BIOCHEMICAL ASPECTS OF CARDIAC HYPERTROPHY

by

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CHAPTER I INTRODUCTION.

INTRODUCTION

1.0 Adaptive Growth

Adaptive growth can be caused in a variety of ways. In many organs, for example liver, a reduction in mass by surgical excision calls forth a growth response in what remains (Glinos 1958). The same sort of response may often be elicited by creating a functional overload without diminishing the substance of the organ. The nature of this functional overload varies from organ to organ depending upon what physiological activities are normally carried out. Apparently all that is necessary is to create an imbalance between functional demands and the capacity of the organ in question to meet them.

It has been suggested (Goss, 1966-67) that the way in which growth is achieved depends largely on the kind of organ and the level of organisation. Some organs can increase their mass by adding new units at the histological level of organisation. New follicles, for example, can be added to the thyroid (Gray, 1929), the ovary (Greenwald, 1962) and the exocrine glands (Augustine, 1963). Other organs, unable to produce such complicated structures, enlarge solely by cellular proliferation. The kidney for instance, cannot make new nephrons, but can enlarge those it has by multiplying and enlarging their component cells, (Bucher and Malt, 1971).

In like manner, the lungs, which cannot make new alveoli beyond infancy, increase their size by cell division (Goss, 1964). Cell replication is also the mode of growth characteristic/...

characteristic of such renewing tissues as blood, epidermis, intestinal mucosa and the spermatogenic epithelial lining of seminiferous tubules. Finally, there are some tissues and organs which are incapable of multiplying their functional units at the histological or cellular level of organisation. Such a mitotically static tissue mass relies on molecular turnover in order to renew itself. This subcellular renewal is the basis for growth in the nervous system (Messier et al, 1958), skeletal muscle (Goldberg, 1969) and heart muscle (Karsner et al, 1925). Here the capacity for compensatory growth is limited to cellular hypertrophy. Although such a mechanism provides the additional morphological substrate for doing extra work, the extent of compensatory growth in such organs is considerably restricted owing to the impossibility of mitotic proliferation, and the upper limit beyond which a cell cannot enlarge without an increase in DNA.

2.0 General Introduction

2.1 Cardiac Hypertrophy; of the two adaptations of the heart to increased work - acceleration in rate (hyperfunction); and hypertrophy - the last-named is more readily apparent. That hypertrophy results from increased work of the heart was appreciated by Carvisart (1811), who drew an analogy between "active aneurysm", as it was then known, and the massive arm of the blacksmith. Subsequent work has confirmed the belief that the mass of the myocardium is a function of the work it performs. The supporting evidence has/...

has been derived from hearts in the diseased state.

The most universal manifestation of heart disease, whatever its cause, is cardiac enlargement. Although it may not always be present in heart disease, its presence always indicates a diseased heart.

Freidbert and Sohval (1937) have classified the various factors which result in cardiac hypertrophy as:

(1) those which increase the resistance to outflow from the heart (i.e. hypertension, valvular disorder and coarctation of the aorta).

(2) those which increase in flow to the heart (i.e. thyrotoxicosis and anaemia).

(3) those which produce severe myocardial weakness (i.e. inflammatory and toxic diseases and myocardial infarction).

The common factor associated with these three classifications appears to be work.

The process of cardiac hypertrophy can perhaps be divided into two stages; hyperfunction and hypertrophy proper. In compensatory hyperfunction the organism makes use of the mechanisms of adaptation to physical work which have been developed in the process of evolution. At the same time however, there is a clear distinction between physiological hyperfunction of the heart on the one hand and compensatory hyperfunction on the other. This is determined not only by the cause of the hyperfunction but also by a time/...

time factor. Hyperfunction in a healthy organism reacting to a given physiological stress is always temporary and discontinuous. It ceases after the completion or cessation of the physiological task. Compensatory hyperfunction in response to hypertension or valvular disease, on the other hand, is not only long-lasting, but what is more important, continuous; and the continuity of the compensatory hyperfunction which results is a vital necessity.

In spite of its continuity, compensatory hyperfunction of the heart may last for many years without signs of exhaustion. At first it is maintained by the existing myocardium, but this leads quite rapidly to the development of cardiac hypertrophy and the gradual exhaustion of the heart and of its regulatory mechanisms. It is this exhaustion which is manifested as the syndrome of chronic heart failure.

Thus, compensatory hyperfunction, hypertrophy and the subsequent exhaustion of the hypertrophied heart represent links in a single process.

3.0 Structural Changes During Cardiac Hypertrophy

3.1 Histological Changes

The writers of pathology textbooks from the middle of the 19th century regarded cardiac hypertrophy as predominantly hyperplasia (i.e. an increase in the number of fibers, Forster, 1856). However, even at that time, some investigators noted that there was no evidence for muscle fiber multiplication (Hirtl, 1855) and later studies showed that the diameter of normal human heart fiber is 15μ, while in the hypertrophied/...

hypertrophied heart it is 25μ to 30μ (Letull, 1897).

With the development of more accurate methods for counting muscle fibers, Linzback (1947, 1948, 1952, 1955) concluded that no division of muscle fibers occurred when myocardial mass was increased up to 60%. At greater levels of hypertrophy Linzback believed that muscle fibers began to divide, and hyperplasia did take place. These last observations, however, are at variance with the findings of a majority of other investigations, particularly those of Wearn (1939-40) who showed in extensive autopsy and experimental material that muscle fiber size increased in myocardial hypertrophy without any alteration in the number of fibers.

Black-Schaffer and Turner (1958) estimated the frequency of nuclei in the myocardium and concluded that myocardial hypertrophy is associated with a reduction in the frequency of nuclei. This supports the idea that cardiac hypertrophy is not accompanied by multiplication of existing muscle cells.

Wearn, (1939, 1940) studying the hypertrophied human heart, found that the ratio between the number of muscle fibers and the number of capillaries in the myocardium was the same as in normal hearts (1:24) though the diameter of the myocardial fibers increased from 15μ to 26μ. This, of course, would indicate that there had been no multiplication of capillaries to serve the greatly enlarged muscle fibers. In addition, Woods (1961) compared the cross-sectional area of/...

of the right coronary artery with the weight of the total ventricular myocardium which it supplied and concluded that it did not increase as the ventricle hypertrophied. This, like the reduction in the number of capillaries per unit mass of myocardium, may limit the supply of oxygen and other substrates to the central portion of the muscle fibers.

Several authors have described the development of fibrosis in the hypertrophied myocardium and generally considered it to result from connective tissue replacement of necrotic and degenerative myocardial fibers. Vail (1939) described areas of necrosis and foci of fatty degeneration in human hypertrophied myocardium. Krymkii (1962) studied 135 children and adolescents with congenital heart disease, and found diffuse or focal myocardial fibrosis in 134 cases. Earlier case studies by Jacobi (1928) and Moenckebert (1924) had indicated that fibrosis only occurred when dilation accompanied hypertrophy. Schoenmacher (1958) studied the relationship between muscle fibers and connective tissue by photomicrography of transverse sections of the myocardium. They found that muscle fibers in the left ventricle of the normal heart occupied 92% of the area of section and connective tissue only 8%. In chronic aortic insufficiency, connective tissue occupied 48% of the section and muscle fibers only 52%. These results taken together indicate that muscle degeneration with subsequent fibrosis is a characteristic of hypertrophy.

3.2/...

3.2 Morphological Changes in the Myocardial Cell

The morphology of the hypertrophied myocardial cell began to be studied only after the introduction of the electron microscope. There are several main ultrastructural changes in the hypertrophied hearts:-

(1) The sarcolemma. Studied by Poche (1969) and Dowlatshahi et al, (1969) of hypertrophied hearts have shown that, in cases where individual myocardial cells have enlarged to twice their normal size, an increase occurs in the amount of subsarcolemmal sarcoplasm. This increase in sarcoplasm was associated with a high degree of vesiculation of the sarcolemmal membranes. Other ultrastructural investigators of cardiac hypertrophy have noted sarcolemmal vesiculation (Bozner et al, 1966) describing them as "cardiac villi" or "arcade-like diverticulae". Often ribosomes, sarcoplasm and mitochondria filled these diverticulae, and multiple pinocytotic vesicles were seen in close approximation to either surface of the sarcolemmal membranes. Such modifications in the sarcolemma may reflect the need of the hypertrophied cell for an increased surface area. The presence of mitochondria in these villus-like processes, as well as the multiplicity of pinocytotic vesicles, may indicate hyperfunction/...

hyperfunction of active transport mechanisms.

(2) The Sarcotubular System. Most observers of hypertrophied hearts have noted dilatation of the sarcoplasmic reticulum and T-tubules, components of the sarcotubular system (Meessen, 1968). These changes in area may make possible an increase in the level of electrolyte transport to maintain the excitation/ionic conduction relationship of the normal fiber.

(3) Nuclei. In hypertrophy, an increase occurs in the cellular volume supplied by each nucleus. There is a concomitant but smaller increase in nuclear size (Linzback 1962); further, the nuclear membrane has often been seen to have numerous indentations (Poche, 1969).

(4) Intercalated Disc. Increase in the width of the intercellular gap region of the intercalated disc and vesiculation exhibiting a dense matrix have been observed by Bishop et al, (1969) and Sohal et al, (1968). It was suggested that hypertrophy and vesiculation of the gap structure might result in an alteration in the efficiency of electrical excitation affecting ultimately the force of contraction.

(5)/...

(5) Mitochondria. Alterations have been reported in

(a) the mitochondrion/myofibril ratio and

(b) mitochondrial structure.

A significant decrease in the ratio of mitochondria to myofibrils has been observed by Palade (1952), Wollenberger et al, (1963) and Poche (1969). This might lead, it is suggested, to a decline in cardiac efficiency. However, other investigators have failed to detect any such changes and have doubted their occurrence (McCallister et al 1962). The question remains open.

Changes in the structure of the mitochondria have been reported by Kirch (1960), Wollenberger etal.(1961) and Novikoff (1961). These have included increases in the width of the cristae, fewer cristae in each mitochondrion, an increased width of the intercristae spaces, increases in the width of the outer mitochondrial membrane and increases in amorphous osmiophilic structure. The significance of these changes is unknown.

(6) Myofilaments. The increase in diameter of myocardial cells during hypertrophy is accompanied by an increase in the number of myofilaments/...

myofilaments (Carney et al.1964, Bishop et al 1969, Meessen 1968 and Richter et al 1963). This suggests that fiber, and ultimately organ, hypertrophy result from a cellular organelle (i.e. myofilament) hyperplasia. As the hexagonal pattern of thick and thin filaments is reported to be unaltered, the suggestion has been advanced that the new myofilaments are added at the periphery of the myofibrils.

(7) The Z Disc or Band. Structural alteration in the disc has been reported by Bishop et al, (1969) and Fawcett (1968). This manifested itself as either a focal or a general widening of the band. However, these changes are sparse and irregular so that their functional significance is doubtful.

Apart from these major changes, other ultrastructural alterations have been reported including increases in glycogen and lysosomal-like granules (lipofuschin-granule, Pearse 1961, 1971; lipid droplets, Hibbs et al (1965), Alexander (1967)).

4.0 Physiology of Cardiac Hypertrophy

4.1 Factors Surrounding Hypertrophy

When/...

When the heart increases its level of activity as an adaptation to functional overload, the necessary adjustment is brought about by two mechanisms:

(a) the Frank-Starling mechanism and

(b) inotropism

Depending on the type and extent of the hyperfunction, either of these mechanisms may play the leading role.

(1) In response to physical work in the hypoxia of high altitude, in all kinds of valvular insufficiency, in arteriovenous fistulae, and in anaemia and thyrotoxicosis, an increase in cardiac output is obtained by an increase in stroke volume and heart rate. While systolic tension and ventricular pressure also increase , they do so to a lesser extent. This type of hyperfunction is chiefly isotonic.

(2) In systemic and pulmonary hypertension or valvular stenosis, hyperfunction of the heart maintains cardiac output in spite of increased resistance to ventricular ejection. This is accomplished by an increase in myocardial wall tension and systolic pressure with little or no change in stroke volume. This type of hyperfunction is chiefly isometric.

Thus it is generally accepted that isotonic hypertrophy/...

hypertrophy results when ventricular volume is increased primarily by the Frank-Starling mechanism with inotropism playing a secondary role; while isometric hypertrophy occurs when myocardial wall tension is increased, primarily by the inotropic mechanism with the Frank-Starling mechanism in a secondary role (Spiro and Sonnenblick 1965; Sonnenblick et al 1962, 1964 and 1965).

4.2 The Functional Significance of Hypertrophy

For many years it has been questioned whether the development of myocardial hypertrophy affects the maximum strength which the heart is capable of developing (Grant, 1963). Many investigators have claimed that the hypertrophied heart is more efficient than the normal heart (Diechhoff, 1936, Hasenfield and Romberg, 1897). Others, however, have believed, on the basis of clinical observations, including the well known ST and T wave abnormalities on the electrocardiogram, that the hypertrophied heart is always functionally inadequate, and recent investigations have clearly established that there is a decrease in the force and velocity of contraction in the hypertrophied heart muscle (Spann et al, 1966, Beznak, 1958, Korecky, 1966, Geha et al, 1966 and Meerson, 1969).

Meerson (1969) has attempted to frame a comprehensive theory of both types of hypertrophy. He suggests that in isometric hypertrophy the increased wall tension evokes increased energy production and protein synthesis and these in turn lead to rapid hypertrophy. This, however, is succeeded/...

succeeded by a variety of degenerative changes which, Meerson believes, reflect exhaustion of the heart's functional reserve and of its regulatory mechanisms and lead to ultimate failure. In isotonic hypertrophy there is little increase in wall tension and, therefore, little activation of energy metabolism and protein synthesis. Heart failure in this case is, according to Meerson, a result of failure to mobilize a functional reserve.

5.0 Biochemical Aspects of Cardiac Hypertrophy

5.1 Introduction

Perhaps one might say that every disorder of cardiac contractility must find its ultimate explanation in a biochemical lesion. Such a lesion during cardiac hypertrophy and subsequent failure has been the subject of intense investigation during the past few years.

5.20 Energy Production

A defect in energy production was one of the first biochemical lesions suspected to underlie the hypertrophied failing heart.

5.2.1 Oxidative Phosphorylation

Several reports have described abnormalities in oxidative phosphorylation during hypertrophy and subsequent failure, (Schwartz and Lee 1962, Wollenberger 1965, Schien et al, 1959). Other investigators have found normal oxidative phosphorylation in association with heart failure (Sobel et al, 1967, Fox and Reed 1965).

Some/...

Some of these apparent differences may be explained by the differences in the degree of hyperfunction produced by these investigators as well as the presence or absence of overt heart failure. However, in the presence of hypertrophy and heart failure, oxygen consumption remains normal (Bing et al, 1963, Olson, 1959, Levine and Wagman, 1962). In addition Bing (1961) on the basis of coronary sinus catheterisation has found that the uptake of substrate by the myocardium is normal in heart failure.

In comparing experiments in vitro and in vivo it must be remembered that any increase in energy metabolism seen in vivo is presumably brought about by active myocardial hyperfunction. When the heart is removed from the body this hyperfunction naturally ceases, and, therefore, in such cases we are by no means assessing the effects of hyperfunction on energy metabolism but rather the influence of hyperfunction on the enzyme systems which produce energy. Thus it is easy to see that long-lasting hyperfunction might be associated with degeneration of mitochondrial structures and gradual impairment of the mechanisms by which energy is made available for contraction.

However, experiments in vivo are not without their own limitations. Firstly, steady state conditions must be assumed to prevail in measuring coronary blood flow and consequently, in determining myocardial utilisation of a substrate (Zierler, 1961); secondly, the methods provide no direct knowledge of the intermediate metabolism of the utilised/...

utilised substrate (Bing, 1965); and, finally, the quantity of substrate catabolised to CO_2 and H_2O is inferred from an oxygen extraction ratio (Bing et al, 1954). In view of these limitations the metabolism of carbohydrates in the hypertrophied failing heart appears to require more investigation.

5.2.2 Carbohydrate Metabolism

Little work has been done on carbohydrate metabolism in cardiac hypertrophy and heart failure. The few results are confusing. Gudjarnason et al. (1964) found increased activity of glyceraldehyde phosphate dehydrogenase in the myocardium of patients who died with heart failure. This may suggest increased use of the glycolytic pathway. Schwartz and Lee (1962) found a decrease in anaerobic lactate production and glucose utilisation in cell-free supernatant in the hypertrophied ventricles of guinea pigs with heart failure. However, Bishop et al. (1970) found an increase in anaerobic glycolysis as measured by lactate production in dogs. The possible discrepancy between these two results might be explained in the systems used. Schwartz and Lee incubated their homogenates with ATP and glucose; whereas Bishop et al used ADP, glucose and hexokinase. The allosteric effects of adenine nucleotides on the enzymes of glycolysis is well known. Other investigators (Meerson et al, 1955, Larionov, 1967) have found marked increases in vivo in the incorporation of glucose 14C into glycogen during cardiac hypertrophy and increased concentrations of lactate/...

lactate and pyruvate. These results suggest an increase in anaerobic glycolysis during cardiac hypertrophy.

5.2.3 Lipid Metabolism

Studies using isolated perfused hearts have revealed that there is an increase in palmitate uptake with a rise in ventricular pressure. Physiological concentrations of palmitate also inhibited glucose transport and glycogenolysis in hearts with increased ventricular pressure (Neeley et al, 1969). Studies by Opie (1965) support the findings of preferential oxidation of palmitate as compared to glucose over a wide range of isovolumic work loads. Carlsten et al, (1963), however, have shown that in human hypertrophied hearts non-esterfied fatty acids tend to be extracted less well than under normal conditions. The discrepancy between these findings and those of Neeley and Opie quoted above might be explained by a decrease in perfusion in the hypertrophied heart due to an increase in the ratio of capillary to muscle (Wearn 1940).

Wittles et al, (1968) measured fatty acids metabolism in cardiac hypertrophy and found a decrease in the rate of the oxidation of long chain fatty acids by the myocardium. They also found an increase in the synthesis of triglycerides.

Although more studies are needed, the balance of evidence at present suggests that during cardiac hypertrophy there is an increase in glycolysis and a decrease in the oxidation of fatty acids.

5.2.4 Energy Storage

Insufficient/...

Insufficient energy stores for the maintenance of normal myocardial contraction have been thought to underlie the failure of the hypertrophied heart. Several investigators have reported decreased high energy phosphate stores during hypertrophy or heart failure (Mangus and Myers, 1940, Minton et al, 1960 and Goodkind et al, 1959, Vyalykh and Meerson, 1960, Poole et al, 1967). However, the decreasesobserved by most of these investigators were small.

It has been suggested that the failure to find a significant relationship between energy stores and the onset of heart failure may be due to the fact that energy stores are compartmentalised. In this case, a significant deletion of a single compartment of energy stores might lead to heart failure, but this decrease might not be detected when compared to the total store of energy (Meerson, 1969).

5.3 Protein and Nucleic Acid Metabolism

In experimental cardiac hypertrophy and heart failure, the total myocardial contents of DNA and RNA are increased. The increase in RNA content exceeds the increase in myocardial mass and, therefore, myocardial RNA concentration is increased. Myocardial DNA content is increased more or less in proportion to the increase in myocardial size and, therefore, myocardial DNA concentration is unchanged (Meerson, 1969).

The myocardial cell does not proliferate during the process/...

process of hypertrophy. Hence the increase in myocardial DNA content implied an increase in the DNA content of each myocardial cell. In accordance with this, an increase in DNA per cell presumably indicating polyploidy in the nuclei of myocardial cells, has been reported (Sandrilter et al, 1964, Kompmann et al, 1966). On the other hand several investigators have measured DNA content of individual cells within the hypertrophied myocardium and have found that virtually the entire increase in DNA content within the myocardium during hypertrophy occurs in connective tissue cells rather than in myocardial cells (Meerson 1967, Morkind and Ashford 1968, Grove et al,1969). These authors consequently concluded that there was no increase in nuclear DNA synthesis in the myocardial cell during hypertrophy, and that each unit of DNA in the hypertrophied myocardial cell served a greatly expanded cell volume.

The process of transcription is thought to increase during myocardial hypertrophy. Actinomycin D specifically inhibits RNA polymerase and thereby blocks the DNA dependent synthesis of RNA. Experimental coarctation of the aorta following the administration of actinomycin D does not cause hypertrophy. Instead, heart failure ensues rapidly without activation of protein synthesis (Meerson 1969, Zuhlke et al, 1966). On the other hand, in the absence of experimental stress, administration of actinomycin D does not result in an immediate inhibition

of/...

of protein synthesis, indicating that the already synthesised RNA is capable of carrying out protein synthesis for some time. Clearly the acutely overloaded heart must need increased RNA synthesis.

The stimulation of RNA during hypertrophy seems to occur to about the same extent in all species of RNA. The distribution of labelling the various types of RNA, (28s, 18s and 4s) separated by sucrose density gradient centrifugation, is the same for hearts of animals with aortic constriction as for those of the control animals (Koide et al 1969, Posner et al 1966).

The activation of the process of translation during hypertrophy is indicated by an increased incorporation of amino acid into protein. Following an acute overload, there is an increase in the incorporation of amino acids both in the isolated heart (Schreiber et al, 1968) and in the rabbit with experimental aortic stenosis (Gudbjarnason et al, 1964). In addition Meerson (1969) has shown that this increase in amino acid incorporation occurs only during the early stages of acute overload. When heart failure occurred seven months after coarctation of the aorta in the rabbit, incorporation of ^{35}S-methionine into mitochondria protein and actomyosin was decreased. What relation this decrease in amino acid incorporation may have to heart failure is unknown. Meerson, however, has suggested that it could explain the failure to renew cellular structures.

It has been suggested that certain rate-limiting steps/...

steps in normal myocardial energy metabolism may limit the ability of the myocardium to respond to acute stress. For instance, during the early stages of experimental cardiac overload increased nucleic acid and protein synthesis require an increased supply of ribose-5-phosphate which is formed from glucose-6-phosphate in the pentose phosphate shunt. These reactions are catalysed by transketolase and transaldolase and, since transketolase activity may be rate-limiting in the formation of ribose-5-phosphate (Bruns et al, 1958), myocardiac requirements for this substrate may not be met. Indeed, Meerson (1967) has shown that inhibition of transketolase activity with oxythiamine inhibits protein synthesis in myocardial hypertrophy produced by severe coarctation of the aorta.

Several studies have shown that in the early stages of hyperfunction there is an increase in the pentose phosphate shunt (Meerson et al, 1960, 1962). However, during the late stages of cardiac hypertrophy there was a decrease in this pathway (Matsumoto et al, 1965).

5.4.0 Catecholamines

Recently several studies have demonstrated that hypertrophied hearts contain abnormally low concentrations of norepinephrine. Fischer et al, (1965) found both the concentration of endogenous norepinephrine and the binding of exogenous ^{3}H-norepinephrine to be diminished in hypertrophied hearts from rats subjected to constriction of the abdominal aorta for two months. Other investigators have also/...

also found diminished norepinephrine stores in hypertrophy (Chidsey et al, 1964, de Champlain et al, 1968).

Poole et al, (1967) have attributed the decrease in norepinephrine to a deficiency of the enzyme tyrosine hydroxylase. Fischer et al, (1965) suggested that there may be increased turnover of catecholamines resulting from both decreased rebinding of norepinephrine released from sympathetic nerve endings and decreased binding of circulating norephinephrine. Although de Champlain et al, (1968) demonstrated an increased monamine oxidase activity in hypertrophied heart muscle, they concluded that this could not account for the decrease in norepinephrine. Regardless of the cause for the depletion of norepinephrine, it appears from the studies of Span et al, (1966) that it can be associated with normal contractility in the isolated muscle and, hence, that norepinephrine deficiency alone cannot explain an alteration in contractility of hypertrophied muscle-

5.5 Energy Utilisation

(1) "Excitation Contraction Coupling" is the series of events leading from electrical depolarisation of the muscle membrane to contraction of the sarcomere. Electrical depolarisation of the muscle membrane is carried to the interior of the cell via the transverse tubular system (T-system) which then forms a junction with the longitudinal series of tubules which surround the myofilaments-

The/...

The sarcoplasmic reticulum is capable of concentrating calcium ion against a large concentration gradient and the activity of this calcium pump is associated with stoichiometric utilisation of ATP. In the resting state, these membranes are able to decrease the concentration of calcium ion in the area of the myofilaments to less than 10^{-7}M. Contraction is initiated when calcium is released from the sarcoplasmic reticulum into the area of the myofilaments, increasing the calcium concentration to the level of 10^{-6}M.

The nature of the junction between the T-system and the sarcoplasmic reticulum and the means by which electrical depolarisation of the T-system causes release of calcium from the sarcoplasmic reticulum are not completely understood, nor is it clear how calcium itself initiates contraction. However, knowledge in this area has been advanced in the last ten years. It is known that tropomyosin plays an important role in the interaction of Ca^{++} with actin and myosin and that tropomyosin is distributed alone the thin filaments of actin. The active component of tropomyosin relative to Ca^{++} is another protein, troponin. In the absence of Ca^{++}, troponin inhibits the interaction of actin and myosin and prevents muscle contraction. In the presence of Ca^{++} this inhibition is removed, actin and myosin interact and contraction occurs.

The possibility that a defect in excitation contraction/...

contraction coupling could underlie the hypertrophied failing heart has been recently considered. Recent studies by Harigaya and Schwartz (1969) and Siegal et al, (1969) seem to suggest an alteration in calcium transport. These authors suggested that an alteration in calcium metabolism might be an important factor in the hypertrophied failing heart.

5.6 Contraction

The physiological function of the myocardial cell contraction is performed by specialised organelles, the myofibrils. The changes that occur may be explained in terms of theories of muscle contraction where the development of force and power depend on the interaction of myosin, actin, ATP, Mg^{++} and Ca^{++}. The sliding filament hypothesis describes the interaction of actin and myosin by means of cross-bridges which extend from myosin to actin. This interaction produces a force of displacement by cyclic binding of the myosin cross bridges to actin.

A prerequisite for activity in this model is the hydrolysis of ATP in the presence of Mg^{++} and Ca^{++} by the contractile protein complex actomyosin. In this context, the ATPase activity of myosin has been correlated with velocity of shortening and tension development (Barany 1967).

Recent studies by Meerson (1969) on dogs; Alpers and Gordon (1962), in human; Gordon and Brown (1966), in human) have shown decreased ATPase activity in the failing hypertrophied heart. However, studies by Chandler et al (1967), in cat; Olson/...

Olson, (1961), in dogs, revealed no change in ATPase activity. This mechanical-biochemical discrepancy may result from differences in the cationic milieu in which the protein ATPase activity is assayed. This area obviously needs more investigation.

6.0 Pathogenesis of Cardiac Hypertrophy

Many theories have been proposed to explain the development of cardiac hypertrophy (Willius et al 1934, Merrmann et al 1939).

6.1 The Theory of Work Hypertrophy attributes cardiac hypertrophy to an increase in the work of the heart. The size of an animal's heart increases in proportion to the amount of activity necessitated by its mode of life and cardiac hypertrophy can be induced by exercising dogs (Steinhaus 1933). Clinical and pathological observations disclose that hypertrophy of a chamber occurs when its work is increased in compensation for a valvular or circulatory abnormality. A modification of this theory regards a chronic increase in myocardial metabolic rate per beat (oxygen consumption per beat) as a stimulus to cardiac hypertrophy (Badeer, 1964).

6.2 The Nutritional or Hypoxic Theory attributed cardiac hypertrophy to a defective blood supply. Hypertrophy has been demonstrated repeatedly in clinical and experimental anaemia and in experimental coronary ligation (Normal et al, 1960). The cardiac hypertrophy in arteriovenous aneurysm and aortic insufficiency is thought to be due to a deficient coronary circulation/...

circulation (Lewis et al, 1923). Cardiac hypertrophy can be introduced in rats either by exercise (work hypertrophy) or by prolonged intermittent exposure to low O_2 tension. The essential difference is the relatively greater hypertrophy of the right ventricle in the latter group, presumably due to increased pulmonary vascular resistance secondary to hypoxia (Van Liere et al, 1965).

6.3 The Injury Theory suggests that cardiac hypertrophy is a reaction to tissue injury which acts as a "pathologic nutritive" (Albrecht et al, 1903, Eyster et al, 1927). Its validity has been questioned in a critical appraisal by Kerr (1957).

6.4 The Muscular Stretch Theory suggests that the fundamental cause of hypertrophy is the stretching of muscle fibers (Horvath 1897). This is not necessarily contradictory to the other theories, but rather an attempt to define, more clearly and precisely, their application. Stretching of myocardial fibers results whenever this diastolic volume and pressure are increased by enhanced inflow, impaired myocardial contraction or increased resistance to outflow. This is the stage of cardiac dilatation. Increased fiber length may alter both the shape and size of the heart, factors which influence the degree of myocardial tension and oxygen requirement (Burton et al, 1957).

Ultimately increased oxygen utilisation appears important/...

important to the growth process which underlies hypertrophy. It has been suggested that the tension-time index, which is a measure of increased myocardial work, and often the oxygen requirement may serve as the "indicator" to the deoxyribonucleic acid (DNA) genetic coding centre in the myocardial to order a controlled synthesis of ribonucleic acid (RNA) and protein for required growth of hypertrophy (Norman, 1962). The tension-time index can be correlated with dilatation which by itself, or in association with increased heart rate, may be the stimulus to cardiac hypertrophy.

The lengthening of muscle fibers results in greater surface area for an unchanged mass of muscle. This permits a relatively greater nutritive diffusion surface between blood and fiber and, therefore, leads to the fiber's growth. In due time the dilated fibers become hypertrophied. This may explain the development of cardiac hypertrophy following dilatation in cases of valvular and vascular lesions as well as in instances of myocardial disease. Hormonal factors especially thyroxine and growth hormone may be concerned in the development of cardiac hypertrophy (Beznak 1960, 1964).

Progressive heart failure is a potent factor in pronounced cardiac hypertrophy because it is responsible for progressive increase in diastolic volume and consequent muscle stretch. On the other hand, increased work of the heart does not appear to cause hypertrophy if the additional work/...

work is due exclusively to tachycardia while the stroke output is not enhanced, as in most cases of uncomplicated hyperthyroidism. Finally, there may be many unknown extrinsic or intrinsic factors controlling the metabolism of the muscle fiber which may account for marked differences in the degree of hypertrophy experienced by different hearts in response to apparently similar "pathologic" disturbances. Hearts in aortic stenosis have varied from virtually normal weight to over 2000g (Lowe et al 1948) - sometimes without any correlation with the degree of stenosis or duration of illness. Although marked hypertrophy is the rule in cases of hypertension with heart failure, little or no hypertrophy has been found at autopsy in the hearts of some patients with these conditions (Kleinfeld et al, 1952).

The Situation 1969

At the beginning of the present investigation, the situation could be summarised as follows. Histological and morphological studies had generally showed that the hypertrophic heart was not merely larger than usual; it also contained much more fibrous tissue than the normal heart and the fine structure of the myocardial cells was abnormal in a number of important respects.

One might expect that the morphological changes and more especially the loss of efficiency should have a biochemical aspect and might even to the ultimate be capable of explanation in purely biochemical terms. However, minimum biochemical changes have been reported in abundance; Ca^{++} transport is marginally depressed; the concentration of ATP and phosphocreatine are marginally depressed; in spite of the increased functional demand, uptake and, to a greater degree, oxidation of fatty acids is depressed; the glycogen reserve of the myocardial cells is depressed, while the lactate content is increased. Neither individually nor in combination do these changes indicate damage to the myocardium; but they do suggest that it is being pushed beyond its normal physiological limit and is perhaps not coping very successfully with the challenge. There seemed a need for a more detailed investigation on these lines.

CHAPTER II RESULTS.

SECTION I RESULTS

1.0 Introduction

In their attempts to understand the metabolic abnormalities during myocardial hypertrophy and heart failure, most investigators have directed their attention to the mitochondria and oxidative metabolism as the major source of energy production in the heart. However, an additional factor which must be considered in the bioenergetics of the hypertrophied myocardium is the increased demand placed upon the energy resources of the heart by alterations in hemodynamic and mechanical factors. When oxidative metabolism is taxed to its limit, or if inhibitory factors are operating, it is reasonable to assume that reserve sources of energy production will be brought into action. The role of glycolysis in meeting this increased energy demand has received little attention. It has also been argued that a decrease in the ratio of capillary to myofibril during cardiac hypertrophy leads to hypoxia in the myocardium (page 5) as a result of the increase in the diffusion distance from the capillary lumen to the centre of the hypertrophied area. Since direct methods of determining the intracellular distribution of oxygen are not available, indirect means were sought.

The association of different isoenzymes of lactate dehydrogenase (LDH) with aerobic and anaerobic metabolism (Chan et al, 1962, Markert, 1963, Dawson et al, 1964) and the shift in the isoenzymes distribution, which occurs with/...

with altered environmental oxygen availability, (Johansson, 1966, Lindy and Rajasalmi, 1966, Thorling and Jensen, 1966) appeared to afford a means of evaluation myocardial oxygen availability.

Lactate dehydrogenase exists as a tetramer made up of two types of genetically determined subunits - heart (or H) and muscle (or M) corresponding to their respective preponderance in these tissues (Appella and Markert, 1961).

The nomenclature used in this study to describe the various isoenzymes of lactate dehydrogenase will be as follows: LDH I, LDH II, LDH III, LDH IV, and LDH V, LDH I carrying the highest negative change and migrating furthest towards the anode during electrophoresis.

The theory that the pattern of LDH isoenzymes in a tissue is associated with the metabolic activity of that tissue is based mainly on the observation that tissues highly dependent on aerobic metabolism, such as heart and brain, have a predominance of H subunits (Vessell et al, 1962, Dawson et al, 1964), whereas tissues such as white skeletal muscle and uterine smooth muscle which function well under anaerobic conditions have a predominance of M subunits (Vessell et al, 1962, Dawson et al, 1964).

Exposure of heart cells (originally derived from primary explant of monkey heart) in tissue culture to low oxygen tensions provokes an increased synthesis of the LDH V isoenzyme (Goodfried et al, 1966). These findings indicate that cells are capable of adapting their LDH isoenzymes/...

isoenzymes in response to environmental variation in oxygen availability.

Recently, Bello and Messer, (1968) measured the percentage of M chains present in cardiac LDH for patients who had a combination of diminished tissue perfusion and cardiac enlargement as evidenced by coronary artery narrowine and increased heart weight (greater than 325g for females and 375g for males). They found that hearts which were enlarged and poorly perfused had a greater proportion of M chains of LDH as compared to hearts with or without coronary artery disease and/or mild cardiac enlargement. They concluded that these changes in the proportion of M chains of LDH were related to an enhanced utilisation of anaerobic glycolysis in association with cardiac hypertrophy.

The idea that the hypertrophied myocardium is biochemically similar to embryonic myocardium is quite attractive. Embryonic and young growing myocardial tissue, which is less susceptible to the effects of hypoxia than adult myocardium, contains greater amounts of M-LDH (Dawson et al, 1964). The finding by Bishop and Altchuld (1970) of increased rates of lactate production and glucose uptake by anaerobically incubated homogenates of hypertrophied ventricles is additional evidence that hypertrophied myocardium utilises anaerobic glycolytic metabolism to a greater degree than normal myocardium.

Further studies, it was felt, were necessary to determine whether changes in the M chains of LDH were associated/...

associated with cardiac hypertrophy or related to a marked decrease in tissue perfusion (i.e. hypoxia).

If changes in the M subunits were a result of cardiac hypertrophy then marked enlargement should result in the greatest change in M-LDH. However, if the changes in M-LDH were related to tissue perfusion then these hearts in which perfusion was most impaired should show the greatest change in M-LDH.

1.1 Clinical and Morphological Data

Subjects were selected (Table 1) by the attending pathologist, based on the following criteria; the controls, group A, were patients who had died accidentally or by violence. The heart weight in this group averaged 340g for males and 300g for females and coronary arteries were normal; patients in group B all showed marked coronary narrowing with moderate cardiac enlargement (i.e. 50% over the control); the patients in group C had marked cardiac enlargement (i.e. 100% increase over the control) with or without moderate coronary artery disease.

Additional evidence substantiating cardiac hypertrophy was provided by left and right ventricular wall thickness (Table 1). The left and right ventricular walls in group B were increased by 40% and 29%, respectively, while the left and right ventricular wall of group C were increased by 87% and 50% respectively.

1.2 Lactate Dehydrogenase Isoenzymes

Sections of the left ventricular free wall were removed at autopsy within 7 to 24 hours of death. Samples of/...

TABLE 1

Clinical and Morphological characterization of patient groups.

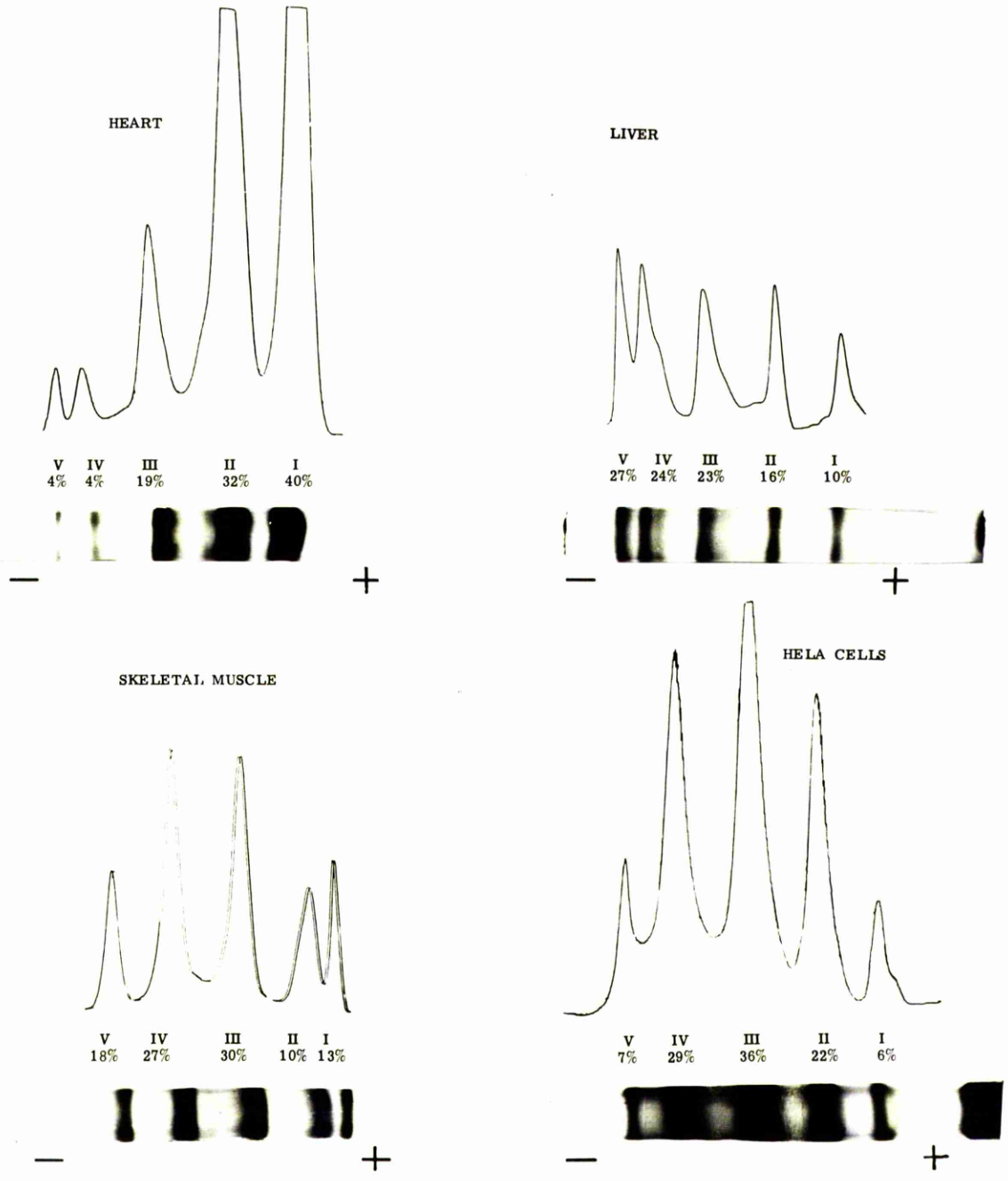
Group I; the control, neither coronary artery disease nor cardiac enlargement. Group II marked coronary artery disease with moderate cardiac enlargement. Group III marked cardiac hypertrophy with or without coronary artery disease.

	Patient Groups		
	A	B	C
Number of Cases	19	14	12
Male/Female	15/4	12/2	10/2
Heart Weight (g)	320 ±40	477 ±50 **	669 ±80 ***
Left ventricular wall thickness (cm)	1.5 ±0.05	2.1 ±0.10 *	2.8 ±0.07 ***
Right ventricular wall thickness (cm)	0.14 ±0.01	0.18 ±0.03 *	0.21 ±0.04 **

Mean ± SEM *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Fig. 1 The percentage of lactate dehydrogenase isoenzymes in normal heart, liver and skeletal muscle from human at autopsy. Also present in Fig. 1 are the percentage of lactate dehydrogenase isoenzymes from Helu cells of the fourth passage.

FIG 1


HEART
V 4%
IV 4%
III 19%
II 32%
I 40%
−
+
LIVER
V 27%
IV 24%
III 23%
II 16%
I 10%
−
+
SKELETAL MUSCLE
V 18%
IV 27%
III 30%
II 10%
I 13%
−
+
HELA CELLS
V 7%
IV 29%
III 36%
II 22%
I 6%
−
+

of normal liver and skeletal muscle were removed to check whether (the slower moving) heat sensitive isoenzymes LDH IV, V were retained. These samples were homogenised and the homogenate were centrifuged at 20,000xg for 20 minutes. The supernatants were then subjected to electrophoresis on polyacrylamide gels.

The results from these experiments are shown in Tables 2 - 3 - 4. The typical LDH isoenzymes distribution in normal human heart, liver and skeletal muscle is shown in Fig. 1.

Relative to the control group A, group B showed a decrease in isoenzyme I, while isoenzymes III, IV and V were significantly increased. Group C showed a modest decrease in isoenzyme I and an insignificant increase in isoenzymes III, IV and V (Table 2).

The relative amounts of heart and muscle subunits were calculated from the percentage of isoenzymes (Appella and Markert 1961, Cahn et al, 1962). The M subunits in group B were significantly increased by 60%, while group C showed a significant increase of 31% over the control (Table 3).

As would be expected, liver and skeletal muscle showed a predominance of isoenzymes III, IV and V and of M subunits of LDH (Plummer et al, 1963; Vessell and Bearn, 1961). These slow moving heat sensitive isoenzymes did not showmuch variation during the experimental period (Table 4a and b).

To corroborate these changes in the isoenzymes and subunits/...

TABLE 2

The percentage of lactate dehydrogenase isoenzymes in the left ventricular free wall from various patients groups.

		LDH Isoenzymes				
		I	II	III	IV	V
Group	No. of patients			%		
A	19	49	36	12	2	1
		±1.9	±1.1	±0.4	±0.030	±0.004
B	14	31	38	21	6	4
		±2.3	±1.3	±0.6	±0.050	±0.005
C	12	28	40	16	4	2
		±2.0	±1.9	±0.6	±0.038	±0.005

Mean ± SEM

TABLE 3

The percentage of M subunits in the left ventricular wall from various patient groups.

		M subunits of LDH
Group	No. of patients	%
A	19	17.5
		±1.18
B	14	28.5 **
		±1.64
C	12	23.0 *
		±1.19

Mean ± SEM *$p < 0.05$, **$p < 0.001$

TABLE 4a

The percentage of lactate dehydrogenase isoenzymes and M subunits in normal liver and skeletal muscle.

		LDH isoenzymes				
		I	II	III	IV	V
Tissue	No. of patients	%				
Liver	6	10 ±0.8	16 ±1.4	23 ±1.6	24 ±1.6	27 ±2.4
Skeletal muscle	7	13 ±1.3	10 ±0.9	30 ±2.3	27 ±2.1	18 ±1.5

Mean ± SEM

4b

		M subunits of LDH
Tissue	No. of patients	%
Liver	6	60.5 ±2.31
Skeletal muscle	7	55.5 ±2.08

Mean ± SEM

subunits of LDH, high and low concentrations of pyruvate were used as a measure of the percentage of H and M - LDH present in the left ventricular free wall from the various patient groups.

1.3 Pyruvate Inhibition

Dawson et al. (1964) have shown that relatively high concentrations of pyruvate inhibit the H subunits of LDH. Gutfreund et al, (1968) have suggested that this inhibition is related to the formation of an abortive ternary complex between NAD^+, pyruvate and LDH.

Kaplan et al, (1960) have advanced the hypothesis that inhibition of LDH by high tissue concentrations of pyruvate may assure a constant flow of pyruvate into pathways of oxidative phosphorylation. They observed that tissues, such as the heart, which rely on oxidative phosphorylation contain predominantly LDH I (which is inhibited by pyruvate) while tissues, such as skeletal muscle, relying partly on anaerobic glycolysis, have a predominance of LDH V (which is pyruvate independent). However, the validity of this hypothesis has been questioned by Vessell and Pool (1966) and Stambaugh and Post (1966), on the grounds that the temperatures and pyruvate concentrations employed were outside the physiological ranges. Although inhibition *in vivo* of LDH has been questioned, studies *in vitro* have established that the amount of substrate inhibition is directly proportional to the percentage of H chains in the enzyme (Plagemann et al, 1960).

In/...

In the present study the relative proportions of H subunits of LDH were estimated by using high ($x10^{-4}M$) and low ($x10^{-3}M$) pyruvate concentrations. Tables 5 - 6 show the results of such estimations on supernatants from heart, liver and skeletal muscle.

The control or group A showed a 59% inhibition at high pyruvate concentration while Bello and Messer (1968) found a 70% inhibition at the same concentration. However, a higher proportion of M subunits were found in the control group in the present study than in the control results of Bello and Messer. Group B showed 44% inhibition which was significantly less than the controls. A 52% inhibition was found with group C. This was not significantly different from the controls. In other terms, group B showed an increase in LDH activity (or less inhibition) at high pyruvate concentration of 37%, while group C showed an increase of 17%. Bellow and Messer found an increase of 33% in patients similar to group B in this experiment.

To sum up: hearts which were moderately hypertrophied and poorly perfused showed a marked increase in M subunits of LDH as evidenced both by electrophoresis, and by pyruvate inhibition; while hearts which were markedly hypertrophied and moderately perfused showed no significant change.

These results tend to support Bellow and Messer's view that severe hypoxia and mild cardiac enlargement result in a shift to a greater proportion of M subunits. This/...

TABLE 5

Lactate dehydrogenase enzyme activity at different concentration of pyruvate in the left ventricular free wall of various patient groups.

		Lactate dehydrogenase activity in the presence of:	
		Low pyruvate con^{c}	High pyruvate con^{c}
		$x10^{-3}$ M	$x10^{-4}$ M
Groups	No. of patients	moles / min / g wet weight	
A	19	216	88
		±22	± 8
B	14	195	110
		±16	±11
C	12	201	96
		±19	± 7

Mean ± SEM

TABLE 6

Lactate dehydrogenase enzyme activity at different concentration of pyruvate in normal liver skeletal muscle.

		Lactate dehydrogenase activity	
		Low pyruvate con^{c}	High pyruvate con^{c}
		$x10^{-3}$ M	$x10^{-4}$ M
Tissue	No. of patients	moles / min / g wet weight	
Liver		168	240
		±12	±17
Skeletal Muscle		219	315
		±19	±26

Mean ± SEM

This shift during severe hypoxia might reflect an altered control of LDH synthesis during cardiac enlargement; on the other hand, marked enlargement with mild hypoxia did not produce this.

An alternative explanation might be that the changes in isoenzymes represent a change in the relative proportions of different cell types in the heart. The attending pathologist observed that group B, which exhibited the greatest change in M subunits also exhibited cardiac fibrosis with recent or old infarctions. Hearts in group C exhibited only small areas of cardiac fibrosis and group A (the controls) exhibited none. He concluded that connective tissue in groups B represented an appreciable amount of the left ventricular free wall as compared to group C or the control group A. Fibrosis involves the proliferation of fibroblasts and the subsequent synthesis of collagen which is laid down in areas which have previously undergone necrosis. Accordingly attempts were made to determine whether there was a relationship between fibrosis and the proportion of M subunits in the heart.

1.4 Hydroxyproline

Hydroxyproline is found exclusively in collagen, in which it forms a constant percentage of 13.4% by weight (Neuman and Logan 1950). The concentration of collagen and thus the connective tissue component of the myocardium can, therefore, be estimated by determination of hydroxyproline.

The/...

The evidence on the abundance of connective tissue in human hypertrophic hearts is conflicting. Oken and Boucek (1957) showed that the collagen concentration in 20 out of 22 human hearts with significant left ventricular hypertrophy was normal. Similar studies by Monfort and Perez-Tamoyo (1962) who measured the ratio of collagen to muscle protein in the hypertrophied human left ventricle showed no change in the collagen content. On the other hand, Blumgart et al, (1940) found increases in the collagen concentration in 9 out of 24 hypertrophied human hearts. Vail (1939) and Krymski (1962) both observed increased areas of fibrosis in the human hypertrophied heart.

These conflicting results might be in part explained by the method used to excise the left ventricle. Montfort and Perez- Tamayo (1962) cut away the endocardial and epicardial layer before estimating hydroxyproline. However, Vail (1939) and Krymski (1962) both noticed it was precisely in these layers that the increase in fibrous tissue was located. Unfortunately, these investigators did not measure the amount of collagen chemically.

In the present study, sections of left ventricle removed at autopsy were homogenised, as in estimation of LDH isoenzymes, without cutting away the endocardial or epicardial layers. The results of hydroxyproline estimations are shown in Table 7. In comparison with group A(the controls), group B showed a significant increase in hydroxyproline, while group C showed a smaller but still significant/...

TABLE 7

The hydroxyproline concentration in the left ventricular free wall from various patient groups.

Groups	No. of patients	Hydroxyproline mg/g dry weight
A	19	2.24 ±0.34
B	14	4.36 ** ±0.71
C	12	2.98 * ±0.39

Mean ± SEM *p 0.05, **p 0.001

significant increase.

These chemical findings appeared to warrant histological comparison.

1.5
Histological Studies

Histological sections were taken from three parts of the left ventricular free wall, the anterior, posterior and lateral.

Determination of the relative amounts of fibrous tissue, myocardial cells and other cellular elements, was achieved with a microscope fitted with a grid in one of the eyepieces. A total of 25 fields was examined in each section and in each field the number of grid intersections that fell upon myocardial cells, fibroblasts or collagen and other cells (i.e. endothelial cells and histocytes) was recorded. These observations were made in areas which were not highly vascularised (i.e. not heavily endowed with arteries or capillaries). Figs. 2a-b - c show typical sections from groups A, B and C.

In group A (the controls) the lateral section of the left ventricular free wall appeared to contain a relatively higher proportion of muscle cells than fibrous tissue; the anterior and posterior showed a greater amount of fibrous tissue and the muscle cells were correspondingly reduced (Table 8 - 9). However, there was no significant difference between these results.

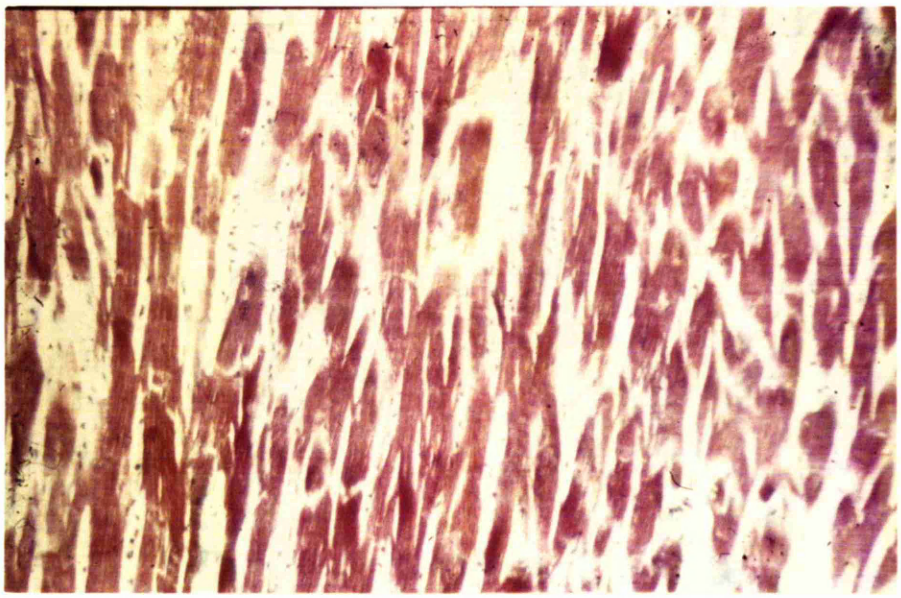
The results from group B showed that, compared to the controls, myocardial cells decreased, while fibrous tissue and other cellular elements significantly increased in all three. Group C showed a small decrease in/...

Fig. 2-a-b-c — Sections of the left ventricular free wall from human at autopsy stained for collagen with Masson's stain which appears green, counterstained with Hamalum.

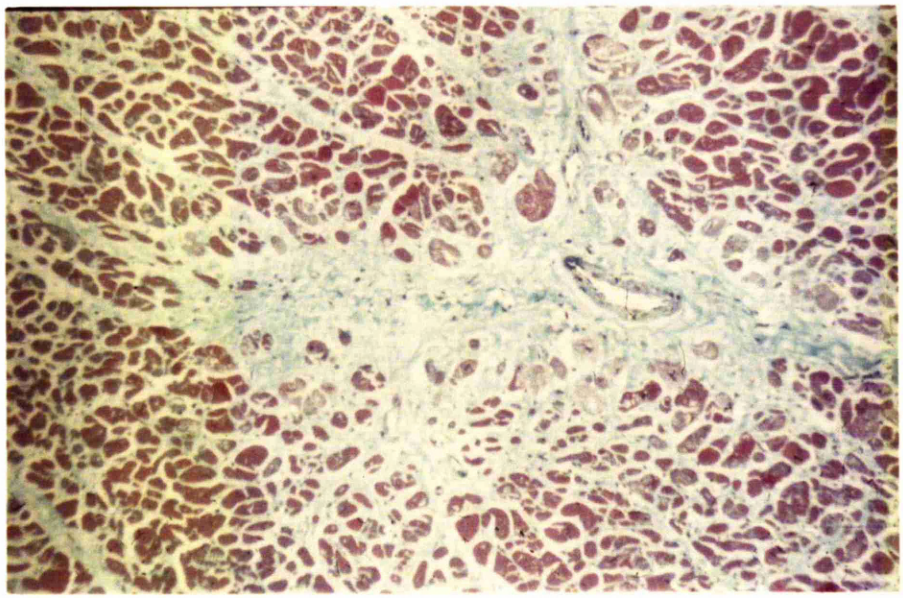
Fib. 2a — Marked left ventricular hypertrophy (Group C) without coronary artery disease with diffuse collagen. (Mag x 250).

Fib. 2b — Moderate left ventricular hypertrophy with marked coronary disease (Group B) with marked increase in collagen. (Mag x 100).

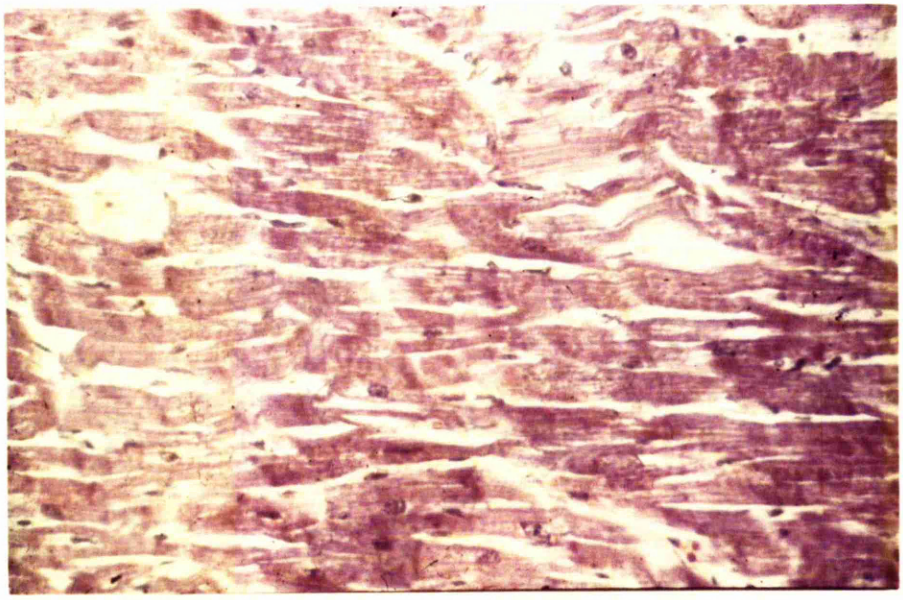
Fib. 2c — Normal left ventricle (Group A) with sparse collagen. (Mag x 250).



A



B



C

TABLE 8

Histological counting in the anterior, lateral and posterior sections of the left ventricular free wall from the various patients groups. The value expressed below represent the number of times the grid intersection fell apond (M) or muscle cells, (F) or fibroblast and collagen and (O) or other cells (i.e. histocytes and endothelial cells).

Groups	No. of patient	Anterior			Lateral			Posterior		
		M	F	O	M	F	O	M	F	O
A	10	1311	271	119	1344	272	108	1300	289	111
		±68	±16	±12	±73	±20	±13	±56	±23	±12
B	8	1060	460	180	1050	470	180	1064	440	195
		±60	±17	±20	±40	±24	±14	±43	±18	±21
C	10	1210	360	130	1320	285	95	1220	380	100
		±85	±18	±13	±57	±21	±16	±74	±15	±14

Mean ± SEM

TABLE 9

The percentage of M or muscle cells, F or fibroblast and collagen and O or other cells (i.e. histocytes and endothelial cells) in the anterior, lateral and posterior from the various patient groups.

Groups	No. of patient	Anterior			Lateral			Posterior		
		M	F	O	M	F	O	M	F	O
		%								
A	10	77	16	7	79	14	7	76	17	7
B	8	62	27	11	62	28	10	63	26	11
C	10	71	21	8	78	16	6	72	22	6

in muscle mass in the anterior and posterior sections, while the lateral section was unchanged. Fibrous tissue and other cellular elements in group C was either unchanged or slightly increased.

The chemical and histological studies both indicate that fibrous tissue and other cellular elements had considerably increased in group B. Although group C showed similar changes they were very much smaller.

1.6 Attempted Isolation of Muscle Cells

It seemed advantageous, therefore, to separate the various cellular elements in the left ventricular free wall and determine their characteristic patterns of LDH isoenzymes. It was thought that this experiment would answer the question whether the new tissue which is added as a result of cardiac hypertrophy has an altered pattern of LDH isoenzyme. Using a modification of the method of Berry et al, (1970) a section of the left ventricular free wall was cut into small 1mm cubes and incubated for one hour in a buffered solutation of collagenase and hyaluronidase. These enzymes should digest the connective tissue (i.e. collagen and ground substance), and release free cells. The different cell types should then be separable on the basis of size and density by sedimentation through a serum gradient under gravity. This experiment was unsuccessful since complete digestion was never achieved. Incubation for as long as 4 hours with various concentrations of collagenase and hyaluronidase did not lead to an appreciable release of the individual cells.

Because of this an alternative experiment was designed/...

designed which involved harvesting Helu cells (human embryonic lung cells) which have been replaced by predominantly fibroblast and determining the percentage of M-LDH.

1.7 Helu cells of the fourth passage were used to determine the M-LDH content. Fig. 1 shows the typical distribution of the LDH isoenzymes found in the Helu cells. The results from these experiments show that Helu cells contain a predominance of the (slower moving) isoenzymes III, IV and V. The predominance of M subunits of LDH was further demonstrated by a pyruvate inhibition experiment (Table 10).

These results clearly demonstrate that fibroblast or Helu cells contain a predominance of M-LDH. Since fibrous tissue was found in appreciable amounts in group B, they do help to offer an alternative explanation to the changes found in M-LDH in groups B and C.

TABLE 10.

The percentage of lactate dehydrogenase isoenzymes and high-low pyruvate kinetics in Helu cells.

	Lactate dehydrogenase isoenzymes				
	I	II	III	IV	V
No. of Experiments			%		
5	6	22	36	29	7
	±0.8	±1.6	±2.1	±1.8	±0.6

Mean ± SEM.

	Lactate dehydrogenase activity in the presence of:	
	Low pyruvate con^{c}	High pyruvate con^{c}
	$x10^{-3}$ M	$x10^{-4}$ M
No. of Experiments	moles / min / g wet weight	
5	290	365
	±24	±39

Mean ± SEM

1.8
Myocardial Enzymes

The reported changes in the structure of the mitochondria (Wollenberger, 1963) and anaerobic glycolysis (Bello and Messer, 1968) associated with congestive heart failure and enlargement, seemed to warrant a study of cardiac enzyme activities, especially since a change in metabolism might be responsible for the failing heart.

The enzymes chosen were selected as representative of three metabolic pathways: the Embden-Meyerhof pathway (lactate dehydrogenase (LDH)); the citric acid cycle (malate dehydrogenase (MDH) and NADP-dependent isocitrate dehydrogenase (ICDH)); and the pentose phosphate pathway (glucose-6-phosphate dehydrogenase (C-6-PDH)). The activities of creatine phosphokinase (CPK) and glutamic-oxaloacetic transaminase (GOT) were also measured.

The enzyme pattern from patients in group B with poorly perfused tissue or marked cardiac enlargement (group C) in general differed from the control group A (Table 11). The activity of ICDH in hearts from group B showed a significant decrease to 44% of control activity while ICDH in group C was decreased by 75% of the control activity. The activities of MDH and LDH were insignificantly decreased in both groups. Finally, G-6-PDH, CPK and GOT were slightly but insignificantly decreased in group B, while these enzymes in group C were the same as the control or slightly elevated.

It appears from the results of M-LDH and the intracellular enzyme activities that group B or those hearts which were moderately hypertrophied and poorly perfused showed the greatest/...

TABLE 11

The specific activities of six intracellular enzymes in heart muscle from the various patient groups.

specific activity (μ moles / min / mg. protein)

Groups	No. of patients	LDH	MDH	GOT	ICDH($\times 10^{-1}$)	G-6-PDH($\times 10^{-3}$)	CPK
A	12	4.63	24	7.4	4.8	5.6	11
		±0.35	±2.4	±1.0	±0.64	±0.78	±0.56
B	10	4.2	18 *	6.3	2.1 **	4.9	9
		±0.10	±1.9	±0.91	±0.44	±0.61	±0.46
	% of normal	(91)	(75)	(85)	(44)	(88)	(81)
C	10	4.4	20	7.4	3.6	5.9	12
		±0.16	±1.0	±1.4	±0.77	±0.58	±0.81
	% of normal	(97)	(88)	(100)	(75)	(105)	(109)

Mean ± SEM *$p < 0.05$, **$p < 0.01$

greatest change as compared to hearts which showed a marked increase in weight, with or without moderate coronary artery disease.

However, it was difficult to conclude from these results whether the changes observed in M-LDH were associated with increases in histocytes, endothelial cells and fibroblasts or related to the myocardial cell, although it would appear that fibroblasts were related to the changes in M-LDH. The changes in the six intra-cellular enzymes activities during the above study might also be explained by decrease in the mass of sarcoplasm due to necrosis and subsequent fibrosis and/or an increase in the autolytic process due to an increase in histocytes and fibroblast which contain high levels of hydrolytic enzymes. This problem could only be circumvented by parallel studies in experimental animals.

SECTION II

2.0 Introduction. The events described in the previous section demonstrated that some of the chemical findings attributed to cardiac hypertrophy are in fact due to the concomitant fibrosis. It was clearly of interest to see whether these findings in autopsy material could be repeated and extended in experimental animals.

Cardiac hypertrophy in animals can be induced in a variety of ways, as is shown in Table 12. For our purpose it was clearly desirable to find a method which would induce hypertrophy in a reasonably short period. It was also desirable to select a method which varied the extent of fibrosis. On these principles three methods were chosen -

1. Coarctation of the aorta.
2. Injection of thyroxine.
3. Injection of isoprenaline.

The effects of these methods in producing enlargement are discussed below.

Coarctation of the abdominal aorta induces cardiac enlargement as a result of increased peripheral resistance, which in a way, may be similar to hypertension. This means that the heart has to pump out a normal amount of blood against an abnormally high resistance. The resulting hypertrophy varies depending on the length of time, and the degree of constriction. When the abdominal aorta is constricted to approximately 60% of its original diameter, marked cardiac enlargement can usually be produced within periods of less than 30 days (Meerson, 1969). This is usually accompanied by/...

TABLE 12

Methods of inducing cardiac hypertrophy in animals.

Methods employed to induce hypertrophy	Species	Degree of Hypertrophy	Period of time
1. Aortic constriction	Dogs, Rabbits, Rats	25 - 400%	5 days to 2 months.
2. Experimental Hypertension			
a) DOCA administration	Rats	56%	28 days
b) constriction of renal artery	Rats	34%	30 days
3. High Altitude Hypoxia	Rats	50%	12 days
4. Anaemia	Rats	44%	3 months
5. Thyroxine injections	Rabbits	79%	15 days
6. Physical Exercise	Rats	35%	8 weeks
7. Aortic Valve Insufficiency	Rabbits	43%	4 weeks
8. Isoprenaline injections	Rats	60%	15 days
9. Coronary Artery Ligation	Rats	34%	6 weeks

by a moderate degree of fibrosis (Bartosova et al, 1969).

Injection of thyroxine generally increases the oxygen utilization in the tissues and subsequently increases the volume load to the heart. Cardiac enlargement is the result of increased work due to increased heart rate and cardiac output (Beznak 1962). Cardiac hypertrophy appears to be variable depending on the dosage, the animals used and the period of administration. Generally it is in the range of 50 to 80%.

Fibrosis does not seem to accompany the hypertrophy (Bartosova et al, 1967) although in the rat Sandler and Wilson (1959) occasionally observed small areas of necrosis in the apex of the ventricles.

Isoprenaline is a catecholamine similar to adrenaline. When injected it stimulates the heart rate, increases the oxygen requirement of the myocardium and at the same time reduces the systemic blood pressure and coronary flow (Beznak and Hacker 1963; Denison et al 1956). The stimulation of heart rate with the subsequent increase in cardiac output may in part be responsible for the resulting hypertrophy. However, since isoprenaline injection induces marked fibrosis, (Judd and Wexler 1969), the resulting hypertrophy may be a result of injury as previously discussed (page25). However that may be, the resulting hypertrophy is substantial and rapid (Rakusan et al, 1965).

RESULTS

2.1.0 Heart and Body Weight

Tables 13a - b show the changes in heart and body weight/...

weight following each of the three treatments. Neither saline injection (Table 13a) nor sham operation (Table 13a) appeared significantly to effect either heart weight or body weight. Coarctation (Table 13b) produced a marked increase in heart weight without effecting body weight. The observed increases were 32% at 10 days and 89% at 21 days of constriction. Similar results have been reported by Meerson (1969).

Thyroxine produced a progressive fall in body weight. This was accompanied by a progressive increase in heart weight so that the ratio of the latter to the former increased by almost 80% (Table 13b). This is in agreement with Inchiosa and Freedberg's (1963) previous observations.

Isoprenaline, in contrast to the other two treatments, produced a 60% increase in heart weight within 5 days and no further increase thereafter. It had no apparent effect on body weight. Similar results had been reported by Judd and Wexler (1969).

2.1.1 Liver and Kidney Weight

Tables 14a - b show the effects of the same treatments on the weights of liver and kidney expressed as a ratio to body weight. Saline injections (Table 14a) and sham operation (Table 14a) had no discernible effect. Coarctation had little, if any, effect on liver, but kidney weight was increased by 25% at 10 days after the operation and it was still at this level at 21 days.

Thyroxine had no effect on either liver or kidney weight/...

TABLE 13a.

The heart and body weights of sham operated and saline controls californian rabbits at various time intervals.

Saline controls

Days	No. of animals	Body weights Initial (g)	Body weights Final (g)	Heart wet weights (g)	Ratio of Heart weight/ body weight
5	9	2,780 ±38	2,950 ±48	5.7 ±0.87	0.19 ±0.013
10	7	2,640 ±29	2,890 ±46	5.6 ±0.66	0.19 ±0.014
15	23	2,700 ±39	3,010 ±54	5.8 ±0.78	0.19 ±0.016

Sham operated

6	2	2,680 ±30	2,798 ±26	5.5 ±0.34	0.19 ±0.011
10	3	2,600 ±29	2,840 ±40	5.6 ±0.79	0.19 ±0.017
21	7	2,740 ±21	3,100 ±48	5.8 ±0.51	0.19 ±0.016

Mean ± SEM

TABLE 13b.

. The effects of coarctation, isoprenaline and thyroxine on Body and Heart weight in californian rabbits at various time intervals.

Treatment	Days	No. of animals	Body weight		Heart Wet Weight	Ratio of Heart weight/ body weight
			Initial (g)	Final (g)	(g)	
Controls	-	49	2690	2958	5.7	0.19
			±31	±47	±0.72	±0.015
Coarctation	10	5	2560	2740	6.7	0.25 *
			±40	±35	±0.70	±0.018
	21	11	2589	2789	9.9	0.36 **
			±32	±47	±0.95	±0.029
Thyroxine	5	10	2577	2489	5.3	0.21
			±38	±31	±0.68	±0.011
	10	7	2701	2535	6.7	0.26 *
			±27	±58	±0.81	±0.026
	15	16	2519	2265	7.8	0.34 **
			±32	±41	±0.48	±0.020
Isoprenaline	5	8	2689	2837	8.5	0.30 **
			±37	±50	±0.68	±0.010
	10	9	2400	2688	8.2	0.30 **
			±29	±44	±0.83	±0.021
	15	19	2654	2950	8.9	0.31 **
			±39	±59	±0.99	±0.031

Mean ± SEM, Body and Heart weight is givin in (g),

*$p < 0.01$, **$p < 0.001$

TABLE 14a.

The ratio of liver and kidney wet weights to body weight in the saline controls and sham operated rabbits at various time intervals.

Saline Controls

Days	No. of animals	Ratio of Liver weight/ body weight	Ratio of Kidney weight/ body weight
5	9	3.9 ±0.55	0.63 ±0.059
10	7	3.9 ±0.73	0.64 ±0.048
15	23	4.0 ±0.47	0.61 ±0.030

Sham Operated

10	3	4.0 ±0.66	0.64 ±0.044
21	7	4.0 ±0.49	0.64 ±0.053

Mean ± SEM

TABLE 14b.

The influence of coarctation, isoprenaline and thyroxine on the ratio of liver and kidney wet weight to body weight in californian rabbits at various time intervals.

Treatment	Days	No. of animals	Ratio of Liver weight/ body weight	Ratio of Kidney weight/ body weight
Controls	-	49	4.0 ±0.69	0.63 ±0.076
Coarctation	10	4	3.9 ±0.60	0.77 * ±0.081
	21	11	4.5 ±0.79	0.76 * ±0.093
Thyroxine	5	10	4.7 ±0.77	0.64 ±0.031
	10	7	3.9 ±0.81	0.67 ±0.073
	15	16	3.2 ±0.50	0.62 ±0.038
Isoprenaline	5	8	3.2 ±0.93	0.70 ±0.033
	10	9	3.7 ±0.94	0.76 * ±0.041
	15	19	5.0 * ±0.81	0.74 ±0.084

Mean ± SEM, *$p < 0.05$

weight, but isoprenaline, like coarctation produced a 25% increase in kidney weight. This, like the increase in heart weight produced by the same drug is not progressive. It was fully developed at 5 days. Isoprenaline also appeared to produce a 25% increase in liver weight but this effect is only apparent after 15 days.

It is quite clear therefore that though all these treatments produce a satisfactory degree of cardiac hypertrophy, they differ widely in their side effects on other organs.

2.1.2 Left Ventricular Weight

Tables 15a - b show the extent to which the cardiac hypertrophy produced by the same three procedures could be attributed to the left ventricle. It is immediately apparent that all three treatments have increased left ventricular weight. In the case of thyroxine and isoprenaline treatment for 5 to 15 days the left ventricle still accounted for approximately a third of the heart weight, indicating that it had increased in the same proportion as the rest of the heart. After coarctation however, the left ventricle accounted for almost half the weight of the heart. The heart weight after 10 days of constriction had increased roughly by 1g while the left ventricular weight increased by 0.95g, indicating that most of the growth occurred in the left ventricle. After 21 days of aortic constriction the heart weight had increased by 4.2g, while the left ventricular weight was increased by 2.45g. These results would seem to indicate that/...

TABLE 15a

The left ventricular wet weight of sham operated and saline injected californian rabbit at various time intervals.

Days	No. of animals	Heart Weight (g)	Left Ventricular Weight (g)	Ratio of Heart Weight/ Left Ventricular Weight
		Saline Injected		
5	5	5.7 ±0.87	2.00 ±0.08	35
10	6	5.6 ±0.66	1.99 ±0.06	35
15	9	5.8 ±0.78	2.03 ±0.108	35
		Sham operated		
10	3	5.6 ±0.79	1.87 ±0.03	33
21	6	5.8 ±0.51	2.06 ±0.100	35

Mean ± SEM, Heart weight is given in (g)

TABLE 15b

The effects of coarctation, thyroxine and isoprenaline on the left ventricular wet weight at various time intervals.

Treatment	Days	No. of animals	Heart Weight (g)	Left ventricular Weight (g)	Ratio of Heart Weight/ Left ventricular Weight
Controls	-	29	5.7 ±0.72	1.99 ±0.06	35
Coarctation	10	4	6.7 ** ±0.70	2.95 ** ±0.118	44
	21	11	9.9 *** ±0.95	4.46 *** ±0.149	48
Thyroxine	5	7	5.3 ±0.68	1.90 ±0.07	36
	10	7	6.7 ** ±0.81	2.36 ±0.09	35
	15	8	7.8 *** ±8.48	2.61 * ±0.06	34
Iso-prenaline	5	8	8.5 *** ±0.68	2.92 ** ±0.100	33
	10	6	8.2 *** ±0.83	2.69 * ±0.110	34
	15	10	8.9 *** ±0.99	3.01 *** ±0.08	36

Mean ± SEM, Heart weight is given in (g), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

that growth had occurred in other chambers of the heart, though not on the same scale as in the left ventricle.

In summary then, isoprenaline and thyroxine affected the whole heart whereas coarctation primarily affected the left ventricle.

2.1.3 Left Ventricular Wet and Dry Weight

It was obviously desirable at this time to ascertain whether left ventricular growth was due to increases in the wet or dry weight or both. An increase in dry weight would be a crude index of cellular growth. Tables 16a - b show to what extent increases in dry weight of the left ventricle paralleled the corresponding increase in wet weight. It is clear that the two are closely related to one another and the increases in dry weight did therefore represent a real increase in cellular growth and not merely an increase in water. These changes are found with the three methods used to induce left ventricular hypertrophy.

TABLE 16a

The Wet and Dry weight of the left ventricle in the sham operated and saline injected rabbits at various time intervals.

Saline Injected

Days	No. of animals	Wet Weight	Dry Weight	% Wet Weight
5	5	1.60 ±0.050	0.40 ±0.009	80
10	6	1.55 ±0.049	0.40 ±0.013	78
15	9	1.71 ±0.036	0.39 ±0.010	81

Sham Operated

10	3	1.50 ±0.44	0.40 ±0.008	79
21	6	1.64 ±0.053	0.38 ±0.009	81

Mean ± SEM

TABLE 16b

The effects of coarctation, thyroxine and isoprenaline on left ventricular wet and dry weight at various time intervals.

Treatment	Days	No. of animals	Wet Weight (g)	Dry Weight (g)	% Wet Weight
Controls	-	29	1.60 ±0.046	0.39 ±0.010	80
Coarctation	10	4	2.35 ±0.054	0.60 ±0.010	80
	21	11	3.78 ±0.078	0.68 ±0.015	84
Thyroxine	5	7	1.53 ±0.02	0.37 ±0.006	81
	10	7	1.87 ±0.06	0.49 ±0.008	79
	15	8	2.10 ±0.041	0.51 ±0.007	80
Isoprenaline	5	8	2.40 ±0.039	0.52 ±0.016	82
	10	6	2.20 ±0.068	0.49 ±0.019	82
	15	10	2.47 ±0.071	0.53 ±0.006	81

Mean ± SEM

DISCUSSION:

The experiments just described threw up two unexpected results which call for comment. The first is the increase in kidney size which follows constriction of the aorta. This may be tentatively attributed to liberation of growth hormone (Beznak 1964). The second is the fact that isoprenaline apparently exerts its maximum effect during the first five days of administration, although the dose is continually increased throughout the entire fifteen days of the experiment. This is most easily explained if it can be assumed that the animals respond to the continued injections by increasing their ability in some way to metabolize isoprenaline. In this connection it may be relevant that de Champlin et al, (1968) have shown that in congestive heart failure (which was proceeded by cardiac hypertrophy) monoamine oxidase activity was increased, while the levels of catecholamines was substantially lowered.

2.20 Lactate Dehydrogenase Isoenzymes

The experiments described in the preceeding section established that authentic cardiac hypertrophy could be produced in rabbits. The next step was obviously to see how far this hypertrophy resembled the hypertrophy which was observed in human autopsy material. Tables 17a - b - c show the abundance of the five LDH isoenzymes in the left and right ventricles of californian rabbits in which cardiac hypertrophy had been induced by each of the three techniques described in the preceeding section. Several points call for comment. Although in the controls isoenzyme I predominated to a much greater extent than in human heart there was quite substantial proportions of II and III and though IV and V were present in least amount they were quite measurable (Fig. 3). This is in agreement with Weiland et al (1959) and contrary to Plageman et al (1960) and Markers and Appella (1964) who claimed that only isoenzyme I is present in rabbit hearts.

The controls also showed two other interesting features; there was no progressive change in isoenzyme pattern as the sequence of saline injections was prolonged or as a result of sham operation at various time intervals. This corresponds to the fact (see Table 13a) that neither saline injections nor sham operations affected the heart weight, but there was a small but interesting difference between the ventricles; the predominance of isoenzyme I was slightly, but consistently, more marked in the left ventricle than in the right.

1/...

1- Coarctation. The effect of coarctation of the aorta for either 10 or 21 days is to diminish somewhat the predominance of isoenzyme I, while isoenzymes IV and V was consistently increased in the left ventricle. The pattern in the right ventricle was altered in the same direction but to a lesser extent. This difference between the two ventricles can presumably be related to the fact that coarctation produced hypertrophy much more in the left than in the right. There is a suggestion that the effects in both ventricles were greater at 21 days than at 10 days.

2- Thyroxine. The isoenzyme pattern in both ventricles seemed little effected by thyroxine. There is a suggestion in both ventricles that the predominance of isoenzyme I was diminished to about the same slight degree and that this diminution was progressive, at least in the sense of being most evident at the longest time interval. The isoenzymes IV and V at this point appeared to be increased in both ventricles.

3- Isoprenaline. This produced a very much more marked effect than either thyroxine or coarctation. In both ventricles, and to roughly equal degrees, isoenzyme I was depressed, especially at the expense of isoenzymes IV and V. The effect was fully developed at 5 days and showed no further increase between 10 and 15 days. In this it resembled the effect of isoprenaline on heart weight.

2.2.1 H & M Subunits

From the figures shown in Tables 17a - b it is possible to calculate the percentage of H and M sub-units of/...

Fig. 3 The percentage of lactate dehydrogenase isoenzymes from the left and right ventricle of control rabbit and the left ventricle of rabbits subjected to isoprenaline treatment for 5 days or coarctation of the aorta for 10 days.

Fig 3

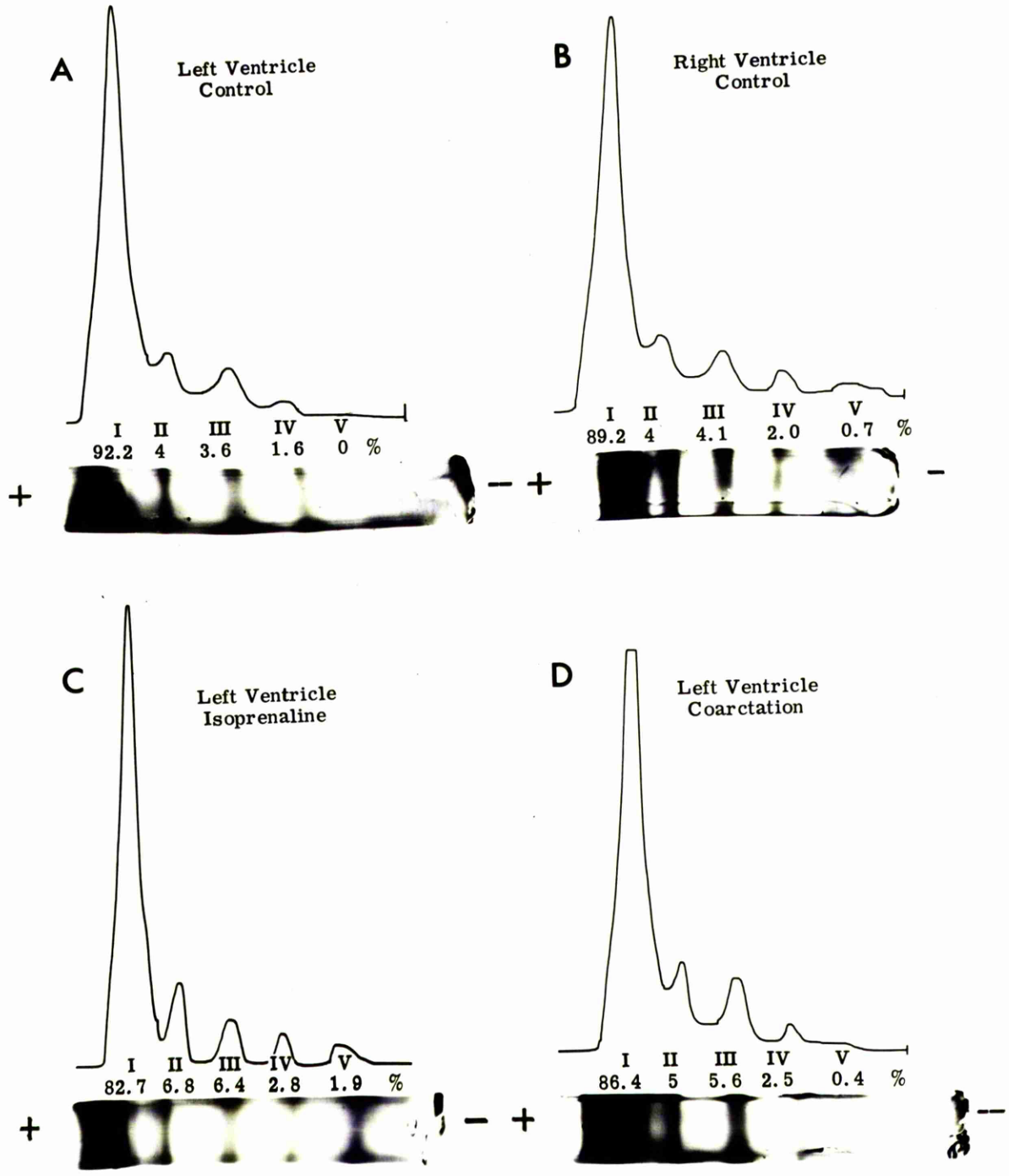

A
Left Ventricle
Control
I II III IV V
92.2 4 3.6 1.6 0 %
+ −
B
Right Ventricle
Control
I II III IV V
89.2 4 4.1 2.0 0.7 %
+ −
C
Left Ventricle
Isoprenaline
I II III IV V
82.7 6.8 6.4 2.8 1.9 %
+ −
D
Left Ventricle
Coarctation
I II III IV V
86.4 5 5.6 2.5 0.4 %
+ −

TABLE 17 a

The percentage of lactate dehydrogenase isoenzymes in the right and left ventricle of sham operated and saline injected californian rabbits at various time intervals.

Days	No. of animals	I	II	III	IV	V
				Saline injected		
				Right ventricle		
				% LDH isoenzymes		
5	7	89.7	4.0	4.0	1.8	0.5
		±1.8	±0.160	±0.110	±0.080	±0.013
10	5	86.6	4.1	4.3	2.3	0.7
		±2.1	±0.180	±0.150	±0.067	±0.018
15	7	90.4	3.9	4.1	2.0	0.6
		±2.6	±0.146	±0.133	±0.059	±0.016
				Left ventricle		
5	7	91.4	4.0	2.9	1.7	0
		±3.0	±0.166	±0.144	±0.041	-
10	5	91.4	4.1	3.1	1.4	0
		±2.7	±0.130	±0.151	±0.033	-
18	7	91.5	4.0	3.0	1.5	0
		±2.7	±0.143	±0.141	±0.066	-
				Sham operated		
				Right ventricle		
10	3	90.4	4.0	4.0	2.0	0.6
		±2.9	±0.160	±0.130	±0.090	±0.011
21	5	90.4	4.1	4.0	2.0	0.5
		±2.7	±0.150	±0.146	±0.065	±0.014
				Left ventricle		
10	3	91.4	4.0	3.0	1.6	0
		±2.6	±0.121	±0.158	±0.049	-
21	5	91.3	4.1	2.9	1.7	0
		±2.8	±0.134	±0.101	±0.041	-

Mean ± SEM

TABLE 17b

The influence of coarctation, thyroxine and isoprenaline on the percentage of lactate dehydrogenase isoenzymes in the left ventricle of californian rabbits at various time intervals

Treatment	Days	No. of animals	% LDH isoenzymes in left ventricle I	II	III	IV	V
Controls	-	27	91.4	4	3	1.6	0
			±2.6	±0.149	±0.133	±0.046	-
Coarctation	10	4	86.1	5	6	2.5	0.4
			±3.9	±0.153	±0.141 **	±0.053 **	±0.013 **
	21	11	86.2	4	6	2.9	0.9
			±2.6	±0.161	±0.149 **	±0.061 **	±0.016 **
Thyroxine	5	5	91.9	3.8	2.9	1.4	0
			±2.1	±0.131	±0.131	±0.059	-
	10	9	91.8	4.2	3.1	1.5	0
			±2.4	±0.129	±0.146	±0.053	-
	15	8	90.6	4.0	3.9	1.9	0.6
			±2.7	±0.140	±0.140	±0.067	±0.017 **
Isoprenaline	5	5	79.0	7.0	6.9	3.2	1.9
			±2.9 *	±0.190 *	±0.183 ***	±0.079 ***	±0.021 ***
	10	5	82.4	6.6	6.6	2.8	1.6
			±2.1 *	±0.200 *	±0.144 ***	±0.077 ***	±0.018 ***
	15	10	82.7	6.4	6.8	3.1	1.4
			±2.7 *	±0.164 *	±0.178 ***	±0.084 ***	±0.020 ***

Mean ± SEM *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

TABLE 17c

The influence of coarctation, thyroxine and isoprenaline on the percentage of lactate dehydrogenase isoenzymes in the right ventricle of californian rabbits at various time intervals.

Treatment	Days	No. of animals	% LDH isoenzymes in right ventricle				
			I	II	III	IV	V
Controls	-	27	89.3	4.0	4.1	2.0	0.6
			±2.4	±0.154	±0.139	±0.061	±0.013
Coarctation	10	4	90.6	4.8	3.9	2.1	0.4
			±2.2	±0.167	±0.181	±0.073	±0.019
	21	11	85.9	4.3	4.9	3.6	1.3
			±3.4	±0.198	±8.179	±0.091 **	±0.021 **
Thyroxine	5	5	88.3	4.1	4.2	1.8	0.5
			±2.9	±0.201	±0.195	±0.066	±0.016
	10	9	86.3	4.4	5.3	2.9	1.1
			±2.6	±0.179	±0.189	±0.073	±0.021
	15	8	86.2	4.2	5.6	2.7	1.3
			±2.7	±0.183	±0.210	±0.114	±1.108 **
Isoprenaline	5	5	81.7	5.0	7.2	4.2	1.9
			±3.0 *	±0.194	±0.718 **	±0.114 ***	±0.022 ***
	10	5	82.9	4.9	6.8	3.8	1.6
			±2.5	±0.210	±0.197 **	±0.088 ***	±0.019 **
	15	10	82.7	4.6	6.9	4.1	1.7
			±2.8	±0.188	±0.201 **	±0.094 ***	±0.019 ***

Mean ± SEM *$p < 0.05$, **$p < 0.01$,

***$p < 0.001$.

of LDH in each ventricle. Tables 18a - b show the results of such calculations and these possibly demonstrate more clearly the conclusion drawn from Tables 17a - b - c. The difference between the left and right ventricles of the controls in respect to M sub-units are readily noticable. After 10 and 21 days of aortic constriction the M sub-units in the left ventricle increased from 40 to 67% at the expense of H sub-units. The proportion of M sub-units in the right ventricle was unaffected after 10 days of aortic constriction, but after 21 days there was a significant increase of 32%. It is interesting that the non-stressed right ventricle, which was presumably not subject to overload, should show a change in M sub-units. Similar changes were observed by SØbel et al, (1969) and Bishop et al, (1969).

The percentage of M sub-units in both ventricles were little if at all effected by thyroxine treatment. The small effect that was observed did not occur until the 15th day. Isoprenaline, by contrast led to an immediate and much greater effect that coarctation or thyroxine. Both ventricles appeared to be effected to about the same degree.

In section I it was shown that in hypertrophied human hearts at autopsy a fall in the percentage of H sub-units and the subsequent increase in M sub-units could be explained by fibrosis (i.e. an increase in the proportion of fibrosis tissue). Although in the rabbit experiment hypertrophy had been induced very much more rapidly than would have been the case in human patients, it is nonetheless tempting/...

TABLE 18a

The percentage of H and M subunits of lactate dehydrogenase in the right and left ventricle of sham operated and saline injected californian rabbits.

Days	No. of animals	% subunits Left ventricle		Right ventricle	
		H	M	H	M
Saline-injected					
5	7	96.3	3.7	95.2	4.8
10	5	96.5	3.5	94.4	5.6
15	7	96.7	3.6	94.9	5.1
Sham-operated					
10	3	96.3	3.7	94.9	5.1
21	5	96.2	3.8	95.0	5.0

TABLE 18b

The percentage of H and M subunits of lactate dehydrogenase in the right and left ventricle following coarctation, thyroxine and isoprenaline treatment in californian rabbits at various time intervals.

Treatment	Days	No. of animals	% subunits Left ventricle		right ventricle	
			H	M	H	M
Control	-	27	96.3	3.7	94.8	5.2
Coarctation	10	4	93.4	6.6	95.0	5.0
	21	11	92.3	7.7	92.5	7.5
Thyroxine	5	5	96.4	3.6	95.0	5.0
	10	9	96.3	3.7	93.1	6.9
	15	8	94.9	5.1	92.7	7.3
Isoprenaline	5	5	90.5	9.5	90.1	9.9
	10	5	91.2	8.8	90.9	9.1
	15	10	91.2	8.8	90.5	9.5

tempting to suppose that the changes in isoenzyme pattern again reflected an increase in fibrous tissue at the expense of heart muscle. This idea seemed particularly attractive since the greatest isoenzyme change was produced by isoprenaline, which would be expected to produce most fibrosis and the least change by thyroxine, which would be expected to produce little or none (page 44). Coarctation, which would be expected to produce an intermediate degree of fibrosis produced an intermediate change in isoenzyme pattern. To establish the correctness of these ideas it was necessary to estimate the extent of fibrosis of rabbit hearts. This was done in the manner already described for autopsy material; it was assumed that the hydroxyproline content of the heart could be taken as a measure of collagen and collagen in turn could be used as a measure of fibrous tissue.

2.3.0 Fibrous Tissue in the Heart

The results are shown in Tables 19a - b. The controls results show that neither repeated saline injections or sham operations had any effect on the hydroxyproline content. There was however a consistently greater content of hydroxyproline in the right ventricle than in the Left. Coarctation for 10 and 21 days increased the hydroxyproline by 50%. The right ventricle showed a marginal increase after 21 days of constriction. Thyroxine appeared to have little effect, though such effect as it did produce was apparent in both ventricles and seemed progressive. The effects of isoprenaline were immediate and very much greater than/...

TABLE 19a

The hydroxyproline content in the left and right ventricle of sham operated and saline injected californian rabbit.

Saline injected

Days	No. of animals	ug. hydroxyproline / mg. dry weight	
		Left ventricle	right ventricle
5	7	2.31 ±0.27	2.93 ±0.33
10	4	2.18 ±0.31	3.09 ±0.28
15	10	2.46 ±0.26	3.15 ±0.39

Sham operated

10	3	2.22 ±0.34	3.00 ±0.20
21	11	2.50 ±0.44	3.11 ±0.41

Mean ± SEM

TABLE 19b

The effects of coarctation, thyroxine and isoprenaline on hydroxyproline in the left and right ventricles of californian rabbits.

Treatment	Days	No. of animals	ug. of hydroxyproline / mg. dry weight	
			Left ventricle	right ventricle
Controls	-	35	2.33 ±0.33	3.06 ±0.31
Coarctation	10	4	3.46 ** ±0.45	3.01 ±0.34
	21	11	3.70 ** ±0.61	3.78 ±0.37
Thyroxine	5	5	2.15 ±0.29	2.81 ±0.31
	10	9	2.20 ±0.31	2.99 ±0.29
	15	8	2.80 ±0.58	3.70 ±0.51
Isoprenaline	5	5	4.49 *** ±0.44	4.10 * ±0.79
	10	10	4.30 *** ±0.51	4.50 ** ±0.63
	15	10	4.59 *** ±0.39	4.49 ** ±0.48

Mean ± SEM, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

than those of either coarctation or thyroxine. The hydroxyproline content of both ventricles almost doubled in 5 days and showed no further increase up to 15 days.

In every respect the parallelism between Tables 18a - b and 19a - b is extraordinary. An increase in M sub-units in Table 18b is paralleled by an almost proportionate increase in hydroxyproline (Table 19b). The conclusion that LDH isoenzyme and the hydroxyproline content are reflections of the amount of fibrous tissue seems ineluctable.

2.31 Histological Examination

To put the issue beyond doubt, sections of left ventricle from animals subjected to coarctation and to thyroxine and isoprenaline treatment were stained by Masson's method which stains collagen green. In addition to our own examination, sections were examined by a pathologist who was asked to score them for fibrosis (estimated on the amount of collagen and fibroblast) and necrosis (judged by the number of histocytes and other blood cells and vacuolisation of myocardial cells).

His report is shown in Table 20. Thyroxine showed perhaps the least effect. It caused little fibrosis and that only after 15 days. Necrosis appeared at 10 days and only more marked after 15 days. Isoprenaline, by contrast, produced marked fibrosis within 5 days which remained constant at 10 and 15 days. Necrosis was maximal at 5 days and thereafter diminished. Coarctation produced effects similar to, but less severe than, isoprenaline; moderate fibrosis was/...

was apparent at 10 days and persisted to 21 days; necrosis was moderate at 10 days and diminished at 21 days.

Two points emerge from these observations. First that the histological appearance of fibrous tissue runs parallel to the hydroxyproline estimations in Table 19a; and second, that in each of the three treatments, fibrosis was preceded by necrosis and presumably represented scarring or repair of the necrotic areas.

The relationship of fibrosis to the preceding necrosis is illustrated in Fig. 4a - b - c - d - e. Fig. 4a and b are taken from control hearts. There is no obvious necrosis and only a moderate amount of collagen. Fig. 4d shows a section taken from an animal treated with isoprenaline for 5 days. There is massive intiltration of the area with leucocytes and fibroblasts. In contrast with the controls, collagen appears to be localized and more prominent. Fig. 4c shows a section taken from an animal treated with isoprenaline for 15 days and therefore represents a later stage of the same process as Fig. 4d. There is less necrosis than in Fig. 4d and more collagen. Fig. 4e from an animal subjected to coarctation for 10 days. Necrosis is indicated by the number of vacuoles in the myocardial cells. At the same time there is a good deal of rather diffusely distributed collagen.

The main conclusions to be drawn from these histological studies is that the increase in fibrous tissue which appears in varying degrees to accompany experimental cardiac hypertrophy in the rabbit, is not merely a change in the relative/...

TABLE 20

The extent of necrosis and fibrosis of the left ventricle following coarctation, thyroxine and isoprenaline treatment at various time intervals.

Treatment	Days	Necrosis	Fibrosis
Coarctation	10	++	++
	21	+	++
Thyroxine	5	0	0
	10	0	+
	15	++	+
Isoprenaline	5	+++	+++
	10	++	+++
	15	+	+++

0 = no change, + = small change, ++ moderate change, +++ marked change. At each time interval three californian rabbits were used.

Fig. 4a-b-c-d-e — Sections of left ventricle from californian rabbits stained for collagen with Masson's stain which appears green, counter-stained with Hamalum.

Fig. 4a — Rabbit which was injected with saline for 15 days. The normal appearance of muscle cells and collagen (Mag x 100).

Fig. 4b — Rabbit which had undergone sham operation for 21 days. The normal appearance of muscle cells and collagen (Mag x 100).

Fig. 4c Rabbit which was injected with isoprenaline for 15 days. Increased collagen and vacuolization of muscle cells (Mag x 100).

Fig. 4d Rabbit which was injected with isoprenaline for 5 days. Increased collagen and round cells. (Mag x 100).

Fig. 4e Rabbit which had undergone coarctation of the aorta for 10 days. Increase in collagen and vacuolization of muscle cells (Mag x 100).

relative proportions of different cell types in the myocardium; on the contrary it represents the replacement by fibrous tissue of more or less extensive areas of necrosis.

2.4.0 Serum Creatine Phosphokinase (SCPK) and Lactate Dehydrogenase (SLDH).

If some degree of necrosis does accompany cardiac hypertrophy, one might reasonably expect it to be signalised by the release of those enzymes which can normally be detected in the blood stream after myocardial damage, as for example, myocardial infarction.

Accordingly, SCPK and SLDH were estimated in the sera of rabbits which had been subjected to coarctation or to thyroxine or isoprenaline treatment. The results are shown in Fig. 5 and 6 for SCPK and SLDH, respectively. To take SCPK first, since it is presumably more responsive to cell damage; coarctation led to a modest but substantial and quite unmistakable elevation throughout the period of the experiment. Thyroxine had little effect at first but after about 14 days the enzyme level showed a modest but significant rise. Isoprenaline, once again, provided a contrast. Within hours of its injection the enzyme showed a marked rise which was sustained for some days; but towards the end of the experimental period there was gradual fall back to the control level.

The results with SLDH ran roughly parallel to those with SCPK. Coarctation gave a sustained elevation starting from the second post-operative day. Thyroxine had no effect/...

Fig. 5 Serum Creatine Phosphokinase levels at various time intervals following sham operation or saline injections (the controls) or isoprenaline and thyroxine treatment or coarctation of the aorta. Each point represents the mean ± SEM of four californian rabbits.

FIG 5.

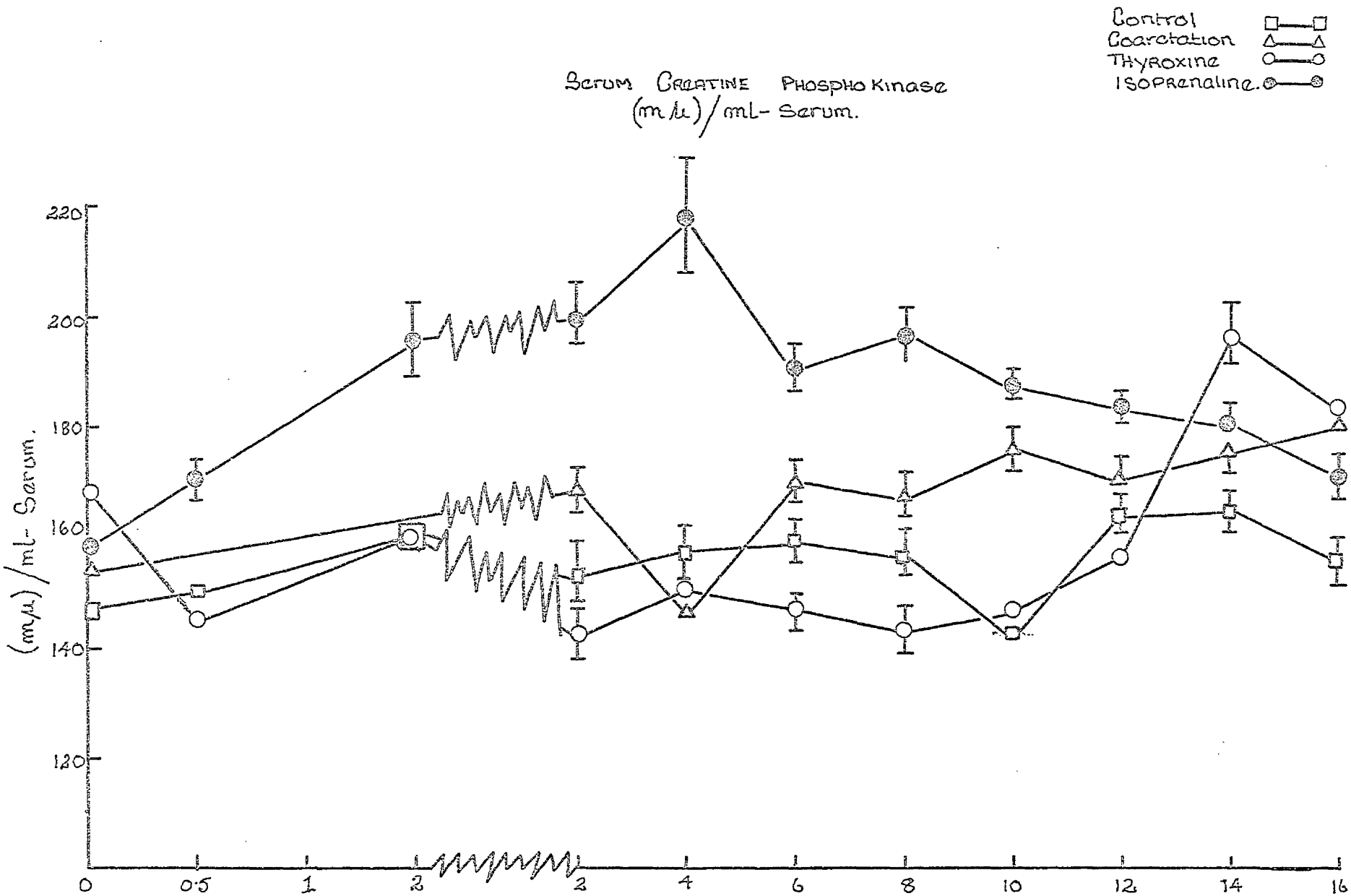
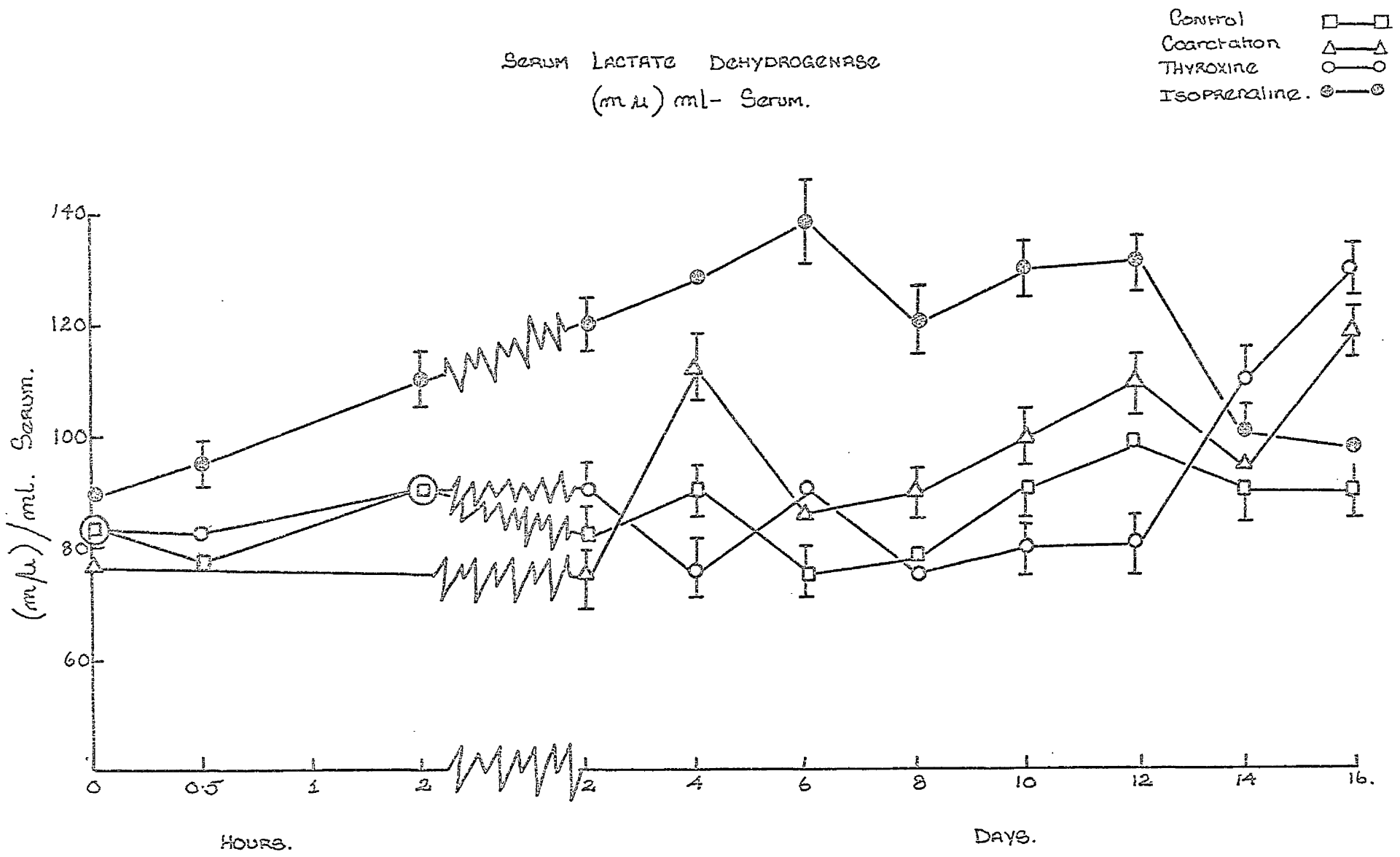

Control
Coarctation
THYROXINE
ISOPRENALINE.
SERUM CREATINE PHOSPHO KINASE
(mμ)/ml- Serum.
(mμ)/ml- Serum.
220
200
180
160
140
120
0
0·5
1
2
2
4
6
8
10
12
14
16
HOURS
DAYS.

Fig. 6 Serum lactate dehydrogenase levels at various time intervals following sham operation of saline injection; (the controls) or isoprenaline and thyroxine or coarctation of the aorta. Each point represents the mean ± SEM of four californian rabbits.

FIG 6


Control
Coarctation
Thyroxine
Isoprenaline.
Serum Lactate Dehydrogenase
(mμ) ml- Serum.
140
120
100
80
60
(mμ)/mL. Serum.
0
0·5
1
2
2
4
6
8
10
12
14
16.
Hours.
Days.

effect initially but produced a rise towards the end of the experimental period. Isoprenaline produced an immediate and dramatic increase followed ultimately by a slow fall toward the control level, although this had not been reached by the end of the experimental period.

The results of these experiments therefore suggest that coarctation causes moderate but continuous damage to the myocardium, that thyroxine causes damage but only after administration for at least 14 days and that the damage caused by isoprenaline, though severe, is largely confined to the earlier part of the experimental period. This is in exact agreement with the histological observation shown in Table 20.

2.5. O Lysosomal enzymes

Since the results of SCPK and SLDH strongly suggested that cell damage accompanied cardiac hypertrophy, it seemed reasonable to look for confirming evidence. If cell damage does take place one would expect that lysosomes would play some part in this process.

The name lysosomes was proposed by de Duve et al, (1955 and 1959) for a group of cytoplasmic particles which contain a variety of acid hydrolases surrounded by membrane which acts as a barrier between the internal enzyme and the susceptible substrates present outside. It is now clear that lysosomes comprise a number of related entities which together form the main components of the digestive system within animal cells. Thus, lysosomes are implicated in the breakdown of material taken into the cell by endocytosis/...

endocytosis, that is, by any form of pinocytosis or phagocytosis. The main forms of lysosomes that can be distinguished are; the so-called primary lysosome, a storage granule for newly synthesized enzymes; the digestive vacuole or secondary lysosome, containing the engulfed material together with the enzyme; and the tertiary lysosome or residual body, characterized by various undigested remnants, usually either polymorphic or dense in appearance. A fourth type of lysosome, the autophagic vacuole, arises by an as yet poorly understood intra-cellular process whereby a portion of cytoplasm, often including mitochondria, endoplasmic reticulum etc., becomes trapped inside a lysosome and undergoes digestion. This general process of digestion is shown in Fig. 11.

Quite apart from their role in the process of digestion, which can be regarded as a normal physiological event, lysosomes may be involved in the destruction of cells of which they form a part. de Duve et al (1959) has shown that lysosomal enzyme release during ischaemia in the liver results in cellular necrosis. However, recent investigations have cast doubt on the part lysosomal enzyme release plays in the onset of necrosis (Goldblatt et al, 1965; Griffin et al, 1945; Bassi and Bernali-Zazzera, 1964; Slater et al, 1967). It is now generally believed that lysosomal enzyme release plays a secondary rather than a primary role, with the one exception of photosensitisation (Slater and Riley, 1966; Allison et al, 1966). However, whether/...

Fig 11

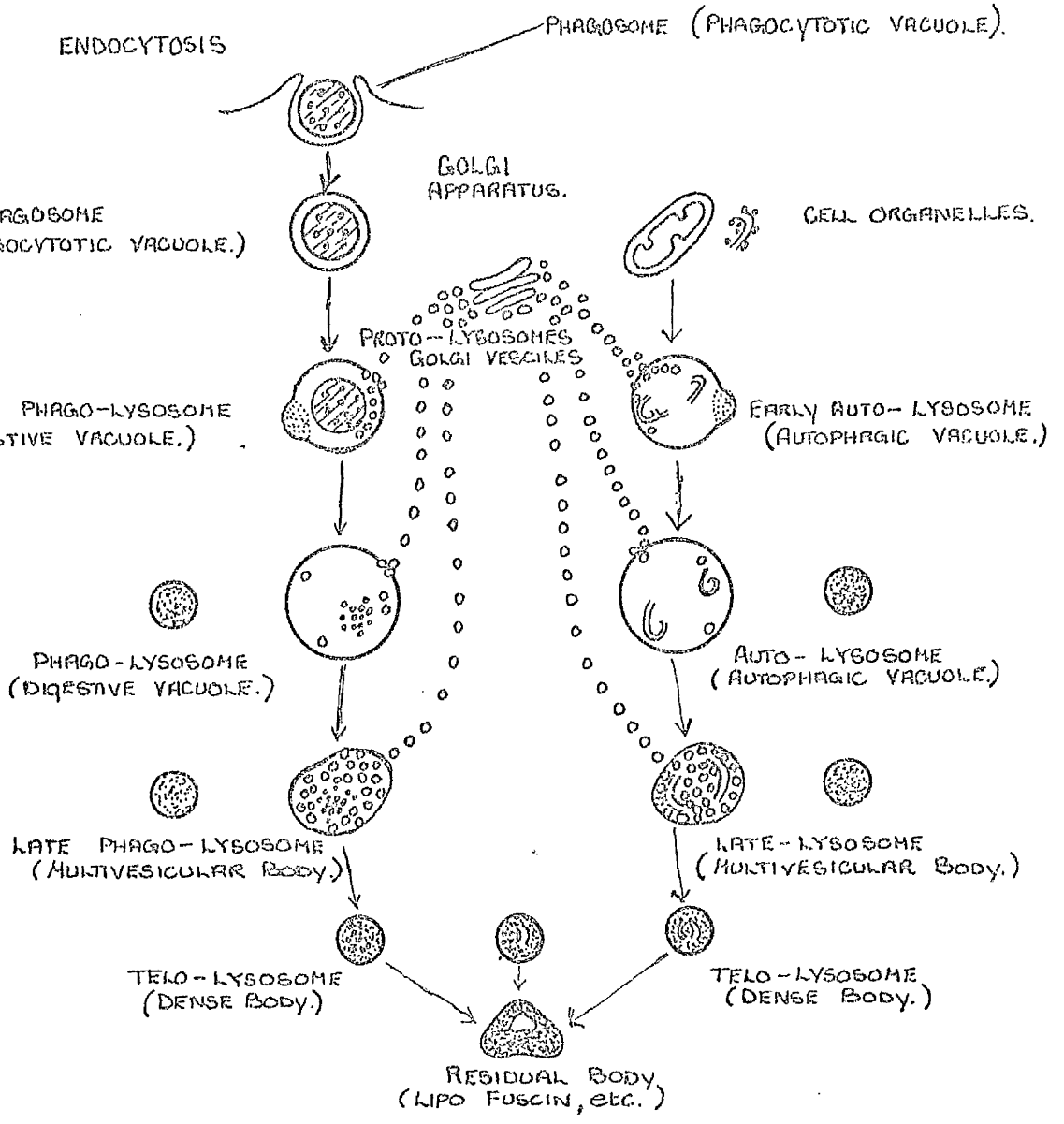


Fig. 11

The inter-relationship of cytoplasmic granules functioning in intracellular digestion of cells, as suggested by Gordon et al, (1965).

whether lysosomal enzyme action is primary or secondary to necrosis, it is generally agreed that changes in lysosomal permeability play an important role. Therefore an increase in the free or unsedimentable form of lysosomal enzymes would constitute prima facie evidence of necrosis.

An experiment was therefore performed to measure the relative proportions of the bound and free forms of ten characteristic lysosomal enzymes in heart homogenates after the induction of cardiac hypertrophy. A high ratio of bound to free enzyme would imply that the lysosomes are still intact and that the enzymes within are inaccessible to substrate; whereas a low ratio would indicate that the enzymes were being released.

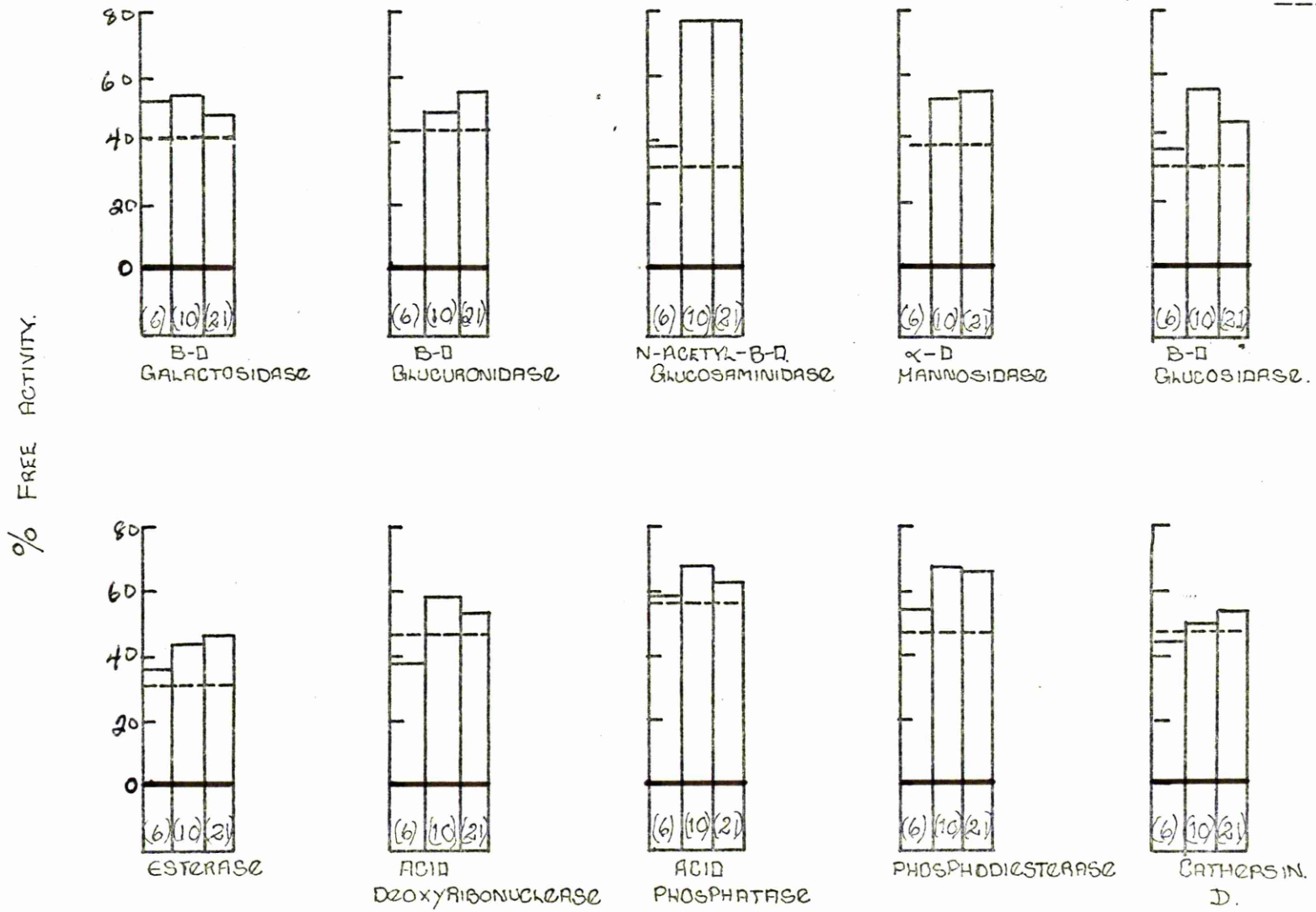
Experiments by Raven et al, (1969) have already shown that if myocardial necrosis is induced in dogs by ligating the descending coronary artery, there is an accompanying increase in free lysosomal enzymes. The present experiments were intended to confirm and extend Raven's observations. There were ten enzymes measured in this experiment which are typical lysosomal enzymes

Tables 22a - b show that the level of each enzyme in the free form was unaffected by saline injection or sham operation at various time intervals. Tables 23 - 24 - 25 and Figs. 7 - 8 - 9 show the changes which took place when hypertrophy was induced by coarctation or by administration of thyroxine or isoprenaline. The pattern was remarkably uniform. Coarctation uniformly increased the/...

Fig. 7 - 8 - 9 The percentage of free lysosomal enzymes in the left ventricle of californian rabbits following coarctation of the aorta or injections of thyroxine or isoprenaline. The numbers in the closed parenthesis represents the number of days of the above treatment. The broken lines represents the percentage of free lysosomal enzymes in the controls.

THE PERCENTAGE OF FREE LYSOSOMAL ENZYMES
DURING COARCTATION OF THE AORTA.

_____ EQUAL THE CONTROL



THE PERCENTAGE OF FREE LYSOSOMAL ENZYMES.
FOLLOWING THYROXINE TREATMENT.

_____ equal the cont

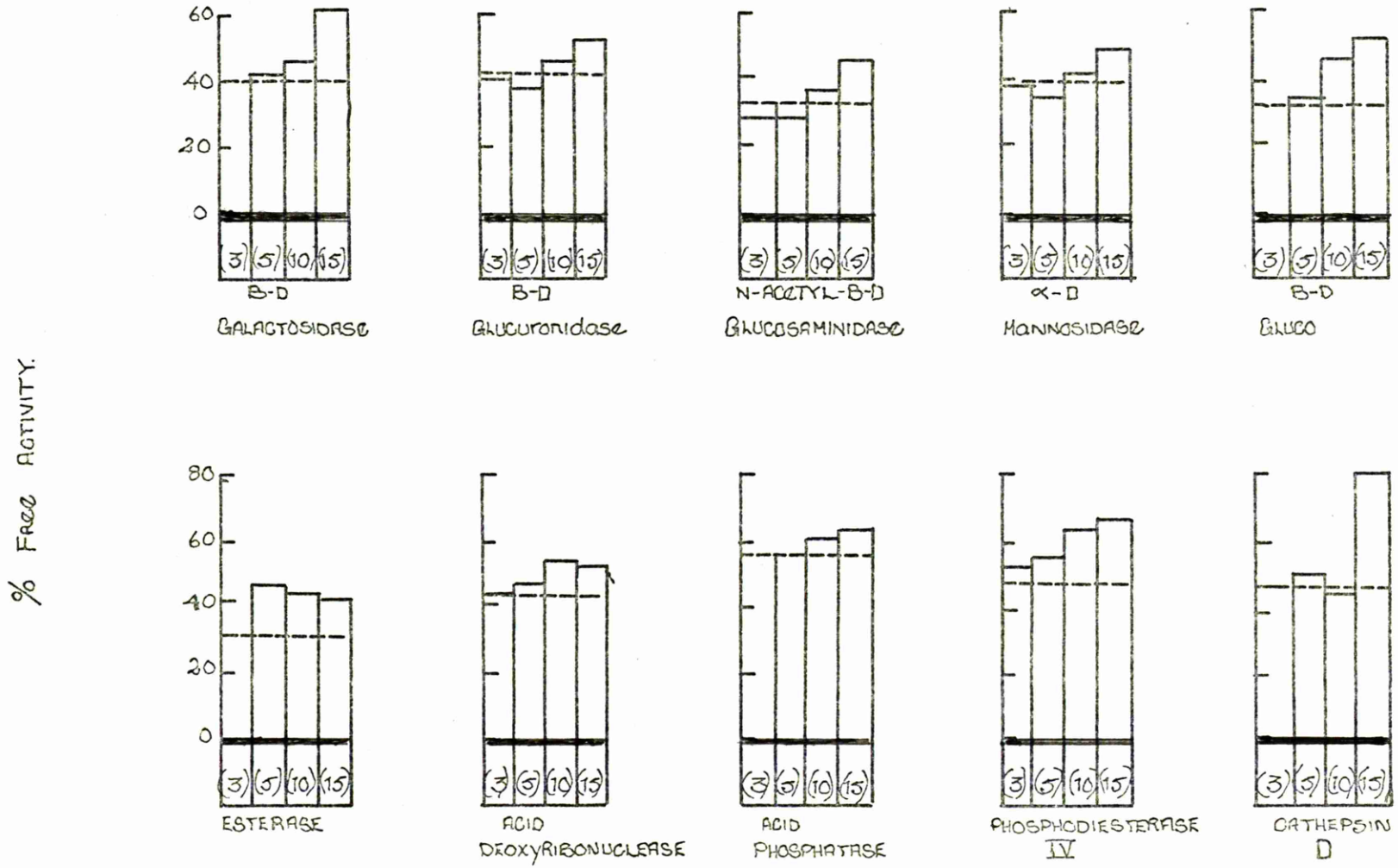
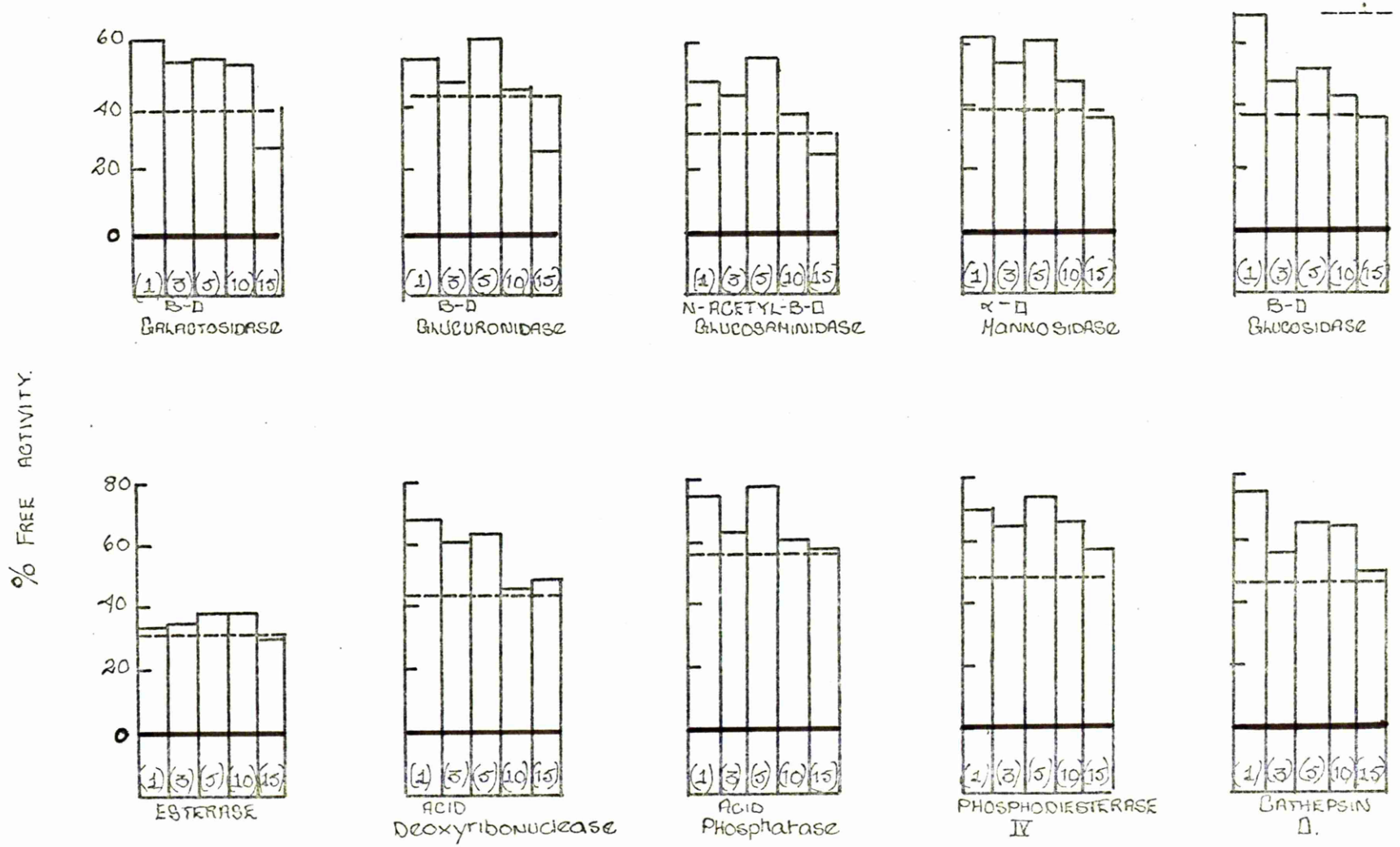


FIG 9

THE PERCENTAGE OF FREE LYSOSOMAL ENZYMES.
FOLLOWING ISOPRENALINE TREATMENT.

-·- equal the CONTROL.



the proportion of each enzyme which was in the free form. The increase was progressive in the same sense that it was more marked at 21 days than at 6 or 10 days. Similarly, thyroxine produced a progressive increase in the proportion of free enzymes. This increase was most noticable after 15 days of injection. By contrast, isoprenaline produced a much more abrupt response. The proportion of each enzyme in the free form increased sharply within 24 hours and the increases in all cases were sustained to the fifth day. Thereafter, there was in most cases a decline toward the control level.

Taken by themselves these observations are clearly consistant with the hypothesis on which the experiment was based. They furnish further evidence of necrosis accompanying hypertrophy and they confirm that while the effect produced by isoprenaline is far more immediate and abrupt than those produced by coarctation and thyroxine, it is not qualitatively different.

Unfortunately this agreeably consistent picture is complicated if one examines the absolute amounts of bound enzyme in each case (i.e. enzyme presumably still locked in the lysosomes). It would be a reasonable assumption that when necrosis is accompanied by a release of lysosomal enzymes the increase in the free level of these enzymes should be accompanied by a fall in the bound level. In the present experiment this assumption was borne out only to a limited degree.

Twenty-four hours after the first isoprenaline injection/...

TABLE 22a.

Lysosomal enzyme activity in the left ventricle of the control californian rabbits at various days.

Enzymes	Days 1 (4) Free	1 (4) Bound	3 (5) Free	3 (5) Bound	5 (9) Free	5 (9) Bound	10 (10) Free	10 (10) Bound	15 (30) Free	15 (30) Bound
B-D-Galactosidase	0.34	0.50	0.32	0.48	0.30	0.56	0.33	0.51	0.30	0.52
(A)	±0.013	±0.020	±0.010	±0.014	±0.010	±0.016	±0.014	±0.011	±0.015	±0.010
B-D-Glucuronidase	0.39	0.51	0.32	0.51	0.40	0.48	0.38	0.50	0.30	0.50
(A)	±0.013	±0.023	±0.014	±0.010	±0.018	±0.016	±0.014	±0.010	±0.016	±0.013
N-Acetyl-B-D Glucosaminidase	0.60	1.3	0.63	1.5	0.60	1.4	0.70	1.6	0.70	1.6
(A)	±0.061	±0.046	±0.081	±0.039	±0.083	±0.060	±0.081	±0.030	±0.064	±0.050
-D-Mannosidase	0.28	0.46	0.24	0.48	0.28	0.45	0.26	0.47	0.30	0.44
(A)	±0.009	±0.008	±0.015	±0.009	±0.013	±0.018	±0.010	±0.010	±0.008	±0.014
B-D-Glucosidase	0.20	0.43	0.21	0.44	0.20	0.45	0.22	0.46	0.20	0.43
(A)	±0.013	±0.010	±0.009	±0.014	±0.010	±0.013	±0.009	±0.015	±0.015	±0.009

Mean ± SEM. Number in the parenthesis equal the number of experiments.

A = mu moles phenol/minute/mg protein

TABLE 22b.

Lysosomal enzyme activity in the left ventricle of control californian rabbits at various days.

Enzymes	Days 1 (4) Free	1 (4) Bound	3 (5) Free	3 (5) Bound	5 (9) Free	5 (9) Bound	10 (10) Free	10 (10) Bound	15 (30) Free	15 (30) Bound
Esterase	0.80	1.7	0.85	1.7	0.78	1.9	0.80	1.6	0.90	1.8
(A)	±0.090	±0.100	±0.040	±0.103	±0.140	±0.120	±0.067	±0.091	±0.110	±0.120
Deoxyribonuclease acid	0.25	0.31	0.27	0.29	0.24	0.32	0.25	0.30	0.24	0.30
(B)	±0.020	±0.040	±0.16	±0.019	±0.024	±0.015	±0.020	±0.026	±0.019	±0.030
Acid Phosphatase	2.0	1.6	2.1	1.9	2.0	1.9	2.0	1.7	2.0	1.6
(A)	±0.178	±0.140	±0.135	±0.136	±0.146	±0.199	±0.175	±0.095	±0.100	±0.114
Phosphodiesterase IV	0.60	0.63	0.59	0.61	0.60	0.60	0.61	0.49	0.63	0.57
(A)	±0.051	±0.043	±0.054	±0.041	±0.040	±0.030	±0.036	±0.019	±0.030	±0.032
Cathepsin "D"	15.0	17.0	16.0	18.0	14.0	16.0	13.0	17.0	16.0	19.0
(C)	±1.4	±1.0	±1.1	±1.4	±1.4	±2.1	±1.3	±2.9	±2.1	±2.4

Mean ± SEM. The number in the parenthesis equal the number of experiments.

A = mu moles phenol/minute/mg protein.

B = increase in optical density (unit = 0.100 OD) per hour/mg protein

C = ug tyrosine/30 minutes/mg protein

TABLE 23a.

The effect of coarctation of the aorta on lysosomal enzyme activity in the left ventricle of californian rabbits at various days.

	Days					
	6 (2)		10 (4)		21 (11)	
Enzymes	Free	Bound	Free	Bound	Free	Bound
B-D-Galactosidase	0.48	0.43	0.53	0.45	0.59	0.62
	±0.014	±0.011	±0.019	±0.017	±0.019	±0.024
(A)			*		**	
B-D-Glucuronidase	0.40	0.50	0.52	0.46	0.50	0.41
	±0.013	±0.021	±0.010	±0.026	±0.023	±0.019
(A)			**		*	
N-Acetyl-B-D Glucosaminidase	0.81	1.5	1.6	1.5	2.0	1.6
	±0.067	±0.095	±0.103	± 0.094	±0.136	±0.148
(A)			**		***	
-D-Mannosidase	0.29	0.46	0.44	0.40	0.43	0.36
	±0.009	±0.014	±0.013	±0.023	±0.021	±0.014
(A)			**		*	
B-D-Glucosidase	0.25	0.43	0.40	0.31	0.33	0.40
(A)	±0.016	±0.021	±0.024	±0.010	±0.016	±0.029

Mean ± SEM. Number in the parenthesis equal number of experiments.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

A = mu moles phenol/minute/mg. protein.

TABLE 23b.

The effects of coarctation of the aorta on lysosomal enzyme activity in the left ventricle of californian rabbits at various days.

	Days					
	6 (2)		10 (4)		21 (11)	
	Free	Bound	Free	Bound	Free	Bound
Esterase	0.90	1.6	1.5	1.9	1.8	2.1
	±0.091	±0.094	±0.014	±0.160	±0.189	±0.175
(A)			**		**	*
Deoxyribonuclease Acid	0.24	0.39	0.40	0.31	0.39	0.34
	±0.019	±0.040	±0.018	±0.023	±0.021	±0.032
(B)			*		*	
Acid Phosphatase	2.4	1.8	3.3	1.6	3.4	2.0
	±0.140	±0.170	±0.180	±0.134	±0.201	±0.099
(A)			**		**	
Phosphodiesterose IV	0.78	0.64	1.3	0.60	1.3	0.66
(A)	±0.041	±0.038	±0.067	±0.051	±0.074	±0.066
			**		**	
Cathepsin "D"	14	18	20	20	23	20
(C)	±1.0	±0.94	±1.6	±1.0	±1.3	±1.8

Mean ± SEM. Numbers in the parenthesis equal number of experiments. *$p < 0.05$, **$p < 0.01$.

A = mu moles phenol/minute/mg protein

B = increase in optical density (unit = 0.100 OD) per hour/mg protein

C = ug tyrosine/30 minutes/mg protein

TABLE 24a.

The effects of thyroxine on lysosomal enzyme activity in the left ventricle of californian rabbits at various days.

	Days							
	3 (2)		5		10		15	
Enzymes	Free	Bound	Free	Bound	Free	Bound	Free	Bound
B-D-Galactosidase	0.33	0.48	0.35	0.49	0.41	0.50	0.69	0.41
(A)	±0.011	±0.018	±0.010	±0.014	±0.020	±0.011	±0.013	±0.006
							**	
B-D-Glucuronidase	0.36	0.52	0.31	0.50	0.40	0.48	0.48	0.42
(A)	±0.013	±0.018	±0.008	±0.016	±0.013	±0.021	±0.007	±0.008
							*	
N-Acetyl-B-D Glucosaminidase	0.66	1.6	0.60	1.6	1.0	1.8	1.6	1.9
(A)	±0.036	±0.071	±0.060	±0.084	±0.098	±0.007	±0.100	±0.125
					*		**	*
-D-Mannosidase	0.20	0.46	0.27	0.50	0.33	0.47	0.38	0.40
(A)	±0.009	±0.016	±0.006	±0.012	±0.019	±0.021	±0.020	±0.010
B-D-Glucosidase	0.20	0.43	0.20	0.40	0.32	0.38	0.34	0.32
(A)	±0.010	±0.008	±0.009	±0.013	±0.010	±0.015	±0.021	±0.026
								*

Mean ± SEM. Numbers in the parenthesis equal the number of experiments. *p< 0.05, **p< 0.01

A = mu moles phenol/minute/mg protein

TABLE 24b.

The effect of thyroxine on lysosomal enzyme activity in the left ventricle of californian rabbits at various days.

	Days							
	3 (2)		5		10		15	
	Free	Bound	Free	Bound	Free	Bound	Free	Bound
Esterase	0.88	2.0	1.5	1.6	1.8	2.1	2.0	2.8
(A)	±0.061	±0.094	±0.060	±0.073	±0.120	±0.160	±0.098	±0.125
			*	*	**	**	**	**
Deoxyribonuclease Acid	0.26	0.29	0.28	0.31	0.34	0.30	0.37	0.34
(B)	±0.018	±0.031	±0.016	±0.023	±0.021	±0.016	±0.016	±0.021
Acid phosphatase	2.1	1.6	2.0	1.6	2.8	1.8	3.1	1.8
(A)	±0.170	±0.100	±0.091	±0.131	±0.121	±0.181	±0.188	±0.231
					*		**	
Phosphodiostarase IV	0.66	0.61	0.60	0.50	0.90	0.50	1.0	0.50
(A)	±0.040	±0.061	±0.052	±0.030	±0.066	±0.073	±0.101	±0.048
					*		**	
Cathepsin "D"	16	18	18	19	17	20	25	16
(C)	±1.6	±1.3	±1.2	±1.9	±2.1	±1.9	±3.1	±1.6

Mean ± S.E.M. Number in the parenthesis equal number of experiment.

*p < 0.05, **p < 0.01, ***p < 0.001

A = mumoles phenol/minute/mg protein. B = increase in optical density (unit = 0.100 OD) per hour/mg. protein.

TABLE 25a.

The effect of isoprenaline on lysosomal enzyme activity in the left ventricle of californian rabbits at various days.

Enzymes	Days 1 (3) Free	1 (3) Bound	3 (3) Free	3 (3) Bound	5 (6) Free	5 (6) Bound	10 (9) Free	10 (9) Bound	15 (19) Free	15 (19) Bound
B-D-Galactosidase	0.51	0.30	0.69	0.53	0.80	0.60	0.59	0.52	0.40	1.0
(A)	±0.019	±0.010	±0.011	±0.013	±0.018	±0.030	±0.009	±0.016	±0.018	±0.038
	**	**	**		***		**			**
B-D-Glucuronidase	0.48	0.40	0.55	0.50	0.78	0.49	0.41	0.49	0.38	0.46
(A)	±0.016	±0.013	±0.018	±0.016	±0.031	±0.026	±0.021	±0.031	±0.011	±0.014
	*		**		***					
N-Acetyl-B-D Glucosaminidase	0.98	1.0	1.3	1.6	2.2	1.7	1.3	2.1	1.0	3.2
(A)	±0.048	±0.051	±0.066	±0.071	±0.071	±0.160	±0.070	±0.180	±0.050	±0.088
	**		***		***	*	**	**	*	***
-D-Mannosidase	0.50	0.30	0.51	0.39	0.57	0.37	0.40	0.41	0.26	0.44
(A)	±0.009	±0.015	±0.018	±0.011	±0.013	±0.008	±0.016	±0.019	±0.009	±0.016
	**	*	**		**		**			
B-D-Glucosidase	0.44	0.21	0.40	0.41	0.47	0.44	0.30	0.42	0.22	0.41
(A)	±0.013	±0.014	±0.014	±0.010	±0.016	±0.015	±0.008	±0.010	±0.008	±0.009

Mean ± SEM. Number in parenthesis equal number of experiments. *p 0.05, . **p< 0.01, ***p< 0.001

A = mu moles phenol/minute/mg protein.

TABLE 25b.

The effect of isoprenaline on lysosomal enzyme activity in the left ventricle of californian rabbits at various days.

	days									
	1 (3)		3 (3)		5 (8)		10 (9)		15 (19)	
Enzymes	Free	Bound	Free	Bound	Free	Bound	Free	Bound	Free	Bound
Esterase	0.81	1.7	0.76	1.4	0.60	1.0	0.60	1.0	1.1	2.4
(A)	±0.090	±0.100	±0.040	±0.103	±0.110	±0.169	±0.088	±0.134	±0.093	±0.160
				*		*		*		**
Acid-Deoxyribo-Nuclease	0.41	0.18	0.44	0.29	0.59	0.34	0.40	0.48	0.30	0.33
	±0.020	±0.009	±0.016	±0.008	±0.019	±0.014	±0.012	±0.020	±0.010	±0.010
(B)	**	*	**		***					
Acid phosphatase	2.9	1.0	3.1	1.8	4.0	1.5	3.2	2.1	3.2	2.4
(A)	±0.170	±0.100	±0.130	±0.141	±0.201	±0.387	±0.186	±0.211	±0.213	±0.198
	**		**		***		***	*	***	***
Phosphodiesterase IV	1.0	0.40	1.0	0.53	1.4	0.50	0.94	0.46	0.90	0.70
(A)	±0.040	±0.030	±0.036	±0.019	±0.100	±0.067	±0.066	±0.073	±0.053	±0.061
	*		*		**		**		*	
Cathepsin "D"	28	12.0	26	20	36	19	25	14	18	18
(C)	±1.6	±1.0	±1.9	±2.0	±3.1	±1.8	±2.1	±1.1	±0.98	±1.0

Mean ± SEM. Number in the parenthis equal the number of experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

A = mu moles phenol/minute/mg protein. B = increase in optical density (unit = 0.100 OD) per hour/mg. protein.
C = ug tyrosine/30 minutes/mg protein.

injection the rise in the free form of most of the enzymes studied was accompanied by an equivalent fall in the bound enzymes, so that there was no change in the total amount of enzyme. These changes presumably represent the "autolytic" process (i.e. change in lysosomal enzyme distribution of the existing cells). In every other case any increase in the free form of the enzyme was accompanied by an increase (albeit smaller) in the bound form. These changes would probably represent the "heterolytic" process (i.e. increased infiltration of phagocytic cells). So therefore, the increase in total lysosomal enzyme activity may be related to an increase in other cell types which are rich in lysosomal enzymes, such as phagocytic cells (Cohn and Hirsch, 1960).

2.5.1. Histochemical Observations

There is histochemical evidence however, that increases in lysosomes and their enzymes are associated with cardiac hypertrophy. Studies by Pearse (1964) and Van Noorden and Pearse (1970) have revealed histochemically that two lysosomal enzymes, acid phosphatase and non-specific esterase, were increased in myocardial cells from patients with cardiac hypertrophy.

In an attempt to resolve this problem, histochemical experiments were designed to find out where the increases in total lysosomal enzyme activity occurred (i.e. in myocardial cells or in other cell types). The two enzymes chosen for this purpose were acid phosphatase and N-Acetyl-B-D-Glucosaminidase. They were chosen because they are representative of lysosomes and because they showed/...

showed an increase in total activity in the present experiments.

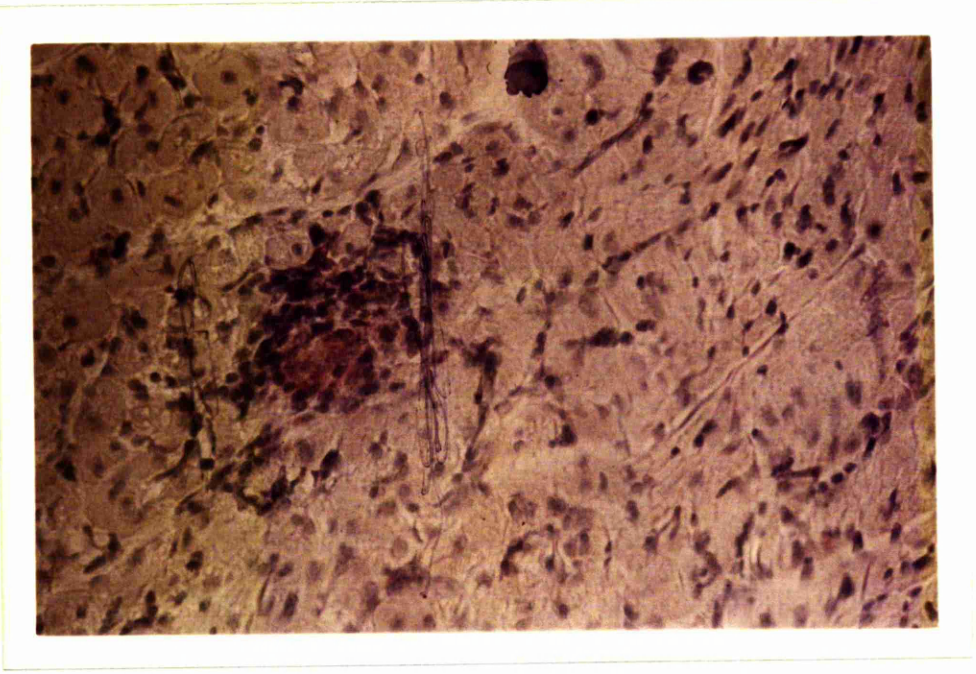
Preliminary histochemical studies with acid phosphatase showed that the enzyme was increased in myocardiac cells during 10 and 21 days of coarctation and 10 and 15 days of isoprenaline injections and 15 days of thyroxine injections. However, it was difficult to measure these changes. The other enzyme N-Acetyl-B-D-glucosaminidase was not found at all in normal heart tissue, (fig. 10a) which is similar to the results of Hayashi (1964). These results may be related to the low levels of enzyme found chemically in heart tissue as compared to other tissue (i.e. liver and kidney) where this enzyme is found in appreciable amounts both histochemically and chemically. However, after coarctation for 10 days or 21 days the enzyme could be demonstrated in the heart, but only in fibroblasts or cells of similar appearance which could not be unequivocally identified (fig. 10b). None was demonstrable in myocardial cells. After thyroxine treatment the enzyme was again found only in fibroblasts and similar cells and only after 15 days (fig. 10c). After isoprenaline treatment it was demonstrable at 5 and 15 days, once again only in fibroblasts and similar cells (fig.10gh). Fibroblasts are, of course, much less numerous than myocardial cells, and it is quite conceivable that, although the individual myocardial cell might contain much less N-Acetyl-B-D-glucosaminidase than the individual fibroblast, all the myocardial cells taken together might contain a total more

Fig.16a-b-c-d-e-f-g-g Sections of the left ventricle from californian rabbits incubated for N-Acetyl-B-D-Glucosaminidase activity with Naphthol AS-BI-N-Acetyl-B-D-Glucosaminide as substrate and hexazotized p-rosaniline as coupler for 60 min. at 37°C pH 4.5. The reaction product appears red at the site of enzyme activity.

Fig.16a Rabbits which were injected with saline for 15 days. Note no reaction product inside myocardial cells. However there appears to be some reaction product in or near an arteriole (Mag x 250).

Fig.16b Rabbit which had undergone sham operation for 21 days. Note no reaction product inside the myocardiac cell. (Mag x 1000).

Fig 10



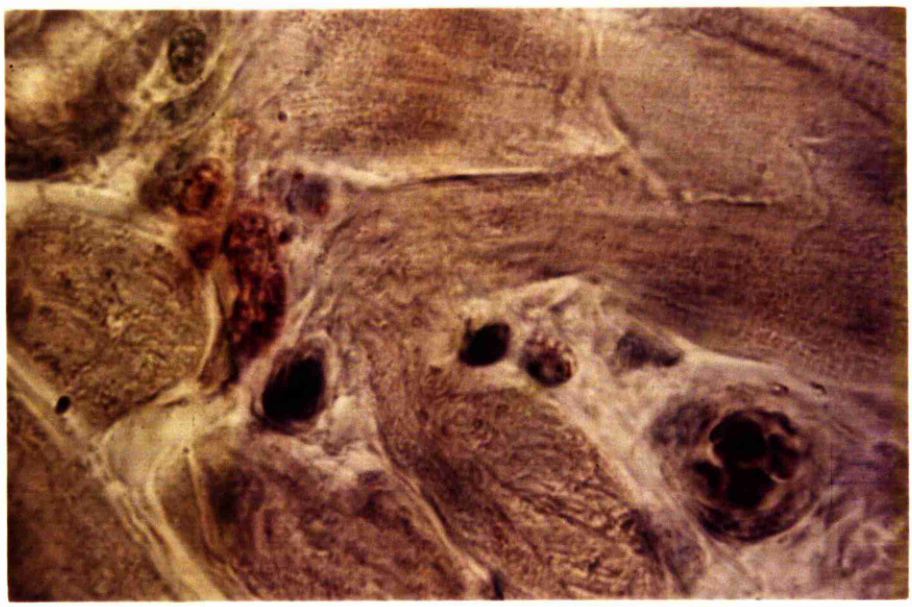
A

B

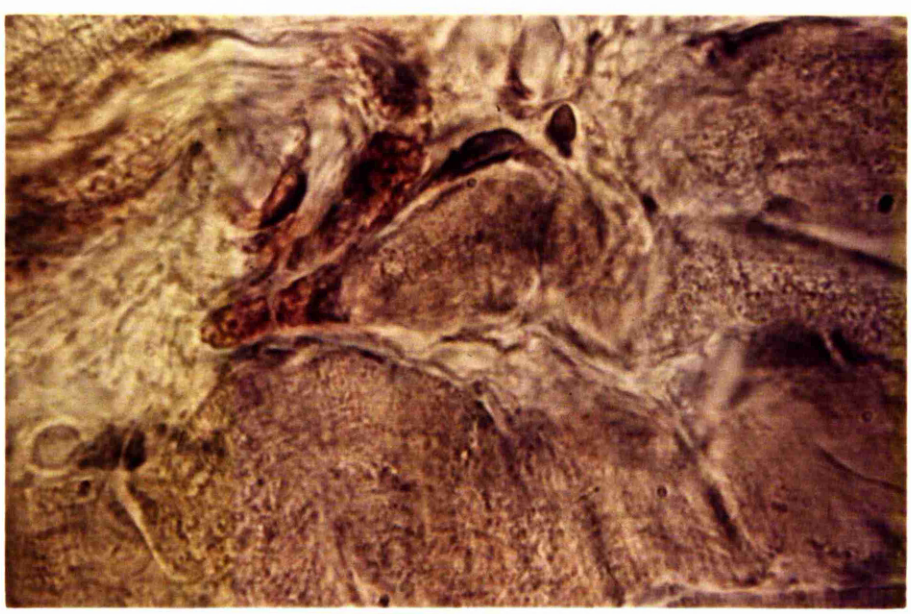
Fig.10c Rabbit which had undergone coarctation of the aorta for 10 days. Note the reaction product appears in fibroblasts or cells with similar appearance (Mag x 1000).

Fig.10d Rabbit which had undergone coarctation of the aorta for 21 days. Note reaction product only appears in fibroblast or cells with similar appearance (Mag x 1000).

Fig 10



C



D

Fig. 19e-f

Rabbits injected with thyroxine for 15 days. Note reaction product appears only in fibroblast or cells of similar appearance (Mag x 250).

Fig 10

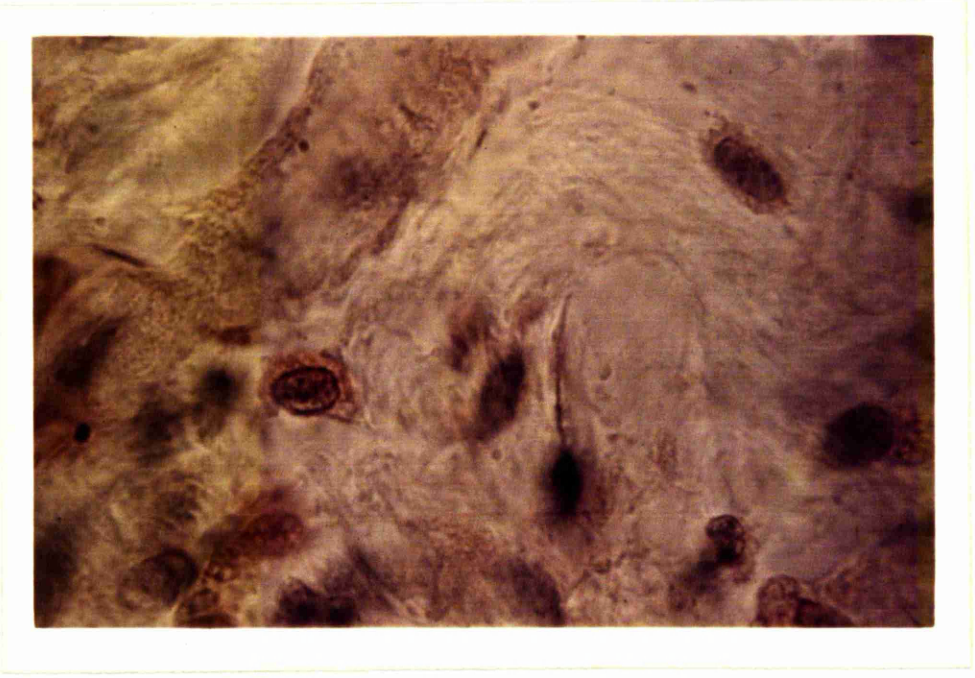
E

F

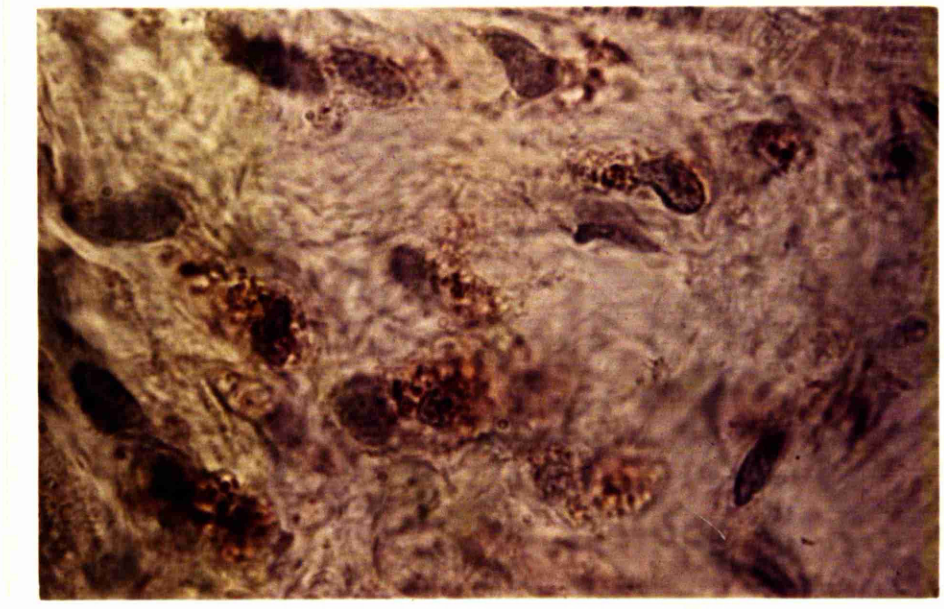
Fig.10g Rabbit injected with isoprenaline for 5 days. Note reaction product only appears in fibroblast or cells of similar appearance (Mag x 1000).

Fig.10h Rabbit injected with isoprenaline for 15 days. Note reaction product only appears in fibroblast or cells of similar appearance (Mag x 1000).

Fig 10



G



H

of the enzyme than all the fibroblasts taken together.

2.5.2 Ground Substance

Since N-Acetyl-B-D-glucosaminidase was found to be increased it seemed reasonable to determine if its presumptive substrate changed in cardiac hypertrophy. In the myocardium, ground substance, which generally contains mucopolysaccharides and/or glycoproteins are presumed to be the substrate of N-Acetyl-B-D-glucosaminidase *in vivo* (Fibian 1966). An attempt was therefore made to detect chemically and histochemically mucopolysaccherides and glycoproteins in the heart following experimentally induced cardiac hypertrophy.

Histochemically, acid mucopolysaccharides stain blue using the method of Hale (1948). In the normal heart it was demonstrable in the intra-cellular ground substance, between collagen fibers but in very small amounts (fig. 12a); after coarctation it was clearly increased at 10 and not at 21 days (fig. 12b and Table 27): after 5 days of thyroxine treatment no change was apparent but after 15 days there was a small increase (Table 27); isoprenaline produced a marked increase at 5 days which had diminished at 15 days (fig. 12c and Table 27).

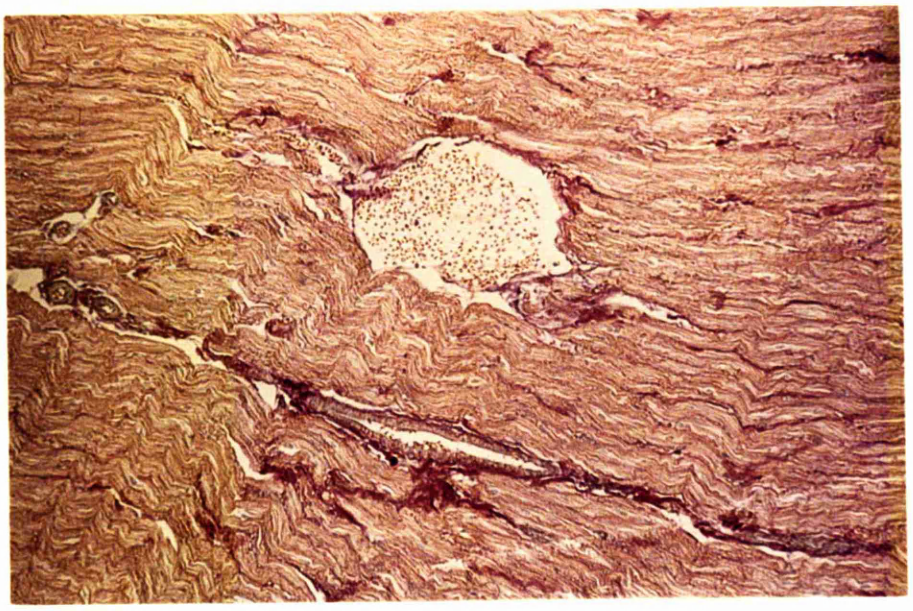
A parallel chemical estimation involved measuring glucosamine, which is normally a major constituant of mucopolysaccharides and glycoproteins. The results found in Tables 26a - b are roughly parallel to those found histochemically. There were slight increases at 6 and 10 days with coarctation and at 15 days with thyroxine; a much more dramatic/...

Fig. 12a-b-c — Sections of the left ventricle of californian rabbits stained for acid mucopolysaccherides with Hale stain which appears blue, and stained with Van Gieson's stain, counterstained with Hamalum.

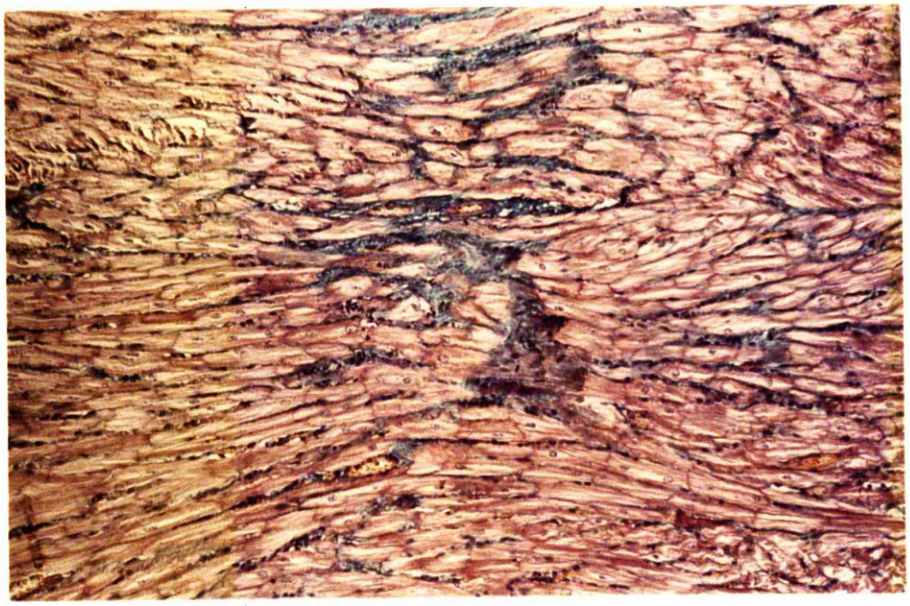
Fig. 12a — Rabbit injected with saline for 15 days. Note spearse acid mucopolysaccherides (Mag x 100)

Fig. 12b — Rabbit which had undergone coarctation of the aorta for 10 days. Note accumulation of acid mucopolysaccherides (Mag x 100).

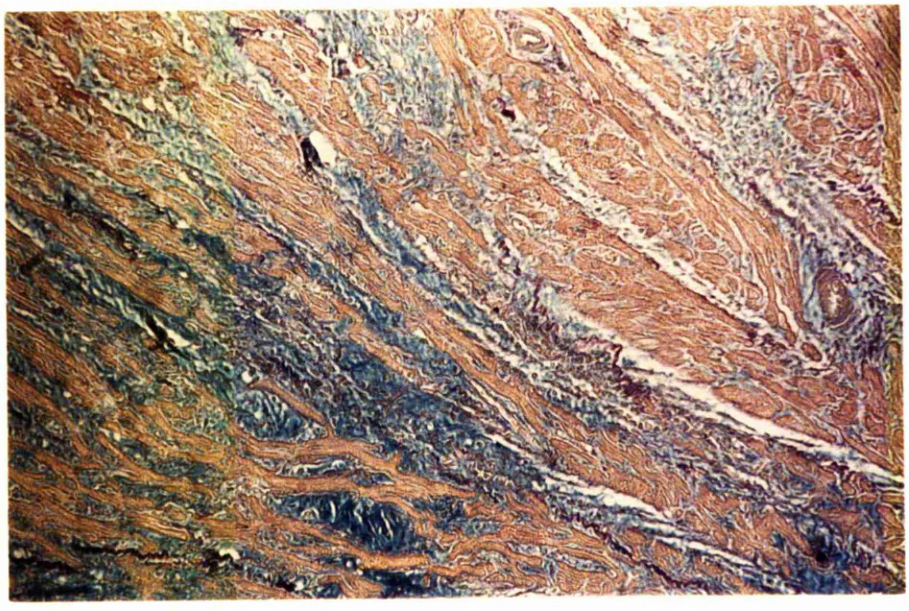
Fig. 12c — Rabbit injected with isoprenaline for 3 days. Note the marked increase of acid mucopolysaccherides (Mag x 100).



A



B



C

TABLE 27

The extent or change in mucopolysaccharide of the left ventricle following coarctation, thyroxine and isoprenaline treatment at various time intervals.

Treatment	Days	Mucopolysaccharides
Coarctation	10	++
	21	0
Thyroxine	5	0
	10	0
	15	+
Isoprenaline	5	+++
	10	+
	15	+

0 = no change, + = small change, ++ = moderate change, +++ = marked change. At each time interval three californian rabbits were used.

TABLE 26a

The hexosamine content in the left ventricle of sham operated and saline injected californian rabbits at various time intervals.

Saline injected

Days	No. of animals	Hexosamine in mg. (as Glucosamine HCL)/g. Dry Weight
3	3	2.1 ±0.20
5	8	2.2 ±0.22
10	4	2.2 ±0.23
15	10	2.2 ±0.18

Sham operated

6	2	2.2 ±0.24
10	4	2.2 ±0.19
21	4	2.1 ±0.22

TABLE 26b

The effects of coarctation, thyroxine and isopreanline treatment on the amount of hexosamine in the left ventricle at various time intervals.

Treatment	Days	No. of animals	Hexosamine in mg. (as Glucosamine HCL)/g. Dry Weight
Controls	-	30	2.2 ±0.21
Coarctation	6	2	2.6 ±0.26
	10	4	3.1 * ±0.21
	21	11	2.0 ±0.29
Thyroxine	5	8	2.1 ±0.24
	10	8	2.3 ±0.20
	15	10	2.7 ±0.19
Isopreanline	3	3	3.7 ** ±0.28
	5	5	3.5 ** ±0.31
	10	8	2.4 ±0.26
	15	13	1.9 ±0.20

Mean ± SEM *$p < 0.01$, **$p < 0.001$

dramatic effect at 3 and 5 days with isoprenaline, which subsequently diminished.

These results by themselves are interesting but difficult to interpret. They follow a pattern which we have already noted repeatedly in these experiments in that isoprenaline showed a large and immediate effect which subsequently diminished, while thyroxine showed a smaller and progressive effect and coarctation same somewhere in between.

The histochemical results for N-Acetyl-B-D-glucosaminidase seemed to have nothing to do with the myocardial cell, but reflected the behaviour of fibroblasts or similar cells. This threw doubt on the whole idea that changes in lysosomal enzymes in cardiac hypertrophy have anything to do with myocardial cells. This doubt could only be resolved by the electron microscope.

2.6.0 Electron Microscope Observation

The early phase of lysosomal research was based mainly on the biochemical analysis, however in recent years cytochemical methods and electron microscopy have contributed to our understanding of the subject. Such observations have mostly been confined to liver, although Abraham et al, (1967) observed lysosome-like veiscles in the perinuclear region of heart muscle. These vesicles were subsequently isolated by Hendley et al, (1965) and shown to contain a typical lysosomal enzyme, Cathepsin D. In the present study, sections of the left ventricle were prepared for electron microscopic observation. Figures 13a - b - c - d show the normal appearance of the left ventricle/...

Fig. 13a — Myocardial fiber from left ventricle of rabbit injected with saline for 15 days. (MY) myofibril, (ID) intercalated disc, (M) mitochondria, the arrow points to sarcotubular system (Mag x 25,000).

Fig. 13b — Myocardial fiber from left ventricle of rabbit which had undergone sham operation for 21 days. (ID) intercalated disc, (M) mitochondria, (ER) endoplasmic reticulum, (Z) Z band, (S) S zone, the arrow points to pinocytic vacuoles (Mag x 25,000).

Fig. 13c — Myocardial fiber from the left ventricle of rabbit which had undergone sham operation for 10 days. (F) fat droplets, (DV) dense vesicle, (GV) golgi vesicle, (N) nucleus, (ER) rough sarcoplasmic reticulum (Mag x 25,000).

Fig 13

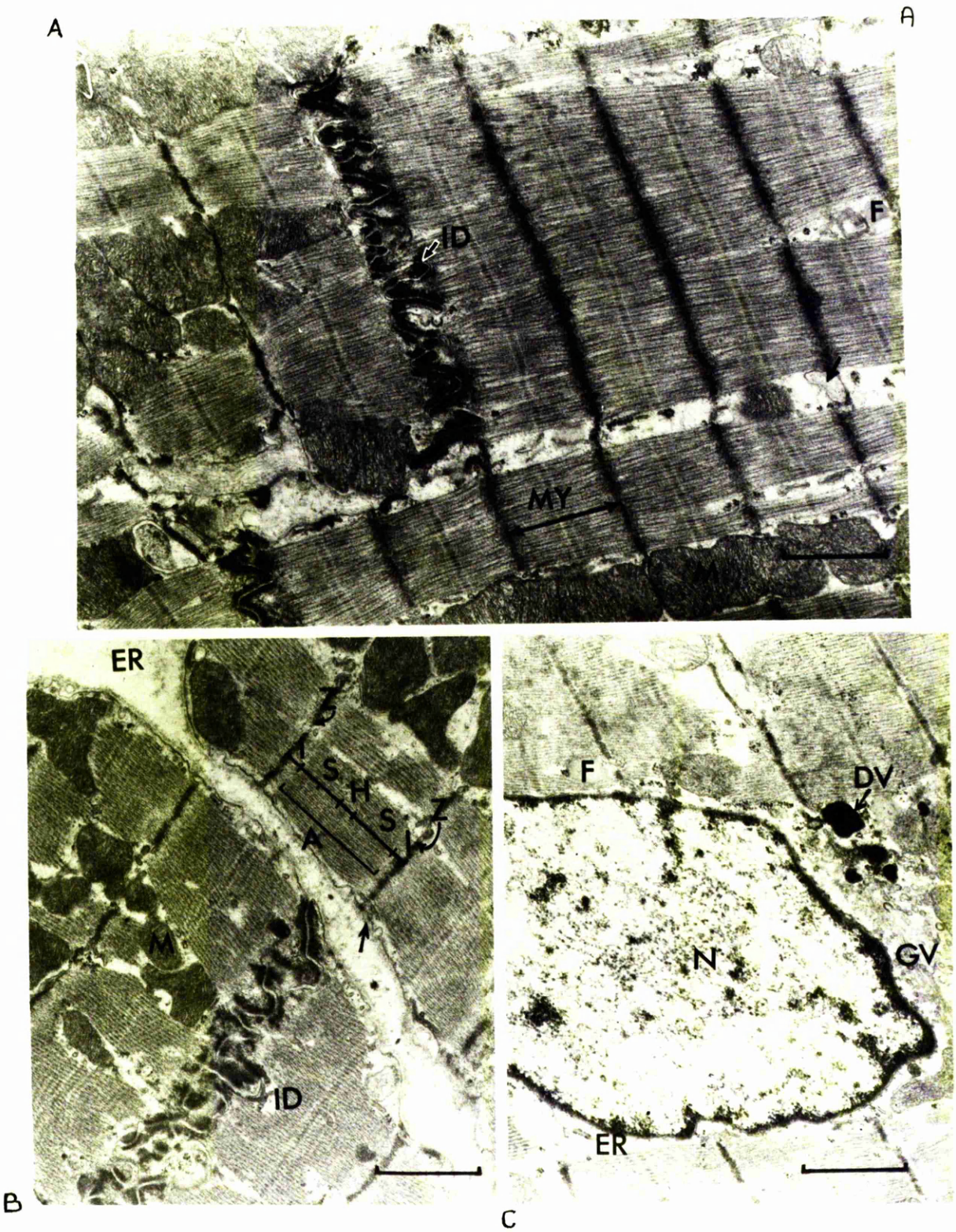
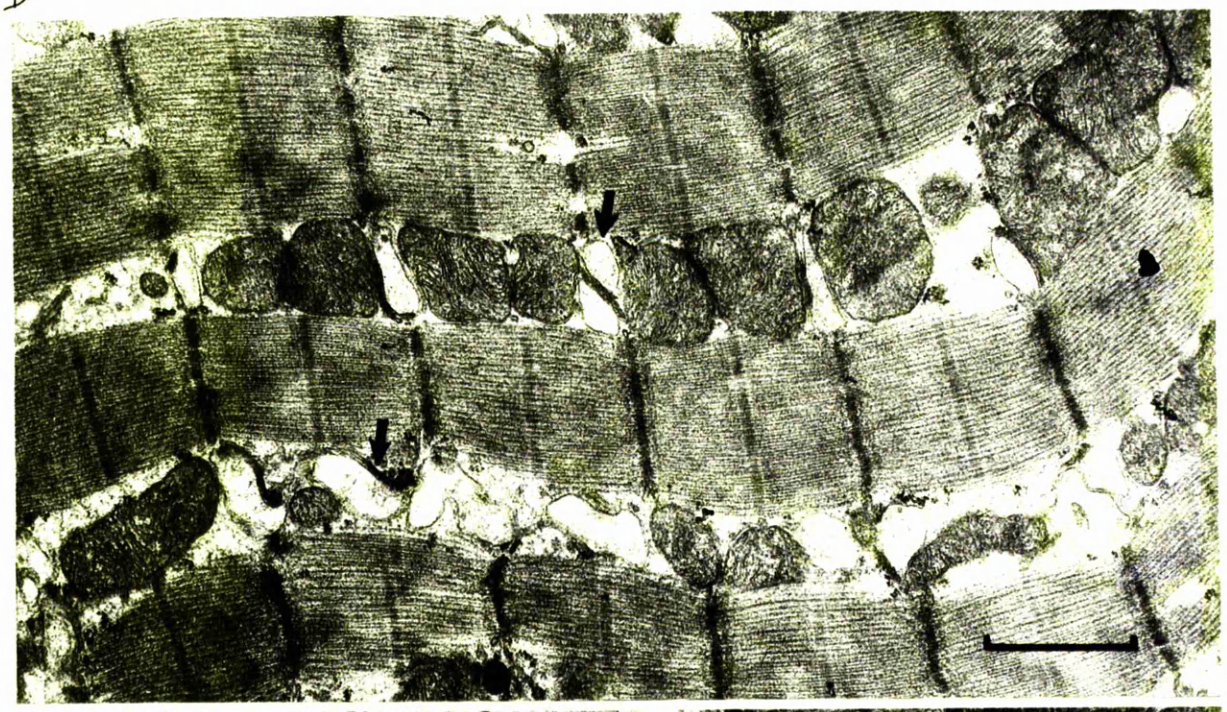


Fig. 13d Myocardial fiber from the left ventricle of rabbit injected with saline for 5 days. Arrows point to the sarcotubular system (Mag x 25,000).

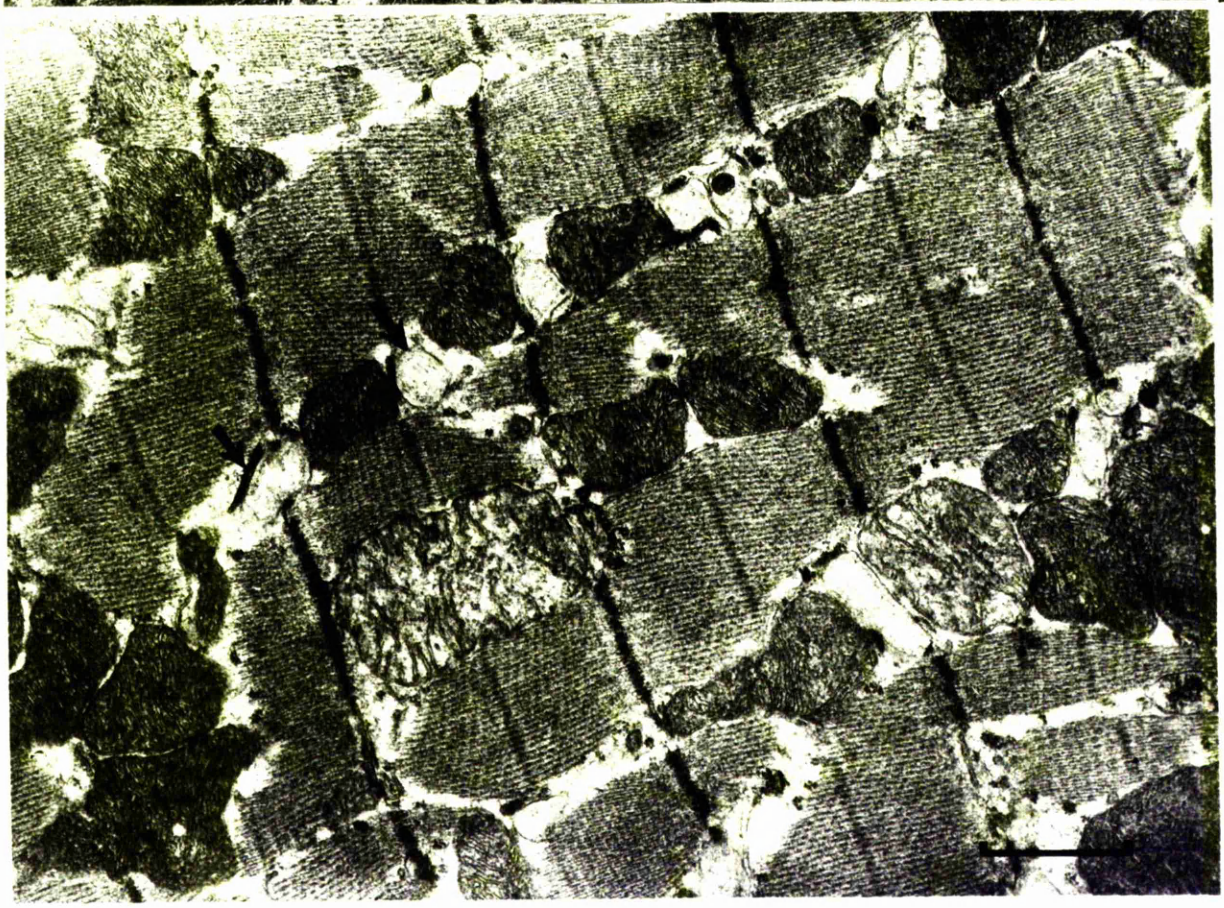
Fig. 13e Myocardial fiber from left ventricle of rabbit which had undergone sham operation for 21 days. Arrows point to the sarcotubular system (Mag x 25,000).

Fig 13

D



D



E

E

ventricle. Only a few structures could reasonably be identified as lysosomes, one such is shown in Fig. 13c. It has a dense matrix surrounded by a membrane. Similar structures have been described in a variety of tissues under the names "dense vesicles" or "lipofuchsin granules," (Novikoff, 1963). It is generally accepted that they are tertiary lysosomes or residual bodies as defined above. However, for the present, it is perhaps better to use the more non-committal description term "dense vesicle." In the normal myocardial cell the few dense vesicles observed were confined to the perinuclear region.

After coarctation of the aorta for either 10 or 21 days, many more dense vesicles could be seen in myocardial cells, not only around the nucleus but throughout the cytoplasm. No obvious difference could be seen between sections taken at 10 days and 21 days. Some examples are shown in fig. 14b - d. It was observed that about a fifth of the dense vesicles contained what looked like fat droplets (fig. 14d).

After thyroxine treatment for 5 days no increase in dense vesicles could be detected. However, 15 days of thyroxine treatment led to an increase similar to that seen after coarctation. As after coarctation, the vesicles were found throughout the cytoplasm and not merely around the nucleus (fig. 14a) and about a fifth contained fat droplets.

After isoprenaline treatment for 5 days there was an increase in dense vesicles greater than that produced by either coarctation or thyroxine. Again about one fifth contained/...

Fig. 14a — Myocardial fiber from the left ventricle of rabbits injected with thyroxine for 15 days. Arrows point to dense vesicle (Mag x 25,000).

Fig. 14b — Myocardial fiber from the left ventricle of rabbit which had undergone coarctation of the aorta for 10 days. Arrows point to dense vesicle (Mag x 25,000).

Fig. 14c — Myocardial fiber from the left ventricle of rabbit injected with isopreanline for 5 days. Arrows point to dense vesicle (Mag x 25,000).

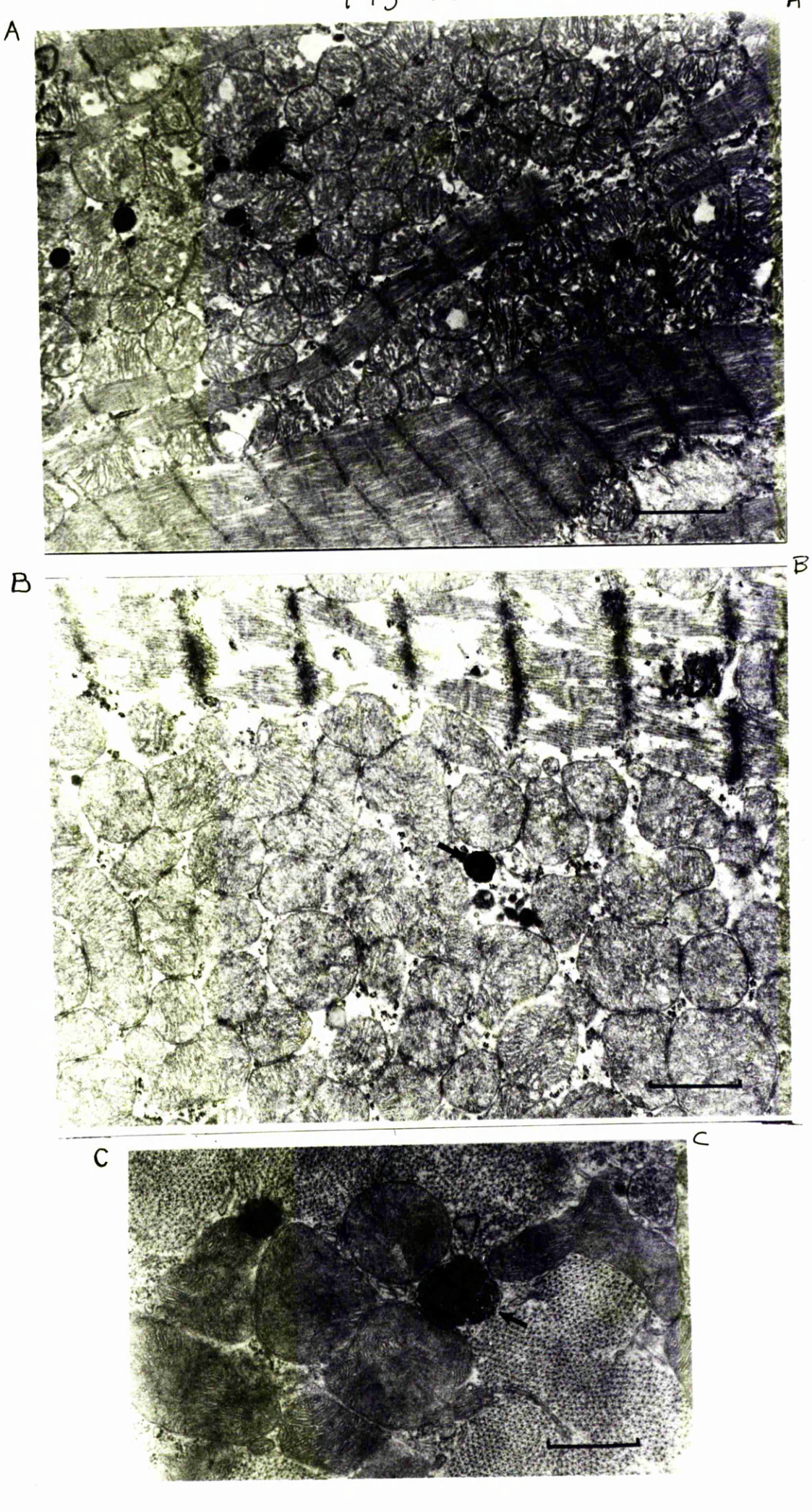
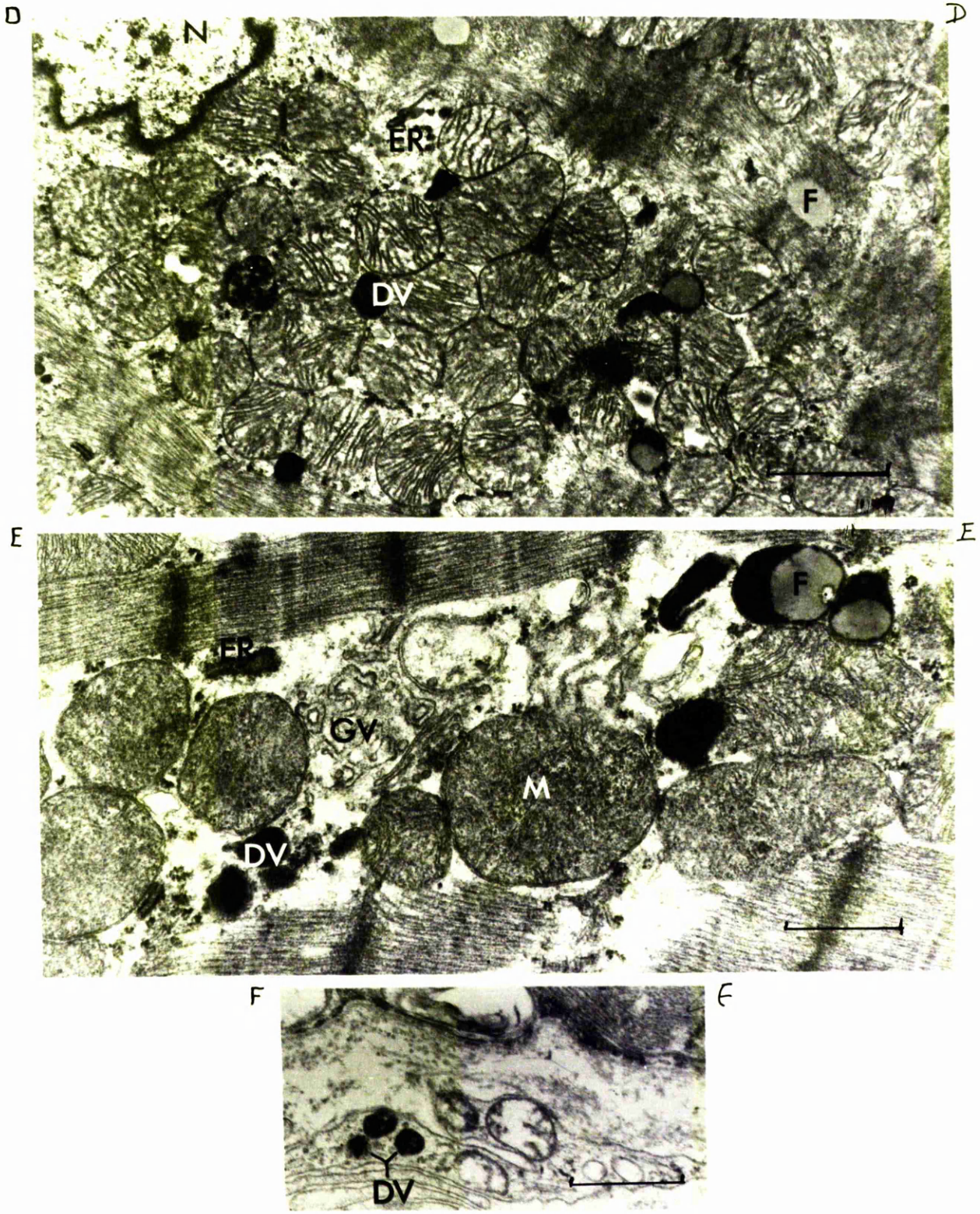

A
B
C

Fig. 14d — Myocardial fiber from the left ventricle of rabbit which had undergone coarctation for 21 days (DV) dense vesicle, (ER) rough sarcoplasmic reticulum, (F) fat droplets (Mag x 25,000).

Fig. 14e — Myocardial fiber from the left ventricle of rabbit injected with isoprenaline for 15 days. (DV) dense vesicle, (M) mitochondria, (F) fat droplets, (ER) rough sarcoplasmic reticulum, (GV) golgi vesicle (Mag x 25,000).

Fig. 14f — Fibroblast from the left ventricle of rabbit injected with isoprenaline for 5 days. (DV) dense vesicle (Mag x 25,000).

Fig 14



contained fat droplets, and again they were distributed throughout the cytoplasm and not merely around the nucleus (fig. 14c - e). The picture after 15 days of isoprenaline treatment was not essentially different.

Because the electron microscope can be used to examine only an infinitesimally small sample of tissue, it can seldom by itself be the basis of quantitive conclusions. In the present instance however, the marked increase in the number of structures which can safely be identified as lysosomes is sufficient to justify the view that the changes observed in lysosomal enzymes in the heart are largely explicable in terms of events in the myocardium. Although other cell types may play some role in altering the enzyme pattern of the heart as a whole, there is no reason to suppose that it is a predominant one.

In the course of the search for electron microscopic evidence of lysosomes in the myocardium, a number of other structural changes were observed to accompany hypertrophy. Thus, although the appearance of dense vesicles was the most striking evidence for the presence of lysosomes, a smaller number of autophagosomes or secondary lysosomes were also observed after hypertrophy. These structures were never encountered in the normal myocardium. As was explained above, autophagosomes are vesicles which contain part of the cytoplasm which, apparently, they digest. Autophagosomes are normally found in cells which are involved in cellular necrosis (Deter and de Duve, 1967; Ericsson/...

Ericsson et al, 1967; Trump et al, 1962, 1965). After coarctation they were seen in almost equal numbers at 10 and 21 days (fig. 15a). After thyroxine none were seen at 5 days and only a few at 15 days. After isoprenaline they were seen at 5 days in greater numbers than after coarctation, (fig. 15b- c -) but they had disappeared at 15 days. If the autophagosome can be regarded as a lysosome actively engaged in cell destruction, these observations would fit in with the view derived from the earlier experiment in the present work, that all three methods used to produce hypertrophy cause cell destruction; that it is slow and progressive in the case of thyroxine; and the effects of coarctation lie between these extremes.

One might perhaps expect that this cell destruction should be evident from electron microscope studies and it is true that in general the hypertrophic hearts, no matter how the hypertrophy had been produced, seem to show disorientation of myofibrils and swelling of mitochondria. It is however difficult to be certain that both of these are not due to artefact in preparing the tissue for the electron microscope (Maser et al, 1967).

There is on the other hand clear evidence of increased protein synthesis.

Rough sarcoplasmic reticulum, which is rare in normal myocardium, (Fawcett and McNutt, 1967) shows a marked increase 10 or 21 days after coarctation (fig. 16a - b), after 5 or 15 days of isoprenaline treatment (fig. 16c - d) and/...

Fig. 15a

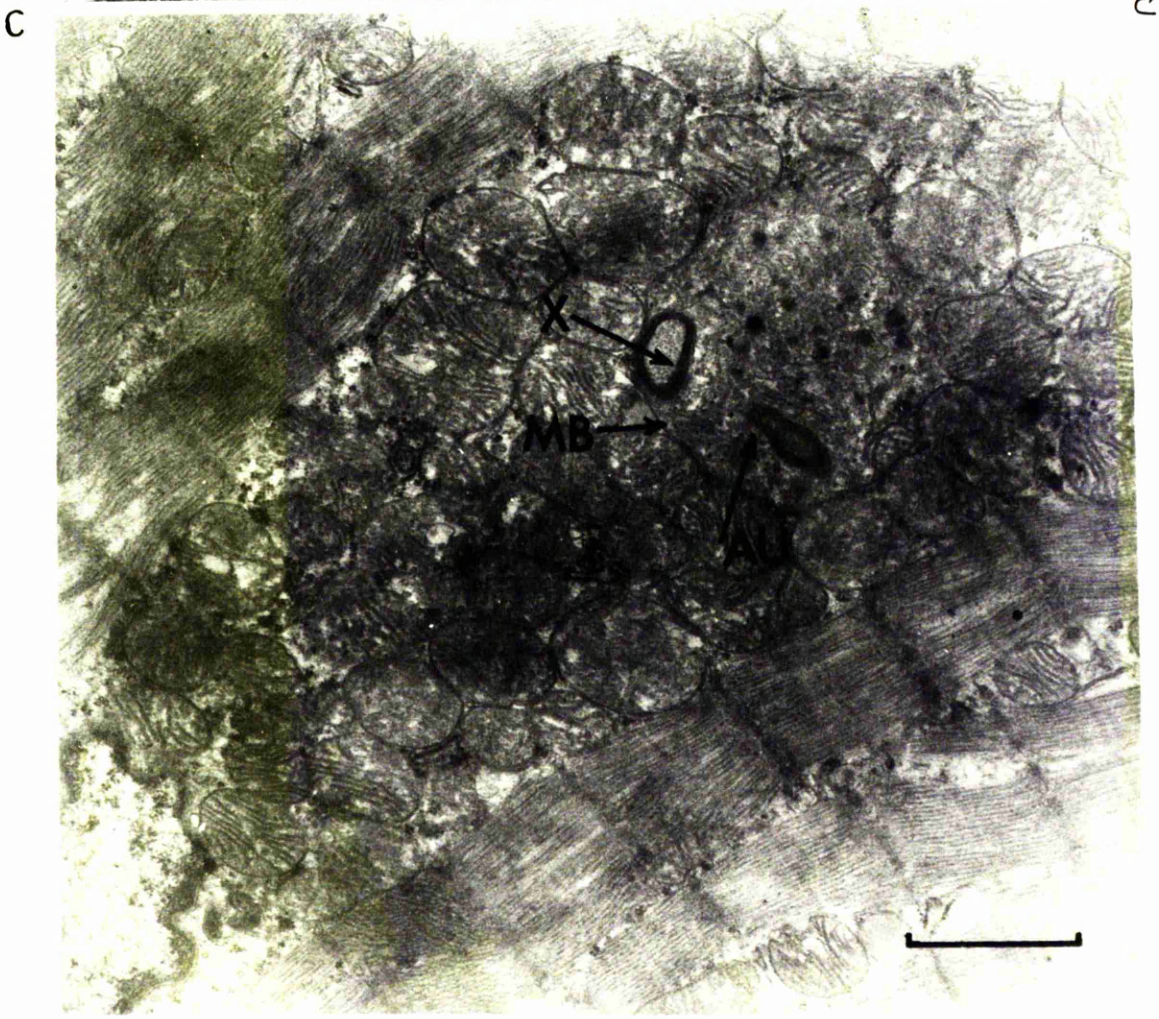
Myocardial fiber from the left ventricle of rabbit which had undergone coarctation of the aorta for 10 days. Arrows point to autophagosome (Mag x 25,000).

Fig 15

Fig. 15b Myocardial fiber from the left ventricle of rabbit injected with isoprenaline for 5 days. (AU) autophagosome (Mag x 25,000).

Fig. 15c Myocardial fiber from the left ventricle of rabbit injected with isoprenaline for 15 days (X) an autophagocytic vacuole inside an autophagosome, (MB) membrane of the autophagosome, (AU) autophagosome (Mag x 25,000).


B
B
F
DV


C
C
X
MB

and after 15 days of thyroxine injection (fig. 16e). It is not however increased after 5 days of thyroxine- What proteins are synthesized by this additional machinary is, of course, an open question; it may be for new myofibrils or it may be for new lysosomes.

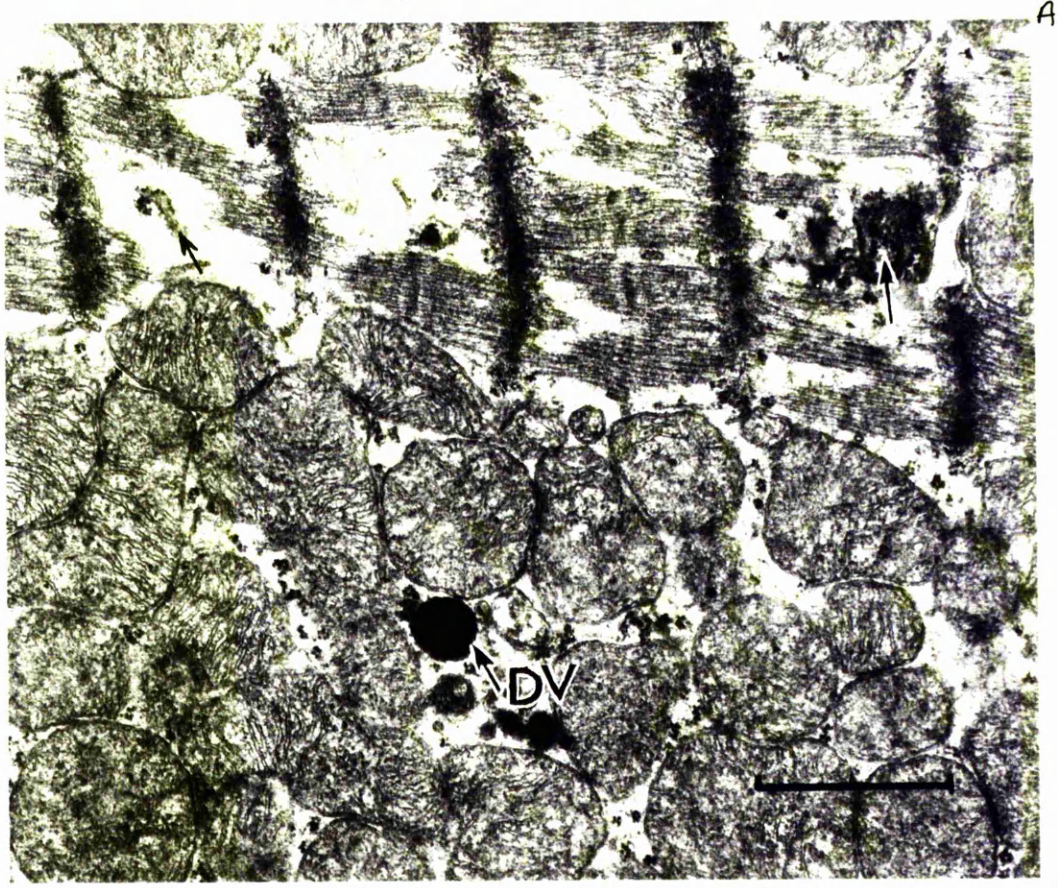
In parallel with these changes in the myocardial cells the fibroblasts and endothelial cells showed increases in rough endoplasmic reticulum. As compared to the control this was found in increased amounts at 10 and 21 days of coarctation (fig. 17b), after 5 and 15 days of isoprenaline (fig. 17a) or 15 days of thyroxine (fig. 17d) but not after 5 days of thyroxine injections. In fibroblasts, rough endoplasmic reticulum can perhaps be implicated in the synthesis of glycoprotein and/or collagen. It is more difficult however, to understand what may be happening in the endothelial cells (fig. 17c).

All the electron microscope findings discussed up to this point can plausibly be related to either cell destruction or cell growth, but there is one outstanding feature of the electron microscope evidence which cannot be completely related to either; all the hypertrophic hearts showed a substantial increase in the number of lipid droplets in the myocardial cells- This was visible to a roughly equivalent extent at 10 and 21 days after coarctation (fig. 18a). In many cases these lipid droplets were in contact with one or more mitochondria. Sometimes they were actually inside the mitochondron and in these cases there was invariably a contiguous/...

Fig. 16a Myocardial fiber from left ventricle of rabbit which had undergone coarctation of the aorta for 10 days. (DV) dense vesicle, the arrow points to rough sarcoplasmic reticulum (Mag x 25,000).

Fig. 16b Myocardial fiber from left ventricle of rabbit which had undergone coarctation of the aorta for 21 days. (F) fat droplet, the arrows point to rough sarcoplasmic reticulum (Mag x 25,000).

A



A

B

B

Fig. 16c — Myocardial fiber from left ventricle of rabbit injected with isoprenaline for 15 days. Arrow points to rough sarcoplasmic reticulum (Mag x 25,000).

Fig. 16d — Myocardial fiber from left ventricle of rabbit injected with isoprenaline for 5 days. Arrow points to rough sarcoplasmic reticulum (Mag x 25,000).

Fig. 16c — Myocardial fiber from left ventricle of rabbit injected with thyroxine for 15 days. Arrow points to rough sarcoplasmic reticulum. (Mag x 25,000).

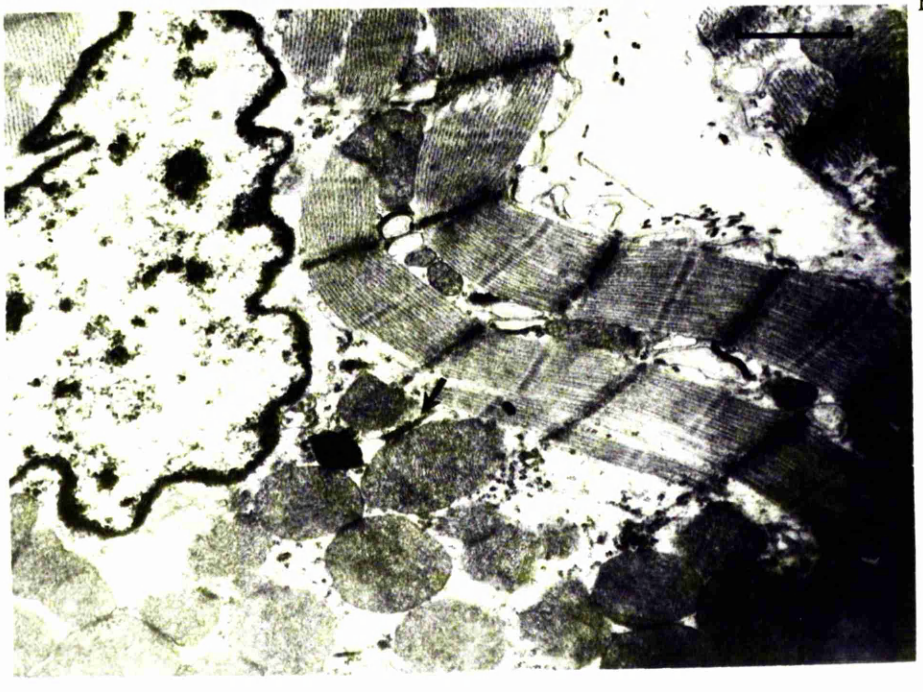
C

C

D

D

E



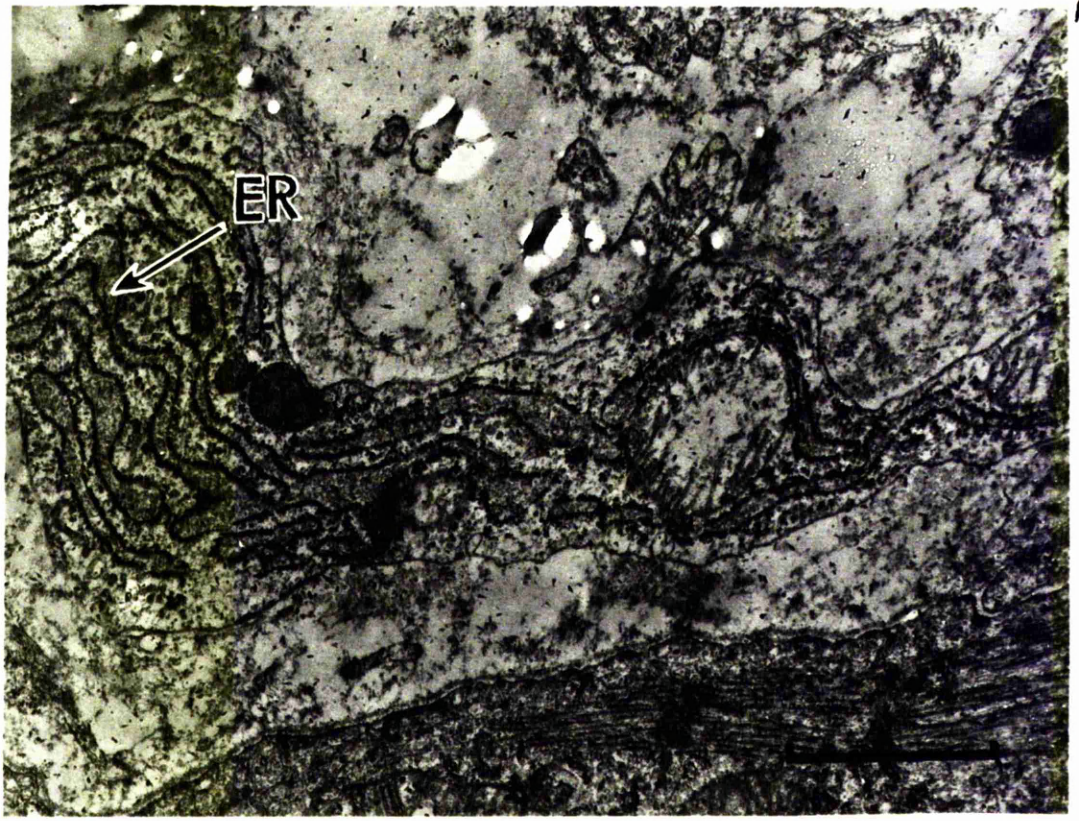
E

Fig. 17a Fibroblast from the left ventricle of rabbit injected with isoprenaline for 5 days. Arrow points to rough endoplasmic reticulum (Mag x 25,000).

Fig. 17b Fibroblast from the left ventricle of rabbit which had undergone coarctation of the aorta for 10 days. Arrow points to rough endoplasmic reticulum (Mag x 25,000).

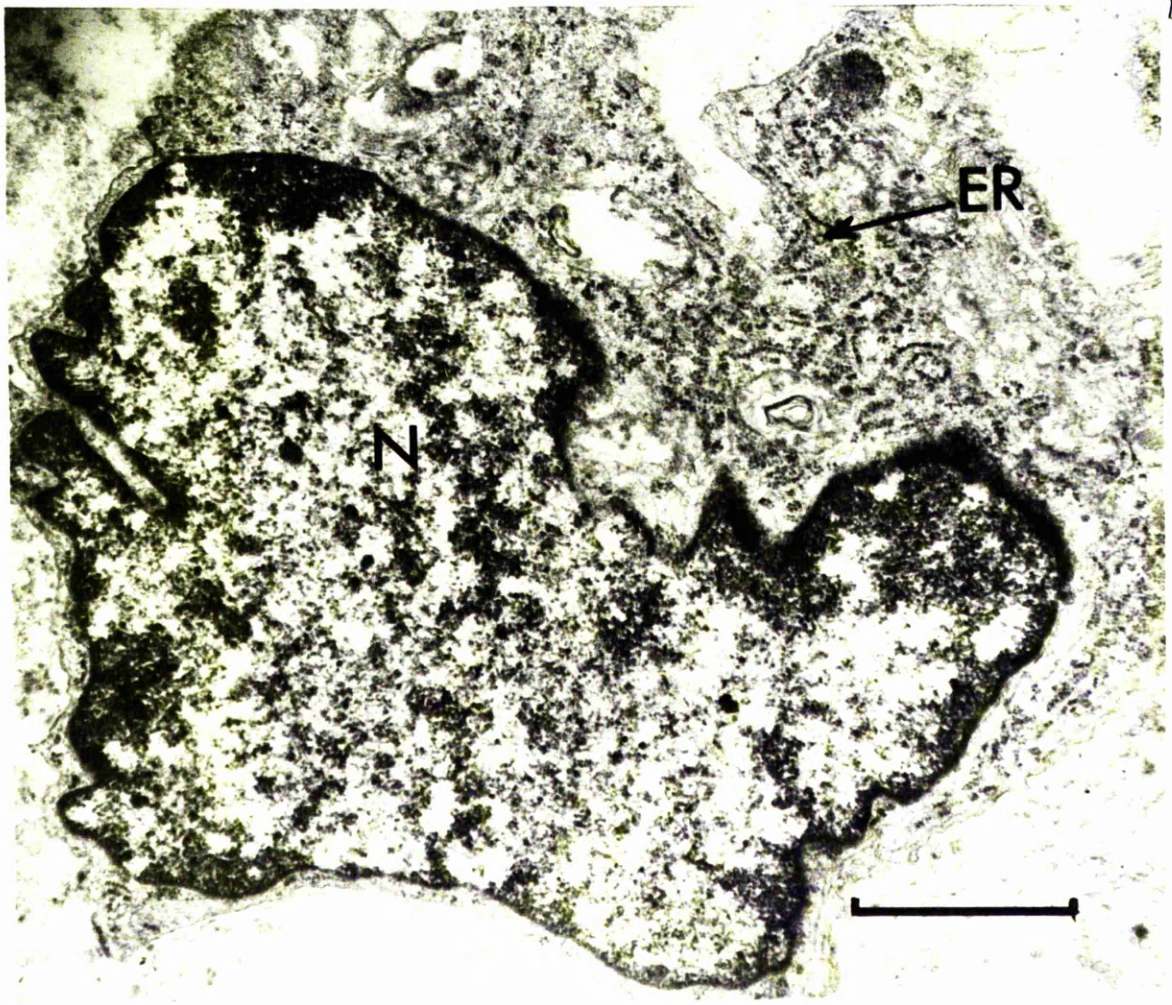
Fig 17

A



A

B



B

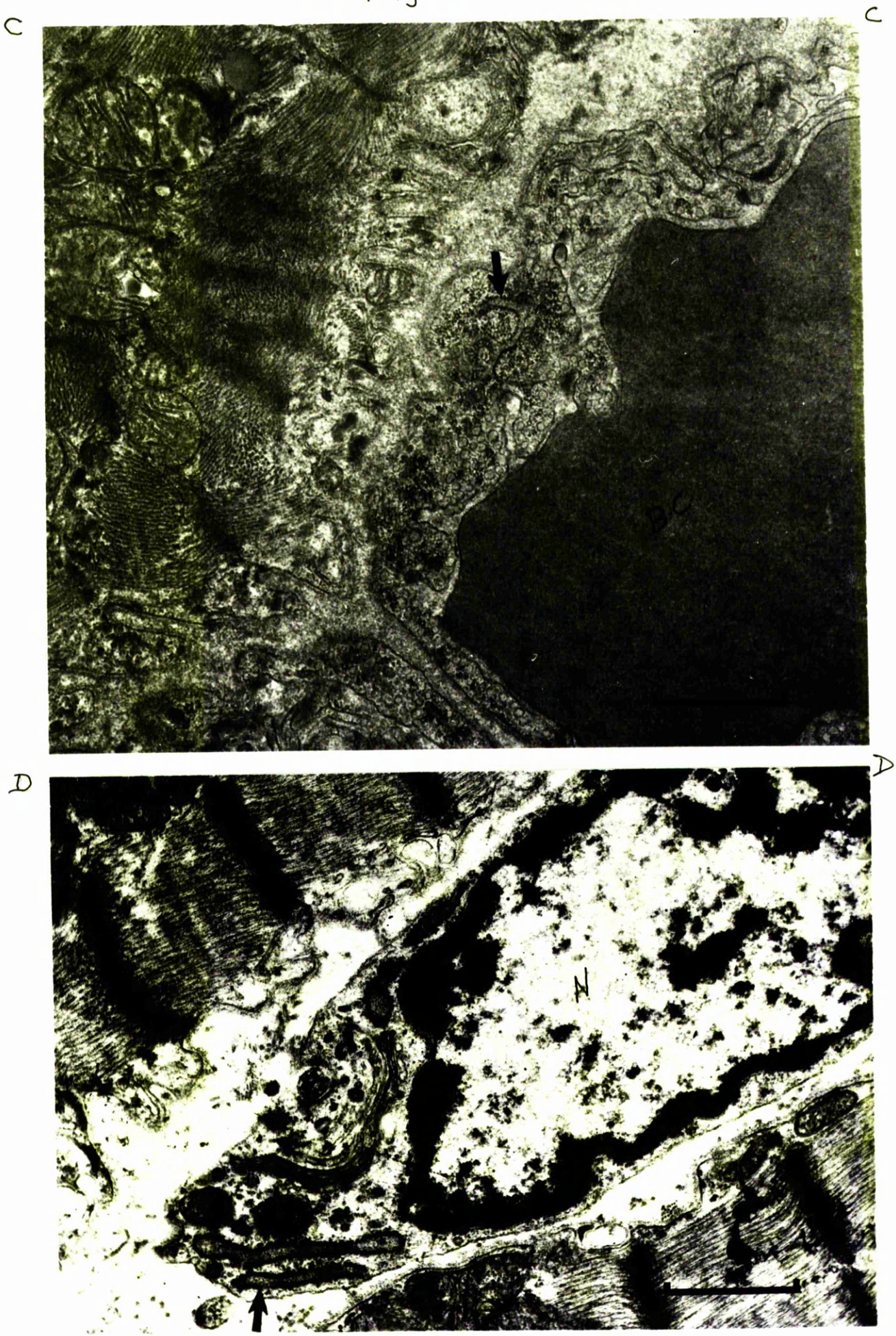
Fig. 17c

Endothelial cell from left ventricle of rabbit injected with isoprenaline for 5 days. (BC) red blood cell. The arrow points to rough endoplasmic reticulum (Mag x 25,000).

Fig. 17d

Fibroblast from left ventricle of rabbit injected with thyroxine for 15 days. (N) nucleus, arrow points to rough endoplasmic reticulum (Mag x 25,000).

Fig 11



contiguous clear space, sometimes indeed completely surrounding them, as though the cristae had been destroyed. After 5 days of thyroxine administration there appeared to be fewer lipid droplets than after coarctation and correspondingly fewer within mitochondria (fig. 18c). Qualitatively, however, the picture was the same as after coarctation. After 15 days of thyroxine treatment the number of lipid droplets had increased the the general picture was idential with that seen after coarctation (fig. 18b). The effects of isoprenaline at 5 and 15 days were again qualitatively similar to those of coarctation but seemed even more marked (fig. 18d - e). The significance of these findings will be discussed later.

Apart from the features common to all three, coarctation, thyroxine and isoprenaline, each produced its own peculiar electron microscope pattern. Thus, coarctation produced after 10 and 21 days a characteristic deformation of the Z line from its normal rectilinear appearance and a corresponding deformation of the intercalated disc (fig. 19a-b). Similar findings have been reported by Bishop and Cole, (1969). Thyroxine treatment at 5 and 15 days produced a marked increase in the numbers of mitochondria (fig. 20a - b). Perhaps, in view of the known action of thyrxoine on metabolism in general this is hardly unexpected. Isoprenaline treatment at 5 and 15 days led to the appearance in some myocardial nuclei of what might be called inclusion bodies (fig. 21a - b). These were never seen in the myocardial nuclei of the control animals or of animals which had undergone coarctation or been treated with thyroxine. The significance of these findings is obscure.

Fig. 18a Myocardial fiber from left ventricle of rabbit which had undergone coarctation of the aorta for 21 days. Arrows point to lipid droplets inside and outside the mitchondria. (Mag x 25,000).

Fig 18

Fig. 18b Myocardial fiber from left ventricle of rabbit injected with thyroxine for 15 days. (F) fat droplets, (N) nucleus (Mag x 25,000).

Fig. 18c Myocardial fiber from left ventricle of rabbit injected with thyroxine for 5 days. (F) fat droplets, (M) mitochondria (Mag x 30,000).

Fig 18

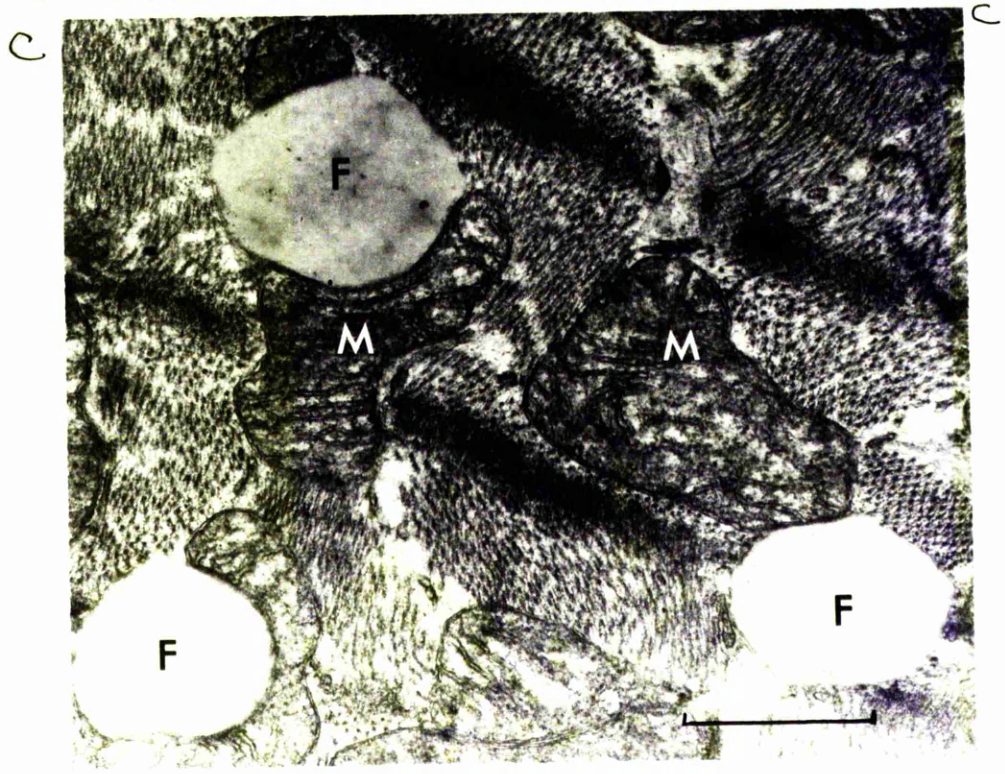


Fig. 18d

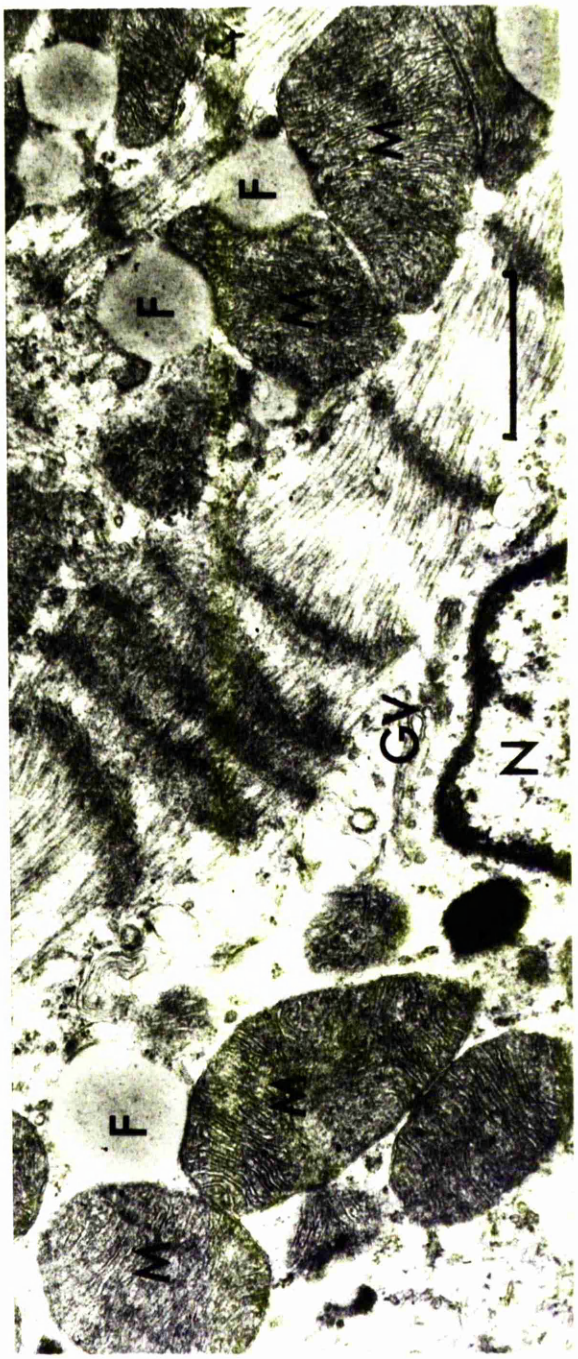
Myocardial fiber from left ventricle of rabbit injected with isoprenaline for 15 days (F) fat droplets, (M) mitochondria (Mag x 25,000).

Fig. 18e

Myocardial fiber from left ventricle of rabbit which had undergone coarctation of the aorta for 10 days. (F) fat droplets, (M) mitochondria, (GV) Golgi vesicles, (N) nucleus (Mag x 30,000).

Fig 18

A

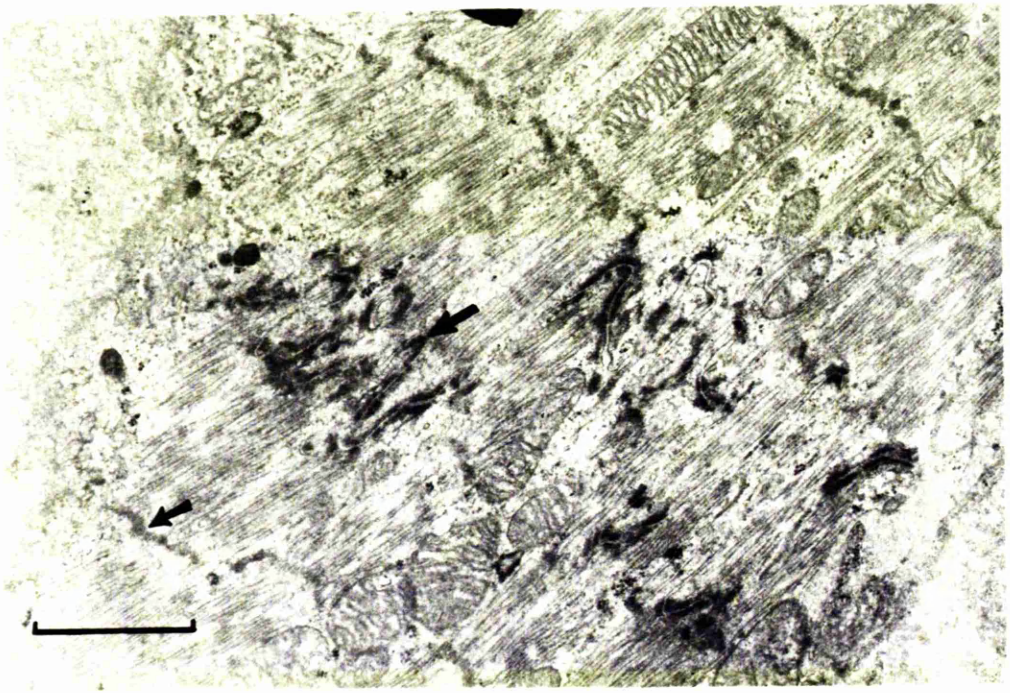


E

Fig. 19a Myocardial fiber from left ventricle of rabbit which had undergone coarctation of the aorta for 21 days. Arrows point to altered Z bands and intercalated disc (Mag x 25,000).

Fig. 19b Myocardial fiber from left ventricle of rabbit which had undergone coarctation of the aorta for 10 days. Arrow points to disorientation of myofibril (Mag x 25,000).

A



B

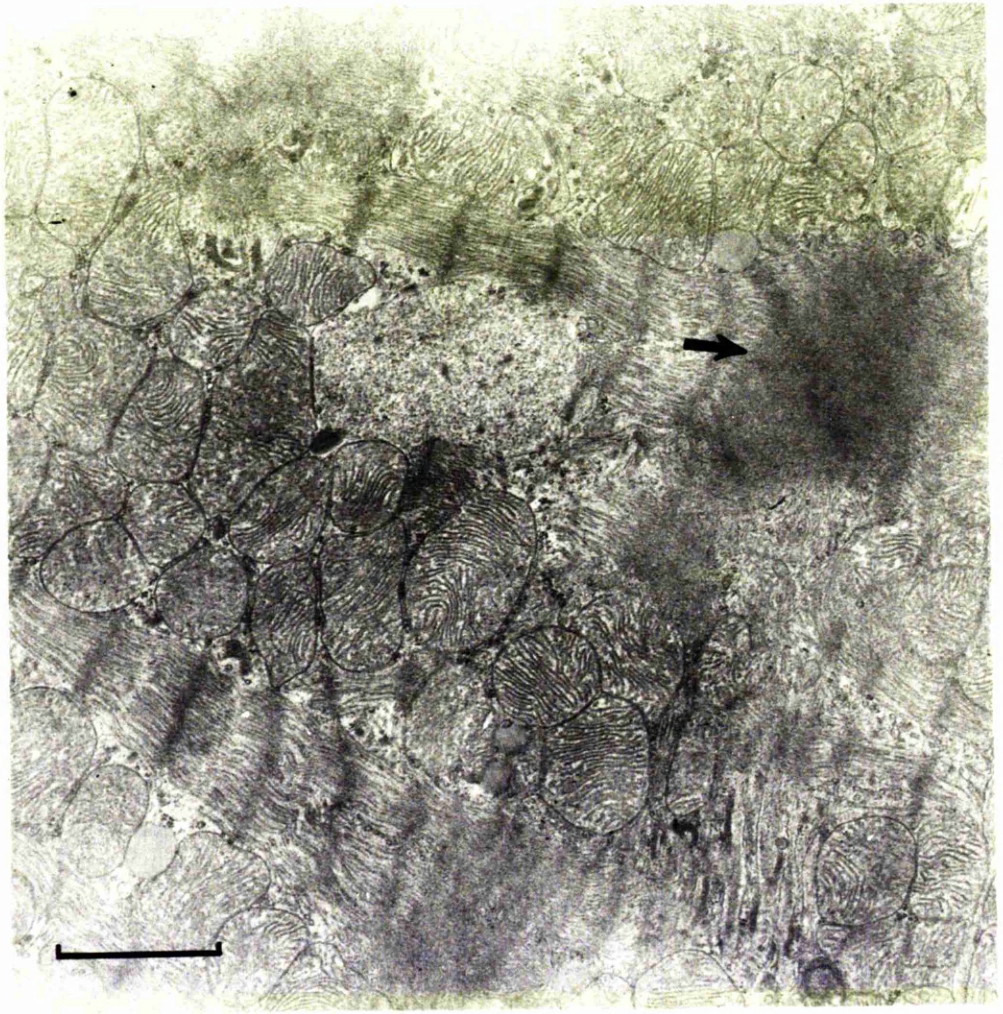


Fig. 20a

Myocardial fiber from left ventricle of rabbit injected with thyroxine for 5 days. (F) fat droplets, (M) mitochondria. Arrows point to pinocytic vacuoles in myocardial and fibroblast (Mag x 20,000).

Fig. 20b

Myocardial fiber from left ventricle of rabbit injected with thyroxine for 15 days. (N) nucleus, (L) lipid, (ME) mitochondria, (DV) dense vesicle (Mag x 15,000).

Fig 20

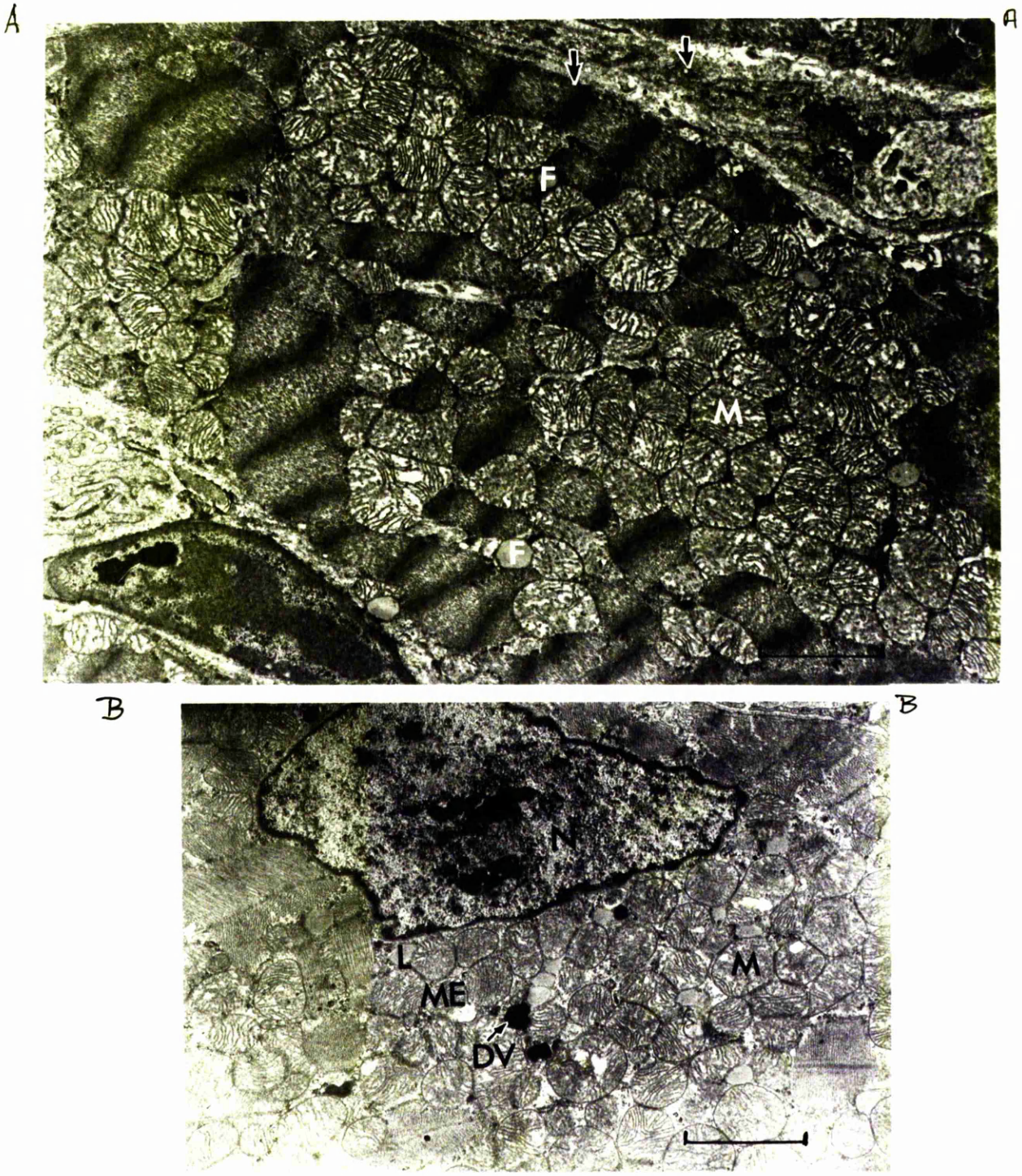
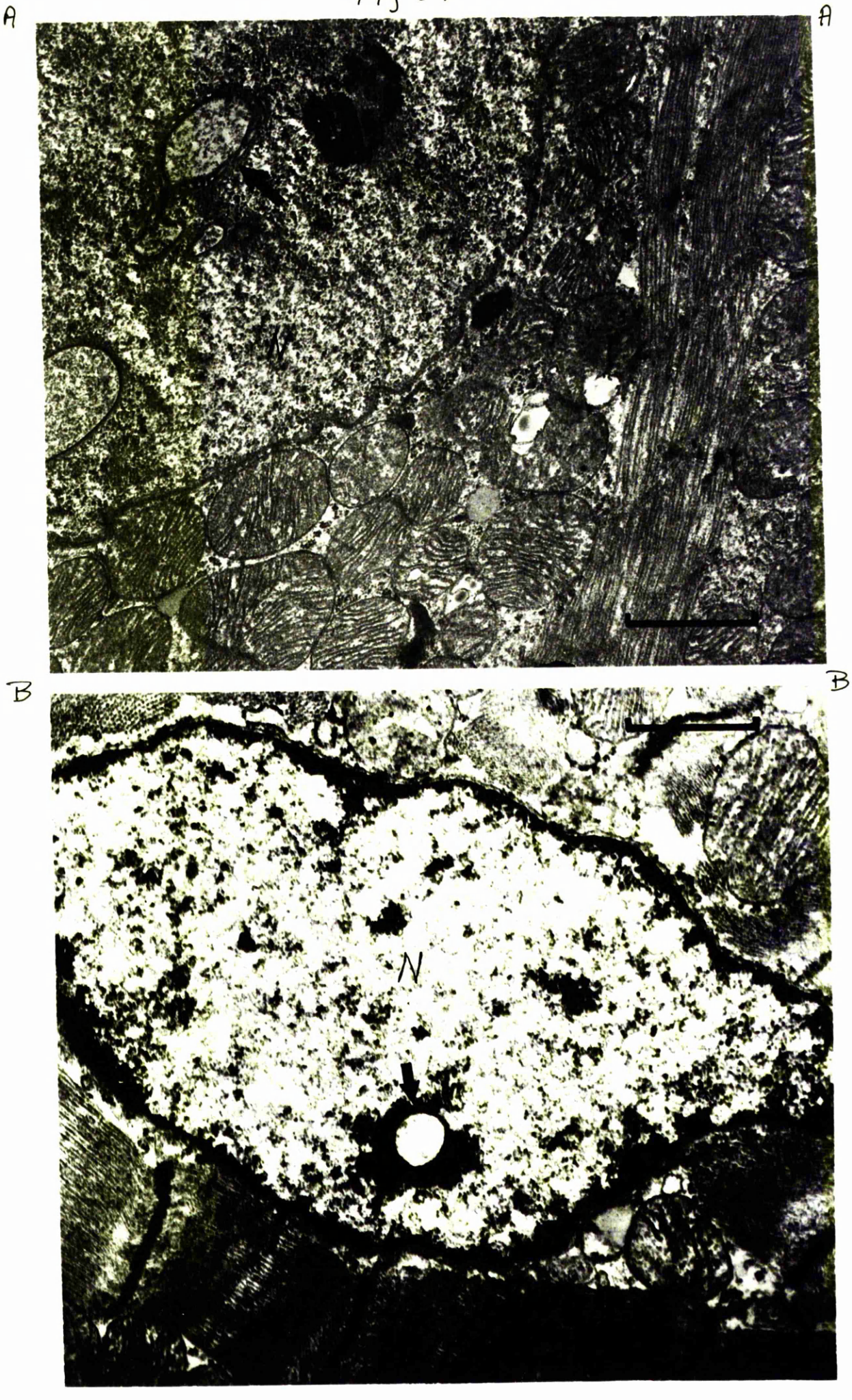


Fig. 21a Myocardial fiber from left ventricle of rabbit injected with isoprenaline for 5 days. (N) nucleus, arrow points to inclusion body inside the nucleus (Mag x 25,000).

Fig. 21b Myocardial fiber from left ventricle of rabbit injected with isoprenaline for 15 days. (N) nucleus, arrow points to inclusion body inside the nucleus.


A
A
N
B
B
N

2.7.0
Lipid Metabolism During Cardiac Hypertrophy

The increase in fat or lipid droplets seen in the electron microscope in hypertrophy produced by all three procedures obviously required confirmation and extension by other methods. Tables 28a - b show the total lipid concentration in the left ventricle. As with all the other measurements it is unaffected by injections with saline and by sham operation. Coarctation produces a marked increase after 10 and 21 days. Thyroxine produces a progressively greater increase as treatment is prolonged for 5 days to 15 days, and isoprenaline produces an immediate increase at 5 days which gradually diminishes. This is analogous to the pattern of response shown in many of the other parameters measured in the present study, but there is an unusual feature. Normally the effects of thyroxine not only developed more slowly than those of coarctation and isoprenaline but at the end of the 15 days experimental period they were generally smaller than the maximum effects seen with either coarctation or isoprenaline. In the present instance, however, thyroxine produces a greater increase than either coarctation or isoprenaline.

Tables 29a - b show the corresponding figures for glyceride glycerol which may be taken to be proportionate to triglyceride. It is clear that these results are in parallel to those for total lipids.

Figures 22a - b - c - d show the results of an experiment which was intended to provide histochemical confirmation/...

TABLE 28a

The total lipid in the left ventricle of sham operated and saline injected californian rabbits at various time intervals.

Saline injected

Days	No. of animals	Mg. lipid / g. wet weight
5	4	20 ±1.6
10	4	20 ±2.4
15	4	20 ±2.0

Sham operated

10	4	20 ±1.9
21	4	21 ±2.4

Mean ± SEM

TABLE 26b

The influence of coarctation, thyroxine and isoprenaline on total lipid in the left ventricle of californian rabbits at various time intervals.

Treatment	Days	No. of animals	Mg. lipid / g. wet weight
Controls	-	20	20 ±2.1
Coarctation	10	4	32 * ±2.6
	21	5	33 * ±2.4
Thyroxine	5	5	28 ±1.9
	10	7	41 ** ±3.4
	15	10	45 ** ±3.6
Isoprenaline	5	5	40 ** ±3.8
	10	8	38 * ±2.6
	15	9	30 * ±2.3

Mean ± SEM *$p < 0.01$, **$p < 0.001$

TABLE 29a

. Glyceride Glycerol in the left ventricle of sham operated and saline injected californian rabbits at various time intervals.

Saline injected

Days	No. Of animals	μ moles Glyceride Glycerol/g. wet weight
5	4	1.66 ±0.034
10	4	1.60 ±0.045
15	4	1.62 ±0.029

Sham operated

10	4	1.60 ±0.033
21	4	1.62 ±0.030

Mean ± SEM

TABLE 29b

T he effects of coarctation, thyroxine and isoprenaline on Glyceride Glycerol in the left ventricle of californian rabbits at various time intervals.

Treatment	Days	No. of animals	μmoles Glyceride Glycerol/g. wet weight
Controls	-	20	1.62 ±0.033
Coarctation	10	4	2.98 * ±0.1310
	21	5	2.89 * ±0.0967
Thyroxine	5	5	2.34 ±0.063
	10	7	4.53 ** ±0.173
	15	10	4.73 ** ±0.166
Isoprenaline	5	5	4.80 ** ±0.213
	10	8	3.86 ** ±0.101
	15	9	3.87 ** ±0.096

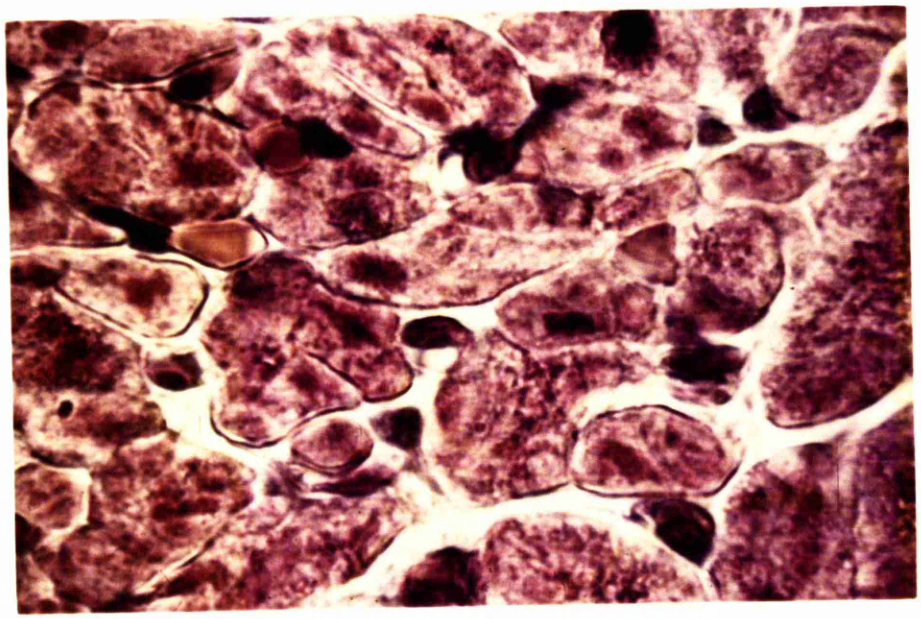
Mean ± SEM *p 0.01, **p 0.001

Fig. 22a-b-c-d — Sections of the left ventricle of californian rabbits stained for lipid droplets with Oil Red "O" which appears as red droplets inside the myocardial cell, counterstained with Hamalum.

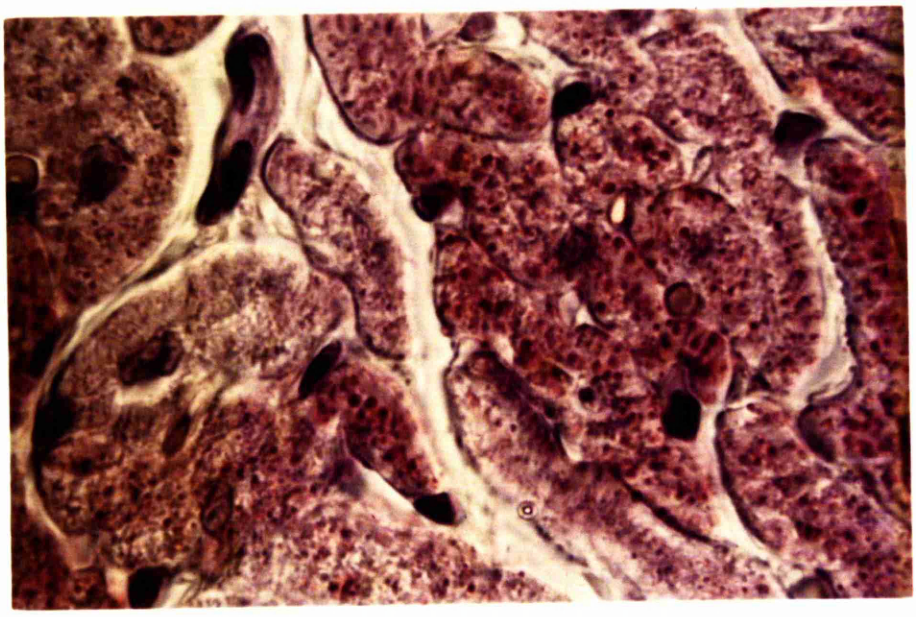
Fig. 22a — Rabbit injected with saline for 15 days. Note the occasional lipid droplet inside the myocardial cell (Mag x 100).

Fig. 22b — Rabbit which had undergone coarctation of the aorta for 21 days. Note the accumulation of lipid droplets (Mag x 400)

Fig 22



A

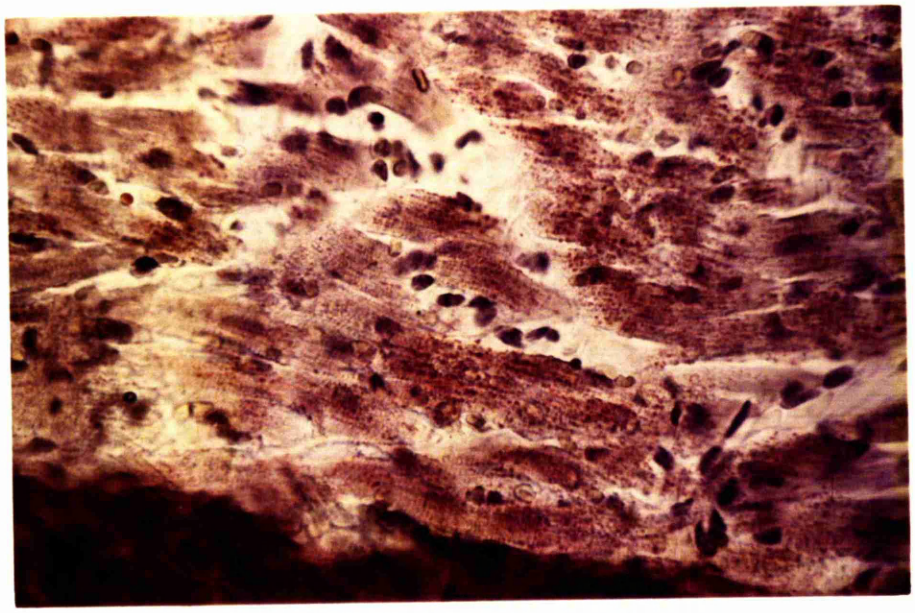


B

Fig. 22c Rabbits injected with thyroxine for 15 days. Note marked increase in lipid droplets inside the myocardial cell. (Mag x 250).

Fig. 22d Rabbits injected with isoprenaline for 5 days. Note marked lipid accumulation inside the myocardial cell (Mag x 400).

Fig 22



C

D

confirmation of the chemical results shown in Tables 28a - b and of the electron microscope findings of lipid droplets in myocardial cells. For this purpose, sections of left ventricle were stained with Oil Red "O" which shows lipid as discrete red droplets. It is quite clear that all three treatments have caused accumulation of lipid in the myocardial cells. This was moderate in the case of coarctation (fig. 22b); progressively becoming more marked with thyroxine (fig. 22c) and initially severe but becoming less with isoprenaline (fig. 22d).

2.7.1 Serum Free Fatty Acids

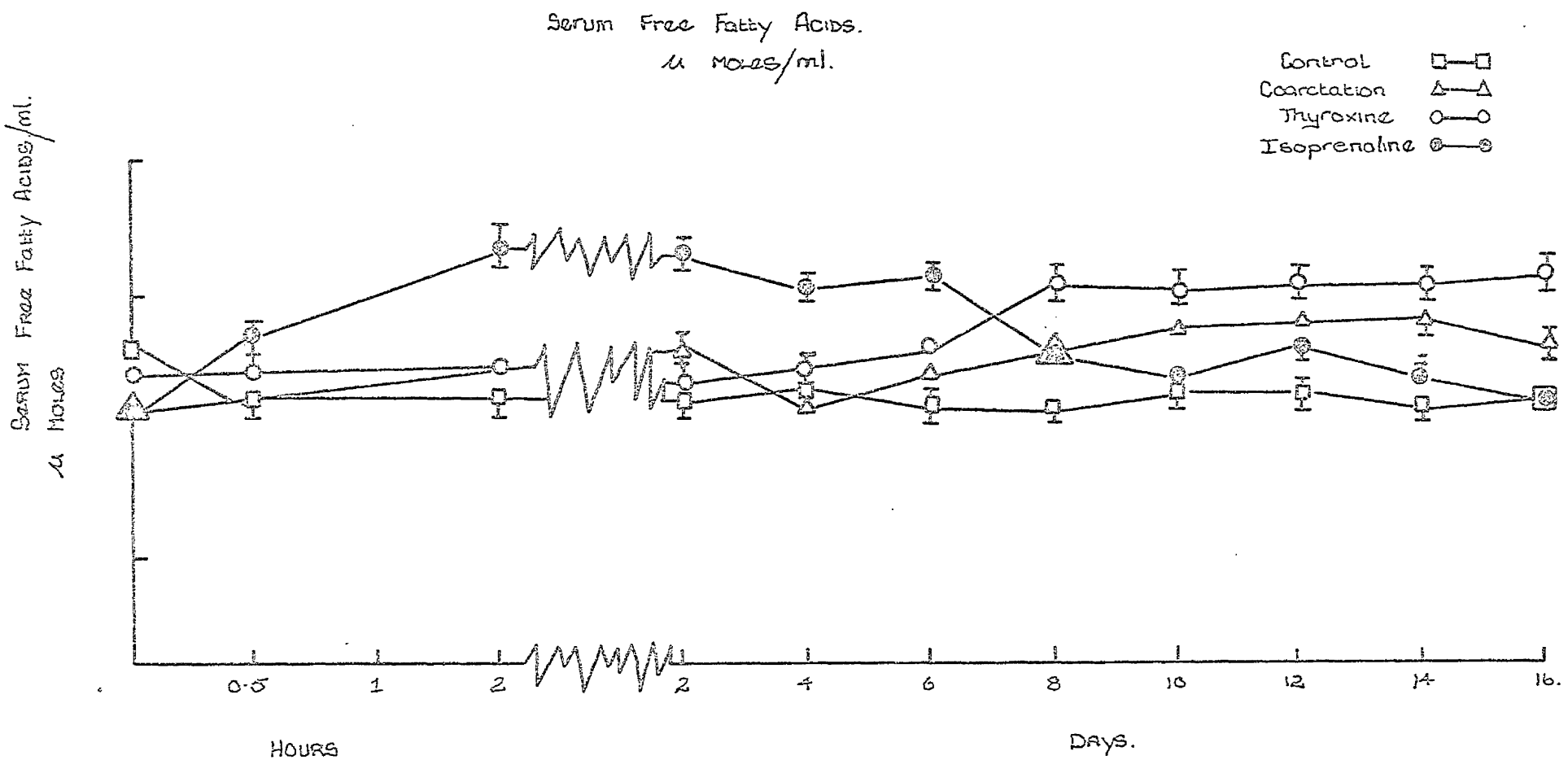
Since lipid in the form of free fatty acids is the major energy source for the heart increase in lipid in the hypertrophic myocardium seemed to warrant an investigation of the levels of free fatty acid in the serum. The results are shown in Fig. 23. Coarctation had no significant effect in either the short term or the long term. Thyroxine had no effect for the first six days but thereafter produced a modest but consistent increase. Isoprenaline produced an increase in a matter of hours. This was sustained up to the sixth day, after which there was a return to the control level.

The fact that thyroxine and isoprenaline both, though in different ways, raise FFA, whereas coarctation does not, may be related to the previous observation (Table 28b) that though all three treatments cause lipid accumulation in the heart, thyroxine and isoprenaline d- so to a greater extent than coarctation. However that may be/...

Fig. 23

Serum free fatty acids at various time intervals following sham operation or saline injections (the controls) or isoprenaline and thyroxine treatment or coarctation of the aorta. Each point represents the mean ± SEM of four californian rabbits.

Fig 23.


Serum Free Fatty Acids.
u Moles/ml.
Control
Coarctation
Thyroxine
Isoprenaline
Serum Free Fatty Acids/ml.
u Moles
0·5
1
2
2
4
6
8
10
12
14
16.
Hours
Days.

may be, the fact that lipid accumulation does occur after coarctation shows that it is not entirely a result of raised FFA levels but is to some extent a feature of the process of hypertrophy itself.

2.8.0
Lactate Acid in the Myocardium

The results obtained up to this point provide a fairly detailed picture of the differences, structural and chemical, between normal and hypertrophic hearts. They throw little light however on the mechanism by which these differences are produced. A starting point in the search for such a mechanism might be the demonstration (fig. 7 - 8 - 9) that liberation of lysosomal enzymes is a feature common to the hypertrophy produced by all three treatments. Such a liberation might result from hypoxia and consequent accumulation of lactic acid with a concomitant fall in pH (De Hann and Field, 1959). This hypothesis is open to simple experimental test. Table 20a - b shows the level of lactate in the right and left ventricles.

All three treatments appear to have characteristic effects; coarctation approximately doubled the lactate concentration in the left ventricle within 5 days of the operation and this increase is sustained at 10 and 21 days There is little or no corresponding increase in the right ventricle, which probably reflects the fact that coarctation, as we have seen (Table 15b), causes hypertrophy primarily of the left ventricle.

The effects of thyroxine are quite different.

From/...

From day 3 to day 15 there is a slow parallel increase in the lactate concentration in both ventricles.

Finally isoprenaline, as usual, produces the most dramatic effects. After one day there was significant increases in both ventricles though the increase in the left is much greater (200%) that that of the right (50%). Thereafter the levels in both ventricles gradually returned towards the normal level.

TABLE 30a

The lactate concentration in the left and right ventricle of sham operated and saline injected californian rabbits.

Saline injected

Days	No. of animals	mg. of lactic acid / 100g. wet weight Left ventricle	Right ventricle
1	3	12.1	8.6
		±1.3	±0.85
3	3	9.3	10.3
		±0.95	±1.4
5	4	10.6	9.2
		±1.0	±0.99
10	4	14	7.8
		±1.6	±0.91
15	5	11.1	10.0
		±1.3	±0.87

Sham operated

5	2	10.9	11.3
		±1.4	±1.9
10	3	11.4	10.6
		±1.2	±1.6
21	6	11.8	9.8
		±1.0	±0.96

Mean ± SE.

TABLE 30 b

The effects of coarctation, thyroxine and isoprenaline on lactate concentration in the left and right ventricle of californian rabbits at various time intervals.

Treatment	Days	No. of animals	mg. of lactic acid / 100g. wet weight	
			Left ventricle	Right ventricle
Controls	-	30	11.4 ±1.2	9.7 ±1.9
Coarctation	5	2	23.0 ** ±2.3	9.0 ±0.88
	10	3	20.8 ** ±1.8	7.0 ±0.91
	21	5	20.6 ** ±2.6	13.0 ±0.99
Thyroxine	1	3	8.3 ±1.1	7.6 ±1.3
	3	5	8.0 ±0.98	6.3 ±1.6
	5	3	9.1 ±1.3	9.4 ±1.9
	10	3	10.4 ±1.8	13.3 ±0.94
	15	5	14.3 ±0.96	13.0 ±1.6
Isopreanline	1	4	38.6 *** ±3.1	17.4 ** ±3.2
	3	3	29.4 ** ±4.1	18.3 ** ±2.6
	5	4	26.3 ** ±2.2	13.6 ±3.1
	10	5	14.4 ±3.1	12.3 ±2.4
	15	5	16.0 ±2.8	12.8 ±3.6

Mean ± SEM, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

CHAPTER III SUMMARY AND CONCLUSIONS.

SUMMARY AND CONCLUSION

1. The present investigation started from observations reported in the literature that in hypertrophy the myofibrils increase in diameter without a concomitant increase in blood supply as indicated by the number of capillaries and the diameter of coronary artery i.e. hypertrophy and impaired perfusion are usually concomitant.

2. Comparison of hearts which were markedly hypertrophic, but essentially well perfused, with hearts which were poorly perfused, but only moderately enlarged, made it apparent that the changes in LDH which have been thought characteristic of hypertrophy are really characteristic of poor perfusion.

3. Such poorly perfused hearts were also characterised by a high content of fibrous tissue which could be demonstrated both histologically and chemically. The LDH pattern of fibroblast in pure culture suggested that the altered LDH pattern in poorly perfused hearts may merely reflect their high content of fibrous tissue.

4. A survey of several metabolic enzymes in hypertrophy and poorly perfused hearts showed that in the latter there was a notable decrease in two mitochondria enzymes (malate dehydrogenase and NADP-dependent isocitrate dehydrogenase) which seemed too large to be explained merely by the change of the relative proportions of muscle cells and fibroblasts. This, if true suggested that poor perfusion with possible/...

possible hypoxia might have brought about degeneration of the mitochondria, but it was dangerous to put too much confidence in enzyme patterns from autopsy material due to autolysis between death and post mortem dissection.

5. Hence, there was the need for experiments on animals.

6. The first and in some ways the most striking result obtained from these experiments was that, although in man cardiac hypertrophy develops slowly over months and perhaps years, in animals it can, by a variety of agents, be produced in a matter of weeks and even days. However the degree of hypertrophy that can be reached in man is never achieved in animals.

7. This experimental hypertrophy was characterised by the same change in LDH isoenzyme patterns with the same changes in fibrous tissue as the cardiac hypertrophy encountered in human autopsy material. When marked changes in LDH isoenzymes occurred they were accompanied by marked changes in amount of fibrous tissue (i.e. isoprenaline treatment). When moderate changes in LDH isoenzymes occurred, they too were accompanied by moderate changes in fibrous tissue (i.e. coarctation of the aorta). Or, when small changes in LDH isoenzymes occurred they were also accompanied by small changes in fibrous tissue, (i.e. thyroxine treatment). These results clearly establish that changes/...

changes in the LDH isoenzyme pattern are closely related to the changes in fibrous tissue and are not associated with the new material which is added to the myocardial cell as a result of cardiac hypertrophy.

8. The fibrosis found in the hypertrophic hearts at autopsy or in experimental animals can most readily be explained on the assumption that it represents the replacement of damaged or destroyed myocardial cells. In an attempt to confirm or refute this supposition, CPK and LDH were determined in the blood serum of experimental animals in which hypertrophy was being artificially induced. The levels of both enzymes showed modest, but unmistakable increases at precisely the time when destruction of muscle cells would have been predicted. Isoprenaline treatment led to an early increase in both of these enzymes which, during the latter stages of the experiment, return to the control. Thyroxine treatment led to progressive increases in both of these enzymes during the latter stages of the experiment. Coarctation led to intermittent changes in both enzymes. The changes in fibrous tissue more or less proceeded or occurred during these changes in serum enzymes.

9. If necrosis did indeed accompany or precede hypertrophy, it would be reasonable to suppose that lysosomal enzymes would play some role (though not necessarily a primary role) in the process of cellular necrosis. Estimation /...

Estimation of free lysosomal enzymes in the hearts of experimental animals did indeed show their liberation. The increase in free lysosomal enzymes following a single injection of isoprenaline was paralleled by an increase in serum CPK and LDH. This sort of parallelism remained throughout the experimental period. Thyroxine treatment led to progressive change in free lysosomal enzymes and a progressive change in both serum enzymes. Coarctation of the aorta led to intermittent changes in free lysosomal enzymes and intermittent changes in both serum enzymes. This remarkable parallelism clearly establishes the association between myocardiac necrosis and lysosomal enzymes liberation. This clear picture however, was complicated by the fact that the bound forms of these enzymes also increased (with the exception of isoprenaline treatment where a single injection resulted in an increase in the free form and concomitant fall in the bound form). The increase in the bound form of lysosomal enzymes could most easily be explained on the assumption that the concentration of free and bound lysosomal enzymes in the heart as a whole were being altered as fibroblasts and macrophages displaced the necrotic muscle cells. Since these cells are rich in lysosomal enzymes they would obscure the true change in the ratio of bound to free lysosomal enzymes. Histochemical evidence did indeed/...

indeed show that the lysosomal enzyme N-Acetyl-B-D-Glucosaminidase was localized only in fibroblasts or cells of similar appearance. There also appeared to be more enzymes in these cells as compared to the control. However, histochemical evidence also showed that the enzyme acid phosphatase was increased in the myocardial cell. The increase in acid phosphatase suggested de novo synthesis which may be an alternate explanation of why the bound form of lysosomal enzymes increases.

10. This question of whether hypertrophy was accompanied by a change in lysosomal activity in the myocardial cell proper was tackled by electron microscopy. The results of examining the hearts of experimental animals in the electron microscope showed, among other changes, abundant evidence that dense veiscles, which appeared to be tertiary lysosomes, were much more numerous in hypertrophic hearts than in normal hearts. Autophagosomes (secondary lysosomes), which were never seen in normal hearts, were frequently observed in the hypertrophic hearts. This seems strong circumstantial evidence for lysosomal activity in the myocardium during cardiac hypertrophy. Thus, the increases in the bound lysosomal enzymes may represent two distinct processes; one which is related to change in the cell types; the other related to de novo synthesis of lysosomal enzymes.

11/...

11. This evidence of cell destruction in the hypertrophic hearts of experimental animals was accompanied by an increase in rough sarcoplasmic reticulum in the myocardial cells. These results would tend to suggest increased protein synthesis in response to hypertrophy or cellular damage. Also observed were increases in rough sarcoplasmic reticulum in fibroblasts, which may be related to collagen synthesis in response to myocardial necrosis.

12. The least expected of the electron microscope findings was the abundance of lipid droplets in the hypertrophic myocardial cells. These lipid droplets were quite often found within mitochondria and in such cases there was often a contiguous empty zone as though the cristae had been destroyed. It is hardly fanciful to see a connection here with the observations, noted above, that in hearts obtained at autopsy the activities of two characteristic mitochondrial enzymes (malate dehydrogenase and NADP dependent isocitrate dehydrogenase), were consideraly depressed.

13. This association of lipid droplets with hypertrophy was confirmed histochemically and by chemical analysis. The increase in lipids might very well be explained by the parallel increase in serum free fatty acids. However, the accumulation of lipids may also be related to decrease in the oxidation/...

oxidation fatty acids as a result of hypoxia or a decrease in carnitine. A decrease in carnitine has been associated with cardiac hypertrophy (Wittle and Spann, 1968).

14. It should be emphasized that all the changes described above as associated with experimental cardiac hypertrophy form a common pattern quite regardless of the agency by which the hypertrophy was induced. This suggests that all the methods which were used to produce hypertrophy bring about some common effect which triggers all the other changes. A possible candidate for this common effect is lactic acid, the concentration of which increases quite sharply in the hypertrophic myocardium despite the fact that it diffuses so readily in and out of the cells. A local increase in lactic acid would presumably produce a local fall in pH. This would be expected to result in the release of lysosomal enzymes, and they in turn might evoke the whole pattern of changes described above. Such an effect was observed following a single injection of isoprenaline. When the levels of lactic acid increase there was a concomitant increase in the free lysosomal enzyme and a decrease in the bound lysosomal enzymes. These changes were accompanied by an increase in both CPK and LDH. These results would appear to substantiate the above hypothesis.

15. The results described above, give a detailed and/...

and consistant description of the changes, chemical, histological and cytological, which take place in experimental cardiac hypertrophy. They also offer some reason for thinking that such experimental hypertrophy, in spite of its rapid development, resembles the slowly developing hypertrophy seen in human patients. But they throw little light on the sequence of cause and effect which leads from hyperfunction to hypertrophy and ultimately to failure. It is, however, quite clear that the hypertrophic heart is neither chemically nor morphologically merely a large version of the normal heart. Corvisart's celebrated comparison with the blacksmith's forearm is fundamentally false. Since the fine structure of the hypertrophic heart shows evidence of disorganization long before failure is clinically apparent. The dense vesicles and the autophagosomes which appear so early in the hypertrophic process can surely be related to the failure in which that process ultimately ends. Therefore, the processes of hypertrophy and degeneration must have a common origin.

16. It is this which distinguishes cardiac hypertrophy from most examples of what has been called "adaptive" growth. If part of the liver is removed the remainder grows until it is as large as the original intact organ (Bucher and Malt, 1971). Histologically and functionally this hypertrophic fragment is indistinguishable from normal liver, and it will sustain the animal perfectly adequately/...

adequately throughout its normal life span. Again it is well known that if one kidney is surgically removed its partner slowly doubles in size. This enlarged single kidney seems to be an entirely adequate substitute for the original pair of kidneys.

Both these phenomena are presumably examples of an organ responding to a disproportionate functional load by increasing in size. The muscles of a blacksmith or an athlete are a more familiar example of the same phenomena. Even the heart can respond to exercise or hypoxia in the same way. Why cannot it respond equally well to the overload imposed, say, by hypertension? Only a very tentative answer can be offered. One of the most striking and surprising findings of the present study was the high lactate concentrations in the hypertrophic hearts. This may indicate a degree of local hypoxia: such hypoxia would not be unexpected given that heart muscle under normal circumstances extracts a very high proportion of the oxygen from the blood circulating through it. The continued unremitting exertion required to maintain a normal twenty-four hours a day cardiac output, against the increased aortic pressure of hypertension might be too much for the oxygen supply available to the myocardium. If one accepts that hypoxia could be produced in this way, some at least of the changes found in the present study would find an explanation: not only the/...

the high lactate concentrations and the liberation of lysosomal enzymes; but perhaps also the accumulation of lipid, which the hypoxic mitochondria, which may be damaged, cannot utilise.

17. But all this is highly speculative. Whatever else may be said of cardiac hypertrophy, it is unquestionably a highly complex process. It seems unlikely that we shall establish its underlying mechanism until we know much more than at present about the way in which it manifests itself. The present study is, I hope, a small contribution to that end.

APPENDIX - METHODS AND MATERIALS.

METHODS

1) General

1.1 Animals: Male californian rabbits from the departmental colony were maintained on the diet of Short and Gammage (1959). The animals were housed in a temperature controlled unit, given water and food ad-libitum and used when these were three months' old and weighed 2,500 to 2,800 g.

1.2 Injection procedure:

1.21 Isoprenaline: Isoprenaline sulphate was dissolved in 0.9% saline and injected subcutaneously at an initial dose of 1 mg/kg body weight, on the first day. On each subsequent day the dose was increased 1 mg/kg, so that on the 15th day the dose was 15 mg/kg body weight. Control rabbits were injected with 0.9% saline for 15 days.

1.22 Thyroxine: Sodium L-thyroxine was dissolved in 0.01N NaOH, brought to pH 10 with 0.1N HCl and daily subcutaneous injections of 88 ug/kg body weight were administered for 15 days. Control rabbits were injected daily with 0.9% saline adjusted to pH 10.

All injections were performed at 11.00 a.m.

1.3 Surgical procedure:

1.31 Coarctation of the abdominal aorta: Animals were anaesthetized with nembutal and a silver clamp (1 mm. in width) was placed around the descending aorta just beneath the diaphragm, according to the/...

the original method of Beznak (1958), as modified by Kogan (1961). This procedure created a diminution of the transverse section of the aorta to about three fourths of its original area. Sham operated animals were treated identically except that aortic constriction was omitted.

1.4 Autopsy material: Human cadavers at death were placed in a refrigerator at 10°C until the attending pathologist performed the post mortem. Autopsies were generally performed within 7 to 24 hours of death. The dissected hearts were rinsed with 0.25 M. sucrose, blotted and weighed. After weighing, the thickness of the left and right ventricular walls was recorded and the left ventricular free wall was removed and placed into ice cold 0.25 M. sucrose.

The subjects selected for this study were chosen as follows: Group I (the control) consisted of patients without cardiac enlargement or coronary artery disease. Group II, consisted of patients who had suffered moderate cardiac hypertrophy and marked coronary disease as indicated by coronary artery narrowing with recent or old infarction. Group III, consisted of patients with marked cardiac enlargement with or without moderate coronary artery disease. The selection of the various groups was made by the attending pathologist from the Western Infirmary of Glasgow.

1.5 Weight measurements: Rabbits were killed by cervical/...

cervical dislocation and their hearts were dissected out and flushed with 0.25 M. sucrose then blotted and weighed.

The left ventricle of the excised hearts was dissected according to the procedure of Fulton et al, (1952), then blotted and weighed.

1.6 Wet and dry weight: The dissected left ventricle was cut into small pieces, blotted and weighed and then placed on a clean weighed watch glass. The tissue was then dried in a hot air oven at 100°C for 24 hours. The watch glass with the dried tissue was then reweighed. The weight of the dried tissue minus the weight of the watch glass was taken as the dry weight. While the difference in the original weight was taken as the wet weight.

2) Chemical Methods

2.1 Lactate dehydrogenase isoenzymes:

A) A 1 g sample of either the left or right ventricle was cut into thin slices and homogenised in 20 ml. ice-cold 0.25 M sucrose using an "Ultra Turrax" (Janke and Kunkel KG, Stauffen i.BR., Germany) homogeniser (setting of 90). The homogenate was centrifuged at 20,000 xg for 20 minutes. The supernatant was decanted off, diluted 1:50 and 10 ul aliquots were used to determine the LDH isoenzymes.

The same procedure was used for rabbits and human autopsy material, except for the left ventricular free wall from autopsy material where 4 g of tissue was/...

was homogenised in 80 ml of 0.25 M sucrose.

Method:

LDH isoenzymes were separated by disc gel electrophoresis on a 5.5% acrylamide/bis acrylamide gels as described by Dietz and Lubrano (1967). Gels were run in a Canalco chamber at $0^{o}C$. A constant current (2.5 m.a. per tube) was maintained with a Beckman Duostat power supply. Isoenzyme separation was usually complete in 40 minutes. The separated isoenzymes were stained with nitro-blue tetrazolium according to Van der Helm (1961). The isoenzyme content was quantitated using a Joyce-Lobel microdensitometer fitted with a photovolt gel scanner and an integrator to measure the area under each peak.

Helu cells:

(Human embroyoic lung cells or fibroblast) of the fourth passage were kindly supplied by the tissue culture unit of the department of Biochemistry, Glasgow.

Approximately 10^{8} cells were suspended in ice-cold 0.25 M sucrose and centrifuged at 300 x g for 20 minutes and the supernatants were decanted. This process was repeated three times. After the third wash the cells were suspended in ice-cold 0.25 M sucrose and homogenized using a Dounce (B) homogeniser by ten up and down strokes. The homogenate was centrifuged at 20,000 x g for 20 minutes. The supernatant was used to measure the LDH isoenzymes as described above.

2.2/...

2.2 Tissue lactic acid determination: Samples of the left and right ventricles were quickly removed, weighed and frozen in liquid nitrogen. The liquid nitrogen was allowed to evaporate and 5 ml. of ice-cold perchloric acid (6% w/v) was added to the frozen tissue. The tissue was homogenised using an "Ultra Turrax" homogeniser (setting of 90) for 4 minutes at $0^{o}C$. The homogenate was centrifuged at 600 x g for 15 minutes. The residue was re-extracted with 3 ml. of ice-cold perchloric acid and again centrifuged as described above. The supernatants were combined and neutralized with 5 M potassium carbonate, (approximately 0.2 ml) using 0.02 ml. of methyl orange as indicator. This solution was allowed to stand in an ice-bath for 10 minutes, then centrifuged at 600 x g for 10 minutes. The resulting supernatant was used to determine lactic acid by the method of Hohorst (1959).

2.3 Hydroxyproline estimation: Dried tissue (0.1 to 0.5 g) from the left ventricle was hydrolysed with 3 ml. 6N HCl for 24 hours in sealed test tubes at $105^{o}C$. After hydrolysis, humin was removed by filtration. The resulting suspension was dried using a rotary evaporator and diluted to a known volume. Aliquots of 2 ml. (1-5 ug hydroxyproline) were taken for the determination of hydroxyproline by the modified Stegeman (1958) method described by Woessner (1961). Hydroxyproline was also measured using the Beckman amino acid auto analyzer according to the method/...

method of Benson et al, (1965) and Spackman et al, (1958).

2.4 Hexosamine estimation: Dried tissue (0.1 to 0.2 g) from the left ventricle was hydrolysed in 5 ml. 2N HCl and heated at 95°C for 5 minutes in screw capped cultured tubes. The tubes were then flushed with nitrogen, sealed with teflon-lined caps and heating at 95°C was continued for a further 3 hours. Following this the tissue was broken-up with a stirring rod and the tubes were again flushed with nitrogen, re-sealed and heated at 95°C for a further 15 hours. After hydrolysis, humin was removed by filtration and the hydrolysate was diluted to a known volume.

Aliquots of 2 ml. (equivalent to 5-50 ug of amino sugar) were taken for the determination of total hexosamine by the method of Cessi et al (1960).

2.5 Protein estimation: Protein was estimated according to the method of Lowery et al (1951) except that sodium citrate was used instead of sodium potassium tartrate as it gives a more stable reagent (Eggstein 1955).

Standard curves were prepared using 10-100 ug of Bovine serum albumin (Fraction IV) in the appropriate homogenising medium.

2.6 Lipid extraction: Lipid were extracted using the method of Folch, Lees and Stanley (1956).

Approximately 1 g portion of the left ventricle was/...

was cut into small slices and placed in a volumetric flask containing 20 ml. of chloroform-methanol (2:1 v/v). After homogenising for 8 minutes using an "Ultra Turrax" homogeniser (setting of 120) the homogenate was filtered through Whatman No. 1 paper into a glass stoppered test tube. The residue was re-homogenised in 10 ml of chloroform-methanol mixture and again filtered. The combined extracts were shaken with 6 ml 0.9% saline and the two phases separated by centrifugation at 400 x g for 30 minutes.

The upper phase was aspirated off and the lower phase rinsed three times with 4.5 ml of a chloroform-methanol-water mixture (3:48:47 v/v). After each rinse the two phases were allowed to separate and the upper phase was removed by aspiration.

The lower phase was finally evaporated to dryness at 37°C under a steam of Nitrogen.

After drying the sample was taken up in 7 ml of pure chloroform filtered and brought to a final volume of 10 ml.

Total lipids was measured by pipeing 2 ml aliquots of the chloroform extract in weighed tubes, evaporating the chloroform by heating at 37°C for 4 hours and determining the weight of the residue.

2.7 Glycerol estimation: A 2 ml sample of chloroform extract was evaporated to dryness at 37°C with a steam of nitrogen. The residue was saponified in centrifuge tubes for 30 minutes in a 70% water bath, using/...

using 0.5 ml of 0.5N alcoholic potassium hydroxide. After saponification 1 ml of 0.15 M magnesium sulphate was added and the content of the tubes were mixed and centrifuged. Glycerol was then determined using 0.5ml aliquots of the clear supernatant.

Boerhinger kits were used to measure glycerol, according to the method of Eggstein et al (1966).

Myocardial enzymes

Method of extraction: A 1.5 g sample of the left ventricular free wall from human hearts obtained at autopsy was rinsed with ice-cole 0.25 M sucrose and blotted. It was then cut into small slices and homogenised using an "Ultra Turrax" homogeniser, (setting of 85), for 5 minutes in 10 ml of ice-cold 0.25 M sucrose buffered with 0.005 M triethanolamine pH 7.5. The homogenate was centrifuged at 14,000 x g for 30 minutes at $0^{o}C$. The supernatant was decanted and the residue re-extracted with 5 ml of the same buffered medium. The two supernatants were combined and used for measuring enzyme activity.

3.2 Estimation of enzyme activity

3.21 Glutamate - oxaloacetate transaminase

E.C. 2.6.1.1: Glutamate-oxaloacetate transaminase (GOT) catalyses the reaction:

L-Glutamate + oxaloacetate $\rightleftharpoons$ L-aspartate + α-oxoglutarate.

The activity of the transaminase was measured by the increase of oxaloacetate with time. The

oxaloacetate/...

oxaloacetate was determined with the indicator reaction catalysed by malate dehydrogenase (MDH).

$$\text{Oxaloacetate} + \text{NADH} + H^+ \rightleftharpoons \text{malate} + NAD^+$$

The oxidation of NADH, which is proportional to the amount of oxaloacetate formed, was measured by decrease in the optical density at 340nm.

In addition to enzyme the reaction mixture contained:

1) 0.1M phosphate buffer pH 7.4
   0.04M L-Aspartate
2) 0.012 M NADH
3) 0.25 mg each of MDH and LDH/ml.
4) 0.25 M oxoglutarate

The description for measuring GOT is essentially that of Karmen (1955).

3.22 Creatine phosphokinase: E.C. 2.7.3.2: The basic forward reaction catalysed by creatine phosphokinase (CPK) was coupled to a dehydrogenase reaction as can be seen from the following. This method is essentially that of Tanzer Gilvarg (1959).

$$\text{Creatine phosphate} + \text{ADP} \xrightleftharpoons{\text{CPK}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \xrightleftharpoons{\text{hexokinase}} \text{Glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + \xrightleftharpoons{\text{G-6-PDH}} \text{6-phosphogluconase} + \text{NADPH} + H^+$$

An increase in optical density at 340 nm per unit or time was a measure of CPK activity Glutathione was used as an activator in this reaction.

In addition to enzyme the reaction mixture contained/...

contained:

1) 0.11 m triethanolamine buffer pH 7.0; 22 mM glucose; 11 mM Mg-acetate; 1.1 mM ADP; 11 mM AMP; 0.65 mM NADP.

2) 1 mg Hexokinase/ml; 1 mg Glucose-6-phosphate dehydrogenase/ml.

3) 0.25 M glutathione.

3.23 Malate dehydrogenase: E.C.1.1.1.37: Malate dehydrogenase (MDH) catalyse the reaction:

L-Malate + $NAD^+ \rightleftharpoons$ oxaloacetate + NADH + H^+

The amount of oxoloacetate converted per unit of time as determined by the decrease in the optical density of NADH is a measure of MDH activity (Ordell, 1957).

In addition to enzyme the reaction mixture contained:

1) 0.01 M phosphate buffer pH 7.4; 0.042 M aspartate

2) 0.085 M oxoglutarate

3) 0.031 M NADH

4) 0.1 mg Glutamatic-oxaloacetate transaminase/ml.

3.24 Glucose-6-phosphate dehydrogenase: E.C.1.1.1.49.

Glucose-6-phosphate dehydrogenase 6-6-PDH catalyses the reaction. Glucose-6-phosphate + $NADP^+ \rightleftharpoons$ 6-phosphogluconate + NADPH + H^+.

The/...

The increase in NADPH at 340 nm is a measure of the enzyme activity. The method used to measure G-6-PDH is that of Schmidt et al (1958).

In addition to enzyme the reaction mixture contained:

1) 0.05 M triethanolamine buffer pH 7.6;
   0.005 M ethylenediamine-tetraacetate
2) 0.01 M NADP
3) 0.031 M glucose-6-phosphate

3.25 Lactate dehydrogenase: E.C.1.1.1.27 (LDH) catalyses the reaction:

pyruvate + NADH + $H^+ \rightleftharpoons$ lactate + NAD

The activity is measured by the rate of consumption of pyruvate and NADH. The decrease of optical density at 340 nm is due to the oxidation of NADH. The method used is that of Wroblewski and La Due (1955).

In addition to enzyme the reaction mixture contained:

1) 0.05 M phosphate buffer pH 7.5;
   0.00031 M pyruvate
2) 0.009 M NADH

3.26 Isocitrate dehydrogenase: E.C.1.1.1.42 The enzyme ICDH catalyses the hydrogen transfer reaction;

Isocitrate + NADP $\rightleftharpoons$ oxoglutarate + CO_2 + NADPH + H^+.

The ICDH activity is determined from the rate of increase of absorption at 340 nm due to NADPH produced by the reaction. The method is that of Wolfson et/...

et al (1957).

In addition to enzyme the reaction mixture contained:

1) 0.1 M triethanolamine buffer pH 7.5;
0.0046 M DL isocitrate; 0.052 M NaCl.

2) 0.0091 M NADP; 0.12 M MNSO4

All the enzyme activities listed above were determined using the test combination kits manufactured by the Boehringer Corporation London Ltd.

Appropriate dilutations of the supernatant were made with the stock buffer to ensure that all reactions were linear with time.

The reactions were carried out in 3 ml silca cuvettes with a 1 cm ligh path. The course of the reaction was followed on a unicam S-P 500 series-2-spectrophotometer with a S-P 508 programme controller and a thermostatically controlled cuvette carriage.

4) Serum Studies

4.1 Extraction method: A small cut was made into the marginal ear vein of californian rabbits and the blood was allowed to flow freely into test tubes which were chilled in an ice-bath. The blood was then centrifuged at 600 x g at 0°C. and the serum removed and placed in an ice-bath. Analyses were carried out with a minimum of delay after blood samples were withdrawn.

Blood samples were always drawn two hours after the injections (normally 11 a.m.) of isoprenaline/...

isoprenaline, thyroxine or saline. Samples of blood were drawn at 11 a.m. each day following aortic constriction.

4.2 Serum enzyme activities: Serum creatine phosphokinase and lactate dehydrogenase were measured using Boehringer test kits as previously described.

Enzyme activities were expressed in the units of enzyme activity (u) recommended by the International Union of Biochemistry. Thus, one unit of any enzyme is that amount which will catalyse the transformation of 1 micro mole (u mole of substrate per minute under standard conditions). 1 milli-unit (mu) = 0.001u.

4.3 Serum free fatty acids: Serum free fatty acids were extracted in chloroform. After extraction the amounts of free fatty acids was measured according to the method of Duncomb (1962).

5) Attempted Isolation of muscle cells

The left ventricle of a rabbit was removed and cut into small slices (1 mm thick). The slices were then incubated in Krebs-Ringer bicarbonate medium pH 7.4 which contained 0.40% collagenase and 0.15% hyaluronidase for 0-4 hours at 37^{o}C. After this period of incubation the suspension was carefully removed from the undigested section and placed on a serum gradient (20-50%). The cells were allowed to sediment under gravity, fractions were collected and centrifuged 100 x g for 5 minutes. The resulting pellets were examined under a light microscope to determine the purity/...

purity of each fraction.

6) Myocardial lysosomal enzymes

6.1 Method of homogenisation: Hearts removed from rabbits were rinsed with ice-cold 0.25 M sucrose to remove any residual blood. Sample of the left and right ventricles were cut into small slices and homogenised using an "Ultra Turrax" homogeniser (setting of 42) for 15 seconds in ice-cold 0.25 M sucrose (1.5 g of tissue/10 ml). This suspension was re-homogenised using a Dounce (A) homogeniser using 3 up and down strokes. This homogenate was centrifuged as shown in Fig. 24.

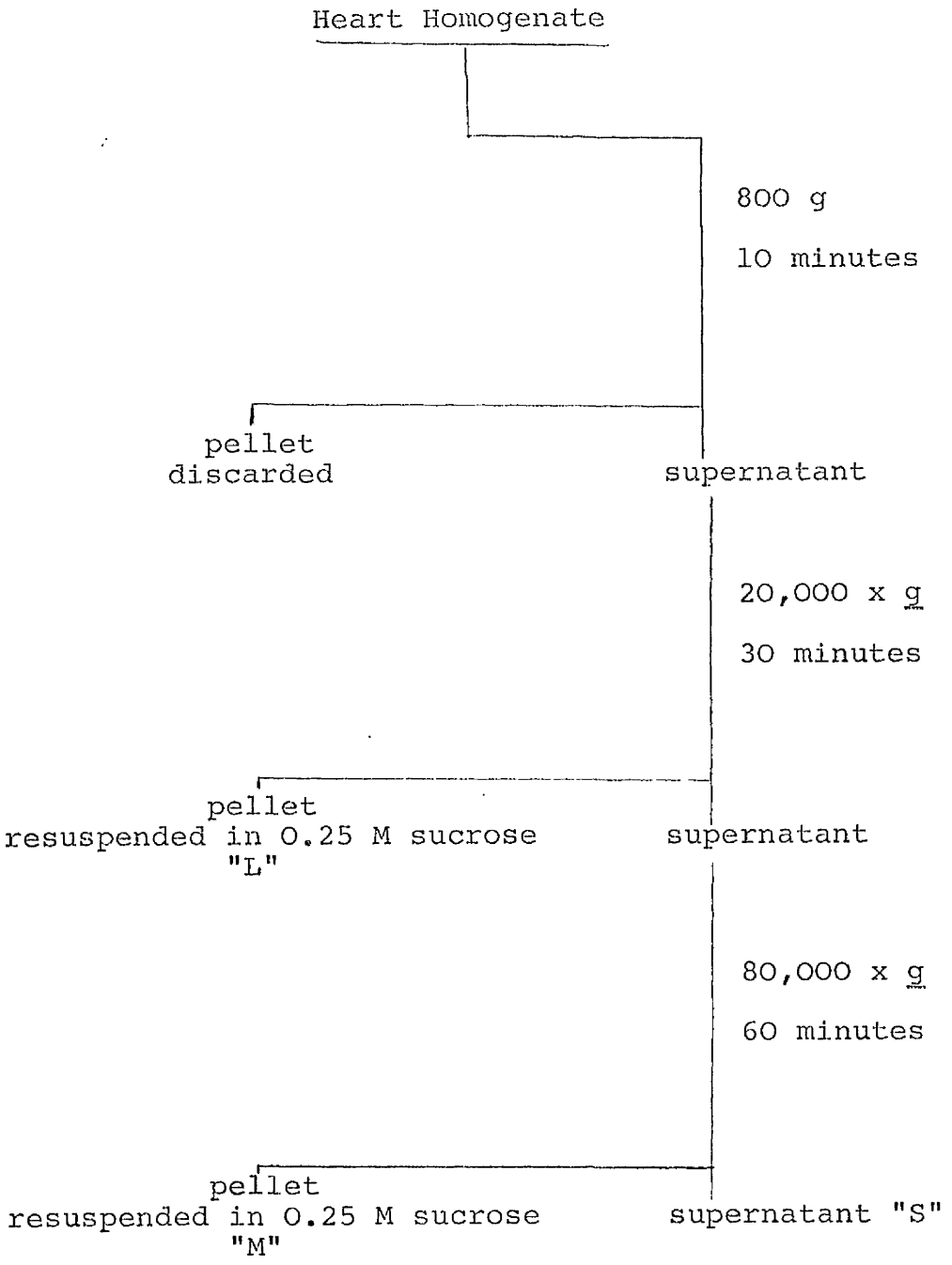
The supernatant from the second centrifugation was called the S-fraction and the activity of the free or soluble enzymes was determined in this fraction. The 20,000 x g pellet was resuspended in 10 ml. of 0.25 M sucrose and was called the L-fraction. The release of particulate lysosomal enzymes in this fraction was brought by freezing and thawing ten times or by addition of triton-x-100 to a final concentration of 0.14%. Triton-x-100 was found to have a greater effect on the release of lysosomal enzymes (Table 3).

The above homogenisation scheme was chosen based on the distribution of lysosomal enzyme activity (Table 3).

6.2 Estimation of enzyme activity: Ten lysosomal hydrolase were measured on the S and P fraction, eight of/...

Fig. 24

Method of separating bound and soluble lysosomal enzyme.



L = lysosomal fraction

M = microsomal fraction

S = supernatant

TABLE 3

The effect of triton-x-100 and freezing and thawing on the release of enzyme from 20,000 x g pellet.

Enzymes	Triton-x-100 (0.1%)	Freezing and thawing (10 times)
B-D-Galactosidase	0.50	0.34
*	±0.019	±0.010
B-D-Glucuronidase	0.51	0.50
*	±0.061	±0.018
N-Acetyl-B-D-Glucosaminidase	1.41	1.00
*	±0.049	±0.063
-D-Mannosidase	0.46	0.40
*	±0.014	±0.023
B-D-Glucosidase	0.43	0.44
*	±0.021	±0.016
Esterase	1.82	0.90
*	±0.110	±0.086
Acid deoxyribonuclease	0.30	0.21
**	±0.010	±0.016
Acid phosphatase	1.9	1.9
*	±0.135	±0.199
Phosphodiesterase IV	0.60	0.61
*	±0.048	±0.063
Cathepsin D	19	16
***	±1.9	±2.1

Mean ± SEM

* = mu moles phenol/minute/mg protein

** = increase in optical density (unit = 0.100 OD) per hour/mg protein

*** = ug tyrosine/30 minutes/mg protein

TABLE 3

Lysosomal enzyme distribution in the left ventricle of californian rabbits. in the presence of 0.4 % Triton X-100

Enzymes	Lysosomal	Microsomal	Supernatant
B-D-Galactosidase	0.50	0.05	0.34
*	±0.016	±0.001	±0.010
B-D-Glucuronidase	0.40	0	0.40
*	±0.020	-	±0.016
N-Acetyl-B-D-Glucosaminidase	1.4	0.11	0.63
*	±0.046	±0.014	±0.023
-D- Mannosidase	0.40	0	0.29
*	±0.009	-	±0.013
B-D-Glucosidase	0.44	0	0.19
*	±0.011	-	±0.006
Esterase	1.8	0.30	0.81
*	±0.194	±0.006	±0.073
Acid deoxyribonuclease	0.30	0	0.25
**	±0.008	-	±0.013
Acid phosphatase	1.9	0.9	2.1
*	±0.164	±0.094	±0.187
Phosphodiesterase IV	0.60	0.05	0.63
*	±0.035	±0.009	±0.046
Cathepsin D	18	0	16
***	±1.6	-	±1.3

Mean ± SEM

* = mu moles phenol/minute/mg protein

** = increase in optical density (unit = 0.100 OD) per hour/mg protein

*** = ug tyrosine/30 minutes/mg protein

of them were measured using the automated method of Bradley et al (1964) modified as detailed later, the other two were measured manually.

6.21 Manual method:

1) Acid deoxyribonuclease E.C. 3.1.4.6

In this reaction deoxyribonucleic acid was hydrolyzed by acid deoxyribonuclease to various oligonucleotides and mononucleotides. The products of this reaction were then measured spectrophotometrically at a U.V. absorption of 260 nm. The method used in this study was that of de Duve et al (1955).

In addition to enzyme each reaction mixture contained:

0.05 M acetate buffer pH 5.0 and 2.5 mg of denatured DNA.

2) Cathepsin D: E.C. 3.4.4.23

The hydrolysis of acid-denatured haemoglobin was used to measure the activity of cathepsin D. The aromatic degradation products were measured by means of the Folin-Ciocaltease reaction as described by Anson (1937). The exact method used to measure cathepsin D was that of de Duve et al (1955).

In addition to enzyme the reaction mixture contained:

2% denatured haemoglobin in 0.1 M lactate Buffer pH 5.0.

6.22 Automated method: Eight lysosomal enzymes were measured using an automated method. The principle/...

principle involved the hydrolysis of p-Nitrophenyl substrates to p-Nitrophenol. The p-Nitrophenol was developed in base to give a yellow colour, the absorbance of which was measured at 420 nm. The conditions chosen, i.e. substrate concentration and pH were essentially those reported by Bradley et al (1964) Table 3 and were not radically different from those of the manual method (Tappel 1968, Comprehensive Biochemistry).

Procedure: From the sampler II substrate and enzyme were simultaneously sampled through separate lines. The solutions were passed through a peristaltic pump (pump II technicon) and then connected to a mixing coil on the effluent side before the stream was segmented with air bubbles. The segmented stream passed into a heating bath and underwent incubation for a period of time proportional to the flow rate and heating bath column volume. This was usually 10 minutes. On the effluent side of the bath the reaction mixture was mixed with 1.93 M NH_4 OH pH 10.7 to terminate the reaction. The solution then passed through a filter and the chromophoric product was determined in a flow cell colorimeter. The absorbance at 420 nm was recorded on a strip chart (Fig. 25).

A one minute buffered wash (0.001% triton -x-100 in 0.01 M acetate buffer pH 5.0) followed each test sample.

Enzyme/...

TABLE 32

Substrates and buffer for enzyme determinations.

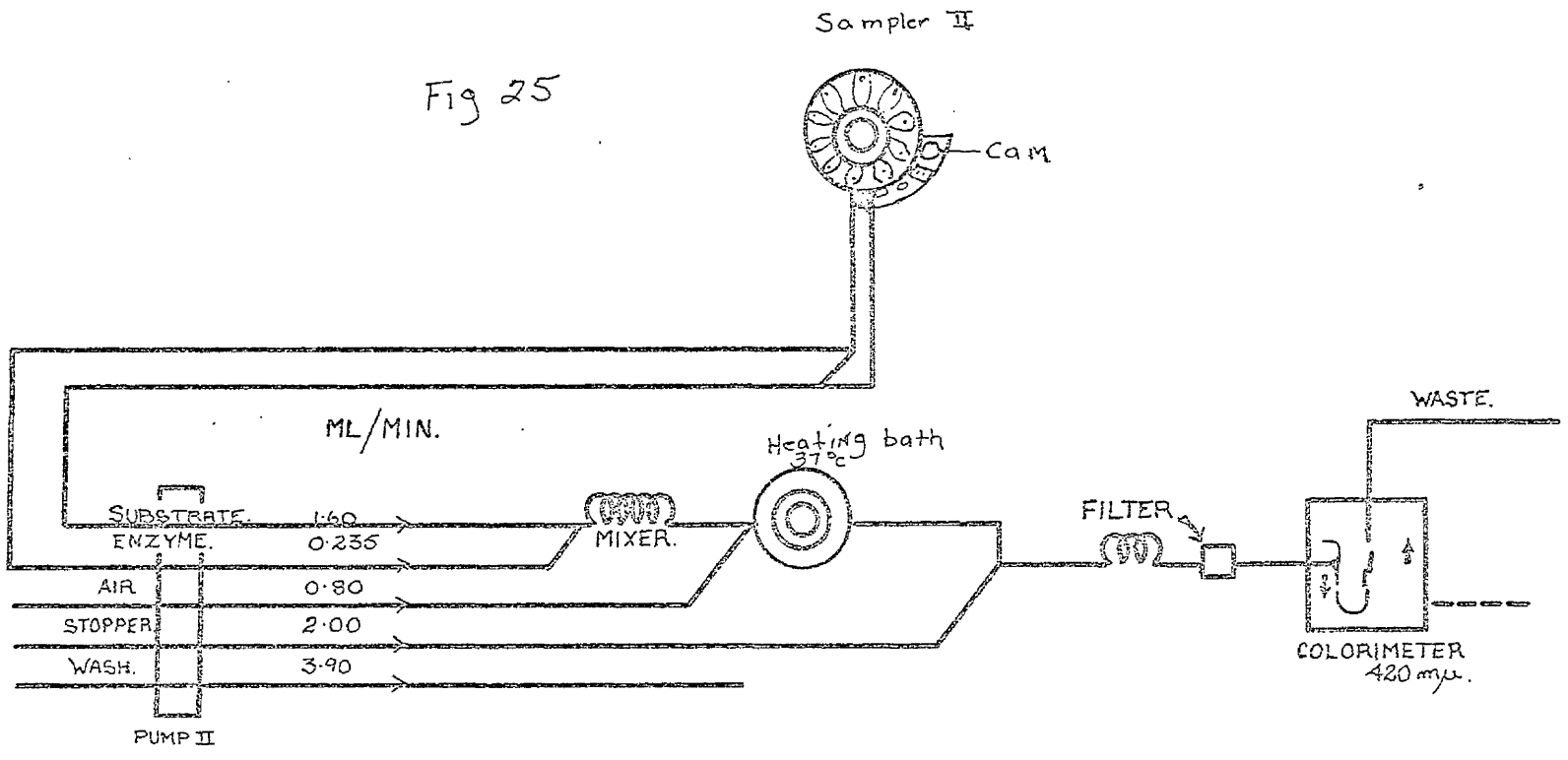
Enzymes	Substrate	Final con[c] in reaction mixture mM	Buffer and pH
B-D-Galactosidase E.C. 3.2.1.23	P-Nitrophenyl B-D-Galectoside	1.05	pH 3.0 (B)
B-D-Glucuronidase E.C. 3.2.1.31	P-Nitrophenyl B-D-Glucuronide	1.90	pH 5.0 (A)
N-Acetyl-B-D-Glucosaminidase E.C. 3.2.1.30	P-Nitrophenyl-N-Acetyl B-D-Glucosaminide	3.00	pH 4.0 (B)c 0.1M NaCl
∝-D-Mannosidase E.C. 3.2.1.24	P-Nitrophenyl ∝-D-Mannoside	6.00	pH 5.0 (A)
B-D-Glucosidase E.C. 3.2.1.21	P-Nitrophenyl B-D-Glucoside	1.00	pH 5.0 (B)
Esterase E.C. 3.1.1	P-Nitrophenyl Myristate	8.3	pH 4.6 (A)
Acid phosphatase E.C. 3.1.3.2	P-Nitrophenyl Phosphate	15.0	pH 5.2 (A)
5^1-Phosphodiesterase IV E.C. 3.1.4.1	Bis-P-Nitrophenyl Phosphate	2.9	pH 5.0 (A)

A = 0.1 m Sodium Acetate

B = 0.2 m Sodium Citrate, 0.4 m Sodium phosphate

Fig. 25 Flow diagram for the determination of eight lysosomal enzymes.

Fig 25


Sampler II
Cam
ML/MIN.
Heating bath
37°c
WASTE.
SUBSTRATE.
ENZYME.
1·60
0·235
MIXER.
FILTER
AIR
0·80
STOPPER
2·00
WASH.
3·90
PUMP II
COLORIMETER
420 mμ.

Enzyme and substrate blanks were run separately and their absorbance values were subtracted from those of the test sample. In addition p-Nitrophenyl standards (0-20 ug in 1.93 M NH_4OH pH 10.7) were run daily for calibration.

This method incorporated the following modifications of the original method of Bradley et al (1964).

1) System for filtering: Measurement of enzyme activity on the 20,000 x g pellet after resuspension and homogenisation, proved difficult due to the formation of a precipitate during incubation of substrate and enzyme. This precipitation often obscured the enzyme activity. Apparently this problem was not encountered by Bradley et al (1964) using liver. However, the low levels of lysosomal enzymes in heart tissue necessitate the use of larger amounts of tissue.

The introduction of system to filter the reaction mixture, (detailed in Fig. 26) prior to its entering the recorder resolved this proglem. Fig. 27 illustrates the results before and after this system was introduced.

2) The second modification involved introducing substrate and enzyme simultaneously as described in Fig. 28a. This was incorporated due to the cost of two technicon samplers. Bradley et al (1964) had found that the simultaneous introduction of substrate and/...

Fig. 26

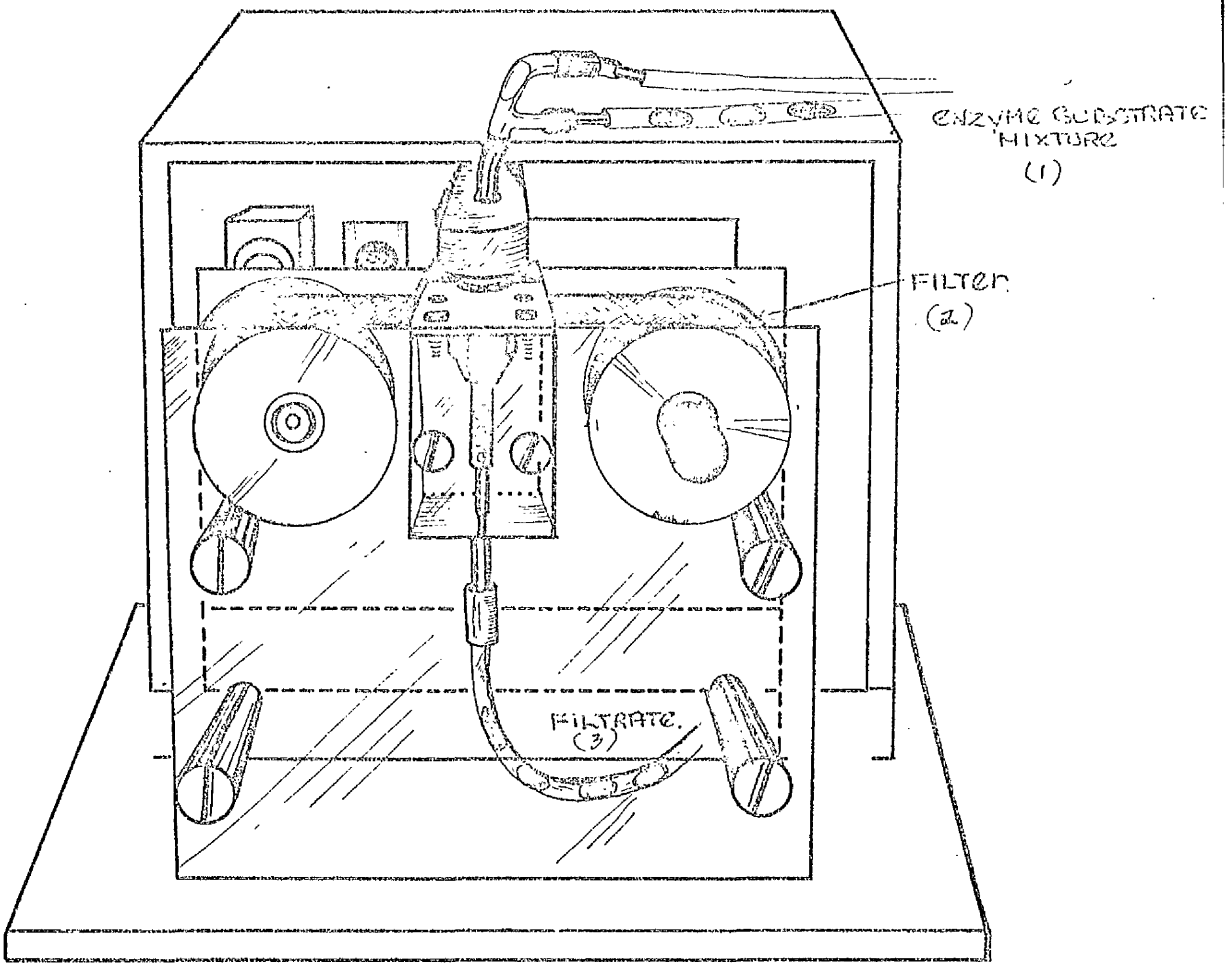
a) Details of filter:

1) unfiltered enzyme and substrate mixture:

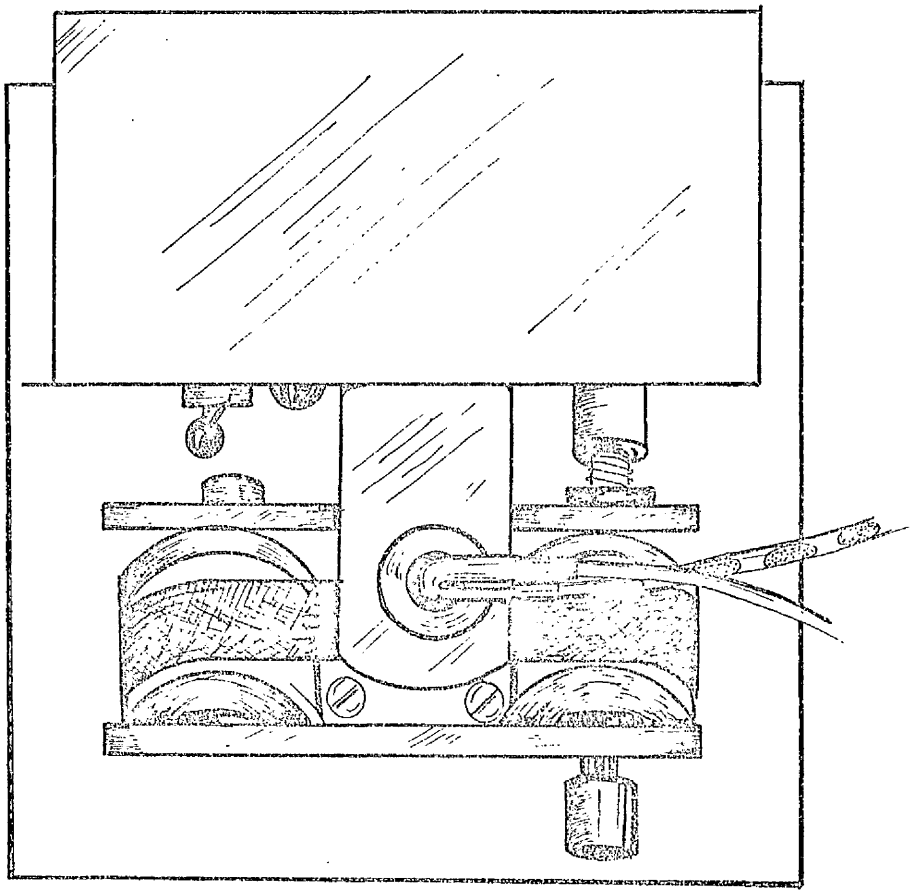
2) wire mesh used to filter enzyme substrate mixture.

3) filtered substrate and enzyme mixture.

b) Top view of filter.



FRONT. VIEW. A



TOP VIEW. B

Fig. 27

a) The direct recorder readout of eight lysosomal enzymes after introducing the filter.

b) The direct recorder readout of eight lysosomal enzymes before the filter was introduced.

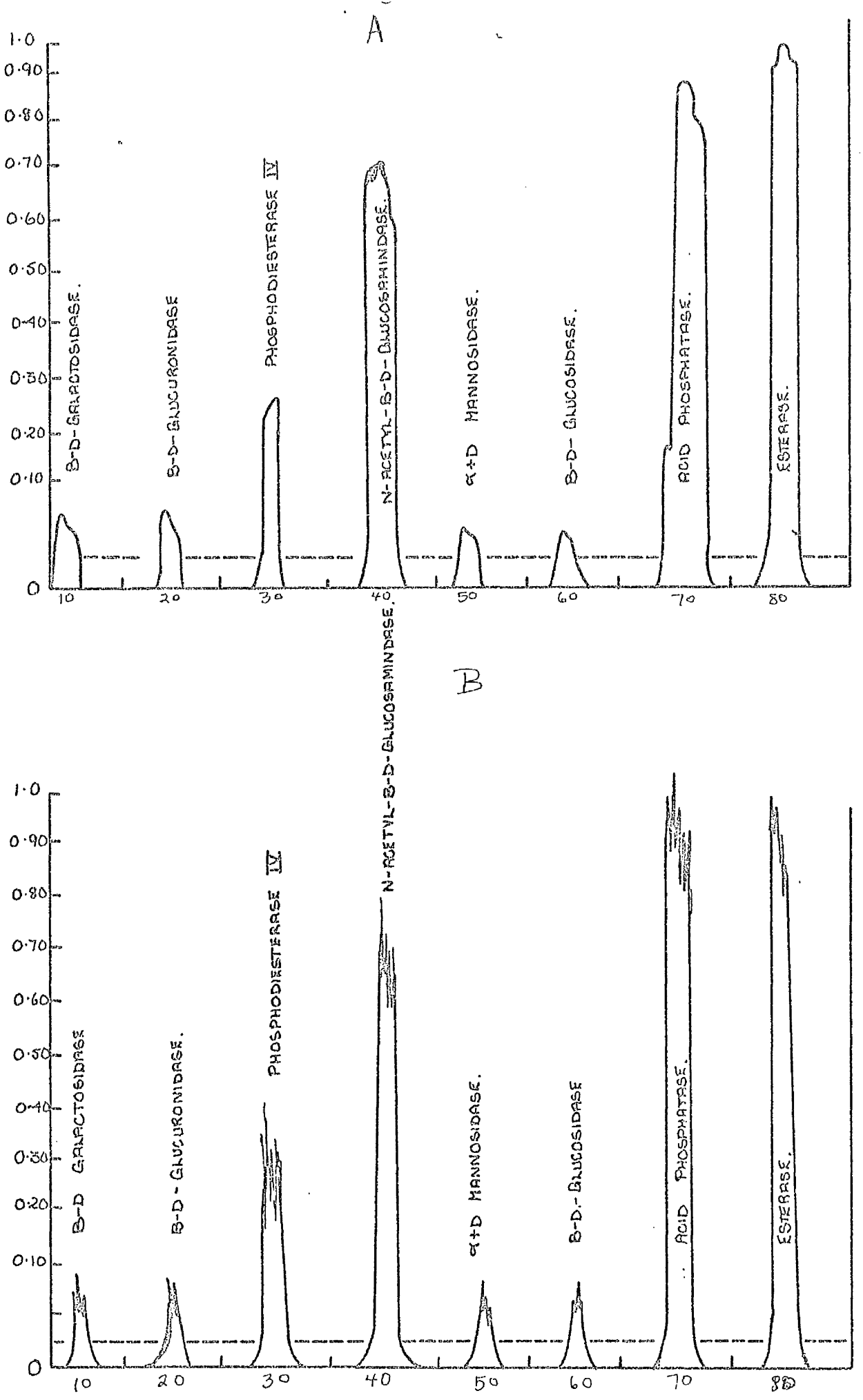
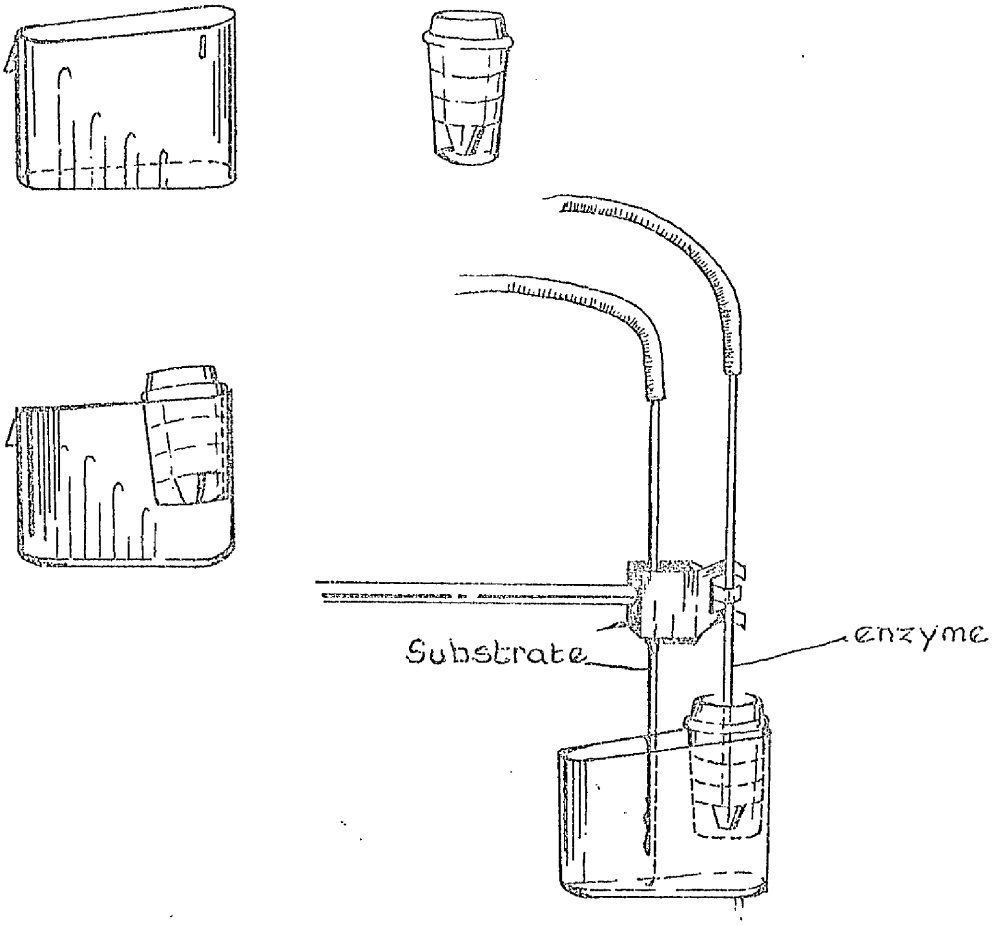

A
1·0
0·90
0·80
0·70
0·60
0·50
0·40
0·30
0·20
0·10
0
β-D- GALACTOSIDASE.
β-D- GLUCURONIDASE
PHOSPHODIESTERASE IV
N- ACETYL- β-D- GLUCOSAMINIDASE.
α+D MANNOSIDASE.
β-D- GLUCOSIDASE.
ACID PHOSPHATASE.
ESTERASE.
10
20
30
40
50
60
70
80
B
N-ACETYL-β-D-GLUCOSAMINDASE.
1·0
0·90
0·80
0·70
0·60
0·50
0·40
0·30
0·20
0·10
0
B-D GALACTOSIDASE
B-D - GLUCURONIDASE.
PHOSPHODIESTERASE IV
α+D MANNOSIDASE.
B-D.- GLUCOSIDASE
ACID PHOSPHATASE.
ESTERASE.
10
20
30
40
50
60
70
80
TIME

Fig. 28

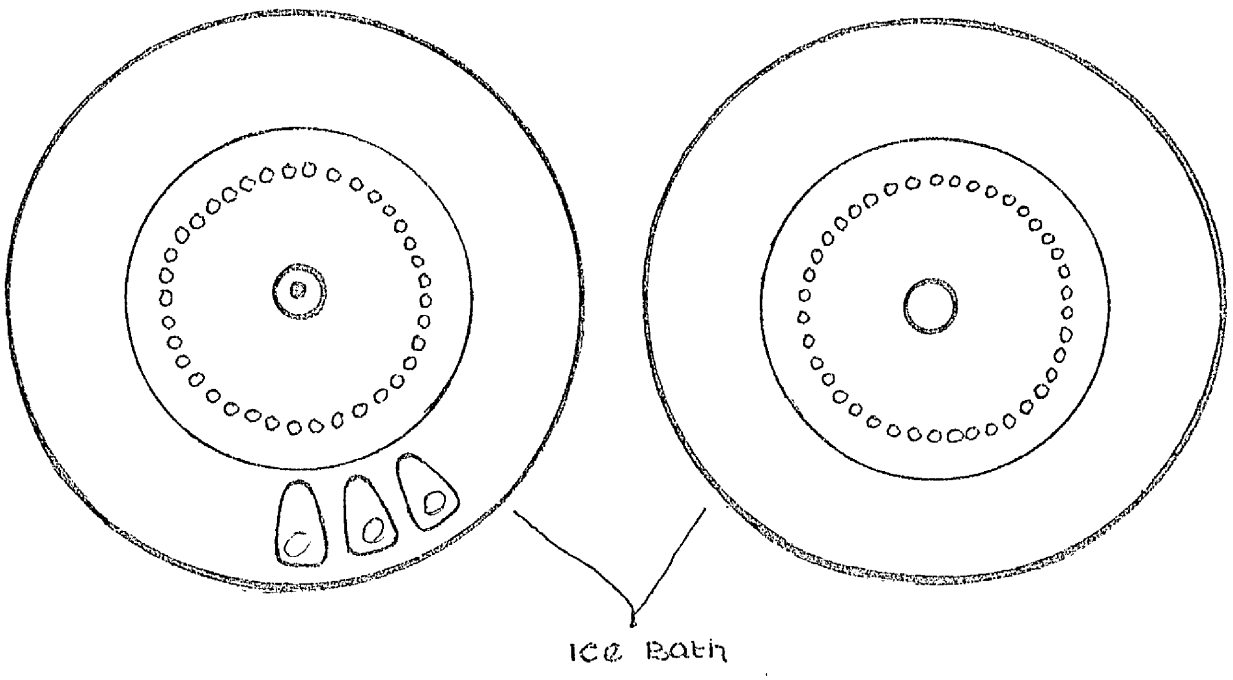
a) Illustration of how enzyme and substrate were sampled simultaneously.

b) Illustration of ice bath.

A.



B.



and enzyme often led to ill defined peaks. This difficulty was not experienced in the present work.

3) An ice-cold water bath was fitted onto the sampler II to keep the enzyme and substrate cold prior to their mixing (Fig. 28b).

7) Histochemical studies

7.1 Histological examination for collagen and mucopolysaccharides.

A section of the left ventricle was removed as quick as possible and placed in 10% buffered formol solution (Lillie 1954). After fixation, usually for 24 hours, sections were dehydrated through increasing alcohol, embedded in paraffin and 6μ sections were cut, stained and mounted on glass slides.

The left ventricular free wall from human autopsy was divided into three parts, anterior, posterior and lateral. The 6μ sections from each part were stained for collagen using the Masson trichrome strain as described by Masson (1928).

After staining, sections were examined under a binocular Watson Barnett light microscope (magnification x 100) to determine the relative proportions of muscle cells, fibrous tissue (i.e. collagen and fibroblast and other cellular elements i.e. blood and endothelial cells), in each section. This was done by inserting a grid into one of the eyepieces of the light microscope. Twenty-five fields were examined in each section noting in each field/...

field how many grid intersections fell upon muscle cell, fibrous tissue and other cellular elements. There were 64 possible intersections in the grid used for this study. In some rabbit experiments three serial sections were made of the left ventricle; one was stained with Hematoxylin eosin, (as described by Schleider 1953); one with Masson trichrome, and one with Hale colloidal iron stain (as described by Hale 1946).

Hale stain gave a blue colour for acidic mucopolysaccherides, while Masson trichrome stain gave a green colour for collagen.

7.2 Histochemical examination for, acid phosphatase, N-Acetyl-B-D-Glucosaminidase and lipids.

A section of the left ventricle of californian rabbits were cut into 1 mm slices and fixed in formol calcium fixative containing 4% w/v formaldehyde and 1% anhydrous calcium chloride for 24 hours at 4°C. Without washing, the tissue was blotted on filter paper and transferred to hypertonic gum sucrose medium, (0.88 M sucrose containing 1% gum acacia) for 24 hours at 4°C (Holt, 1959). A 5u section was then cut using a cold microtome as described by Adamstone and Taylor (1948). The sections were stained as described below and mounted in glycerine jelly on glass slides.

7.21 Acid phosphatase: In the above sections were demonstrated according to the method of Gomori (1952)/..

(1952).

Principle: The substrate Na-B-Glycero-phosphate is hydrolysed releasing phosphate groups which immediately combine with lead ions. Lead phosphate is insoluble in water and precipitates in the tissue at sites of enzyme activity. The colourless precipitate is converted to lead sulphide using hydrogen sulphide, which forms a brown or black stain.

7.22 N-Acetyl-B-D-Glucosaminidase was measured according to the method of Hayashi (1964).

Principle: The substrate Naphthol-AS-Bl-N-Acetyl-B-D-Glucosaminide (7-bromo-3-hydroxy-2-Naphth-O-anisidine) is hydrolysed releasing naphthol which combine with p-rosaniline at the site of enzyme activity. The reaction product appears red at the sit of enzyme activity.

7.23 Lipids were stained using either Sudan Black B as described by Chiffelle and Putt (1951) or Oil Red "O" according to Lillie (1944). Sections stained with sudan black B were counter-stained with haemalum while those sections stained with Oil Red "O" were counter-stained with Nuclear fast green.

8) Electron Microscope Studies

Portions of the left ventricle (1 mm thick) were fixed in ice-cold 2.5% gluteraldehyde in 3% sucrose buffered with 0.1 M sodium cacodylate at pH 7.2 for 2 hours; washed with cold 6% sucrose buffered with 0.1 M sodium cacodylate pH 7.2 for 2 hours; post-fixed/...

postfixed with cold 1% osmium tetroxide in 5% sucrose buffered with 0.1 M sodium calcodylate pH 7.2; washed in the above buffered sucrose; dehydrated in ethanol and propylene oxide and embedded in araldite.

Silver-gray sections, cut with glass knives on a ultra-microtome and suspended on 200 mesh copper grids, were stained with uranyl (saturated solution) and lead citrate (3% solution), and examined with AEl EM6 and EM6B electron microscope.

9) Statistical Method

In the experiments in which statistical analysis was performed, results were analysed by the students' t-test. The expressions $p<0.05$ and $p<0.01$ are used in the conventional sense to indicate significance at the 5 per cent and 1 per cent levels respectively.

Materials

All chemicals when possible, were "analar grade" or its equivalent; substrates for lysosomal enzymes, nitro blue tetrazolium, methyl phenenazonium and Methosulphate, Collagenase and Hyaluronidase, Bovine serum albumin (Fraction IV), Glucosamine, were obtained from SIGMA Chemical Co. London; isoprenaline and thyroxine was obtained from Burroughs Wellcome Co. London.

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