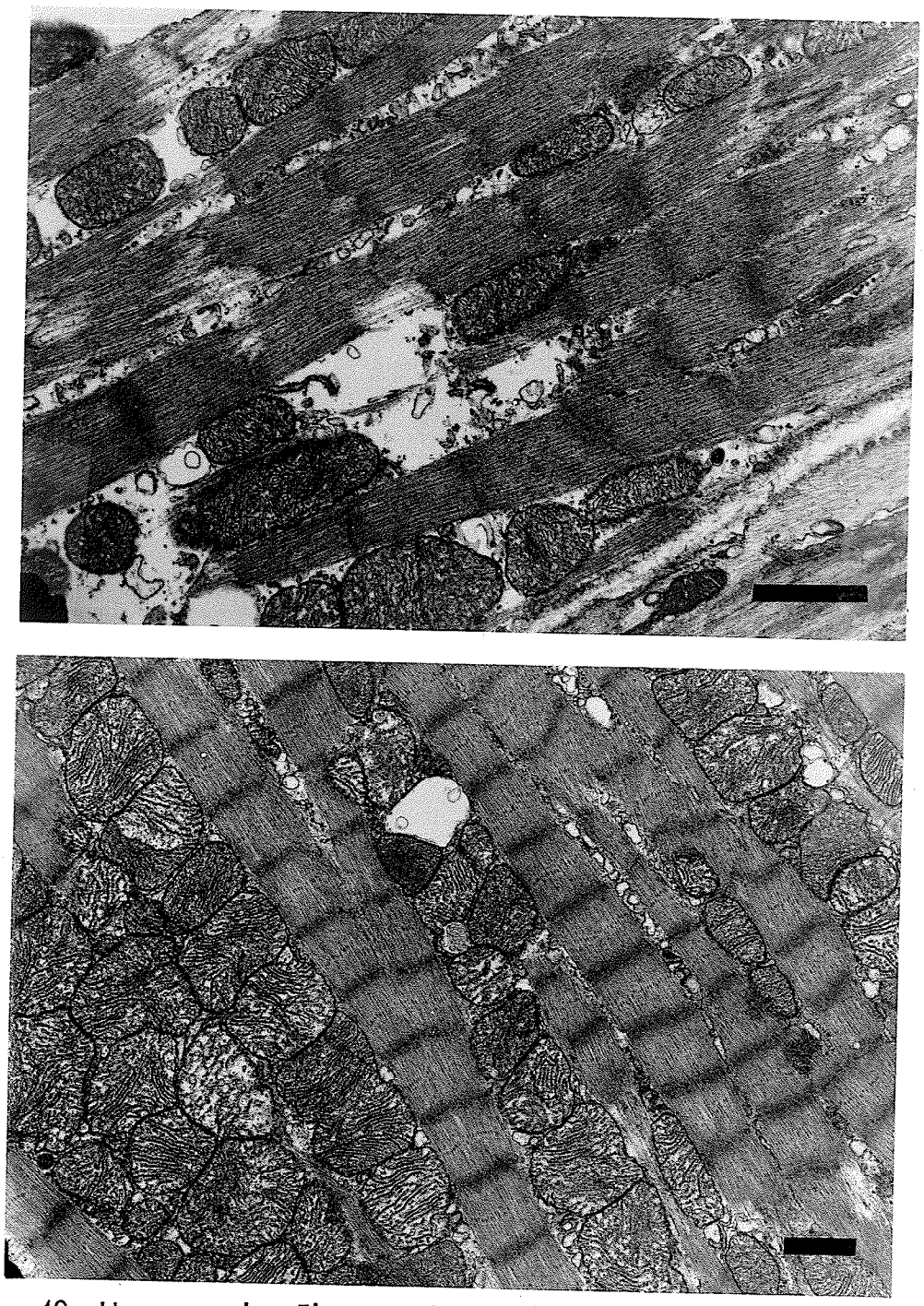

Figure 40: Upper panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (50 mg/l) in the presence of guanethidine (2 mg/l). Lower panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (50 mg /l) in the presence of bretylium (2 mg/l). These micrographs are representative of sections examined from four hearts perfused under the conditions described. Black bar indicates one micron.

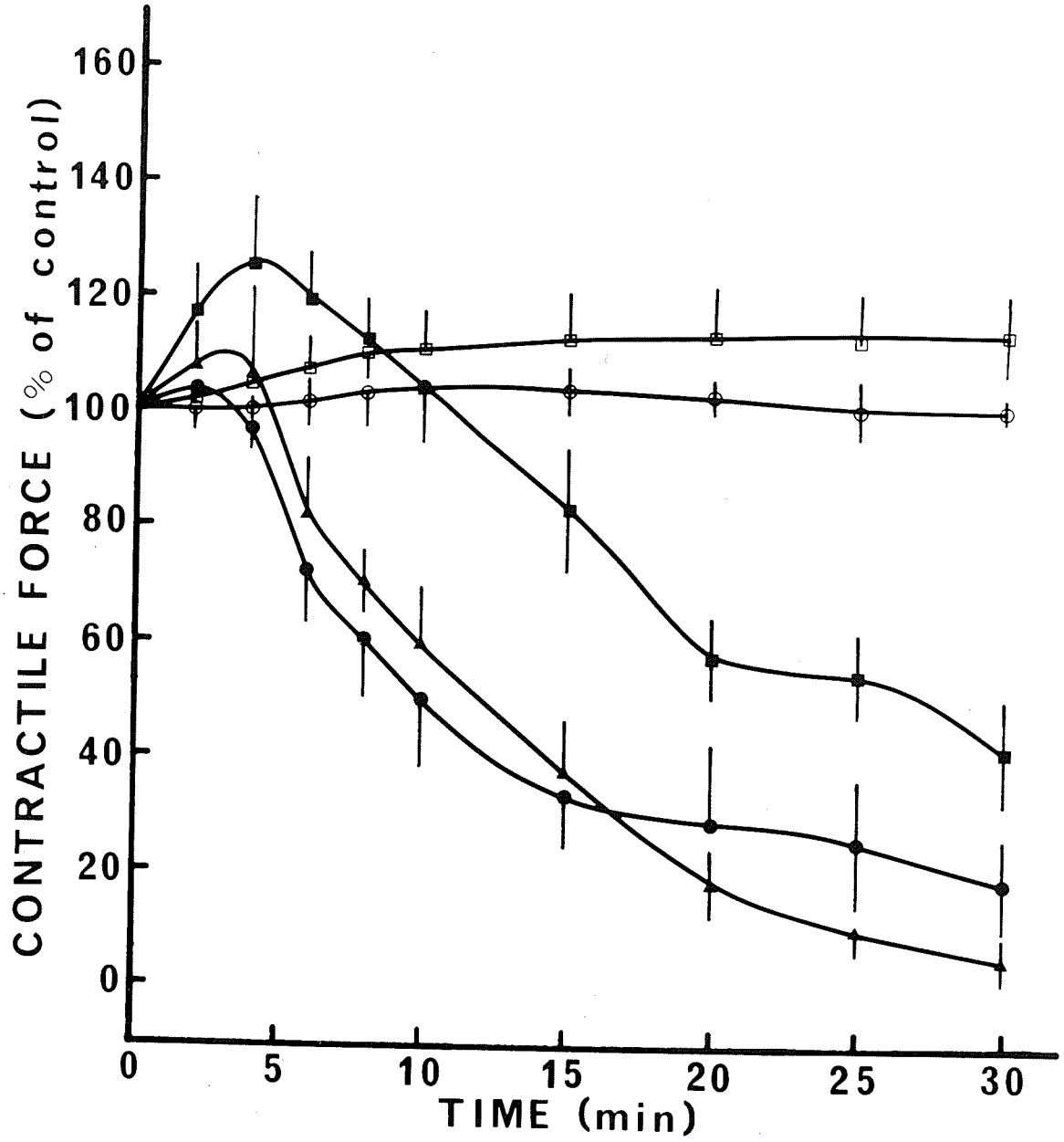

Figure 41: Effect of iproniazide (25 mg/l) and tranylcypromine (25 mg/l) on the time course of failure of contractile force development of isolated rat hearts due to perfusion with adrenochrome (25 mg/l). Iproniazide ⊡—⊡ , iproniazide plus adrenochrome ■—■, tranylcypromine ⊙—⊙ , tranylcypromine plus adrenochrome ●—●, adrenochrome ▲—▲. Each point is the mean ± standard error of four experiments. Mean initial contractile force values in grams were : iproniazide - 7.8 ± 0.6; tranylcypromine - 8.3 ± 0.5; and control - 8.4 ± 0.3 at a resting tension of 2.5 gm.

Iproniazide (25 mg/l) was also found to be completely effective in protecting myocardial structure from necrotic changes due to 30 minutes of perfusion with 50 mg/l of adrenochrome (Figure 42, upper panel). Although tranylcypromine (25 mg/l) greatly reduced the severity of ultrastructural damage and completely prevented development of contracture and disruption of contractile elements due to adrenochrome (50 mg/l), it was not as effective in preserving the integrity of mitochondria (Figure 42, lower panel). Mitochondrial swelling was found to be less pronounced, but disruption of cristae was evident throughout.

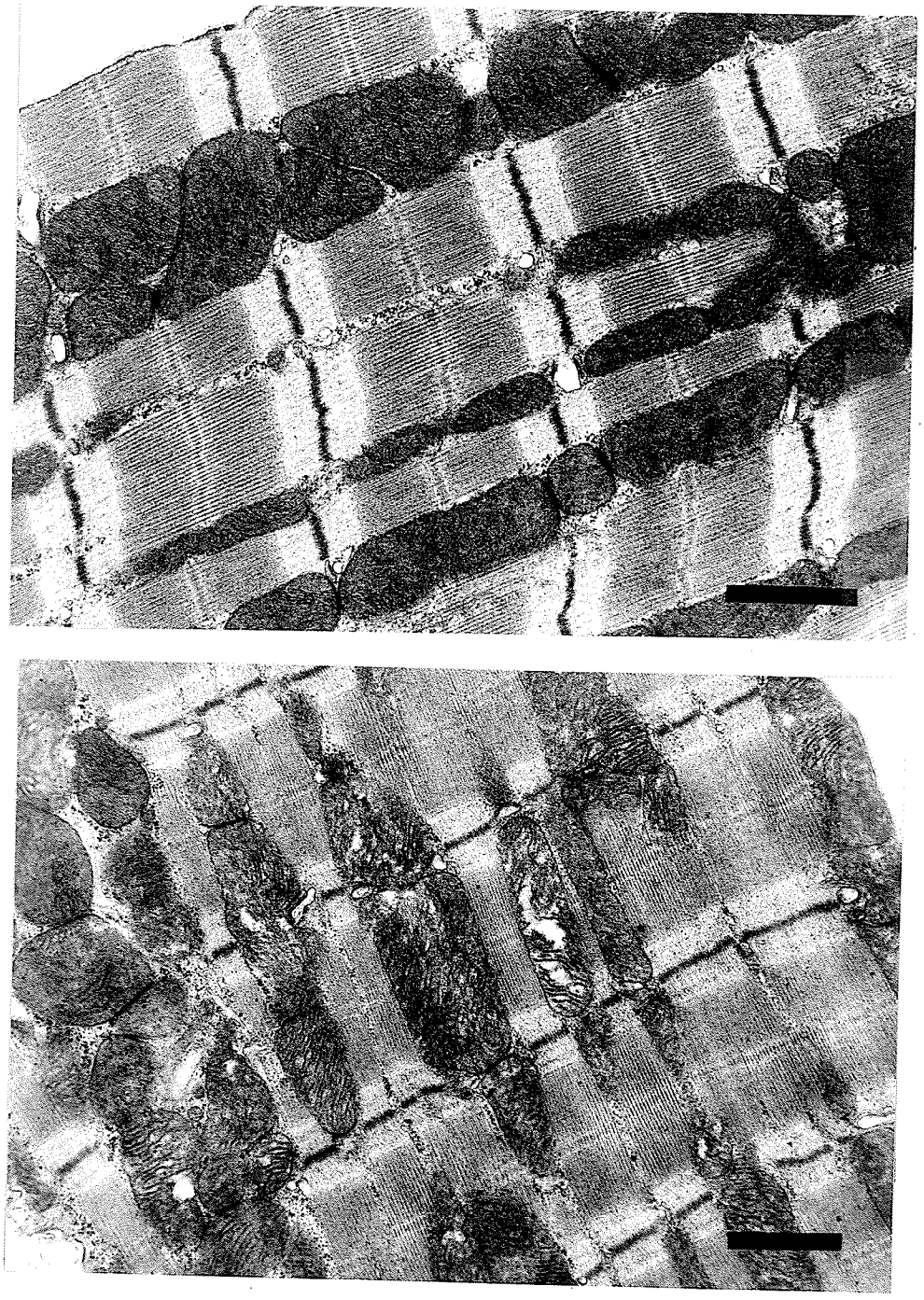

Figure 42: Upper panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (50 mg/l) in the presence of iproniazide (25 mg/l). Lower panel - Electron micrograph of a typical section from an isolated rat heart perfused for 30 minutes with adrenochrome (50 mg/l) in the presence of tranylcypromine (25 mg/l). These micrographs are representative of sections examined from four hearts each perfused under the conditions described. Black bar indicates one micron.

V. DISCUSSION

A. Effects of Isoproterenol and Oxidized Isoproterenol

The results presented here clearly demonstrate that (a) unaltered isoproterenol in concentrations greatly in excess of those likely to occur in the coronary circulation following injection of high doses fails to produce any of the typical ultrastructural changes associated with myocardial necrosis, and (b) that isoproterenol which has undergone a degree of spontaneous oxidation produces massive ultrastructural lesions involving the entire ventricle. The observed ultrastructural changes in the isolated hearts perfused with oxidized isoproterenol were qualitatively identical of those seen after high doses of catecholamines in the intact animal (11, 51 - 53, 56). Furthermore, the occurrence of necrotic damage is associated with a dramatic impairment of the functional capacity of cardiac muscle leading in a short time to complete failure of contractility. The conclusion which is suggested by these observations is that the injection of high doses of catecholamines results in formation of toxic quantities of oxidation products, within myocardial cells or in the circulation, which are at least partly responsible for the lesions in the heart. In support of this view, catecholamines have been reported to be oxidized enzymatically by a variety of enzymes present in mammalian tissues (161 - 174) and catecholamines oxidation products have been identified in the heart and other organs of rabbits (152 - 154). Furthermore, evidence for the extraneuronal uptake of isoproterenol has been recently demonstrated in isolated perfused rat and guinea pig hearts (228 - 230).

The absorption spectrum of the oxidized isoproterenol solution which produced cardiac necrosis in the isolated rat heart is very similar to that of adrenochrome (216), thus suggesting that the toxic substance is an analogue of adrenochrome resulting from the spontaneous oxidation of isoproterenol. As in the case of adrenochrome (216), this analagous aminochrome is apparently unstable and undergoes further oxidation, losing its cardiotoxicity and giving a different absorption spectrum. Thus it is possible that myocardial necrosis in patients with elevated levels of catecholamines is due to the formation of substances such as adrenochrome.

Recent research strongly suggests that the primary irreversible step leading

to cell necrosis following exposure to high dosages of catecholamines is a failure to maintain adequate high energy phosphate levels in the face of increased energy consumption, resulting in a severe deficiency of energy stores required for essential cell processes (77). It is likely that the oxidation products of catecholamines may impair mechanisms for energy production and thereby decrease the energy state of the myocardium. In this regard it is of interest to note that adrenochrome has been shown to be a potent inhibitor of mitochondrial oxidative phosphorylation (147), whereas epinephrine, norepinephrine, and isoproterenol do not affect normal rat heart mitochondria in vitro (17). The possibility that the mechanism by which catecholamines produce myocardial lesions is related to the effects of aminochromes on mitochondrial respiration is an intriguing one and deserves further investigation. The observed alteration in contractile function of the heart upon exposure to oxidized isoproterenol may reflect changes in calcium transport by the sarcotubular system of necrotic hearts as well (81).

There are several reasons for which the isolated rat heart appears to be an ideal preparation for further studying the underlying molecular changes which ultimately result in cell death. These include elimination of hemodynamic factors, neural mechanisms, availability of exogenous lipids, and other physiological parameters which tend to complicate the model for producing heart lesions in the intact animals. In this regard it should be noted that different factors such as a profound fall in blood pressure (4, 9, 10) and coronary vascular changes resulting in a diminished perfusion of subendocardial layers (5 - 8, 10, 135) have been suggested to induce relative cardiac hypoxia and subsequently necrosis due to isoproterenol in the intact animal. These considerations for the pathogenesis of isoproterenol induced necrosis are of particular interest in view of the well known β - adrenergic stimulatory effects such as increased frequency and amplitude of cardiac contractions which result in increased myocardial oxygen demands. However, other workers have provided evidence against the participation of hemodynamic changes (11, 14, 15) and coronary vascular changes (12, 13) in the pathophysiology of lesions produced by catecholamines in the intact animal.

Bajusz (140) has pointed out that catecholamine induced necrosis must be considered to be of a mixed pathogenesis, probably involving a direct toxic effect

on cellular metabolism as well as factors secondary to vascular, hemodynamic and β - adrenergic stimulatory effects. Although hemodynamic and vascular effects of catecholamines can be conceived to greatly influence the extent and severity of lesions in the intact animal, the results reported in this study demonstrate that the direct toxic effects of catecholamines are due to their oxidation products. It is therefore our view that the biochemical events underlying catecholamine cardiotoxicity should be investigated by employing the metabolites and oxidation products of catecholamines rather than catecholamines per se .

B. Effects of Epinephrine, its Metabolites, and Adrenochrome.

As in the case of isoproterenol, perfusion of isolated rat hearts with fresh epinephrine solutions fails to elicit any evidence of necrotic changes in the myocardium such as are seen following injection or infusion of large doses of epinephrine in intact animals, although a strong positive inotropic influence is readily apparent from various parameters of contractile function. On the other hand, adrenochrome produces severe and extensive ultrastructural lesions throughout the left ventricle associated with a decline and dissappearance of contractility. These findings strongly support the opinion that similar effects obtained with oxidized isoproterenol solutions are due to formation, by oxidation of isoproterenol, of an aminochrome which is an adrenochrome analogue, and further support the suggestion made previously that cardiac necrosis induced by injection of catecholamines is a result of the formation of toxic quantities of catecholamine oxidation products.

The enzymatic inactivation of epinephrine by monoamine oxidase (MAO) and catechol - O - methyltransferase (COMT) proceeds along a very different route from the spontaneous oxidation pathway which produces adrenochrome, and the principle metabolites formed by the action of these enzymes are dihydroxymandelic acid, metanephrine, and vanilmandelic acid (231). In the present study these physiological metabolites have been shown to have no damaging effect whatsoever on the myocardium, which confirms the previous findings of Muller (18). It would thus appear that the physiological degradation of catecholamines normally proceeds via two essential structural modifications, namely deamination and O-methylation, which prevent the formation of the 2, 3 - dihydroindole - 5, 6 - quinone ring system of

adrenochrome (216) and yield non-toxic metabolites instead. It may be further postulated that the presence of unusually high levels of catecholamines which exceed the capacity of the MAO and COMT systems will result in the formation of adrenochrome by way of other enzyme systems known to convert epinephrine to adrenochrome (161 - 174), through spontaneous oxidation and autocatalysis (155), and catalytically via cytochrome C (175). It is worth mentioning in this regard, that blocking the pathway of catecholamine metabolism via COMT by means of pyrogallol results in an increase in the extent and severity of myocardial lesions due to subsequent administration of isoproterenol (18).

The ultrastructural damage which was produced by adrenochrome in this study is qualitatively indistinguishable from that seen following injection of large doses of isoproterenol (11, 51 - 53, 55, 56), or epinephrine (55, 57, 59), although the altered appearance of mitochondrial cristae observed with 10 and 25 mg/l of adrenochrome has not been reported previously in studies on catecholamine - induced necrosis. Damage to the contractile elements ranging from occasional regions of contracture to fusion of sarcomeres and fragmentation of myofibrils seen in these hearts is also typical of the classical isoproterenol necrosis model. As has been reported to be the case in time course studies with isoproterenol (53), an advanced degree of swelling and disruption of mitochondria is only evident in hearts in which disorganization and disruption of the contractile elements are prominent as well. There appears to be no other correlation between mitochondrial damage and disruption of myofilaments, since relatively normal mitochondria appear among severely affected sarcomeres, and badly damaged mitochondria are seen among apparently normal sarcomeres in both the adrenochrome - perfused and the catecholamine - injected (52, 54) models of cardiac necrosis.

The adrenochrome - induced decrease in contractile ability of the heart, which has been demonstrated previously (157, 158, 162, 180 - 182), is clearly a dose and time dependent phenomenon, but does not correlate well with the occurrence of ultrastructural damage. A significant reduction in contractile force development occurs with 30 minutes of perfusion at an adrenochrome concentration of 5 mg/l and with 5 minutes of perfusion at an adrenochrome concentration of 50 mg/l without any ultrastructural evidence of myocardial damage. A further decrease in the force of

contraction occurs with 30 min of perfusion at an adrenochrome concentration of 10 mg/l and is associated with a subtle alteration of the intramitochondrial membranes, and contractile activity is virtually abolished in hearts perfused with 25 mg/l in which these mitochondrial changes are much more pronounced. These observations do suggest the possibility that mitochondrial changes play an important role in the loss of contractile function due to adrenochrome. Interestingly, adrenochrome and related oxidation products have been shown to have profound effects on mitochondrial function (147, 197, 200, 201), tissue oxygen consumption (191 - 198) and various stages in glucose metabolism (172, 199, 200, 202 - 207). It is not, therefore, unlikely that the reduction in contractile force reflects a decrease in the availability of high energy phosphate stores to the contractile apparatus, which would also correspond well with the reduction of high energy phosphates resulting from catecholamine injection (76, 77). On the other hand, adrenochrome has also been found to inhibit myosin ATPase activity as well (209 - 211), and the contractility changes may be due to a defect in the ability of the contractile apparatus to utilize whatever high energy phosphates are available.

C. Influence of Epinephrine and Various Reducing Agents on Adrenochrome Induced Necrosis and Failure.

Although epinephrine clearly did not potentiate the toxic influence of adrenochrome on the myocardium under the conditions of this study, a quite different situation exists in the intact animal, and the potentiation of adrenochrome cardiotoxicity indirectly by the pharmacologic action of epinephrine on the cardiovascular system is not only possible but highly likely. A reduced endocardial perfusion resulting from a fall in diastolic blood pressure (10, 40), shortened diastole (136 - 139), and / or coronary vascular changes (5, 7, 8, 135) due to high levels of epinephrine or isoproterenol in the circulation would favor stagnation of blood flow and accumulation of catecholamine oxidation products. These factors would produce a relative hypoxia as well, aggravating the disruptive effects of these oxidation products on oxidative phosphorylation (147, 197, 200, 201) and glucose metabolism (172, 199, 200, 202 - 207). Increased myocardial oxygen demand related to increased heart rate and cardiac work would further contribute to the development of a relative hypoxia. When these factors are considered together, it appears very probable that cardiac lesions following

injection of large doses of catecholamines arise as a result of the combined effects of catecholamines and aminochromes formed by their oxidation.

Attempts to prevent the further oxidation of adrenochrome in solution by the addition of various reducing agents gave rise to an unexpected result in that a solution of considerably greater cardiotoxic potency was produced which was clearly not due to the presence of adrenochrome alone. Colour changes indicated that a chemical reduction of adrenochrome had occurred following addition of ascorbic acid, dithiothriotol, or cysteine. According to the information available in the literature, reduction of adrenochrome to epinephrine is a highly unlikely event, and the major product of reduction of adrenochrome with ascorbic acid or cysteine is 5, 6 - dihydroxy-N - methylindole (216). These results clearly demonstrate that adrenochrome is not the only compound produced by chemical rearrangement of epinephrine which is capable of inducing necrosis in the myocardium. Further study of the chemical nature and biological activity of catecholamine derived compounds, most particularly those containing an indole ring system, is obviously warranted.

D. Influence of Cation Concentration on Adrenochrome Induced Necrosis and Failure.

A primary object of this aspect of the present study was to determine whether ionic factors which influence the production of necrosis by catecholamine injections have similar influences on necrosis induced by perfusion with adrenochrome. The increase in the severity of necrosis due to ionic modifications which tend to increase calcium influx, such as increased Ca^{++} concentration, decreased Na^{+} concentration and decreased K^{+} concentration (232 - 238) and the protective effect of reduced Ca^{++} concentration shown in these experiments are consistent with reports of Fleckenstein and others (76, 77, 89, 106) of a dramatic influence on necrosis produced by catecholamine injection of factors affecting movements of calcium across the myocardial cell membrane. The protective effect of high Mg^{++} concentration may also be a result of a reduction in Ca^{++} influx due to competition between Ca^{++} and Mg^{++} for membrane sites, or due to some other effect of increased availability of Mg^{++}. In this regard, administration of $Mg\,Cl_2$ (77, 109) or K^{+} - Mg^{++} aspartate (111) has been previously found to decrease the severity of ultrastructural damage following isoproterenol injections.

The increased severity of necrotic damage resulting from increased K^+ concentration of the perfusion medium was an unexpected finding in view of reports that diets high in potassium (89, 90, 107) or administration of KCl (65, 77, 109) reduce the severity of lesions produced by isoproterenol injections. This discrepancy may be due to the very high K^+ concentration used in the present experiments as compared to plasma K^+ levels achieved by other workers. An increased Ca^{++} uptake by the myocardium is associated with high extracellular K^+ concentrations (233), and K^+ has also been implicated in the regulation of intracellular Ca^{++} movements (87, 239). Interestingly, Emberson and Muir (240) have reported that perfusion of isolated hearts with K^+ concentrations of 20 mM or more resulted in development of localized areas of contracture. Although no evidence of contracture was seen in controls at 18 mM K^+ in this study, their findings suggest that some alteration of myocardial Ca^{++} metabolism occurs in the presence of high K^+ concentrations which may not be evident in the form of overt structural changes at the K^+ concentration and perfusion duration employed in the present study. If higher than normal extracellular K^+ levels do in fact alter Ca^{++} metabolism, interaction of these effects with those of adrenochrome could explain the increased severity of myocardial damage reported here.

It is evident from the observation of severely damaged cells adjacent to comparatively normal cells in experiments involving decreased Na^+ and increased Ca^{++} concentrations that some cells are considerably more susceptible to potentiation of adrenochrome induced necrosis by increased Ca^{++} influx than others. No explanation for this phenomenon is readily apparent, although it may have a direct parallel in the micro-focal distribution of necrotic damage induced by catecholamine injections since catecholamines are also known to increase Ca^{++} influx in myocardial cells (95).

There is no simple correlation between the severity of cell damage and the time course of failure of contractile force. The severity of structural damage may be either decreased, as in the case of reduced Ca^{++} or elevated Mg^{++} concentrations, or increased, as in the case of reduced K^+ concentration, without any significant change in the pattern of contractile failure due to adrenochrome. Furthermore, an improved ability to maintain contractile force can be accompanied by more severe effects upon structure, as with increased Ca^{++}, decreased Na^+, and increased K^+.

The maintenance of contractile force in the presence of high Ca^{++}, low Na^{+}, and possibly high K^{+} concentrations as well, is probably due to an increased availability of Ca^{++} to the contractile elements which are still functional.

The significant increase in contractile force during the first 8 minutes of perfusion with adrenochrome in the presence of 6 mM K^{+} at a stimulation rate of 180/min suggests that the smaller increase observed at a stimulation rate of 360/min is a genuine phenomen which is less obvious at the higher rate. As was mentioned previously, this may be due to epinephrine contamination of the adrenochrome preparation, especially since addition of exogenous epinephrine also maintained a higher level of contractile force development during the early stages of perfusion with adrenochrome. Similarly, more dramatic increases in contractile force occur during the initial stages of adrenochrome perfusion in the presence of factors like high Mg^{++} and high K^{+} concentrations. This may reflect improved Ca^{++} entry due to the same factor, probably epinephrine contamination, which produces a similar but less pronounced effect in the presence of normal Mg^{++} and K^{+} concentrations of the Krebs-Henseleit solution. The absence of such an effect in the low Ca^{++} experiments where Ca^{++} influx is limited by the availability of Ca^{++} in the extracellular fluid is consistent with this view.

These results are therefore consistent with the hypothesis that catecholamine oxidation products are the primary agents responsible for the direct toxic component of catecholamine - induced necrosis. The importance of calcium metabolism in determining the severity of necrosis is similar in both models.

E. Influence of Various Pharmacological Interventions on Adrenochrome - Induced Necrosis and Failure.

Perfusion of isolated rat hearts with the calcium antagonist D - 600 blocks excitation - contraction coupling to such an extent that contractile force soon becomes unmeasurable. This is a more dramatic effect than has been previously reported with other mammalian cardiac muscle preparations (241) but is consistent with the very great dependence of rat myocardium on extracellular Ca^{++} for contraction (242). The prevention of the usual ultrastructural damage due to adrenochrome by D-600 is consistent with the protective effect of reduced Ca^{++} concentration of the perfusion medium, and paralells the protection afforded by Ca^{++} antagonists against catecholamine injections (76, 77, 104). The unusual disalignment of contractile filaments within

the sarcomeres of hearts perfused with adrenochrome in the presence of D-600 is quite different from any of the ultrastructural changes which usually characterize adrenochrome induced necrosis. Whether these changes are indicative of an incomplete prevention of certain effects of adrenochrome or represent some unique effect of adrenochrome on the D-600 arrested heart is not clear and further studies on the effects of adrenochrome on various types of non-contracting hearts are warranted.

Although α - adrenergic receptor blocking agents have been reported to somewhat reduce the severity of lesions due to epinephrine injection (70, 82, 99, 100), they were completely ineffective in protecting the myocardium from adrenochrome - induced damage in this study. This suggests that the moderating influence of these agents in intact animals is due to their prevention of α - receptor stimulation by epinephrine and its consequences on myocardial work load and oxygen demand, whereas the direct toxic influence of adrenochrome is not mediated by α - receptor activation. It should be noted that α - receptor blocking compounds are not very potent by themselves in preventing myocardial necrosis due to epinephrine injection, and were more effective when used in combination with a β - blocker (59, 82, 99). Furthermore, the α- blocking agents are ineffective against necrosis induced by injection of the β - agonist isoproterenol (65, 70, 96, 97, 99). Adrenergic neuron blocking agents are also ineffective against adrenochrome - induced necrosis, just as they have been reported to be against necrosis produced by catecholamine injection.

In contrast to the α- blockers, the β - adrenergic receptor blocking agents propranolol and practolol are very effective in preventing necrosis due to adrenochrome. These results do not necessarily indicate that adrenochrome acts via β - receptors since these drugs are not highly specific as to their site of action. Propranolol has been previously shown to have a negative inotropic effect on human, dog and rabbit cardiac muscle (243, 244), to decrease lipid-facilitated transport of Ca^{++} (243), and to prevent increased myocardial Ca^{++} content due to isoproterenol (95). It is thus not unlikely that the protective effect of β - blockers against the necrotizing influence of both adrenochrome perfusion and catecholamine injection (22, 83, 89, 95 - 97) is a function of their influence on Ca^{++} movements.

Experiments with tranylcypromine and iproniazide clearly demonstrate that monoamine oxidase inhibitors can prevent necrotic damage due to adrenochrome,

although the non-hydrazine type inhibitor tranylcypromine was found to be less effective than the hydrazine - type inhibitor iproniazide. These results parallel those obtained in studies on catecholamine - induced necrosis in intact animals (18, 65, 71, 89 - 93). Although overt evidence of ultrastructural damage is not seen with iproniazide, practolol, or propranolol some degree of contractile failure still occurs suggesting that all the effects of adrenochrome have not been prevented. Reduced contractile force also occurs after 5 minutes with 50 mg/l and 30 minutes with 5 mg/l of adrenochrome although ultrastructural damage was not evident. A similar situation is seen in the presence of low Ca^{++} and high Mg^{++} as well. These contractile force changes are indicative of alterations of cellular function which precede development of structural damage and are not wholly prevented by any of the above mentioned ionic and pharmacologic interventions. It is interesting to note in this regard that the mono-amine oxidase inhibitor tranylcypromine failed to alter the time course of contractile force changes even though the only subcellular structures in which damage was evident were the mitochondria. This tends to support the possibility, discussed earlier in connection with contractile force and ultrastructural changes seen with 10 and 25 mg/l of adrenochrome, that mitochondrial changes play an important role in the loss of contractile function during perfusion with adrenochrome.

In conclusion, the toxic influence of adrenochrome on the myocardium, and its modification by various ionic and pharmacologic agents can adequately account for most of the data in the literature pertaining to necrosis resulting from injection or infusion of catecholamines. Furthermore, it has been clearly demonstrated that catecholamines *per se* are not capable of producing necrotic lesions by means of any direct effects on myocardial cell metabolism. It is therefore our opinion that cardiac lesions resulting from massive doses or prolonged infusion of catecholamines are the result of an accummulation of toxic quantities of adrenochrome and related catecholamine oxidation products within the myocardium.

VI. CONCLUSIONS

In this study the effects of catecholamines, their metabolities, and their oxidation products on the structural and functional integrity of the isolated rat heart have been investigated. From the results obtained the following conclusions are made:

1. The catecholamines, isoproterenol and epinephrine, are not capable of producing necrosis in the isolated perfused rat heart.
2. The products of epinephrine metabolism via catechol - o - methyltransferase and monoamine oxidase are not capable of producing necrosis in the isolated rat heart.
3. The spontaneous oxidation of isoproterenol in solution results in the formation of a substance capable to producing severe myocardial necrosis similar to that observed following injection of large doses of catecholamine in intact animals.
4. The above mentioned substance is probably the N - isopropyl analogue of adrenochrome.
5. Adrenochrome is capable of producing myocardial necrosis in the isolated rat heart which is qualitatively identical with necrosis produced by catecholamine injection in intact animals.
6. Necrosis produced by adrenochrome is associated with contractile failure.
7. The severity of necrosis and degree of contractile failure due to adrenochrome are time and dose dependent phenomena.
8. No simple relationship exists between the occurrence of ultrastructural damage and contractile failure, although mitochondrial changes correlate more closely with the degree of contractile failure than any other structural alteration.
9. Alterations of the mitochondrial cristae precede any other structural change in the development of adrenochrome - induced necrosis.
10. Epinephrine does not directly potentiate the effects of adrenochrome on the isolated perfused rat heart.

11. The products of chemical reduction of adrenochrome by ascorbic acid, dithiothriotol or cysteine are more potent than adrenochrome in producing myocardial necrosis in the isolated rat heart.
12. The calcium antagonist D-600 and ionic factors affecting Ca^{++} influx and metabolism alter the severity of necrosis produced by perfusion with adrenochrome in a manner similar to the influence of these factors on the severity of necrosis produced by catecholamine injection.
13. Adrenergic neuron blocking agents and α - receptor blocking agents do not alter the production of necrosis and contractile failure by adrenochrome.
14. β - receptor blocking agents protect the myocardium from necrotic damage due to adrenochrome in a similar manner to the protection afforded by these agents against catecholamine - induced necrosis.
15. Monoamine oxidase inhibitors protect the myocardium from adrenochrome induced necrosis.
16. The hydrazine type monoamine oxidase inhibitor iproniazide is more effective than the non-hydrazine type inhibitor tranylcypromine against adrenochrome induced myocardial necrosis.
17. Necrosis resulting from catecholamine injection is probably of a mixed pathogenesis involving direct toxic effects of catecholamine oxidation products as well as factors secondary to vascular, hemodynamic and β - adrenergic stimulatory effects of catecholamines themselves.

VII. REFERENCES

1. Pearce, R.M. Experimental myocarditis; a study of the histological changes following intravenous injections of adrenalin. J. Exp. Med. 8: 400 - 409 (1906).

2. Ziegler, K. Uber die Wirkung intravenoser Adrenalininjektion auf das Gefasssystem und ihre Beziehungen zur Arteriosclerose. Beitr. z. Path. Anat. u.z. allg. Path. 38: 229 - 254 (1905).

3. Szakacs, J.E., and Cannon, A. *l* - norepinephrine myocarditis. Amer. J. Clin. Path. 30: 425 - 430 (1958).

4. Rona, G., Chappel, C.I., Balazs, T., and Gaudry, R. An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. A.M.A. Arch. Path. 67: 443 - 455 (1959).

5. Boutet, M., Huttner, I., and Rona, G. Aspect microcirculatoire des lesions myocardiques provoquees par l'infusion de catecholamines. Etude ultrastructural a l'aide de traceurs de diffusion. I. Isoproterenol. Pathologie - Biologie 21: 811 - 825 (1973).

6. Boutet, M., Huttner, I., and Rona, G. Aspect microcirculatoire des lesions myocardiques provoquees par l'infusion de catecholamines. Etude ultrastructurale a l'aide de traceurs de diffusion. II. Norepinephrine. Pathologie - Biologie 22: 377 - 387 (1974).

7. Handforth, C.P. Isoproterenol - induced myocardial infarction in animals. Arch. Path. 73: 161 - 165 (1962).

8. Handforth, C.P. Myocardial infarction and necrotizing arteritis in hamsters, produced by isoproterenol (Isuprel). Med. Serv. J. Can. 18: 506 - 512 (1962).

9. Rona, G., Boutet, M., Huttner, I., and Peters, H. Pathogenesis of isoproterenol induced myocardial alterations: functional and morphological correlates. In: Myocardial Metabolism: Recent Advances in Studies on Cardiac Structure and Metabolism, N.S. Dhalla, Ed. 3: 507 - 525, Baltimore, University Park Press (1973).

10. Rona, G., Kahn, D.S., and Chappel, C.I. Studies on infarct-like myocardial necrosis produced by isoproterenol: a review. Rev. Can. Biol. 22: 241 - 255 (1963).

11. Maruffo, C.A. Fine structural study of myocardial changes induced by isoproterenol in Rhesus monkeys (Macaca mulatta). Amer. J. Path. 50: 27 - 37 (1967).

12. Ostadal, B., Rychterova, V., and Poupa, O. Isoproterenol - induced acute experimental cardiac necrosis in the turtle. Amer. Heart J. 76: 645 - 649 (1968).

13. Regan, T.J., Markov, A., Kahn, M.I., Jesrani, M.J., Oldewurtel, H.A. and Ettinger, P.O. Myocardial ion and lipid exchanges during ischemia and catecholamine induced necrosis: relation to regional blood flow. In: Myocardiology: Recent Advances in Studies on Cardiac Structure and Metabolism, E. Bajusz and G. Rona, Eds. 1: 656 - 664, Baltimore, University Park Press (1972).

14. Rosenblum, I., Wohl, A., and Stein, A.A. Studies in cardiac necrosis. I. Production of cardiac lesions with sympathomimetic amines. Toxicol. Appl. Pharmacol. 7: 1 - 8 (1965).

15. Rosenblum, I., Wohl, A., and Stein, A.A. Studies in cardiac necrosis. II. Cardiovascular effects of sympathomimetic amines producing cardiac lesions. Toxic. Appl. Pharmacol. 7: 9 - 17 (1965).

16. Raab, W. Pathogenic significance of adrenalin and related substances in heart muscle. Exper. Med. Surg. 1: 188 - 225 (1943).

17. Sobel, B., Jequier, E., Sjoerdsma, A. and Lovenberg, W. Effect of catecholamines and adrenergic blocking agents on oxidative phosphorylation in rat heart mitochondria. Circ. Res. 19: 1050 - 1061 (1966).

18. Muller, E. Histochemical studies on the experimental heart infarction in the rat. Naunyn - Schmiedebergs Arch. Pharmok. u. exp. Path. 254: 439 - 447 (1966).

19. Josue, O. Hypertrophie cardiaque causee par l'adrenaline et la toxine typhique. Compt. Rend. Soc. Biol. 63: 285 (1907).

20. Fleisher, M.S., and Loeb, L. Experimental myocarditis. Arch. Int. Med. 3: 78 - 91 (1909).

21. Fleisher, M.S., and Loeb, L. The later stages of experimental myocarditis. J. Am. Med. Assn. 53: 1561 (1909).

22. Fleisher, M.S., and Loeb, L. Uber experimentelle Myocarditis. Centralbl. f. allg. Path. u. path. Anat. 20: 104 - 106 (1909).

23. Fleisher, M.S., and Loeb, L. Further investigations in experimental myocarditis. Arch. Int. Med. 6: 427 - 438 (1910).

24. Christian, H.A., Smith, R.M., and Walker, I.C. Experimental cardiorenal disease. Arch. Int. Med. 8: 468 - 551 (1911).

25. Anitschkow, N. Uber die Histogenese der Myokardveranderungen bei einigen Intoxikationen. Virchows Arch. Pathol. Anat. Physiol. Klin. Med. 211: 193 - 232 (1913).

26. Franz, G. Eine seltene Form von toxischer Myokardschadeging. Virchow's Arch. Path. Anat. 298: 743 - 752 (1937).

27. Gormsen, H. Case of fatal epinephrine intoxication. Ugesk. laeger. 101: 242 - 245 (1939).

28. Samson, P.C. Tissue changes following continuous intravenous injection of epinephrine hydrochloride into dogs. Arch. Path. 13: 745 - 755 (1932).

29. Blacket, R.B., Pickering, G.W., and Wilson, G.M. The effects of prolonged infusion of noradrenaline and adrenaline on arterial pressure of rabbit. Clin. Sci. 9: 247 - 257 (1950).

30. Catchpole, B.N., Hackel, D.B., and Simeone, F.A. Coronary and peripheral blood flow in experimental hemorrhagic hypotension treated with *l* - norepinephrine. Ann. Surg. 142: 372 - 380 (1955).

31. Hackel, D.B. and Catchpole, B.N. Pathologic and electrocardiographic effects of hemorrhagic shock in dogs treated with *l* - norepinephrine. Lab. Invest. 7: 358 - 368 (1958).

32. Vishnevskaya, O. P. Reflex mechanisms in the pathogenesis of adrenalin myocarditis. Bull. Exper. Biol. Med. 41: 27 - 31 (1956).

33. Nahas, G.G., Brunson, J.G., King, W.M., and Cavert, H.M. Functional and morphological changes in heart lung preparations following administration of adrenal hormones. Amer. J. Path. 34: 717 - 729 (1958).

34. Szakacs, J.E., and Mehlman, B. Pathologic changes induced by *l* - norepinephrine. Quantitative aspects. Amer. J. Cardiol. 5: 619 - 627 (1960).

35. Maling, H.M., and Highman, B. Exaggerated ventricular arrythmias and myocardial fatty changes after large doses of norepinephrine and epinephrine in unanaesthetized dogs. Amer. J. Physiol. 194: 590 - 596 (1958).

36. Highman, B., Maling, H.M., and Thompson, E.C. Serum transaminase and alkaline phosphatase levels after large doses of norepinephrine and epinephrine in dogs. Am. J. Physiol. 196: 436 - 440 (1959).

37. Maling, H.M., Highman, B. and Thompson, E.C. Some similar effects after large doses of catecholamines and myocardial infarction in dogs. Amer. J. Cardiol. 5: 628 - 633 (1960).

38. Chappel, C.I., Rona, G., Balazs, T., and Gaudry, R. Severe myocardial necrosis produced by isoproterenol in the rat. Arch. Int. Pharmacodyn. 122: 123 - 128 (1959).

39. Chappel, C.I., Rona, G., Balazs, T., and Gaudry, R. Comparison of cardiotoxic actions of certain sympathomimetic amines. Canad. J. Biochem. Physiol. 37: 35 - 42 (1959).

40. Rona, G., Zsoter, T., Chappel, C., and Gaudry, R. Myocardial lesions, circulatory and electrocardiographic changes produced by isoproterenol in the dog. Rev. Canad. Biol. 18: 83 - 94 (1959).

41. Kline, I.K. Myocardial alterations associated with pheochromocytomas. Amer. J. Path. 38: 539 - 551 (1961).

42. Szakacs, J.E., Dimmette, R.M., Cowart, E.C., Jr. Pathologic implication of the catechol amines, epinephrine and norepinephrine. U.S. Armed Forces Med. J. 10: 908 - 925 (1959).

43. Van Vliet, P.D., Burchell, H.B., and Titus, J.L. Focal myocarditis associated with pheochromocytoma. New Eng. J. Med. 274: 1102 - 1108 (1966).

44. Connor, R.C.R. Focal myocytolysis and fuchsinophilic degeneration of the myocardium of patients dying with various brain lesions. Ann. N.Y. Acad. Sci. 156: 261 - 270 (1969).

45. Greenhoot, J.H., and Reichenbach, D.D. Cardiac injury and subarachnoid hemorrhage. A clinical, pathological and physiological correlation. J. Neurosurg. 30: 521 (1969).

46. Reichenbach, D.D., and Benditt, E.P. Catecholamines and cardiomyopathy: The pathogenesis and potential importance of myofibrillar degeneration. Hum. Path. 1: 125 - 150 (1970).

47. Smith, R.P., and Tomlinson, B.E. Subendocardial hemorrhages associated with intracranial lesions. J. Path. Bact. 68: 327 - 334 (1954).

48. Kaye, M.P., McDonald, R.H., and Randall, W.C. Systolic hypertension and subendocardial hemorrhages produced by electrical stimulation of the stellate ganglion. Circulation Res. 9: 1164 - 1170 (1961).

49. Klouda, M.A., and Brynjolfsson, G. Cardiotoxic effects of electrical stimulation of the stellate ganglia. Ann. N.Y. Acad. Sci. 156: 271 - 279 (1969).

50. Melville, K.I., Garvey, H.L., Sluster, H.E., and Knaack, J. Central nervous system stimulation and cardiac ischemic changes in monkeys. Ann. N.Y. Acad. Sci. 156: 241 - 260 (1969).

51. Bloom, S., and Cancilla, P.A. Myocytolysis and mitochondrial calcification in rat myocardium after low doses of isoproterenol. Amer. J. Path. 54: 373 - 391 (1969).

52. Csapo, Z., Dusek, J., and Rona, G. Early alterations of cardiac muscle cells in isoproterenol induced necrosis. Arch. Path. 93: 356 - 365 (1972).

53. Kutsuna, F. Electron microscopic studies on isoproterenol - induced myocardial lesions in rats. Jap. Heart J. 13: 168 - 175 (1972).

54. Ferrans, V.J., Hibbs, R.G., Cipriano, P.R., and Buja, L.M. Histochemical and electron microscopic studies of norepinephrine - induced myocardial necrosis in rats. In: Myocardiology: Recent Advances in Studies on Cardiac Structure and Metabolism, E. Bajusz and G. Rona, Eds. 1: 495 - 525, Baltimore, University Park Press (1972).

55. Ferrans, V.J., Hibbs, R.G., Walsh, J.J., and Burch, G.E. Histochemical and electron microscopic studies on the cardiac necrosis produced by sympathomimetic agents. Ann. N.Y. Acad. Sci. 156: 309 - 332 (1969).

56. Ferrans, V.J., Hibbs, R.G., Black, W.C., and Weilbaecher, D.G. Isoproterenol - induced myocardial necrosis. A histochemical and electron microscopic study. Amer. Heart J. 68: 71 - 90 (1964).

57. Ferrans, V.J., Hibbs, R.G., Weily, H.S., Weilbaecher, D.G., Walsh, J.J., and Burch, G.E. A histochemical and electron microscopic study of epinephrine - induced myocardial necrosis. J. Molec. Cell. Cardiol. 1: 11 - 22 (1970).

58. Lehr, D. Healing of myocardial necrosis caused by sympathomimetic amines. In: Myocardiology: Recent Advances in Studies on Cardiac Structure and Metabolism, E. Bajusz and G. Rona, Eds. 1: 526 - 550, Baltimore, University Park Press (1972).

59. Lehr, D., Krukowski, M., and Chau, R. Acute myocardial injury produced by sympathomimetic animes. Israel J. Med. Sci. 5: 519 - 524 (1969).

60. Schenk, E.A., and Moss, A.J. Cardiovascular effects of sustained norepinephrine infusions. II. Morphology. Circ. Res. 18: 605 - 615 (1966).

61. Regan, T.J., Moschos, C.B., Lehan, P.H., Oldewurtel, H.A. and Hellems, H.K. Lipid and carbohydrate metabolism of myocardium during the biphasic inotropic response to epinephrine. Circulat. Res. 19: 307 - 316 (1966).

62. Wexler, B.C., Judd, J.T., and Kittinger, G.W. Myocardial necrosis induced by isoproterenol in rats: changes in serum protein, lipoprotein, lipids and glucose during active necrosis and repair in arteriosclerotic and nonarteriosclerotic animals. Angiology 19: 665 - 682 (1968).

63. Wexler, B.C., Judd, J.T., Lutmer, R.F., and Saroff, J. Pathophysiologic changes in arteriosclerotic and nonarteriosclerotic rats following isoproterenol-induced myocardial infarction. In: Myocardiology: Recent Advances in Studies on Cardiac Structure and Metabolism, E. Bajusz and G. Rona, Eds. 1: 463 - 472, Baltimore, University Park Press (1972).

64. Wexler, B.C., and Kittinger, G.W. Myocardial necrosis in rats: serum enzymes, adrenal steroid and histopathological alterations. Circulat. Res. 13: 159 - 171 (1963).

65. Zbinden, G., and Moe, R.A. Pharmacological studies on heart muscle lesions induced by isoproterenol. Ann. N.Y. Acad. Sci. 156: 294 - 308 (1969).

66. Wexler, B.C. Serum creatine phosphokinase activity following isoproterenol-induced myocardial infarction in male and female rats with and without arteriosclerosis. Amer. Heart J. 79: 69 - 79 (1970).

67. Regan, T.J., Passannante, A.J., Oldewurtel, H.A., Burke, W.M., and Ettinger, P.O. Metabolism of C^{14} labelled triglycerides by the myocardium during injury induced by norepinephrine. Circulation 38: Suppl. VI: 162 (1968).

68. Judd, J.T., and Wexler, B.C. Myocardial connective tissue metabolism in response to injury: histological and chemical studies of mucopolysaccharids and collagen in rat hearts after isoproterenol - induced infarction. Circulation Res. 25: 201 - 214 (1969).

69. Wenzel, D.G., Chau, R.Y.P. Dose-time effect of isoproterenol on aspartate amino-transferase and necrosis of the rat heart. Toxic. Appl. Pharmacol. 8: 460 - 463 (1966).

70. Wenzel, D.G., and Lyon, J.P. Sympathomimetic amines and heart lactic dehydrogenase isoenzymes. Toxic Appl. Pharmacol. 11: 215 - 228 (1967).

71. Stanton, H.C., and Schwartz, A. Effects of hydrazine monoamine oxidase inhibitor (phenelzine) on isoproterenol - induced myocardiopathies in the rat. J. Pharmacol. Exp. Ther. 157: 649 - 658 (1967).

72. Vorbeck, M.L., Malewski, E.F., Erhart, L.S., and Martin, A.P. Membrane phospholipid metabolism in the isoproterenol - induced cardiomyopathy of the rat. Pathophysiology and Morphology of Myocardial Cell Alteration: Recent Advances in Studies on Cardiac Structure and Metabolism, A. Fleckenstein and G. Rona, Eds. 6: 175 - 181, Baltimore, University Park Press (1975).

73. Hattori, E., Yatsaki, K., Miyozaki, T., and Nakamura, M. Adenine nucleotides of myocardium from rats treated with isoproterenol and / or Mg - or K - deficiency. Jap. Heart J. 10: 218 - 224 (1969).

74. Kako, K. Biochemical changes in the rat myocardium induced by isoproterenol. Can. J. Physiol. Pharmacol. 43: 541 - 549 (1965).

75. Robertson, W.V.B., Peyser, P. Changes in water and electrolytes of cardiac muscle following epinephrine. Am. J. Physiol. 166: 277 - 283 (1951).

76. Fleckenstein, A. Specific inhibitors and promoters of calcium action in the excitation - contraction coupling of heart muscle and their role in the prevention or production of myocardial lesions. In: Calcium and the Heart, Harris, P. and Opie, L.H., eds., Academic Press, London and New York, (1971).

77. Fleckenstein, A., Janke, J., and Doering, H.J. Myocardial fiber necrosis due to intracellular Ca - overload. A new principle in cardiac pathophysiology. In: Myocardial Biology: Recent Advances in Studies on Cardiac Structure and Metabolism, N.S. Dhalla, Ed. 4: 563 - 580, Baltimore, University Park Press (1974).

78. Pelouch, V., Deyl, Z., Poupa, O. Experimental cardiac necrosis in terms of myosin aggregation. Physiol. Bohemoslov. 17: 480 (1968).

79. Pelouch, V., Deyl, Z., Poupa, O. Myosin aggregation in cardiac necrosis induced by isoproterenol in rats. Physiol. Bohemoslov. 19: 9 - 13 (1970).

80. Fedelesova, M., Ziegelhoffer, A., Luknarova, O., and Kostolansky, S. Prevention by K^+, Mg^{++} - Aspartate of isoproterenol - induced metabolic changes in the myocardium. Pathophysiology and Morphology of Myocardial Cell Alteration: Recent Advances in Studies on Cardiac Structure and Metabolism, A. Fleckenstein and G. Rona Eds, 6: 59 - 73, Baltimore, University Park Press (1975).

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

82. Lehr, D. Tissue electrolyte alteration in disseminated myocardial necrosis. Ann. N.Y. Acad. Sci. 156: 344 - 378 (1969).

83. Lehr, D., Krukowski, M., and Colon, R. Correlation of myocardial and renal necrosis with tissue electrolyte changes. J. Am. Med. Assn. 197: 105 - 112 (1966).

84. Melville, K.I., and Korol, B. Cardiac drug responses and potassium shifts. Studies on the interrelated effects of drugs on coronary flow, heart action and cardiac potassium movement. (Section I). Am. J. Cardiol. 2: 81 - 94 (1958).

85. Melville, K.I., and Korol, B. Cardiac drug responses and potassium shifts. Studies on the interrelated effect of drugs on coronary flow, heart action and cardiac potassium movement. (Section II). Am. J. Cardiol. 2: 189 - 199 (1958).

86. Nasmyth, P.A. The effect of corticosteroids on the isolated mammalian heart and its response to adrenaline. J. Physiol. 139: 323 - 336 (1957).

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

88. Daggett, W.M., Mansfield, P.B., Sarnoff, S.J. Myocardial K^+ changes resulting from inotropic agents. Fed. Proc. 23: 357 (1964).

89. Kahn, D.S., Rona, G., and Chappel, C.I. Isoproterenol - induced cardiac necrosis. Ann. N.Y. Acad. Sci. 156: 285 - 293 (1969).

90. Rona, G., Chappel, C.I., Kahn, D.S. The significance of factors modifying the development of isoproterenol - induced myocardial necrosis. Amer. Heart J. 66: 389 - 395 (1963).

91. Zbinden, G. Inhibition of experimental myocardial necrosis by the monoamine oxidase inhibitor isocarboxazid (Marplan). Amer. Heart J. 60: 450 - 453 (1960).

92. Zbinden, G. Effects of anoxia and amino oxidase inhibitors on myocardial necrosis induced by isoproterenol. Fed. Proc. 20: 128 (1961).

93. Zbinden, G., and Bagdon, R.E. Isoproterenol - induced heart necrosis, an experimental model for the study of angina pectoris and myocardial infarct. Rev. Can. Biol. 22: 257 - 263 (1963).

94. Leszkovsky, G.P., and Gal, G. Observations on isoprenaline - induced myocardial necroses. J. Pharm. Pharmacol. 19: 226 - 230 (1967).

95. Bloom, S., and Davis, D. Isoproterenol myocytolysis and myocardial calcium. In: Myocardial Biology: Recent Advances in Studies on Cardiac Structure and Metabolism, N.S. Dhalla, Ed. 4: 581 - 590, Baltimore, University Park Press (1974).

96. Dorigotti, L., Gaetani, M., Glasser, A.H., and Turollia, E. Competitive antagonism of isoproterenol - induced cardiac necrosis by β - adrenereceptor blocking agents. J. Pharm. Pharmacol. 21: 188 - 191 (1969).

97. Mehes, G., Rajkovits, K. and Papp, G. Effect of various types of sympathicolytics on isoproterenol - induced myocardial lesions. Acta Physiol. Acad. Sci. Hung. 29: 75 - 85 (1966).

98. Kako, K. The effect of a beta - adrenergic blocking agent on chemical changes in isoproterenol - induced myocardial necrosis. Can. J. Physiol. Pharmacol. 44: 678 - 682 (1966).

99. Mehes, G., Papp, G., and Rajkovits, K. Effect of adrenergic - and β - receptor blocking drugs on the myocardial lesions induced by sympathomimetic amines. Acta Physiol. Acad. Sci. Hung. 32: 175 - 184 (1967).

100. Waters, L.L., and deSuto - Nagy, G.I. Lesions of the coronary arteries and great vessels of the dog following injection of adrenalin. Their prevention by Dibenamine. Science 111: 634 - 635 (1950).

101. Wenzel, D.G., and Chau, R.Y. Effect of adrenergic blocking agents on reduction of myocardial aspartate - amino transferase activity by sympathomimetics. Toxic. Appl. Pharmacol. 9: 514 - 520 (1966).

102. Dhalla, N.S., Balasubramanian, V., and Goldman, J. Biochemical basis of heart function. III. Influence of isoproterenol on the norepinephrine stores in the rat heart. Can. J. Physiol. Pharmacol. 49: 302 - 311 (1971).

103. Bajusz, E., and Jasmin, G. Protective action of serotonin against certain chemically and surgically induced cardiac necroses. Rev. Canad. Biol. 21: 51 - 62 (1962).

104. Mjos, D.D. Effect of inhibition of lipolysis on myocardial oxygen consumption in the presence of isoproterenol. J. Clin. Invest. 50: 1869 - 1873 (1971).

105. Wenzel, D.G., and Stark, L.G. Effect of nicotine on cardiac necrosis induced by isoproterenol. Amer. Heart J. 71: 368 - 370 (1966).

106. Sigel, H., Janke, J., and Fleckenstein, A. Restriction of isoproterenol - induced myocardial Ca uptake and necrotization by a new Ca - antagonistic compound (ethyl - 4 (3, 4, 5 - trimethoxycinnamoyl) piperazinyl acetate (Vascoril). Pathophysiology and Morphology of Myocardial Cell Alterations: Recent Advances in Studies on Cardiac Structure and Metabolism, A. Fleckenstein and G. Rona, Eds. 6: 121 - 126, Baltimore, University Park Press (1975).

107. Rona, G., Chappel, C.I., and Gaudry, R. Effect of dietary sodium and potassium content on myocardial necrosis elicited by isoproterenol. Lab. Invest. 10: 892 - 897 (1961).

108. Chappel, C.I., Rona, G., and Gaudry, R. The influence of adrenal cortical steroids on cardiac necrosis produced by isoproterenol. Acta Endocrinol. 32: 419 - 424 (1959).

109. Lehr, D., Chau, R., and Kaplan, J. Prevention of experimental myocardial necrosis by electrolyte solutions. In: Myocardiology: Recent Advances in Studies on Cardiac Structure and Metabolism, E. Bajusz and G. Rona, Eds. 1: 684 - 698, Baltimore, University Park Press (1972).

110. Janke, J., Fleckenstein, A., Hein, B., Leder, O., and Sigel, H. Prevention of myocardial Ca overload and necrotization by Mg and K salts

or acidosis. Pathophysiology and Morphology of Myocardial Cell Alteration: Recent Advances in Studies on Cardiac Structure and Metabolism, A. Fleckenstein and G. Rona, Eds. 6: 33 - 42, Baltimore, University Park Press (1975).

111. Slezack, J., and Tribulova, N. Morphological changes after combined administration of isoproterenol and K^{+}, Mg^{++} - Aspartate as a physiological Ca^{2+} antagonist. Pathophysiology and Morphology of Myocardial Cell Alteration: Recent Advances in Studies on Cardiac Structure and Metabolism, A. Fleckenstein and G. Rona, Eds. 6: 75 - 84, Baltimore, University Park Press (1975).

112. Balazs, T. Cardiotoxicity of isoproterenol in experimental animals. Influence of stress, obesity, and repeated dosing. Myocardiology: Recent Advances in Studies on Cardiac Structure and Metabolism, E. Bajusz and G. Rona, Eds. 1: 770 - 778, Baltimore, University Park Press (1972).

113. Balazs, T., Sahasrabudhe, M.R., and Grice, H.C. The influence of excess body fat on the cardiotoxicity of isoproterenol in rats. Tox. Appl. Pharmacol. 4: 613 - 620 (1962).

114. Rona, G., Chappel, C.I., Balazs, T., and Gaudry, R. The effect of breed, age and sex on myocardial necrosis produced by isoproterenol in the rat. J. Gerontol. 14: 169 - 173 (1959).

115. Balazs, T., Arena, E., and Barron, C.N. Protection against the cardiotoxic effect of isoproterenol HCl by restricted food intake in rats. Toxic. Appl. Pharmac. 21: 237 - 243 (1972).

116. Dusek, J., Rona, G., and Kahn, D.S. Myocardial resistance. A study of its development against toxic doses of isoproterenol. Arch. Pathol. 89: 79 - 83 (1970).

117. Dusek, J., Rona, G., and Kahn, D.S. Myocardial resistance to isoprenaline in rats: variations with time. J. Path. 105: 279 - 282 (1971).

118. Selye, H., Veilleux, R., and Grasso, S. Protection by coronary ligation against isoproterenol - induced myocardial necroses. Proc. Soc. Exp. Biol. Med. 104: 343 - 345 (1960).

119. Balazs, T., Ohtake, S., and Noble, J.F. The development of resistance to the ischemic cardiopathic effect of isoproterenol. Tox. Appl. Pharmacol. 21: 200 - 213 (1972).

120. Turek, Z., Kalus, M. and Poupa, O. The effect of isoprenaline pretreatment on the size of acute myocardial necrosis induced by the same drug. Physiol. Bohemoslov. 15: 353 - 356 (1966).

121. Wexler, B.C., Kittinger, G.W., and Judd, J.T. Responses to drug - induced myocardial necrosis in rats with various degree of arteriosclerosis. Circulat. Res. 20: 78 - 87 (1967).

122. Faltova, E., and Poupa, O. Temperature and experimental acute cardiac necrosis. Can. J. Physiol. Pharmacol. 47: 295 - 299 (1969).

123. Kennedy, J.H., Alousi, M., and Homi, J. The protective effect of hyperbaric oxygenation upon isoproterenol - induced myocardial necrosis in Syrian hamsters. Med. Thorac. 23: 169 - 175 (1966).

124. Poupa, O., Krofta, K., Prochazka, J., and Turek, Z. Acclimation to simulated high altitude and acute necrosis. Fed. Proc. 25: 1243 - 1246 (1966).

125. Poupa, O., Turek, Z., Kaluz, M., and Krofta, K. Acute infarct-like necrosis in high altitude adapted rats. Physiol. Bohemoslov. 14: 542 - 545 (1965).

126. Balazs, T., Murphy, J.B., and Grice, H.C. The influence of environmental changes on the cardiotoxicity of isoprenaline in rats. J. Pharm. Pharmacol. 14: 750 - 755 (1962).

127. Raab, W., Bajusz, E., Kimura, H., and Henlich, H.C. Isolation, stress, myocardial electrolytes, and epinephrine cardiotoxicity in rats. Proc. Soc. Exp. Biol. Med. 127: 142 - 147 (1968).

128. Hatch, A.M., Wiberg, G.S., Zawidzka, Z., Cann, M., Airth, J.M., and Grice, G.C. Isolation syndrome in the rat. Toxicol. Appl. Pharmacol. 7: 737 - 745 (1965).

129. Jasmin, G. Morphologic effects of vasoactive drugs. Can. J. Physiol. Pharmacol. 44: 367 - 372 (1966).

130. Hoak, J.C., Warner, E.D., and Connor, W.E. New concept of levarterenol - induced acute myocardial ischemic injury. Arch. Path. 87: 332 - 338 (1969).

131. Raab, W. Myocardial electrolyte derangement: Crucial feature of pluricausal, so-called coronary, heart disease. Ann. N.Y. Acad. Sci. 147: 627 - 686 (1969).

132. Rosenblum, I., Wohl, A., Stein, A. Studies in cardiac necrosis. III. Metabolic effects of sympathomimetic amines producing cardiac lesions. Toxicol. Appl. Pharmacol. 7: 344 - 351 (1965).

133. Rona, G., and Dusek, J. Studies on the mechanism of increased myocardial resistance. In: Myocardiology: Recent Advances in Studies on Cardiac Structure and Metabolism, E. Bajusz and G. Rona, Eds. 1: 422 - 429, Baltimore, University Park Press (1972).

134. Strubelt, O. and Siegers, C.P. Role of cardiovascular and ionic changes in pathogenesis and prevention of isoprenaline - induced cardiac necrosis.

Pathophysiology and Morphology of Myocardial Cell Alteration: Recent Advances in Studies on Cardiac Structure and Metabolism, A. Fleckenstein and G. Rona, Eds. 6: 135 - 142, Baltimore, University Park Press (1975).

135. Ostadal, B., and Poupa, O. Occlusion of coronary vessels after administration of isoprenaline, adrenalin and noradrenalin. Physiol. Bohemoslov. 16: 116 - 119 (1967).

136. Brandi, G., and McGregor, M. Intramural pressure in the left ventricle of the dog. Cardiovasc. Res. 3: 472 - 475 (1969).

137. Cutarelli, R., and Levy, M.N. Intraventricular pressure and the distribution of coronary blood flow. Circulation Res. 12: 322 - 327 (1963).

138. Buckberg, G.D., Fixler, D.E., Archie, J.P., and Hoffman, J.I.E. Experimental subendocardial ischemia in dogs with normal coronary arteries. Circulation Res. 30: 67 - 81 (1972).

139. Buckberg, G.D., and Ross, G. Effects of isoprenaline on coronary blood flow: its distribution and myocardial performance. Cardiovasc. Res. 7: 429 - 437 (1973).

140. Bajusz, E. The terminal electrolyte - shift mechanism in heart necrosis; its significance in the pathogenesis and prevention of necrotizing cardiomyopathies. In: Electrolytes and Cardiovascular Diseases, Vol. Bajusz, E., ed., S. Karger, Basel, 1975.

141. Raab, W., van Lith, P., Lepeschkin, E., and Herrlich, H.C. Catecholamine - induced myocardial hypoxia in the presence of impaired coronary dilatability independent of external cardiac work. Am. J. Cardiol. 9: 455 - 470 (1962).

142. Lee, K.S., and Yu, D.H. Effects of epinephrine on metabolism and contraction of cat papillary muscle. Am. J. Physiol. 206: 525 - 530 (1964).

143. Weisfeldt, M.L., and Gilmore, J.P. Apparent dissociation of the inotropic and O_2 consumption effects of norepinephrine. Fed. Proc. 23: 357 (1964).

144. Klocke, F.J., Kaiser, G.A., Ross, J., Jr., and Braunwald, E. Mechanism of increase of myocardial oxygen uptake produced by catecholamines. Am. J. Physiol. 209: 913 - 918 (1965).

145. Challoner, D.R., and Steinberg, D. Metabolic effect of epinephrine on the QO_2 of the arrested isolated perfused rat heart. Nature 205: 602 - 603 (1965).

146. Hauge, A., and Øye, I. The action of adrenaline in cardiac muscle. II. Effect on oxygen consumption in the asystolic perfused rat heart. Acta Physiol. Scand. 68: 295 - 303 (1966).

147. Park, J.H., Meriwether, B.P., Park, C.R., Mudd, S.H., and Lipmann, F. Glutathione and ethylenediamine - tetraacetate antagonism of uncoupling of oxidative phosphorylation. Biochim. Biophys. Acta 22: 403 - 404 (1956).

148. Sordahl, L.A., and Silver, B.B. Pathological accumulation of calcium by mitochondria: modulation by magnesium. Pathophysiology and Morphology of Myocardial Cell Alteration: Recent Advances in Studies on Cardiac Structure and Metabolism, A. Fleckenstein and G. Rona, Eds. 6: 85 - 93, Baltimore, University Park Press (1975).

149. Lossnitzer, K., Janke, J., Hein, B., Stauch, M., and Fleckenstein, A. Disturbed myocardial calcium metabolism: a possible pathogenetic factor in the hereditary cardiomyopathy of the Syrian hamster. Pathophysiology

and Morphology of Myocardial Cell Alteration: Recent Advances in Studies on Cardiac Structure and Metabolism, A. Fleckenstein and G. Rona, Eds. 6: 207 - 217, Baltimore, University Park Press (1975).

150. Jasmin, G., and Bajusz, E. Prevention of myocardial degeneration in hamsters with hereditory cardiomyopathy. Pathophysiology and Morphology of Myocardial Cell Alteration: Recent Advances in Studies on Cardiac Structure and Metabolism, A. Fleckenstein and G. Rona, Eds. 6: 219 - 229, Baltimore, University Park Press (1975).

151. Fleckenstein, A., Janke, J., Doering, H.J., and Leder, O. Key role of Ca in the production of noncoronarogenic myocardial necroses. Pathophysiology and Morphology of Myocardial Cell Alteration: Recent Advances in Studies on Cardiac Structure and Metabolism, A. Fleckenstein and G. Rona, Eds. 6: 21 - 32, Baltimore, University Park Press (1975).

152. Osinskaya, V.O. Catechol amines and compounds with the properties of their oxidation products in the animal organism. Adrenalin: Noradrenalin, Akad. Nauk. SSSR, Lab. Neiro-Gumoralin. Regulyatsii, 118 - 123 (1964); Chem. Abstracts 63: 869 (1965).

153. Osinskaya, V.O. Adrenalin and noradrenalin metabolism in animal tissues. Biokhimiya 22: 537 - 545 (1957); Chem. Abstracts 52: 2211 (1958).

154. Utevskii, A.M., and Osinskaya, V.O. The nature, properties, and significance of fluorescing substances formed in the processes of adrenaline and noradrenaline oxidation. Ukrain. Biokhim. Zhur. 27: 401 - 406 (1955); Chem. Abstracts 50: 1948 (1956).

155. Kisch, B. Die Autokatalyse der Adrenalinoxydation. Biochem. Z. 220: 84 - 91 (1930).

156. Okagawa, M., and Ichitsubo, H. Oxidation and reduction of adrenaline in the body. Proc. Japan Pharmacol. Soc. 9: 155 (1935).

157. Heirman, P. L'adrenoxine, adrenaline oxydee inhibitrice. Compt. Rend. Soc. Biol. 126: 1264 - 1266 (1937).

158. Heirman, P. Action de la tyrosinase sur l'oxidation et les effets cardiaques de l'adrenaline et de la tyramine. Compt. Rend. Soc. Biol. 124: 1250 - 1251 (1937).

159. Hogeboom, G.H., and Adams, M.H. Mammalian tyrosinase and dopa oxidase. J. Biol. Chem. 145: 272 - 279 (1942).

160. Mason, H.S. The chemistry of melanin. II. The oxidation of dihydroxy-phenylalinine by mammalian dopa oxidase. J. Biol. Chem. 168: 433 - 438 (1947).

161. Euler, U.S., von. Noradrenaline - chemistry, physiology, pharmacology and Clinical Aspects. Springfield, Illinois, Charles C. Thomas (1956).

162. Heirman, P. Modifications of the cardiac action of adrenaline in the course of its oxidation by phenolases. Arch. Intern. Physiol. 46: 404 - 415 (1938).

163. Blaschko, H., and Schlóssman, H. The inactivation of adrenaline by phenolases. J. Physiol. 98: 130 - 140 (1940).

164. Derouaux, G. The in vitro oxidation of adrenaline and other diphenol amines by phenol oxidase. Arch. Intern. pharmacodynamie 69: 205 - 234 (1943).

165. Wajzer, J. Oxydation de l'adrenaline. Bull. soc. chim. biol. 28: 341 - 345 (1946).

166. Valerno, D.M., and McCormack, J.J. Xanthine oxidase - mediated oxidation of epinephrine. Biochem. Pharmacol. 20: 47 - 55 (1971).

167. Vercauteren, R. Sur la cytochimie des granulocytes eosinophiles. Bull. soc. chim. biol. 33: 522 - 525 (1951).

168. Odajima, T. Myeloperoxidase of the leukocyte of normal blood. II. Oxidation - reduction reaction mechanism of the myeloperoxidase. Biochim. Biophys. Acta 235: 52 - 60 (1971).

169. Philpot, F.J. Inhibition of adrenaline oxidation by local anaesthetics. J. Physiol. 97 : 301 - 307 (1940).

170. Slater, E.C. The measurement of the cytochrome oxidase activity of enzyme preparations. Biochem. J. 44: 305 - 318 (1949).

171. Iisalo, E., and Rekkarinen, A. Enzyme action on adrenaline and noradrenaline. Studies on heart muscle in vitro. Acta Pharmacol. Toxicol. 15: 157 - 174 (1958).

172. Green, D.E., and Richter, D. Adrenaline and Adrenochrome. Biochem. J. 31: 596 - 616 (1937).

173. Bacq, Z.M. Metabolism of adrenaline. Pharmacol. Revs. 1: 1 - 26 (1949).

174. Axelrod, J. Enzymic oxidation of epinephrine to adrenochrome by the salivary gland. Biochim. Biophys. Acta 85 (2): 247 - 254 (1964).

175. Falk, J.E. The formation of hydrogen carriers by haemitin - catalyzed peroxidations. Biochem. J. 44: 369 - 373 (1949).

176. Chaix, P., and Pallaget, C. Comparative characteristics of the oxidation of adrenaline and noradrenaline. Congr. intern. Biochim., Resumes communs., pg. 54, 2nd Congr., Paris, 1952; Chem. Abstracts 48: 12851 (1954).

177. Chikano, M., and Kominami, M. Uber die Spaltung des Adrenalins im Serum. Biochem. z 205: 176 - 179 (1929).

178. Hoffer, A. Adrenochrome and Adrenolutin and their relationship to mental disease, in Psychotropic Drugs, ed. by S. Garattini and V. Ghetti, p. 10. Elsevier, Amsterdam (1957).

179. Osmond, H., and Hoffer, A. Schizophrenia: a new approach continued. J. Mental Sci. 105: 653 - 673 (1959).

180. Schweitzer, A. Comparative investigations of the action of p - benzo - quinone and omega on the function of the frog heart. Arch. Ges. Physiol. (Pflugers) 228: 568 - 585 (1931).

181. Sanders, E. The inhibiting effect of oxidized adrenalin. Arch. exptl. Path. Pharmakol. 193: 572 - 575 (1939).

182. Webb, W.R., Dodds, R.P., Unal, M.O., Karow, A.M., Cook, W.A., and Daniel, C.R. Suspended animation of the heart with metabolic inhibitors. Effect of magnesium sulfate or fluoride and adrenochrome in rats. Ann. Surg. 164: 343 - 350 (1966).

183. Yen, T. The reversal of the effect of adrenaline upon the rabbit intestine and the toad limb vessels. Tôhoku J. Exptl. Med. 14: 415 - 465 (1930).

184. Raab, W., and Lepeschkin, E. Pressor and cardiac effects of adrenochrome (omega) in the atropinized cat. Exptl. Med. Surg. 8: 319 - 329 (1950).

185. Titaev, A.A. Relation of some metabolites to the sympathetic nervous system in newborn animals. Chem. Abstracts 60: 8438 (1964).

186. Green, S., Mazur, A., and Shorr, E. Mechanism of catalytic oxidation of adrenalin by ferritin. J. Biol. Chem. 220: 237 - 255 (1956).

187. Parrot, J.L. Decrease of capillary permeability under the influence of adrenochrome. Compt. rend. soc. biol. 143: 819 - 822 (1949); Chem. Abstracts 44: 5008 (1950).

188. Lecomte, J., and Fischer, P. The effect of trihydroxy - 1 - methylindole on bleeding time and capillary permeability. Arch. intern. pharmacodynamie 87: 225 - 231 (1951).

189. Marquardt, P. The pharmacology and chemistry of the adrenochromes. Z. ges. exptl. Med. 114: 112 - 126 (1944); Chem. Abstracts 44: 2657 (1950).

190. Snyder, F.H., Leva, E., and Oberst, F.W. An evaluation of adrenochrome and iodoadrenochrome based on blood - sugar levels in rabbits. J.Am. Pharm. Assoc.,Sci. Ed. 36: 253 - 255 (1947).

191. Kaulla, K.N.V. Uber die Beeinflussung des O_2 - Verbrauches durch Adrenochrom beim Meerschweinchen. Biochem. Z. 319: 453 - 456 (1949).

192. Parrot, J.L., and Cara, M. Action of adrenochrome on oxygen consumption of man and guinea pig. Compt. rend. soc. biol. 145: 1859 - 1862 (1951); Chem. Abstracts 46: 10415 (1952).

193. Kisch, B. Katalytische Wirkungen oxydierten Adrenalins. Klin. Wochshr. 9: 1062 - 1064 (1930).

194. Gaisinska, M.Y., and Utevskii, A.M. Effect of catecholamines on oxidative processes under normal conditions and in experimental hypertension. Ukr. Biokhim. Zh. 34: 237 - 242 (1962); Chem. Abstracts 57: 3986 (1962).

195. Radsma, W., and Golterman, H.L. Influence of adrenaline and adrenochrome on oxygen consumption of liver homogenates. Biochim et Biophys. Acta 13: 80 - 86 (1954).

196. Kisch, B., and Leibowitz, J. Die Beeinflussung der Gewebsatmung der Omega und durch Chinon. Biochem. 220: 97 - 116 (1930).

197. Issekutz, B., Jr., Lichtneckert, I., Hetenyi, G., Jr., and Bedo, M. Metabolic effects of noredrenaline and adrenochrome. Arch. intern. pharmacodynamie 84: 376 - 384 (1950).

198. Itasaka, K. Tissue respiration and substrate utilization of heart muscle slices. Fukuoka Igaku Zasshi 49: 3347 - 3367 (1958); Chem. Abstracts 53: 7436 (1959).

199. Wieland, O., and Suyter, M. Der Stoffwechselwirkung des Adrenochroms. Klin. Wochschr. 34: 647 - 648 (1956).

200. Krall, A.R., Siegel, G.J., Gozanski, D.M., and Wagner, F.L. Adrenochrome inhibition of oxidative phosphorylation by rat brain mitochondria. Biochem. Pharmacol. 13: 1519 - 1525 (1964).

201. Sudovtsov, V.E. Effect of adrenoxyl on the oxidative phosphorylation and potassium and sodium permeability of mitochondria. Mitokhondrii, Bioklium. Funkts, Sist. Kletochnykh Organell, Mater, Simp., 4th. 83 - 88 (1969). Severin, S.E., ed. Moscow; Chem. Abstracts 74: 2436 (1971).

202. Wajzer, J. Effet Pasteur du muscle de grenouille et adrenochrome. Bull. soc. chim. biol. 28: 345 - 349 (1946).

203. Wajzer, J. Synthese du glycogene en presence d'adrenochrome. Bull. soc. chim. biol. 29: 237 - 239 (1947).

204. Meyerhof, O., and Randall, L.O. Inhibitory effects of adrenochrome on cell metabolism. Arch. Biochem. 17: 171 - 182 (1948).

205. Walaas, E., Walaas, O., and Jervell, K. Action of adrenochrome on glucose phosphorylation in the rat diaphragm. Congr. intern. biochim., Resumes connums., pg. 67, 2nd Congr., Paris, 1952; Chem. Abstracts 49: 5632 (1955).

206. Pastan, I., Herring, B., Johnson, P., and Field, J.B. Mechanisms by which adrenaline stimulates glucose oxidation in the thyroid. J. Biol. Chem. 237: 287 - 290 (1962).

207. Dumont, J.E., and Hupka, S. Action of neuromimetic amines on the metabolism of thyroid incubated in vitro. Compt. Rend. Soc. Biol. 156: 1942 - 1946 (1962); Chem. Abstracts 59: 5434 (1963).

208. Dickens, F., and Glock, G.E. Mechanism of oxidative inhibition of myosin. Biochim et Biophys. Acta 7: 578 - 584 (1951).

209. Inchiosa, M.A., Jr., and Van Demark, N.L. Influence of oxidation products of adrenaline on adenosinetriphosphatase activity of a uterine muscle preparation. Proc. Soc. Exptl. Biol. Med. 97: 595 - 597 (1958).

210. Denisov, V.M. Effect of the oxidation products of catecholamines on the ATPase activity of myosin. Ukr. Biokhim. Zh. 36: 711 - 717 (1964); Chem. Abstracts 62: 2930 (1965).

211. Denisov, V.M. and Rukavishnikova, S.M. Influence of adrenoxyl on the activity of heart adrenosine-triphosphatase and phosphorylase. Ukr. Biokhim. Zh. 40: 384 - 387 (1968); Chem. Abstracts 69: 105000 (1968).

212. Friedenwald, J.S., and Heinz, H. Inactivation of amine oxidase by enzymic oxidative products of catechol and adrenaline. J. Biol. Chem. 146: 411 - 419 (1942).

213. Usdin, V.R., Su., and Usdin, E. Effects of psychotropic compounds on enzyme systems. II. In vitro inhibition of monoamine oxidase (MAO). Proc. Soc. Exptl. Biol. Med. 108: 461 - 463 (1961).

214. Petryanik, V.D. Effect of adrenochrome and vikasol on monoamine oxidase of some rabbit organs. Ukr. Biokhim. Zh. 43: 225 - 228 (1971); Chem. Abstracts 75: 47117 (1971).

215. Anderson, A.B. Inhibition of alkaline phosphatase by adrenaline and related dihydroxyphenols and quinones. Biochim. et Biophys. Acta 54: 110 - 114 (1961).

216. Heacock, R.A. The chemistry of adrenochrome and related compounds. Chem. Revs. 59: 181 - 237 (1959).

217. Shebeko, G.S. Effect of adrenaline and adrenoxyl on the level of coenzyme A in some organs of albino rats. Ukr. Biokhim. Zh. 42: 596 - 599 (1970); Chem. Abstracts 74: 72333 (1971).

218. Frederick, J. Cytologic studies on the normal mitochondrion and those submitted to experimental procedures in living cells cultured in vitro. Arch. Biol. 69: 167 - 349 (1958).

219. Blix, G. Adrenaline as an oxidation catalyzer. Oxidative deamination of amino acids by means of adrenaline or some simple hydroxybenzenes. Skand. Arch. Physiol. 56: 131 - 172 (1929).

220. Kisch, B., and Leibowitz, J. Die Omegakatalyze der oxydativen Glykokollspaltung. Biochem. Z. 220: 370 - 377 (1930).

221. Kisch, B. Die Omegakatalyze der oxydativen Glykokollspaltung. Neue Untersuchungen. Biochem. Z. 236: 380 - 386 (1931).

222. Kisch, B. O-Chinone als Fermentmodell. II. Mitteilung: Versuche mit verschiedenen Substraten. Biochem. Z. 244: 440 - 450 (1932).

223. Abderhalden, E., and Baertich, E. Deamination of glycine by "omega" (adrenaline oxidation product). Fermentforschung 15: 342 - 347 (1937); Chem. Abstracts 32: 3431 (1938).

224. Fischer, P., and Landtsheer, L. The metabolism of adrenochrome and trihydroxymethylindole in the rabbit. Experientia 6: 305 - 306 (1950).

225. Fischer, P., and Lecomte, J. Metabolisme de l'adrenochrome, de ses produits de reduction et du trihydroxy - N - methylindol chez le lapin, le chat et le chien. Bull. soc. chim. biol. 33: 569 - 600 (1951).

226. Noval, J.J., Sohler, A., Stackhouse, S.P., Bryan, A.C. Metabolism of adrenochrome in experimental animals. Biochem. Pharmacol. 11: 467 - 473 (1962).

227. Schayer, R.W., and Smiley, R.L. The metabolism of epinephrine containing isotropic carbon. III. J. Biol. Chem. 202: 425 - 430 (1953).

228. Bonisch, H. and Trendelenburg, V. Extravenronal removal, accumulation and O-methylation of isoprenaline in the perfused heart. Naunyn - Schmiedeberg's Arch. Pharmacol. 283: 191 - 218 (1974).

229. Bonisch, H., Uhlig, W. and Trendelenburg, V. Analysis of the compartments involved in the extraneuronal storage and metabolism of isoprenaline in the perfused heart. Nannyn - Schmiedeberg's Arch. Pharmacol. 283: 223 - 244 (1974).

230. Uhlig, W., Bonisch, H. and Trendelenberg, V. The O-methylation of extraneuronally stored isoprenaline in the perfused heart. Naynyn - Schmiedeberg's Arch. Pharmacol. 283: 245 - 261 (1974).

231. Axelrod, J., Whitby, G., Hertting, G., and Kopin, I.L. Studies on the metabolism of catecholamines. Circulat. Res. 9: 715 - 720 (1961).

232. Niedergerke, R. Calcium and activation of contraction. Experientia 15: 128 - 130 (1959).

233. Niedergerke, R. Movements of Ca in frog heart ventricles at rest and during contractures. J. Physiol. 167: 515 - 550 (1963).

234. Niedergerke, R., and Harris, E.J. Accumulation of calcium (or strontium) under conditions of increasing contractility. Nature 179: 1068 - 1069 (1957).

235. Langer, G.A. Kinetic studies of calcium distribution in ventricular muscle of the dog. Circ. Res. 15: 393 - 405 (1964).

236. Legato, M.J., Spiro, D., and Langer, G.A. Ultrastructural alterations produced in mammalian myocardium by variation in perfusate ionic composition. J. Cell. Biol. 37: 1 - 12 (1968).

237. Reuter, H.,and Seitz, N. The dependence of Ca efflux from cardiac muscle on temperature and external ionic composition. J. Physiol. 195: 451 - 470 (1968).

238. Langer, G.A., Sato, E., and Seraydarian, M. Calcium exchange in a single layer of rat cardiac cells studied by direct counting of cellular activity of labeled calcium. Circ. Res. 24: 589 - 597 (1969).

239. Arora, H.R.K. Calcium, sodium, and potassium interactions during cardiac contractions. Am. J. Physiol. 222: 333 - 338 (1972).

240. Emberson, J.W., and Muir, A.R. Changes in the ultrastructure of rat myocardium induced by hyperkalemia. J. Anat. 104: 411 - 421 (1969).

241. Nayler, W.G., and Szeto, J. Effect of verapamil on contractility, oxygen utilization, and calcium exchange - ability in mammalian heart muscle. Cardiovas. Res. 6: 120 - 128 (1972).

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

243. Nayler, W.G., Stone, J., Carson, V., McInnes, I., Mack, V., and Lowe, T.E. The effect of beta adrenergic antagonists on cardiac contractions, myofibrillar ATPase activity, high - energy phosphate stores and lipid - facilitated transport of calcium ions. J. Pharmacol. Exp. Ther. 165: 225 - 233 (1969).

244. Nayler, W.G., Chipperfield, D., and Lowe, T.E. The negative inotropic effect of adrenergic beta-receptor blocking drugs on human heart muscle. Cardiovasc. Res. 3: 30 - 36 (1969).