AN ULTRASTRUCTURAL AND PHARMACOLOGICAL STUDY ON THE EFFECT OF DOBUTAMINE ON THE ISCHEMIC MYOCARDIUM

A THESIS

SUBMITTED TO THE

UNIVERSITY OF MANITOBA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

BY

THOMAS P. THOMAS

DEPARTMENT OF ANATOMY

FACULTY OF MEDICINE

AUGUST, 1984

AN ULTRASTRUCTURAL AND PHARMACOLOGICAL STUDY ON THE EFFECT OF DOBUTAMINE ON THE ISCHEMIC MYOCARDIUM

BY

THOMAS P. THOMAS

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

DOCTOR OF PHILOSOPHY

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. E.J.H. Nathaniel, Professor of Anatomy, for the facilities he provided for my studies under his guidance.

It is with great pleasure I express my gratitude to Dr. Ian R. Innes, Professor of Pharmacology and Therapeutics for providing me the opportunity to pursue my studies in Anatomy and accomplish this endeavour. Without his support, understanding, broadmindedness and magnanimity, I could not have fulfilled my long awaited goal.

Thanks are due to Dr. T.V.N. persaud, Head of the Department of Anatomy, for accepting me into the graduate studies programme in human anatomy.

I am very happy to thank Dr. William J. Davidson, Associate Professor of Psychiatry and Pharmacology for the co-operation, friendship and sincere help he provided, Dr. Felix D. Bertalanffy, Professor of Anatomy, for all the moral support he extended throughout my graduate programme and Dr. Harvey Weisman for the helpful suggestions. I also would like to thank Dr. K. Dakshinamurti and Dr. C.S. Paulose of the Department of Biochemistry for providing the facilities for the enzyme assay.

I thank my family for their understanding and patience and my sister Dr. Aleyamma P. Thomas and her husband Jim for their support.

Thanks a lot Koruthu (Mr. Ken Eapen), for all the help you provided when I needed it.

My sincere thanks to Ms. Barbara Hunt for the prompt, efficient and excellent typing of this manuscript. I very much appreciate the work of Mr. Roy Simpson in doing the excellent photography.

ABSTRACT

Myocardial ischemia in animals and man is known to lead to necrotic changes if the ischemia persists for a critical period of time.

The objective of this investigation was to study the ultrastructural changes of ischemic myocardium and the effects of a cardioselective drug dobutamine on the ultrastructure and the functional correlates of the subcellular organelles of the ischemic myocardium. Light and electron microscopic studies were carried out initially to establish the morphology of normal myocardium in rats. This was followed by observations on the morphological alterations produced by experimentally induced myocardial ischemia. Animals having myocardial ischemia were then treated with dobutamine and the observations and results obtained from this procedure were then compared with the previous two sets of experiments.

Myocardial ischemia was induced experimentally in adult male Long-Evans rats by a single intraperitoneal injection of isoproterenol hydrochloride at a massive dose of 40 mg/kg body weight. Ischemia was confirmed by electrocardiographic changes. Under light ether anesthesia, animals were killed at 15, 30, 60 minutes or 18 hours after the insult, by intracardiac perfusion with Karnovsky's fixative. Pieces of tissues from selected areas of the left ventricle were processed for light and electron microscopy with conventional techniques. From the light microscopic observations, lesioned areas were isolated for electron microscopy. The ultrastructure of ischemic myocardium showed the following features: depletion of glycogen granules, scalloping of

sarcolemma, disappearance of I-band, clumping of Z-lines, minor and major contraction bands, aggregation of chromatin material at the periphery of irregular shaped nucleus, mitochondria with altered definition of cristae and some mitochondria with electron dense bodies on their inner membranes, sarcomeres out of registry, separation of myofilaments from the intercalated discs and swollen sarcoplasmic reticulum. Accumulation of electron dense bodies in the mitochondria is a sign of irreversible ischemic injury. These ischemic changes observed at the ultrastructural level were correlated with the results obtained from the biochemical and hemodynamic studies of the ischemic myocardium.

The influence of dobutamine hydrochloride on the effects of isoproterenol was tested, with appropriate control groups. Dobutamine was infused intravenously at a dose of 30 ug/kg/min for 60 minutes with a Harvard Continuous automatic infusion pump. Blood pressure and electrocardiogram were monitored throughout the experiment. Dobutamine infusion was commenced at different time intervals: immediately after, 15 minutes, and 30 minutes after isoproterenol insult. After 18 hours the animals were killed by perfusion with Karnovsky's fixative through the left ventricle and selected pieces of tissues removed were processed for light and electron microscopy. Serum was collected from the various groups to compare the activity of creatine phosphokinase and lactate dehydrogenase enzymes in these groups, thereby assessing the extent of cardiac damage.

A protective effect of dobutamine was seen in the ultrastructure of the ischemic myocardium after the treatment with dobutamine. In those rats treated with dobutamine immediately after isoproterenol a nearly normal pattern of ultrastructure was observed in contrast to rats given dobutamine at the later time intervals after the cardiac insult. The results were remarkable: less marked myofibrillar degeneration, uniform distribution of chromatin in the nucleus, less irregularity of nuclear contour, significantly fewer electron dense bodies in the mitochondria and intact sarcolemma. Most of the sarcomeres were in proper registry. Mitochondria showed well defined cristae, though some had electron dense bodies. These observations correlated well with a significant reduction in the release of enzymes from the infarcted myocardium into the blood.

Another noteworthy feature observed was the presence of large number of glycogen granules in the sarcoplasm and the conversion of beta type small particles to aggregation of alpha type granules. The maintenance of the structural integrity of mitochondria with dobutamine administration may be the key factor responsible for the significant reduction in the release of enzymes from the ischemic myocardium. The structural integrity of sarcolemma was manifest in the electrocardiographic changes after dobutamine administration.

The subcellular defects produced by ischemia were, in most of the instances, prevented by immediate administration of dobutamine. Since the beneficial effects of dobutamine largely depend upon the dose, concentration and duration of infusion, such parameters are

important criteria for reducing the size of the infarct. More data from the chronic study are warranted about the ultrastructure, biochemistry and pharmacology of the ischemic myocardium after the infusion of dobutamine for longer periods of time. However, time is a critical factor for the maintenance of the viable myocardium in the ischemic area - the earlier the drug therapy, the better the protection and the less damage to ischemic myocardium.

DEDICATED TO MY WIFE

NEENA,

DAUGHTERS REITA AND SHOBA

AND MY SON

PRIYAN.

"MY ANGUISH, MY ANGUISH! I WRITHE
IN PAIN! OH, THE WALLS OF MY HEART!
MY HEART IS BEATING WILDLY; I CANNOT
KEEP SILENT; FOR I HEAR THE SOUND OF
THE TRUMPET, THE ALARM OF WAR".

JEREMIAH 4:19

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS USED IN THE TEXT

A = Anisotropic band
A^{o} = Angstrom unit
Af = Actin filament
BP = Blood pressure
C = Capillary
Ca^{2+} = Calcium
CB = Contraction band
Chr = Chromatin
CPK = Creatine phosphokinase
CF = Collagen fibrils
Des = Desmosome
EDB = Electron dense body
EKG = Electrocardiogram
ES = Extracellular space
F = Fibre
FA = Fascia adherens
FIB = Fibril
FIL,fil = Filament
G = Golgi complex/bodies
Gap = Gap junction
GC = Glycocalyx
Gly = Glycogen granule
H = H line
HR = Heart rate
I = Isotropic band
ID = Intercalated disc
IC = Intracristal space
IS = Intercellular space
INC = Intercristal space
Kg = Kilogram
L = Lipid droplet
Lu = Lumen
M = M zone
MA = Macula adherens
MF,mf = Myofilament
mg = Milligram
Mit = Mitochondrion
ug = Microgram
u = Micron
mat = Matrix
N = Nucleus
Nm = Nuclear membrane
nm = Nanometer
Nu = Nucleolus
Ne = Nexus
PV = Pinocytotic vesicle
Pl = Platelets
S = Sarcomere
SL = Sarcolemma
Sp = Sarcoplasm
SR = Sarcoplasmic reticulum
T = Transverse tubule
Tj = Tight junction
V = Vacuole
Ve = Vesicle
Z = Z line

I

INTRODUCTION

INTRODUCTION

Myocardial ischemia is defined as being present in the myocardium whenever the arterial flow is depressed to the point that the oxygen demand of the tissue exceeds the oxygen supply available from collateral arterial flow (Jennings, 1981).

Ischemic heart disease is one of the major health problems confronting humanity as a whole. The predominant symptom of myocardial ischemia is angina pectoris (Greenspon and Goldberg, 1983). It was the English clinician William Heberden who introduced the term "angina pectoris" and provided a clear description of it when he delivered his classic paper entitled "Some Account of a Disorder of the Breast" to the Royal College of Physicians in 1768. The ancient Greeks recognized that chest pain could be associated with serious disease. Galen, in the second century A.D., referred to chest pain as "kardialgia". He recognized that chest pain could come from the heart, and postulated that pain from the heart was very serious (Weintraub and Helfant, 1983). Despite the clear description of the clinical features of angina pectoris, Heberden and his contemporaries were not aware of the pathological basis of this disorder.

However, it was the Chicago physician James B. Herrick who provided the classic description of coronary thrombosis and correlated the clinical and postmortem features of myocardial infarction in 1912. When William Osler delivered 'The Lumleian Lectures' on angina pectoris on the 15th of March, 1910 before the Royal College of Physicians, he gave a full description of the clinical aspects of the pathology of

angina pectoris. He mentioned the coronary artery disease and acute infarct of the left ventricle in one of his case studies. By coronary artery spasm he meant "persistent contraction leading to ischemia, with disturbance of function of the parts supplied". However, Keefer and Resnick (1928) were the first authors to have recognized clearly that angina was due to a relative oxygen lack in the myocardium. Now angina pectoris is known to occur by two broad mechanisms: 1) There may be a primary increase in myocardial oxygen consumption, and 2) there may be an abrupt reduction in myocardial oxygen supply (Greenspon and Goldberg, 1983).

In the last two decades, major advances have been made to obtain a better understanding of myocardial infarction by the use of different animal models. To develop a rational mode of therapy for the treatment of myocardial infarction in humans, it is essential we understand the morphology of ischemic myocardium and get a better knowledge of the nature of cell death caused by this entity.

During the early phase of ischemia there are ultrastructural, biochemical, contractile and electrocardiographic abnormalities which are complex and difficult to study in humans (Kloner and Braunwald, 1980). Studies have been carried out in primates (Smith et al., 1980), rats (Innes and Weisman, 1981; Koltai et al., 1983; Campbell et al., 1983), pigs (Fujiwara et al., 1982; Pashkow et al., 1977), cats (Beckman et al., 1983) and dogs (Nagi et al., 1983). The method used was the invasive technique - occluding the left anterior descending (LAD) or circumflex coronary artery. The pathological (Jennings et al.,

1969), electrocardiographic (Hillis et al., 1976) and hemodynamic (Boden et al., 1978) observations were more or less similar in dog and man. There are non-invasive methods as well (using catecholamines) of producing myocardial ischemia in experimental animal models (Wexler et al., 1968, 1970, 1972; Ferrans et al., 1969).

The objective of this study was four fold. The first objective was to re-establish the ultrastructure of the normal myocardium in rats. Some aspects of the hemodynamics and biochemistry of the normal myocardium were also investigated. The second objective was to re-establish the ultrastructural, hemodynamic and biochemical parameters of the ischemic myocardium after ischemia being induced by the administration of a catecholamine, isoproterenol hydrochloride. The third objective was to observe and establish the morphological, biochemical and hemodynamic alterations in the ischemic myocardium treated with a cardioselective drug, dobutamine hydrochloride (dobutrex). Other than some hemodynamic studies, there has not been much work done at the ultrastructural and biochemical levels with dobutamine. The final objective was to compare and contrast the results obtained from the three experimental observations and to establish the structural and functional correlates of experimentally induced myocardial ischemia after the administration of the cardioselective drug dobutamine.

II

REVIEW OF LITERATURE

A. ANATOMY OF THE HEART

The rat heart is a cone-shaped organ which lies within the pericardium - a dense fibrous sac - in the middle mediastinum of the thoracic cavity between the lungs. The heart weighs about 1 g (wet weight) in an adult male rat of about 250 g body weight. The heart is composed of three major types of cardiac muscle namely the atrial muscle, the ventricular muscle and specialized excitatory and conductive muscle fibers (Guyton, 1981). The walls of the heart are composed of a thick layer of cardiac muscle, the myocardium, covered externally with a serous pericardium, the epicardium, and lined internally with a layer of endothelium, the endocardium. Blood supply to the myocardium is provided by the right and left coronary arteries which arise from the aorta immediately above the aortic valve. The coronary arteries, after branching to supply the myocardium with oxygenated blood, become continuous with cardiac veins that empty into the coronary sinus, which eventually opens and empties into the right atrium (Jenson, 1980).

Collateral coronary arteries provide alternate routes for blood supply to the myocardium distal to major coronary artery obstruction (Wechsler, 1977). Intercoronary collateral vessels constitute the major class of naturally occurring collateral arteries. These arteries have an anatomic point of origin termed the stem, a mid-zone, and a reentry point (Wechsler, 1977). Genetic and hereditary variations in different species and within the same species have been observed as far as the presence, number, and transmural distribution of coronary collaterals

are concerned (James, 1970). In dogs there are an extensive epicardial collaterals and in pigs there is a dense subendocardial network of collateral arteries (Wechsler, 1977). In man collateral vessels have been demonstrated at every depth of myocardium, but subendocardial connections are more numerous. However, goats do not generally develop collateral vessels (Schaper et al., 1967) when the coronary arteries are occluded.

Sympathetic and parasympathetic fibers of the autonomic nervous system innervate the heart. Sympathetic stimulation increases the heart rate and parasympathetic stimulation decreases the heart rate (Guyton, 1981). Fine structure observations made of the autonomic innervations of the heart have revealed the presence of both adrenergic (excitatory) and cholinergic (inhibitory) axons lying within the same Schwann Cell sheath in the rat heart (Ehinger et al., 1970) and mouse and guinea pig heart (Chiba, 1973).

B. MORPHOLOGY OF THE NORMAL MYOCARDIUM

Cardiac muscle fibers are made up of individual cellular units that branch and anastomose and branch again (McNutt and Fawcett, 1974). The fibers are roughly parallel to each other. Because of the branching and anastomosing network of these fibers, there are intercellular spaces between them which are provided with blood and lymphatic capillaries as well as nerve fibers (Ham, 1974).

In the adult rat, the length of the ventricular myocardial cell varies from 35 u to 130 u which corresponds to about 20 to 80 functional subunits. The diameter of these myocardial cells varies between 12 u and 90 u (Muir, 1965). The nuclei are elongated and usually lie deep in the middle of the myocardial cells. In the isolated myocardial cells of rat, it has been reported that usually there are two nuclei in every cell, although occasionally only one is present (Muir, 1965). There is uniform distribution of chromatin material in the nucleus. The morphological characteristics of the myocardium depend upon the type of myocardial cell. The ventricular cells are primarily contractile and have a cytoplasm well packed with the contractile (filamentous) proteins and a good amount of glycogen granules. The conductive cells are usually larger in diameter with very few contractile proteins, but with larger amounts of glycogen granules in the sarcoplasm (Langer and Brady, 1974).

The cell membrane of myocardial fibers, known as the sarcolemma, is very thin when observed under the light microscope. The cytoplasm termed the sarcoplasm contains numerous mitochondria. The contractile proteins constituting the myofibrils show cross striations because of the A (anisotropic) and I (isotropic) bands and Z lines. Along the length of the myocardial fibers, at regular intervals, there are dark-stained heavy transverse lines called the intercalated discs which form the base for the attachment of individual myocardial cells. Though we cannot observe the intercalated discs under the light microscope with hematoxylin-eosin stain, these discs can clearly be seen after staining with iron-hematoxylin or phosphotungstic acid

staining (Bloom and Fawcett, 1975). The fine structure of myocardium gives the true nature of the sarcolemma and the sarcoplasmic organelles and inclusions.

C. ULTRASTRUCTURE OF THE NORMAL MYOCARDIUM

1. SARCOLEMMA

The sarcolemma of the myocardial cell is a trilaminar unit membrane with a thickness of about 7.5 to 9 nm which encloses the cardiac muscle cell (McNutt and Fawcett, 1974). This unit membrane is extremely thin but tenaciously stable film of lipid and protein molecules. A lipid molecule of the lipid bilayer is amphipathic (hydrophilic and hydrophobic portions). The bulky head of a lipid molecule is hydrophilic and faces outward to interact with the intercellular space and inward facing the cytoplasm of a cell or the interior of an organelle. The hydrophobic tails are pointed away from the water interacting with one another in the interior of the bilayer. The amino acids of proteins embedded in the lipid bilayer are linked by peptide bonds to form long polypeptide chains. The water soluble proteins of the polypeptide chain are alpha helix and beta sheet (Unwin and Henderson, 1984). Sarcolemma has the plasma membrane (unit membrane), the laminar coat and a third layer of collagen fibers. The terms basal lamina, basal membrane, basement membrane and glycocalyx are used interchangeably (Sommer and Johnson, 1979). The peripheral sarcolemma is scalloped, the valleys being at the Z lines and the humps reaching over mitochondria that are present between the Z lines on the

outer surface of myofibrils and beneath the plasmalemma. The plasmalemma is about 9 nm thick, the outer surface of which is covered with a layer of fluffy or feltlike electron-dense material of about 50 nm thick, the laminar coat (basal lamina), which is composed of glycoproteins and collagen.

Peripheral and interior plasmalemma show a number of small surface invaginations, which are called caveolae with an outer diameter of about 50-80 nm. These caveolae were once referred to as pinocytotic vesicles but since they do not have that type of function they are called caveolae or sarcolemmal vesicles. The caveolae serve to increase the surface area of the peripheral plasmalemma (Sommer and Johnson, 1979).

2. INTERCALATED DISC

Based on the observations with electron microscope it has been established that intercalated discs consist of the opposed membranes of adjacent myocardial cells and represent regions of cell-to-cell attachment (Sjostrand and Anderson, 1954; Fawcett & McNutt, 1969). Different types of specialized regions can be seen within the disc and include: fascia adherens, macula adherens and nexus (gap junction).

Fascia adherens (intermediate junction) is the predominant component of the transverse segments of the intercalated disc. Here the two plasma membranes are interdigitated but lie parallel and are separated by an interspace of about 20 to 30 nm. Proteinaceous material

in this interspace binds the membranes together (McNutt and Fawcett, 1974). The thin myofilaments of the I-band enter the filamentous mat at the fascia adherens and attach the myofilaments very strongly to the plasma membrane.

Macula adherens (desmosome) is a round-to-ovoid area of the intercalated disc with a diameter of about 0.2 to 0.5 u. Here the plasma membranes are parallel and separated by a 25 to 30 nm interspace filled with a dense proteinaceous material. The desmosomes serve for the attachment of cytoplasmic filaments called the tonofilaments. Desmosomes do not act as sites for the attachment of myofilaments (Simpson, Rayns & Ledingham, 1973).

The nexus of cardiac muscle as a component of the intercalated disc was first described by Sjostrand et al. (1958). It is usually found on the longitudinal segment of the intercalated disc. Since nexus seems to be the site at which the wave of depolarization can pass from one cell to another cell, this junctional complex is important from the physiological standpoint. Though we do not have enough information about the internal architecture of this specialized segment, it is important to note that the nexus represents a specialized transport site in the plasma membrane. Narrow channels may exist in the centre of the nexus subunits and allow passage of small ions from cell to cell (McNutt and Fawcett, 1974). According to the observations of Barr et al., (1965) nexus represents a site of low electrical resistance between cells.

3. SARCOPLASMIC RETICULUM

In the myocardial sarcoplasm, there is a network of membrane-bound tubules which has been known as the sarcoplasmic reticulum. The sarcoplasmic reticulum of myocardial cells is analogues to the endoplasmic reticulum of other cells. Though the general architecture of the sarcoplasmic reticulum in cardiac and skeletal muscle is similar, there are clear anatomical differences between the two. These structural differences presumably are important functionally too (Sommer and Johnson, 1979).

The sarcoplasmic reticulum can be divided into two components namely 1) free sarcoplasmic reticulum and 2) junctional sarcoplasmic reticulum. The junctional SR is a synonym for the terminal cisterna of skeletal muscle (Porter and Palade, 1957). Although the junctional SR in the myocardium is further modified into extended junctional SR, lamellar junctional SR and corbular SR as far as their geometry is concerned, the principal architecture is the same (Sommer and Johnson, 1979). The free SR tubules of the cardiac muscle have a width of about 20 to 35 nm and the thickness of SR membrane is about 9 nm according to the observations of Sommer et al. (1979). Myocardial SR has specialized areas of contact with the sarcolemma or its extensions which form the T (tubular) system known as couplings. The term coupling was introduced by Sommer and Johnson (1968). In myocardial cells which have a well developed T system, the couplings are mainly on the extensions of the plasmalemma which form the T tubules, but some are found on the plasmalemma proper and a few are on the intercalated disc (Simpson,

Rayns and Ledingham, 1973). In those cells which have no T system, like the conductive type cells and some atrial cells, the couplings are present at the periphery of the cell on the sarcoplasmic surface of the sarcolemma proper (Sommer and Johnson, 1968; McNutt and Fawcett, 1969).

In the mammalian myocardium the coupling is a flattened sac of SR closely opposed to the plasmalemma or its extensions. The lumen of the sac is about 15 to 25 nm in width and contains a layer of electron dense material (Johnson and Sommer, 1967; Simpson and Rayns, 1968; Fawcett and McNutt, 1969). These couplings have been shown to be the sites of ATPase activity (Sommer and Spach, 1964; Rostgaard and Behnke, 1965; Essner et al., 1965; Ferrans et al., 1969). They are generally regarded as the sites of accumulation of calcium ions, which are released when they receive suitable impulses from the sarcolemma (Page, 1968).

4. TRANSVERSE TUBULES (T System)

The transverse tubules (T tubules) are invaginations of the sarcolemma, penetrating in a transverse direction, into the interior of the myocardial cell at the Z band level (Simpson et al., 1973). The main difference between the skeletal muscle and cardiac muscle is in the geometry of the T systems. The T tubules are slender in the skeletal muscle with an approximate internal diameter of 40 nm and they do not have the glycoprotein surface coat. Usually there are 2 transverse tubules present for each sarcomere, situated at the boundary of the A and I bands. In the mammalian cardiac muscle the T-tubules are

associated with the Z bands and thus there is only one T-tubule for each sarcomere. The internal diameter is between 100 and 200 nm and they do have the glycoprotein surface coat continuing down into the T-tubules (McNutt and Fawcett, 1974).

The transverse tubules have numerous longitudinal connections as well, between the adjacent T-tubules along the length of the cell (McNutt et al., 1974). In the rat myocardium, it has been shown that slender tubules of about 30 to 50 nm in diameter may interconnect larger T-tubules (Forssmann and Girardier, 1970). Whether these slender tubules have glycoprotein surface coat or not has not been determined yet. Cardiac muscle cells of lower vertebrates (amphibians and reptiles) lack a T-system (McNutt and Fawcett, 1974).

Lindner (1957) suggested for the first time the existence of a communication between the extracellular space and the lumen of the T-system in the mammalian myocardium. Forssmann and Girardier (1966) confirmed this observation in the rat myocardium. They also showed some narrow tubules, similar in size to the tubules of sarcoplasmic reticulum, at the myocardial cell surface of rat heart, having communications with the extracellular space.

Histochemical studies have shown that the T-systems and surface caveolae are the sites of enzymes hydrolyzing nucleoside monophosphate (Sommer and Spach, 1964; Rostgaard and Behnke, 1965; Essner et al., 1965; Ferrans et al., 1969). It has been generally accepted that the function of the T-system is to act as a pathway for conduction of electrical impulses from the sarcolemma into the interior of the cell.

5. MYOFIBRILS

The most distinctive feature of the cytoplasm of myocardial and skeletal muscle cells is the presence of an alternating array of transverse bands (McNutt and Fawcett, 1974). One of these bands is anisotropic to polarized light and hence is called the A-band. The other band is isotropic to polarized light and is called the I-band. During muscule contraction the A-band does not shorten whereas the I-band does as contraction proceeds. The I-band is always bisected by a thin dense line called the Z-line. The region between adjacent Z-lines is called the sarcomere, the functional unit of cardiac muscle or any other type of muscle per se. The sarcomere length of a fully shortened muscle is 1.5 u and fully stretched muscle is between 3.0 to 3.5 u (McNutt and Fawcett, 1974). Ultrastructurally, it has been shown that A- and I- bands are composed of an array of protein filaments called myofilaments, which lie parallel to one another (Hanson and Huxley, 1953; Hanson and Lowy, 1963; Huxley, 1963).

The A-band consists of thick filaments which are 15 nm in diameter and 1.55 u in length and consist mainly of the protein myosin (Huxley, 1963). The A-band also contains the thin filaments which slide in between the thick filaments from both ends of the A-band (Simpson et al., 1973). The I-band consists of thin filaments, which are 5 to 8 nm in diameter and about 1 u in length. The regulatory proteins of these actin filaments are troponin and tropomyosin (Sommer and Johnson, 1979). The central region of the A-band, in which there are only thick filaments, appears lighter and is called the H-zone. During full

contraction the H-zone disappears. The thick filaments are arranged in a hexagonal pattern and are held together in the middle of the A-band and form the M-line (Simpson et al., 1973). Each thick filament is surrounded by six thin filaments, which can be very well seen in the transverse section of myocardial cells. The basic contractile event is due to the cyclic attachment and detachment of crossbridges between thick and thin filaments, which cause the thin filaments to slide along the thick filaments toward the center of the A-band (Huxley, 1973).

6. NUCLEUS

The nuclei of mammalian myocardial cells are elongated, situated usually in the centre of the cell and oriented along the long axis of the cells (McNutt and Fawcett, 1974). According to the investigations of Muir (1965) on isolated rat myocardial cells, there are usually two nuclei in each cell. Simpson et al. (1973) and McNutt and Fawcett (1974) could not find examples of paired nuclei in the rat myocardium. The percentage of binucleate cells in other mammalian species is not known. Since myocardial cells cease to divide shortly after birth thereby ending proliferative growth, it would be interesting to note if there are binucleate cells. The demand for growth of the heart is met by an increase in size (hypertrophy) of the existing cells (McNutt and Fawcett, 1974).

The Nuclear chromatin is predominantly dispersed (euchromatin) and stains lightly with heavy metals. Usually the nucleus is enclosed by a flattened membraneous saccule called the nuclear envelope which is

usually smooth in outline (Bloom and Cancilla, 1969; Fawcett and McNutt, 1969). The nuclear envelope appears as two membrane profiles separated by a narrow space, the perinuclear cisterna. The nuclear envelope is interrupted at intervals by nuclear pores which are about 80 nm in diameter. The thin diaphragm separating the sarcoplasm and nucleoplasm at the nuclear pores is responsible for the selective exchange of substances across (McNutt and Fawcett, 1974).

At the nuclear pole in the sarcoplasm there is an array of membrane bound saccules and vesicles which constitutes the Golgi complex. Little is known about the function of the Golgi complex in myocardial cells (McNutt and Fawcett, 1974).

7. MITOCHONDRIA

These are structures of variable size and geometry, located in the sarcoplasm of the myocardial cells, arranged longitudinally along the long axis of myofibrils. They measure from 0.3 to 1.7 u in length and from 0.2 to 1 u in width (Moore and Ruska, 1957). They are either freely mobile or immobilized by attachment to other cellular structures. According to Segretain et al., (1981) the mitochondria in the ventricular myocardium of adult rat are squarish, flattened and of the approximate size of a sarcomere. They also observed accumulation of spheroidal mitochondria in clusters in the perinuclear cytoplasm.

Each mitochondrion is enclosed by two membranes, an outer and inner one. The space between the outer and inner membranes is called

the intracristal space and the space within the inner membrane is referred to as the matrix space. The matrix space and the inner membrane contain the full set of enzymes required for mitochondrial function (Green, 1983).

8. GLYCOGEN AND LIPID

Glycogen is more abundant in cardiac than in skeletal muscle. They are present as small dense granules (beta-particles) of about 15 to 35 nm in diameter usually individually distributed in the sarcoplasm. Glycogen is plentiful near the nuclear pole, in the clefts of sarcoplasmic reticulum and between and among myofibrils. The function of the large amount of stored glycogen is not clear yet. This may have evolved to ensure against prolonged substrate deprivation (McNutt and Fawcett, 1974).

Lipid droplets, spherical in shape with a diameter of about 0.1 u are frequently found in close association with the outer membrane of mitochondria. These droplets represent the store of triglycerides which might be used during acute exogenous substrate deprivation in the presence of adequate oxygen (McNutt and Fawcett, 1974).

D. ISCHEMIC MYOCARDIUM

MORPHOLOGY OF THE MAMMALIAN ISCHEMIC MYOCARDIUM

The morphological changes which occur in myocardial cells during the early phase of ischemia can be appreciated by electron microscopy (Caulfield and Klionsky, 1959). These alterations have been studied in both dog and rat models of regional ischemia by Henderson et al., (1965), Jennings et al., (1965), Kloner et al., (1974) and Banka et al., (1978). In the dog, within 15 minutes of ischemia, the nucleus shows margination of chromatin, the myofibrils are relaxed and the number of glycogen granules becomes reduced. These changes are believed to be reversible. Within 20 to 40 minutes of ischemia, there is a swelling of mitochondria, edema of the sarcoplasmic reticulum (SR) and appearance of electron dense bodies in the mitochondria. As time passes, the mitochondria show increased swelling and disruption of their cristae and outer membranes. By 60 minutes the sarcolemma lifts off the myofilaments because of intracellular edema and breaks in the membrane can be seen as well. According to Jennings et al., (1977), this type of altered membrane permeability can result in cell swelling. These changes are irreversible and result in necrosis at least in the subendocardium. Ischemia damages the myocardial cells initially and followed by damage of the microvasculature (Kloner et al., 1979). After coronary artery ligation in the dog, the subendocardial tissue becomes irreversibly injured rapidly, within 20 to 60 minutes. As time passes a wavefront of cell death progresses from subendocardium to subepicardium and the whole process is completed within 6 to 9 hours (Reimer et al.,

1977). Thus myocardial cell death progresses at different rates in different regions of the heart.

E. SUBCELLULAR PATHOPHYSIOLOGY OF THE ISCHEMIC MYOCARDIUM

It has been known that a significant goal of research in protecting the ischemic myocardium is the understanding of the nature of ischemic myocardial cell death due to irreversible cell injury. In general, most of the studies have indicated that damage to the cellular membrane is one of the crucial factors of the process of irreversible injury.

a. IMPAIRMENT OF THE INTEGRITY OF SARCOLEMMA

An early event in the irreversible phase of myocardial ischemia is the loss of integrity of sarcolemma. Damage to the sarcolemma results in an entry of excess Ca^{2+} into the sarcoplasm from the extracellular space causing disruption of the internal metabolic machinery of the myocardial cell (Jennings et al., 1975). Breaks in the membrane also result in the loss of important intracellular components such as enzymes and cofactors to the extracellular space (Kaltenbach and Jennings, 1960). The cause for the actual disruption of the cell membrane is still not clear. Several theories have been proposed and they are: 1) activation of endogenous phospholipases of sarcolemma as a result of increased intracellular Ca^{2+} (Nayler et al., 1979); 2) depressed phosphorylation of membrane proteins by the reduced ATP of the ischemic cell (Jennings et al., 1978); 3) release of acid

hydrolases and phospholipases from lysosomes (Wildenthal, 1978) resulting in the degradation of membrane phospholipids (Sobel et al., 1978).

b. MITOCHONDRIAL ALTERATIONS

Trump et al. (1976) are of the opinion that ischemia or anoxia of the myocardium sets into motion four subcellular pathological processes which include release of hydrolases from lysosomes, defective synthesis of protein and nucleic acids, defects in the sarcolemmal function, and defects in mitochondrial ATP synthesis. Of these pathological changes, Trump and his associates (1976) think the mitochondrial dysfunction as the most important subcellular change. Depending upon the severity of ischemia, cellular levels of PO_2 rapidly approach zero and ATP synthesis, electron transport and other mitochondrial functions stop instantaneously. The integrity of mitochondrial membranes is crucial to the role of mitochondria in the process of energy production as well as calcium sink mechanisms. According to Trump et al. (1976), the earliest morphological change in mitochondria because of ischemia is the loss of mitochondrial granules which leads to condensation of mitochondria. This is followed by swelling of mitochondria associated with the appearance of flocculent densities culminating in the fragmentation. The lack of ATP inhibits the membrane transport which causes the inhibition of membrane pumps resulting in abnormal influx of calcium into the mitochondria. The increased calcium influx activates the mitochondrial phospholipase, thereby increasing the permeability of the inner mitochondrial

membrane. When the permeability increases, the mitochondria will undergo swelling. Jennings and Ganote (1974) and Jennings and Reimer (1981) consider mitochondrial defects as a characteristic feature of the irreversible phase of myocardial ischemia. The alterations in mitochondria consequent to drug induced heart disease has been reviewed by Dhalla et al., (1980).

c. CALCIUM OVERLOAD IN MYOCARDIAL ISCHEMIA

Calcium overload in the myocardial cells occurs because of the impaired Ca^{2+} exchange mechanism present in the sarcolemma, the inner membrane of mitochondria and the sarcoplasmic reticulum (Wolkowicz et al., 1983). Shen and Jennings (1972a,b) observed massive calcium overload in myocytes irreversibly injured by ischemia and subsequently reperfused with arterial blood. This was observed in canine myocardium after occluding the left anterior descending coronary artery. Calcium overload has also been observed in catecholamine induced myocardial lesions in rat myocardium (Fleckenstein et al., 1973; 1975).

The calcium paradox phenomenon described by Zimmerman and Hulsmann (1966) and Zimmerman et al., (1967) points out the effect of calcium influx into the myocardium. When isolated rat hearts were perfused with a modified Tyrode solution, containing calcium, no significant amounts of lactate dehydrogenase, creatinephosphokinase, myoglobin and adenosine triphosphates were detectable in the effluent. When the same hearts were perfused with a calcium-free medium these intracellular components were absent in the effluent. After 3 minutes

of calcium-free perfusion, when a calcium containing medium was introduced, the intracellular components appeared immediately, simultaneously and abundantly in the effluent. Electrical activity disappeared when calcium ions were reintroduced and the heart became irreversibly contracted, at this point there has been a substantial influx of calcium. The essential feature in all these situations is the massive influx of calcium into the myocardial cells and the accumulation within the mitochondria. There is loss of tissue high-energy phosphates (Boink et al., 1976; Bulkley et al., 1978) as a result of calcium influx. According to Fleckenstein et al. (1973), intracellular calcium overload initiates a deleterious breakdown of adenosine triphosphate and creatine phosphate in the myocardium of rats subjected to myocardial lesions by isoproterenol administration. This calcium-induced high energy phosphate exhaustion may be the crucial point in the etiology of myocardial fibre necroses.

In an in vitro study on the interventricular septum of rabbit heart muscle, Rich and Langer (1982) observed contracture development, loss of developed tension, and loss of potassium and creatine kinase as a result of myocardial necroses. A number of factors attenuate the extent of damage occurring during the calcium overload in the myocardium. On the basis of some of the previous observations, results and data, Whalen et al., (1974) speculated that calcium overload per se is the lethal event in ischemic injury.

d. MYOCARDIAL LESIONS INDUCED BY CATECHOLAMINES

As early as 1906, Pearce described hyaline necrosis in the myocardial cells of rabbit, following the injection of adrenaline. Since then numerous experiments have been done in different species of animals by a number of investigators in different parts of the world, utilizing catecholamines to induce myocardial lesions (Chappel et al., 1959; Rona et al., 1959; Szakes and Mehlman, 1960; Shenk and Moss, 1966; Ferrans et al., 1969; Eliot et al., 1979).

Isoproterenol, a synthetic catecholamine, is the most active sympathomimetic amine that acts almost exclusively on β-receptors. First studied by Konzett (1940), it has since been the subject of extensive animal and clinical research. Based on the reports of the aforesaid investigators it is well known that the myocardial cells show both reversible and irreversible changes. Changes which are considered reversible include cytoplasmic areas with myofilaments out of register, loss of density of the Z-lines, increase in ribosomes and prominent Golgi apparatus. Within 24 hours, tubules and vesicles containing electron dense material appear and are considered as belonging to the tubular system or proliferation of the sarcoplasmic reticulum. Calcium deposits also have been observed by many authors (Richenbach and Benditt, 1970). These changes resemble the morphological alterations observed in the ischemic myocardium of humans. Recent biochemical studies have established the presence of elevated levels of cyclic AMP, in response to large doses of catecholamine administration (Shahab et al., 1972). Lubbe et al. (1983) have illustrated the relationship

between ventricular fibrillation threshold levels and concentrations of cyclic AMP. In general, in animal models of myocardial ischemia, ventricular fibrillation is prevalent when myocardial levels of cyclic AMP are elevated. Cyclic AMP is the major determinant of myocardial cell membrane permeability to Ca^{2+}, (Watanabe et al., 1974).

e. ELECTROCARDIOGRAPHIC CHANGES AS AN INDEX OF MYOCARDIAL INJURY

Electrocardiogram recordings serve to indicate the extent of ischemic damage in patients with myocardial infarction and are important in assessing the prognosis and the beneficial effects of hemodynamic, pharmacological and clinical interventions in the management of myocardial ischemia.

(i). Significance of ST-segment

Epicardial electrodes mapping used to assess the area of ischemic damage in open-chest animals subjected to coronary artery ligation, is a valuable and simple technique. Within one minute of occlusion, the epicardial electrocardiograms show ST segment elevation congruent with the area of visible damage or visible epicardial cyanosis, (Pardee, 1920; Braunwald et al., 1976). It appears that the altered ion transport mechanism across the sarcolemma is responsible for the ST segment elevation during myocardial ischemia. It has been postulated that ischemia limits the availability of energy necessary for the sodium-potassium exchange across the sarcolemma (Kloner et al., 1980). Consequently, ischemia results in the accumulation of

intracellular sodium accompanied by chloride and water, and potassium leaks into the extracellular space (Nayler et al., 1971; Opie et al., 1973). Because of this, the cells lose their ability to regulate volume and tend to develop the electrolyte concentration similar to that of the extracellular fluid. Small changes in the ratio between potassium ions inside and outside the cell have a marked effect on the polarity of cell membranes (Holland and Brooks, 1976) and the alteration in this ratio, induced by ischemia, seems to play the critical role in producing ST segment elevation (Johnson, 1976).

ST segment elevation during the early phase of myocardial ischemia, identifies the tissue of canine heart that eventually became necrotic as a result of coronary artery ligation, the assessment being based on gross pathological observation, measurement of creatine kinase depletion from tissues and histological evaluations (Braunwald and Maroko, 1976). The height of the epicardial ST segment elevation after coronary artery ligation correlates with the severity of ischemic injury as assessed by electronmicroscopy (Kloner et al., 1977). Recently, Daniel (1979), in a study on the relationship between ST segment changes and the extent of infarction of the left ventricle, 15 minutes after ligation of the left anterior descending coronary artery in dogs, concluded that a greater portion of the ventricular wall remained viable at sites with less than 8 millivolts elevation. He considered that sites with less than 8 millivolts elevation in the epicardial ST segment area are most susceptible to beneficial effects of pharmacologic interventions designed to limit the infarct size.

(ii). Significance of R-wave

Ribeiro et al. (1979), have analyzed the early increase in R wave voltage to see whether it could be correlated with myocardial injury and predict the extent of necrosis 24 hours after coronary artery ligation. On the basis of their studies on dogs, they concluded that the increase in R wave, which occurs early after coronary artery ligation, reflects the reduction in blood flow to the subjacent myocardium and predicts the eventual extent of necrosis in the underlying myocardium. Alterations in R wave amplitude during 15 minutes after coronary artery ligation may be used as an index to determine the efficacy of pharmacologic interventions used to limit ischemic injury. Though the R wave rises only initially following coronary artery ligation, this change is reversed rapidly. The R wave may decline, may disappear and Q or QS waves develop (Shugold, 1967). Though the ST segment elevations and increases in the height of R wave are electrocardiographic expressions of myocardial ischemia and are reversible when the occlusion is released, loss of R waves and development of Q waves are the expressions of irreversible injury (Kloner et al., 1980).

F. THE BORDER ZONE CONCEPT

The border zone of a myocardial infarct is defined as an area of reversibly injured ischemic cells that can be salvaged by proper pharmacological interventions (Kloner et al., 1980). If the tissue in the border zone is left without any protective treatment, that tissue

would become necrotic. Reperfusion, a drug, or a hemodynamic intervention might salvage the cells in the reversibly injured border zone (Kloner et al., 1980).

Reimer and Jennings (1979), have used the canine circumflex coronary artery occlusion model and postmortem injection of a dye through the same artery to see the circumflex coronary bed. They showed a wavefront phenomenon of ischemic cell death from the subendocardium to the subepicardium depending upon the duration of occlusion. Coronary occlusion for 18 minutes followed by reflow did not cause any necrosis according to Sommers et al., (1964), Jennings and Reimer (1973) and Reimer et al., (1977). Fishbein et al. (1977, 1981) observed a lateral border zone in the rat myocardium after coronary artery occlusion and defined histochemically as a zone where glycogen is depleted, but oxidative enzymes are still present. As duration of ischemia progressed, the zone became gradually reduced in size and disappeared 9 hours after occlusion. All these studies suggest that the salvageable border zone is the largest zone immediately after coronary ligation and becomes progressively smaller until it disappears between 6 and 9 hours after the ligation (Kloner et al., 1980). Some of the previous histochemical (Cox et al., 1968) and biochemical (Bilheimer et al., 1978) studies also provide evidence for a border zone. Recently Gottlieb et al. (1981) studied the border zone in adult mongrel dogs following ligation of the left anterior descending coronary artery. Regions of infarct area, which were unstained by nitroblue tetrazolium chloride gross histochemical staining technique, revealed under electron microscopic observation, features of irreversible damage.

Areas stained by this method were adjacent to the infarct area and showed varying degrees of reversible ischemic injuries. On the basis of semiquantitative evaluation, they established the existence of a border zone, which showed mild to moderate ischemic changes and absence of electron-dense deposits in the mitochondria. However, Janse et al. (1979), using histochemical, biochemical and electrophysiological parameters, were unable to show the existence of a border zone in the porcine heart, following 2 hours of ischemia by the coronary artery ligation method. So the border zone concept is still controversial, largely a semantic issue and depends on how the border zone is defined (Kloner et al., 1980).

G. BIOCHEMICAL CHANGES

(i). Enzyme alterations

In acute myocardial infarction, many kinds of myocardial enzymes are rleased into the circulation. By determining the activities of these enzymes in the blood, important information can be obtained about the degree and the process of destruction of myocardial cells and intracellular organelles (Nagi et al., 1983). In order to quantitate the relation between the extent of myocardial infarction and the impairment of left ventricular function, serum enzyme changes are correlated with left ventricular hemodynamics (Mathey et al., 1984).

According to a study conducted by Hearse (1977), the injured myocardial cells release creatine phosphokinase, lactate dehydrogenase,

glutamic oxalacetic transaminase, glutamic pyruvate transaminase and citrate synthetase, enzymes into circulation. Some of the proteins released during acute myocardial infarction are myoglobin, light chain of myosin, cathepsin C, beta-glucuronidase and beta-galactosidase. In addition to these, cytochrome C from the respiratory chain of mitochondria is also released during myocardial infarction. Kiss and Reinhart (1956), reported for the first time the abnormal serum concentrations of myoglobin 10 to 12 hours after acute myocardial infarction. Stone et al. (1977), Sylven and Bendz (1978) and Freeman et al. (1981) have confirmed that most patients with acute myocardial infarction have a raised serum myoglobin concentration. McComb et al. (1984) showed that the serum myoglobin concentration rises in most patients within 4 hours of the onset of symptoms of acute myocardial infarction and in almost all patients within 6 hours. They also found that serum creatine kinase activity was slower to rise within the early hours of infarction, the peak activity being between 20 and 28 hours after the onset of symptoms.

A highly sensitive radioimmunoassay method for the measurement of myocardial myoglobin has been developed recently for human serum, and McMurtry et al. (1979) have modified that method to measure the circulating myoglobin levels in rats subjected to isoproterenol-induced myocardial infarction. Myoglobinemia offers excellent promise as a diagnostic aid in detecting early myocardial infarction in rats, dogs and humans, since there are only small differences between the immunological specificity of human and rat myoglobin (McMurtry et al., 1979).

Toleikis (1983) measured the activity of cytochrome C oxidase in mitochondria isolated from rabbit hearts which were subjected to coronary ligation and observed that there was a decrease in the activity of this enzyme. Cytochrome C oxidase is the terminal member of the electron-transport chain and an integral part of the inner membrane of mitochondria (Toleikis, 1983).

Kluge (1969), Wexler (1970), Oliver et al. (1972) Sobel et al. (1977), Willerson et al. (1982) and Horder et al. (1983) have clearly demonstrated that there are changes in the serum enzyme levels in patients with myocardial ischemia and in animals subjected to coronary artery ligation or treated with massive doses of isoproterenol. The elevated plasma or serum levels of creatine kinase, lactate dehydrogenase, serum glutamic oxalacetic transaminase, myoglobin, light chain of myosin, and serum glucose, following infarction correlate with their depletion in infarcted tissue (Willerson et al., 1982).

(ii). Creatine phosphokinase

This enzyme has 3 isoenzymes namely CK-MM, CK-BB and CK-MB (Roberts and Sobel, 1973; 1976; Wagner et al., 1973). Brain and kidney predominantly have the BB isoenzyme, skeletal muscle the MM isoenzyme and heart muscle the MM and MB isoenzymes. Measurement of the CK-MB isoenzyme is useful in the detection of myocardial infarction. After the onset of myocardial ischemia, the serum CK activity increases within 4 to 6 hours, peaks at 12 to 24 hours, and returns to normal within 2 to 3 days. If there is skeletal muscle damage, alcoholic

intoxication, intramuscular injection or vigorous exercise etc., there may be "false positive" results and the quantitative measurement of CK isoenzyme can specifically detect the organ injury (Willerson et al., 1982).

(iii). Lactate dehydrogenase

The isoenzymes of lactate dehydrogenase represent the combination of the 2 subunits, H (heart) and M (muscle), into 5 distinct molecular forms classified as isoenzymes 1, 2, 3, 4 and 5 (Oliver, et al., 1972). The serum lactate dehydrogenase 1 activity increases within 24 to 48 hours after the onset of myocardial infarction, peaks at 3 to 6 days, and returns to normal in 7 to 14 days after the infarction (Hearse, 1977; Vasudevan et al., 1978). LDH1 moves most rapidly, and LDH5 most slowly, toward the anode. Heart contains primarily LDH1, liver and skeletal muscle LDH4 and LDH5 (Vasudevan et al., 1978). Acute myocardial infarction can be determined most accurately by detecting an increase in serum LDH1 activity (Vasudevan et al., 1978).

Hematologic alterations:-

During acute myocardial infarction, in response to tissue necrosis, the white blood cell count increases within 2 hours, peaks at 2 to 4 days, and returns to normal within 1 week (Alpert and Braunwald, 1980). The number of polymorphonuclear leukocytes increases as well.

H. PHARMACOLOGIC MANAGEMENT OF THE ISCHEMIC HEART

Pharmacologic management of the ischemic myocardium is important to reduce the myocardial infarct size in a variety of experimental models, (Kloner and Braunwald, 1980). Prognosis in patients with acute myocardial infarction is mainly dependent on infarct size (Willerson et al., 1982). On the basis of the documented evidence for the existence of viable myocardium surrounding the infarct, it has been believed that if proper pharmacologic intervention is applied those cells in the so called border zone could be salvaged.

Various pharmacologic agents administered have different mechanism of action on the ischemic myocardium. Some of these agents have improved the relation between oxygen supply and demand, by increasing myocardial oxygen supply, decreasing oxygen demand or both. Maroko et al. (1975) have shown that they could reduce the extent of myocardial infarction in anesthetized open-chest dogs by giving them 40% and 100% oxygen to inhale. Madias et al. (1976) administered 100% oxygen to patients who had anterior wall myocardial infarction and noted a reduction in ST segment elevation, suggesting that the severity of ischemia was reduced. Coronary vasodilators like nitroglycerin and sodium nitroprusside have reduced ischemia or infarction in experimental studies, as shown by epicardial ST segment mapping, (Myers et al., 1975).

Maroko et al. (1972; 1973), Braunwald and Maroko (1976), Hillis et al. (1976) and Kloner et al. (1978) have shown that hyaluronidase

decreased the extent of myocardial damage in open-chest dogs and Maclean et al (1976; 1978) showed a similar improvement in the rat after coronary artery ligation. Their observations were based on the electrocardiographic, biochemical and morphological techniques. Electron microscopic observations showed that during the early phase of ischemia, hyaluronidase diminished myocardial cellular edema and had a disproportionate sparing effect on the glycogen granules within the ischemic myocardial cells. In effect, hyaluronidase improves substrate delivery (Kloner et al., 1977).

Agents which reduce oxygen demand of the myocardium during ischemia, tend to protect the heart and cause a reduction in the size of the infarct. The beta-adrenergic blockers appear to have beneficial effects on ischemic myocardium, both in experimental animals and in patients (Braunwald et al., 1983). Several investigators have used different beta-adrenergic blockers, especially propranolol, practolol, atenolol, metoprolol, oxeprenolol and timolol, in experimental attempts to reduce infarct size. Many, though not all, have found these agents to be beneficial (Reimer et al., 1973; 1976; Rasmussen et al., 1977). Initial studies by Reimer and Jennings (1973) using the canine model, showed that propranolol significantly reduced the necrosis as evaluated by histological analysis of the cardiac muscle.

The action of propranolol in reducing the infarct size is related presumably to the reduction of myocardial oxygen consumption, resulting from the blocking of sympathetic stimulation on heart rate and contractility (Kloner and Braunwald, 1980). Though the method of

action of these beta-blockers is not known, suggested mechanisms of action are: (1) blockade of beta-adrenergic receptors, which results in decreased myocardial oxygen consumption; (2) blockade of the sympathetic activity; (3) alteration of substrate utilization; (4) increase in collateral blood flow; (5) decrease of microvascular damage; and (6) stabilization of cell membranes (Braunwald et al., 1983).

Adrenergic agents

These agents have 3 major effects on the heart, namely the inotropic, chronotropic and arrhythmogenic effects (Farah et al., 1984). Although epinephrine, norepinephrine and isoproterenol have powerful positive inotropic effects, their usefulness in the therapy of heart failure has been limited because of their arrhythmogenic properties (Farah et al., 1984). Since the ventricles of the heart (cat and guinea pig) contain mostly the beta 2 receptors and the atria about 75% beta 2 receptors, the adrenergic agents have different effects on the heart rate (beta 2) and contractile force (beta 1) (Hedberg et al., 1980).

Dobutamine is a synthetic catecholamine with potent inotropic properties (Tuttle and Mills, 1975). It acts directly on beta 1 adrenergic receptors to increase the cardiac contractility and heart rate (Goldberg et al., 1977). Though it acts on beta 2 receptors as well, the chronotropic effect is less significant. A separation of the inotropic and chronotropic effects of dobutamine has been demonstrated

(Tuttle and Mills 1975, Tuttle et al., 1976) and so this agent has potential therapeutic value in the management of cardiovascular failure associated with low cardiac output after myocardial infarction (Jewitt et al., 1974). However, Kenakin (1981) and Kenakin and Beek (1982) have not been able to demonstrate any selectivity for beta adrenergic receptors with either dobutamine or prenalterol.

The data presented by Unverferth et al. (1980) have clearly illustrated the ultrastructural effects of dobutamine on the electron dense particles in the mitochondria of human ischemic myocardium. The electron-dense bodies in the mitochondria were significantly reduced after the continuous infusion of dobutamine.

The crucial problem in the treatment of myocardial ischemia is to provide the pharmacologic intervention while the myocardial cells around the infarct are still viable. Once these viable cells - salvageable cells - of the ischemic myocardium reach the "point of no return" no clinical or pharmacological intervention can save them.

III

MATERIALS AND METHODS

MATERIALS AND METHODS

A. General plan and method of procedure.

B. Drugs, dosages and routes of administration.

C. Design of the experiment.

D. Blood pressure and electrocardiogram.

E. Measurement of enzyme activity.

F. Light and electron microscopy.

G. Statistical analysis.

A. GENERAL PLAN AND METHOD OF PROCEDURE

The animals used in this study were 6-8 weeks old male Long-Evans hooded rats weighing between 225-250 g, obtained from the CBF (Canadian Breeding Farm) Laboratories in Montreal, Quebec. They were maintained on normal Purina rat chow and water ad libitum. Before the experiment, electrocardiograms of all rats were recorded under ether anesthesia to exclude those rats with cardiac abnormalities since most colonies have a few rats showing such abnormalities. Animals with normal electrocardiogram (EKG) were selected for the subsequent experiments.

The first phase of this study was to re-establish the ultrastructure of the normal myocardium in rats.

The second phase was to study the morphology of ischemic myocardium in rats. Myocardial ischemia was induced experimentally by the non-invasive method of using a catecholamine and confirmed by the EKG changes. Details of the procedure are described elsewhere.

The last phase of this study was to establish the morphological, hemodynamic, and biochemical features of catecholamine induced ischemic myocardium after pharmacological intervention.

B. DRUGS, DOSAGES AND ROUTES OF ADMINISTRATION

1. Isoproterenol hydrochloride.

a) DL-Isoproterenol hydrochloride was obtained from Sigma Chemical Company, (USA).

b) Different doses of isoproterenol hydrochloride, 5 mg/kg, 10 mg/kg, 20 mg/kg, 40 mg/kg and 80 mg/kg were tried; 40 mg/kg was selected for this experiment. Since isoproterenol hydrochloride in solution oxidises, the solution was prepared in ice-cold normal saline, kept in the ice bucket, and used within 30 minutes. The concentrations used was 40 mg/ml. Fresh solution was prepared for each experiment.

c) Isoproterenol hydrochloride was given intraperitoneally because the rate of absorption is faster than by the subcutaneous route. Sublingual or oral route is unreliable and is not recommended.

2. Dobutamine hydrochloride.

a) Dobutamine hydrochloride (Dobutrex) was obtained from Eli Lilly and Company (Canada) in 20 ml vials that contained 250 mg of the drug.

b) Different dose levels, ranging from 5 ug to 40 ug/kg/minute, were tested. At higher dose levels, the heart rate was increased significantly. The dose selected was 30 ug/kg/min for 60 minutes, because with this dose level the extent of

myocardial damage caused by dobutamine was significantly less. The parent compound was dissolved in 10 ml sterile water. The reconstituted dobutamine hydrochloride was further diluted with sterile normal saline, which was stable for up to 24 hours. If the reconstituted solution turned pink, due to oxidation, that was discarded. The concentration of drug used was between 200 and 225 ug/ml depending upon the weight of the animal. c) The route of administration was intravenous and for continuous slow infusion, a Harvard infusion pump was used.

3. Sodium pentobarbital.

a) Sodium pentobarbital was obtained from Allen & Hanburys, Toronto, (Canada).

b) Sodium pentobarbital was dissolved in propylene glycol and ethyl alcohol and diluted with normal saline. The concentration of the solution was 40 mg/ml and the dose selected was 40 mg/kg.

c) Route of administration was intraperitoneal.

C. DESIGN OF THE EXPERIMENT

Animals were divided into several groups on the basis of their body weights. Care was taken so that the body weights were equal among the control and experimental groups. Control groups of animals in all the experiments, selected randomly, received normal saline injection intraperitoneally. Myocardial ischemia, induced by a single

intraperitoneal injection of isoproterenol hydrochloride, was confirmed by changes in electrocardiogram recordings and established by the ultrastructural changes in the ischemic myocardium. In each group there were four animals, all adult males. A total of four experiments were conducted.

EXPERIMENT I

THE OBJECTIVE OF THIS EXPERIMENT WAS TO ESTABLISH THE CYTOLOGY OF THE MYOCARDIUM OF CONTROL AND ISOPROTERENOL INSULTED GROUPS OF ANIMALS. THERE WERE 5 GROUPS IN THIS EXPERIMENT.

Group 1. Control. The four animals in this group received equivalent volume of normal saline injection as those who received isoproterenol injection intraperitoneally.

Groups 2-5. These were experimental animals. Each group consisted of 4 animals. Each animal was administered isoproterenol hydrochloride intraperitoneally in a dosage of 40 mg/kg, irrespective of the group and killed at different time intervals following the injections, as follows:

Group 2 - killed after 15 minutes.

Group 3 - killed after 30 minutes.

Group 4 - killed after 60 minutes.

Group 5 - killed after 18 hours.

All animals, in the control and experimental groups, were killed under light ether anesthesia, by intracardiac perfusion with

Karnovsky's fixative through the left ventricle. Selected pieces of tissues from the same ventricular wall were processed for light and electron microscopy.

EXPERIMENT II

THE OBJECTIVE OF THIS EXPERIMENT WAS TO ESTABLISH THE HEMODYNAMIC AND ELECTROCARDIOGRAPHIC FEATURES IN THE CONTROL AND EXPERIMENTAL GROUPS OF ANIMALS. THERE WERE SIX GROUPS OF ANIMALS IN THIS EXPERIMENT. THE DOSAGES OF DRUGS USED WERE GIVEN UNDER B. BLOOD PRESSURE AND ELECTROCARDIOGRAM WERE RECORDED FOR 1 HOUR AFTER THE INSULT AND TREATMENT ACCORDING TO THE PROCEDURAL METHOD GIVEN UNDER D, AFTER WHICH THE RATS WERE KILLED AND DISCARDED.

Group 1. Control. These rats received normal saline injection intraperitoneally after they were given sodium pentobarbital injection. Blood pressure and electrocardiogram were recorded for 1 hour after which the rats were killed and discarded.

Group 2. The four animals in this group were given isoproterenol injection after being anesthetized with sodium pentobarbital. Blood pressure and electrocardiogram were recorded for 1 hour.

Group 3. The four animals in this group were given dobutamine infusion after being anesthetized with sodium pentobarbital. Blood pressure and electrocardiogram were recorded for 1 hour.

Group 4. These rats received isoproterenol injection and dobutamine infusion immediately after the insult under pentobarbital anesthesia. Blood pressure and electrocardiogram were recorded for 1 hour.

Group 5. The animals in this group received dobutamine infusion 15 minutes after isoproterenol insult under pentobarbital anesthesia. Blood pressure and electrocardiogram were recorded for 1 hour.

Group 6. These rats received dobutamine infusion 30 minutes after isoproterenol insult under anesthesia. Blood pressure and electrocardiogram were recorded for 1 hour.

All animals were killed and discarded after recording of the blood pressure and electrocardiogram.

EXPERIMENT III

THE OBJECTIVE OF THIS EXPERIMENT WAS TO ESTABLISH THE ULTRASTRUCTURAL FEATURES OF DOBUTAMINE ON ISOPROTERENOL INDUCED ISCHEMIC MYOCARDIUM. THERE WERE FIVE GROUPS IN THIS EXPERIMENT. ANIMALS IN ALL GROUPS OF THIS EXPERIMENT WERE ANESTHETIZED WITH SODIUM PENTOBARBITAL. EXCEPT GROUP 1, ALL OTHER GROUPS RECEIVED ISOPROTERENOL INSULT AND DOBUTAMINE TREATMENT OR DOBUTAMINE ALONE AT DIFFERENT TIME INTERVALS. AFTER THE INSULT AND TREATMENT, THE SURGICAL WOUNDS WERE SUTURED WITH SUTURE CLIPS (IREX SURGICAL SUTURES) AND BLEEDING IF THERE WAS ANY WAS PREVENTED BY THE APPLICATION OF STERILE SURGICEL, THE ABSORBABLE HEMOSTAT (JOHNSON & JOHNSON), AND THE RATS RETURNED TO THEIR CAGES FOR 18 HOURS. AT THE END OF 18 HOURS THEY WERE KILLED UNDER ETHER ANESTHESIA BY INTRACARDIAC PERFUSION WITH KARNOVSKY'S FIXATIVE AND TISSUES FROM THE LEFT VENTRICLE WERE PROCESSED FOR LIGHT AND ELECTRON MICROSCOPY.

Group 1. The four animals in this control group received normal saline injection. After 18 hours, the perfusion fixed pieces of tissues were processed for light and electron microscopy.

Group 2. These four rats received normal saline injections intraperitoneally and dobutamine infusion intravenously. They were killed after 18 hours.

Group 3. Animals in this group received isoproterenol insult and immediately after the insult they were given dobutamine infusion. They were killed at the end of 18 hours.

Group 4. These rats received isoproterenol insult and 15 minutes later dobutamine administration. They were killed after 18 hours.

Group 5. These four rats received isoproterenol insult and 30 minutes later dobutamine infusion. They were killed after 18 hours.

EXPERIMENT IV

THIS EXPERIMENT WAS CONDUCTED TO STUDY THE ACTIVITY OF CREATINE PHOSPHOKINASE AND LACTATE DEHYDROGENASE ENZYMES RELEASED INTO THE BLOOD IN THE CONTROL AND EXPERIMENTAL GROUPS OF ANIMALS. THERE WERE 10 GROUPS IN THIS EXPERIMENT WITH FOUR ANIMALS IN EACH GROUP. EXCEPT GROUP 1, WHICH WAS GIVEN NO INSULT OR TREATMENT, ALL OTHER GROUPS WERE GIVEN INSULT AND TREATMENT EITHER BEFORE OR AFTER. BLOOD SAMPLES WERE TAKEN 18 HOURS AFTER THE INSULT AND KEPT AT ROOM TEMPERATURE FOR 30 MINUTES FOR SERUM SEPARATION. EVERY PRECAUTION WAS TAKEN DURING SAMPLE COLLECTION TO AVOID ANY HEMOLYSIS. ONCE THE SERUMS WERE PIPETTED OUT INTO PREVIOUSLY NUMBERED VIALS, THEY WERE IMMEDIATELY STORED AT $-70^{o}C$ UNTIL ALL THE SAMPLES FROM ALL THE GROUPS WERE AVAILABLE FOR THE ENZYME ANALYSIS. THE METHOD OF MEASURING THE ACTIVITIES OF CREATINE

PHOSPHOKINASE AND LACTATE DEHYDROGENASE ENZYMES IS DISCUSSED IN SECTION E.

Group 1. Normal animals, no insult and no treatment.

Group 2. Control animals. Normal saline injection only.

Group 3. Isoproterenol insult.

Group 4. Propranolol injection intraperitoneally at a dose of 40 mg/kg.

Group 5. Propranolol injection first and 30 minutes later isoproterenol insult.

Group 6. Dobutamine infusion.

Group 7. Dobutamine infusion first and 30 minutes later isoproterenol insult.

Group 8. Isoproterenol insult first and 30 minutes later dobutamine infusion.

Group 9. Isoproterenol insult first and 15 minutes later dobutamine infusion.

Group 10. Isoproterenol insult first and immediately after that dobutamine infusion.

Groups 6-10 needed surgery for dobutamine infusion. After the surgery and infusion of the drug the wounds were sutured and the animals returned to their cages for 18 hours. At the end of 18 hours, blood samples were collected under ether anesthesia.

D. BLOOD PRESSURE AND ELECTROCARDIOGRAM

Animals were anesthetized with sodium pentobarbital injection intraperitoneally at a dose of 40 mg/kg. To record the blood pressure a catheter was introduced into the common carotid artery. A statham pressure transducer model number P23 Dc was used to monitor the blood pressure. This was connected to a Low Level DC preamplifier unit of the Grass model polygraph. Before recording the blood pressure, the unit was calibrated with a mercury manometer; 0.5 ml of sodium heparin (1000 units/ml) was used in the catheter and pressure transducer head to avoid clotting of blood in the catheter. Chart speed used for recording the blood pressure was 5 mm/second. Systolic, diastolic and mean blood pressures were recorded.

The electrocardiogram was recorded with a Grass Model 5D polygraph. The limb leads from the right foreleg, left foreleg and left leg of the anesthetized animal were connected to the D.C. Driver amplifier of the polygraph. Lead II electrocardiograms were recorded on the graph paper where the chart speed was set at 100 mm/sec. Heart rate was recorded as beats/minute.

E. MEASUREMENT OF ENZYME ACTIVITY

Enzyme activity was measured with the creatine phosphokinase and lactate dehydrogenase assay kits provided by Boehringer Mannheim of Canada Limited, Montreal.

The numbered vials containing the serum samples kept at -70^{o}C were taken out and brought to room temperature. 100 ul of the sample

was mixed with the reagents to measure the rate of absorbence. Beckman DB-G Grating Spectrophotometer was used and the rate of absorbence was measured at the ultraviolet wavelength of 340 nm. Cuvettes used had 1 cm light path and measurement was against air at room temperature. Creatine phosphokinase activity in the sample was calculated as UI at ($25^{o}C$) = 4127 x A_{340}/min and lactate dehydrogenase activity in the sample was calculated as UI at ($25^{o}C$) = 4921 x A_{340}/min.

F. LIGHT AND ELECTRON MICROSCOPY

Selected pieces of tissues, from the left ventricle of perfusion fixed hearts, were taken from an area between the apex and base of the heart, about 3 mm above the apex. Every precaution was taken to select the pieces of tissues consistently from the same areas of each heart. The 1 mm thick sections were divided into small pieces under a dissection microscope, and further fixed in fresh Karnovsky's fixative followed by washing in Millonig's phosphate buffer and final fixation in buffered osmium tetroxide fixative. After dehydration the tissues were embedded in araldite. Sectioning was done on a Reichert ultramicrotome with glass knives and half micron thick sections were stained with toluidine blue and observed under a light microscope. This was to localize the lesioned areas. Thin sections from the lesioned areas were cut and after being collected on 300 mesh copper grids, were stained with uranyl acetate and lead citrate for electron microscopy. A Philips 300 electron microscope was used to observe the fine structure of myocardium.

G. STATISTICAL ANALYSIS

Statistical analysis was accomplished by Bartlett's homogeneity of variance test or multiple comparison by Duncan's test using PDT 11 computer. If the P value was less than 0.05, it was considered to reflect a significant difference.

IV

OBSERVATIONS AND RESULTS

CHAPTER

IV OBSERVATIONS AND RESULTS

A. MORPHOLOGY OF THE NORMAL MYOCARDIUM

B. ELECTRON MICROSCOPY

a) Ultrastructure of the ischemic myocardium.

b) Ultrastructural changes in the ischemic myocardium after drug administration.

C. HEMODYNAMICS OF:-

a) Normal

b) Ischemic Animals.

c) Treated

D. BIOCHEMICAL RESULTS

A. MORPHOLOGY OF THE NORMAL MYOCARDIUM (FIGS. 1-7)

The cytoplasm of the cardiac muscle cell, known as the sarcoplasm, contains nucleus, mitochondria, sarcomeres, glycogen granules, Golgi complex, lipid droplets, lysosomes etc. (Fig. 1). The sarcoplasm is continuous with the extracellular space or interstitium by way of the transverse tubular system (Fig. 2). Since the ventricular cells of the myocardium are primarily of the contractile type, the sarcoplasm is packed with the contractile elements namely the myofilaments (actin and myosin) (Fig. 7). Numerous glycogen granules which are present in the sarcoplasm serve as an emergency source of metabolic fuel (Figs. 1-6).

The lateral boundaries of a myocardial cell are related to the extensive extracellular space which separates the parallelly oriented muscle fibers. Each of these fibers is formed by the end to end junction of a series of single myocardial cells. At these functions, the cells are closely attached to each other to form the intercalated discs (Fig. 6).

Ultrastructurally, the surface membrane on the side of the cell is seen to be composed of an osmiophilic lamina which separates it from an outer amorphous layer which is less dense and has no sharp boundaries (Fig. 2). The trilaminar structure of the plasma membrane (sarcolemma), usually seen after permanganate fixation (Robertson, 1960) was not observed in myocardial tissues fixed in osmium tetroxide fixative. The osmiophilic lamina represents the inner dense layer of

the three components of the sarcolemma. So the sarcolemma of osmium tetroxide fixed myocardial tissue consists of a plasma membrane, an intervening gap and the basement membrane (Fig. 2).

At the intercalated disc, the basement membrane passes without interruption or invagination onto the surface of the adjacent cell (Fig. 6). In this figure, the plasma membrane can be seen turning inwards to traverse the fiber with a clear space between the opposed membranes. The intercalated disc crosses the myofibrils at the Z-band level. The section of the intercalated disc with its membranes running parallel to the myofibrils is known as the lateral portion, which is devoid of electron dense bodies. The other portion of the disc which runs tranversely across the fibril, known as the transverse portion, has three different types of specialized regions viz: the desmosomes (macula adherens), tight junctions (fasciae adherentes) and gap junctions (nexuses), which can be observed in figure 6. Fascia adherens, also called the intermediate junction, is the predominant component of the transverse portion (segment) of the disc. The thin myofilaments of the I-band of the sarcomere enter the filamentous mat at the fascia adherens and serve to attach the myofilaments very strongly to the plasma membrane of the intercalated disc (Fig. 6). The desmosomes, which are believed to be the sites of attachment for the cytoplasmic filaments, the tonofilaments, and nexuses, thought to be sites of low electrical resistance, have also been observed.

Between the myofibrils, the mitochondria are arranged in rows along the long axis of the fibrils (Fig. 2) on either side of the

Z-bands. They measure from 0.3 to 1.7 microns in length and from 0.2 to 1 micron in width. One to three rows of mitochondria have been observed in the micrographs beneath the sarcolemma (Fig. 4). The size and shape of the mitochondria vary greatly depending upon the functional state. Lipid droplets are located contiguous to the mitochondria.

The sarcoplasmic reticulum, which surrounds the sarcomeres at the level of the Z-bands, has also been observed between the myofibrils (Fig. 3). Blood capillaries have been observed in the interstitial space (Fig. 5). In this micrograph a number of pinocytotic vesicles are also seen.

The nucleus is situated centrally deep in the myocardial cell. The nuclear chromatin is well dispersed (euchromatic) within the nucleoplasm and stains lightly with the heavy metals like uranium and lead (Fig. 4). In this micrograph the presence of two nuclei of two adjacent myocardial cells have been observed. In one of the nuclei, because of the sectioning, a nucleolus has also been observed. A prominant nucleolus indicates the functional state of the myocardial cell. The nucleus is enclosed by a flattened membraneous saccule, the nuclear envelope (Fig. 4), which is very smooth in appearance. The nuclear envelope has two membrane profiles which are separated by a narrow space, the perinuclear cisterna, and may be seen in subsequent micrographs. At the nuclear pole in the sarcoplasm is an array of membrane bound saccules and vesicles which constitute the Golgi complex (Fig. 11). The functional significance of the Golgi complex in adult myocardium has not been understood clearly.

One of the significant observations is the presence of numerous glycogen granules in the sarcoplasm of myocardial cells (Figs. 1,2,3,4 and 5). Since they are dispersed in the sarcoplasm and have been observed as small dense granules, they are believed to be the beta-type particles. Another feature is the presence of the glycogen granules in large quantities near the nuclear pole, in the clefts of sarcoplasmic reticulum, and between and among myofibrils (Figs. 1 to 6). Lipid droplets, spherical in shape, have also been frequently observed in close association with the outer membrane of mitochondria. The significance of these lipid droplets has already been established, since they represent the store of triglycerides which might be used during acute exogenous substrate deprivation.

The functional subunit of cardiac muscle - sarcomere - can be observed in Fig. 7 with the classical bands which give the cardiac muscle a striated appearance. The anisotropic A band, isotropic I bands, H zone, and M line are seen in this micrograph. The I band is always bisected by a thin dense line, the Z line, and the region between the adjacent Z lines forms the sarcomere (Fig. 7). Depending upon the functional state of the myocardium the length of the sarcomere varies 3 microns when fully relaxed (Fig. 7) and 1.5 microns when fully contracted (Fig. 8).

The A and I bands are composed of an array of protein filaments, the myofilaments, which are arranged parallel to each other (Fig. 7). The A band consists of thick (myosin) and thin (actin) filaments and is dark. The I band consists of only thin filaments and

stains lightly. In cross section, each thick filament is surrounded by six thin filaments in a hexagonal pattern (Fig. 40).

ELECTRON MICROGRAPHS OF NORMAL, ISCHEMIC AND TREATED MYOCARDIUM

1. CONTROL GROUP (SALINE INJECTION)

FIGS: 1-7

FIG. 1. This low power electronmicrograph shows the sarcomeres in proper registry. Glycogen granules, intercellular space (arrows) and mitochondria are seen. Vacuole (V) is also seen.

Mag. 11, 036 X

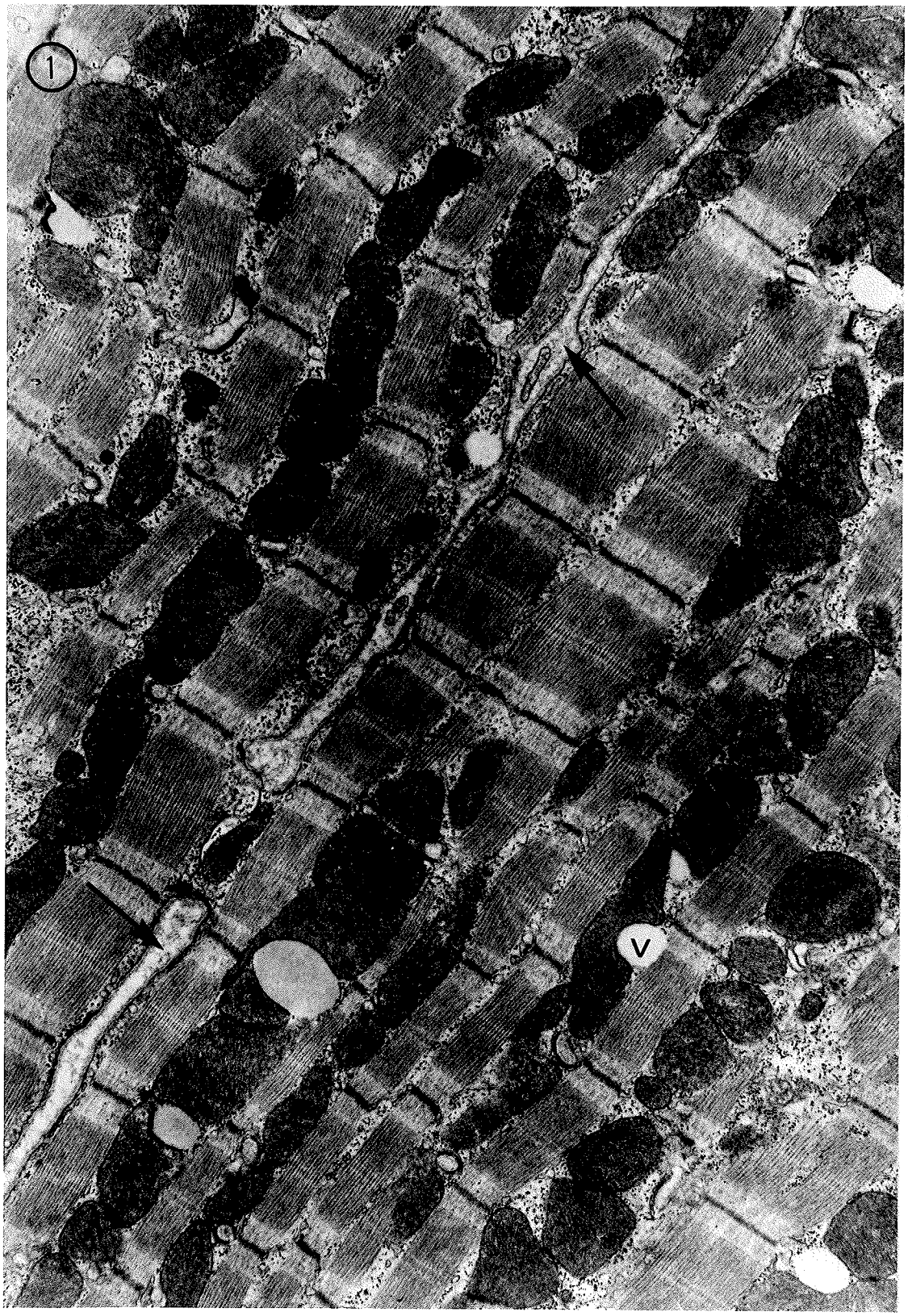

1
V

FIG. 2. This micrograph shows the fine structure of normal myocardium. Glycogen (arrowheads), Sarcomeres (S) and intercellular space (Is) are seen.

Mag. 19, 210 X

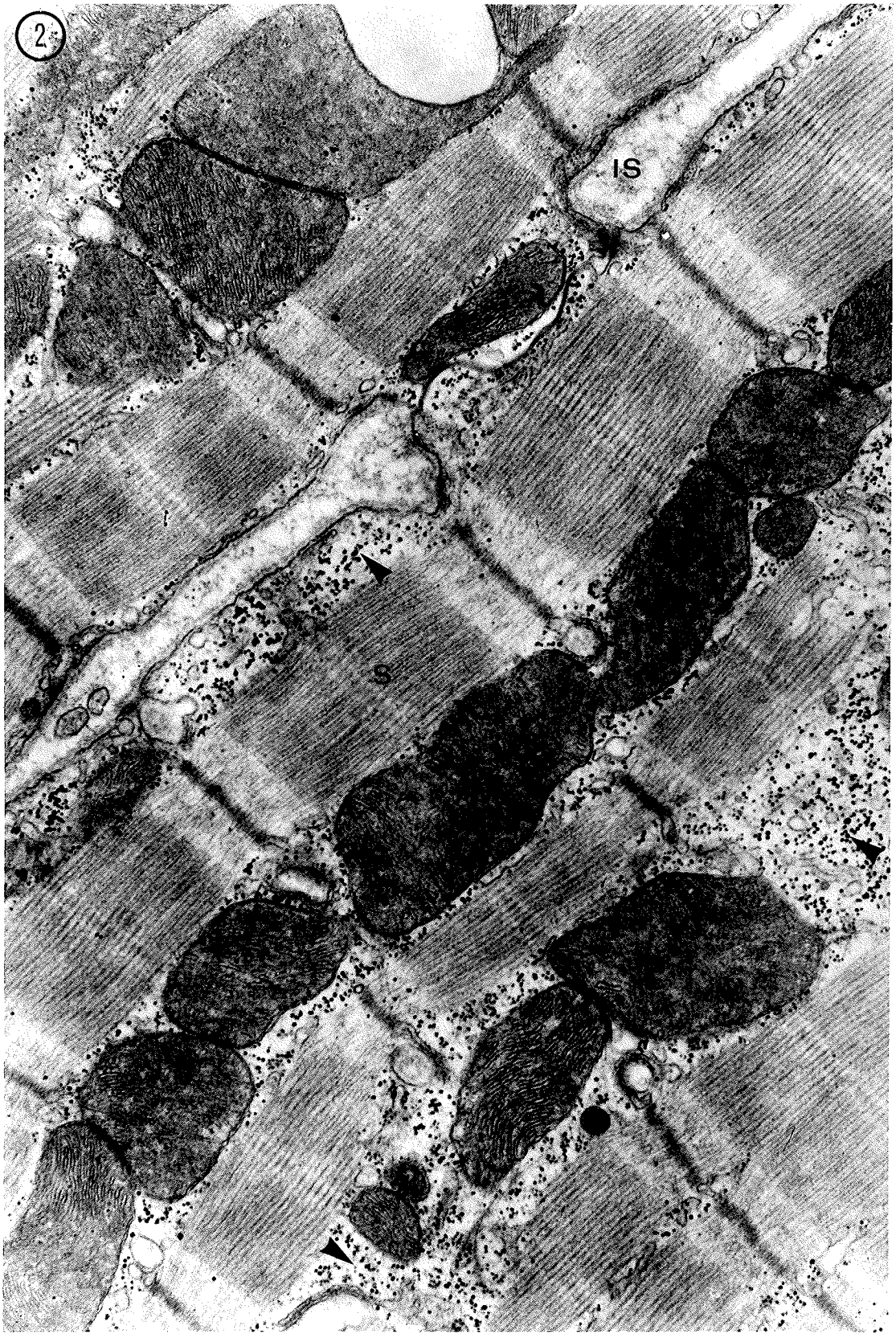

2
IS
S

FIG. 3. This micrograph shows the general registry. Observe the sarcomere (S), mitochondria (Mit) and Z line.

Mag. 9576 X

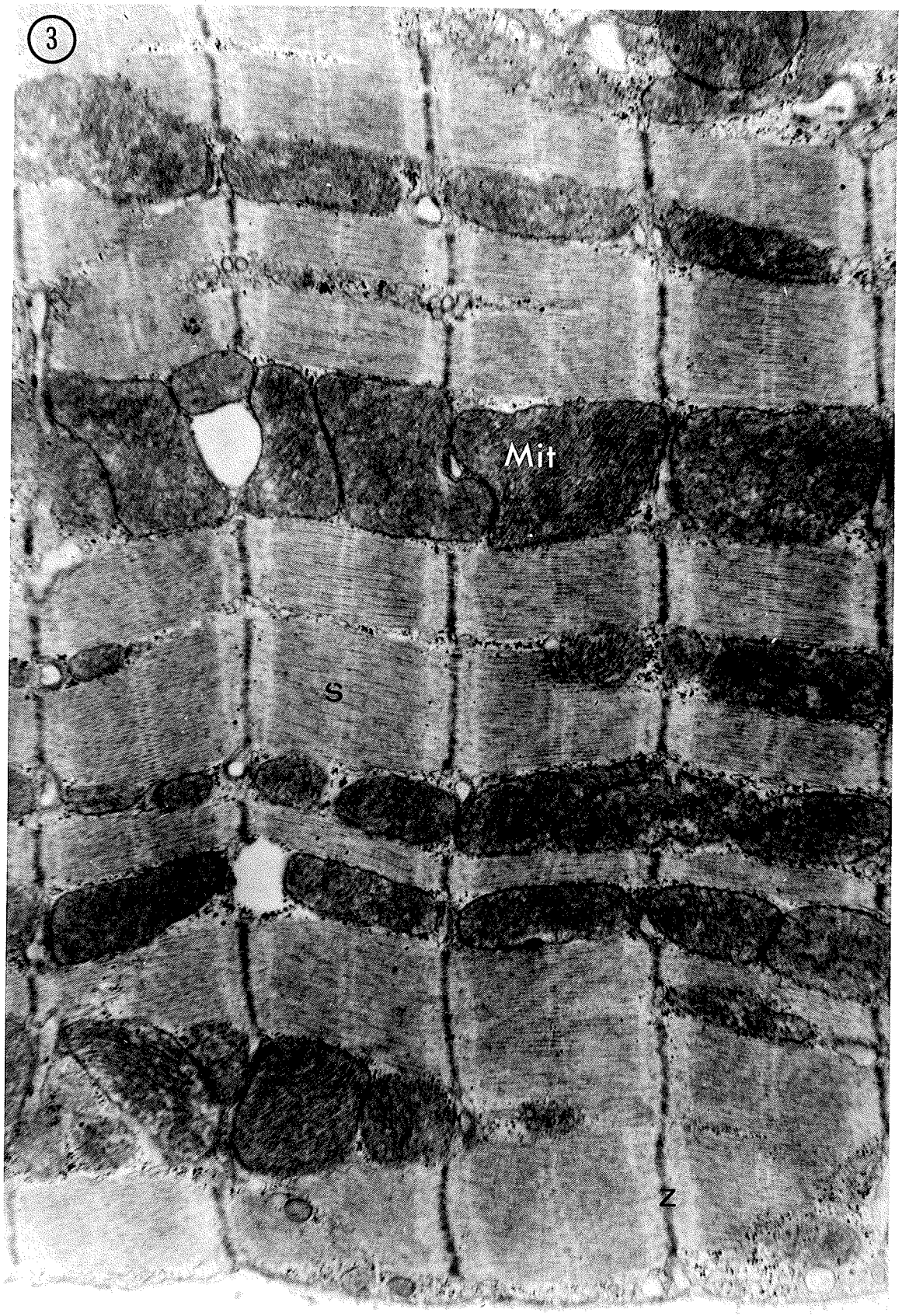

3
Mit
S
Z

FIG. 4. Two nuclei (N) of adjacent cells are seen in this micrograph. Nucleolus (Nu) is seen in one of the nuclei. Chromatin (Chr), Glycogen (Gly) and Sarcolemma (SL) are also seen.

Mag. 22, 732 X

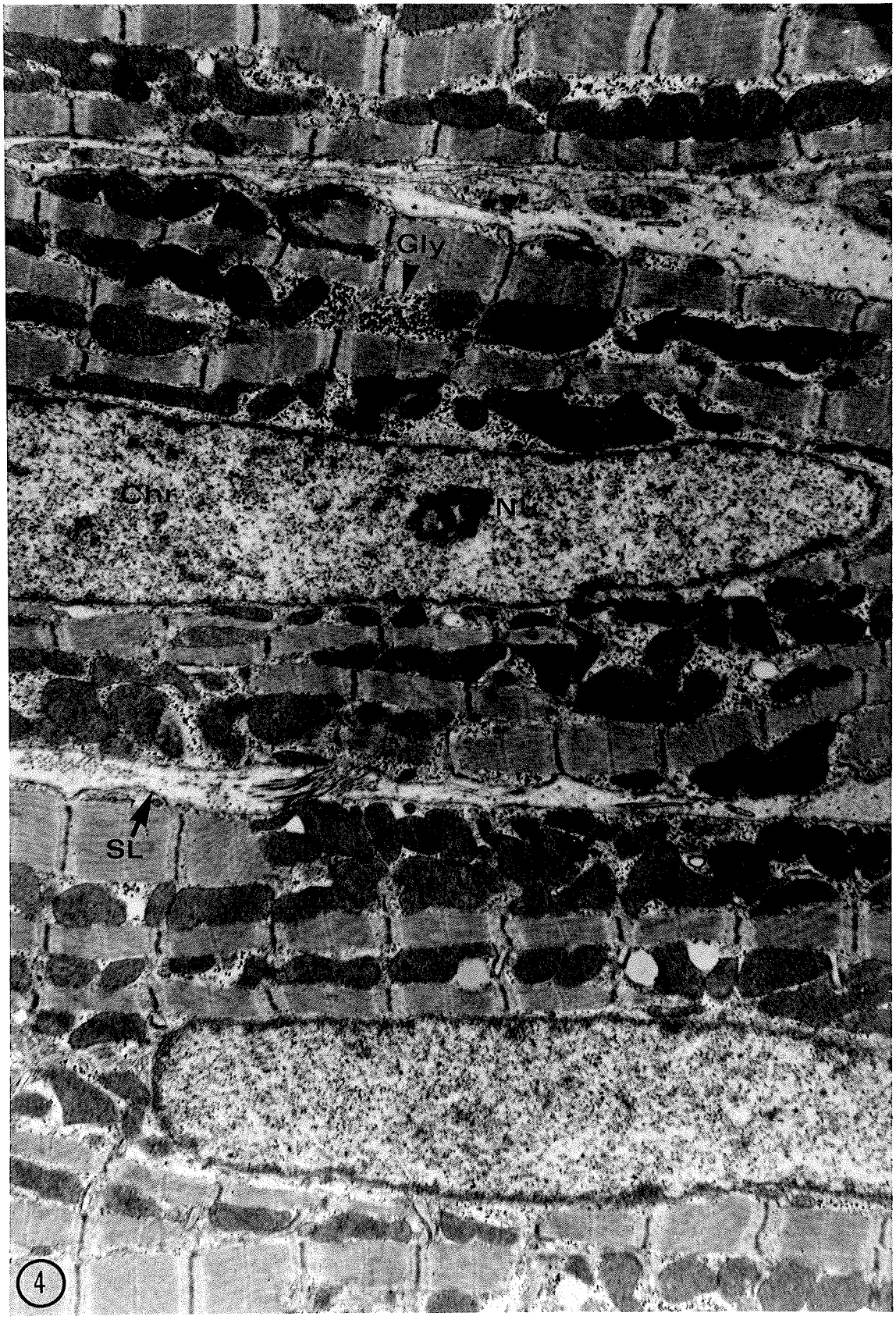

Gly
Chr
N
SL
4

FIG. 5. This micrograph shows general registry, with lots of glycogen granules. Endothelial nucleus (arrow) can be seen.

Mag. 19, 281 X

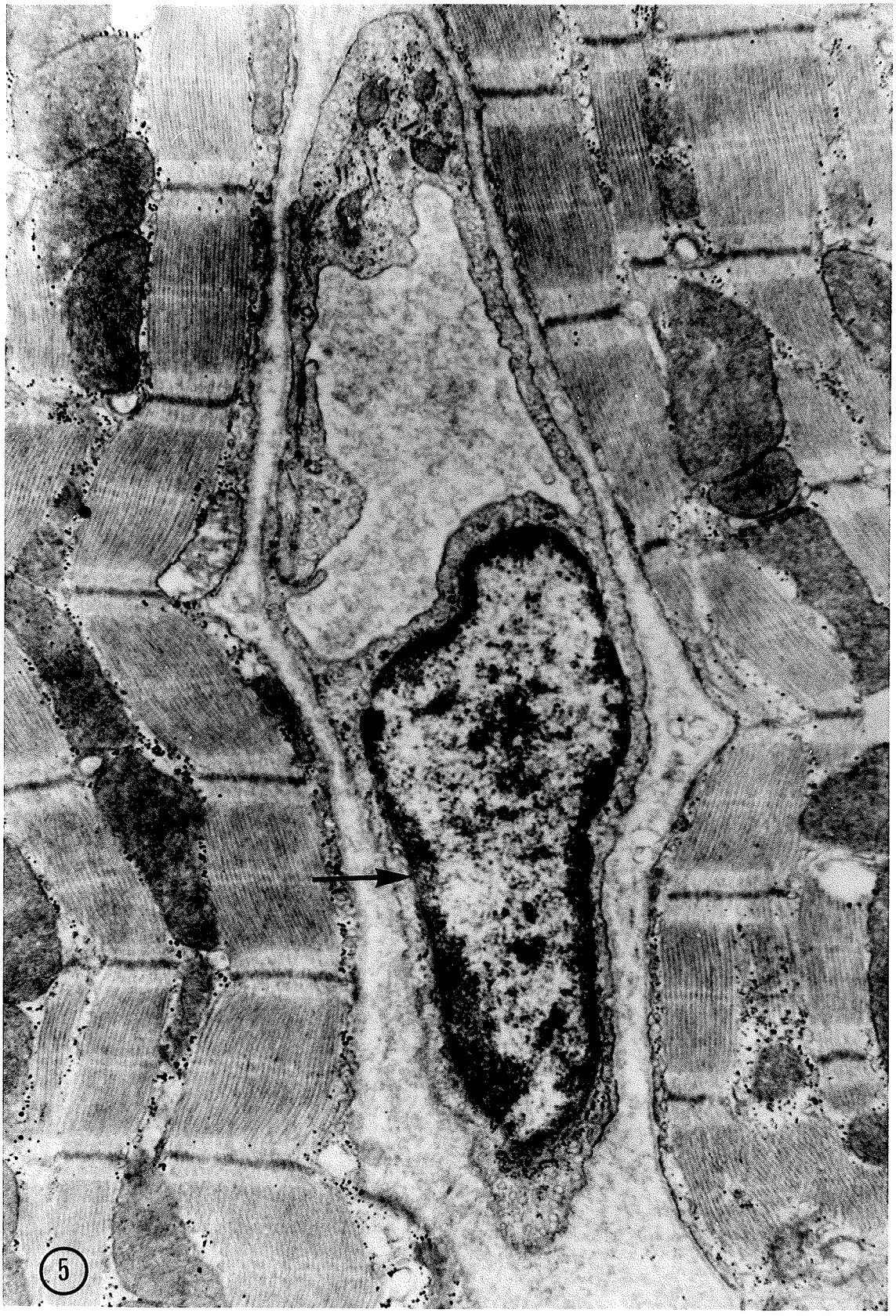

5

FIG. 6. This electronmicrograph shows the Intercalated disc (ID) and the attachment of myofilaments on to the tight junctions of the transverse portion of the disc. Glycogen granules, mitochondria and sarcomeres (S) are seen in this micrograph.

Mag. 28, 924 X

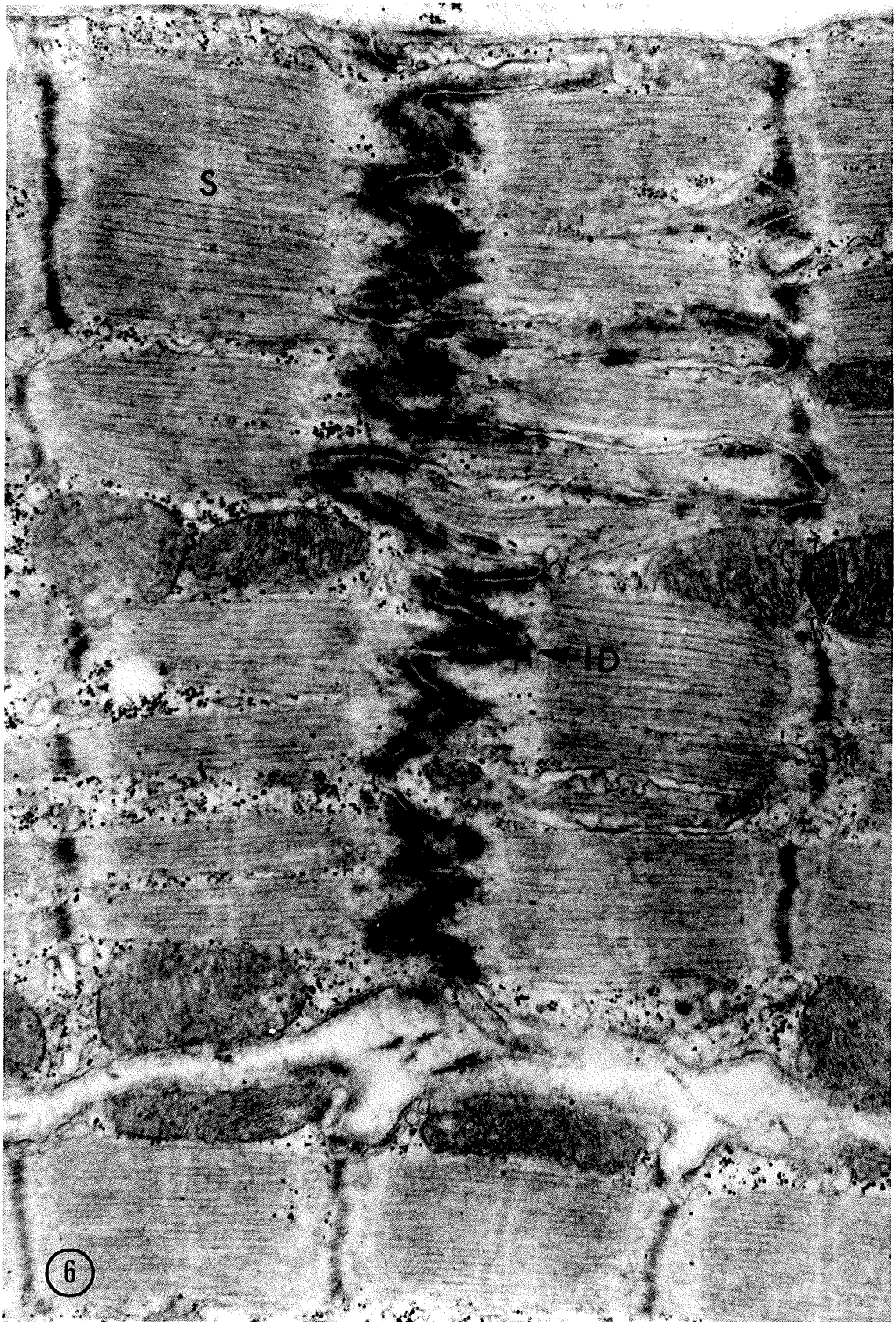
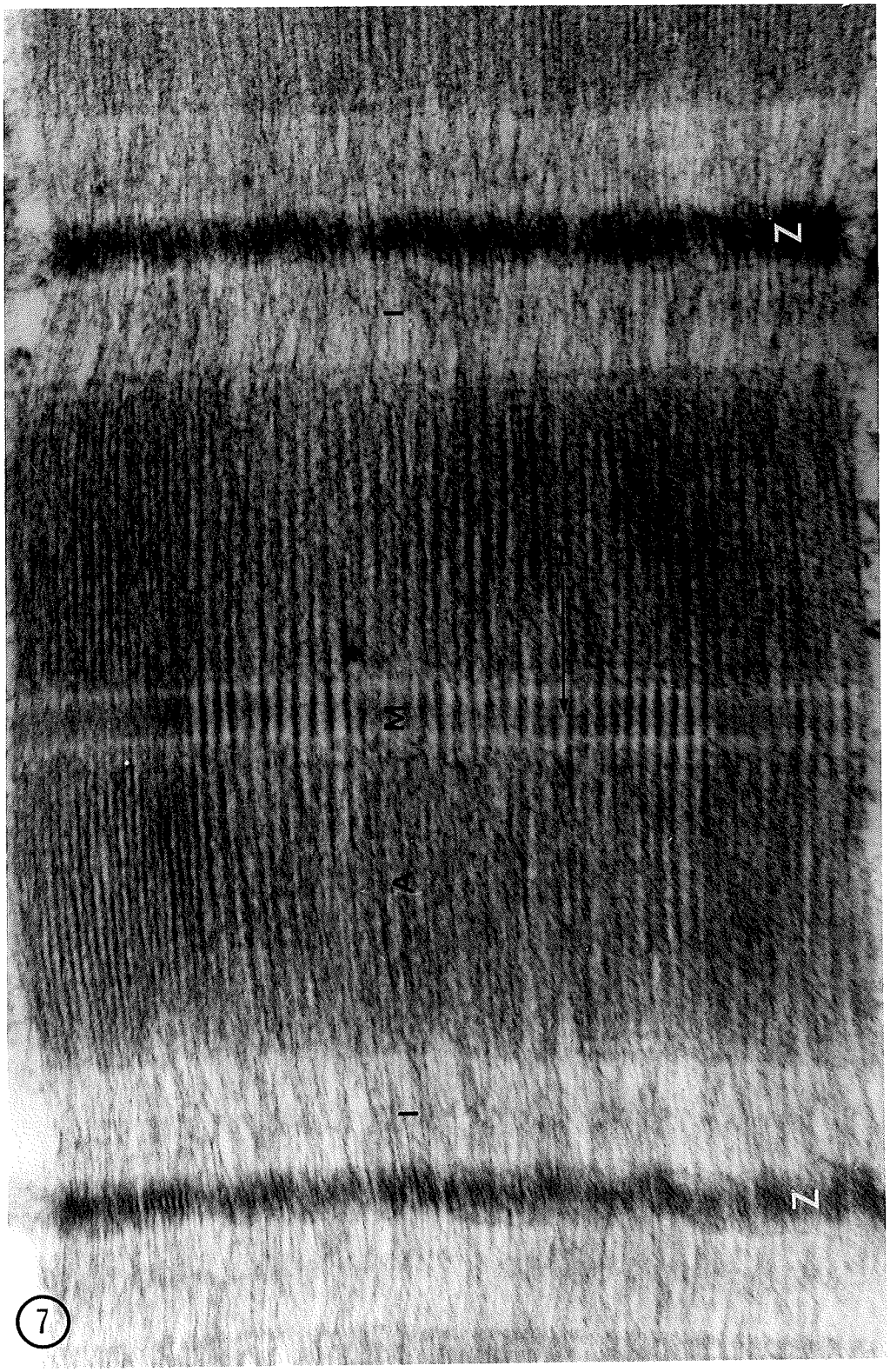

S
ID
6

FIG. 7. This is a typical sarcomere between the 2 Z lines. Notice the Z line bisecting the I band, which consists of thin filaments. A band consists of thick (myosin) and thin (actin) filaments. M line & H zone can also be seen in the centre of the A band.

Mag. 74, 706 X



(7)

B

ELECTRON MICROSCOPY

a) Ultrastructure of the ischemic myocardium

b) Ultrastructural changes observed in the ischemic myocardium after drug administration

a) ULTRASTRUCTURE OF THE ISCHEMIC MYOCARDIUM.

The ultrastructural changes and the extent of damage produced by experimentally induced ischemia have varied depending upon the duration of ischemia.

i) STRUCTURAL ALTERATIONS OBSERVED IN THE MYOCARDIAL CELL AFTER 15 MINUTES OF ISCHEMIA (Figs. 8 to 13) include: depletion of glycogen granules, scalloping of sarcolemma, disappearance of I band, swelling of sarcoplasmic reticulum (Figs. 8,9); vesicular Golgi complex, minor contraction bands or clumping of Z lines (Fig. 10); irregular shape of nuclear membrane with aggregation of chromatin material at the periphery of nucleoplasm (Fig. 11); degeneration of myofilaments (Figs. 12,13) and mitochondria with electron dense bodies (Fig. 13). These changes during the early phase of ischemia are reversible if the increased oxygen requirement of the myocardium is met. The percentage of mitochondria with electron dense bodies was 81.01% (Table I, Fig. 52).

ii) ISCHEMIA - 30 MINUTES (Figs. 14 to 19).

The structural abnormalities observed in the ischemic myocardium, where ischemia was for 30 minutes, are as follows:- irregular shape of the nucleus with accumulation of chromatin at the periphery (Fig. 14), myofibrillar degeneration (Figs. 15,16A), swollen sarcoplasmic reticulum (Figs. 16B,17) and mitochondria with altered definition of cristae (Fig. 17). The features peculiar to Fig. 18 are as follows: increased number of lipid droplets in close association

with the mitochondria, glycogen granules in the sarcoplasm, sarcomeres almost in proper registry, mitochondria without flocculent densities still arranged longitudinally along the long axis of myofibrils and nucleus with uniform distribution of chromatin even though there are nuclear indentations. These ultrastructural features observed in Fig. 18 are significant because usually we do not see such structural features unless we specifically isolate, process and observe tissues from the border (transition) zone.

Compared to the 15 minutes ischemia the extent of damage is significantly greater in this group where ischemia persisted for 30 minutes. The presence of electron dense bodies is a feature of irreversible ischemic injury (Fig. 19). The percentage of mitochondria with electron dense bodies was 84.28% (Table I, Fig. 52), which was not significantly different from the previous group. Though the number of mitochondria with electron dense bodies did not increase significantly, the number of dense bodies in the mitochondria increased as ischemia progressed.

iii) ISCHEMIA - 60 MINUTES (Figs. 20 to 25).

The ultrastructural features of the ischemic myocardium in rats where ischemia lasted for 60 minutes, showed irreversible changes and very extensive lesions. The structural alterations observed are: nuclear margination with chromatin aggregation at the periphery of nucleoplasm (Fig. 20), contraction bands (Fig. 21) and mitochondria with electron dense bodies, and electron dense longitudinal crystalline structures (Figs. 22). In addition to the structural alterations

observed in the above two experimental situations, platelet aggregation also was observed in the blood vessels (Fig. 23). The presence of contraction bands indicate the advanced stage of degeneration of the myofibrils (Fig. 24). In addition to the development of electron dense bodies in the mitochondria, there are also electron dense longitudinal crystalline structures (Fig. 25) which have not been observed in the mitochondria during the early stages of ischemia. Another feature observed was the presence of increased number of electron dense bodies in each mitochondrion, though the number of mitochondria with such bodies was not increased substantially.

iv) ISCHEMIA - 18 HOURS (Figs. 26 to 30).

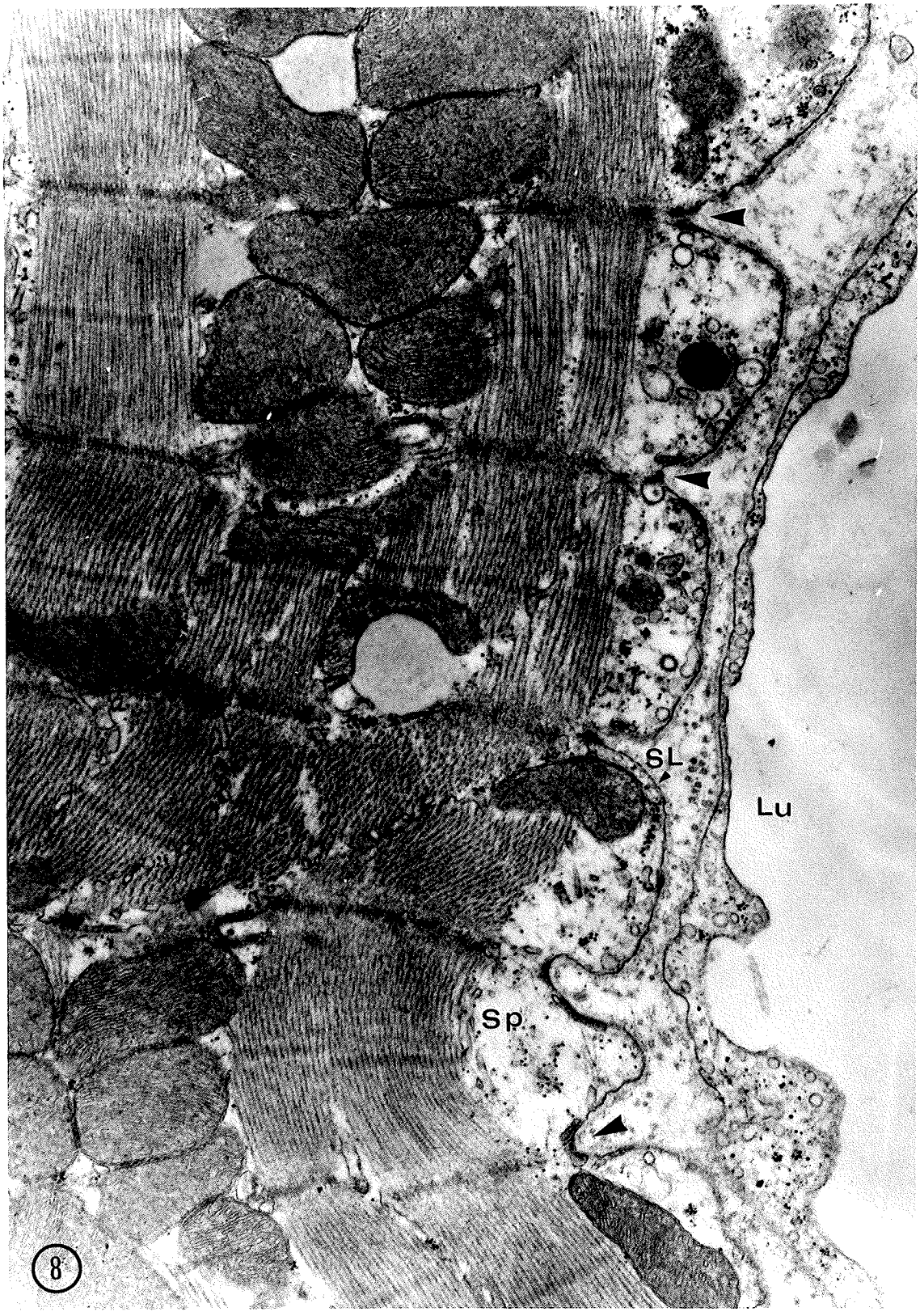
All the micrographs in this subsection show the advanced stage of lesion as a result of prolonged ischemia which has led to infarction. The morphological changes observed are: contraction bands and mitochondria with electron dense bodies (Fig. 26), numerous lipid droplets, vacuoles and degenerating mitochondria with few cristae (Fig. 27), presence of polymorphonuclear leukocytes in the severely damaged regions of the myocardium (Fig. 28), and deposition of numerous developing collagen fibrils (Fig. 29). The whole cytoarchitecture of the myocardium is disrupted (Fig. 30). These irreversible changes represent the "point of no return".

1. ISCHEMIA - 15 MINUTES

FIGS: 8-13

FIG. 8. In this micrograph, the arrowhead shows sarcolemmal scalloping. Lumen (Lu), Sarcolemma (Sl) and Sarcoplasm (Sp) can be seen. Notice the disappearance of I band.

Mag. 77, 769 X



8

FIG. 9. Here we can observe the swollen sarcoplasmic reticulum (SR), depletion of glycogen granules (Gly), vesicular Golgi complex, and mitochondria (Mit).

Mag. 58, 286 X

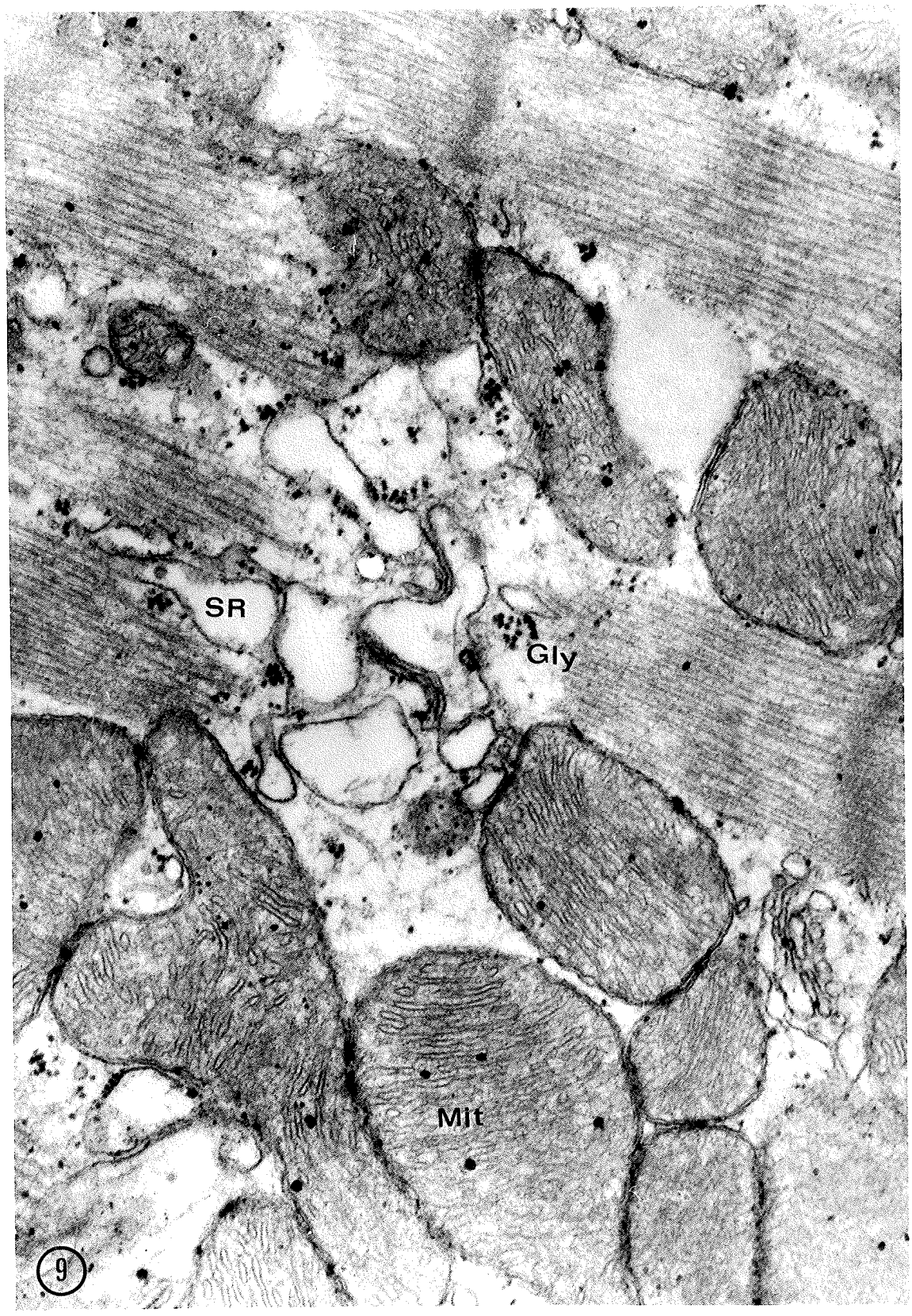

SR
Gly
Mit
9

FIG. 10. In this electronmicrograph we can see minor contraction bands (Caulfield-Klionsky type) where Z bands are wide. Arrow shows Pinocytic vesicles (PV), Mitochondria (Mit) and Lipid (L) droplets.

Mag. 73, 130 X

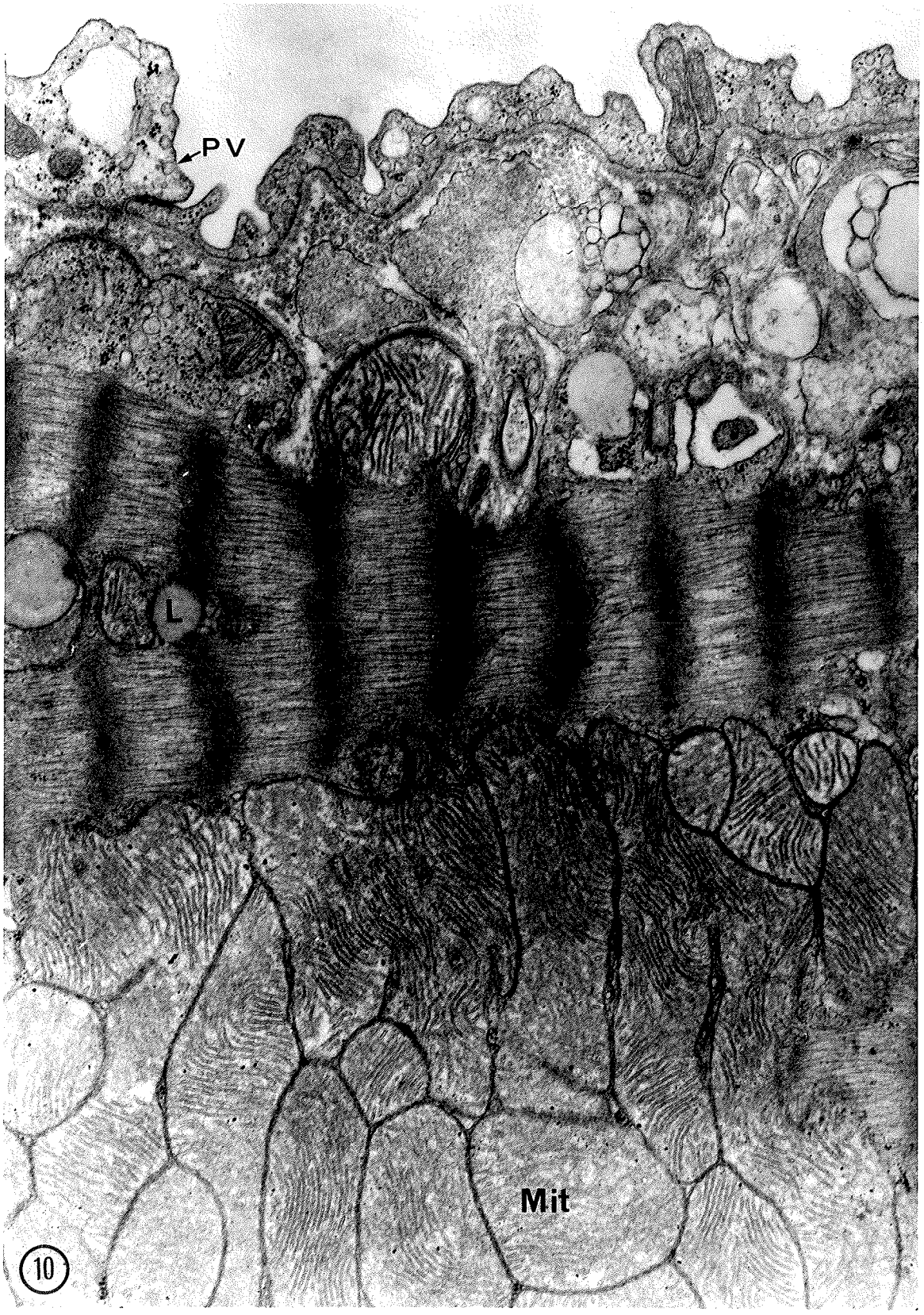

PV
L
Mit
10

FIG. 11. This electromicrograph shows the irregular shape of the nucleus (N) with chromatin (Chr) aggregation at the periphery. Some sarcomeres are contracted. Golgi complex (G) is seen at the nuclear pole.

Mag. 16, 996 X

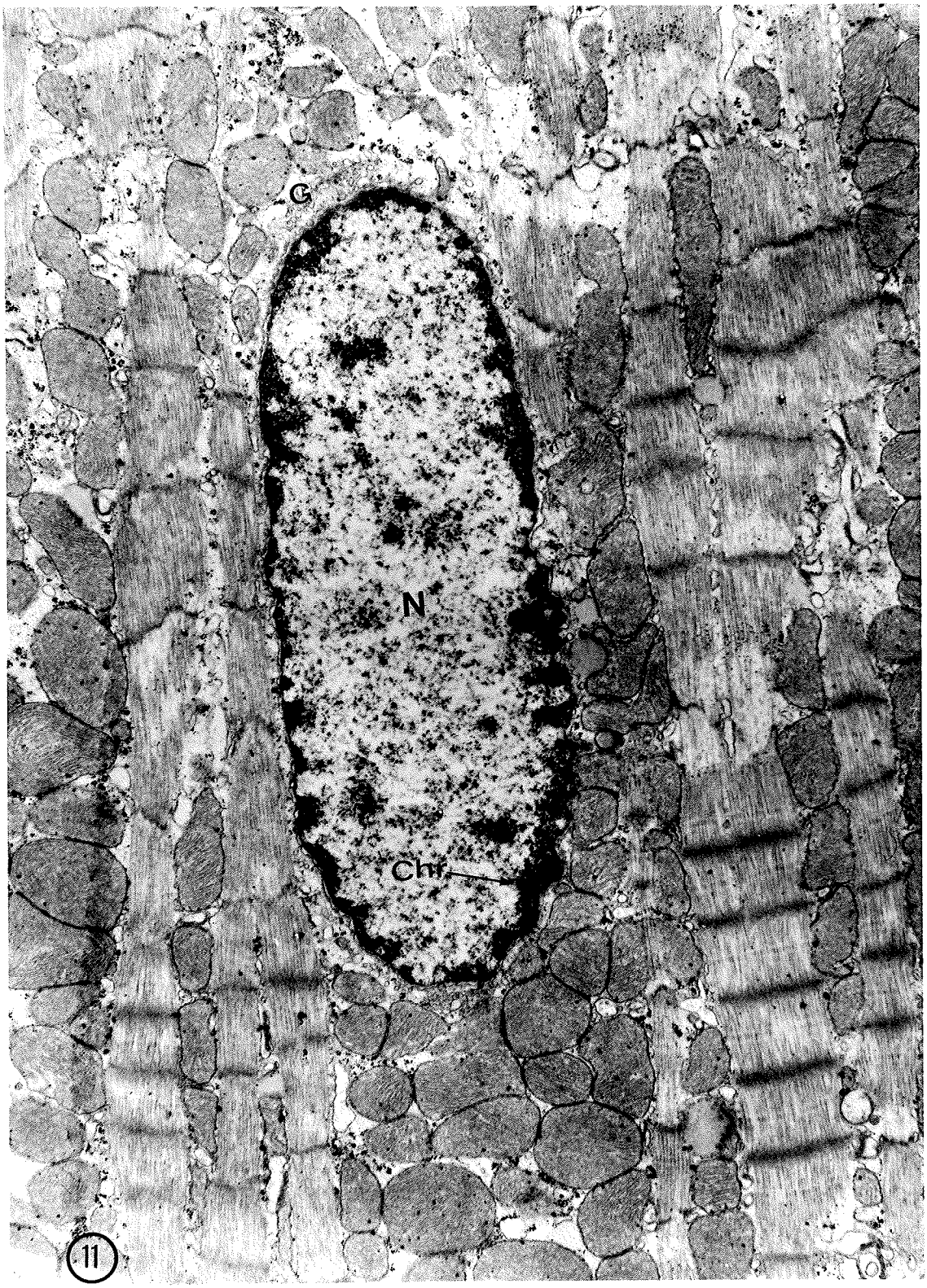
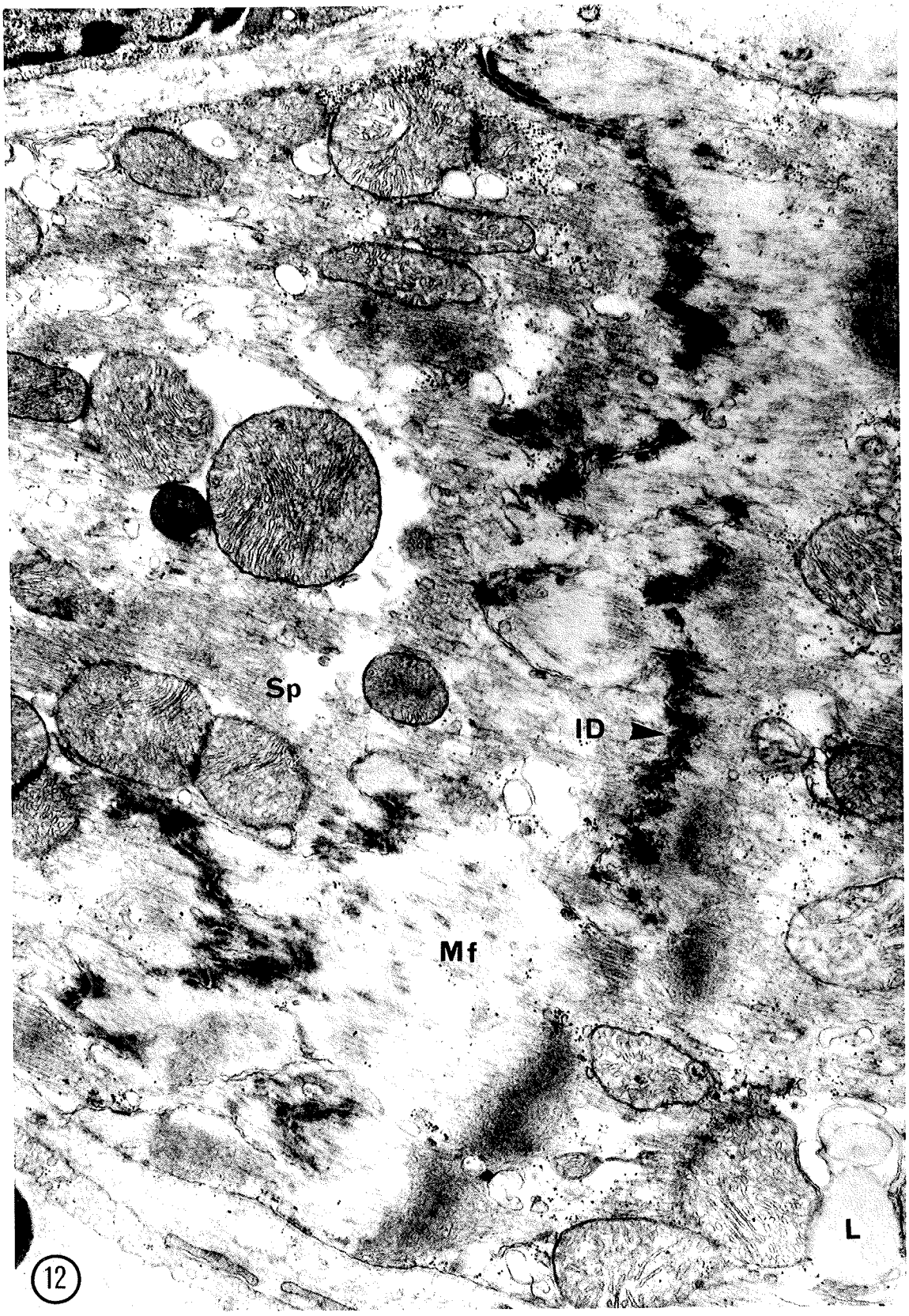

C
N
Chr
11

FIG. 12. This micrograph shows the Intercalated Disc (ID) with detached myofilaments (Mf). Some mitochondria with disrupted cristae are also observed. Lipid droplets (L) are seen as well.

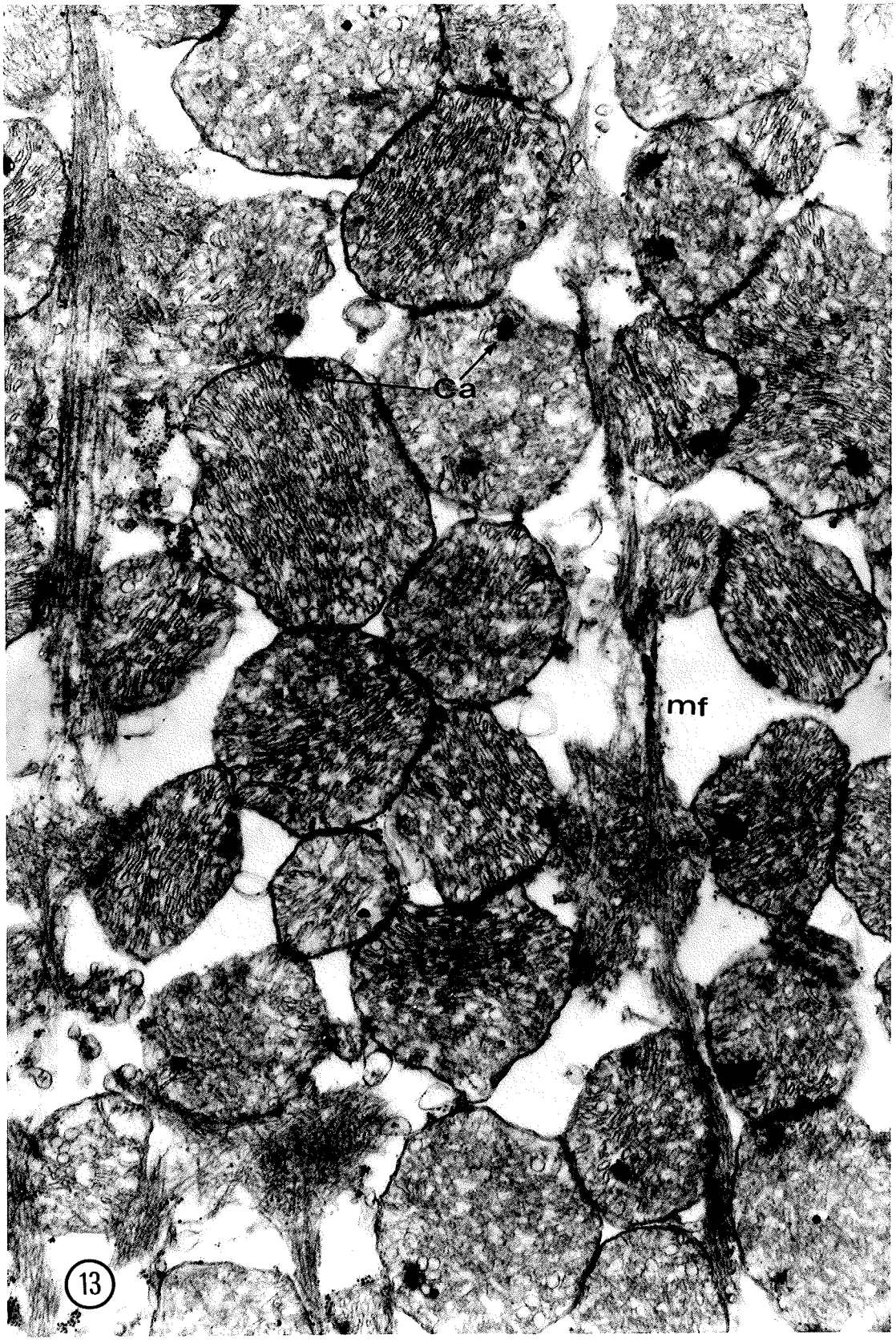
Mag. 21, 830 X



12

FIG. 13. This micrograph shows the electron dense bodies in the mitochondria. Myofilaments (mf) in the process of degeneration are seen among the mitochondria. The electron dense bodies are believed to be Calcium (Ca) deposits.

Mag. 27, 942 X


Ca
mf
13

2. ISCHEMIA - 30 MINUTES

FIGS: 14-19

FIG. 14. In this micrograph, vacuoles (V), Chromatin (Chr), Nucleus (N) and Sarcomere (S) can be observed. Nuclear margination with chromatin aggregation at the periphery of nucleoplasm are observed. Some sarcomeres show contracture. Note the depletion of glycogen granules.

Mag. 13, 970 X

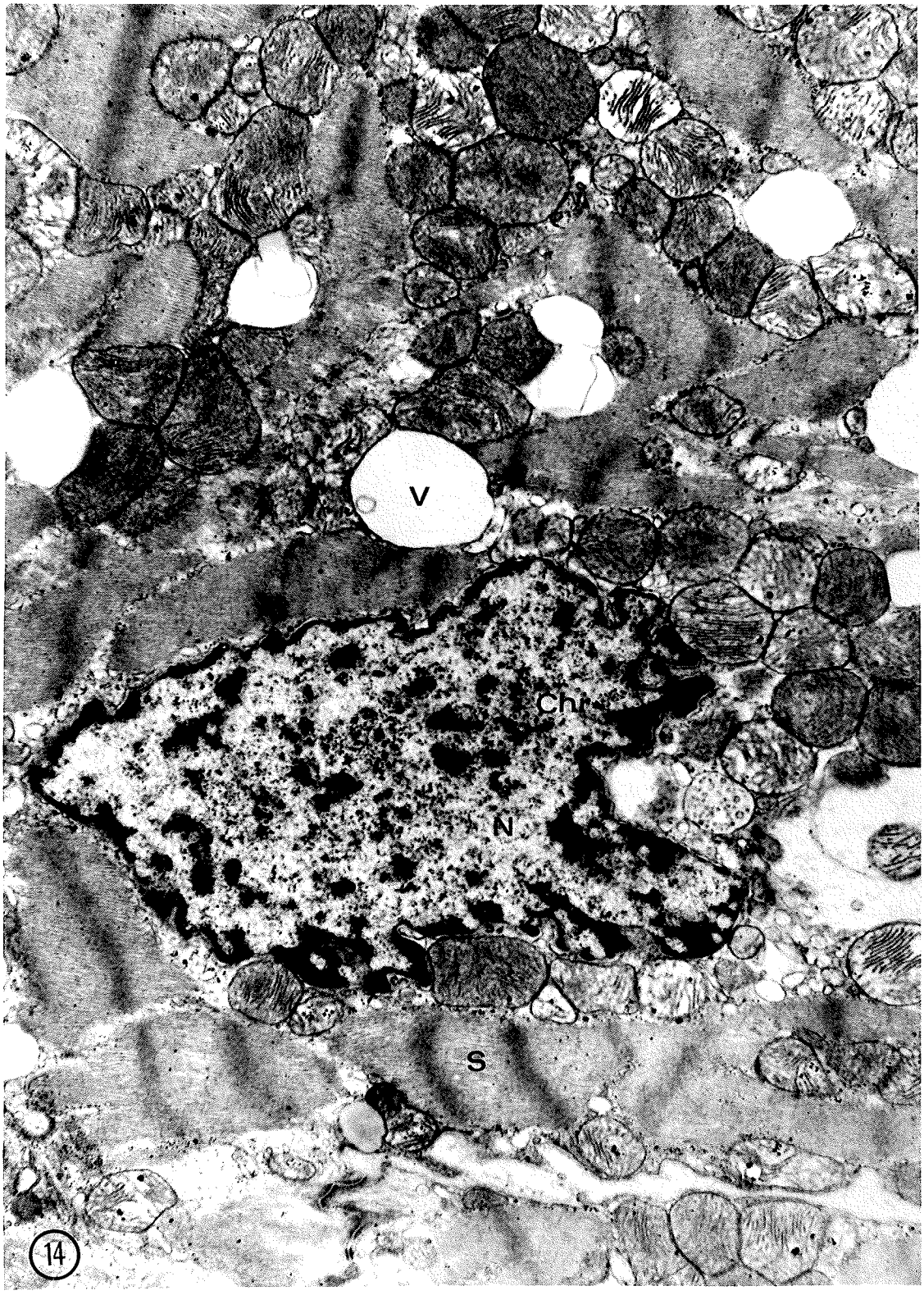

V
Chl
N
S
14

FIG. 15. This micrograph shows myofibrillar degeneration (arrow heads). Sarcoplasm (Sp) Z-line (widened) can be observed.

Mag. 58, 940 X

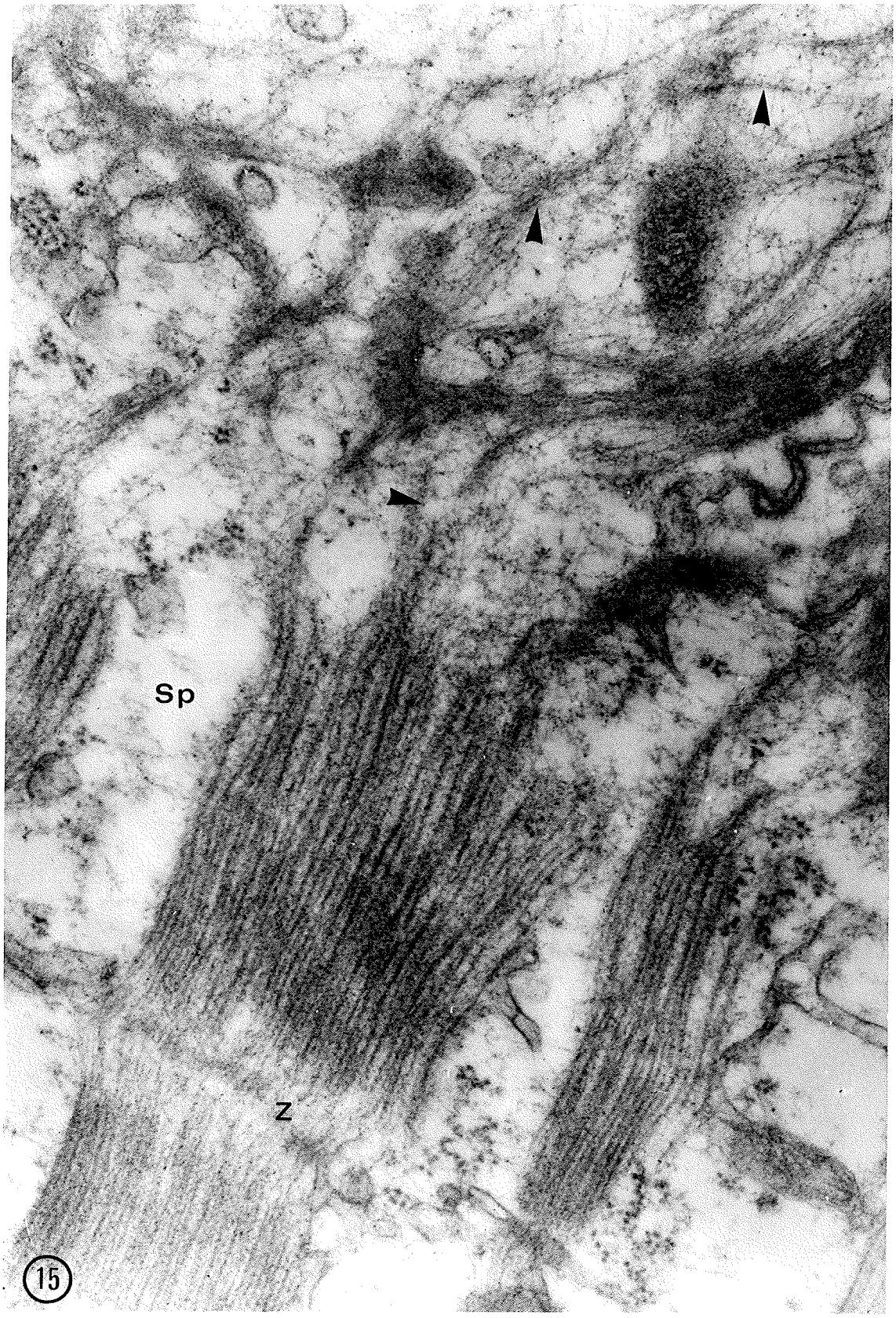
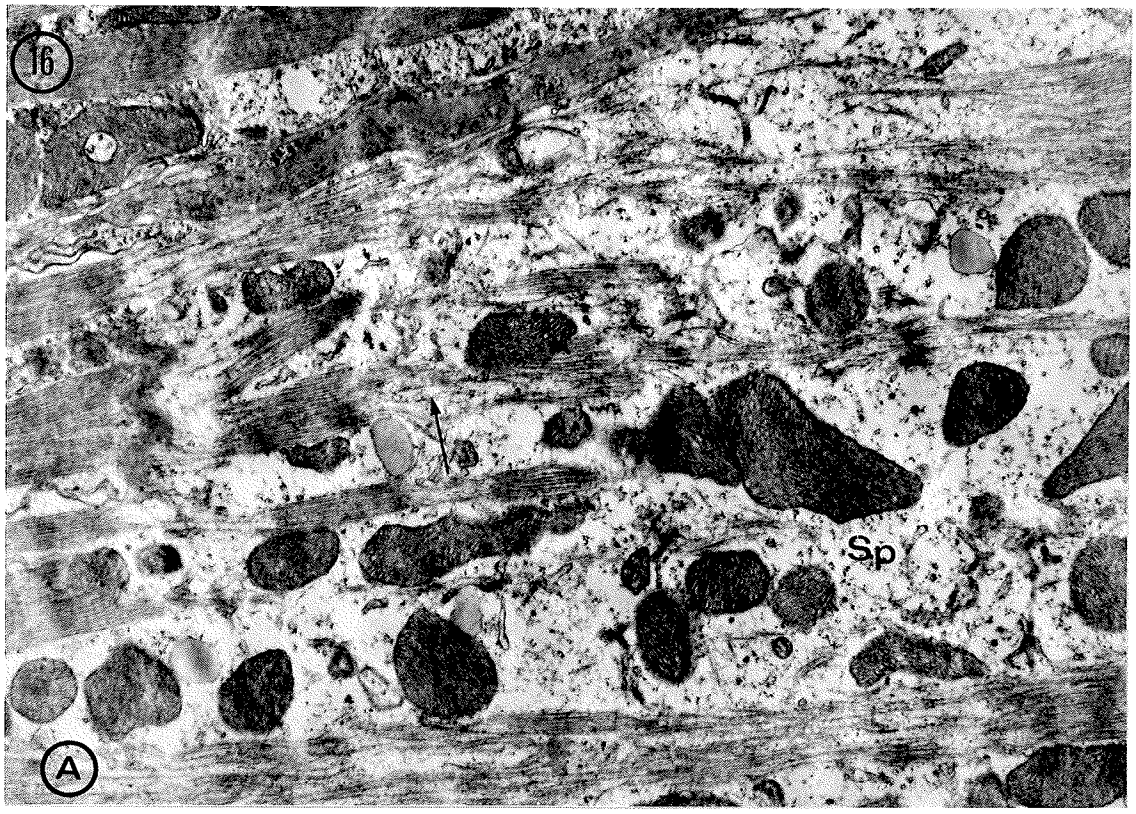

Sp
Z
15

FIG. 16. A. In this micrograph the arrow shows damaged filaments. The cardiac cell shows edema of the sarcoplasm (Sp) as well.

Mag. 12, 612 X

B. This half plate shows swollen sarcoplasmic reticulum in the sarcoplasm (Sp). Arrow shows damaged filaments which are degenerating.

Mag. 25, 650 X


16
Sp
A

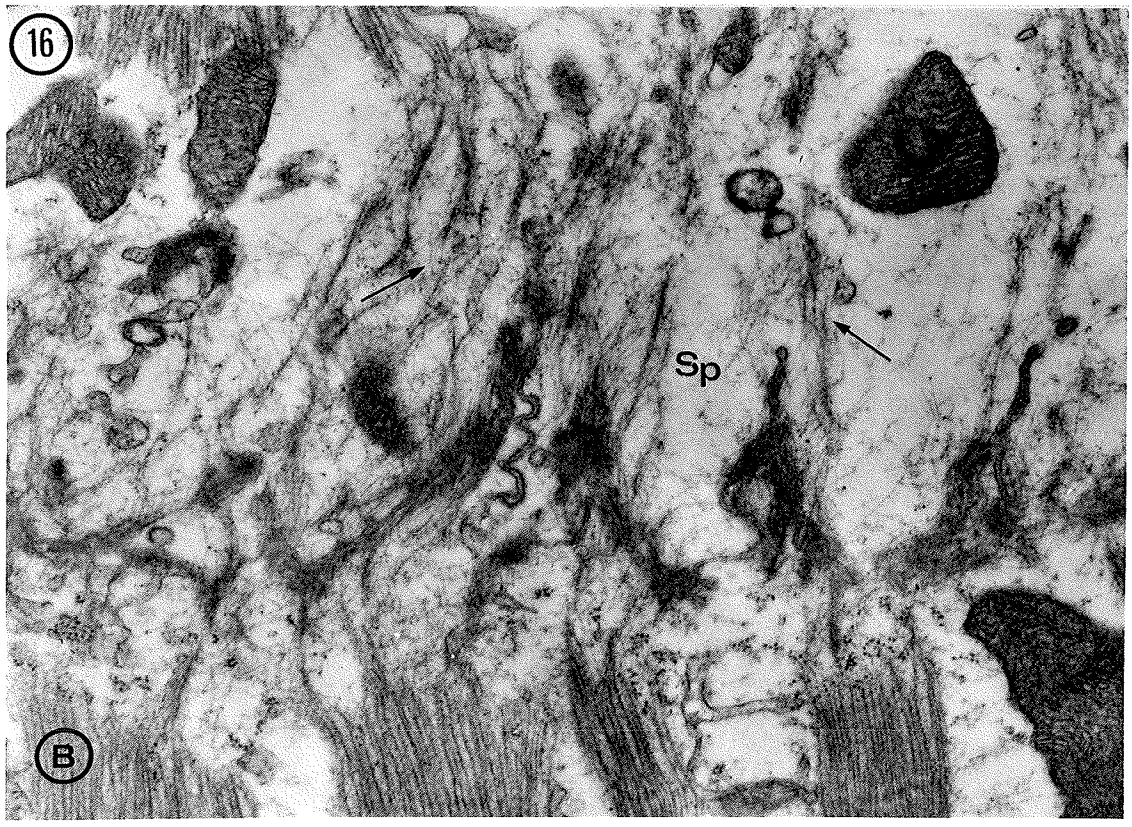

16
Sp
B

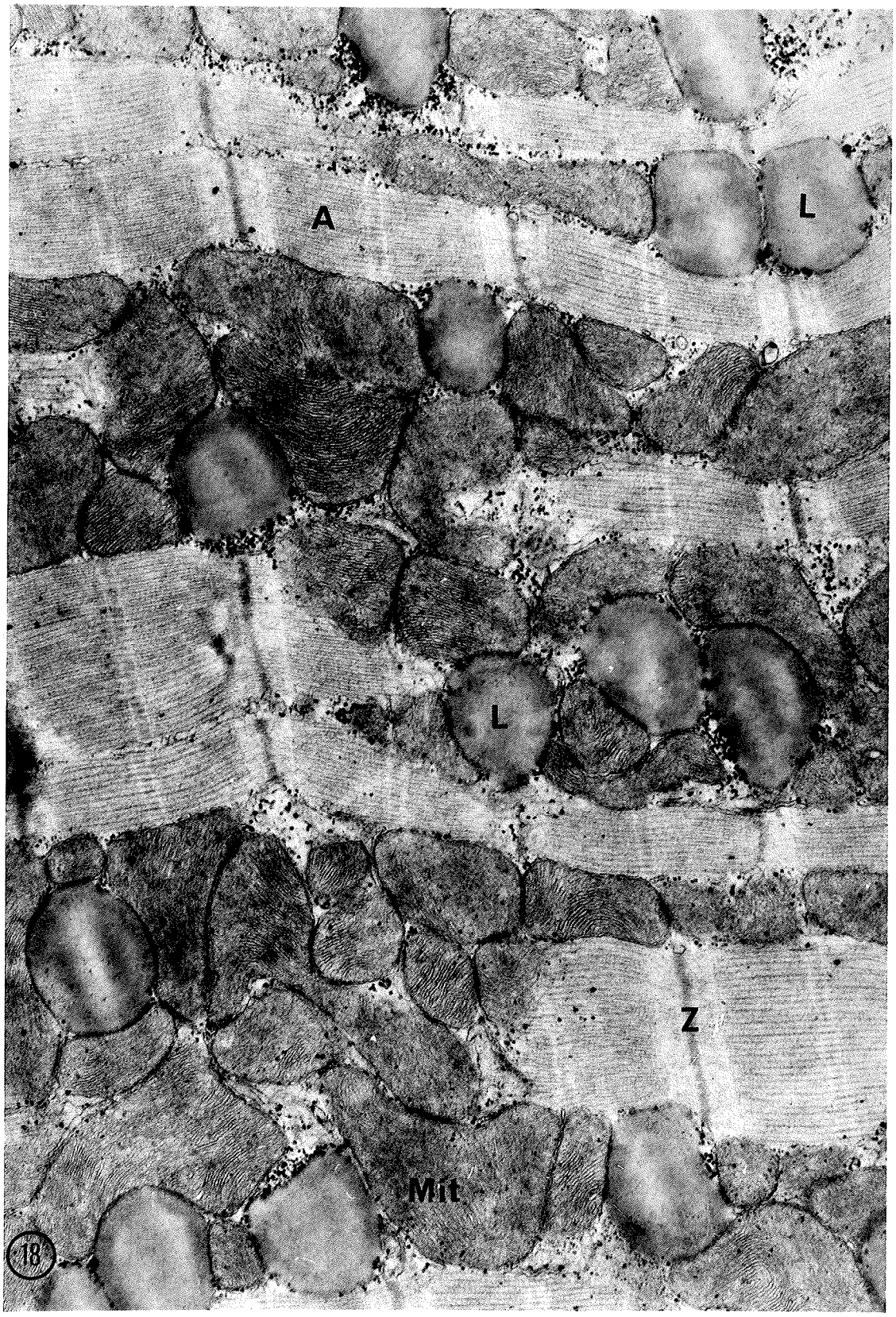
FIG. 17. In this micrograph, the arrowheads point to the swollen sarcoplasmic reticulum (SR). The arrows point the mitochondria with disrupted cristae.

Mag. 31, 107 X


SR
17

FIG. 18. Here in this electronmicrograph, there are large numbers of lipid droplets which show the impaired lipid metabolism (L). A band and Z lines can be observed. This micrograph is from the border zone because glycogen is still present in the myocardium. The lipid droplets (L) are found in close association with mitochondria (Mit).

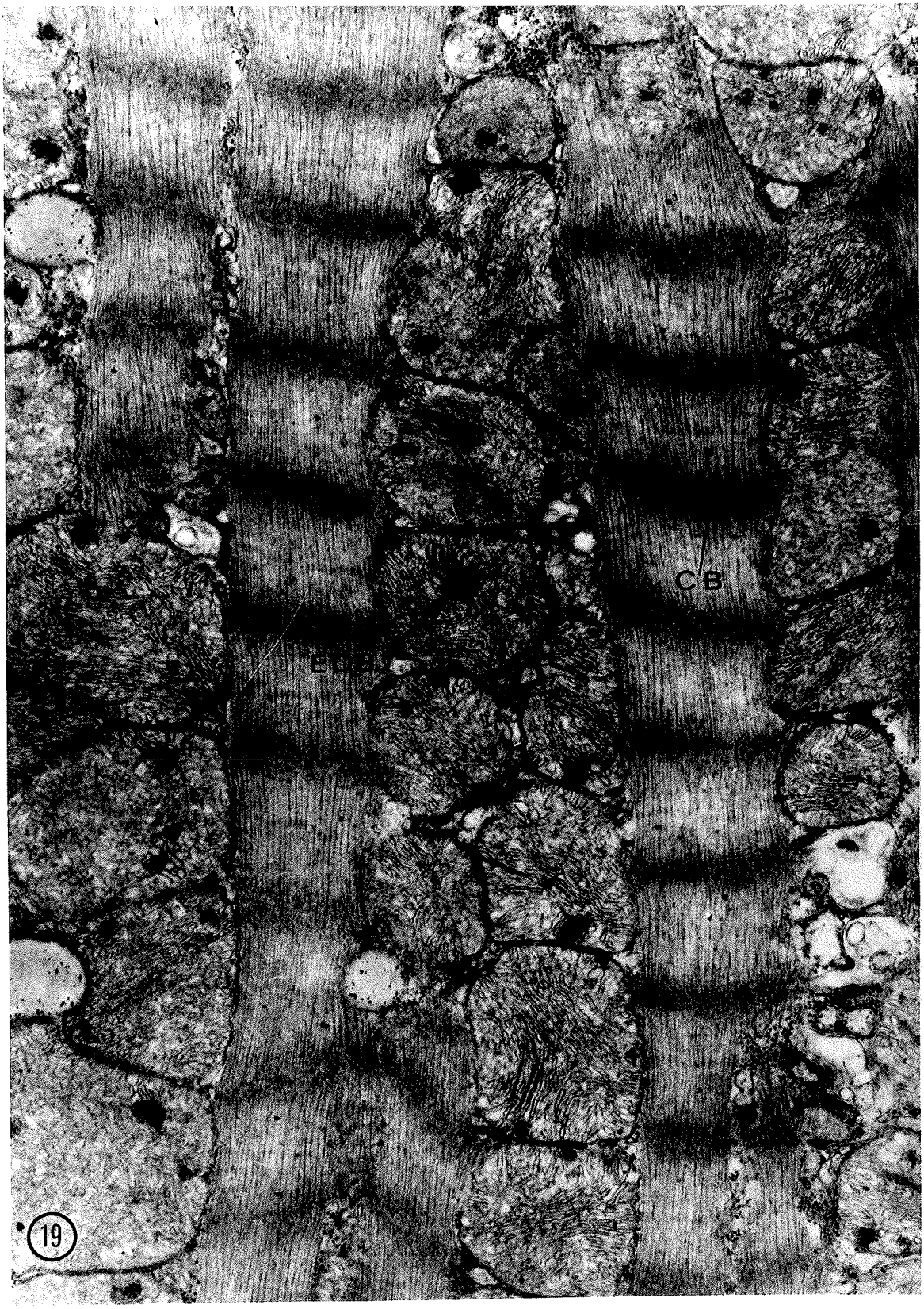
Mag. 30, 562 X



18

FIG. 19. In this micrograph we can see the prominent contraction bands (CB), and Electron Dense Bodies (EDB) in the mitochondria. Clumping of Z lines forms the minor contraction bands (CB).

Mag. 71, 765 X

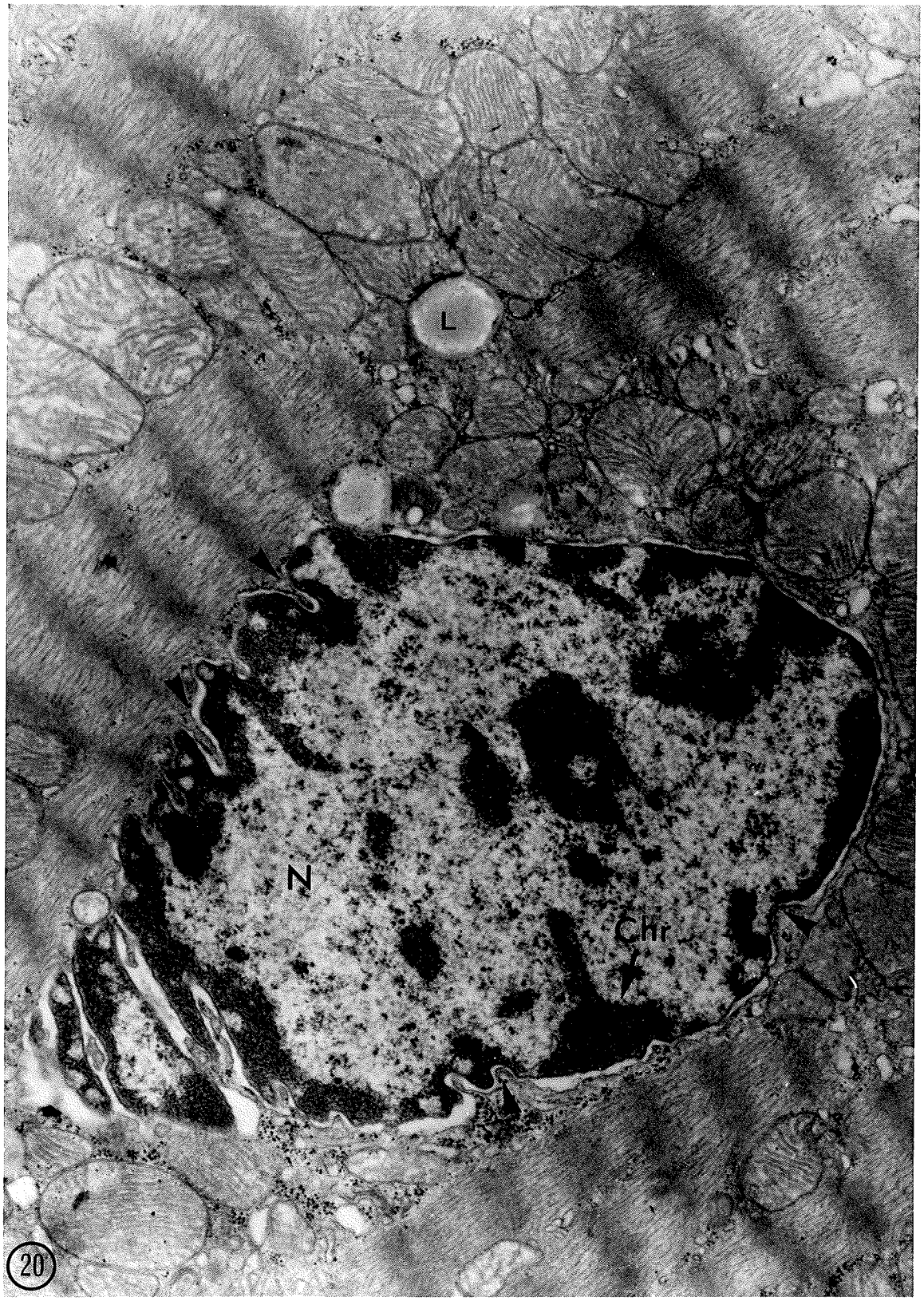

CB
EL
19

3. ISCHEMIA - 60 MINUTES

FIGS: 20-25

FIG. 20. In this electronmicrograph, the arrowheads show the nuclear marginations. Aggregation of chromatin (Chr) at the periphery of the nucleus (N) can be observed. There are lipid (L) droplets in the sarcoplasm.

Mag. 68, 764 X



20

FIG. 21. This micrograph shows the advanced stage of myocardial necrosis. Contraction Bands (CB) are seen. Arrowheads show mitochondria with electron dense bodies. On the right hand corner observe the endothelial nucleus. Swollen sarcoplasm (Sp) is observed as well.

Mag. 9508 X

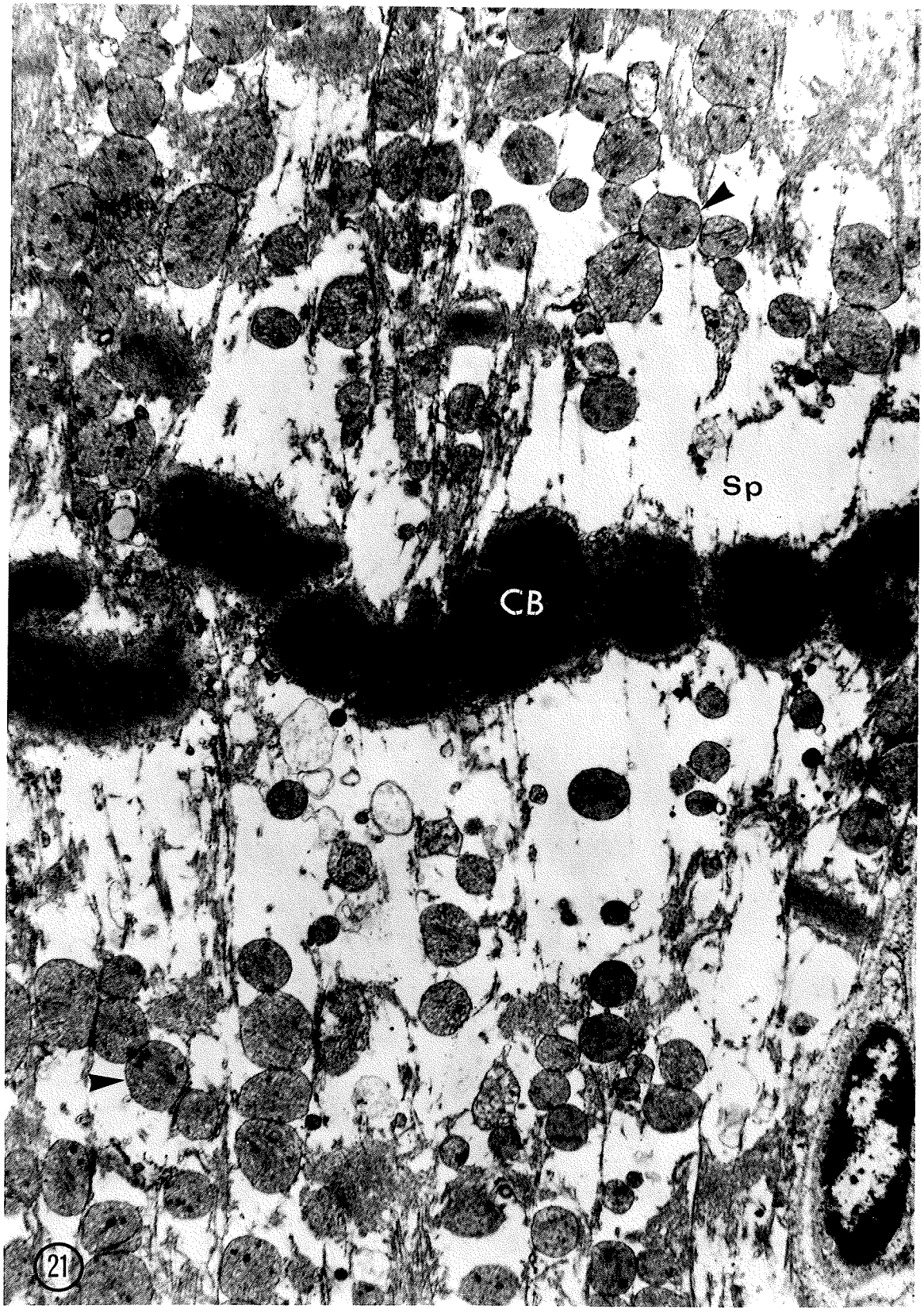
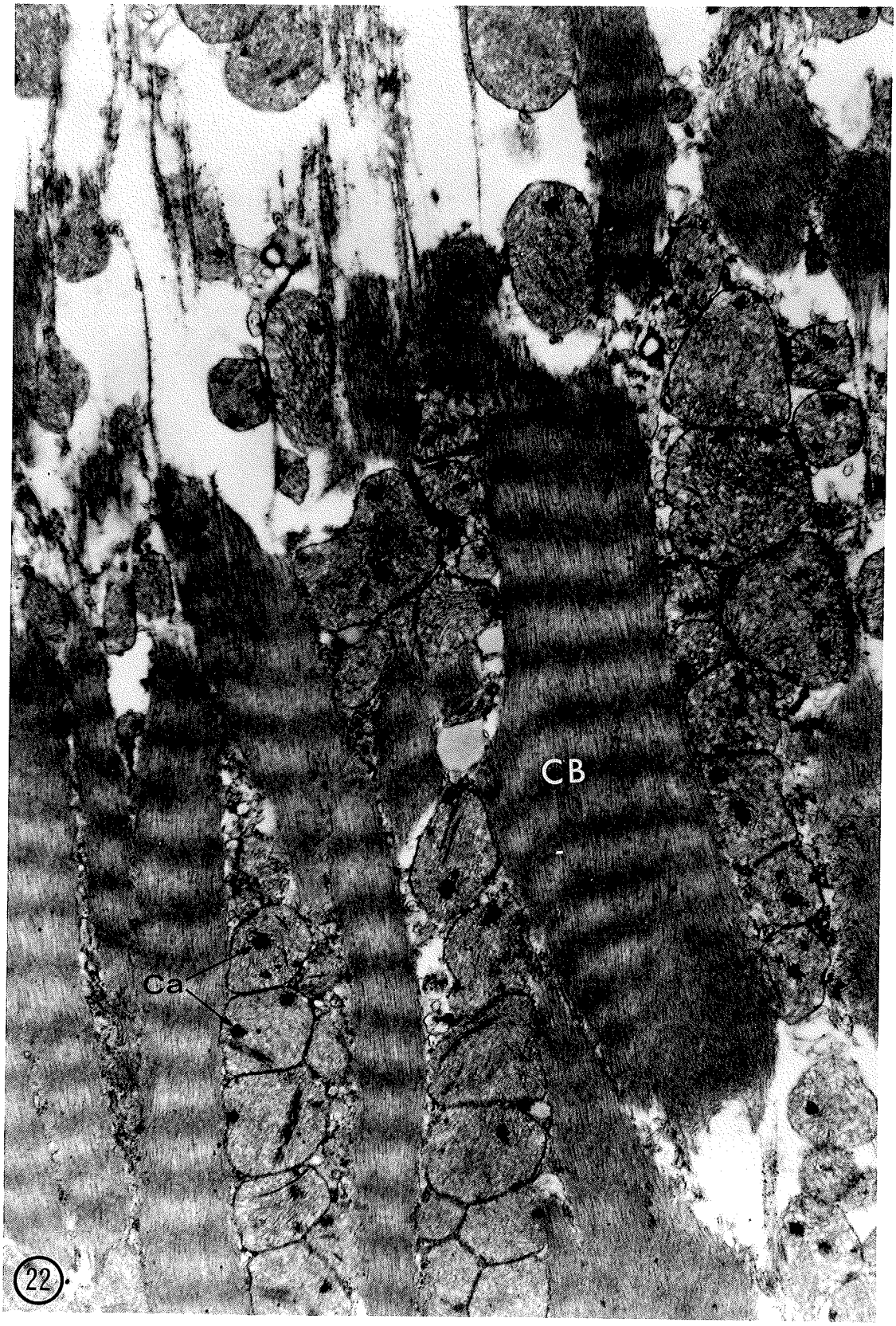

Sp
CB
21

FIG. 22. Here we can see the prominant contraction bands (CB). In addition to the electron dense bodies in the mitochondria, as Calcium (Ca) deposits, elongated parallel lines are also observed. This feature depicts the irreversible phase of ischemic injury.

Mag. 15, 213 X



22.

FIG. 23. In this micrograph we can see the aggregation of platelets (Pl) in a blood vessel. Arrowhead points the sarcolemma (SL) just below the endothelium.

Mag. 7, 3130 X

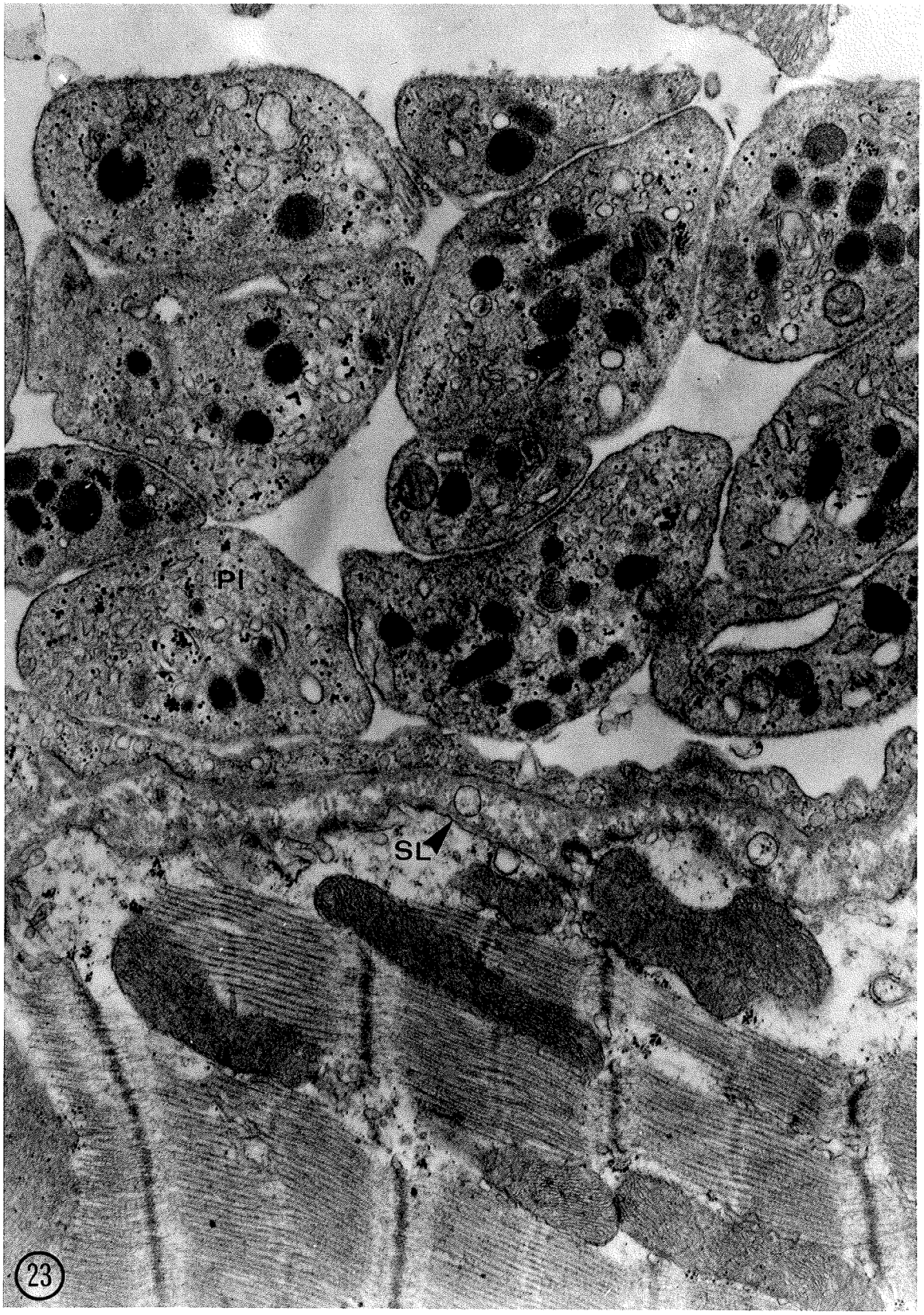
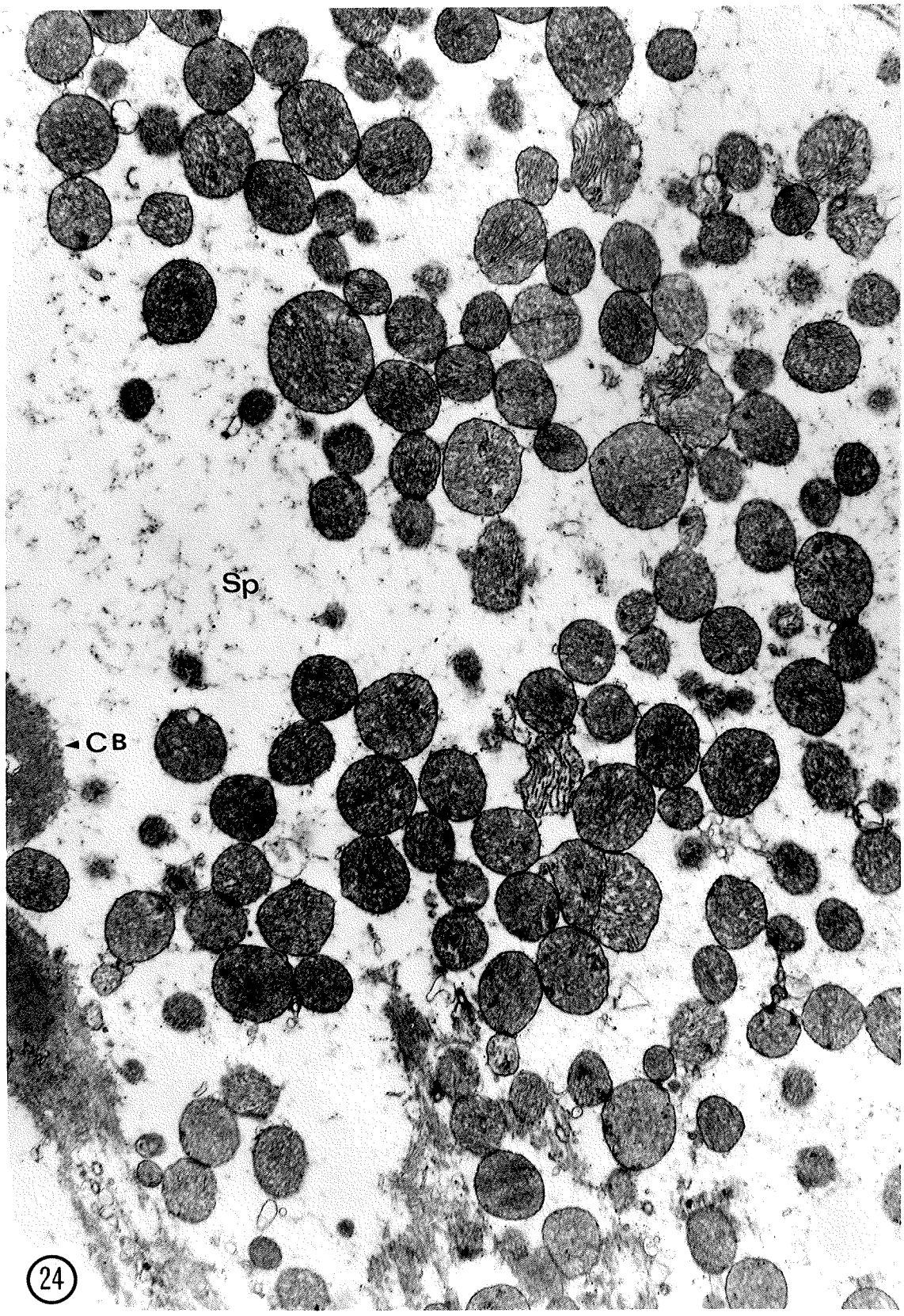

PI
SL
23

FIG. 24. In this electronmicrograph, there are numerous mitochondria in the sarcoplasm (Sp) and the arrowhead points the contraction bands (CB). Degenerating myofilaments are observed as well in the sarcoplasm.

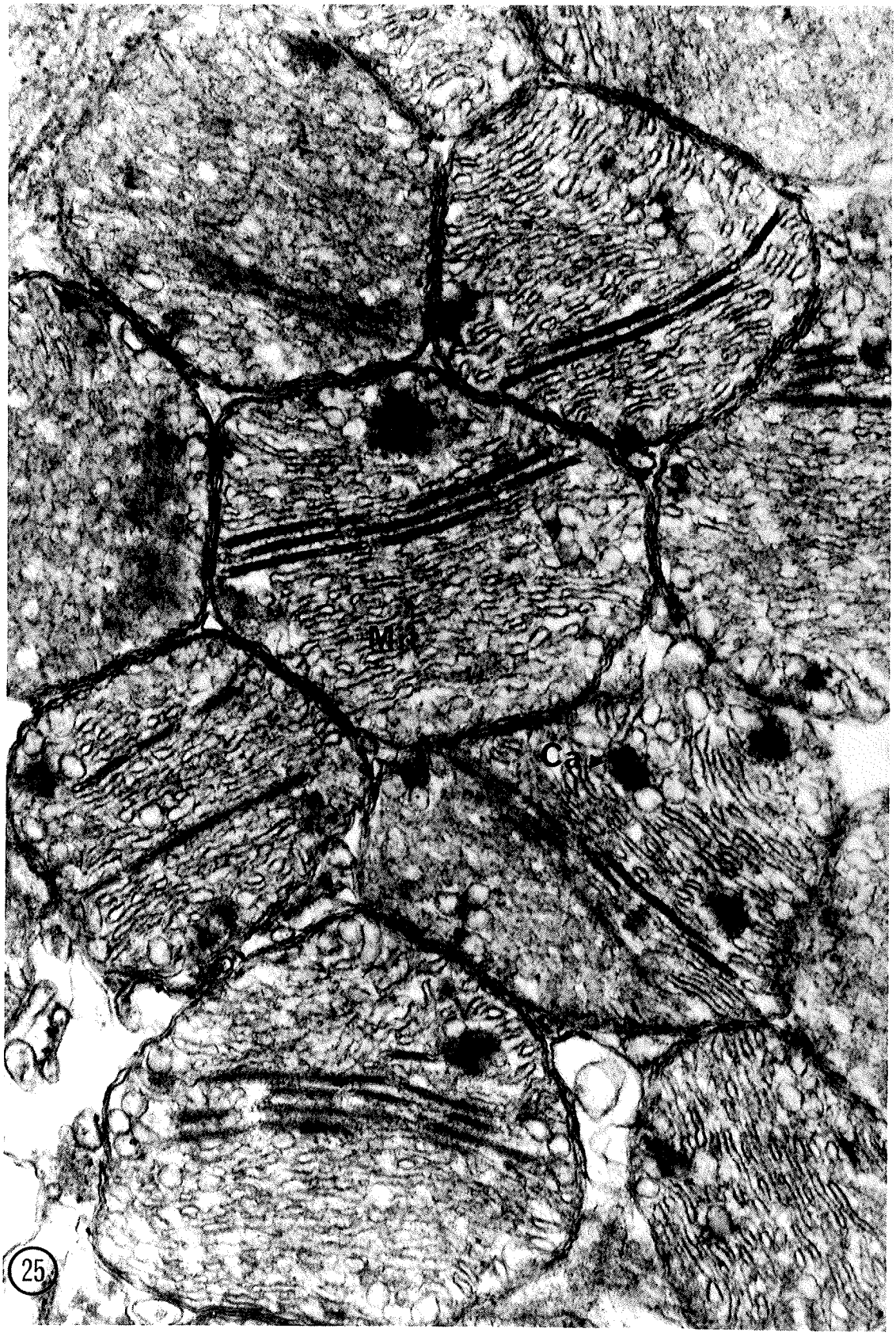
Mag. 10, 187 X



24

FIG. 25. Here we can see the mitochondria (Mit) with electron dense bodies as Calcium (Ca) deposits. Vesicles are seen as well in the mitochondria. These features show the functional impairment of the mitochondria. Parallel crystalline lines are observed as well, in the mitochondria.

Mag. 59, 814 X


M
C
25

4. MYOCARDIAL INFARCTION - 18 HOURS

FIGS: 26-30

FIG. 26. This low power electronmicrograph shows the advanced stage of degeneration of the myocardium. Here we can see Contraction Bands (CB), mitochondria (Mit) with electron dense bodies. Blood vessel (lu) and an endothelial nucleus (En) can also be observed.

Mag. 9780 X

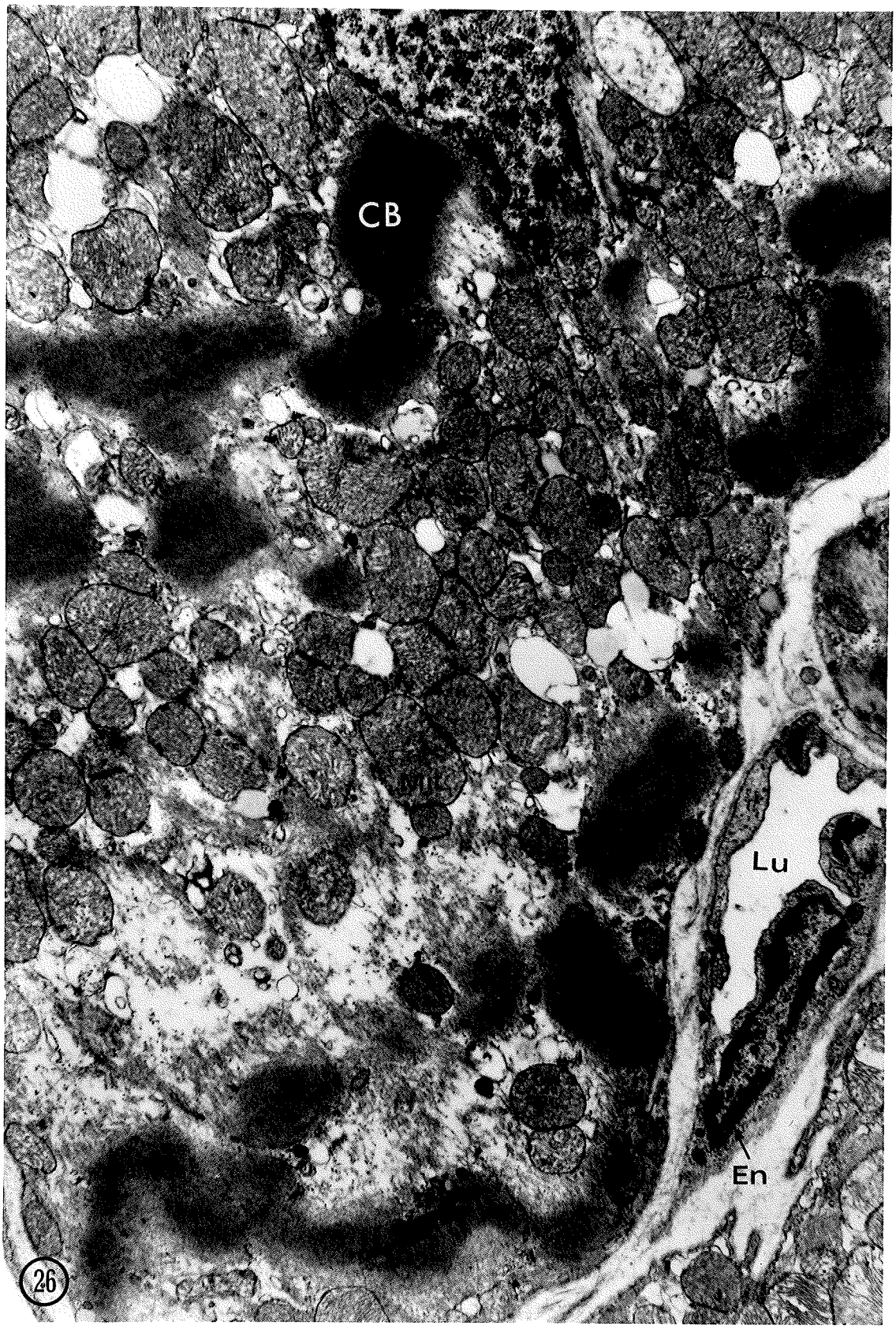

CB
Lu
En
26

FIG. 27. In this electron micrograph, we can observe a lot of lipid (L) droplets and Vacuoles (V). In the sarcoplasm (Sp), arrowheads show degenerating mitochondria. Very few cristae are seen in some mitochondria.

Mag. 21, 102 X

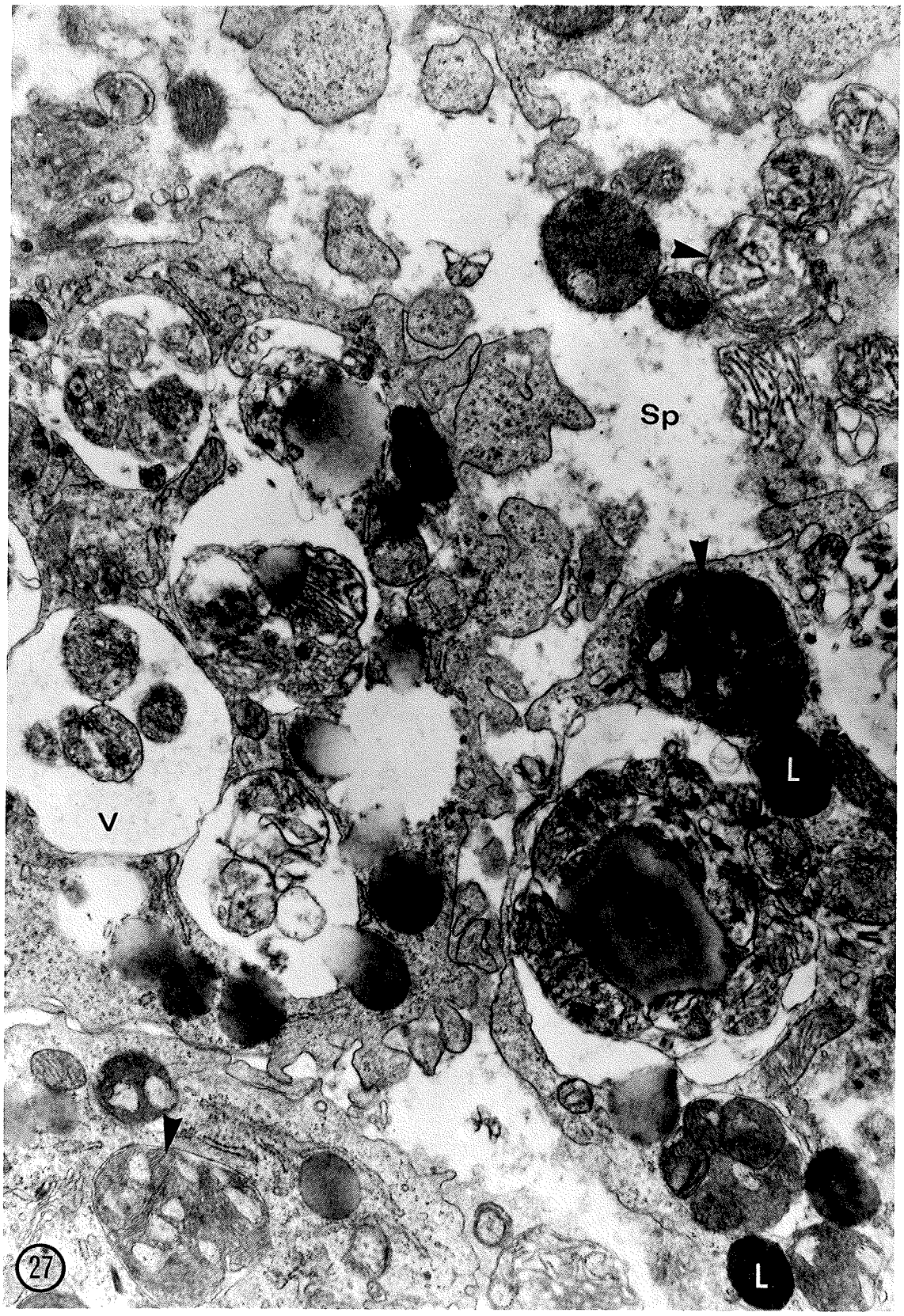

Sp
L
V
L
27

FIG. 28. This low power electronmicrograph shows some polymorphonuclear leukocytes, contraction bands (CB) and degenerating mitochondria (arrowhead). Ruptured sarcolemma can also be seen with a macrophage invading the sarcoplasm. Some vesicles (V) are also seen.

Mag. 12, 419 X

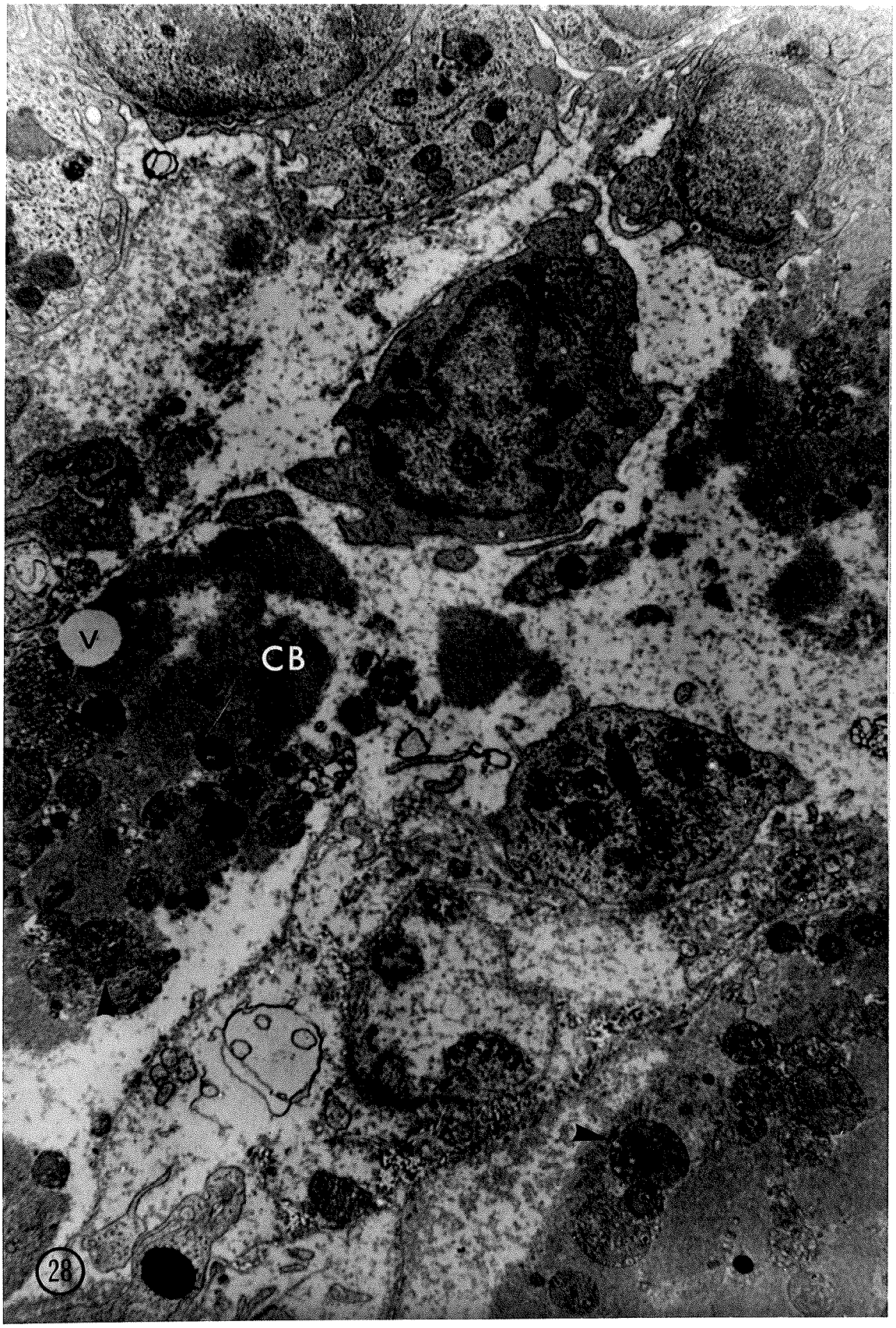
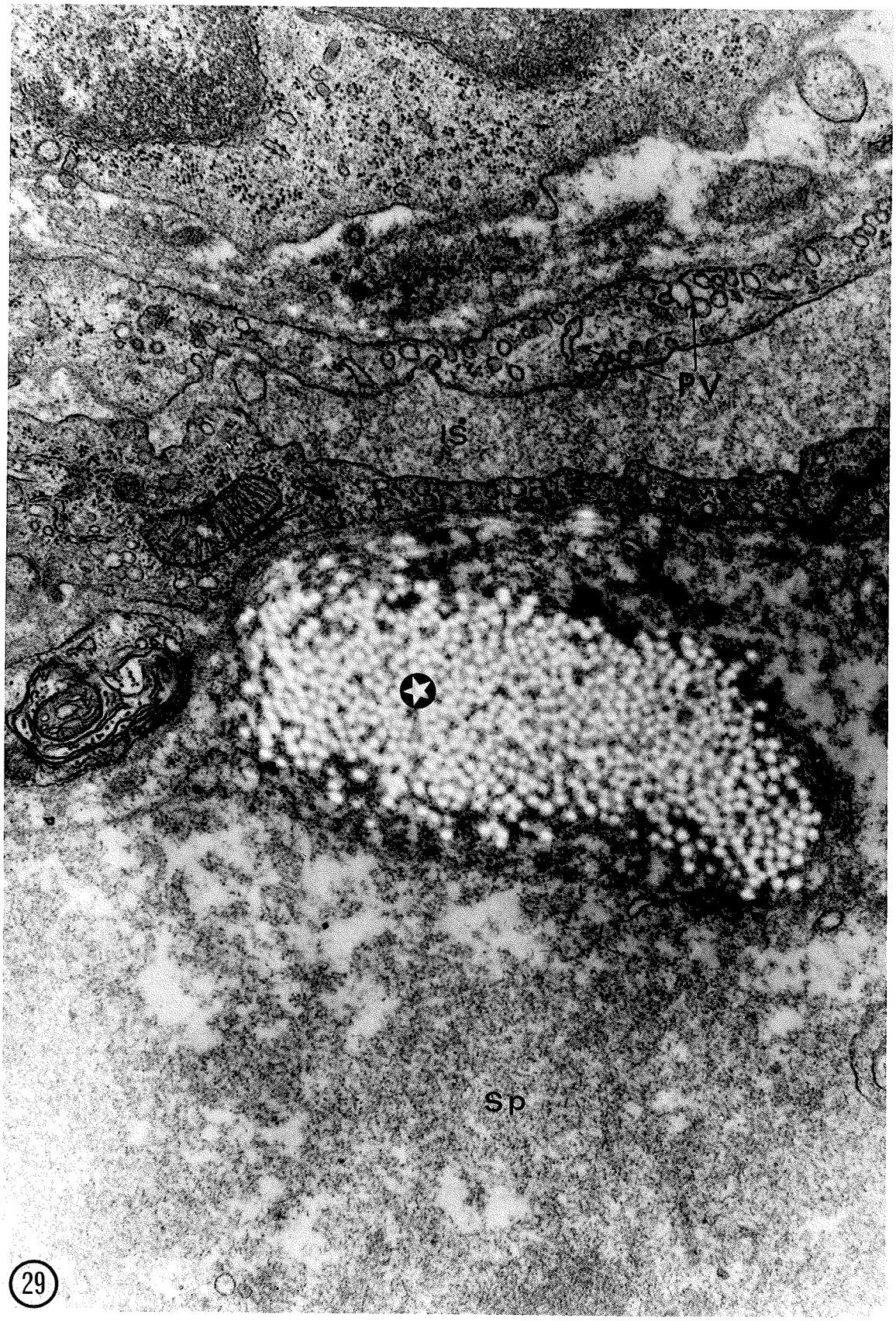

V
CB
28

FIG. 29. This micrograph shows the degenerated myocardium with procollagen fibrills (astrik) in the sarcoplasm (SP). Numerous pinocytotic vesicles (PV) are seen on the endothelial wall, near the intercellular space (Is).

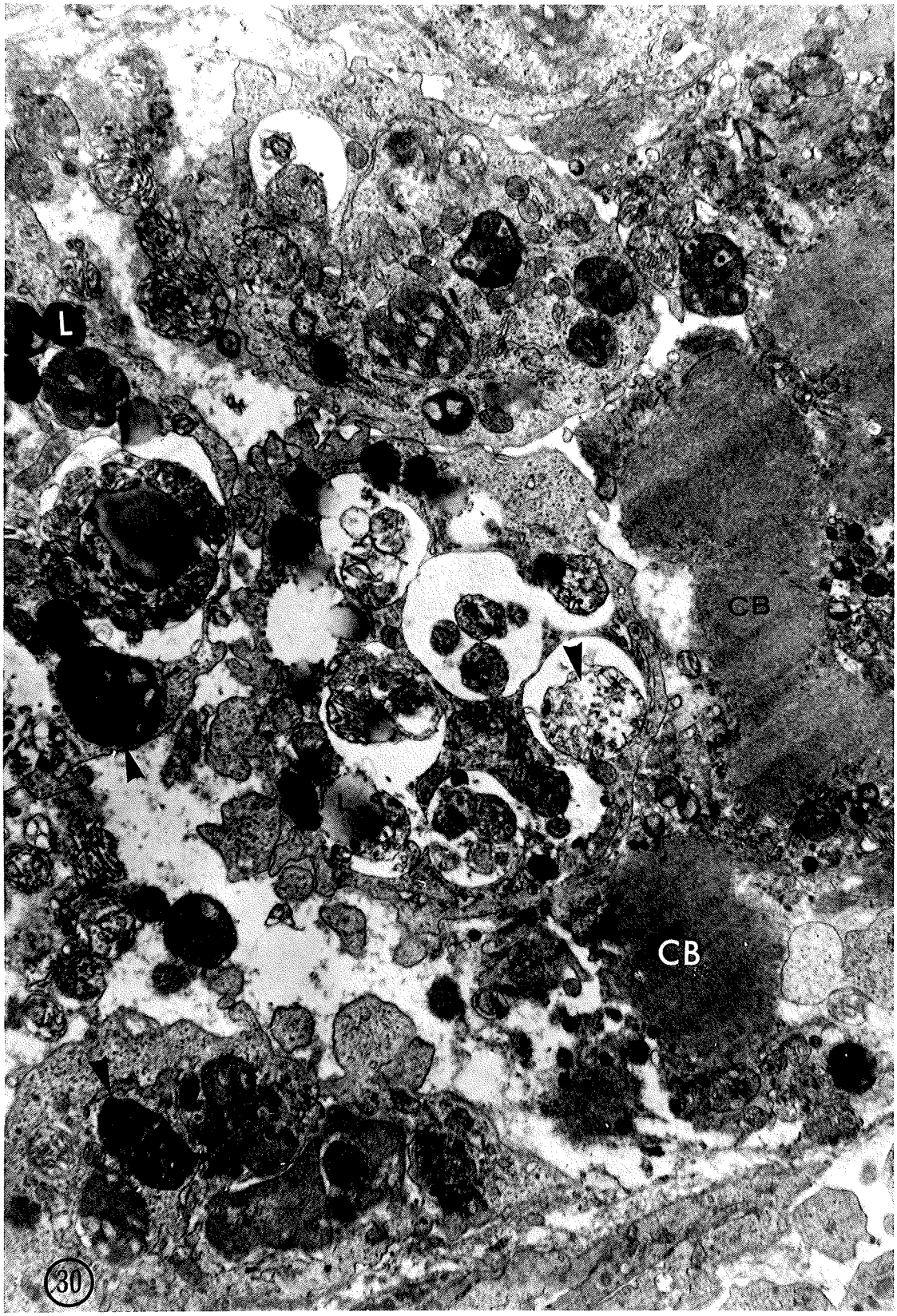
Mag. 34, 152 X



29

FIG. 30. In this low power micrograph we can observe contraction bands (CB) and numerous lipid (L) droplets. Mitochondria are condensed (arrowheads). This is an example of a typical irreversible damage ("the point of no return"). Notice the depletion of glycogen granules.

Mag. 14, 068 X


L
CB
CB
30

b) ULTRASTRUCTURAL CHANGES IN THE ISCHEMIC MYOCARDIUM AFTER DOBUTAMINE ADMINISTRATION.

Two studies were conducted under this section: In the first subsection, the effect of dobutamine administration alone on the myocardium was studied. Since dobutamine is a synthetic catecholamine and since almost all catecholamines in large quantities cause myocardial damage to some extent, dobutamine alone was administered deliberately to observe whether this drug caused any damage to the myocardium. In the second subsection, the protective mechanism of action of dobutamine on isoproterenol insulted myocardium was studied.

i) EFFECT OF DOBUTAMINE ON THE MYOCARDIUM (Figs. 31 to 35).

Observations made on the ultrastructure of dobutamine treated myocardium showed no structural changes. The nucleus and the pattern of chromatin distribution in the nucleus were essentially normal (Fig. 31). Sarcomeres maintained proper registry (Figs. 31,32). The attachment of actin filaments to the filamentous mat on the intermediate junctional complex of the transverse portion of the intercalated disc was found to be essentially normal (Figs. 33, 35B). There was no structural change in the sarcolemma (Fig. 34). Fig. 35A shows two sarcomeres with normal A and I bands, and Z lines.

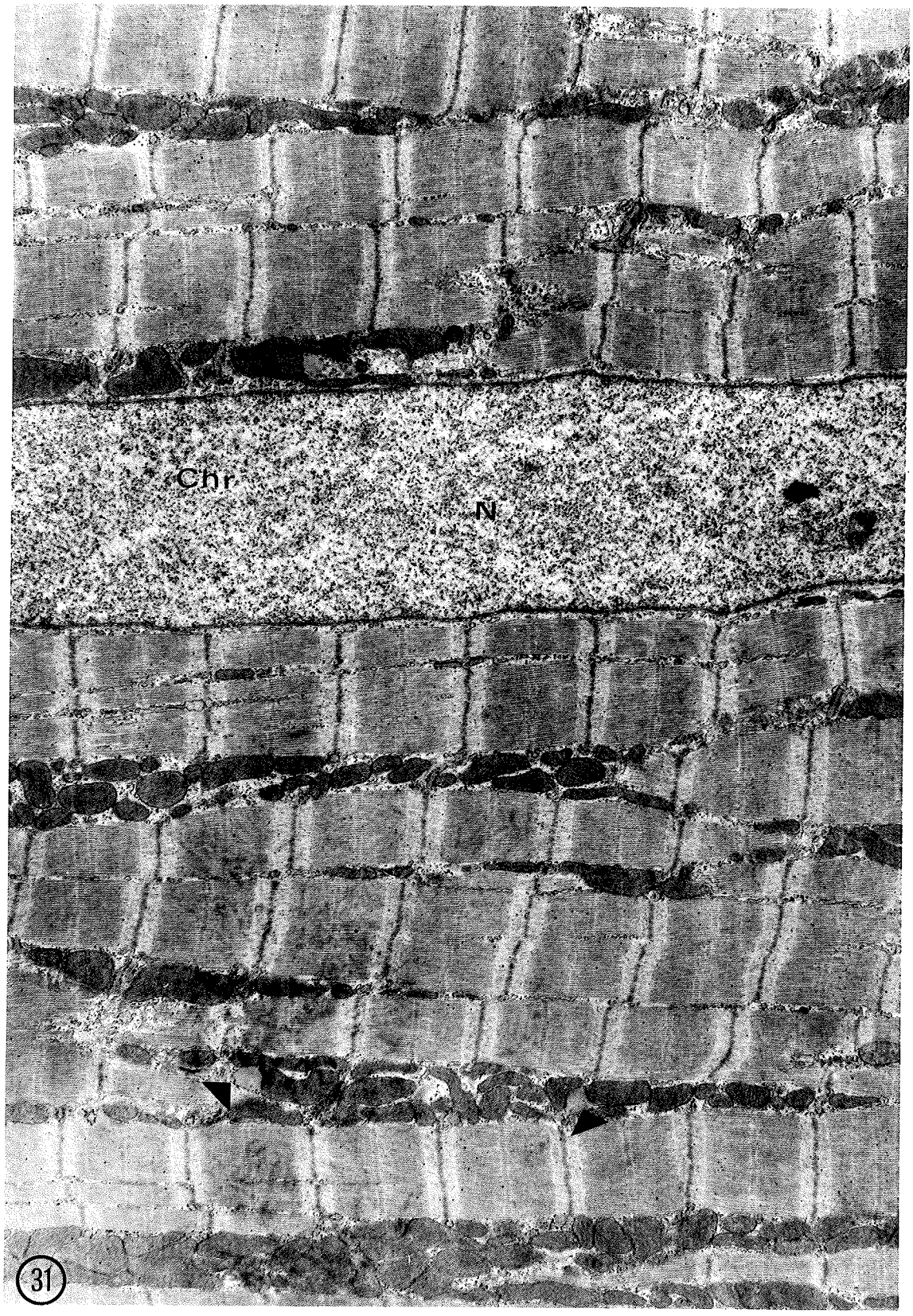
The only two features, different from the control, observed were numerous glycogen granules in the sarcoplasm with quite a few vacuoles (Figs. 32,33). The glycogen granules observed seemed to be larger than those in the control group.

1. DOBUTAMINE TREATED GROUP

FIGS: 31-35

FIG. 31. This low power micrograph shows a nucleus (N) with uniform distribution of chromatin (Chr) material. Sarcomeres and mitochondria are essentially normal. Arrowheads show lipid droplets.

Mag. 8999 X



(31)

FIG. 32. This is a higher power electronmicrograph showing the sarcomeres in proper registry. Arrows point the sarcoplasmic reticulum. The T-tubules join with the sarcoplasmic plasm at the Z level of each fibril. Observe the glycogen granules on the upper left hand corner, among the mitochondria.

Mag. 30, 016 X

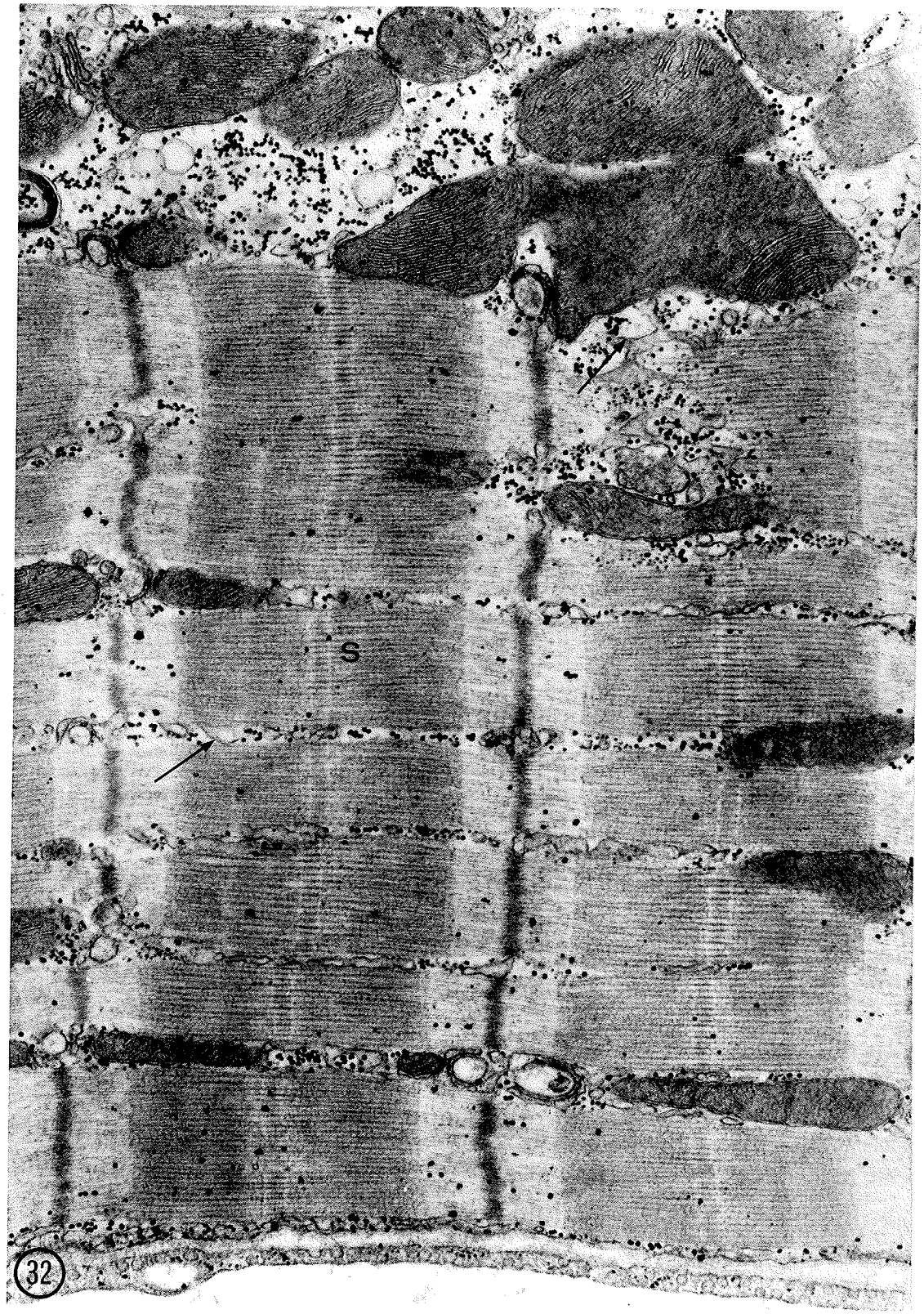

S
32

FIG. 33. The attachment of the myofilaments on to the intercalted disc (ID) is seen in this micrograph. A prominent Golgi (G) complex is seen in the sarcoplasm. Numerous glycogen (Gly) grnaules are also observed in this figure.

Mag. 24, 108 X

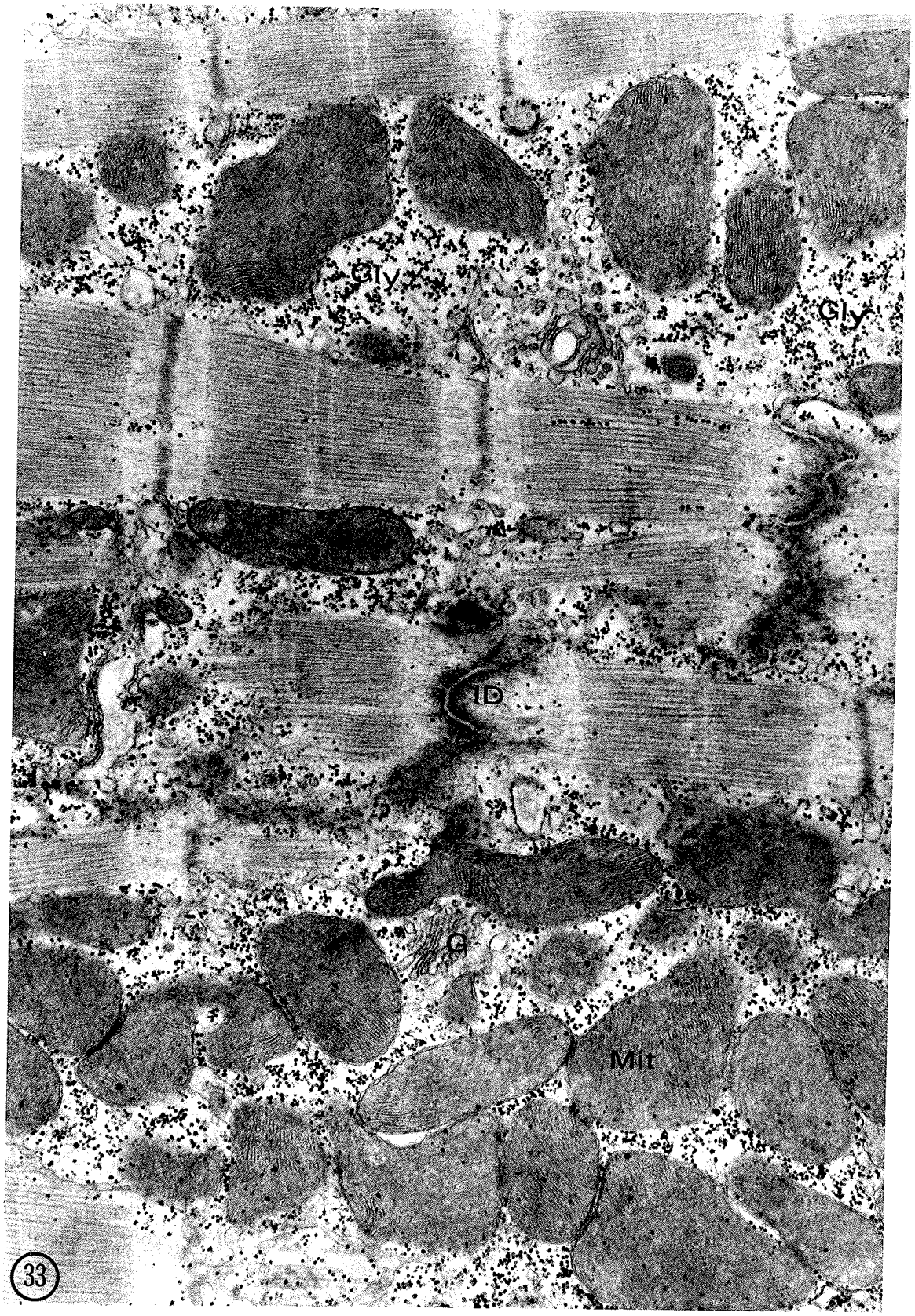

Gly
Gly
ID
G
Mit
33

FIG. 34. In this electronmicrograph, the endothelial lumen (Lu), sarcolemma (SL), the A, Z, I and M lines of the sarcomere are essentially normal. mitochondria also seem to be normal.

Mag. 20, 011 X

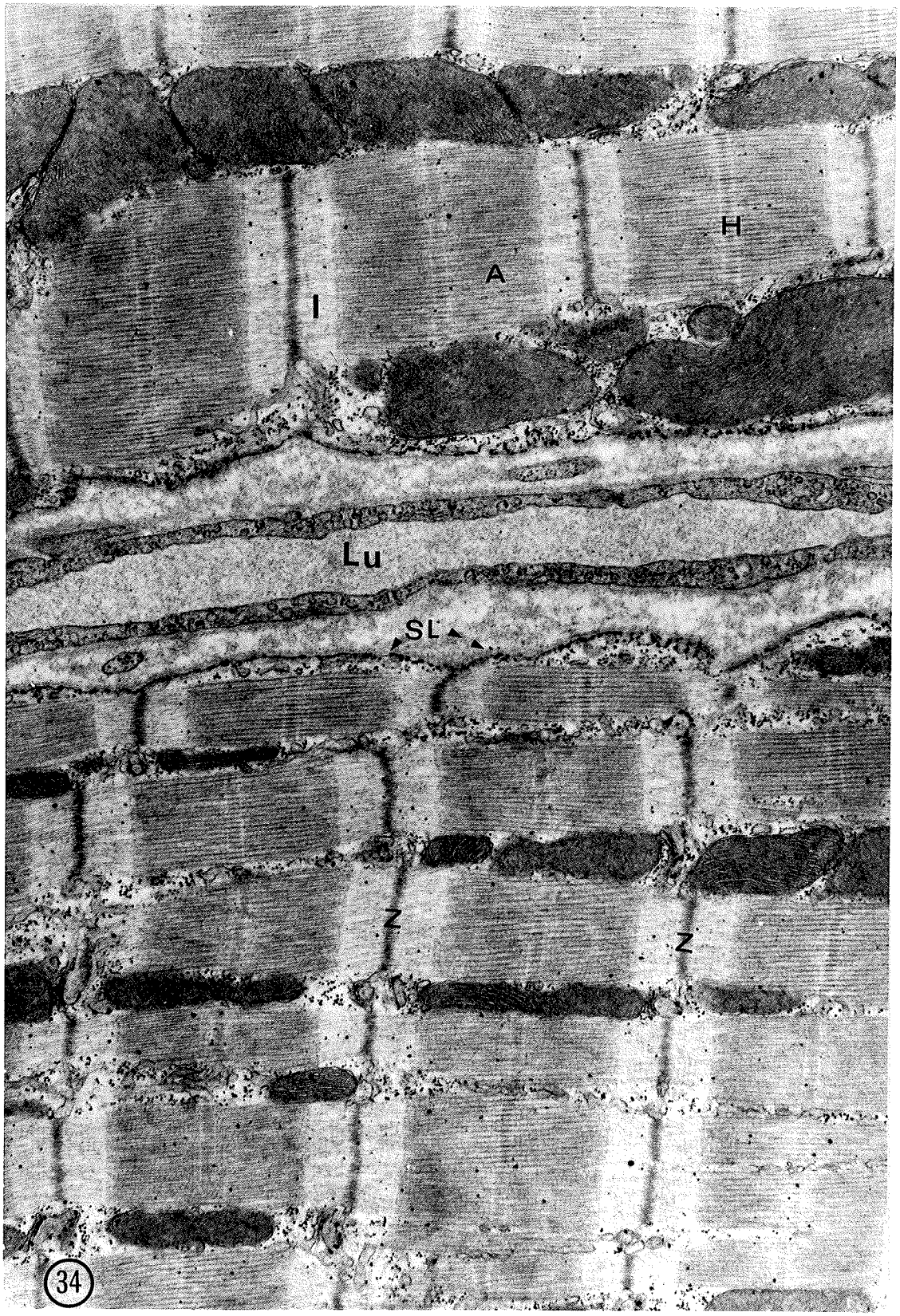
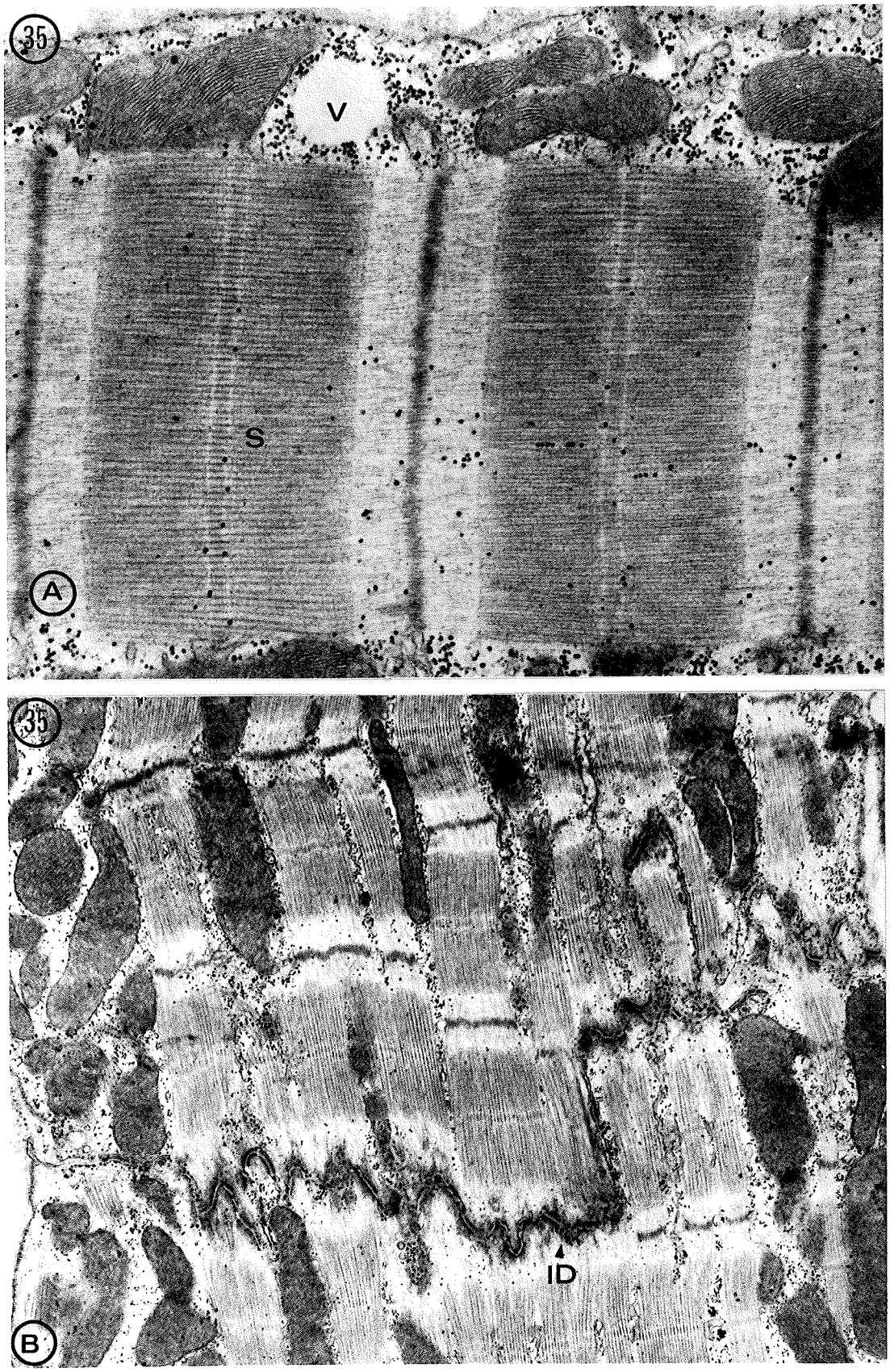

H
A
I
Lu
SL
Z
Z
34

FIG. 35 A. This micrograph shows a vacuole (V) surrounded by glycogen granules. The two sarcomeres (S) and mitochondria observed are essentially normal. Numerous glycogen granules are also present in the sarcoplasm.

Mag. 30, 016 X

FIG. 35 B. In this micrograph, we can observe the intercalated disc (ID) and the attachment of myofilaments. Transverse and lateral portions of the intercalated disc are seen here. Sarcomeres and mitochondria are normal.

Mag. 16, 164 X


35
V
S
A
35
ID
B

ii) ISOPROTERENOL AND DOBUTAMINE (Figs. 36 to 40).

After isoproterenol insult to induce ischemia, dobutamine was administered immediately and procedures for electron microscopic observation were carried out 18 hours after isoproterenol insult.

The nucleus and cytoplasmic features were found to be essentially normal (Fig. 36). The structure of intercalated disc and arrangement of myofilaments were quite normal (Fig. 37). Some mitochondria were slightly swollen and only a few had electron dense bodies (Fig. 38). The percentage of mitochondria with electron dense bodies was 12.62% (Table I, Fig. 52) which was significantly lower when compared with other insulted and treated groups (Table I, Fig. 52). Development of vacuoles and abundance of glycogen granules have been observed (Fig. 39). The micrograph in Fig. 40 shows the transverse section of two myocardial cells. The one on the right hand side shows edema. Though the animals were killed after 18 hours of isoproterenol insult, there were no significant ultrastructural changes because of the immediate administration of dobutamine.

iii) ISOPROTERENOL AND DOBUTAMINE (Figs. 41 to 45).

Dobutamine was administered 15 minutes after isoproterenol insult. Animals were killed for electronmicroscopy, 18 hours after the insult.

Some nuclear indentations were observed (Fig. 41). A few sarcoplasmic reticula were swollen (Fig. 42). Myofilaments were essentially normal, still attached to the intercalated disc (Fig. 43).

Minor contraction bands and a few disintegrating mitochondria were also observed (Figs. 44, 45). In a few mitochondria, electron dense bodies were observed and the percentage of mitochondria with such electron dense bodies was found to be 61.31% (Table I, Fig. 52) which was significant when compared with the isoproterenol insulted group without dobutamine treatment, which had 81.01% (Table I).

iv) ISOPROTERENOL AND DOBUTAMINE (Figs. 46 to 51).

The delay time for dobutamine intervention after isoproterenol insult was 30 minutes. Animals were killed 18 hours after the insult.

The myocardial damage was more extensive when compared with the previous two subgroups. Fig. 46 shows a nucleus with less chromatin material dispersed in the nucleoplasm. Some mitochondria contained electron dense bodies (Figs. 47, 48A) and the percentage of such mitochondria was 72.64% which was significant when compared with the isoproterenol insulted but not dobutamine treated groups which had 84.28% (Fig. 52, Table I) of mitochondria with electron dense bodies in them. Fig. 48B shows a degenerating mitochondrion with vesicles in the matrix space. The arrow heads in Fig. 49 show numerous ribosomes on the endoplasmic reticulum. A prominent nucleolus is seen in the nucleus which shows the active protein synthesis (Fig. 50). Though some mitochondria are devoid of cristae, the mitochondrial membranes are still intact (Fig. 51).

2. ISOPROTERENOL INSULTED AND DOBUTAMINE TREATED (IMMEDIATELY) GROUP

FIGS: 36-40

FIG. 36. In this micrograph, the nucleus (N) and chromatin (Chr) distribution seem to be quite normal. At one end of the nuclear pole, a slight edema is observed. Vacuoles (V), lipid droplets, sarcomeres and numerous glycogen granules are also seen in the sarcoplasm. Arrowheads show the aggregation of glycogen granules.

Mag. 14, 554 X


V
N
Chr
S
36

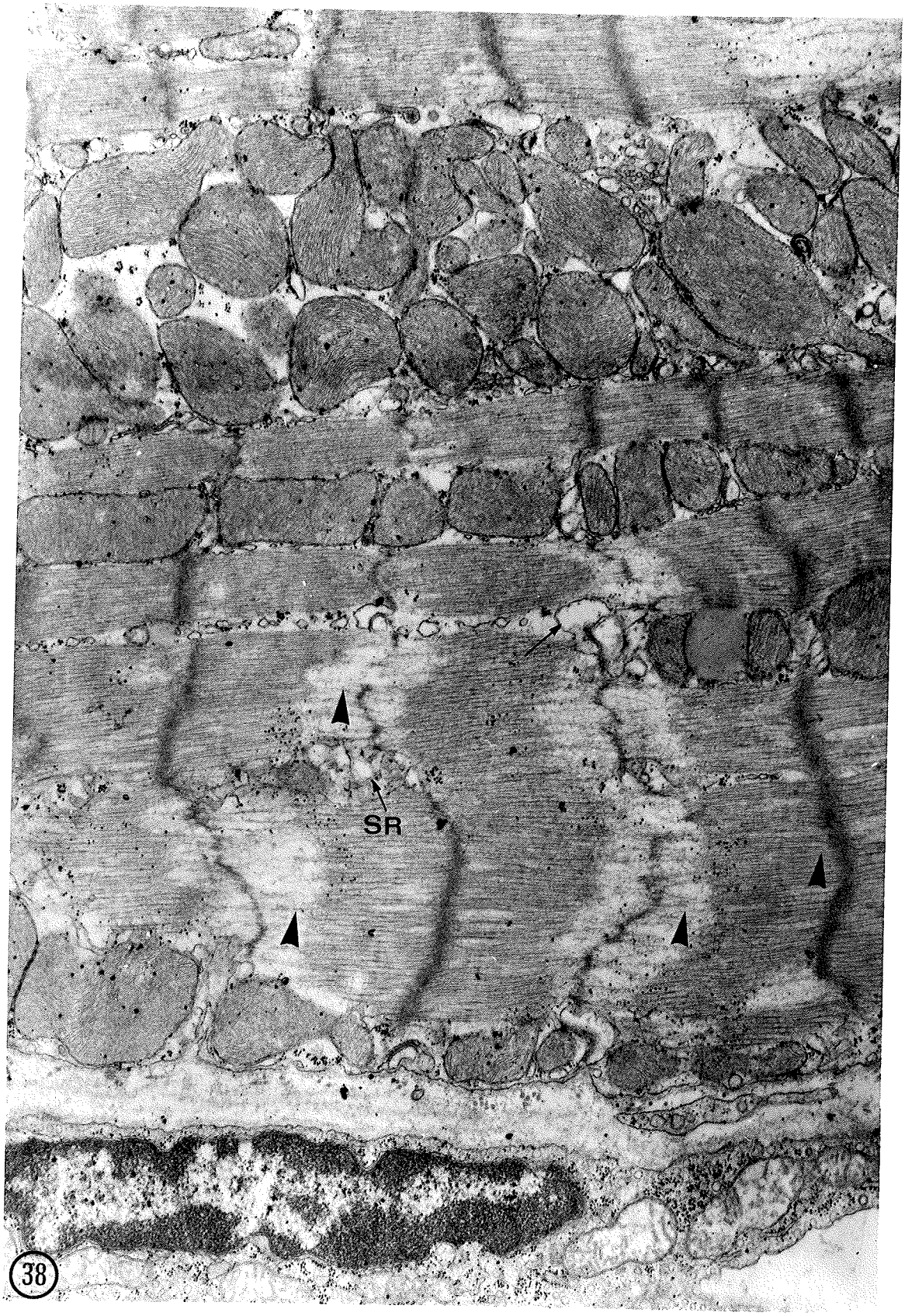
FIG. 37. In this low power electronmicrograph, we can observe numerous glycogen granules, sarcomeres (S) in proper registry, intercalated discs (ID) with myofilaments attached to them and mitochondria between the myofibrils. No lesions are seen here.

Mag. 19, 016 X


V
ID
S
37

FIG. 38. This micrograph shows some edema (swollen) of the sarcoplasmic reticulum (arrow pointing the SR). Sarcomeres are in a relaxed state (arrowheads). Sarcolemma is still intact. No change in mitochondrial ultrastructure. An endothelial nucleus is observed at the lower left hand corner.

Mag. 23, 250 X



38

FIG. 39. This micrograph shows large vacuoles surrounded by numerous glycogen granules (Gly). The asterik (star) shows vesicular appearance of the Golgi complex. Other than this, the ultrastructure appears to be normal.

Mag. 58, 940 X


V
Gly
39

FIG. 40. Micrograph of two adjacent cells in cross section. The one on the right hand side shows edema in the sarcoplasm (Sp) and the one on the left side appears to be normal. The intercellular space (Is) separates them.

Mag. 31, 107 X

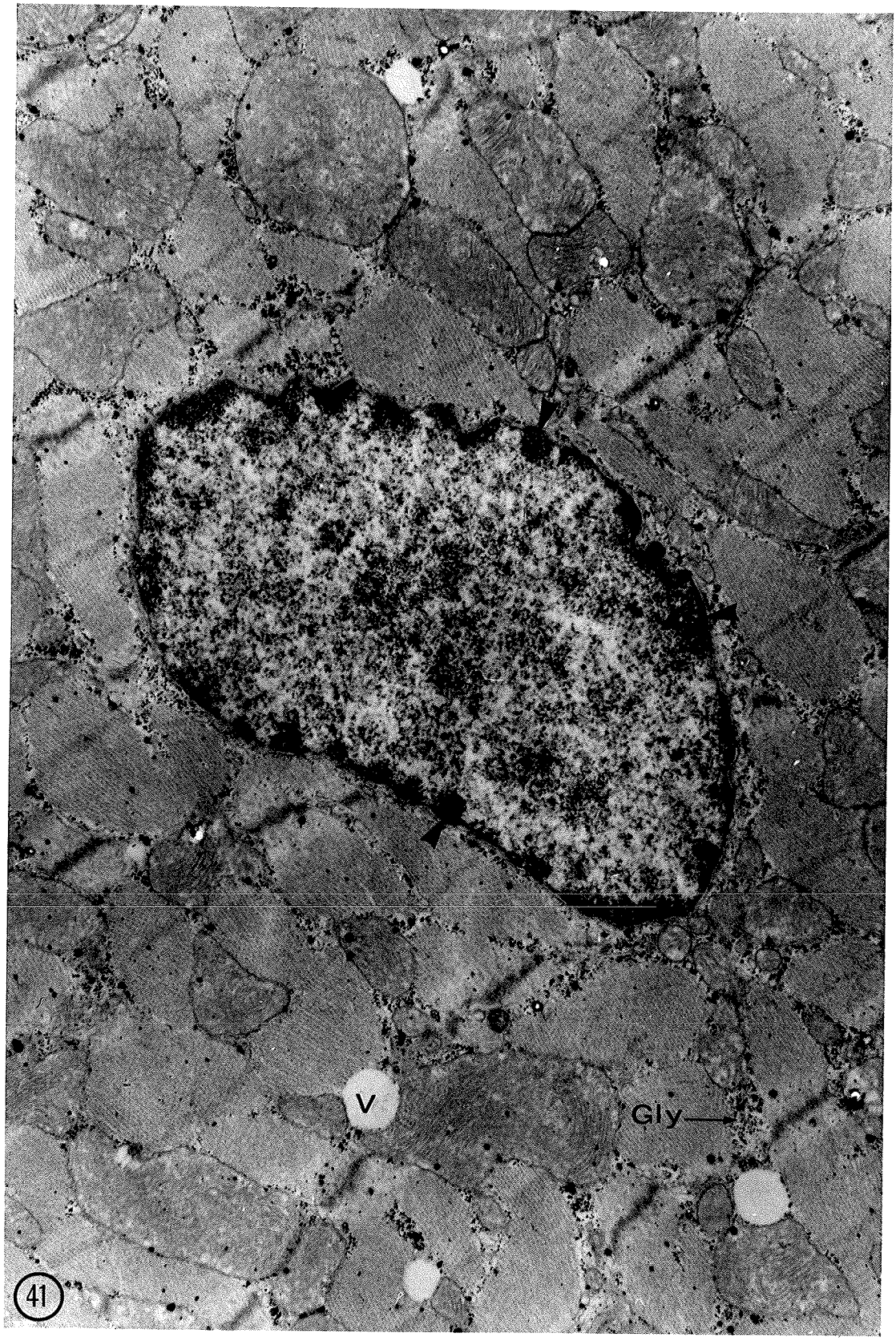

IS
Sp
40

3. ISOPROTERENOL-INSULTED AND DOBUTAMINE TREATED (15 MIN. INTERVAL) GROUP

FIGS: 41-45

FIG. 41. This figure shows a nucleus (N) with uniform distribution of chromatin material (Chr). But the aggregation of chromatin material at the periphery of the nucleus is noteworthy. Nuclear margination is also observed here (arrowheads). Sarcomeres are in a contracted state. Vesicles (V) and Glycogen (Gly) particles are observed.

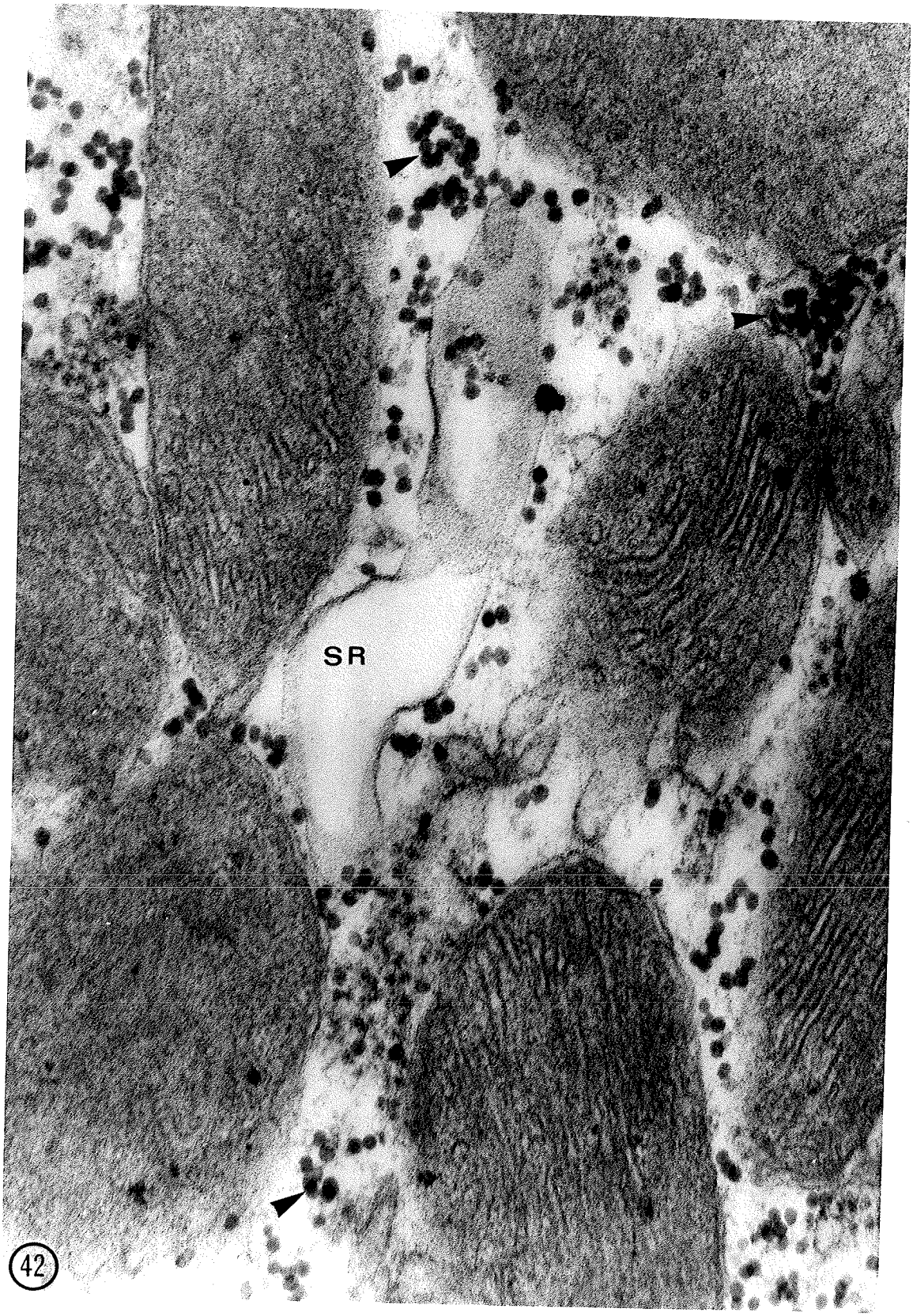
Mag. 20, 810 X



41

FIG. 42. In this micrograph, swollen sarcoplasmic reticulum (SR) is the prominent feature. Arrowheads show the abundance of glycogen granules, which are slightly larger than the beta-type glycogen particles observed in the normal myocardium.

Mag. 91, 685 X



42

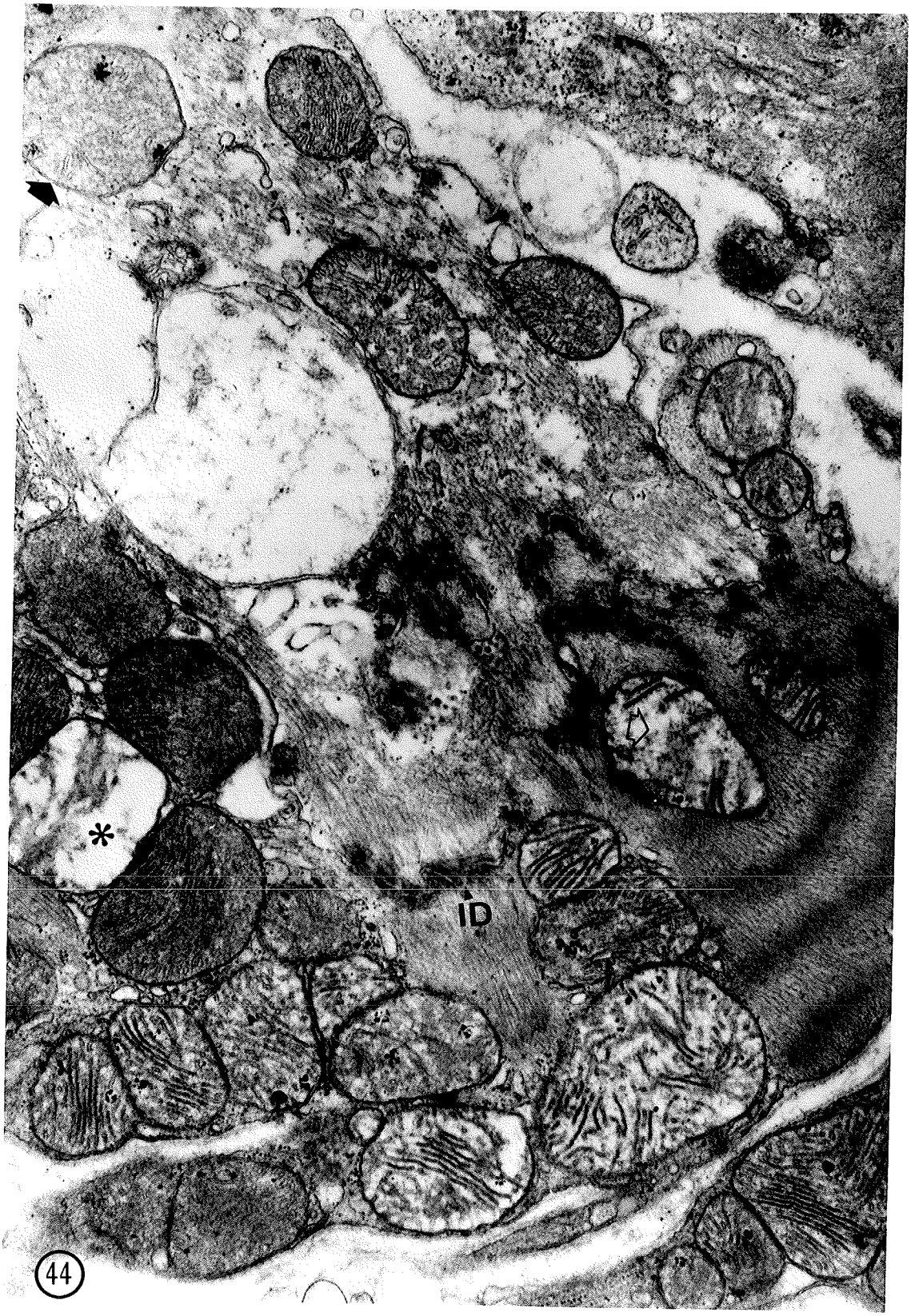
FIG. 43. This micrograph shows the intercalated disc (ID) and the attachment of myofilaments (AF). Mitochondria (Mit) are essentially normal. Glycogen granules are still present in the sarcoplasm, though this group was insulted with isoproterenol. The treatment was with dobutamine.

Mag. 65, 490 X

(43)

FIG. 44. This figure shows some minor contraction bands. Mitochondria are swollen with altered definition of cristae (hollow arrowhead). Some mitochondria are devoid of cristae (asterik) whereas some are condensed. Intercalated disc (ID) is still intact, which means cells are not separated. Thick arrowhead shows mitochondria with electron dense bodies.

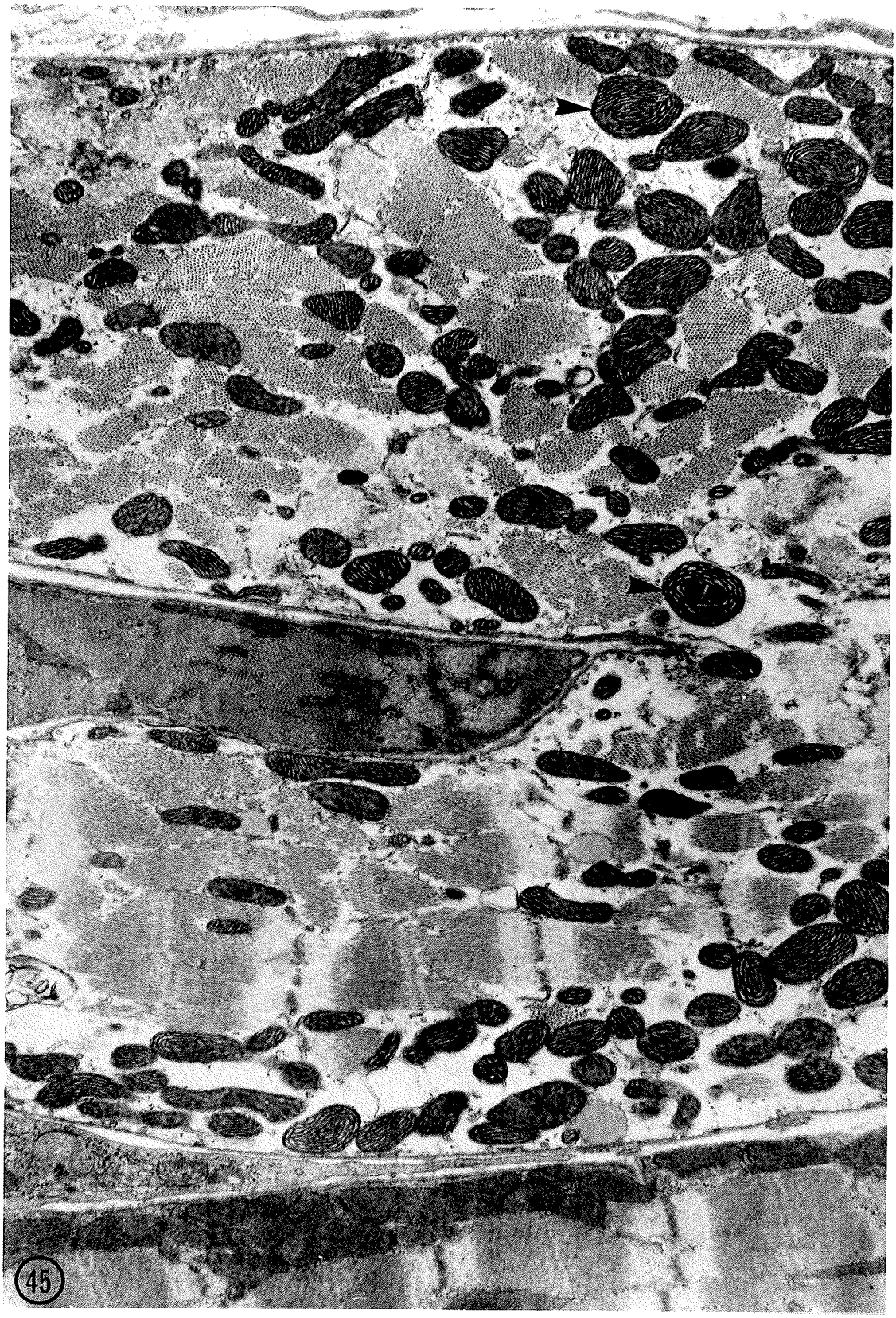
Mag. 24, 540 X



44

FIG. 45. Myocardial cells in cross section. The mitochondria show intracristal space and matrix without any damage (arrowheads). Some mitochondria have a few electron dense bodies which show the functional state of the mitochondria. The cells are slightly swollen. Sarcolemma is still intact.

Mag. 13, 825 X


45

4. ISOPROTERENOL-INSULTED AND DOBUTAMINE TREATED (30 MIN. INTERVAL) GROUP

FIGS: 46-51

FIG. 46. This figure shows the vesicular nucleus (N) with very few chromatin material in the nucleoplasm. Mitochondria are swollen. Sarcomeres show contraction bands as well. Though treated with dobutamine, the treatment was 30 minutes after the isoproterenol insult. Mitochondrial damage is evident in this micrograph.

Mag. 13, 874 X


L
S
N
46

FIG. 47. This electronmicrograph shows the myocardial cellular edema. The sarcolemma (SL) shows the typical scalloping because of the separation from the fibrils. Contraction bands and mitochondria with electron dense bodies can also be observed in this fig. In the sarcoplasm (SP) some mitochondria are with electron dense bodies in them.

Mag. 61, 366 X

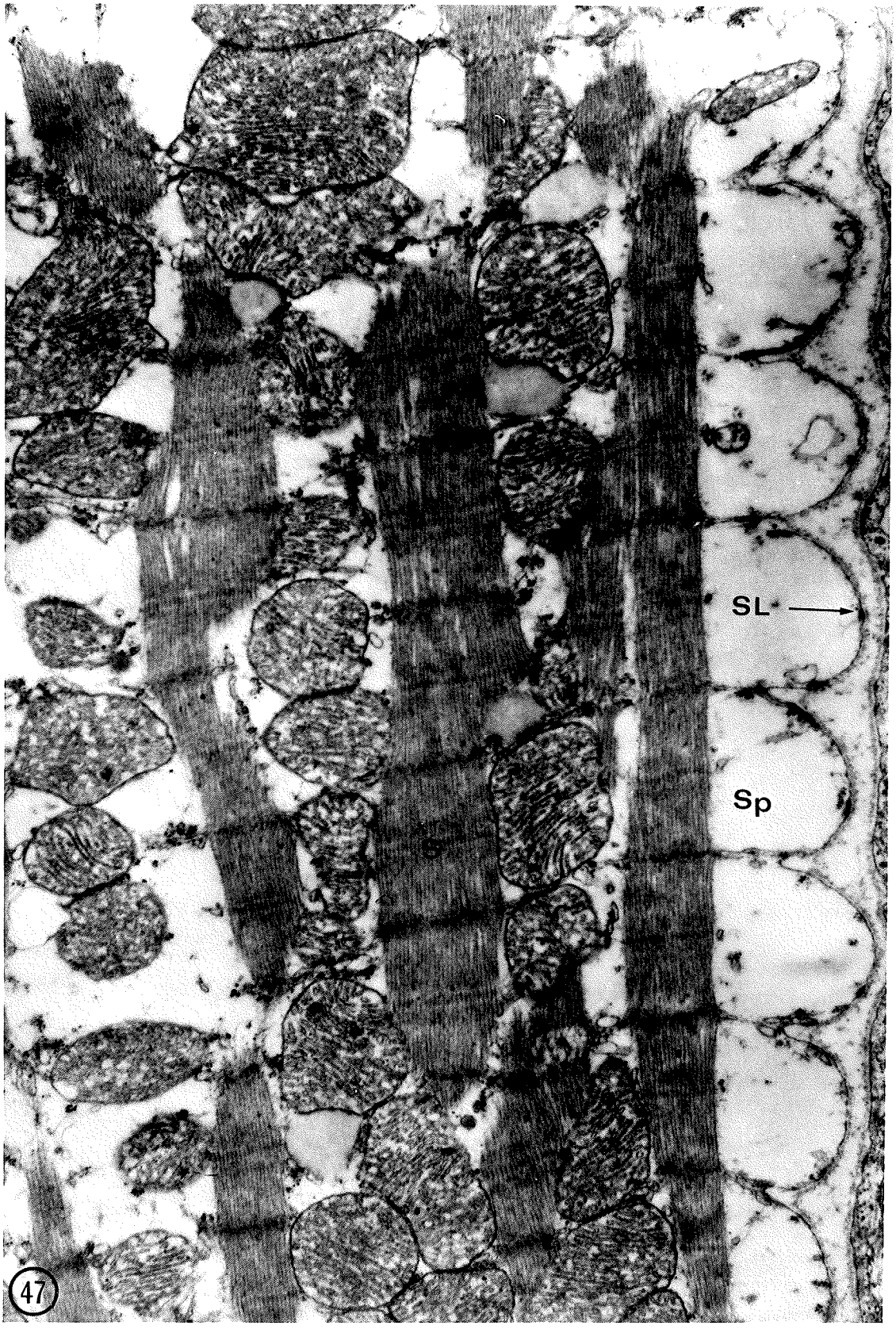
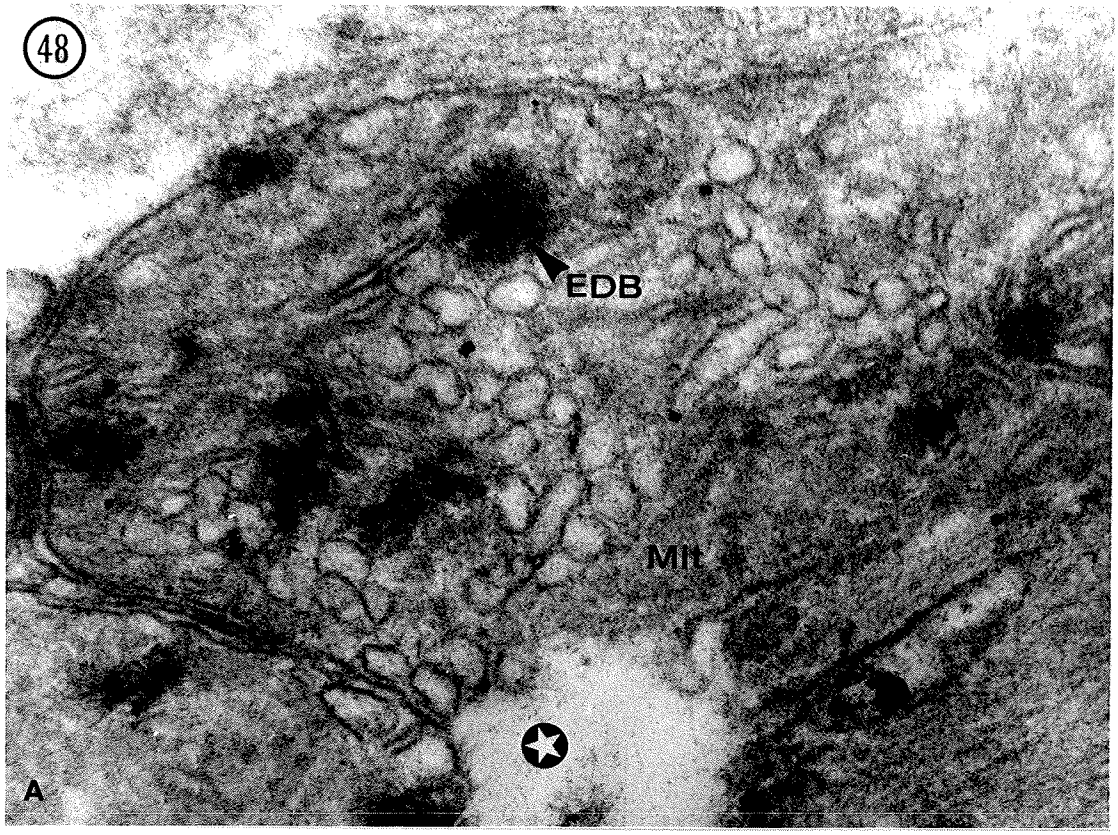

SL
Sp
47

FIG. 48 A. This figure shows a high power electronmicrograph of a mitochondrion with mitochondrial membrane rupture (asterik). Electron dense bodies (EDB) are seen in this mitochondrion. Cristae assume vesicular shape.

Mag. 61, 366 X

FIG. 48 B. Another mitochondrion with vesicles (Ve) and electron dense bodies is seen in this figure. The ultrastructural features observed in this micrograph represent the irreversible damage caused by the isoproterenol insult. Though treated, treatment was after 30 minutes of the insult.

Mag. 60, 300 X


48
EDB
Mit
A

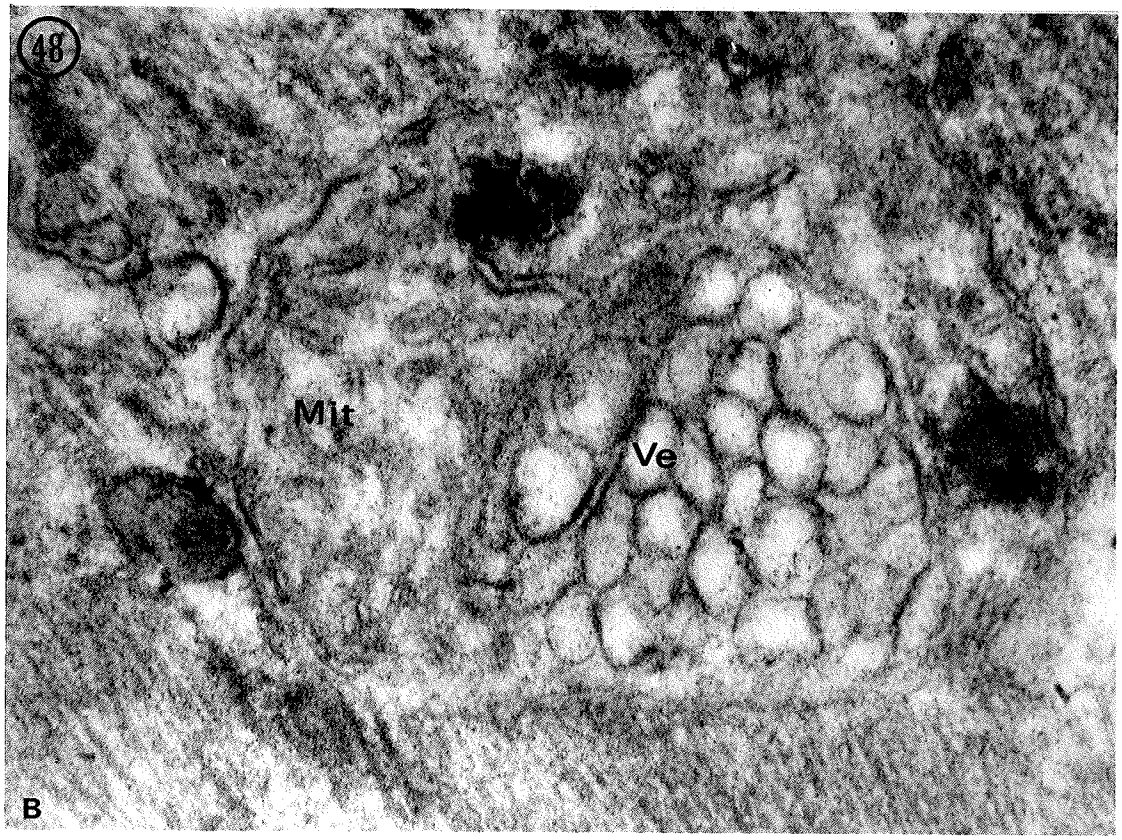
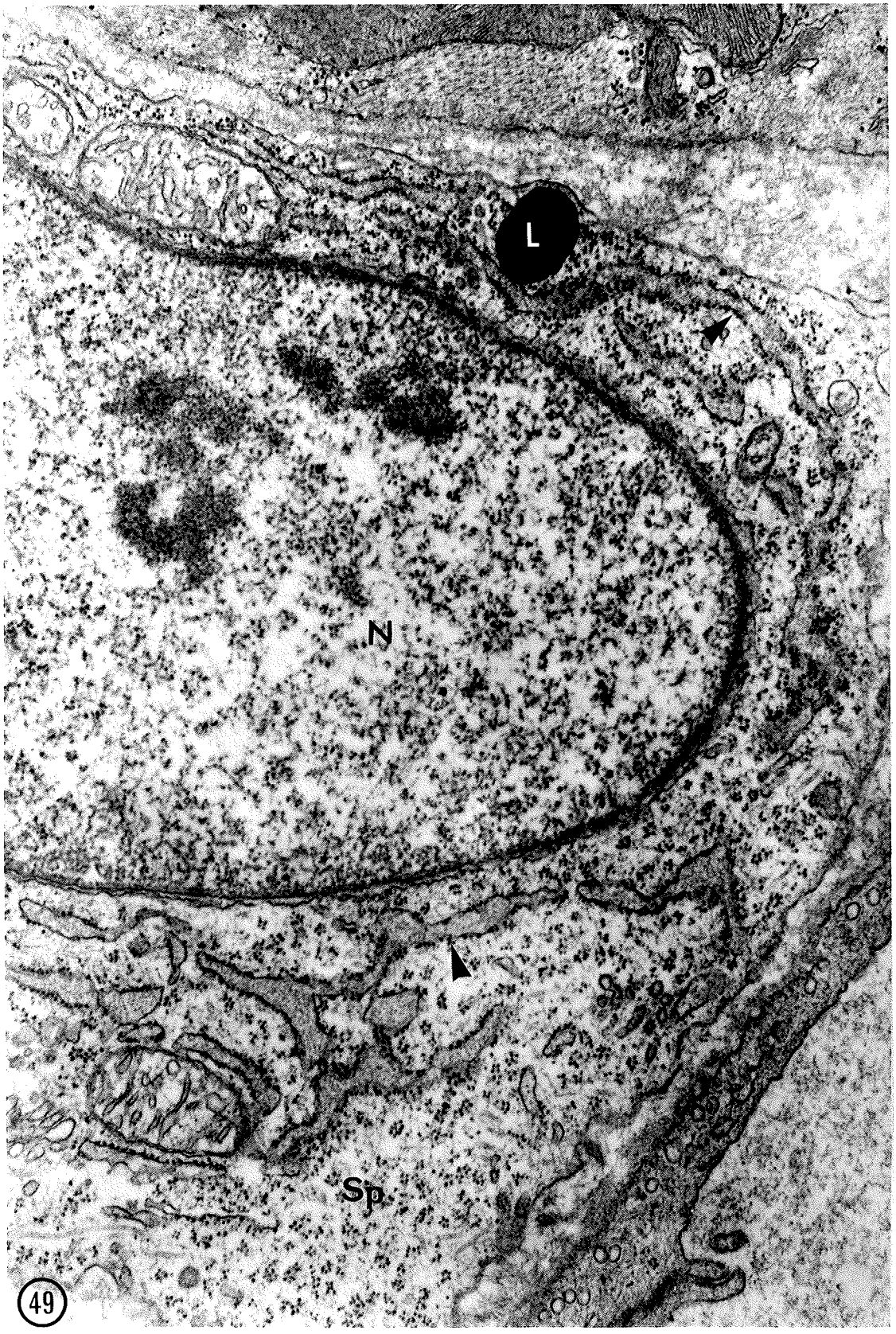

48
Mit
Ve
B

FIG. 49. This figure shows the higher power profile of part of the nucleus (N) with prominent nucleolus. This shows the active functional state of the nucleus. There are numerous endoplasmic reticula with ribosomes attached to them seen outside the nucleus (arrowheads). Mitochondria are with very few cristae. Endothelium is swollen and has numerous pinocytotic vesicles, at the lower right hand side corner.

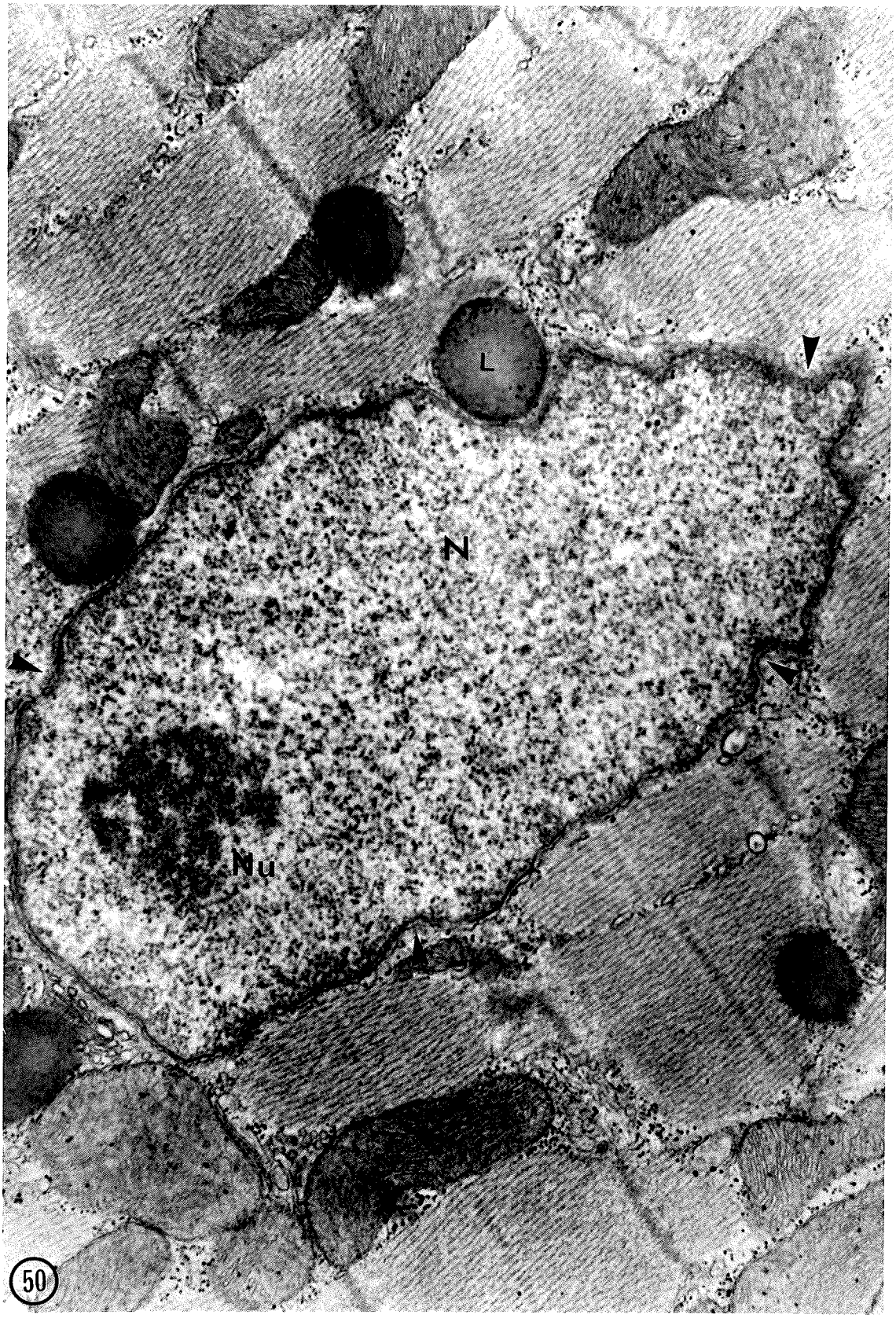
Mag. 28, 025 X



49

FIG. 50. In this figure a nucleus (N) with prominent nucleolus (Nu) is seen. Though there are indentations (arrowheads), the chromatin distribution is uniform. The I-bands of the sarcomeres have disappeared which show the sarcomeres in a state of contracture. Numerous lipid droplets (L) are also seen in the sarcoplasm. This is a typical border zone (transition zone) which shows reversible changes, because of dobutamine administration. Details are given in the text.

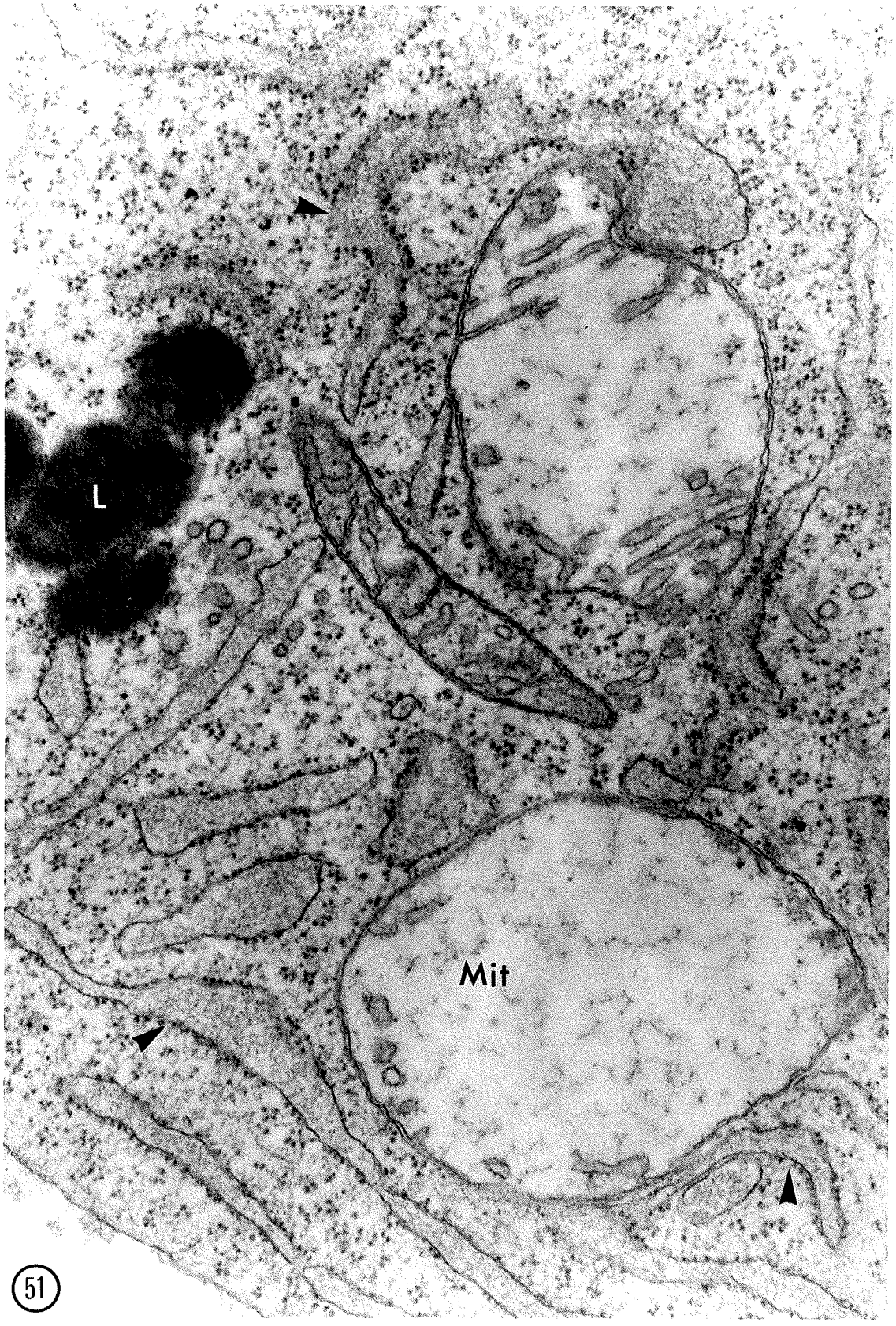
Mag. 32, 150 X



50

FIG. 51. In this electronmicrograph, two mitochondria (Mit) with very few cristae are observed. Aggregation of lipid droplets are also seen in the sarcoplasm. Well developed endoplasmic reticulum with ribosomes on them (arrowheads) is a noteworthy feature of this micrograph.

Mag. 28, 031 X



51

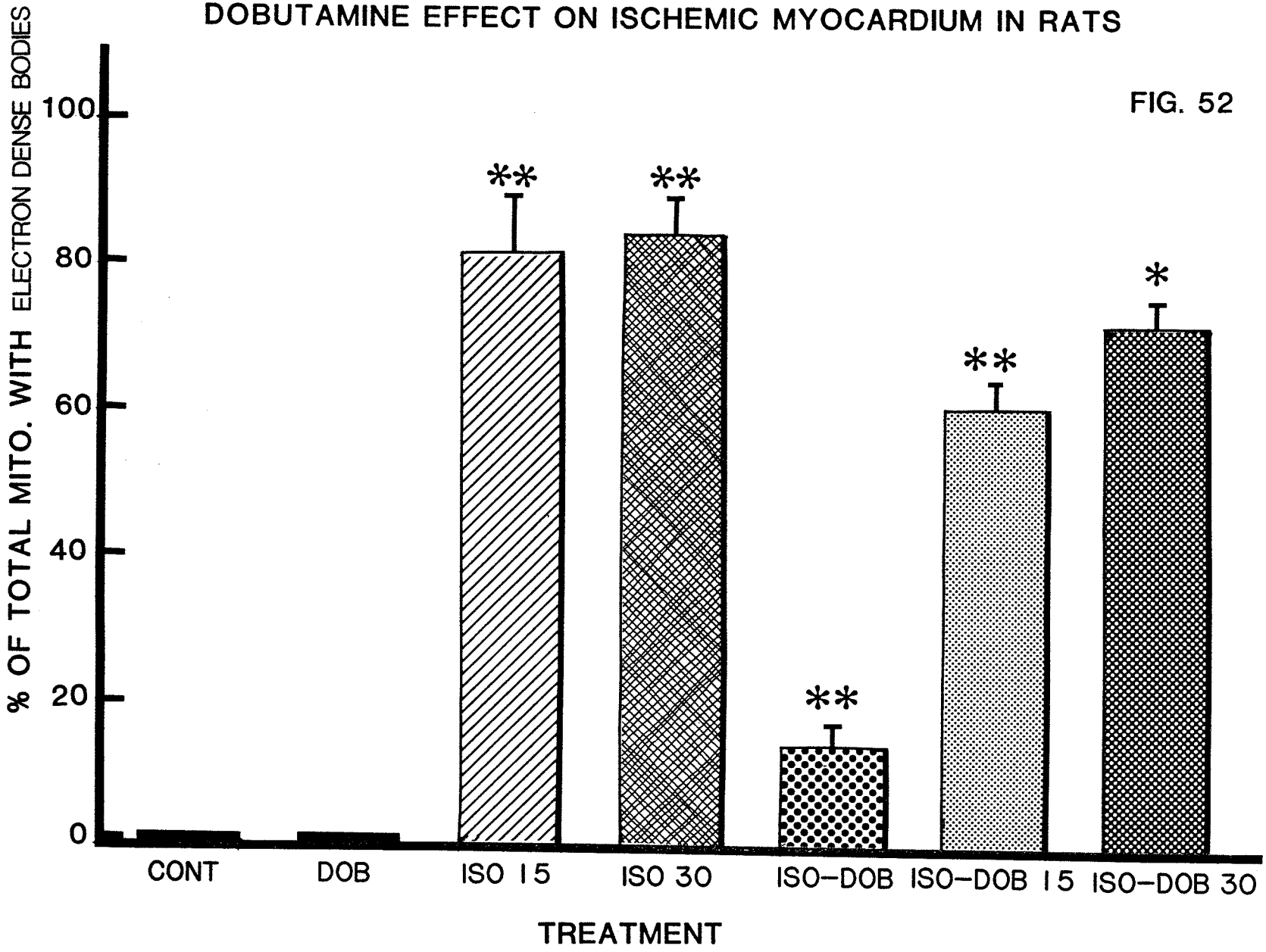

DOBUTAMINE EFFECT ON ISCHEMIC MYOCARDIUM IN RATS
% OF TOTAL MITO. WITH ELECTRON DENSE BODIES
100
80
60
40
20
0
**
**
**
**
*
CONT
DOB
ISO 15
ISO 30
ISO-DOB
ISO-DOB 15
ISO-DOB 30
TREATMENT

FIG. 52

TABLE I

EFFECT OF DOBUTAMINE ON THE FLOCCULENT DENSITIES OF MITOCHONDRIA IN THE ISCHEMIC MYOCARDIUM OF RATS INSULTED WITH ISOPROTERENOL.

GROUP	n	TREATMENT	NUMBER OF MITO. MEAN ± S.E.M.	NUMBER OF MITO. WITH FLOCC DENSITIES. MEAN ± S.E.M.	%
1	4	Control	521.5 ± 4.8	00.00	00.00
2	4	Dob alone, i.v.	472.5 ± 3.7	00.00	00.00
3	4	Iso 40 mg/kg, i.p. (15 min)	545.2 ± 9.8	441.7 ± 7.6	81.01**
4	4	Iso 40 mg/kg, i.p. (30 min)	474.0 ± 5.7	399.5 ± 5.2	84.28**
5	4	Iso 40 mg/kg, i.p. (15 min-Dob)	578.5 ± 7.1	354.5 ± 2.7	61.31**
6	4	Iso 40 mg/kg, i.p. (30 min-Dob)	557.5 ± 4.1	405.1 ± 6.7	72.64*
7	4	Iso 40 mg/kg, i.p. (0 min-Dob)	491.2 ± 6.2	62.0 ± 2.9	12.62**

ANOVA/Duncan's Test for multiple comparisons showed significant ($P<0.01$) differences as follows:- Groups 5&6 differ from groups 3&4. Group 7 differs from all other groups. Groups 3&4, 5&6, 4&6 and 3&6 were NS ($P>0.05$).

C

HEMODYNAMICS OF

a) Normal

b) Ischemic ANIMALS

c) Treated

a) HEMODYNAMICS OF NORMAL ANIMALS.

Blood pressure and electrocardiogram of control group of animals were recorded every 5 minutes for 1 hour during the experiment according to the procedure described in section D of materials and methods. The mean values of blood pressure were 115.25 $\pm$ 3.91 mm Hg. The chart speed was 100 mm/sec, and the heart rate was calculated by multiplying the number of beats/sec by 60. The mean heart rate was 395.00 $\pm$ 15.00 beats/min. These values are illustrated in a tabulated form in Table II.

b) HEMODYNAMICS OF ISCHEMIC ANIMALS.

Blood pressure and electrocardiogram of isoproterenol insulted group were recorded every 5 minutes for 1 hour during the experiment. As a result of ischemia, induced by isoproterenol, there were a significant fall ($P < 0.01$) in blood pressure and a significant increase ($P < 0.01$) in heart rate when compared with the control values. The mean values of blood pressure and heart rate were 76.50 $\pm$ 5.67 mm Hg and 525.75 $\pm$ 17.97 beats/min respectively (Table II). When compared with the control values, the differences were striking.

c) HEMODYNAMICS OF TREATED ANIMALS.

Blood pressure and electrocardiogram were recorded every 5 minutes for 1 hour in all the 4 experimental groups described below.

i) DOBUTAMINE ALONE.

When dobutamine alone was administered, the mean blood pressure dropped to 106.00 ± 1.68 mm Hg from the control value of 115.25 ± 3.91 mm Hg. This was not significant. The mean heart rate increased to 540.00 ± 8.16 beats/min from the control value of 395.00 ± 15.00 which was significant ($P < 0.01$). These results are given in Table II.

ii) ISOPROTERENOL AND DOBUTAMINE (0 TIME).

After isoproterenol insult, dobutamine was administered immediately. Blood pressure and electrocardiogram were recorded every 5 minutes for 1 hour during the experiment. The mean values of blood pressure and heart rate were 89.75 ± 2.32 mm Hg and 552.75 ± 5.85 beats/min respectively. The drop in blood pressure was significant ($P < 0.01$). The increase in heart rate also was significant ($P < 0.01$). The data and tracings are illustrated in Table II.

iii) ISOPROTERENOL AND DOBUTAMINE (15 MINUTES).

Dobutamine was administered 15 minutes after isoproterenol insult. The mean blood pressure was 85.00 ± 2.65 mm Hg and heart rate, 566.25 ± 14.34 beats/min respectively. When compared with the control values, the difference was striking and it was significant, $P < 0.01$, (Table II).

iv) ISOPROTERENOL AND DOBUTAMINE (30 MINUTES).

Dobutamine was administered 30 minutes after isoproterenol insult. The mean blood pressure and heart rate were 80.50 ± 2.02 mm Hg and 570.00 ± 5.67 beats/min respectively. When compared with the control values, the differences were significant ($P < 0.01$).

By multiple comparison between the treated groups, no significance was observed ($P > 0.05$), (Table II).

Note: All mean values of blood pressure and heart rate are expressed as $\pm$ SEM of 4 animals in each group.

TABLE II

EFFECTS OF DOBUTAMINE ON BLOOD PRESSURE AND ELECTROCARDIGRAM OF ISOPROTERENOL INSULTED RATS

GROUP	n	TREATMENT	BLOOD PRESSURE (mm Hg)	HEART RATE (Beats/min)
1	4	Control - normal saline	115.25 ± 3.91	395.00 ± 15.00
2	4	Isoproterenol 40 mg/kg, i.p.	76.50 ± 5.67 **	525.75 ± 17.97 **
3	4	Dobutamine 30 µg/kg/min i.v.	106.00 ± 1.68	540.00 ± 8.16 **
4	4	Isoproterenol - 0 min- Dobu.	89.75 ± 2.32 **	552.75 ± 5.85 **
5	4	Isoproterenol -15 min- Dobu.	85.00 ± 2.65 **	566.25 ± 14.34 **
6	4	Isoproterenol -30 min- Dobu.	80.50 ± 2.02 **	570.00 ± 5.67 **

ANOVA / Duncan's Test for multiple comparisons showed significant differences as follows:-

Blood Pressure ..... Group 1 Vs groups 2, 4, 5, 6 (**); Group 3 Vs groups 4, 5, 6 (**)
Group 2 Vs group 3 (**); Group 2 Vs group 4 (*); All other groups NS

Heart Rate ..... Group 1 Vs groups 2, 3, 4, 5, 6 (**); Group 2 Vs group 5 (*);
Group 2 Vs group 6 (*); All other groups were NS.

* = $P < 0.05$ ** = $P < 0.01$ NS = $P > 0.05$

D

BIOCHEMICAL RESULTS

D. BIOCHEMICAL RESULTS.

i) CREATINE PHOSPHOKINASE ACTIVITY.

Table III and the bar graph in Fig. 53 show that the activity of creatine phosphokinase varies significantly in different groups of animals. However, the activity of this enzyme in the control (normal saline injection) and normal (no injection) groups was not different. The activity of creatine phosphokinase is measured as UI/min. The enzyme activity in the normal and control groups were 375.68 $\pm$ 6.48 UI/min and 364.32 $\pm$ 30.25 UI/min respectively (nonsignificant). In the isoproterenol insulted group, the activity was highest, 490.08 $\pm$ 26.03, significantly different from the control and normal groups ($P < 0.01$).

Dobutamine alone when administered, reduced the enzyme activity significantly ($P < 0.01$) to 277.79 $\pm$ 9.16. Between the treated groups there was significance ($P < 0.05$). All the treated groups showed significant differences, $P < 0.01$, when compared with control or insulted groups (Table III, Fig. 53). An interesting observation was seen when dobutamine was given first and isoproterenol insult 30 minutes later. In this group, the enzyme activity was significantly lower, $P < 0.01$, when compared with control as well as with the insulted groups. Two groups treated with propranolol were included in the experiment because of the well established results obtained from propranolol studies. However, though the corresponding bar graphs of propranolol treated groups are not included in the Fig. 53, the corresponding data are presented in Table III.

TABLE III

EFFECTS OF DOBUTAMINE TREATMENT ON SERUM CK IN ISOPROTERENOL INSULTED RATS, CK ACTIVITY:

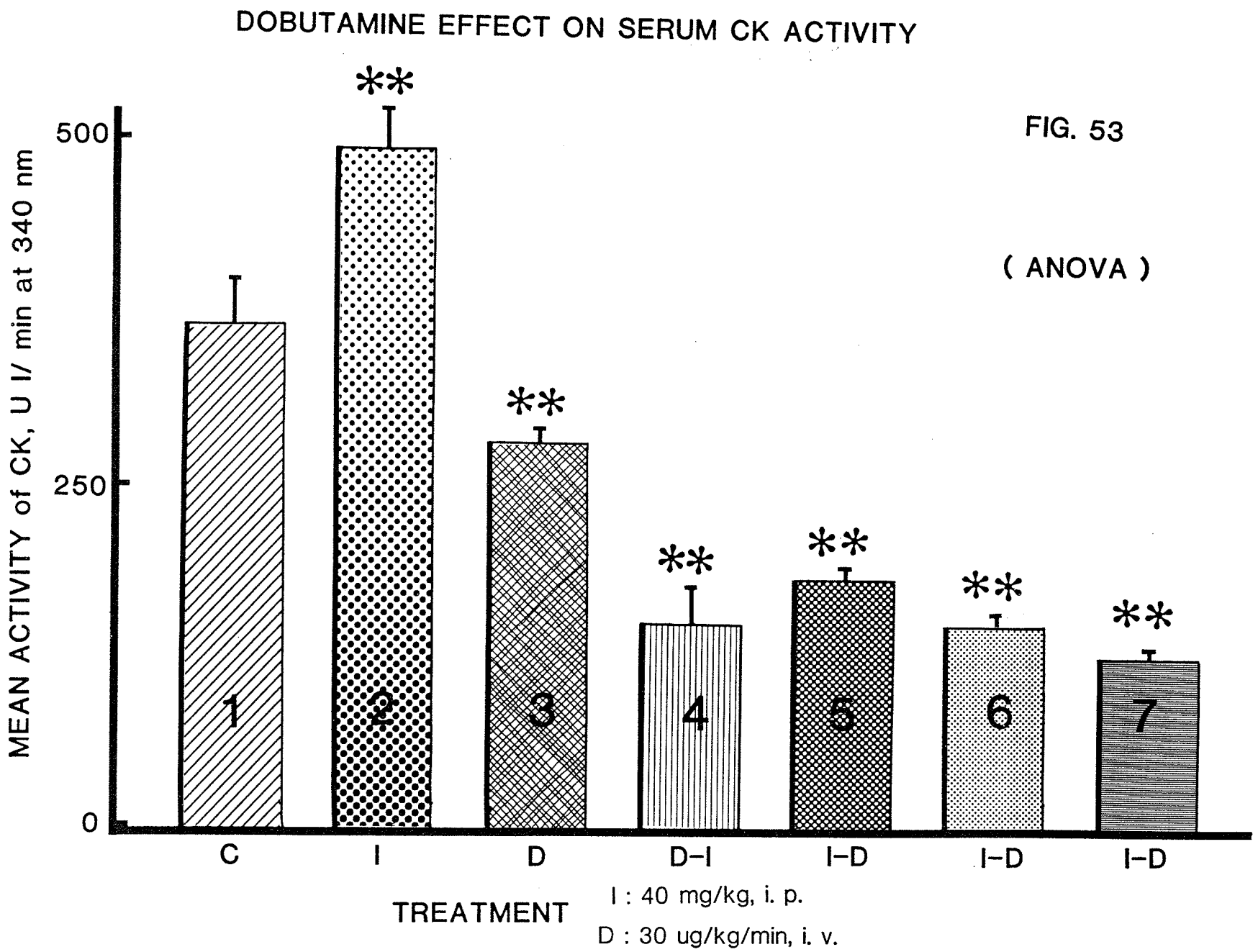
GROUP	n	TREATMENT	DOSE	ROUTE	SERUM AFTER	CK ACTIVITY, MEAN ± S.E.M.
1	4	Normal	::	::	::	375.68 ± 6.48
2	4	Control	Saline	i.p.	18 HRS	364.32 ± 30.25
3	4	Isoproterenol	40 mg/kg	i.p.	18 HRS	490.08 ± 26.03 **
4	4	Propranolol	40 mg/kg	i.p.	,,	342.33 ± 17.65
5	4	Pro-30 min Iso	,, ,,	,,	,,	172.95 ± 4.65 **
6	4	Dobutamine	30 µg/kg	i.v.	,,	277.79 ± 9.16 **
7	4	Dob-30 min Iso	,, + 40	i.v. + i.p.	,,	147.79 ± 15.43 **
8	4	Iso-30 min Dob	40 mg/kg 30 µg/kg	i.p. i.v.	,,	177.56 ± 6.61 **
9	4	Iso-15 min Dob	,, ,,	,,	,,	151.46 ± 6.04 **
10	4	Iso-0 min Dob	,, ,,	,,	,,	124.44 ± 3.28 **

ANOVA/Duncan's Test for multiple comparisons showed significant ($P<0.01$) difference as follows: Group 1 differs from 3,5,6,7,8,9,10. Group 2 differs from 3,5,6,7,8,9,10. Group 3 differs from all other groups. Groups 5&10 and 8&10 were significant ($P<0.05$) as well. Other groups were NS ($P>0.05$)

FIG. 53. Effect of dobutamine on the creatine phosphokinase (CPK or CK) activity in the serum of control, isoproterenol insulted, isoproterenol insulted and dobutamine treated groups of animals. Note *: Blood samples were collected 18 hours after the insult and treatment.

Groups

1. C = Control Group
2. I = Isoproterenol Insulted Group
3. D = Dobutamine Treated Group
4. D-I = Dobutamine First and Isoproterenol 30 min. later
5. I-D = Isoproterenol First and Dobutamine 30 min. later
6. I-D = Isoproterenol First and Dobutamine 15 min. later
7. I-D = Isoproterenol First and Dobutamine immediately after


DOBUTAMINE EFFECT ON SERUM CK ACTIVITY
FIG. 53
(ANOVA)
MEAN ACTIVITY of CK, U I/ min at 340 nm
500
250
0
**
**
**
**
**
**
1
2
3
4
5
6
7
C
I
D
D-I
I-D
I-D
I-D
TREATMENT
I : 40 mg/kg, i. p.
D : 30 ug/kg/min, i. v.

ii) LACTATE DEHYDROGENASE ACTIVITY.

The data presented in Table IV and bar graph in Fig. 54 show that the activity of lactate dehydrogenase enzyme varies in different groups of animals. The mean activity of control group was 831.65 $\pm$ 58.49 UI/min. The serum samples from the isoproterenol insulted group showed the highest activity (1431.39 $\pm$ 37.69), which was significantly greater ($P < 0.01$). All the insulted and treated groups showed significant differences ($P < 0.01$). The significance between the treated groups varied depending upon the time of drug administration before or after the induced ischemia (Table IV, Fig. 54). Dobutamine has a profound influence in restricting the leakage of enzymes from the ischemic or normal myocardium as does propranolol.

Though the data on the effect of propranolol on the lactate dehydrogenase activity in the serum are given in Table IV, these data are omitted in Fig. 54. Since the effects of propranolol have already been established by other investigators, we included the propranolol group in the present study to confirm two aspects: 1) To establish the effects of propranolol in the release of LDH in normal animals. 2) To substantiate the validity on the effect of dobutamine in the release of LDH in normal and isoproterenol insulted groups of animals.

TABLE IV

EFFECTS OF DOBUTAMINE TREATMENT ON SERUM LDH IN ISOPROTERENOL INSULTED RATS

GROUP	n	TREATMENT	DOSE	ROUTE	SERUM AFTER	ACTIVITY: MEAN ± S.E.M.
1	4	Normal	::	::	18 HRS	836.96 ± 83.65
2	4	Control	Saline	i.p.	,, ,,	831.65 ± 58.49
3	4	Isoproterenol	40 mg/kg	,,	,, ,,	1431.39 ± 37.69**
4	4	Propranolol	,, ,,	,,	,, ,,	707.17 ± 26.77*
5	4	Prop-30 min Iso	,, ,,	,,	,, ,,	358.64 ± 15.03**
6	4	Dobutamine	30 ug/kg	i.v.	,, ,,	607.42 ± 18.60**
7	4	Dob-30 min Iso	,, + 40	,, + i.p.	,, ,,	366.21 ± 12.50**
8	4	Iso-30 min Dob	40 mg/kg 30 ug/kg	i.p. i.v.	,, ,,	692.65 ± 13.36*
9	4	Iso-15 min Dob	,, ,,	,,	,, ,,	521.95 ± 11.31**
10	4	Iso-0 min Dob	,, ,,	,,	,, ,,	299.85 ± 6.79**

ANOVA/Duncan's Test for multiple comparisons showed significant ($P<0.01$) differences as follows: Group 1 differs from 3,4,5,6,7,8,9,10; group 2 differs from 3,4,5,6,7,8,9,10; groups 1&4, 1&8, 2&4, 2&8 showed significant ($P<0.05$) differences. All other groups 10&5, 5&7, 9&6, 6&8, 8&4, 1&2, 10&7 and 6&4 were NS ($P>0.05$).

FIG. 54. Effect of dobutamine on the lactate dehydrogenase (LDH) activity in the serum of control, isoproterenol insulted, isoproterenol insulted and dobutamine treated groups of animals. Note *: Blood samples were collected 18 hours after the insult and treatment.

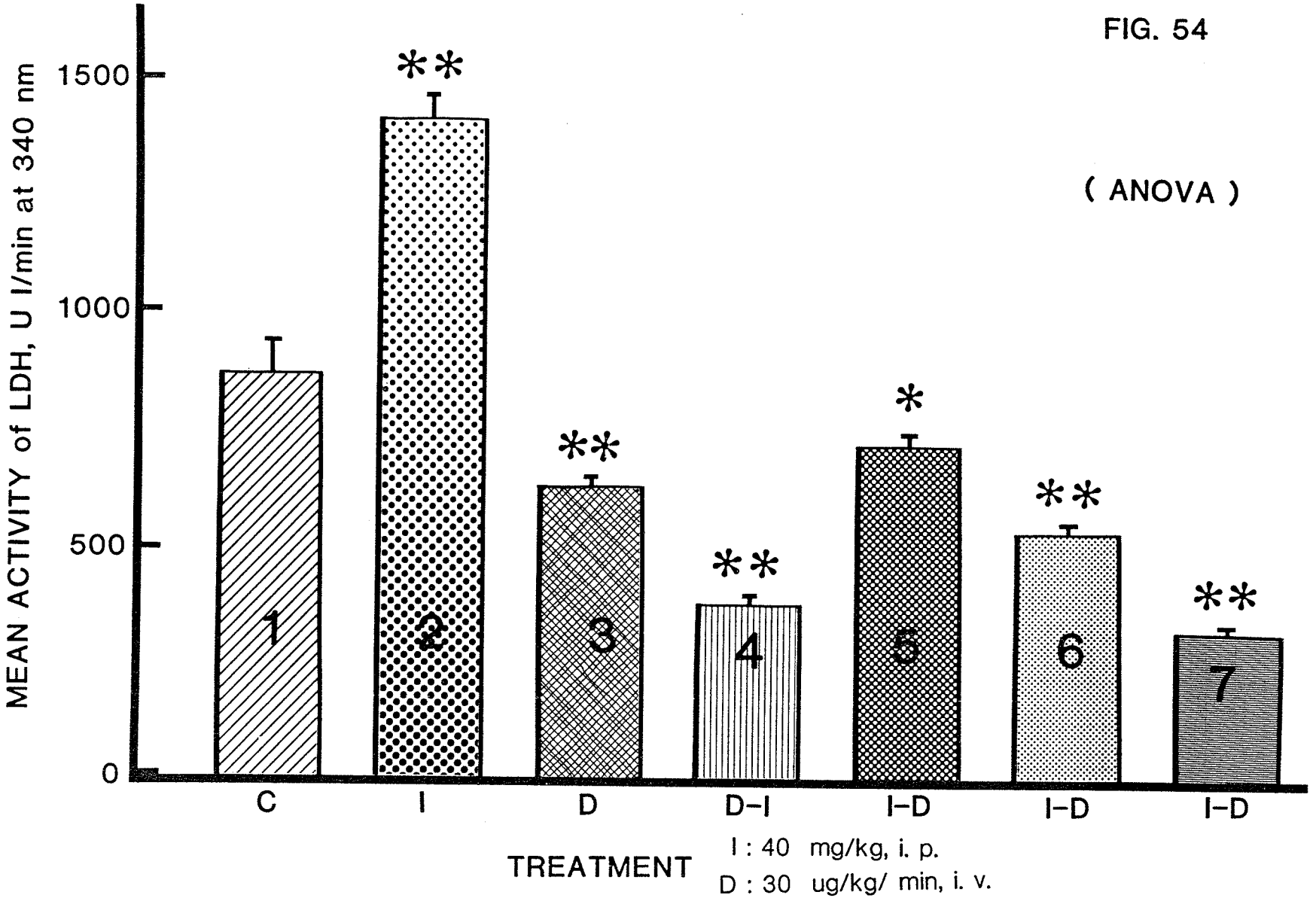
Groups:

1. C = Control Group
2. I = Isoproterenol Insulted Group
3. D = Dobutamine Treated Group
4. D-I = Dobutamine First and Isoproterenol 30 min. later
5. I-D = Isoproterenol First and Dobutamine 30 min. later
6. I-D = Isoproterenol First and Dobutamine 15 min. later
7. I-D = Isoproterenol First and Dobutamine immediately after

DOBUTAMINE EFFECT ON SERUM LDH ACTIVITY

FIG. 54

(ANOVA)



V

DISCUSSION

DISCUSSION

A. General Features of the Ischemic Myocardium.

B. Ultrastructural Features of the Myocardium of Isoproterenol Insulted and Dobutamine Treated Animals.

1) Glycogen

2) Mitochondria

3) Lipid metabolism

C. Biochemical Aspects

Release of enzymes

1) Creatine phosphokinase

2) Lactate dehydrogenase

D. Hemodynamic Evaluations

Effects of dobutamine on the hemodynamics of normal and isoproterenol insulted groups of animals.

A. GENERAL FEATURES OF THE ISCHEMIC MYOCARDIUM

Myocardial ischemia means deficiency of blood in a part of the myocardium, due to functional constriction or actual obstruction of a blood vessel (Jennings, 1981). This is true because of the fact that ligation of the left anterior descending or circumflex coronary arteries in an experimental animal model results in the reproducible ischemic cell death of the entire free wall of the left ventricular myocardium (Chien et al., 1979).

Since myocardial lesions produced by large doses of a catecholamine (isoproterenol) have resemblances to myocardial ischemia produced by coronary artery ligation, the catecholamine-induced lesions may be called myocardial ischemic alterations. So the isoproterenol-induced damage may be interpreted as ischemic. The most generally accepted mechanism of myocardial necrosis produced by isoproterenol is through the positive inotropic and chronotropic actions of this catecholamine, which increases the workload of the heart to such an extent that myocardial oxygen demand exceeds the supply which in turn results in a relative myocardial ischemia (Rona et al., 1959; 1963; Handforth, 1962; and Balazs et al., 1972).

It has been suggested by Beamish et al. (1984) that the initial vasodilation due to catecholamines was associated with the activation of β_2-adrenergic receptors of the vasculature by the exogenous or endogenous catecholamines and implicated this in the development of a coronary artery spasm that might cause myocardial lesions. The direct

action of the naturally occuring catecholamine norepinephrine, the level of which increases during ischemia, on the coronary arteries is through the activation of β-adrenergic receptors. Similar conclusions have also been drawn by Braunwald (1981), who employed in vivo dog heart preparations and indicated that the coronary vasoconstriction by norepinephrine is associated with the activation of α-adrenergic receptors in the coronary smooth muscles. Functional hypoxia may attenuate the nor-epinephrine-induced coronary constriction initially, but prolonged hypoxia itself was observed to produce a coronary constriction (Beamish et al., 1984).

It is now well established that administration of large doses of the synthetic catecholamine, isoproterenol, or the naturally occuring catecholamines, epinephrine or norepinephrine produces myocardial lesions in experimental animals (Rona et al., 1959; 1963; Ferrans et al., 1964; Rosenblum et al., 1965; and Bloom and Davis, 1972). Though the mechanism by which catecholamines cause myocardial necrosis is not clear yet, several suggestions have been offered, such as intracellular calcium overload (Fleckenstein et al., 1973; 1974), mobilization of free fatty acids (Corr and Sobel, 1983), depletion of high energy phosphates (Fleckenstein et al., 1974; and Takenaka, 1975), and imbalance of electrolytes in the myocardial cell (Nayler, 1981). The elevated intracellular calcium appears to be due to enhanced calcium influx resulting from catecholamine-induced opening of membrane Ca^{++} channels and increased membrane permeability caused by membrane damage (Mallov, 1984).

In the present study, the ultrastructural features observed were consistent with the findings of other investigators. The remarkable reduction in glycogen granules, swollen myocardial cells, minor contraction (Caulfield - Klionsky type) bands with wide Z lines, scalloping of sarcolemma, irregular shape of the nucleus with aggregation of chromatin at the periphery of nucleus, some mitochondria with altered definition of cristae and some with electron dense bodies, have been observed in the myocardium where ischemia lasted for 15 minutes. These observations I made substantiate the findings of Bloom and Cancilla (1969), Bloom and Davis (1972), and Nirdhinger and Bramante (1974). The extent of myocardial damage produced by isoproterenol administration was dose and time dependent. Yeager and Iams (1981) tried different dose levels of isoproterenol in several groups of rats and concluded that the extent of cardiac lesions was dose dependent. Depending on the dosage and dose regimen, subcutaneous injections of isoproterenol have been shown to reliably produce either a multiple disseminated necrosis or a massive necrosis that resembles experimental myocardial infarction produced by coronary artery ligation (Rona et al., 1963). It has been observed that as time progresses, the severity of ischemic damage increases. Yeager and Iams (1981), based on the dose response study of isoproterenol (2.5 mg/kg to 250 mg/kg subcutaneously) on the hemodynamics of cardiac failure, supported the existing theory that isoproterenol-induced myocardial damage was due to a relative myocardial hypoxia produced by arterial hypotension and myocardial hyperactivity.

According to a study conducted by Yates and Dhalla (1975), and Dhalla et al. (1978) on the isolated rat heart preparation, it has been suggested that the cardiotoxic effects of catecholamines may be due to the oxidation products of catecholamines formed in the blood or tissues. When the hearts were perfused with fresh isoproterenol, they could not demonstrate any necrotic changes, whereas with oxidized isoproterenol perfusion they could demonstrate extensive ultrastructural damage. This is controversial since there are evidences to show that fresh isoproterenol solution can cause lesions in isolated myocardial cells in vitro. There is strong evidence that adrenochrome and other oxidation metabolites of catecholamines can cause myocardial necrosis and contractile failure in the rat heart (Beamish et al., 1981; Singal et al., 1981). It has also been demonstrated that the autoxidation of catecholamines results in the formation of free radicals which are highly cytotoxic (Cohen and Heikkila, 1974; Sachs and Jonsson, 1975; Graham et al., 1978). Therefore the free radicals may play an important role in catecholamine-induced cardiotoxicity by causing peroxidation of membrane phospholipids which can result in permeability changes in the sarcolemma thereby causing intracellular calcium overload (Singal et al., 1982).

B. ULTRASTRUCTURAL FEATURES OF THE MYOCARDIUM OF ISOPROTERENOL INSULTED AND DOBUTAMINE TREATED ANIMALS

The ultrastructural features observed in the myocardium of isoproterenol-insulted and dobutamine treated groups of animals showed striking differences. The rats treated with dobutamine alone showed myocardium much more close to the normal.

1) GLYCOGEN

Glycogen granules are found in large amounts in cardiac muscle (Figs. 1, 2, 3). They are located in the interfibrillary spaces and also between myofilaments. These granules (15-35 nm in diameter) which stain heavily with lead, are uniformly dispersed (β-particles) but here and there they occur in groups (α-particles), (Sommer and Johnson, 1979).

There was a marked reduction in the amount of glycogen granules in the myocardium of isoproterenol-insulted groups of animals. Reduction of glycogen granules provides further evidence for the suggestion that glycogen is used heavily as fuel during ischemia. These observations agree with the earlier findings of Ferrans et al. (1969) and substantiate those of Fishbein et al. (1981).

The group which received dobutamine infusion alone, showed increased mobilization of glycogen granules in the sarcoplasm. Some of these granules appeared in clusters as well (α-particles). The increased mobilization of these granules may be due to the differential effects of dobutamine on the contractility of myocardium, since dobutamine is a direct-acting inotropic agent whose activity results from stimulation of the β_1-receptors of the myocardium.

When compared with the groups given isoproterenol alone, the glycogen particles in the groups with isoproterenol insult and dobutamine treatment were far more numerous. This observation provides

evidence for the suggestion that glycogen is not used heavily and the actual mechanism of action of dobutamine in preserving glycogen is not understood clearly at this time. During ischemia or hypoxia the myocardium depends upon anaerobic metabolism and consequently glycogen is broken down to form substrate for the glycolytic pathway. But this has not happened in the present situation. So the retention of glycogen granules in the ischemic myocardium of rats treated with dobutamine is an important finding which has not been observed or established before by other investigators.

Improved subendocardial blood flow and increased subendocardial to subepicardial flow ratio in patients with severe congestive cardiomyopathy after dobutamine administration has been demonstrated by Unverferth et al. (1983). The hypothesized mechanism of action of dobutamine may be the improved hemodynamics with improved subendocardial blood flow leading into improved myocardial energetics (represented by the ATP/creatine ratio of 0.36 ± 0.24 to 0.62 ± 0.26) which prolongs the clinical manifestations in cardiac patients (Unverferth et al., 1983).

2) MITOCHONDRIA

Mitochondrial alterations observed in the ischemic myocardium of different groups of animals showed varying stages of structural damage like swollen mitochondria with altered definition of cristae, some with electron dense bodies, some without any cristae and some with autophagic vacuoles (Figs. 13, 19, 24, 25, 26 and 27). These results

confirm the observations of Ferrans et al. (1969), Bloom and Davis (1974), Nirdlinger and Bramante (1974), and Gottlieb et al. (1981).

In the normal myocardium electron dense bodies are very rare in the mitochondria and even when there are some, the proportion is less than 1% of mitochondria. The percentage of mitochondria with electron dense bodies is an important criterion because of the fact that such electron dense bodies are seen in degenerating or ischemic cells (Jennings and Ganote, 1974; Jennings et al., 1978; Kawamura et al., 1978) and an improved myocardial cell would have fewer such bodies (Unverferth et al., 1980). These results confirm and strengthen our observations.

Different investigators have different concepts about the origin and composition of the electron dense bodies in the mitochondria of ischemic myocardium. Bloom and Cancilla (1969) are of the opinion that the electron dense bodies are made up of calcium. According to Jennings and Ganote (1972), the electron dense bodies are calcium phosphate deposits. Kawamura et al., (1978) think that these bodies probably result from the precipitation or denaturation of protein in the matrix. Neely and Feuvray (1981) said that the amorphous densities would represent a rearrangement of the normal myocardial lipids. Because of Ca^{2+} influx and ATP depletion, these electron dense bodies may be $CaPO_4$ deposits. More work is warranted to establish the validity of these propositions.

The extent of mitochondrial damage in the ischemic myocardium, treated with dobutamine, depended upon the time of drug therapy after the isoproterenol insult. The earlier the drug was administered, the lesser was the extent of damage (Figs. 36-51). Dobutamine significantly decreased the percentage of mitochondria with electron dense bodies, from 81% in the isoproterenol-insulted group to 61% in the insulted and dobutamine treated group (Table I, Fig. 52). These results agree with the only other data, available at present, obtained from the endomyocardial biopsy study of Unverferth et al. (1980) in the myocardium of patients with congestive cardiomyopathy treated with dobutamine, where they showed a decrease in the mean electron dense bodies in the mitochondria, from 84 $\pm$ 14 to 65 $\pm$ 12. These investigators administered dobutamine intravenously for 72 hours, at a starting dose of 2.5 ug/Kg/min, increasing every 30 min by 2.5 ug/Kg/min, up to a maximum dose of 15 ug/Kg/min. The results published by Unverferth et al. (1980) prompted us to investigate the effects of dobutamine on the electron dense bodies in the mitochondria of ischemic myocardium, with higher dose, 30 ug/Kg/min, and shorter duration of infusion time, 60 min. We found the same results as Unverferth et al. (1980). Though we examined the mitochondria in the myocardium of dobutamine alone treated group, we could not find any electron dense bodies.

The mechanism by which dobutamine induces the morphological and pharmacological changes in the isoproterenol-induced ischemic myocardium is not known yet. The possible mechanism for the structural improvement observed may be related to the increased coronary blood

flow. Dobutamine not only increases the cardiac output, but dilates the coronary arteries also (Baron et al., 1972). The increased coronary perfusion pressure and subsequent increase in blood flow might have enalbed the ischemic regions of the subendocardium from the hypoxic damages.

3) LIPID METABOLISM

Under aerobic conditions, the heart preferentially utilizes free fatty acids as substrate for energy production (Entmann and McMillin-Wood, 1983). When the free fatty acids enter the myocardial cell, they are either oxidized or esterified into phospholipids or neutral lipids that form a structural component of sarcolemma. The phospholipids and neutral lipids contain coenzyme A (acyl-CoA). The high activity of the acyl-CoA-carnitine transferase system assures transport of acyl-CoA from the cytosol into the mitochondria where this coenzyme is incorporated into the tricarboxylic acid cycle and oxidized to CO_2 and H_2O.

In the presence of myocardial ischemia marked changes in fatty acid metabolism occur (Kloner and Braunwald, 1980). Beta-oxidation of fatty acids is inhibited by limited oxygen availability and eventually there will be inhibition or loss of long-chain acyl-carnitine transferase enzyme activity necessary for the transport of acyl-CoA into the mitochondria for oxidation (Wood et al., 1973). Consequently the concentration of acyl-CoA in the cytosol increases which leads to increased synthesis and accumulation of triglycerides in the cell.

Accumulation of acyl-CoA may also be deleterious because it inhibits further formation of CoA-esters of fatty acids. So the fatty acids which enter the cell cannot be esterified and are trapped (Brachfeld et al., 1972).

Recently, the accumulation of harmful phospholipids within myocardial cells have been implicated in the development of arrhythmias during myocardial ischemia. Corr and Sobel (1983) showed the increase in the concentration of lysophosphoglycerides (lysophosphatidyl choline and lysophosphatidylethanolamine), the phospholipid metabolites, in the ischemic myocardium of isolated canine purkinje fibers and suggested the role of these metabolites in the development of malignant arrhythmias.

The presence of large number of lipid droplets observed (Figs. 18, 27 and 30) indicates the high level of circulating free fatty acids and the impairment of fatty acid metabolism during myocardial ischemia. However, such lipid droplets were not seen in large numbers in the dobutamine treated groups of animals. This may be due to the increased blood flow in the subendocardium because of the increased coronary perfusion pressure. Further investigation is needed to understand the role of dobutamine in the fatty acid metabolism of ischemic heart.

C. BIOCHEMICAL ASPECTS

The comparative studies of Rosenblum et al. (1965) on the cardiovascular effects of lesion-producing doses of sympathomimetic

agents have led to the recognition of 3 types of pathogenic mechanisms: 1) metabolic changes, 2) hemodynamic disturbances and 3) myogenic alterations related to a reduction in myocardial contractile force. The final common pathway for the production of myocardial lesions by large doses of catecholamines, according to Rona et al. (1959), Rona et al. (1963) and Rosenblum et al. (1965), is a transcellular electrolytic imbalance being the unifying factor which promotes irreversible derangement of cellular metabolism with resultant necrosis. Irreversible damage refers to permanent damage where reoxygenation or reperfusion cannot revert the changes to normal structure.

RELEASE OF ENZYMES

The mechanism of enzyme release from myocardial cells during irreversible damage has not been established yet (Ganote et al., 1983). Some of the enzymes have been demonstrated to be rapidly depleted when permanent damage occurs to the myocardium during myocardial infarction (Entman and McMillin-Wood, 1983). The enzyme release is associated with morphological changes observed in the sarcolemma and subcellular organelles like the mitochondria (Figs. 26-30). It appears that the enzymes escape into the lymph and finally into the circulation after myocardial damage. Measuring the activity of enzymes in the plasma or serum has become a very valuable indicator in the diagnosis of myocardial infarction (Wexler, 1970) and detection and quantitation of enzymes will show the extent of myocardial damage. Some of the enzymes released into the circulation during myocardial infarction are:- creatine phosphokinase, lactate dehydrogenase, serum glutamic

oxalacetic transaminase, glutamic pyruvate transaminase and citrate synthetase.

1) CREATINE PHOSPHOKINASE

The creatine phosphokinase activity measured in the serum of isoproterenol-insulted group of animals in this study corresponds to the data established by Kluge (1969) in the human and Wexler (1970) in rats. Wexler (1970) showed remarkably higher activity because of the fact that he used 50 mg of isoproterenol/100 g of body weight of Sprague-Dawley rats. The creatine phosphokinase activity as shown by Kluge (1969) in patients with myocardial infarction was 60-70 units more than in our current finding. Depending upon the severity of damage, the enzyme activity measured in the serum or plasma may vary.

It is evident from the data in Table III and corresponding bar graph in Fig. 53 that there is a significant reduction in the activity of creatine phosphokinase released into the blood from the isoproterenol-induced lesioned areas of the heart, treated with dobutamine. The significance varied in different groups, depending upon the time of dobutamine administration after isoproterenol insult. There are no data available at present for comparison with the results of this study. The only available information is from the work of Unverferth et al. (1983), who observed a significant increase in the measurement of the ATP/creatine ratio in patients with congestive cardiomyopathy who had dobutamine treatment for 3 days. It has been established that during myocardial infarction, there is ATP and

creatine phosphate depletion from the infarcted myocardial tissue (Hearse, 1979). In an isolated rat heart preparation, where anoxia was induced experimentally, Hearse (1979) showed with a high speed freeze-clamp technique that the ATP levels decreased by 25% and creatine phosphate by 50%. The elevated level of enzyme activity in the serum corresponds to the depletion of the substrates of such enzymes in the tissues.

The creatine phosphokinase activity obtained from the group treated with dobutamine alone also showed a significant decrease in the enzyme activity when compared with the values of normal or control groups. The normal leakage of enzyme into the blood also has been reduced by dobutamine. This may be due to the maintenance of sarcolemmal integrity and the increased utilization of creatine and inorganic phosphate for the production of ATP.

2) LACTATE DEHYDROGENASE

Lactate dehydrogenase (LDH) activity in the serum of isoproterenol-insulted and dobutamine treated groups of animals in this study varied significantly when compared with the value obtained from the isoproterenol-insulted group as shown in Table IV and the corresponding bar graph in Fig. 54. In the isoproterenol-insulted group, the LDH activity observed was 1431 $\pm$ 38 which corresponds to the LDH activity in patients with myocardial infarction, as established by Klugue (1969).

The variation in the LDH activity in the different groups of isoproterenol-insulted and dobutamine treated animals reflected the time difference in the administration of dobutamine after the insult. When the drug was administered immediately after the myocardial insult, less LDH activity was measured, indicating less damage to the myocardium.

The effect of dobutamine on the activity of these two enzymes, CPK and LDH in the serum of experimentally induced myocardial ischemia may be similar to the effect of propranolol where propranolol improves mitochondrial function in ischemic myocardium (Nayler et al. 1978). Animals treated with propranolol before coronary artery ligation, showed a reduction in mitochondrial edema (Kloner et al., 1978). These observations are consistent with the results we obtained in our study where the propranolol treated group showed a significant reduction in the CPK and LDH activites in the serum. This reduction in the enzyme activity may be due to the preservation of the functional and structural aspects of mitochondria in the infarcted area by the drug dobutamine or propranolol. The action of propranolol in reducing the size of myocardial infarct is related to the reduction of myocardial oxygen consumption resulting from the blocking sympathetic influences on heart rate and contractility (Maroko et al., 1971; Reimer et al., 1976; and Hillis et al., 1979).

The reduction of CPK and LDH levels in the serum of isoproterenol-insulted and dobutamine treated groups of animals in this study points to the same conclusion. Dobutamine may have improved: 1)

the mitochondrial structure, 2) structure of the other organelles in the cytosol and 3) the sarcolemmal integrity, as evidenced from the Figs. 36-51.

D. HEMODYNAMIC EVALUATION

The mean values of blood pressure and heart rate recorded in Table II illustrate the significant fall in blood pressure and increase in heart rate in the isoproterenol-insulted group of animals when compared with the control group. The blood pressure fell from 115 mm Hg in the control to 76 mm Hg in the insulted group. The heart rate increased from 395 beats/min in the control to 525 beats/min in the insulted group. These values are consistent with the values obtained by Strubelt and Siegers (1975) which were 117 to 72 mm Hg and 326 to 497 beats/min, the blood pressure and heart rate in the control and insulted groups respectively.

The fall in blood pressure and increase in heart rate in the insulted group were due to the positive chronotropic effects of isoproterenol on the β_1-receptors of the myocardium and its vasodilating effect by its action on the β-2 receptors of the peripheral vasculature. This vasodilation lowers peripheral resistance and this lowers the blood pressure. When β-1 receptors of the myocardium are stimulated, the contractility (inotropic effect) and rhythmicity (chronotropic effect) increase, which result in increased heart rate.

Rona et al. (1959) showed that large doses of isoproterenol produced an infarct-like myocardial necrosis in the rat. The authors postulated that such an effect of isoproterenol was due to the positive inotropic and chronotropic actions that would increase oxygen demand by the heart, while the fall in blood pressure would reduce coronary blood flow, thereby reducing myocardial oxygen supply. The consequence of these changes would be myocardial hypoxia. Fleckenstein et al. (1973, 1974) have put forward the theory that cardiotoxic effects of -adrenergic catecholamines are due to myocardial calcium overload. Mallov (1984) has also suggested that the elevated intracellular calcium due to enhanced calcium influx resulting from catecholamine-induced opening of Ca^{++} channels in the membrane has increased the membrane permeability.

The ultrastructural features of the myocardium observed in Figs. 8-30 are the results of isoproterenol induced lesions brought about by the fall in blood pressure and increase in heart rate causing an imbalance in the oxygen supply - demand mechanism in the myocardium. This imbalance leads to ischemia where metabolic acidosis occurs as a result of accumulation of metabolites (Corr and Sobel, 1983). These investigators have suggested that the accumulation of metabolites during ischemia might be the important factor in the genesis of electrophysiological alterations underlying ventricular arrhythmias.

The catecholamine-induced myocardial damage has been well established (Pearce, 1906; Rona et al., 1959; Bloom and Cancilla, 1969). Several mechanisms have been proposed to explain the structural

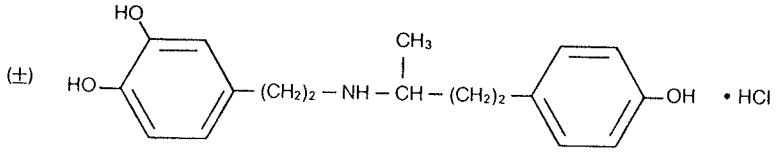
and functional changes produced by catecholamine administration. Recently, Dhalla et al. (1978) have shown the structural damage in the myocardium of isolated rat hearts perfused with spontaneously oxidized isoproterenol solution. Since myocardial damage was not observed in isolated hearts perfused with fresh catecholamines, they suggested that the cardiotoxic effects of catecholamines might be due to the oxidation products such as adrenochrome.

The in vivo study of Singal et al. (1982) by the intravenous administration of adrenochrome (4-32 mg/Kg) in rats showed ultrastructural changes in the myocardium. The extent of damage observed was dose dependent. In an in vitro study they suggested that the cardiotoxic effects of adrenochrome might involve increased free radical activity and the interaction of the oxidation products with the biologically active sulfhydryl (SH) groups (Singal et al., 1981).

During acute myocardial ischemia, production of free radicals and lipid peroxides as metabolic intermediates has been shown by Rao et al (1983). The observation was based on the coronary artery ligation study in dogs. They suggested that the free radicals are produced by (a) oxidation of catecholamines and ascorbic acid in the presence of metalloproteins (Cu, Fe) and (b) reaction of O_2 with proteins and lipids. The free radicals thus produced react with polyunsaturated fatty acids and lipoproteins to yield peroxy radicals and lipid peroxides. The free radicals produced by the ischemic myocardium might trigger later secondary events such as depletion of protective antioxidants and enzymes.

EFFECTS OF DOBUTAMINE ON THE HEMODYNAMICS OF NORMAL AND ISOPROTERENOL INSULTED GROUPS OF ANIMALS.

Dobutamine Hydrochloride



Molecular Formula: $C_{18}H_{23}NO_3 \cdot HCl$ Molecular Weight: 337.84

Dobutrex (dobutamine hydrochloride, Lilly) is (±)-4[2-[[3-(p-hydroxyphenyl)-1-methylpropyl]amino]ethyl]-pyrocatechol hydrochloride. It is a synthetic catecholamine.

Dobutamine, a synthetic catecholamine, is a cardioselective β-1 adrenergic agonist developed by Tuttle and Mills (1975). It is a derivative of dopamine exhibiting minimal β-2 and α-adrenergic effects. It has potent positive inotropic properties and minimal positive chronotropic, vasoconstrictive and arrhythmogenic effects.

Dobutamine can be classified as an adrenergic agent with β-1, β-2 and α-adrenergic actions (Kenakin, 1981). If the inotropic and chronotropic effects are blocked by β-blocking agents, the α-effects would show increase in blood pressure and peripheral vascular resistance because α-receptors when stimulated would constrict the vasculature (Farah et al., 1984). The β effects are best seen after the administration of an α-blocking agent (Bodem et al., 1974; Vatner et al., 1974; Robie and Goldberg, 1975; Tuttle and Mills, 1975; Lumley et al., 1977; and Ruffolo et al., 1981).

In the intact vagotomized dog and in the dog treated with a catecholamine depleting agent (syrosingopine), dobutamine produced a dose-related increase in contractility with minimal increases in heart rate (Vatner et al., 1974; Tuttle and Mills, 1975; and Lumley et al. 1977). Hinds and Hawthorne (1975), administered dobutamine intravenously to dogs at infusion rates of 5-20 ug/Kg/min and observed the linear increase in cardiac contractility without change in mean arterial pressure or heart rate.

However, Willerson et al. (1976), Kirlin et al. (1981), Liang et al. (1981) and Rude et al. (1982) have studied the different aspects of the effect of dobutamine in dogs and reported an increase in heart rate, myocardial contractility and ST-segment elevation, although blood flow to the heart had increased and blood pressure had not changed. Liang et al. (1981) have reported that dobutamine administration in dogs during coronary artery ligation did not increase the area of ischemic injury.

Clinical studies have shown that when dobutamine is given intravenously to patients with heart failure there is increased cardiac output corresponding to the dose, decreased pulmonary capillary wedge pressure and no significant increase in heart rate or arrhythmias (Farah et al., 1984). Gillespie et al. (1977) have evaluated the effects of dobutamine on cardiac performance and myocardial injury in patients with myocardial infarction. The dose used was 1 to 40 ug/kg/min for 24 hours. Dosage was titrated so that maximal systolic blood pressure did not exceed 130 mm Hg and heart rate did not exceed

90 beats/min. In these doses, dobutamine significantly increased the cardiac output and decreased pulmonary arterial wedge pressure without altering the heart rate or systemic arterial blood pressure significantly.

Holloway and Frederickson (1974) have shown the vasodilating and vasoconstricting effects of dobutamine on the blood vessels in dogs. Their dose response study showed a mild peripheral α-adrenergic stimulation of low doses (10 ug/kg/min) and with larger doses (20 ug/kg/min and 40 ug/kg/min) β-adrenergic vasodilation occurred. This dual response prevented extreme changes in blood pressure, heart rate and total peripheral resistance over a wide range of doses. Thus with lower doses, blood pressure, heart rate and peripheral vascular resistance were all elevated and with two larger doses, peripheral vascular resistance fell and blood pressure and heart rate remained approximately the same.

The mean values of heart rate and blood pressure of the insulted group in Table II observed in the present study agree with the results of Holloway and Frederickson (1974). The dobutamine alone treated group showed a significant increase in the heart rate in rats whereas Holloway and Frederickson's (1974) study in dogs showed such a similar increase in a lower dose of dobutamine, 10 ug/kg/min. Dobutamine has improved the blood pressure of isoproterenol insulted groups of animals and it was significant in that group which received dobutamine infusion immediately after isoproterenol injection. Though the heart rates were significantly higher, corresponding

ultrastructural damage was not observed in the myocardium of insulted and treated groups. The earlier the administration of dobutamine to the ischemic myocardium, the less the ultrastructural damage caused by isoproterenol. The mechanism by which dobutamine causes this return of the ischemic myocardium toward a near normal state of ultrastructure is not understood yet (Unverferth et al., 1980). The increased blood flow to the myocardium and subsequent biochemical changes may trigger the repair process in cardiac cells, especially those cells situated in the borderzone of an infarcted area. The border zone or transition zone is an area adjacent to the infarcted myocardium, where the ultrastructural alterations are not permanent. Such cells are in a viable state. If proper blood supply and subsequent oxygenation are attained within the critical period or proper pharmacological intervention is obtained such transient alterations could be reversed. Without this, those changes would lead to irreversible damage.

Jewitt et al. (1974) carried out an important study on patients with aortic ball-valve prostheses (7 patients) and on patients with coronary artery disease or congestive cardiomyopathy (10 patients) when dobutamine was infused in different doses (2.5 to 10 ug/Kg/min) and observed significantly increased stroke volume, cardiac output and left ventricular dp/dt max, without significant changes in heart-rate, diastolic arterial pressure or left ventricular filling pressure. The heart-rate did not change significantly, the control value being 81.8 $\pm$ 9 beats/min and at the highest concentration (10 ug/Kg/min for 10 min) was 89.2 $\pm$7 beats/min. The systolic arterial pressure rose at each successive infusion concentration from a mean control value of 126 $\pm$ 10

to 155 ± 21 mm Hg at 10 ug. In contrast, the diastolic pressure did not change with any of the infused dobutamine concentrations. Though the pulmonary artery pressure fell from a control value of 23.6 ± 5 to 20.8 ± 3 mm Hg at the highest concentration, the change was not significant. The left ventricular dp/dt max changed from the control value of 1203 ± 27 mm Hg/sec, an increase being occured at each infusion concentration, to 3043 ± 620 mm Hg/sec after 10 ug/Kg/min infusion. Thus they demonstrated a separation of inotropic and chronotropic effects of dobutamine. According to their observation, dobutamine has potential therapeutic value in the management of cardiovascular failure associated with low cardiac output after myocardial infarction.

Tuttle and Mills (1975) showed the inotropic efficacy of dobutamine in dogs to be as great as that of epinephrine due to a direct action on β-1 receptors of the myocardium. They also showed, unlike epinephrine, dobutamine's effect on and β_2 vascular receptors was slight. In ischemic dog hearts, dobutamine lacked significant arrhythmic activity whereas dopamine, norepinephrine and isoproterenol caused severe ectopic activity. Unlike dopamine, dobutamine does not indirectly stimulate the heart by the release of norepinephrine.

Dobutamine does not act on dopamine receptors (Robie et al., 1975). Injection of dobutamine into the renal artery after administration of phenoxybenzamine caused a slight increase in renal blood flow, but this effect was eliminated by propranolol. They showed the dual vasoconstrictor-vasodilator response of dobutamine at higher doses and a slight vasoconstriction at low doses by injections of

dobutamine into the femoral vascular bed of anesthetized dogs. Phenoxybenzamine blocked the vasoconstrictor component and propranol blocked the vasodilator component.

The experiments comprising dobutamine and epinephrine before and after blocking the adrenergic receptors showed that dobutamine had the required strong inotropic activity, but had no effect on blood pressure. This was because the activity of dobutamine on the and β-2 adrenergic receptors that control arterial resistance was weak relative to its activity on the β-1 receptors that control myocardial contractility (Tuttle and Mills, 1975).

The results shown in Table II are consistent with the results presented by Jewitt et al. (1974), in the sense that they also showed increased heart rate and blood pressure, though the increase was not significant. However the study of Sakamoto and Yamada (1977) in patients following open heart surgery showed a significant increase (13.6%) in the systolic aortic pressure with dobutamine infusion. There were also significant increases in the mean aortic pressure (4.8%) and in heart rate (24.3%). They showed the improvement of the left ventricular function after dobutamine infusion at a dose of 8 ug/Kg/min for 10 minutes. The hemodynamic data these invetigators presented are consistent with the present observations.

On the basis of the results obtained from the hemodynamic studies and the ultrastructural alterations observed from the electron-microscopic studies, we conclude that dobutamine may be a

potentially useful clinical and pharmacological agent that can be of some value to limit the extent of myocardial infarct.

VI

SUMMARY

SUMMARY

The effects of dobutamine on the ultrastructure of ischemic myocardium have clearly been shown in the present study. The beneficial effects of dobutamine depended upon the time of administration of the drug after myocardial ischemia induced experimentally.

The highlights of this study on the fine structural features of the ischemic myocardium after dobutamine administration were: less marked myofibrillar degeneration, uniform distribution pattern of chromatin in the nucleus, less nuclear marginations, significantly less electron dense bodies in the mitochondria, intact mitochondrial and sarcolemmal membranes, more sarcomeres in registry, and overall near normal pattern of the whole cytoarchitecture. The physiological and pharmacological effects of dobutamine are thus observed in the ultrastructure of ischemic myocardium.

The significant reduction in the depletion of enzymes from the mitochondria and sarcoplasm may be because of the maintenance of the structural integrity of the membranes of organelles and cells as a whole by dobutamine. The presence of glycogen granules in the sarcoplasm of ischemic myocardium treated with dobutamine was noteworthy because of the fact that these glycogen granules were not utilized during the emergency period since mitochondria seemed to be functional and met the energy requirements. This feature was observed in the mitochondria of the ischemic area, where though a few electron dense bodies were present, mitochondrial cristae were clearly defined.

The subcellular defects produced by the administration of isoproterenol looked like prevented, in most of the instances, upon the early administration of dobutamine. The dose, concentration and duration of dobutamine infusion are important parameters to be considered seriously. However, time is the critical factor for the maintenance of the viable myocytes in the ischemic and border zones. The earlier the drug administration, the better the protection and the less damage to ischemic myocardium.

The fact that this cardioselective, synthetic β-1 adrenergic agonist dobutamine, actually improves the ischemic myocardial ultrastructures, energetics and function is equally important as the potential therapeutic benefit of dobutamine is the concept.

VII

REFERENCES

REFERENCES

Akhtar, N., Mikulic, E., Cohn, J.N. and Chaudhry, M.H. (1975). Hemodynamic effects of dobutamine in patients with severe heart failure. Am. J. Cardiol. 36, 202-205.

Alpert, J.S., Braunwald, E. (1980). Pathological and clinical manifestations of acute myocardial infarction. In: Heart Disease. A Textbook of Cardiovascular Medicine, Braunwald, E., Saunders, W.B. (editors), Saunders, Philadelphia, 1309-1352.

Andrew, S., Wechsler (1977). Development of coronary collateral circulation. Ann. Rev. Med. 28, 341-348.

Anggard, E. and Sedvall, G. (1969). Gas chromatography of catecholamine metabolites using electron capture detection and mass spectrometry. Anal. Chem. 41, 1250-1255.

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

Banka, V.S., Bodenheimer, M.M., Ramanathan, K.B., Hermann, G.A. and Helfant, R.H. (1978). Progressive transmural electrocardiographic, myocardial potassium ion/sodium ion ratio and ultrastructural changes as a function of time after acute coronary occlusion. Am. J. Cardiol. 42, 429-443.

Bar, L., Dewey, M.M. and Berger, W. (1965). Propagation of action potentials and the structure of the nexus in cardiac muscle. J. Gen. Physiol. 48, 797-823.

Baron, G.D., Speden, R.N. and Bohr, D.F. (1972). Beta-adrenergic receptors in coronary and skeletal muscle arteries. Am. J. Physiol. 223, 878-881.

Beamish, R.E., Dhillon, K.S., Singal, P.K. and Dhalla, N.S. (1981). Protective effect of sulfinpyrazone against catecholamine metabolite adrenochrome-induced arrhythmias. Am. Heart J. 102, 149-152.

Beamish, R.E., Karmzyn, M., Panagia, V. and Dhalla, N.S. (1984). Mechanism of coronary artery spasm in stress induced heart disease. In: Smooth Muscle Contraction by Newman L. Stephens (editor). Marcel Dekker, Inc., N.Y., 537-550.

Beckman, R., Gallenkamper, W., Mannesmann, G., Schror, K., Smith, E.F. and Thomsen, T. (1983). Early and late administration of a PGI2-analogue, ZK 36373: Effects on cardiac preservation, collateral blood flow and infarct size. Brit. J. Pharmacol. 78, 29.

Bilheimer, D.W., Buja, L.M., Parkey, R.W., Bonte, F.J. and Willerson, J.T. (1978). Fatty acid accumulation and abnormal lipid deposition in peripheral and border zones of experimental myocardial infarcts. J. Nucl. Med. 19, 276-283.

Bloom, S. and Cancilla, P.A. (1969). Conformational changes in myocardial nuclei of rats. Circ. Res. 24, 189-196.

Bloom, S. and Davis, D.L. (1972). Calcium as mediator of isoproterenol-induced myocardial necrosis. Am. J. Pathol. 69, 459-470.

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

Bloom, W. and Fawcett, D.W. (1975). Cardiac muscle. In: A textbook of histology, ed. William Bloom and Don W. Fawcett. W.B. Saunders Company, 315-328.

Bodem, R., Skelton, C.L., Sonnenblick, E.H. (1974). Inotropic and chronotropic effects of dobutamine on isolated cardiac muscle. Eur. J. Cardiol. 2, 181-189.

Boden, W.E., Liang, C.S., Apstein, C.S. and Hood, W.B. (1978). Experimental myocardial infarction. XVI. The detection of inotropic contractile reserve with post extrasystolic potentiation in acutely ischemic canine myocardium. Am. J. Cardiol. 41, 523-530.

Boink, A.B.T.J., Ruigrok, T.J.C., Maas, A.H.J. and Zimmerman, A.N.E. (1976). Changes in high-energy phosphate compounds of isolated rat heart during Ca^{2+}-free perfusion and reperfusion with Ca^{2+}. J. Mol. Cell. Cardiol. 8, 973-979.

Brachfeld, N., Ohtake, Y., Klein, I. and Kawade, M. (1972). Substrate preference and metabolic activity on the aerobic and the hypoxic turtle heart. Circ. Res. 31, 453-467.

Braunwald, E. and Maroko, P.R. (1976). ST segment mapping: realistic and unrealistic expectations. Circulation 54, 529-532.

Braunwald, E. and Maroko, P.R. (1976). Effects of hyaluromidase and hydrocortisone on myocardial necrosis after coronary occlusion. Am. J. Cardiol. 37, 550-556.

Braunwald, E. (1981). Coronary artery spasm as a cause of myocardial ischemia. J. Lab. Clin. Med. 97, 299-312.

Braunwald, E., Mull, J.E., Kloner, R.A. and Maroko, P.R. (1983). Role of beta-adrenergic blockade in the therapy of patients with myocardial infarction. Am. J. med. 74, 113-123.

Bulkley, B.H., Nunnally, R.L., Hollis, D.P. (1978). "Calcium paradox" and the effect of varied temperature on its development. A phosphorus nuclear magnetic resonance and morphologic study. Lab. Invest. 39, 133-140.

Burch, W.M. and Lebovitz, H.E. (1979). Specific nuclear binding of 3',5' cyclic adenosine monophosphate-protein complex with induction of RNA synthesis (Abstract). Clin. Res. 27, 482A.

Campbell, C.A. and Parratt, J.R. (1983). The effect of beta-adrenoceptor blocking agents, with differing ancillary properties on the arrhythmias resulting from acute coronary artery ligation in anaesthetized rats. Br. J. Pharmac. 79, 939-946.

Caulfield, J. and Klionsky, B. (1959). Myocardial ischemia and early infarction: An electron microscopic study. Am. J. Pathol. 35, 489-501.

Chahine, R.A. (1983). Pathophysiology of ischemic heart disease. In: Rehabilitation in ischemic heart disease, edited by William P. Blocker and David Cardus, Spectrum Publications, New York, 69-75.

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

Chiba, T. (1973). Electron microscopic and histochemical studies on the synaptic vesicles in mouse vas deferens and atrium after 5-hydroxydopamine administration. Anat. Record. 176, 35-48.

Chien, K.R., Pfau, R.G. and Farber, J.L. (1979). Ischemic myocardial cell injury. Prevention by chlorpromazine of an accelerated phospholipid degradation and associated membrane dysfunction. Am. J. Pathol. 97, 505-530.

Cohen, G. and Heikkila, R.E. (1974). The generation of hydrogen peroxide, superoxide radical and hydroxyl radical by 6-hydroxydopamine, dialuric acid and related cytotoxic agents. J. Biol. Chem. 249, 2447-2452.

Corr, P.B. and Sobel, B.E. (1983). Arrhythmogenic properties of phospholipid metabolites associated with myocardial ischemia. Fed. Proc. 42, 2454-2459.

Cox, J.L., McLaughlin, V.W., Flowers, N.C. and Horan, L.G. (1968). The ischemic zone surrounding acute myocardial infarction. Its morphology detected by dehydrogenase staining. Am. Heart J. 76, 650-658.

Daniell, H.B. (1979). Studies on the relationship between ST-segment elevations and extent of infarction following coronary artery occlusion in dogs. Res. Comm. Chem. pathol. Pharmacol. 23, 333-340.

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

Dhalla, N.S., Singal, P.K. and Dhillon, K.S. (1980). Mitochondrial functions and drug induced heart disease. In: Drug induced heart disease, ed. M.R. Bristow. Elsevier/North Holland Biomedical Press, 39-61.

Dixon, S.H., Limbird, L.E., Roe, C.R., Wagner, G.S., Oldham, N. and Sabiston, D.C. (1973). Recognition of postoperative acute myocardial infarction. Application of isoenzyme techniques. Circ. Suppl. III, 47 & 48, III, 137-140.

Ehringer, B., Falck, B. and Sporrong, B. (1970). Possible axo-axonal synapses between peripheral adrenergic and cholinergic nerve terminals. Z. Zellforsch. Mikrosk. Anat. 107, 508-521.

Eliot, R.S., Todd, G.L., Pieper, G.M. and Clayton, F.C. (1979). Pathophysiology of catecholamine-mediated myocardial damage. J.S.C. Med. Assoc. 75, 513-518.

Entman, M. and McMillin-Wood, J. (1983). Biochemical manifestations of ischemic heart disease. In: Rehabilitation in ischemic heart disease. Eds. Blocker, W.P. and Cardus, D. Spectrum Publications, 63-68.

Essner, E., Novikoff, A.B. and Quintana, N. (1965). Nucleoside phosphatase activities in rat cardiac muscle. J. Cell Biol. 25, 201-215.

Farah, A.E., Alousi, A.A. and Schwarz, Jr. R.P. (1984). Positive inotropic agents. Ann. Rev. Pharmacol. Toxicol. 24, 275-328.

Fawcett, D.W. and McNutt, N.S. (1969). The ultra structure of the cat myocardium. I. Ventricular papillary muscle. J. Cell Biol. 42, 1-45.

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

Ferrans, V.J., Hibbs, R.G. and Buja, L.M. (1969). Nucleoside phosphatase activity in atrial and ventricular myocardium of the rat: a light and electron microscopic study. Am. J. Anat. 125, 47-86.

Fishbein, M.C., Hare, C., Gissen, S., Maclean, D. and Maroko, P.R. (1977). Histochemical identification and quantification of border zones during the evolution of myocardial infarction in the rat. Circulation 56 (Suppl. III), III-71.

Fishbein, M.C., Meerbaum, S., Rit, J., Lando, U., Kanmatsuse, K., Mercier, J.C., Corday, E. and Ganz, W. (1981). Early phase actue myocardial infarct size quantification: Validation of the triphenyl tetrazolium chloride tissue enzyme staining technique. Am. Heart J. 101, 593-600.

Fleckenstein, A., Janke, J., Doring, H.J. and Pachinger, O. (1973). Ca overload as the determinant factor in the production of catecholamine induced myocardial lesions. In: E. Bajusz and G. Rona (eds.), Recent Advances in Studies on Cardiac Structure and Metabolism. Cardiomyopathies 2, 455-466.

Fleckenstein, A., Janke, J., Doring, H.J. and Leder, O. (1974). Myocardial fiber necrosis due to intracellular Ca overload- a new principle in cardiac pathophysiology. In: N.S. Dhalla (ed.), Recent Advances in Studies on Cardiac Structure and Metabolism. Myocardial Biology 4, 563-580.

Fleckenstein, A., Janke, J., Doring, H.J. and Leder, O. (1975). Key role of Ca^{2+} in the production of noncoronarogenic myocardial necroses. In: Recent Advances in Studies on Cardiac Structure and Metabolism. (Eds) A. Fleckenstein and G. Rona, Baltimore, University Park Press, 6, 21-32.

Forssmann, W.G. and Girardier, L. (1966). Untersuchungen zur ultrastruktur des rattenherzmuskels mit besonderer berucksichtigung des sarcoplasmatischen retikulums. Z. Zellforsch. Mikrosk. Anat. 72, 249-275.

Forssmann, W.G. and Girardien, L. (1970). A study of the T system in rat heart. J. Cell. Biol. 44, 1-19.

Freeman, A.P., Fatches, K.R., Carter, I.W., Cloonan, M.J. and Wilcken, D.E.L. (1981). Comparison of serum myoglobin and creatine kinase MB isoenzyme in early diagnosis of acute myocardial infarction. Br. Heart J. 45, 389-392.

Fujiwara, H., Ashraf, M., Sato, S. and Millard, R.W. (1982). Transmural cellular damage and blood flow distribution in early ischemia in pig hearts. Circ. Res. 51, 683-693.

Ganote, C.E., Liu, S.Y., Safavi, S. and Kaltenbach, J.P. (1983). Hypoxia, calcium and contracture as mediators of myocardial enzyme release. Adv. Myocardiol. 4, 327-331.

Gillespie, T.A., Ambos, H.D., Sobel, B.E. and Roberts, R. (1977). Effects of dobutamine in patients with acute myocardial infarction. Am. J. Cardiol. 39, 588-594.

Goldberg, L.I., Hsieh, Y.Y. and Resnekov, L. (1977). Newer catecholamines for treatment of heart failure and shock: An update on dopamine and a first look at dobutamine. Prog. Cardiovasc. Dis. 19, 327-334.

Gottlieb, G.J., Kubo, S.H. and Alonso, D.R. (1981). Ultrastructural characterization of the border zone surrounding early experimental myocardial infarcts in dogs. Am. J. Pathol. 103, 292-303.

Graham, D.G., Tiffany, S.M., Bell Jr., W.R. and Gutknecth, W.F. (1978). Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells in vitro. Mol. Pharmacol. 14, 644-653.

Green, D.E. (1983). Mitochondria-structure, function and replication. (Editorial). N. En. J. Med. 310, 188-189.

Greenspon, A.J. and Goldberg, S. (1983). Pathophysiology of angina pectoris due to coronary artery spasm. In: Coronary artery spasm and thrombosis, edited by Sheldon Goldberg, F.A. Davis Co., Philadelphia, 17-29.

Gunnar, R.M., Loeb, H.S., Klodnycky, M., Sinno, M.Z. and Towne, W. (1973). Abstract. Hemodynamic effects of dobutamine in man. Circ. VII-VIII. (Supp. IV), IV-132.

Guyton, A.C. (1981). Heart muscle; the heart as a pump. In: Textbook of medical physiology, edited by Arthur C. Guyton, W.B. Saunders Company, 150-159.

Ham, A.W. (1974). Cardiac muscle. In: Histology, edited by Arthur W. Ham, 7th Edition, J.B. Lippincott Co., 544-548.

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

Hanson, J. and Huxley, H.E. (1953). Structural basis of the cross-striations in muscle. Nature 172, 530-532.

Hanson, J. and Lowy, J. (1963). The structure of F-actin and of actin filaments isolated from muscle. J. Mole. Biol. 6, 46-60.

Hearse, D.J. (1977). Myocardial enzyme leakage. J. Mol. Med. 2, 185.

Hearse, D.J. (1979). oxygen deprivation and early myocardial contractile failure: A reassessment of the possible role of adenosine triphosphate. Am. J. Cardiol. 44, 1115-1121.

Hearse, D.J. and Yellon, D.M. (1983). Pathophysiology of irreversible ischemic injury - the border zone controversy. Adv. Myocardiol. 4, 347-361.

Heberden, W. (1772). Some account of a disorder of the breast, read before Royal College of Physicians, July 21, 1768. Med. Trans. Royal College of Physicians, London 2, 59.

Hedberg, A., Matsson, H. and Carlsson, E. (1980). Prenalterol, a nonselective beta adrenoceptor ligand with absolute beta$_1$ selective partial agonist activity. J. Pharm. Pharmacol. 32, 660-661.

Henderson, P.B., Sommers, H.B. and Jennings, R.B. (1965). A comparative study of the fine structure of normal and ischemic dog myocardium with special reference to early changes following temporary occlusion of a coronary artery. Am. J. Pathol. 46, 367-369.

Herrick, J.B. (1912). Clinical features of sudden obstruction of the coronary arteries. JAMA 59, 2015-2020.

Hillis, L.D., Askenazi, J., Braunwald, E., Radvany, P., Muller, J.E., Fishbein, M.C. and Maroko, P.R. (1976). Use of changes in the epicardial QRS complex to assess interventions which modify the extent of myocardial necrosis following coronary artery occlusion. Circ. 54, 591-598.

Hillis, L.D., Khuri, S.F., Braunwald, E. and Maroko, P.R. (1979). The role of propranolol's negative chronotropic effect on protection of the ischemic myocardium. Pharmacol. 19, 202-208.

Hinds, J.E. and Hawthorne, E.W. (1975). Comparative cardiac dynamic effects of dobutamine and isoproterenol in conscious instrumented dogs. Am. J. Cardiol. 36, 894-901.

Holland, R.P. and Brooks, H. (1976). The QRS complex during myocardial ischemia: an experimental analysis in the porcine heart. J. Clin. Invest. 57, 541-550.

Holloway, G.A. and Frederickson, E.L. (1974). Dobutamine, a new beta agonist. Anesth. Analg. 53, 616-623.

Horder, M., Petersen, P.H. and Thygesen, K. (1983). Clinical evaluation of models and markers of myocardial ischemia. Upsala J. Med. Sci. 88, 169-184.

Huxley, H.E. (1963). Electron microscopic studies on the structure of natural and synthetic protein filaments from striated muscle. J. Mol. Biol. 7, 281-308.

Huxley, H.E. (1973). Molecular basis of contraction in cross-striated muscles. In: The structure and function of muscle. Structure (2nd ed.), edited by G.H. Bourne, New York, Academic Press, 1, 301-387.

Innes, I.R. and Weisman, H. (1981). Reduction in the severity of myocardial infarction by sulfinpyrazone. Am. Heart J. 102, 153-157.

James, T.N. (1970). The delivery and distribution of coronary collateral circulation. Chest. 58, 183-203.

Janse, M.J., Cinca, J., Morena, H., Fiolet, J.W.T. et al. (1979). The "border zone" in myocardial ischemia. An electrophysiological, metabolic and histochemical correlation in the pig heart. Circ. Res. 44, 576-588.

Jennings, R.B., Wartman, W.B., Zudyk, Z.E. and Kaltenbach, J.P. (1957). Production of an area of homogeneous myocardial infarction in the dog. Arch. Pathol. 63, 580-585.

Jennings, R.B., Baum, J.H. and Henderson, P.B. (1965). Fine structural changes in myocardial ischemic injury. Arch. Pathol. 79, 135-143.

Jennings, R.B., Sommers, H.M., Henderson, P.B. and Kaltenbach, J.P. (1969). Ischemic injury of myocardium. Ann. N.Y. Acad. Sci. 156, 61-78.

Jennings, R.B. and Ganote, C.E. (1972). Ultrastructural changes in acute myocardial ischemia: Effect of acute ischemia on myocardial functions. Eds.: M.F. Oliver, D.G. Julian and K.W. Donald. Churchil Livingston, 50-74.

Jennings, R.B. and Reimer, K.A. (1973). The fate of the ischemic myocardial cell. In: Corday, E., Swan, H.J.C., eds. Myocardial infarction, new perceptives in diagnosis and managements. Baltimore: Williams and Wilkins, 13-25.

Jennings, R.B. and Ganote, C.E. (1974). Structural changes in myocardium during acute ischemia. Circ. Res. 34-35, (Suppl. III), 156-172.

Jennings, R.B., Ganote, C.E. and Reimer, K.A. (1975). Ischemic tissue injury. Am. J. Pathol. 81, 179-198.

Jennings, R.B. and Hawkins, H.K. (1977). Myocardial cell-volume control in ischemic injury. In: Pathophysiology and therapeutics of myocardial ischemia. Spectrum Publications, 351-365.

Jennings, R.B., Hawkins, H.K., Lowe, J.E., Hill, M.L., Klotman, S. and Reimer, K.A. (1978). Relation between high energy phosphate and lethal injury in myocardial ischemia in the dog. Am. J. Pathol. 92, 187-214.

Jennings, R.B. and Reimer, K.A. (1981). Lethal myocardial ischemic injury. Am. J. Pathol. 102, 241-255.

Jenson, D. (1980). Functional anatomy of the cardiovascular system. In: The principles of physiology. Edited by David Jenson, Appleton-Century-Crofts, New York, 539.

Jewitt, D., Birkhead, J., Mitchell, A. and Dollery, C. (1974). Clinical cardiovascular pharmacology of dobutamine. A selective inotropic catecholamine. Lancet 17, 363-367.

Johnson, E.A. and Sommer, J.R. (1967). A strand of cardiac muscle. Its ultrastructure and the electrophysiological implications of its geometry. J. Cell Biol. 33, 103-129.

Johnson, E.A. (1976). First electrocardiographic sign of myocardial ischemia: an electrophysiological conjecture. Circulation 53:1, 82-84.

Kaltenbach, J.P. and Jennings, R.B. (1960). Metabolism of ischemic cardiac muscle. Circ. Res. 8, 207-213.

Kawamura, K., Cowley, M.J., Karp, R.B., Mantle, J.A. et al. (1978). Intramitochondrial inclusions in the myocardial cells of human hearts with coronary disease. J. Mol. Cell. Cardiol. 10, 797-811.

Keefer, C.S. and Resnick, W.H. (1928). Angina pectoris: a syndrome caused by anoxemia of the myocardium. Arch. Int. Med. 41, 769-807.

Kenakin, T.P. (1981). An in vitro quantitative analysis of the alpha adrenoceptor partial agonist activity of dobutamine and its relevance to inotropic selectivity. J. Pharmacol. Exp. Ther. 216, 210-219.

Kenakin, T.P. and Beek, D. (1982). In vitro studies on the cardiac activity of prenalterol with reference to use in congestive heart failure. J. Pharmacol. Exp. Ther. 220, 77-85.

Kirlin, P.C., Pitt, B. and Lucchesi, B.R. (1981). Comparative effects of prenalterol and dobutamine in a canine model of acute ischemic heart failure. J. Cardiovasc. Pharmacol. 3, 896-905.

Kiss, A. and Reinhart, W. (1956). Ueber den nachweis des myoglobins im serum und im harn nach herzinfarkt. Wien Klin Wochenschr. 68, 154-155.

Kloner, R.A., Ganote, C.E., Whalen, D.H. Jr. and Jennings, R.B. (1974). Effect of a transient period of ischemia on myocardial cells II. Fine structure during the first few minutes of reflow. Am. J. Pathol. 74, 399-413.

Kloner, R.A., Fishbein, M.C., Cotran, R.S., Braunwald, E. and Maroko, P.R. (1977). The effect of propranolol on microvascular injury in acute myocardial ischemia. Circulation 55, 872-880.

Kloner, R.A., Fishbein, M.C., Maclean, D., Braunwald, E. and Maroko, P.R. (1977). Effect of hyaluronidase during the early phase of acute myocardial ischemia: an ultrastructural and morphometric analysis. Am. J. Cardiol. 40, 43-49.

Kloner, R.A., Fishbein, M.C., Maroko, P.R. and Braunwald, E. (1978). Effect of propranolol on mitochondrial morphology during acute myocardial ischemia. Am. J. Cardiol. 41, 880-886.

Kloner, R.A., Braunwald, E. and Maroko, P.R. (1978). Long-term preservation of ischemic myocardium in the dog by hyaluronidase. Circulation 58, 220-226.

Kloner, R.A., Fishbein, M.C., Hare, C.M. and Maroko, P.R. (1979). Early ischemic ultrastructural and histochemical alterations in the myocardium of the rat following coronary artery occlusion. Exptl. Mole. Pathol. 30, 129-143.

Kloner, R.A., Rude, R.E., Maroko, P.R. and Braunwald, E. (1979). Microvascular damage and myocardial cell injury following coronary artery occlusion: which comes first? Circulation 60, Suppl. II, 11-42.

Kloner, R.A. and Braunwald, E. (1980). Observations on experimental myocardial ischemia: Review. Cardiovasc. Res. 14, 371-395.

Kluge, W.F. (1969). Prognostic value of serum creatine phosphokinase levels in myocardial infarction. Northwest Med. 847-853.

Konzett, H. (1940). Neue broneholytisch hochwirksame korper der adrenalinreihe. Naunyn Schmiedebergs Arch. Exp. Pharmakol. Pathol. 197 27-40.

Koltai, M., Kepran, I., Nemecz, Gy. and Szekeres, L. (1983). The possible mechanism of protection induced by dexamethasone against sudden death due to coronary ligation in conscious rats. Br. J. Pharmac. 79, 327-329.

Langer, G.A. and Brady, A.J. (1974). Myocardial ultrastructure. In: The mammalian myocardium, ed. by Glenn A. Langer and Allan J. Brady, a Wiley Biomedical-Health Publication, N.Y., 1-49.

Liang, C., Yi, J.M., Sherman, L.G., Black, J., Garvas, H., et al. (1981). Dobutamine infusion in conscious dogs with and without acute myocardial infarction. Circ. Res. 49, 170-180.

Lindner, E. (1957). Die submikroskopische morphologie des herzmuskels. Z. Zellforsch. mikrosk. Anat. 45, 702-746.

Lubbe, W.F., Nguyen, T. and West, E.J. (1983). Modulation of myocardial cyclic AMP and vulnerability to fibrillation in the rat heart. Fed. Proc. 42, 2460-2464.

Lumley, P., Broadley, K.J. and Levy, G.P. (1977). Analysis of the inotropic: chronotropic selectivity of dobutamine and dopamine in anesthetized dogs and guinea-pig isolated atria. Cardiovasc. Res. 11, 17-25.

Maclean, D., Fishbein, M.C., Braunwald, E. and Maroko, P.R. (1976). Hyaluronidase-induced reductions in myocardial infarct size. Science, 194, 199-200.

Maclean, D., Fishbein, M.C., Braunwald, E. and Maroko, P.R. (1978). Long term preservation of ischemic myocardium after experimental artery occlusion. J. Clin. Invest. 61, 541-551.

Madias, J.E., Madias, N.E. and Hood, B.W. (1976). Precordial ST segment mapping. II. Effect of oxygen inhalation on ischemic injury in patients with acute myocardial infarction. Circulation 53, 411-417.

Mallov, S. (1984). Effect of cardiotoxic concentrations of catecholamines on Na^+-Ca^{2+} exchange in cardiac sarcolemmal vesicles. Exptl. Mole. Pathol. 40, 206-213.

Maroko, P.R., Kjekshus, J.K., Sobel, B.E., Watanabe, T., et al. (1971). Factors influencing infarct size following coronary artery occlusions. Circulation 43, 67-82.

Maroko, P.R., Libby, P., Bloom, C.M., Sobel, B.E. and Braunwald, E. (1972). Reduction by hyaluronidase of myocardial necrosis following coronary occlusion. Circulation 46, 430-437.

Maroko, P.R. and Braunwald, E. (1973). Modification of myocardial infarction size after coronary occlusion. Ann. Intern. Med. 79, 720-733.

Maroko, P.R., Radvany, P., Braunwald, E. and Hale, S.L. (1975). Reduction of infarct size by oxygen inhalation following acute coronary occlusion. Circulation, 52, 360-368.

Maroko, P.R., Ribeiro, L.G.T. and Goldberg, S. (1983). Acute myocardial infarction-coronary thrombosis and salvage of the ischemic myocardium. In: Coronary artery spasm and thrombosis. Ed. Sheldon Goldberg, F.A. Davis Company, Philadelphia, 191-201.

McComb, J.M., McMaster, E.A., MacKenzie, G. and Adgey, A.A.J. (1984). Myoglobin and creatine kinase in acute myocardial infarction. Br. Heart J. 51, 189-194.

McMurtry, J.P. and Wexler, B.C. (1979). Detection of early myocardial infarction by radioimmunoassay of myoglobin. Angiol. 30, 806-815.

McNeill, J.H. (1978). The effect of dobutamine on rat cardiac cyclic AMP phosphorylase and force of contraction. Res. Commun. Chem. Pathol. Pharmacol. 20, 597-600.

McNutt, N.S. and Fawcett, D.W. (1969). The ultrastructure of the cat myocardium. II. Atrial muscle. J. Cell Biol. 42, 46-67.

Moore, D.H. and Ruska, H. (1957). Electron microscopic study of mammalian cardiac muscle cells. J. Biophysic. Biochem. Cytol. 3, 262-267.

Most, A.S., Capone, R.J. and Mastrofrancesco, P.A. (1976). Failure of hyaluronidase to alter the early course of acute myocardial infarction in pigs. Am. J. Cardiol. 38, 28-33.

Muir, A.R. (1965). Further observations on the cellular structure of cardiac muscle. J. Anat. 99, 27-46.

Myers, R.W., Scherer, J.L., Goldstein, R.A., Goldstein, R.E., Kent, K.M. and Epstein, S.E. (1975). Effects of nitroglycerin and nitroglycerin-methoxamine during acute myocardial ischemia in dogs with pre-existing multivessel coronary occlusive disease. Circulation 51, 632-640.

Nagai, R., Chiu, C.-C., Yamaoki, K., Ueda, S., Iwasaki, Y., Ohkubo, A. and Yazaki, Y. (1983). Serial changes in cytosolic, mitochondrial and lysosomal enzymes and cardiac myosin light chain II in plasma following coronary ligation in conscious closed-chest dogs. Adv. Myocardiol. 4, 473-478.

Nayler, W.G., Stone, J., Carson, V. and Chipperfield, D. (1971). Effect of ischemia on cardiac contractility and calcium exchangeability. J. Mol. Cell. Cardiol. 2, 125-143.

Nayler, W.G., Yepez, C.E., Fassold, E. and Ferrari, R. (1978). Prolonged protective effect of propranolol on hypoxic heart muscle. Am. J. Cardiol. 42, 217-225.

Nayler, W.G., Poole-Wilson, P.A. and Williams, A. (1979). Hypoxia and calcium. J. Mol. Cell. Cardiol. 11, 683-706.

Neely, J.R. and Feuvray, D. (1981). Metabolic products and myocardial ischemia. Am. J. Pathol. 102, 282-291.

Nirdlinger, E.L. and Bramante, P.O. (1974). Subcellular myocardial ionic shifts and mitochondrial alterations in the course of isoproterenol induced cardiopathy of the rat. J. Mole. Cell. Cardiol. 6, 49-60.

Oliver, M.F. (1972). Methods of inducing myocardial ischemia. In: M.F. Oliver, D.G. Julian and K.W. Donald (eds.), Effect of acute ischemia on myocardial function. Churchill Livingston, 11-18.

Opie, L.H., Owen, P., Thomas, M. and Samson, R. (1973). Coronary sinus lactate measurements in assessment of myocardial ischemia: comparison with changes in lactate/pyruvate and beta-hydroxybutyrate/acetoacetate ratios and with release of hydrogen, phosphate and potassium ions from the heart. Am. J. Cardiol. 32, 295-305.

Opie, L.H. and Thandroyen, F.T. (1983). Calcium antagonists, ventricular fibrillation and enzyme release in ischemic rat hearts. Fed. Proced. 42, 2465-2469.

Osler, W. (1910). The lumleian lectures on angina pectoris. The Lancet 697-702; 839-844; 973-977.

Page, E. (1968). Correlations between electron microscopic and physiological observations in heart muscle. J. Gen. Physiol. 51, 211S-220S.

Pardee, H.E.B. (1920). An electrocardiographic sign of coronary artery obstruction. Arch. Int. Med. 26, 244-257.

Pashkow, F., Holland, R. and Brooks, H. (1977). Early changes in contractility and coronary blood flow in the normal areas of the ischemic porcine heart. Am. Heart J. 93, 349-357.

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

Porter, K.R. and Palade, G.E. (1957). Studies on the endoplasmic reticulum. III. Its form and distribution in striated muscle cells. J. Biophysic. Biochem. Cytol. 3, 269-299.

Rao, P.S., Cohen, M.V. and Mueller, H.S. (1983). Production of free radicals and lipid peroxides in early experimental myocardial ischemia. J. Mol. Cell. Cardiol. 15, 713-716.

Rasmussen, M.M., Reimer, K.A., Kloner, R.A. and Jennings, R.B. (1977). Infarct size reduction by propranolol before and after coronary ligation in dogs. Circulation 56, 794-798.

Reimer, K.A., Rasmussen, M.M. and Jennings, R.B. (1973). Reduction by propranolol of myocardial necrosis after temporary coronary occlusion in dogs. Circ. Res. 33, 353-363.

Reimer, K.A., Rasmussen, M.M. and Jennings, R.B. (1976). On the nature of protection by propranolol against myocardial necrosis after temporary coronary occlusion in dogs. Am. J. Cardiol. 37, 520-527.

Reimer, K.A., Lowe, J.E., Rasmussen, M.M. and Jennings, R.B. (1977). The wavefront phenomenon of ischemic cell death. Myocardial infarct size vs duration of coronary occlusion in dogs. Circulation 58, 786-793.

Reimer, K.A. and Jennings, R.B. (1979). The "wave front phenonemon" of myocardial ischemic cell death. II. Transmural progression of necrosis within the frame work of ischemic bed size (Myocardium at risk) and collateral flow. Lab. Invest. 40, 633-644.

Ribeiro, L.G.T., Louie, E.K., Hillis, L.D., Davis, M.A. and Maroko, P.R. (1979). Early augmentation of R-wave voltage after coronary artery occlusion: a useful index of myocardial injury. J. Electrocardiol. 12, 89-95.

Rich, T.L. and Langer, G.A. (1982). Calcium depletion in rabbit myocardium. Calcium paradox protection by hypothermia and cation substitution. Circ. Res. 51, 131-141.

Richenbach, D.D. and Benditt, E.P. (1970). Catecholamines and cardiomyopathy: The pathogenesis and potential importance of myofibrillar degeneration. Human Pathol. 1, 125-150.

Robie, N.W., Nutter, D.O., Moody, C. and McNay, J.L. (1974). In vivo analysis of adrenergic receptor activity of dobutamine. Circ. Res. 34, 663-671.

Robie, N.W., Goldberg, L.I. (1975). Comparative systemic and regional hemodynamic effects of dopamine and dobutamine. Am. Heart J. 90, 340-345.

Roberts, R. and Sobel, B.E. (1973). Isoenzymes of creatine phosphokinase and diagnosis of myocardial infarction. Ann. Intern. Med. 79, 741-743.

Roberts, R., Sobel, B.E. and Parker, C.W. (1976). Radioimmunoassay for creatine kinase isoenzymes. Science 194, 855-857.

Robertson, J.D. (1960). The molecular structure and contact relationships of cell membranes. Progr. Biophys. 10, 343-418.

Rona, G., Chappel, C.I., Balazs, T. and Gaudry, R. (1959). An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. Am. Med. Asso. Archi. Pathol. 67, 443-455.

Rona, G., Kahn, D.S. and Chappel, C.I. (1963). Studies on infarct-like necrosis produced by isoproterenol: A review. Revue Canadienne de Biologie 22, 241-255.

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

Rostgaard, J. and Behnke, O. (1965). Fine structural localization of adenine nucleoside phosphatase activity in the sarcoplasmic reticulum and the T system of rat myocardium. J. Ultrastruct. Res. 12, 579-591.

Rude, R.E., Izquierdo, C., Buja, M.L. and Willerson, J.T. (1982). Effects of inotropic and chronotropic stimuli on acute myocardial ischemic injury. I: Studies with dobutamine in the anesthetized dog. Circulation 65, 1321-1328.

Ruffolo, Jr. R.R., Spradlin, T.A., Pollock, G.D., Waddell, J.E. and Murphy, P.J. (1981). Alpha and beta adrenergic effects of stereoisomers of dobutamine. J. Pharmacol. Exp. Ther. 219, 447-452.

Sachs, C. and Jonsson, G. (1975). Mechanism of action of 6-hydroxydopamine. Biochem. Pharmacol. 24, 1-8.

Sakamoto, T. and Yamada, T. (1977). Hemodynamic effects of dobutamine in patients following open heart surgery. Circ. 55, 525-533.

Schaper, W., Jageneau, A. and Xhonneux, R. (1967). The development of collateral circulation in the pig and dog heart. Cardiologia 51, 321-335.

Schenk, E.A. and Moss, A.J. (1966). Cardiovascular effects of sustained norepinephrine infusions. II. Morphology. Circ. Res. 18, 605-616.

Segretain, D., Rambourg, A. and Clermont, Y. (1981). Three dimensional arrangement of mitochondria and endoplasmic reticulum in the heart muscle fiber of the rat. Anat. Rec. 200, 139-151.

Shahab, L., Wollenberger, A., Krause, E.-G. and Genz, S. (1972). The effect of acute ischemia on catecholamines and cyclic AMP levels in normal and hypertrophied myocardium. In: Effect of acute ischemia on myocardial function. Ed. Oliver, M.F. et al., Churchill Livingston, London, 97-108.

Shen, A.C. and Jennings, R.B. (1972). Myocardial calcium and magnesium in acute ischemic injury. Am. J. Pathol. 67, 417-440.

Shen, A.C. and Jennings, R.B. (1972). Kinetics of calcium accumulation in acute myocardial ischemic injury. Am. J. Pathol. 67, 441-452.

Shugold, G.E. (1967). Transient QRS changes simulating myocardial infarction associated with shock and severe metabolic stress. Am. Heart J. 74, 402-409.

Simpson, F.O. and Rayns, D.G. (1968). The relationship between the transverse tubular system and other tubules at the Z disc levels of myocardial cells in the ferret. Am. J. Anat. 122, 193-208.

Simpson, F.O., Rayns, D.G. and Ledingham, J.M. (1973). Ventricular and atrial myocardium. In: C.E. Challice and S. Viragh (Eds.), Ultrastructure of the mammalian heart. Ultrastructure of the biological systems, Academic Press, New York, 6, 1-41.

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

Singal, P.K., Yates, J.C., Beamish, R.E. and Dhalla, N.S. (1981). Influence of reducing agents on adrenochrome-induced changes in the heart. Arch. Pathol. Lab. Med. 105, 664-669.

Singal, P.K., Kapur, N., Dhillon, K.S., Beamish, R.E. and Dhalla, N.S. (1982). Role of free radicals in catecholamine-induced cardiomyopathy. Can. J. Physiol. Pharmacol. 60, 1390-1397.

Singal, P.K., Dhillon, K.S., Beamish, R.E., Kapur, N. and Dhalla, N.S. (1982). Myocardial cell damage and cardiovascular changes due to i.v. infusion of adrenochrome in rats. Br. J. Exp. Pathol. 63, 167-176.

Sjostrand, F.S. and Anderson, E. (1954). Electron microscopy of the intercalated discs of cardiac muscle tissue. Experientia, 10, 369-370.

Sjostrand, F.S., Anderson-Cedergren, E. and Dewey, M.M. (1958). The ultrastructure of the intercalated disc of frog, mouse and guinea pig cardiac muscle. J. Ultrastruct. Res. 1: 271-287.

Sjostrand, F.S. and Anderson-Cedergren, E. (1960). Intercalated discs of heart muscle. In: The structure and function of muscle, structure, edited by G. Bowne, New York: Academic, 1, 421-445.

Smith, G.T., Geary, G., Ruf, W., Fore, F.N., Oyama, M. and McNamara, J.D. (1980). Quantitative effect of a single large dose of methylprednisolone on infarct size in baboons. Cardiovas. Res. 14, 408-418.

Sobel, B.E., Markam, J. and Roberts, R. (1977). Editorial: Factors influencing enzymatic estimates of infarct size. Am. J. Cardiol. 39, 130-132.

Sobel, B.E., Corr, P.B., Robison, A.K., Goldstein, R.A., Witkowski, F.X. and Klein, M.S. (1978). Accumulation of lysophosphoglycerides with arrhythmogenic properties in ischemic myocardium. J. Clin. Invest. 62, 546-553.

Sommer, J.R. and Spach, M.S. (1964). Electron microscopic demonstration of adenosine triphosphatase in myofibrils and sarcoplasmic membranes of cardiac muscle of normal and abnormal dogs. Am. J. Pathol. 44, 491-505.

Sommer, J.R. and Johnson, E.A. (1968). Cardiac muscle. A comparative study of Purkinje fibers and ventricular fibers. J. Cell Biol. 36, 497-526.

Sommer, J.A. and Johnson, E.A. (1979). Ultrastructure of cardiac muscle. In: Handbook of physiology, Ed. Robert M. Berne, American Physiological Society, 113-186.

Sommers, H.M. and Jennings, R.B. (1964). Experimental acute myocardial infarction. Histologic and histochemical studies of early myocardial infarcts induced by temporary or permanent occlusion of a coronary artery. Lab. Invest. 13, 1491-1503.

Stone, M.J., Waterman, M.R., Harimoto, D., et al. (1977). Serum myoglobin levels as diagnostic test in patients with acute myocardial infarction. Br. Heart J. 39, 375-380.

Strubelt, O. and Siegers, C.P. (1975). Role of cardiovascular and ionic changes in pathogenesis and prevention of isoprenaline-induced cardiac necrosis. In: Recent Advances in studies on cardiac structure and metabolism. Albrecht Fleckenstein and George Rona, University Park Press, Baltimore, 6, 135-142.

Sylven, C. and Bendz, R. (1978). Myoglobin, creatine kinase and its isoenzyme MB in serum after acute myocardial infarction. Eur. J. Cardiol. 8, 515-521.

Szakacs, J.E. and Mehlman, B. (1960). pathologic changes induced by l-norepinephrine. Quantitative aspects. Am. J. Cardiol. 5, 619-627.

Takenaka, F. (1975). Effects of isoproterenol and some other drugs on high energy phosphate metabolism in rat myocardium. In: Recent Advances in Studies on Cardiac Structure and Metabolism. Albrecht Fleckenstein and George Rona (Editors), University Park Press, Baltimore, 6, 151-158.

Toleikis, A. (1983). Cytochrome oxidase activity of mitochondria from ischemic and reperfused myocardium. Adv. Myocardiol. 4, 409-418.

Trump, B.F., Mergner, W.J., Kahng, M.W. and Saldino, A.J. (1976). Studies on the subcellular pathophysiology of ischemia. Circ. 53: Suppl. 1, 1-17.

Tuttle, R.R., Pollock, G.D., Todd, G. and Tust, R. (1973). Dobutamine: containment of myocardial infarction size by a new inotropic agent. Circ. VII-VIII (Supp. IV), IV-132.

Tuttle, R.R. and Mills, J. (1975). Dobutamine. Development of a new catecholamine to selectively increase cardiac contractility. Circ. Res. 36, 185-196.

Tuttle, R.R., Hillman, C.C. and Toomey, R.E. (1976). Differential beta adrenergic sensitivity of atrial and ventricular tissue assessed by chronotropic, inotropic and cyclic AMP responses to isoprenaline and dobutamine. Cardiovas. Res. 10, 452-458.

Tuttle, R.R., Pollock, G.D., Todd, G., MacDonald, B., Tust, R. and Dusenberry, W. (1977). The effect of dobutamine on cardiac oxygen balance, regional blood flow and infarction severity after coronary artery narrowing in dogs. Circ. Res. 43, 357-364.

Unverferth, D.V., Leier, C.V., Magorien, R., Kolibash, A.J. and Baba, N. (1979). Abstract: Ultrastructural analysis of endomyocardial biopsies before and after dobutamine. Circ. 59-60 (Supp. II), II-43.

Unverferth, D.V., Leier, C.V., Magorien, R.D., Croskery, R., Svirbely, J.R., et al. (1980). Improvement of human myocardial mitochondria after dobutamine: A quantitative ultrastructural study. J. Pharmacol. Exp. Ther. 215, 527-532.

Unverferth, D.V., Magorien, R.D., Lewis, R.P. and Leier, C.V. (1980). Long-term benefit of dobutamine in patients with congestive cardiomyopathy. Am. Heart J. 100, 622-630.

Unverferth, D.V., Magorien, R.D., Altschuld, R., Kolibash, A.J., Lewis, R.P. and Leier, C.V. (1983). The hemodynamic and metabolic advantages gained by a three-day infusion of dobutamine in patients with congestive cardiomyopathy. Am. Heart J. 106, 29-34.

Unwin, N. and Henderson, R. (1984). The structure of proteins in biological membranes. Sci. Am., 78-94.

Vasudevan, G., Mercer, D. and Varat, M. (1978). Lactic dehydrogenase isoenzyme determination in the diagnosis of acute myocardial infarction. Circulation, 57, 1055-1057.

Vatner, S.F., McRitchie, R.J. and Braunwald, E. (1974). Effects of dobutamine on left ventricular performance, coronary dynamics, and distribution of cardiac output in conscious dogs. J. Clin. Invest. 53, 1265-1273.

Wagner, G.S., Roe, C.R., Limbird, L.E., et al. (1973). The importance of identification of the myocardial specific isoenzyme of creatine phosphokinase (MB form) in the diagnosis of acute myocardial infarction. Circulation, 47, 263-269.

Watanabe, A.M. and Besch, H.R. (1974). Cyclic adenosine monophosphate modulation of slow calcium influx channels in guinea pig hearts. Circ. Res. 35, 316-324.

Wechsler, A.S. (1977). Development of coronary collateral circulation. Ann. Rev. Med. 28, 341-348.

Weintraub, W.S. and Helfant, R.H. (1983). Coronary artery spasm: Historic aspects. In: Coronary artery spasm and thrombosis, ed. Sheldon Goldberg, F.A. Davis Company, Philadelphia, 1-4.

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

Wexler, B.C., Judd, J.T. and Kittinger, G.W. (1968). Myocardial necrosis induced by isoproterenol in rats. Angiol. 19, 665-682.

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

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

Whalen, Jr. D.A., Hamilton, D.G., Ganote, C.E. and Jennings, R.B. (1974). Effect of a transient period of ischemia on myocardial cells. Am. J. Pathol. 74, 381-398.

Wildenthal, K. (1978). Editorial: Lysosomal alterations in ischemic myocardium: Result or cause of myocellular damage. J. Mol. Cell. Cardiol. 10, 595-603.

Willerson, J.T., Hutton, I., Watson, J.T., Platt, M.R. and Templeton, G.H. (1976). Influence of dobutamine on regional myocardial blood flow and ventricular performance during acute and chronic myocardial ischemia in dogs. Circulation 53, 828-833.

Willerson, J.T., Hillis, L.D. and Buja, L.M. (1982). Editors. Serum enzymatic, hematologic and metabolic alterations with acute myocardial infarction. In: Ischemic Heart Disease: Clinical and Pathophysiological Aspects. Raven Press, 245-250.

Willerson, J.T., Hillis, L.D. and Buja, L.M. (1982). Detection and quantitation of ischemic and necrotic myocardium: Electrocardiographic techniques. In: Ischemic Heart Disease: Clinical and Pathophysiological Aspects. Raven Press, 233-243.

Willerson, J.T., Hillis, L.D. and Buja, L.M. (1982). Pathogenesis and pathology of ischemic heart disease. In: Ischemic heart Disease: Clinical and Pathophysiological Aspects. Raven Press, 7-83.

Williamson, J.R. and Rich, T.I. (1983). Mitochondrial function in normal and hypoxic states of the myocardium. Adv. Myocardiol. 4, 271-285.

Wolkowicz, P.E., Michael, L.H., Lewis, R.M. and McMillan-Wood, J. (1983). Sodium-calcium exchange in dog heart mitochondria: effects of ischemia and verapamil. Am. J. Physiol. 244 (Heart Circ. Physiol. 13), H644-H651.

Wood, J.M., Sordahy, L.A., Lewis, R.M. and Schwartz, A. (1973). Effect of chronic myocardial ischemia on the activity of carnitine palmitylcoenzyme a transferase of isolated canine heart mitochondria. Circ. Res. 32, 340-347.

Yates, J.C. and Dhalla, N.S. (1975). Induction of necrosis and failure in the isolated perfused rat heart with oxidized isoproterenol. J. Mol. Cell. Cardiol. 7, 807-816.

Yates, J.C., Beamish, R.E. and Dhalla, N.S. (1981). Ventricular dysfunction and necrosis produced by adrenochrome metabolite of epinephrine: relation to pathogenesis of catecholamine cardiomyopathy. Am. Heart J. 102, 210-221.

Yeager, J.C. and Iams, S.G. (1981). The hemodynamics of isoproterenol-induced cardiac failure in the rat. Circ. Shoc. 8, 151-163.

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

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

Zypen, E.v.d. (1974). On catecholamine-containing cells in the rat interatrial septum. Enzymatic-histochemical and electron microscopical study. Cell. Tissue Res. 151, 201-218.