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THE EFFECTS OF VOLUNTARY EXERCISE ON THE ULTRASTRUCTURE OF THE LEFT VENTRICLE OF THE RAT HEART

By

Brian Curry

A DISSERTATION

Submitted to
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ABSTRACT

THE EFFECTS OF VOLUNTARY EXERCISE ON
THE ULTRASTRUCTURE OF THE LEFT
VENTRICLE OF THE RAT HEART

By

Brian Curry

Ultrastructural changes were examined in the left ventricular myocardium of rats permitted 24-hour access to voluntary running wheels. Prepubertal (30 days old) and young adult (70 days old) male Sprague-Dawley rats were randomly assigned to sedentary control or to voluntary activity groups. All animals were sacrificed after 12 weeks of the treatments and tissue samples from the subepicardial region of the middle portion of the left ventricular free wall were routinely prepared for light and electron microscopy.

Stereologic analysis of electron micrographs by the point-counting method revealed a significantly greater percent volume of interstitium in the hearts of both groups of active animals that resulted from an increase in the extravascular space and a lesser increase in the capillary component. The myocytes of the active animals showed a significantly greater mitochondrial/myofibrillar ratio than those of the sedentary animals. This was due to a

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significant decrease in the percent volume of the myofibrils, an increase in the percent volume of the mitochondria that was significant in the young adult animals, and a slight increase in the matrix (sarcoplasmic reticulum, T-system and free sarcoplasmic space). The increased percent volume of mitochondria observed in the active animals resulted from a greater numerical density of the organelles. The size of the mitochondrial profiles did not differ between the sedentary and the active animals. Histometric analysis revealed that the capillary density was unaltered in the active animals. However, the capillary/fiber ratio was increased in both groups of active animals.

This study suggests that chronic voluntary running 1) increases the ratio of interstitium to myocytes and increases the ratio of energy producing structures to contractile elements in the myocytes; 2) does not compromise the microcirculation of the left ventricle; 3) has similar effects on the myocardium whether activity is initiated before or immediately after puberty.

DEDICATION

To Debbie, Rose and Roy

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CHAPTER I

THE PROBLEM

There is a great deal of information available concerning the effects of acute and chronic exercise on the human heart. The use of various techniques, both invasive and non-invasive, has enabled investigators to evaluate hemodynamic, physiologic and gross morphologic changes induced by physical activity.

Electron microscopy has permitted researchers to examine the ultrastructure of the mammalian myocardium and to correlate morphologic alterations with various changes in the physiologic and hemodynamic state of the organism. The subcellular changes occurring during normal postnatal development (Legato, 1979a, 1979b; David et al., 1979; Anversa et al., 1980a), from pathologic volume (Hatt et al., 1970, 1979; Papadimitriou et al., 1974; Winkler et al., 1977) and pressure (Anversa et al., 1971, 1973, 1975, 1976, 1978, 1979; Loud et al., 1978) overloads, and with varying degrees of ischemia (McCallister et al., 1979; Tomanek et al., 1981) have been examined. Studies also have been conducted to examine the effects of enforced swimming (Kleitke et al., 1966; Bozner and Meessen, 1969; Guski, 1980; Guski et al., 1980a, 1980b, 1981) and running

(Edington and Cosmos, 1972; Kainulainen et al., 1979) on myocardial ultrastructure. The information gained has enabled researchers to further evaluate both the acute and the chronic effects of physical activity on the heart.

Most of the preceding investigations have demonstrated that the ultrastructure of the cardiac myocyte and the myocardial interstitium may undergo various alterations depending on the nature of the stimulus to which the heart is exposed. There is an attempt to compensate for the altered hemodynamic state by a disproportionate biosynthesis of specific subcellular components in order to return the myocardium to a state of relatively normal function under the altered conditions.

Chronic enforced exercise programs have been shown to result in a greater synthesis of mitochondria relative to the contractile component of the myocyte (Kleitke et al., 1966; Bozner and Meessen, 1969; Guski, 1980; Guski et al., 1980a, 1980b, 1981). Changes have been observed in the relative volumes of the membranous tubular organelles of the myocyte (Arcos et al., 1968; Bozner and Meessen, 1969; Sarkisov et al., 1969; Guski et al., 1981) and in the size of the microcirculatory component (Leon and Bloor, 1968, 1976; Bloor and Leon, 1970; Tomanek, 1970; Bell and Rasmussen, 1974). Attempts have been made to correlate these morphologic findings with enhanced mechanical and functional states of the myocardium (Meerson and Breger, 1977; Page, 1978; Guski et al., 1981). The goal has been

to further establish the efficacy of exercise in maintaining a normal healthy heart and in retarding or preventing the onset of various cardiomyopathies in man.

There is, however, a dearth of information concerning the effects of voluntary exercise on the ultrastructure of the myocardium. It remains to be established whether the heart of a voluntarily active animal will demonstrate any ultrastructural differences from a sedentary animal of the same age and species.

Purpose of the Study

The overall objective of this investigation was to determine the effects of voluntary running upon the ultrastructure and microvasculature of the left ventricular myocardium of the rat.

Prepubertal and young adult male albino rats were permitted 24-hour access to voluntary running wheels. After 12 weeks the animals were sacrificed and tissue samples of the left ventricular free wall were fixed and prepared for transmission electron microscopy. Stereologic analyses of the electron micrographs were performed to permit quantitative ultrastructural comparisons to be made between the hearts of the active animals and those of animals that remained sedentary during the experimental period.

Significance of the Study

Participation in various programs of physical activity for both the prevention of, and rehabilitation from, cardiovascular illness is on the increase. However, ultrastructural changes induced in the myocardium by exercise can be evaluated only in animal models at present. Furthermore, enforced exercise programs for animals have the inherent problem of confounding the effects of activity with the effects of the stimulus employed to ensure participation in the program. The psychological stress induced by the stimulus, or the combined effects of psychological stress and exercise, may be the factor that induces the morphological changes observed in the hearts of animals conditioned by enforced activity programs.

The use of voluntary exercise in this investigation is an attempt to isolate the effects of activity and to provide some insight into any ultrastructural adaptations that may occur in the heart of an active, as opposed to a non-active, animal.

Limitations of the Study

1. The results of this study are restricted to normal prepubertal and young adult male albino rats of the Sprague-Dawley strain. Absolute values may prove to be specific to species and/or strain.

2. The results are restricted to the subepicardial region of the middle portion of the left ventricular free wall.

3. No attempt was made to ascertain whether the tissue of the myocardium was fixed in a systolic or diastolic state. Whether the results of stereologic analyses differ between the two states is uncertain.

4. The only quantitative evaluation of voluntary activity was the daily recording of the total revolutions run by each animal during the preceding 24-hour period. No attempt was made to establish the pattern of activity in terms of the duration or intensity of any exercise session. Although a specific animal would engage in approximately the same amount of activity from day to day, there was a lack of homogeneity within the group as regards the total distance run and probably with regard to the individual pattern of activity.

CHAPTER II

REVIEW OF RELATED LITERATURE

This review is concerned with the ultrastructure of the normal mammalian left ventricle and the subcellular changes that occur when the hemodynamics of the ventricle are altered during postnatal development, pathologic overload and chronic enforced exercise programs. The final section discusses the effects of chronic endurance exercise on left ventricular performance.

Ultrastructure of the Ventricular Myocardium

The ultrastructure of ventricular myocytes in the hearts of normal adult mammals has been well documented (Simpson and Rayns, 1968; Sommer and Johnson, 1968; Fawcett and McNutt, 1969; McNutt and Fawcett, 1974). The contractile fibers demonstrate similar morphology in various species of mammals.

The myocytes are elongated, branching cylinders with specialized junctions between adjacent cells. These junctions, the intercalated discs, represent the specialized attachment of two plasma membranes. Each disc is made up of a number of morphologically discrete regions that may be categorized as fascia adherens, macula adherens

(desmosomes), and nexus (gap junctions). The nexus represents a site at which ionic exchange may occur readily, facilitating the transmission of the wave of membrane depolarization from one cell to another.

Each myocyte normally possesses a single fusiform nucleus that is situated centrally along the longitudinal axis of the fiber. The nuclear chromatin is predominantly euchromatic, although the nuclear envelope has a thin lining of the heterochromatic (inactive) form. At the poles of the nucleus, the sarcoplasm contains flattened membranous saccules comprising the Golgi complex.

The major portion of the extranuclear space is occupied by longitudinally oriented myofibrils composed of the contractile proteins actin and myosin. In longitudinal section, the myofibrils appear as a series of alternating light (I or isotropic) and dark (A or anisotropic) bands formed from the regular interdigitation of the actin and myosin protein myofilaments. Each I-band is bisected transversely by a thin electron dense region, the Z-line. A sarcomere is defined as the area between adjacent Z-lines, and a myofibril comprises a number of such sarcomeres arranged in series.

The work of Huxley (1963) and Hanson and Lowry (1963) provided much insight into the ultrastructural organization of the contractile elements of striated muscle. Their work demonstrated that the myosin filaments extend the length

of the A-band. The actin filaments and associated regulatory proteins, troponin and tropomyosin, originate at the Z-lines and extend through the I-band into the A-band. In transverse section, the A-band presents a hexagonal array of actin filaments around a myosin filament.

Numerous elongated mitochondria are found lined up between adjacent myofibrils. When observed in transverse section, the contractile region of the myocyte appears to lose its fibrillar organization and presents itself as a large mass of myofilaments that possesses continuity around clefts in the sarcoplasm. Within these clefts are found the mitochondria. Each mitochondrion has a double unit membrane with the inner structure being formed into numerous cristae which greatly increase its surface area. McNutt and Fawcett (1974) observed that in more rapidly beating hearts a greater proportion of the cross-sectional area of the myocyte is occupied by mitochondria than is the case in hearts that beat more slowly. Thus the myofilamentous areas are smaller in the faster hearts. The shape of the mitochondria appear to be in a constant state of flux. The organelles may appear to branch, fuse, enlarge or constrict. Apparently these alterations are dependent upon the oxygen tension, the availability of substrate and inorganic phosphate, and the energy demands of the myocyte (McNutt and Fawcett, 1974).

The sarcolemma, or plasma membrane, demonstrates a structure of protein inclusions within a phospholipid bilayer that is typical of the cytological unit membrane. At many points, the sarcolemma is deeply invaginated into the myocyte to form the transverse or T-tubules at the level of the Z-lines. Forssman and Girardier (1970) have suggested the presence of longitudinal tubules that may interconnect adjacent T-tubules in heart cells of the rat. The T-system represents a large extension of the external sarcolemma into the myocyte providing a pathway for the wave of membrane depolarization into the deeper regions of the cell.

The endoplasmic reticulum of cardiac myocytes is predominantly agranular, although a small portion possesses ribosomes on the outer surface of the membrane. The smooth sarcoplasmic reticulum comprises numerous longitudinal tubules that anastomose along their length. These tubules coalesce at the Z-line to form a transversely oriented terminal cisterna that is apposed to the T-tubule, forming the diad. In addition, cisternae are located in the subsarcolemmal region at the periphery of the cell.

The interstitial space between myocytes contains a small amount of loose connective tissue. This endomysium forms a thin basal lamina around the sarcolemma and an overlying fine network of thin collagenous and reticular fibers. Within the endomysium are found fibroblasts, mast

cells, macrophages, and lymphocytes, and a rich network of nonfenestrated, longitudinally oriented capillaries that are supplied by branches of the coronary arterial tree.

Quantitative Ultrastructure of the Ventricular Myocardium

With the increasing use of animal experimental models to evaluate the effects of altered hemodynamics on the heart, many researchers saw the need to correlate morphological changes in the myocardium with the various physiological stimuli that were being imposed. The early work of Elias (1951, 1954) and Hennig (1956, 1957) led to the eventual development of stereologic techniques within the biological sciences. Stereology is the use of geometric probability to extrapolate from two dimensional images, such as histological sections, into three dimensional space (Elias et al., 1971). The use of stereologic analysis permitted quantitative evaluation of tissue examined at the light or electron microscope level and resulted in many studies that reported relative values for the various subcellular components of the myocardium.

In reviewing the data reported in these studies, it is apparent that there is a lack of general agreement between various workers as to the relative composition of the myocardium, even within the same species of normal adult animals. As suggested by Singh et al. (1981), these intraspecies differences may result from variety in the

ages of the animal model used, the orientation of the section with respect to the myocyte, the number and magnification of the micrographs used for the analysis, or the methods used for fixation and embedding of the tissue. In addition, experimental group sample size and the strain of animal species may contribute to the variability.

As can be seen from Table 1, the most commonly evaluated components of the left ventricular myocyte have been the relative volumes of the contractile elements and mitochondria. In the rat, percent volumes for myofibrils range from 47% (Page et al., 1974) to 63% (Tomanek et al., 1979), with an extreme value of 42% (Reith and Fuchs, 1973). Mitochondrial values range from 25% (Mall et al., 1977, 1980) to 42% (Imamura, 1978). Less variability is seen when one examines the values reported by a single author or laboratory group. Anversa and his coworkers report values of 56% and 31% (1976) and 54% and 32% (1978) for myofibrils and mitochondria respectively. Tomanek presents values of 62% and 31% (1978) and 63% and 32% (1979). Page reports 48% and 34% (1971), 50% and 34% (1972), 48% and 36% (1973) and 47% and 35% (1974). The more consistent values reported by a single laboratory lend much credence to the view that the methods employed in tissue preparation, sectioning and stereologic analysis contribute greatly to the variability demonstrated between different laboratories.

Table 1. Relative Volumes of Selected Myocardial and Myocyte Components in Various Animal Species

Species	Age/Weight	Mitochon-dria	Myofibrils	T-System	SR	Myocytes	Interstitium	Reference
Rat	100g					81.5	18.5	Anversa et al. '75
Rat	100g	31.4	56.0	1.2	2.9	83.7	16.3	Anversa et al. '76
Rat epi	266g	32.9	54.5	0.9	4.9	79.4	20.6	Anversa et al. '78
end		33.8	53.8	0.9	4.9	84.9	15.1	
Rat	100g	34.8	53.6	0.95	4.7			Anversa et al. '79
Rat	90 days	38.0	52.0					Datta & Silver '75
Rat	90 days	30.4	60.3					David et al. '79
	180 days	32.8	55.7					
Rat	180 days	25.2	56.1					Guski et al. '80
Rat	180 days					84.7	15.3	Guski '80
Rat	90 days	31.4	48.4	1.1	2.7			Guski et al. '81
Rat	200-260g	31.1	58.7					Hatt et al. '78
Rat LV	60 days	34.1	61.1					Hirakow et al. '80
RV		36.4	59.1					
Rat	77 days	41.6	49.9					Imamura '78
	147 days	38.7	55.5					
	365 days	37.2	57.1					
Rat LV		36.0						Laguens & Gomez-Dunn '67
RV		40.0						
Rat epi		31.3	62.0					Lund & Tomanek '78
end		32.5	62.7					
Rat pap		25.3	61.2	0.7				Mall et al. '77
Rat	150g	25.3	61.2					Mall et al. '80
Rat	200-350g	29.0	55.0	0.85	2.2			McCallister et al. '79
Rat	200g	34.0	48.1	1.2	3.5			Page et al. '71
Rat	84 days	34.0	50.0					Page et al. '72
Rat	230g	35.1	46.7					Page et al. '74

Table 1. (Continued)

Species	Age/Weight	Mitochon-dria	Myofibril	T-System	SR	Myocytes	Intersti-tium	Reference
Rat	200-220g	35.8	47.6	1.0	3.5			Page & McCallister '73
Rat	200-240g	37.0	42.0					Reith & Fuchs '73
Rat epi	112 days	31.8	63.1					Tomanek et al. '79
end		33.0	61.8					
Mouse		37.5	54.3	0.81	0.88			Bossen et al. '78
Mouse	90 days	43.7	44.4					Kainulainen et al. '79
	180 days	45.2	40.95					
Hampster		28.2	45.9		3.2			Lazarus et al. '76
Hampster		31.8	37.3		2.0			Colgan et al. '78
Rabbit	2kg/1year	34.4	59.9	2.3				Anversa et al. '71
Rabbit septum	2-3kg	42.0						Goldstein et al. '74
Rabbit pap		20.0	62.4					Mall et al. '77
Dog		24.9						Vitali-Mazza & Anversa'72
Dog		26.0	54.0	0.87				McCallister et al. '78
Dog LV		29.6	59.4					Legato '75
RV		24.7	61.5					
Dog LV	150 days	28.4	59.9					Legato '79b
RV		31.1	63.3					
Cat		22.2	47.8					Sheridan et al. '77
Swine LV	45kg	22.2	65.9	1.6	2.3			Singh et al. '81
RV		21.1	67.9	1.7	2.4			
SEPT		21.9	65.6	1.6	2.3			

All values are percentages, and are for the left ventricle unless otherwise stated.

Studies that compared subepicardial with subendocardial regions of the left ventricle demonstrate a slightly greater mitochondrial/myofibrillar ratio in the endocardium (Anversa et al., 1978; Tomanek, 1979). The myocytes of the endocardium also have slightly greater cross-sectional areas (Anversa et al., 1978; Gerdes et al., 1979: Tomanek, 1979; Stoker et al., 1982).

Other subcellular components that have been quantitatively evaluated in the rat left ventricle are the T-system and the sarcoplasmic reticulum. The values for the T-system range from 0.73% (Mall et al., 1977) to 1.9% (Kawamura et al., 1976); those for the sarcoplasmic reticulum range from 2.2% (McCallister et al., 1979) to 4.9% (Anversa et al., 1978).

Tissue samples for electron microscopic examination and stereologic analyses are most frequently taken from the middle portion of the left ventricular free wall. There is apparently only one study available that reports comprehensive data for the entire heart. Singh et al. (1981) conducted stereologic analyses on tissue samples from 20 different sites in the hearts of normal Yukatan swine. The 12 sites sampling the left ventricle were from both the lateral and posterior free wall, each portion of the wall being further subdivided into basal, apical and middle areas and also into epicardial and endocardial regions. The

results indicate relative homogeneity between the 20 sites. In general, the mitochondrial/myofibrillar percent volume ratios were significantly greater in the left ventricular free wall and the interventricular septum than in the free wall of the right ventricle. Similar findings have been reported by Legato (1975, 1979b) in the dog. These results would appear to be a reflection of the greater workload and rate of oxidative metabolism of the left heart (Weiss et al., 1978). The endocardial regions also demonstrated greater mitochondrial/myofibrillar ratios than the epicardium, which is in agreement with the data reported by Anversa et al. (1978) and Tomanek et al. (1979) in the rat. Singh et al. (1981) also showed that the sarcoplasmic reticulum/myofibrillar volume ratios are greater in the endocardial regions and that this ratio is generally higher in regions that demonstrate increased mitochondrial/myofibrillar ratios.

The interstitium has not been analysed extensively by stereologic techniques. Anversa and his coworkers have reported relative volumes for myocytes and interstitium of 81.5% and 18.5% (1975) and 83.7% and 16.3% (1976) for the rat left ventricle. Later studies reported that the interstitium itself was composed of approximately 18-23% endothelial cells, 20-25% capillary lumen, and 55-57% non-capillary structures such as fibroblasts, pericytes, macrophages, ground substance and collagen (Anversa et al., 1978, 1979).

Guski (1980) reported 85% myocytes and 15% interstitium, of which capillary endothelium and lumen combined was 41% and non-capillary components comprised 59%. Frank and Langer (1974) found values of 52% myocytes and 48% interstitium, with vascular lumen and endothelium comprising 59% of the interstitium of the rabbit left ventricle. This relatively high value for the interstitium may be a result of either the high magnification of micrographs used for the analysis or the inclusion of precapillary or postcapillary vessels along with the capillaries.

There is also a great deal of variability in the values reported for the two measures of myocardial capillarization in the rat. Capillary density (CD) represents the number of capillaries per unit area of tissue and is extremely susceptible to the degree of shrinkage of the tissue during preparation for microscopic examination. The ratio of capillaries to myocytes (CF) disregards tissue area or volume and is derived from absolute counts of the two components. Values for CD (#/mm^2) in the rat have been reported as 1300-3500 (Rakusan, 1971), 2700 (Poupa et al., 1970), 3500 (Angelakos et al., 1964), 3600 (Tomanek, 1970), 3830 (Anversa et al., 1979), and 4850 in the endocardium and 4910 in the epicardium (Anversa et al., 1978). Reported values for CF are 1.00 (Poupa et al., 1970, Tomanek, 1970), 0.92 (Angelakos et al., 1964), 0.82 (Anversa et al., 1979), and 0.89 for endocardium and 0.75 for the epicardium (Anversa et al., 1978).

Morphologic Changes with Altered Hemodynamics

The myocardium should not be regarded as a static entity, but rather as a dynamic system that is capable of responding morphologically to varying hemodynamic stimuli. In both the developing and the adult animal, the hemodynamic workload imposed upon the myocardium is a major factor that determines the ventricular mass. Cardiac myocytes rapidly lose the capability of mitosis during early postnatal development (Claycomb, 1977). In the postnatal rat, the total number of cardiac myocytes present at 21-28 days is believed to be very representative of adult values (Enesco and Leblond, 1962; Zak, 1973). Any increase in the muscle mass after the cessation of myocyte proliferative activity therefore must be due to hypertrophy of the existing fibers or to hypertrophy/proliferation of some or all of the interstitial components.

Postnatal Development

The workloads imposed upon the right and left ventricles of the fetus are approximately equal (Rudolph, 1979). However, major hemodynamic changes occur in the neonate. Closure of the foramen ovale and ductus arteriosus, a decrease in pulmonary resistance due to lung inflation (Rudolph, 1979), and an increase in peripheral resistance due to the loss of the placenta (Rudolph, 1970) all combine to exert a pressure load on the left ventricle. The

increased workload induces enhanced growth of the left ventricle resulting in a relatively larger muscle mass that is characteristic of the adult heart.

Anversa et al. (1980a) demonstrated that mitotic activity in left ventricular myocytes of the rat is greater and persists longer than in those of the right ventricle during the first 11 days of postnatal development. Myocyte length and cross-sectional area increased progressively in both ventricles with a nonsignificantly greater increase in the width of the cells from the left ventricle. There is a significant increase in the relative volume of interstitium in both ventricles, due almost entirely to greater capillary densities, and a concomitant decrease in the percent volume of myocytes. The density of patent capillaries increases approximately 3-fold in both ventricles and is comparable to the adult values reported by Henquell and Honig (1976) and Anversa et al. (1979). At the subcellular level, the percent volumes of mitochondria, myofibrils and sarcoplasmic reticulum increase, and that of the remaining sarcoplasm decreases. An increased mitochondrial/myofibrillar ratio was reported only for the left ventricle.

In another extensive study of postnatal development, David et al. (1979) examined ultrastructural changes in the left ventricle of the rat from birth to 6 months. The results showed a gradual increase in the percent volume of

mitochondria until the end of the first month. However, myofibrils decreased until day 6, increased to slightly above neonate values by day 14, and thereafter fluctuated around these values. The relatively constant myofibrillar values are in agreement with Legato (1975) and Hirakow and Gotoh (1975, 1980). These studies also reported increases in the relative volume of mitochondria, whereas Page et al. (1974) demonstrated constant values for both mitochondria and myofibrils in rats weighing between 36-227g.

Rakusan et al. (1978) examined the effects of growth rate on cardiac myocytes by comparing suckling rats from small litters (fast growing), medium litters (normal growth) and large litters (retarded growth). By 21 days postnatal, there were significant differences in body weight, heart weight, left ventricle weight, myocyte diameter and myocyte number among all three groups. The results indicate that food intake not only limits organism and organ growth rates but also decreases the rate and degree of myocyte hypertrophy and hyperplasia.

Korecky and Rakusan (1978) measured normal development in rats from 30 days (80g) to 8 months (650g) using enzymatic disruption of the myocardium to yield undamaged individual myocytes. Fiber length and width increased 64% and 68% respectively. Extrapolation of average myocyte volumes demonstrated similar increases in cell volume and left ventricular mass. The authors concluded

from these data that normal myocardial growth during the period of the study required only hypertrophy of existing myocytes with no proliferative activity.

The most comprehensive examination of normal postnatal development was performed by Legato (1979a, 1979b). She evaluated the development of both ventricles of the mongrel dog over eight postnatal ages from 24 hours to 5 months. Although usually only one animal (2 at 24 and 72 hours) was used for each time period, the results indicated that the percent volumes of myocytes, interstitium and capillaries remain relatively constant during the developmental period in both ventricles. The relative volume of mitochondria demonstrated slight increases, while that of the contractile component remained somewhat constant in both ventricles.

Experimentally Induced Hemodynamic Overload

Chronic hemodynamic overload of the heart may be from increased volume (preload) or increased pressure (afterload). Volume overload may occur pathologically due to mitral or aortic valve insufficiency when the left ventricle is required to pump a normal forward stroke volume plus an abnormal regurgitant volume that either returns through the mitral valve during systole or through the aortic valve during diastole. In either condition, the end-diastolic volume is chronically elevated as a result of the addition of the regurgitant volume to the normal forward stroke volume. The heart also may be subjected to a chronic volume

overload by any condition that results in long-term increases in venous return, as is found in hyperthyroidism and anemia.

Chronic volume overload leads to both hypertrophy and dilatation of the left ventricle. Dilatation of the chamber occurs as a result of the larger end-diastolic volumes experienced during each cardiac cycle. It may also represent involvement of the Frank-Starling relationship to augment the contractility of the overloaded ventricle (Katz, 1977, p. 390). The myocardial hypertrophy that occurs may be due to the elevated wall tension experienced as the radius of the ventricular chamber increases, to myocardial hypoxia, or to an energy imbalance from the greater required workload (Katz, 1977, p. 390).

Pressure overload of the left ventricle may manifest itself pathologically as a result of coarctation of the aorta, aortic valve stenosis, or systemic hypertension. Under such circumstances, the ventricle experiences an increase in wall tension, although the end-diastolic volumes remain at normal levels. The ventricular wall becomes hypertrophied in order to provide a greater contractile mass to cope with the greater wall tension, and dilatation does not occur until the left heart begins to fail. Indeed, if dilatation were to occur in addition to wall hypertrophy, ventricular wall tension would be very much greater due to the larger chamber radius as well as to the elevated systolic pressures (Katz, 1977, p. 393).

In order to gain insight into the morphologic changes associated with these pathologic conditions, researchers have adopted a variety of techniques, both invasive and non-invasive, to induce volume or pressure overload in the hearts of animal models. Volume overload may be induced by thyroxin supplementation (Gerdes et al., 1979), formaldehyde injection into the A-V node to produce blockage (Winkler et al., 1977), or by surgical intervention to produce aortic insufficiency (Hatt et al., 1970) or an aorto-caval fistula (Winkler et al., 1977; Hatt et al., 1979). Pressure overload is most commonly induced by constricting the aorta (Anversa et al., 1971, 1976, 1979, 1980b; Page et al., 1973; Jacob et al., 1977; Korecky and Rakusan, 1978) or a renal artery (Wendt-Gallitelli et al., 1977, 1979; Anversa et al., 1978; Loud et al., 1978). In addition, the temporal changes that occur with ischemia have been examined (Jennings et al., 1974; McCallister et al., 1979; Tomanek et al., 1981).

Hemodynamic Volume Overload

Hatt et al. (1970) surgically induced aortic insufficiency in rabbits. Almost all of the experimental animals demonstrated reductions in resting systolic and diastolic pressures. Animals were sacrificed when they presented symptoms of heart failure (between 21 and 300 days post-surgery). The experimental group had significantly greater relative heart weights, but absolute values for heart and body weights were not reported. There were nonsignificant

decreases in the relative volumes of myofibrils and sarcoplasm and a nonsignificant increase in mitochondrial volume. However, the mitochondrial/myofibrillar ratio increased significantly. Increases in myocyte diameter were mentioned, but no data were reported. All values were for left ventricle papillary muscle.

Using a surgically induced aorto-caval fistula to produce chronic volume overload in dogs, Papadimitriou and his coworkers (1974) showed significant increases in both mass and volume of the left ventricle. Electron microscopy revealed a significant increase in the numerical density of mitochondria. However, the mitochondria were smaller than in the control animals. The authors reported a lowered mitochondrial/myofibrillar ratio but no values for the percent volumes of the two components. It should be noted that the authors reported elevated systolic pressures in the aortic arch and femoral artery, suggesting that the experimental animals were subjected to a combination of pressure and volume overload.

Hatt et al. (1979) also created aorto-caval fistulas and demonstrated increases of 180% for absolute heart weight in rats. After 4 weeks, the myocytes of the left ventricular subendocardium had larger mean diameters than those of control animals. Ultrastructural examination revealed some degenerative changes in these cells. The changes were mainly some focal disorganization of myofibrils and sarcomeres along with smaller but more numerous mitochondria.

Datta and Silver (1975) maintained 21-day-old rats for a period of 10 weeks on a diet of milk and sugar producing sideropenic (iron deficient) anemia. Volume overload induced a 3-fold increase in absolute heart weight. There was a significant increase in the percent volume of mitochondria after both 6 and 10 weeks with the organelles appearing smaller and more numerous. The myofibrillar volume remained unchanged. The authors also noted increases in the percent volume of interstitium but were not explicit as to which of the interstitial components were involved.

Gerdes et al. (1979) supplemented the diet of adult rats with desiccated thyroid for 8 weeks. The hyperthyroid animals had significantly lower body weights and higher heart weights than control animals. Histologic examination revealed significantly greater cross-sectional areas of the myocytes in both the subendocardial (22.5%) and the subepicardial (34.5%) regions of the left ventricle. In addition, there were significantly lower capillary densities in the endocardium (12%) and epicardium (17%).

Based upon increases in relative heart weights, mild hypertrophy of the left ventricle was reported in mongrel dogs subjected to complete A-V block for 10 weeks (Winkler et al., 1977). Ultrastructural examination revealed no changes in the relative volumes of both mitochondria and myofibrils.

Hemodynamic Pressure Overload

Experimentally induced pressure overload of the left ventricle has been utilized more frequently than volume overload to study pathologic morphology of the heart, and more in-depth investigations of ultrastructural changes have been performed using this model. Much of the quantitative subcellular work has been performed by a group headed by Piero Anversa and Alden Loud. Their studies will form the basis of this section.

Using constriction of the ascending aorta to increase afterload, Anversa et al. (1971) produced moderate (30-70%) hypertrophy in the left ventricles of rabbits. Myocyte dimensions were not measured, but the percent volumes of myofibrils and T-system increased significantly, with a concomitant decrease in the mitochondrial volume, after 2-4 months of pressure overload. These changes were noted in all three regions of the myocardium. The mitochondria were smaller and more numerous, which agrees with previous findings (Wollenberger et al., 1962; Poche et al., 1968; Novi, 1968). These investigators all reported lower mitochondrial/myofibrillar ratios for the left ventricle.

Similar decreases in the mitochondrial/myofibrillar ratio were demonstrated in the hypertrophied left ventricles of young adult rats subjected to constriction of the subdiaphragmattic aorta (Anversa et al., 1973). The mean fiber cross-sectional area increased by 58% after 8 days and

by 144% after 13 days, but the percent volumes of myocytes and interstitium remained constant. The relative volumes of myofibrils and sarcoplasmic reticulum increased, and that of mitochondria decreased slightly. This produced a progressive decrease in the ratio of mitochondria to myofibrils over the 40-day postoperative period. Since the decrease in the mitochondrial value was extremely small in comparison to the increases in myocyte volume, the authors postulated substantial increases in the mitochondrial mass that failed to match the increase in the contractile component and the demands of the elevated workload.

The use of radioautography in this same study showed that the major localization of newly synthesized protein was the surface regions of the myofibrils and the areas of the Z-lines. The former focus would tend to support the hypothesis that increases in the contractile component of the myocyte are occurring due to the formation of new sarcomeres, or at least additional myofilaments, at the periphery of the myofibrils (Bishop and Cole, 1969; Morkin, 1970).

In contrast to the chronic changes already discussed, the acute effects of aortic constriction were examined in rats 20 hours after surgery (Anversa et al., 1975, 1976). There were significant increases in myocyte cross-sectional area (20%), mitochondrial percent volume (36%), and the percent volume of interstitium. The relative volume of

myofibrils dropped very slightly (4%), and the mitochondrial/myofibrillar ratio was increased. These results are in agreement with those of Meerson et al. (1964) who demonstrated an elevated ratio after 48 hours of early hypertrophic changes.

The morphometric data, indicating a rapid increase in the percent volume of mitochondria in the very early stages of pressure overload, are substantiated by biochemical studies which demonstrate a greater protein synthesis in the energy producing component of the myocyte at this time (Kaku, 1968; Fizelova and Fizel, 1970).

Utilizing unilateral renal artery constriction to induce a hypertensive pressure overload, Anversa et al. (1978) and Loud et al. (1978) demonstrated increases in the volume of individual myocytes of the magnitude of 21% in the endocardium and 37% in the epicardium, with left ventricle absolute weight increasing by 30% after 4 weeks. The relative volumes of myocytes and interstitium remained constant in the epicardium, but the value for myocytes decreased significantly in the endocardial region. The interstitial components showed no changes in relative volumes, but there were decreases in the capillary densities of both regions concomitant with decreases in myocyte densities. The capillary/fiber ratio was unchanged. At the subcellular level, the endocardial myocytes demonstrated significant increases in the percent volumes of myofibrils and

sarcoplasmic reticulum, a significant decrease in the mitochondrial value, and no change in the T-system. The epicardial fibers showed significant increases in the percent volumes of the T-system and the sarcoplasmic reticulum, a nonsignificant increase in the value for the myofibrils, and a significant decrease in the mitochondrial volume. The mitochondrial/myofibrillar ratio was decreased in both regions of the myocardium with no alteration in mitochondrial size. These results are in agreement with those of Wendt-Gallitelli and Jacob (1977) who also reported decreased mitochondrial/myofibrillar ratios from 4-24 weeks after renal artery constriction in the rat.

A further study (Anversa et al., 1979) using aortic stenosis in the rat provided additional information concerning the effects of pressure overload on the interstitium. The results demonstrated decreases in the percent volume of endothelium, in capillary density and in myocyte density. The capillary/fiber ratio was increased slightly.

Use of the spontaneously hypertensive rat (SHR) has been very extensive in research directed toward the relief of human hypertension. The SHR progressively develops increasing systemic blood pressure and hypertrophy. It provides a model in which the ventricular overload is gradually induced, in comparison to the acute onset of overload in experimentally induced situations. Several researchers have used the SHR as a model for ultrastructural

studies of hypertrophy (Kawamura et al., 1976; Imamura, 1978). These studies reported increased myocyte diameters, an increased myofibrillar percent volume, and a decreased relative volume of mitochondria.

Pressure induced changes in the myocardial interstitium have more commonly been examined using biochemical techniques. A number of papers report that the DNA content of the hypertrophying myocardium increases in proportion to the increase in total heart protein and that the increased DNA content is within the cells of the interstitium (Grimm et al., 1961; Morkin and Ashford, 1968; Grove et al., 1969). In addition, studies measuring the concentration of hydroxyproline, an amino acid specific to collagen in constant relative quantities, have found increased concentrations in pressure overloaded hearts, thus indicating some proliferative activity of connective tissue (Grove et al., 1969; Spann et al., 1971; Cutilletta et al., 1975; Medugorac, 1980).

The elasticity, gaseous exchange properties and other metabolic processes of the cardiac myocytes are directly dependent on the content of connective tissue in the myocardium (Chvapil, 1969). Thus, increases in connective tissue may compromise the ventricular myocardium during overload and hypertrophy.

Summary of Experimentally Induced Ultrastructural Alterations

Cardiac hypertrophy is regarded as one of the more common manifestations associated with chronic ventricular

overload. There is general agreement that increases in size and mass of the adult heart are a function of myocyte hypertrophy rather than hyperplasia. However, proliferation of the cells of the interstitium often is seen.

Cardiac myocytes respond to a chronic increase in hemodynamic workload by an increase in fiber length and diameter and by a disproportionate biosynthesis of organelles. There is general agreement that in volume overload the mitochondrial/myofibrillar ratio of the relative volumes shifts in favor of the energy producing structures. Under conditions of chronic pressure overload there is a very early period during which the mitochondrial/myofibrillar ratio increases, but the long term subcellular alterations result in a decreased ratio with increased relative volumes of the T-system and sarcoplasmic reticulum. The expanded T-system, continuous with the external sarcolemma, affords an increase in the total surface area of the myocyte, possibly minimizing any disturbance in the electrophysiological properties that may occur with the decreased surface area/cell volume ratio of the hypertrophied myocyte (Hodgkin, 1964). The expansion of the sarcoplasmic reticulum, which is directly concerned with the excitation-coupling reaction by the release and uptake/storage of calcium, may be in compensation for the reduced calcium binding capacity of the organelle that has been observed in hypertrophied myocytes (Sordahl et al., 1973; Ito et al., 1974).

The increase in the relative volume of myofibrillar material in pressure overloading indicates sarcomerogenesis in response to the increased stimulus. However, the fact that the mitochondrial component does not increase at the same rate would predispose the myocyte to potential failure due to a compromised energy producing system.

The opposite shifts in the mitochondrial/myofibrillar ratio in pressure and volume overload have not been satisfactorily explained. In each form of overload the wall tension of the ventricle is greater than for the unloaded chamber. Volume overload results in an increased end-diastolic volume and thus an increased chamber radius, whereas pressure overload results in greater intraventricular pressures during systole. In each case, there is an attempt to compensate for the chronic increase in wall tension by myocyte hypertrophy. However, the relative predominance of the contractile elements in pressure overload may be due to the fact that ventricular pressure work is far less efficient than volume work (Sarnoff et al., 1958; Coleman, 1971).

Exercise Induced Hemodynamic Overload

Long duration, low intensity exercise increases aerobic metabolism and heat production in skeletal muscle necessitating a greater blood flow to the working tissues. The heart responds with an increase in the rate and force of contraction mediated by sympathetic outflow and by circulating catecholamines. Augmentation of stroke volume concomitant with a faster rate results in a greater cardiac output to

satisfy the demands for oxygen, heat exchange and metabolite removal in the periphery. The greater venous return results in increased end-diastolic volumes causing volume overload in the ventricles.

The most commonly used animal model in studies of exercise has been the laboratory rat, although its high resting and maximal heart rates and other cardiovascular differences preclude it from being the ideal model. Rats usually are subjected to programs of running, in animal powered or motorized wheels or on motorized treadmills, or to programs of swimming, with or without weights attached to the animal.

The use of avoidance conditioning (electric shock) is the normal stimulus to ensure participation in a running program. However, the use of such stimuli may impose a psychological stress, with associated increases in circulating catecholamines and the hormones of the adrenal cortex, in addition to the physiological stress imposed by the exercise program. Enforced swimming programs place the animal in a life threatening situation which may produce an even more profound psychologic stress condition. Allowing the animals access to voluntary running wheels isolates the physiologic stress, but the intensity and duration of activity become uncontrollable variables. Therefore, the obvious advantages of enforced exercise programs are the control that the investigator has over the intensity and duration of the workload and the relative homogeneity of the work performance between animals.

There are many factors that will affect the degree of hemodynamic overload, both acute and chronic, in any exercise program. These include the intensity, duration and frequency of the exercise sessions, the rate at which the intensity of the program is increased, and the length of the entire program. In addition, the age and sex of the animal, as well as the specific strain of animal, may further affect the results of a study.

Myocardial Morphologic Alterations With Exercise

Left ventricular hypertrophy and dilatation are well-documented phenomena in conditioned athletes (Roeske, 1975; Nishimura et al., 1976) and in subjects engaged in chronic endurance exercise programs (Demaria et al., 1978). However, studies involving animal models have not demonstrated left ventricular hypertrophy in every instance.

Increases in absolute heart weight have been reported for both male rats (Leon and Bloor, 1968, 1976; Hepp, 1973; Steil et al., 1975) and female rats (Crews and Aldinger, 1967; Mandache et al., 1972; Ljungquist and Unge, 1973; Carlsson et al., 1978; Schaible and Scheuer, 1981) subjected to swimming programs. On the other hand, treadmill running has produced no increases in absolute heart weight in male rats (Van Liere and Northup, 1957; Penpargkul et al., 1980; Schaible et al., 1981; Nutter et al., 1981; Tharp and Wagner, 1982). The data reported for female rats subjected

to treadmill running are conflicting (Molé, 1978; Schaible et al., 1981). In the absence of increases in absolute heart weight, many authors report greater relative heart weights in the experimental animals. The ratio of heart weight to body weight frequently has been used as an index of cardiac hypertrophy. However, an increase in relative heart weight with no increase in the absolute value reflects the lesser gains in body weight found during animal training programs, especially in male rats (Oscai et al., 1971b). Female rats tend to increase their food intake to compensate for the greater energy requirements of the exercise program and, therefore, follow a normal growth pattern. Studies that have limited the food intake of sendentary males, so that they have similar growth rates to exercised counterparts, have consistently demonstrated greater absolute heart weights in the exercised animals (Oscai et al., 1971b; Penpargkul et al., 1980; Schaible et al., 1981).

Ultrastructural Alterations With Exercise

Kainulainen et al. (1979) subjected young adult mice to two programs of treadmill running. The workload per minute was the same for each group, but the more intense program comprised 3 bouts totalling 150 minutes/day, 7 days per week, for 30 days, and the more moderate program required a single bout of 60 minutes, 5 days/week, for 120 days. No values for body weight, heart weight or myocyte dimensions were reported. There were no changes in the relative volumes of

mitochondria, myofibrils, or sarcoplasm, or in the size and numerical density of the mitochondria. These results essentially agree with the data of Gollnick et al. (1971) and Paniagua et al. (1977). Arcos et al. (1968) reported no changes in mitochondrial mass in female rats that had swum for up to 6 hours per day for totals of 60-80 hours or 360-490 hours, but a large increase in mitochondria for animals that had accumulated 140-180 hours. These animals had the same oxygen consumption, expressed per milligram of mitochondrial dry weight, as the other two exercise groups indicating an increased oxidative capacity per gram of cardiac tissue. Absolute heart weights were greater in all the exercise groups than in a group of control animals.

Bozner and Meessen (1969) examined ultrastructural changes in the hearts of young female rats that had been subjected to a swimming program which ranged from 4 to 43 days in duration (11-180 hours of activity). Their data showed a large increase in the mitochondrial/myofibrillar ratio at 4 days and a gradual decline in the ratio thereafter. The ratio was still greater than control values after 43 days. The relative volume and numerical density of the mitochondria were increased at the end of the experimental period. They also reported a slight increase in the relative volumes of sarcoplasmic reticulum and the T-system. The absolute heart weights and myocyte diameters were greater in the animals that had exercised for 9 days (35 hours) up to 43 days (180 hours). These results would

appear to indicate that there is rapid adaptation or biogenesis of the energy producing component of the myocyte during the initial stages of the exercise program.

Another study using a relatively heavy swimming program was performed by Guski et al. (1980b) who exercised adult male rats, 3 hours per day, to produce program durations of 45 and 180 hours. In the 45-hour group, which was subjected to a gradually increasing bout duration from 30 to 180 minutes each day, there were significant increases in the mitochondrial/myofibrillar ratio and in the numerical density of the mitochondria as well as nonsignificant increases in mitochondrial size. In the 180-hour group, which swam for a constant amount of time during the second half of the program, the mitochondrial/myofibrillar ratio and the numerical density of mitochondria both decreased, although these values were still slightly greater than for the control animals. Mitochondrial size was slightly larger than that of the 45-hour group. A third group of animals parallelled the 180-hour group, but then had the bout duration gradually increased to 5 hours each day for a total program duration of 360 hours. These animals also demonstrated significantly increased values for both the mitochondrial/myofibrillar ratio and the mitochondrial density, although mitochondrial size returned to control values. The absolute heart weights and myocyte diameters increased in all the exercise groups. The authors postulated that certain myocardial parameters are altered in response

to an increasing workload and that when the workload is maintained at a constant level the values for these parameters regress towards control values. This hypothesis may provide an explanation for the results reported by Bozner and Meessen (1969). In their swimming program, the length of each bout was increased over the first 6 days and then was maintained or decreased slightly until the end of the program. Thus, the fact that the animals were maintained at a constant workload from day 6 until day 43 may explain the regression of the mitochondrial/myofibrillar ratio from the peak value reported at day 4.

Several studies have employed relatively short daily exercise programs to evoke myocardial adaptations. Kleitke et al. (1966) swam young male rats for 60-90 minutes per day. After 16 weeks the ratio of mitochondria/myofibrils was greater than for control animals despite the constant workload throughout the program. Guski et al. (1981) also demonstrated a greater mitochondrial/myofibrillar percent volume ratio and greater absolute heart weights in young male rats (150-170g at onset) that swam 1 hour each day up to an accumulated total of 40 hours. They reported a decrease in the mean size of mitochondria, due to an increase in the number of smaller organelles, and an increase in the numerical density of mitochondria. There were increases in the relative volumes of the sarcoplasmic reticulum and the Golgi apparatus and no change in the T-system.

There is still a great deal of controversy concerning the changes in the mitochondrial component of the cardiac myocyte with endurance exercise. Studies that have analyzed the concentration of cytochrome *c*, a mitochondrial marker, to indicate changes in mitochondrial mass have shown no changes in the hearts of rats subjected to various exercise programs. Oscai et al. (1971a, 1971b) subjected male rats to 12 weeks of running for 2 hours per day, and to 30, 60 or 180 minutes of swimming per day for up to 6 weeks. Female rats were swum for 6 hours each day until they accumulated a total of 162 hours. No changes were observed in the concentration of cytochrome *c*, or in the activity levels of selected respiratory enzymes, in any of the exercise groups. Since the work duration in this study was maintained at constant levels for all groups, it is possible the mitochondrial component increased significantly at the onset of the programs and then regressed to the levels of the control animals by the time the tissue samples were taken and analyzed, as suggested by Guski et al. (1980b). However, the study of Hickson et al. (1979) would appear to refute this theory. These authors subjected adult female rats (6 months at onset) to 6 hours of swimming per day, 7 days per week. Groups of animals were sacrificed after 1,2,3,5, 7,14,21 and 28 days. There were no changes in the mitochondrial content, as reflected by the concentration of cytochrome *c*, in any of the groups, even in those animals

that could be assumed to be adapting to the newly imposed heavy workload.

Edington and Cosmos (1972) exercised adult male rats on a treadmill, 70 minutes/day, for 16 weeks at a constant workload and produced increases in both absolute and relative heart weights. Analysis showed no changes in the total protein content of the heart or in the mitochondrial fraction of protein. However, ultrastructural examination revealed a shift in the size distribution towards smaller mitochondria.

It would appear that mitochondrial biogenesis in cardiac myocytes is an established phenomenon, whether as a transitory phase in pressure overload or as a long term result of experimentally induced chronic volume overload. The contradictory findings when endurance exercise is employed as the stimulus for ventricular overload apparently have not been explained at present.

There are conflicting data concerning the effects of exercise on the capillaries of the ventricular myocardium. Increases in capillary density (CD) and capillary/fiber ratio (CF) have been reported for the conditioned rat heart (Leon and Bloor, 1968, 1976; Bloor and Leon, 1970; Tomanek, 1970; Bell and Rasmussen, 1974). However, Tharp and Wagner (1982) reported decreased CD and CF in young adult male rats exposed to 8 weeks of treadmill running under 8 different training regimens. These latter results support

the data reported by Frank (1950) and Hakkila (1955) in the guinea pig.

A decrease in the *in vivo* capillary density would not appear to be advantageous to the functioning myocardium. The capillary density determines the diffusion distance for substances such as oxygen. A decrease in CD causes an increase in the diffusion distance, which results in a decrease in the myocardial oxygen pressure (Rakusan, 1971). Furthermore, the decrease in oxygen pressure is more profound if the greater diffusion distance is accompanied by any condition that causes an elevation of myocardial oxygen consumption and/or a decrease in myocardial blood flow (Rakusan, 1971). Thus, if the decreased CD and CF reported previously in conditioned animals are a true reflection of the *in vivo* state, then the myocardium of these animals would appear to have been compromised rather than to have benefited from the exercise regimen.

Several investigators have used *in vivo* injections of ^{3}H-thymidine to provide a measure of DNA synthesis in the myocardium. Autoradiographic analyses have demonstrated significantly greater nuclear incorporation of the thymidine into the capillary endothelial cells of rats subjected to swimming programs (Mandache et al., 1972, Ljungquist and Unge, 1973, 1977; Carlsson et al., 1978; Unge et al., 1979). These results would appear to be indicative of capillary

neoformation, which would support the earlier findings of increased CD and CF as demonstrated by histologic techniques.

The work of Guski (1980) provides the only known quantitative stereologic study of the effects of endurance exercise upon the interstitium. Adult male rats were subjected to the swimming program described previously (Guski et al., 1980a). There were progressive increases in absolute and relative heart weights and a moderate increase in the mitochondrial/myofibrillar ratio. The relative volume of the interstitium was increased from an initial value of 15% to 17% after 45 hours, to 19% after 180 hours, and to 22% after 360 hours with concomitant decreases in the relative volume of myocytes. Within the interstitium (100%) the capillaries, comprising lumen and endothelium, demonstrated an increase in percent volume from a control value of 41% to 44% after 45 hours and a subsequent decrease to 32% after 180 and 360 hours. The relative volume of extracellular space showed opposite changes of equal magnitude, and the nonvascular interstitial cells remained at a constant value of 5-6%. When the reference space used was the entire myocardium (100%) rather than just the interstitium, the relative volume and the numerical density of the capillaries both increased after 45 hours of swimming, decreased slightly after 180 hours when the workload was held constant, and increased again in the second half of the program as the work duration was again lengthened.

However, peak values for both measures were obtained after 45 hours during the initial adaptation to the exercise program. The data for capillary percent volume and numerical density appear to substantiate the findings demonstrated by light microscopy and autoradiography with regard to increased capillary densities and capillary neoformation.

The increase in the extracellular space of the interstitium after 180 and 360 hours was determined qualitatively to result from an increase in the ground substance and/or water deposits and not from an increase in the fine collagen fibers. This is in agreement with studies that have assayed exercised hearts for hydroxyproline as an indicator of collagen content. Various programs of running in young male mice (Kiiskinen and Heikkinen, 1976; Kainulainen et al., 1982) and swimming in young male rats (Medugorac, 1980) and adult female rats (Hickson et al., 1979) produced no increase in the relative collagen content of the myocardium, even with the demonstration of increased absolute heart weight.

The Effects of Exercise on Left Ventricular Performance

Recent advances in the fields of echocardiography and radionuclide angiography have led to increased knowledge of the effects of exercise conditioning on resting and exercising ventricular dimensions, wall motion and contractile factors in human subjects.

Increases in left ventricular mass have been found in trained endurance athletes and endurance conditioned subjects (Morganroth et al., 1975; Roeske et al., 1976; Allen et al., 1977; Underwood and Schwade, 1977; Longhurst et al., 1981). At rest, left ventricular end-diastolic volume (LVEDV) and stroke volume are increased with no change in ejection fraction (Morganroth et al., 1975; Gilbert et al., 1977; Underwood and Schwade, 1977; Zoneraich et al., 1977). During moderate exercise, echocardiography revealed no change in LVEDV, but there is an increased stroke volume that is mediated by a decreased end-systolic volume (Sharma et al., 1976; McLaughlin et al., 1977; Stein et al., 1978, 1980). These results suggest that stroke volume during moderate exercise in the trained individual is a function of enhanced myocardial contractility rather than elicitation of the Frank-Starling effect. This hypothesis was confirmed by Bar-Shlomo et al. (1982) using radionuclide angiography. They demonstrated that during graded exercise to exhaustion, well-conditioned athletes augment stroke volume by greater systolic emptying rather than by increasing end-diastolic volume. The authors suggested that well-conditioned athletes may have decreased diastolic compliance, which would be antagonistic to end-diastolic dilatation, and also enhanced myocardial contractility.

The study of the isolated perfused heart, the *in situ* perfused heart and ventricular muscle preparations have enabled investigators to examine additional parameters of

the exercised myocardium. Using the isolated heart preparation, Schaible et al. (1979, 1981) demonstrated increased stroke volume, stroke work, and ejection fraction in male rats conditioned by running. LVEDV was not different from control values. Female rats subjected to the same program did not differ from control animals. However, when female rats were subjected to a swimming program, the isolated heart preparations demonstrated greater ejection fractions and a greater velocity and extent of shortening of the circumferential ventricular fibers under varying preloads and constant atrial pacing (Schaible and Scheuer, 1981).

Using both isolated and *in situ* perfused preparations, Fuller and Nutter (1981) examined the hearts of young and adult male rats trained by a moderate running program. The exercised animals showed no changes in ventricular function parameters. These results are in agreement with those of Cutilletta et al. (1979) for rats trained by running and with those of Carew and Covell (1978) obtained in trained dogs. Examination of the contractile characteristics of isolated papillary muscle failed to show any change in contractility as demonstrated by peak developed isometric tension, maximum rate of developed tension (dT/dt_{max}), and length-active tension relationships (Grimm et al., 1963; Williams and Potter, 1976; Nutter et al., 1981). Conversely, Whitehorn and Grimmenga (1956) and Molé (1978), with isolated papillary muscle, and Crews and Aldinger (1967),

with the *in situ* heart, have reported enhanced contractile properties in the conditioned heart.

Despite the continuing controversy as to whether the chronically exercised myocardium possesses enhanced characteristics of contractility and performance, it would seem certain that the heart is not compromised by chronic exercise even in the presence of hypertrophy and chamber dilatation. In a comprehensive review of cardiac hypertrophy, Wikman-Coffelt (1979) defines nonpathologic hypertrophy as changes in wall and chamber dimensions accompanied by a normal or augmented contractile state in which the maximum velocity of muscle shortening (V_{max}) and the rate of ATP hydrolysis by myosin ATPase are normal or elevated. No decreases in either of these states have been reported by investigators examining the effects of chronic exercise on the normal myocardium. Indeed, Molé and Raab (1973) demonstrated increases in maximum developed tension, V_{max}, and dT/dt_{max} in exercised rats. Increases in myosin ATPase activity have been reported in the hearts of animals conditioned by chronic exercise programs (Barany, 1967; Wilkerson and Evonuk, 1971; Bhan and Scheuer, 1972, 1975; Malhotra et al., 1976).

CHAPTER III

RESEARCH METHODS AND MATERIALS

This study was undertaken to determine the effects of 12 weeks of voluntary exercise on the ultrastructure and microvasculature of the left ventricular myocardium in the male albino rat.

Experimental Animals

Twenty normal male albino rats (Sprague-Dawley strain) were obtained from Harlan Industries, Indianapolis, Indiana. Twelve of the animals were approximately 70 days old (300g) and eight were approximately 30 days old (65g) upon arrival.

Treatment Groups

From each age group, animals were randomly assigned to a sedentary control (SED) group or to a voluntary exercise (VOL) group.

Sedentary Group. The sedentary control animals were housed in standard individual sedentary cages (24cm x 18cm x 18cm) throughout the experimental period and received no special treatment.

Voluntary Exercise Group. The voluntary exercise animals were housed in individual voluntary activity cages throughout the experimental period. Each animal had

24-hour access to a freely revolving running wheel (13cm wide x 35cm diameter). Individual records of the total revolutions run (TRR) during each 24-hour period were taken from revolution counters attached to each wheel.

Animal Care

During the experimental period, all animals were housed in the vivarium of the Human Energy Research Laboratory under relatively constant environmental conditions. The diurnal cycle of the animals was adjusted so that the hours of darkness were from 3:00 p.m. to 3:00 a.m. every day. All routine laboratory procedures such as daily handling and weighing, cage and food maintenance, and recording of TRR were performed in the hour immediately preceding the onset of darkness. Throughout the treatment period, all animals had access to food (Wayne Laboratory Blox) and water *ad libitum*.

In an effort to obviate the effects of light source and door position, the positions of the racks of cages and the location of each animal's cage within a rack were rotated every week. Each animal was handled on a daily basis.

Sacrifice Procedures

Animal sacrifices were performed 12 weeks after the initiation of the study. The voluntary exercise animals were not permitted access to the running wheels for the

24-hour period preceding sacrifice. The animals were randomly assigned to a sacrifice order, alternating between treatment groups, and final body weights were obtained for each animal.

Each animal was anesthetized by an intraperitoneal injection (4mg/100g body weight) of a 6.48% solution of sodium pentobarbital (Nembutal). A midline abdominal incision was performed, the xiphoid process was clamped with a hemostat, and a ventrolateral incision was made on either side of the thorax to permit cranial reflection of the ventral thoracic wall. With the heart and great vessels exposed, a 14-gauge needle connected to a perfusion apparatus was inserted into the left ventricular chamber through the apical wall. A pre-wash solution of 1% heparin in normal saline was administered through the perfusion apparatus. This was followed by perfusion with cacodylate buffered 3% glutaraldehyde (pH 7.2-7.4). At the onset of perfusion, the abdominal aorta was clamped and an incision was made in the right atrium.

The recommended perfusion pressure for the hearts of rats or mice is 105-120mmHg (Hayat, 1981). However, the brains of the animals in the present study were to be used for a companion investigation. Since the recommended perfusion pressure for the rat brain is 150mmHg (Hayat, 1981), the animals in the present study were perfused at 140-150mmHg.

Following 5 minutes of perfusion, the heart was excised and immersed in a beaker of fresh buffered glutaraldehyde solution for 30 minutes. The heart was trimmed of the great vessels, and the weights of the total heart, right ventricular free wall, left ventricle (free wall plus septum), and atria were obtained. Six tissue blocks were cut from the middle portion of the left ventricular free wall and stored in fresh solutions of buffered glutaraldehyde at 4°C overnight.

The tissue samples were washed three times in 0.1M sodium cacodylate buffer (pH 7.2-7.4) and post-fixed for 30 minutes with 1% buffered osmium tetroxide in 0.1M sodium cacodylate. The tissue samples were put through a further cycle of washings in the buffer solution and then dehydrated with graded concentrations of 70, 80, 90, 95 and 100% alcohol. Three immersions of 20 minutes each were performed in 100% alcohol. Two immersions of 15 minutes each were performed in the other concentrations.

Infiltration was achieved by three immersions of 20 minutes each in 100% propylene oxide followed by 2 hours in an equal mixture of propylene oxide and plastic. Further infiltration was performed for 2 hours in a 1:3 mixture of propylene oxide and plastic, after which the tissue samples were left in plastic overnight. Final embedding in plastic was done in flat molds, and the blocks were polymerized at 60°C for 48 hours. The composition of the plastic used

during all stages of infiltration was: Epon 812, 10ml; Araldite 502, 10ml; dodecenylsuccinic anhydride (DDSA), 23ml; tri-(dimethylaminomethyl)-phenol (DMP-30), 2ml.

Electron Microscopy

Two tissue blocks from each animal were randomly selected for microscopic examination. Sections of 1μm thickness were cut with glass knives on a LKB III Microtome, mounted on glass slides and stained with toluidine blue. These sections were examined by light microscopy for suitability and orientation as well as for histometric analysis. Once it had been established that the tissue was cut in cross section, thin sections demonstrating silver or grey interference colors (approximately 750 Å) were cut and mounted on 200-300 mesh copper grids. The sections were stained for 15 minutes in a 1% uranyl acetate solution containing 1.5% potassium ferrocyanide and for 2 minutes in Reynolds lead citrate solution. All sections were taken from the subepicardial region of the tissue. The thick sections for light microscopic evaluation immediately preceded the thin sections cut for electron microscopy.

The following criteria were used to ensure that the tissue had been cut in cross section: myocyte striations were absent or minimal; myocyte mitochondria were distributed randomly and in a relatively homogenous manner; capillaries appeared circular rather than oval.

Electron microscopy was performed on a Philips 201 electron microscope. The method employed for obtaining electron micrographs was the systematic sampling procedure of Weibel (1979, pp. 82-84). The photographic field was aligned in a predetermined corner of a space in the supporting copper grid. The micrographs were taken from successive grid spaces until a minimum of 10 micrographs had been obtained from a section. One section from each tissue block was photographed. This procedure resulted in a minimum total of 20 electron micrographs for each animal. The micrographs were taken at an initial magnification of X3000. Periodic calibration was performed using a Fullam carbon grating replica with 21,600 lines/cm. The micrographs were printed on Kodak Polycontrast rapid II RC-F paper (20.3cm x 25.4cm) at a final magnification of X8250.

Tissue Analyses

The electron micrographs and the histologic slides were examined using stereologic and histometric techniques respectively in order to ascertain any differences that might exist between the sedentary and the voluntary exercise animals.

Stereologic Techniques

Due to practical limitations, a single magnification of the tissue was used for the stereologic analyses. A final magnification of X8250 was selected in order to permit

examination of myocyte components and interstitial compartments from the same micrographs. The relative volumes of various structures were determined by the method of point counting (Weibel, 1979). A transparent sheet with a square lattice imprinted on it was overlaid on each micrograph. The horizontal and vertical line intersections produced a regular array of equally spaced points, as may be seen in Figure 1.

Since only a single magnification was to be used for the analyses, and the myocardial components to be examined varied greatly in size, distribution, and relative volume, it was necessary to select a lattice test system that would achieve the greatest overall precision for the structures in question. The level of precision adopted for the present study was defined as ± 10% of the mean at the 95% level of confidence. From the procedures of Weibel (1979, pp. 96-100, 114-116) it was determined that a test system of 800 points (line spacing = 0.75cm) applied to 10 micrographs would provide a compromise between the precision requirements for the volume estimates of myofibrils, mitochondria, and the interstitial compartments. It should be noted that a higher magnification of micrograph, and possibly a different test system, would have been required in order to provide sufficient precision in the analysis of the matrix (sarcoplasmic reticulum, T-system and free sarcoplasmic space). The complete procedure for

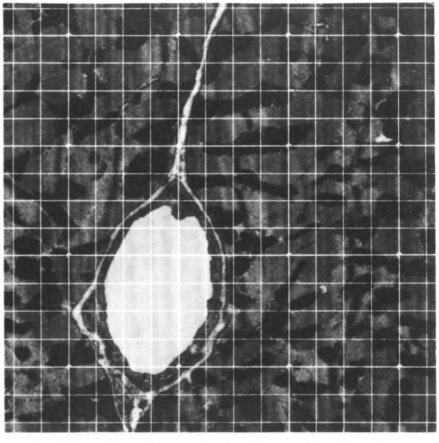

Figure 1. Part of an electronmicrograph with the lattice test system photographically superimposed. (X8250)

selecting the appropriate lattice test system is given in Appendix D.

A point space of 0.75cm corresponds to 0.909μm on electron micrographs printed at X8250. The area of tissue covered by the lattice test system on each micrograph was 661 sq. μm.

Point counting was performed by totalling the number of points falling on profiles of a specific component observed on the micrograph. The relative volume, $V_{Vi,c}$, of a component was calculated from the relationship $V_{Vi,c} = V_i/V_c = P_i/P_c$; where V_i and V_c are the volumes of a component i and a reference space c respectively, P_i is the number of points falling on profiles of the component i, and P_c is the total number of points falling in the reference space c.

The following primary data were obtained from the electron micrographs.

P_{int}, P_{cel}, P_{mit}, P_{mf}, P_{mat}, P_{evs}, P_{endo}, P_{lum}; where int = interstitium, cel = myocyte, mit = mitochondria, mf = myofibrils, mat = matrix (T-system, sarcoplasmic reticulum and sarcoplasm), evs = extravascular space of the interstitium, endo = endothelium, lum = capillary lumen.

From these primary point counts, the following parameters were derived.

$V_{Vint,myo}$ = % volume of interstitium in myocardium

$V_{Vcel,myo}$ = % volume of myocytes in myocardium

$V_{Vevs,int}$ = % volume of extravascular space in interstitium

$V_{Vevs,myo}$ = % volume of extravascular space in myocardium

$V_{Vcap,int}$ = % volume of capillaries in interstitium

$V_{Vlum,int}$ = % volume of capillary lumen in interstitium

$V_{Vendo,int}$ = % volume of capillary endothelium in interstitium

$V_{Vcap,myo}$ = % volume of capillaries in myocardium

$V_{Vmf,cel}$ = % volume of myofibrils in myocytes

$V_{Vmit,cel}$ = % volume of mitochondria in myocytes

$V_{Vmat,cel}$ = % volume of matrix in myocytes

P_{mit}/P_{mf} = mitochondrial/myofibrillar ratio

N_{Amit} = numerical density of mitochondria/unit area of myocyte

Histometric Techniques

Absolute values for both capillaries and myocytes were determined from 1μm thick sections of myocardium mounted on glass slides. The tissue was examined under a Nikon model SBR-Kt light microscope equipped with a mechanical stage. An ocular reticle (Bausch & Lomb 31-16-12) with a square grid (1mm line spacing) delineated a tissue area of 0.0306 sq. mm at a magnification of X400. Successive fields, with no overlap, were brought into view and the absolute numbers of capillary and myocyte profiles were determined in each field.

The following parameters were obtained from the profile counts.

CD = capillary density (# capillaries/unit area of myocardium)

FD = fiber density (# fibers/unit area of myocardium)

CF = capillary/fiber ratio

All of the stereologic and histometric analyses were performed blind by the same individual.

Statistical Analyses

No comparisons were made between the two age groups since the amount of activity performed was not controlled and varied between the two groups. Values for all stereological and histometric parameters were compared between the sedentary and the exercised groups using the Student t-test. The 0.05 level of significance was established for all of the analyses.

Since practical limitations necessitated a small sample size and the use of a single magnification of the tissue under electron microscopy, it should be noted that the probability of making a type II error was increased, thus decreasing the power of some of the statistical comparisons.

CHAPTER IV

RESULTS AND DISCUSSION

Results

The results of this study are presented in the following order; voluntary activity data from the running wheels, data on body weight and heart weight, data from the stereologic analyses of the myocardium, and finally data from the histometric analyses of the myocardium.

Voluntary Activity

The activity of the VOL groups is shown in Figure 2 in terms of the mean distance run per day for each week of the study. It can be seen that each group gradually increased the distance run per day during the course of the investigation.

Individual performances may be found in Table 2. These data show that whereas some animals maintained a relatively low level of activity throughout the study, the remainder generally increased their activity. However, a slight decline in the distance run per day may be seen toward the end of the experimental period in some of these latter animals. It should be noted that the apparent high level of activity demonstrated by the prepubertal group during

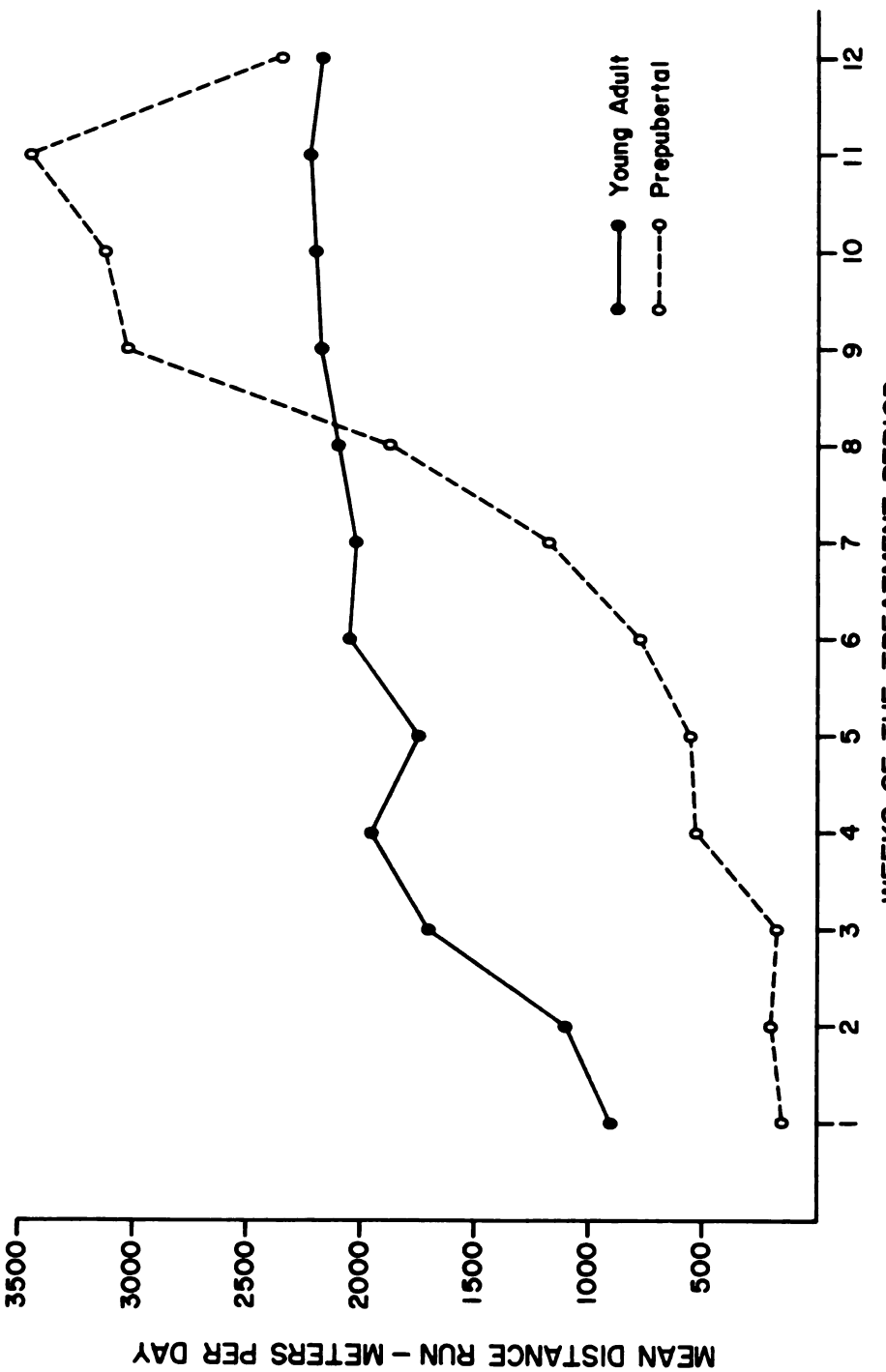

Figure 2. Voluntary activity performed during the study

Table 2. Voluntary Activity Recorded as Mean Distance Run Per Day (Meters) for Each Week.

Week	Prepubertal				Young Adult					
	K1	K2	K4	K5	B1	B2	B3	B4	B5	B6
1	328	238	142	59	997	1304	333	930	580	1258
2	484	112	94	78	888	1576	479	697	1339	1652
3	287	223	127	76	1133	2661	558	2261	1386	2153
4	959	792	310	28	1294	2665	559	3284	1554	2308
5	961	691	376	195	1042	3182	531	2274	1157	2375
6	1090	706	642	693	1151	2584	573	3757	1608	2596
7	1728	754	979	1226	1164	3214	510	3663	1080	2600
8	1301	815	2151	3237	806	5059	362	2959	816	2669
9	3252	979	2357	5526	791	5215	402	3705	661	2224
10	4896	697	2776	4128	706	4375	375	4019	640	2949
11	3029	799	2453	7698	638	3304	381	3340	688	4855
12	2324	845	2041	4186	768	3374	387	3110	669	4716

weeks 10-12 (Figure 2) is actually a reflection of the activity of animal K1 during week 10, and the activity of animal K5 during weeks 9 and 11.

The level of activity performed by the animals in this study would appear to be unrelated to age and is unique to each individual animal.

Body Weight and Heart Weight Results

The results for initial and final body weight and the results for absolute and relative heart weight, ventricle weight and left ventricle weight are shown in Tables 3-4.

Table 3. Body Weights and Heart Weights of Prepubertal Animals.

	SED (n=4)	VOL (n=4)	
Initial Body Weight (g)	65.50± 1.44	67.25± 2.46	
Final Body Weight (g)	411.75±11.98	337.25±33.34	S
Heart Weight (g)	1.300± 0.044	1.339± 0.071	
Ventricle Weight (g)	1.167± 0.039	1.191± 0.061	
Left Ventricle Weight (g)	0.923± 0.046	0.934± 0.038	
Relative Heart Weight ($X10^3$)	3.157± 0.049	4.018± 0.169	S
Relative Left Ventricle Weight ($X10^3$)	2.239± 0.069	2.812± 0.147	S

S = Significance $p < .05$
All values are mean ± SEM

Table 4. Body Weights and Heart Weights of Young Adult Animals

	SED (n=6)	VOL (n=6)	
Initial Body Weight (g)	298.67± 2.74	304.17± 7.93	
Final Body Weight (g)	593.17±11.84	535.67±24.13	S
Heart Weight (g)	1.984± 0.043	2.041± 0.067	
Ventricle Weight (g)	1.852± 0.056	1.905± 0.063	
Left Ventricle Weight (g)	1.185± 0.011	1.196± 0.038	
Relative Heart Weight ($X10^3$)	3.348± 0.070	3.837± 0.163	S
Relative Left Ventricle Weight ($X10^3$)	2.001± 0.040	2.242± 0.059	S

S = Significance $p < .05$
All values are mean ± SEM

Body Weight

The initial body weights did not differ between the SED and the VOL animals in either of the age groups. However, the SED groups weighed significantly more than their VOL counterparts at the end of the experimental period.

Heart Weight

There were no significant differences in total heart weight, ventricle weight, or left ventricle weight between the treatment groups in either the prepubertal or the young adult animals. However, when the data were expressed in terms of body weight, both of the VOL groups had

significantly greater relative heart weights, relative ventricle weights, and relative left ventricle weights, than the SED animals.

Stereologic Analyses

The relative volumes, derived from the primary morphometric data, are summarized here for the total myocardium, the interstitium, and the myocytes.

Myocardium

Similar changes were observed in the relative volume composition of the myocardium in both groups of VOL animals (Tables 5-6). The relative volume of the interstitium was significantly greater in the VOL animals, with a concomitant decrease in the percent volume of the myocytes. The relative volume of the extravascular space was significantly greater in the young adult VOL animals. However, no significant differences were observed in the extravascular space of the prepubertal VOL animals, or in the capillary component for either age group of the active animals.

Interstitium

The relative volumes of the interstitial components (interstitium = 100%) are shown in Tables 7-8. No significant differences were observed between the SED and the VOL groups at either age level.

Table 5. Relative Volume Composition of the Myocardium - Prepubertal Animals

	SED (n=4)	VOL (n=4)	
Total Points Counted	31272	30333	
Tissue Area Sampled (sq.μm)	25840	25064	
Percent Volumes of Myocardial Components:			
Myocytes	86.523±0.872	84.362±0.601	S
Interstitium	13.477±0.872	15.638±0.601	S
Capillaries	6.423±0.532	7.315±0.339	
Extravascular Space	7.055±0.406	8.323±0.529	

S = Significance $p < .05$
All values are mean ± SEM

Table 6. Relative Volume Composition of the Myocardium - Young Adult Animals

	SED (n=6)	VOL (n=6)	
Total Points Counted	47848	47489	
Tissue Area Sampled (sq.μm)	39537	39240	
Percent Volumes of Myocardial Components:			
Myocytes	85.018±0.562	82.185±1.032	S
Interstitium	14.982±0.562	17.815±1.032	S
Capillaries	6.979±0.324	7.960±0.560	
Extravascular Space	8.003±0.383	9.856±0.707	S

S = Significance $p < .05$
All values are mean ± SEM

Table 7. Relative Volume Composition of the Interstitium - Prepubertal Animals

	SED (n=4)	VOL (n=4)
Extravascular Space	51.811±1.375	52.664±1.739
Capillaries	48.190±1.375	47.337±1.739
Endothelium	15.545±0.360	15.104±1.299
Lumen	32.645±1.313	32.212±1.050

All values are mean ± SEM

Table 8. Relative Volume Composition of the Interstitium - Young Adult Animals

	SED (n=6)	VOL (n=6)
Extravascular Space	53.407±1.400	55.496±1.229
Capillaries	46.593±1.400	44.504±1.229
Endothelium	14.814±0.780	12.929±0.777
Lumen	31.780±1.191	31.575±0.783

All values are mean ± SEM

Myocytes

Relative volume composition of the myocytes is shown in Tables 9-10. Similar changes were observed in both groups of VOL animals. The mitochondrial/myofibrillar ratio increased significantly due to a significant decrease in the percent volume of myofibrils and an increase in the relative volume of the mitochondria that was

Table 9. Relative Volume Composition of the Myocytes - Prepubertal Animals

	SED (n=4)	VOL (n=4)	
Myofibrils	57.245±1.015	54.174±0.738	S
Mitochondria	33.956±0.806	36.119±1.035	
Matrix	8.800±0.293	9.707±0.373	
P_{mito}/P_{mf}	0.594±0.024	0.668±0.028	S
Area/Mitochondrial Profile (sq. μm)	0.442±0.052	0.418±0.042	
Mitochondrial Density/ sq. μm	0.766±0.088	0.863±0.068	S

S = Significance $p < .05$
All values are mean ± SEM

Table 10. Relative Volume Composition of the Myocytes - Young Adult Animals

	SED (n=6)	VOL (n=6)	
Myofibrils	58.358±0.528	54.038±0.525	S
Mitochondria	33.133±0.378	36.751±0.660	S
Matrix	8.509±0.192	9.211±0.232	
P_{mito}/P_{mf}	0.568±0.012	0.681±0.019	S
Area/Mitochondrial Profile (sq. μm)	0.453±0.022	0.426±0.035	
Mitochondrial Density/ sq. μm	0.734±0.037	0.812±0.054	S

S = Significance $p < .05$
All values are mean ± SEM

significant in the young adult animals. No significant differences were observed in the matrix, comprising the sarcoplasmic reticulum, the T-system and free sarcoplasmic space, between the treatment groups at either age level.

The increase in the percent volume of the mitochondria manifested itself as an increase in the number of mitochondrial profiles per unit area of sarcoplasm. The size of the organelles did not differ significantly between the treatment groups.

Histometric Results

The densities/mm^2 of both the capillaries and the myocytes, and the capillary/fiber ratios, are shown in Tables 11-12. No significant differences were observed between the SED and the VOL animals for either capillary or fiber density. However, the capillary/fiber ratio was significantly greater in the VOL animals in both age groups.

Table 11. Capillary and Fiber Densities of Prepubertal Animals

	SED (n=4)	VOL (n=4)	
Capillaries/mm^2	3707.52±82.42	3709.97±89.42	
Fibers/mm^2	3486.93±75.53	3459.15±15.92	
Capillary/Fiber Ratio	1.06±0.002	1.08±0.002	S

S = Significance $p < .05$
All values are mean ± SEM

Table 12. Capillary and Fiber Densities of Young Adult Animals

	SED (n=6)	VOL (n=6)	
Capillaries/mm^2	3309.37±34.07	3313.53±38.36	
Fibers/mm^2	3125.27±31.33	3100.76±38.00	
Capillary/Fiber Ratio	1.06±0.003	1.07±0.003	S

S = Significance $p < .05$
All values are mean ± SEM

Discussion

The amount of voluntary activity that each VOL animal performed was relatively consistent on a day-to-day basis throughout the course of the treatment period. However, there was extreme variability between animals. The mean distance run per 24-hour period throughout the study was slightly less than the values reported by Jaweed et al. (1974) and Lamb et al. (1969), but was considerably greater than the values of Jones et al. (1953). The mean daily distance run gradually increased during the course of the investigation for each of the VOL groups. This pattern does not agree with the results of previous studies that demonstrated activity curves with peak values occurring 4-5 weeks after the commencement of voluntary activity (Richter, 1922; Jones et al., 1953) or that reported a continual decline in activity levels from the onset of the treatment period (Hanson et al., 1966). However,

individual animals did demonstrate activity patterns that corresponded to the group curves of Jones and his coworkers.

In the present study the voluntarily active animals failed to increase their body weight at the same rate as the sedentary animals, although heart weight and ventricular weight did not differ between the two groups. These results are in accord with those reported for male rats subjected to enforced running (Whitehorn, 1956; Oscai et al., 1971b; Tomanek, 1970; Dowell et al., 1976; Penpargkul et al., 1980; Nutter et al., 1981; Schaible et al., 1981) and for prepubertal male rats permitted to run voluntarily (Lamb et al., 1969). Thus, the significantly greater relative values for heart weight and ventricle weight that are reported here were due to the lower body weights of the active animals. However, these results are not in agreement with other studies that have subjected male rats to voluntary running. These studies have reported normal body weight gains (Hanson et al., 1966), normal body weight gains and increased absolute heart weights (Hatai, 1915), and greater than normal body weight gains with increased absolute heart weights (Jaweed et al., 1974).

It has been demonstrated that male rats do not increase their food consumption to meet the caloric requirements of increased activity (Nance, 1977; Nutter et al., 1981). This may be the explanation for the lesser body weight gains observed in the active animals in the

present study, although this cannot be substantiated since no measure was made of food intake.

The stereologic analysis of the ventricular myocardium of the sedentary animals revealed lower values for the percent volume of interstitium than those previously reported for young sedentary rats by Anversa et al. (1975, 1976, 1978). However, the values are in close agreement with those of Guski (1980) obtained by electron microscopy and are supported by the results obtained by light microscopy (Laguens, 1971; Reinhold-Richter, 1978). In the active animals the percent volume of interstitium was significantly increased, the greater volume being attributable to an increase in the percent volume of extravascular space that was significant in the young adult animals, and a lesser increase in the relative volume of capillaries. Although the tissue magnification and the lattice test system employed in the present study did not fully meet the precision requirements for the interstitial compartments, thus reducing the power of the statistical comparisons, the changes reported here for the extravascular space and the capillaries are similar to those demonstrated by Guski (1980). This author further suggested that the relative enlargement of the extravascular space was due to an increase in ground substance and/or water deposits rather than an increase in the collagen content of the interstitium. This hypothesis is supported by studies

that assayed conditioned hearts for hydroxyproline as an indicator of collagen content (Kiiskinen, 1976; Hickson et al., 1979; Medugorac, 1980; Nutter et al., 1981; Kainulainen, 1982).

The absence of any significant changes in the percent volume of the capillaries in both groups of active animals in the present study was reflected in the results of the histometric analyses. These data indicated an increased capillary/fiber ratio in the active animals resulting from a slight decrease in the myocyte density and no change in the capillary density. There are two major factors that may cause a decrease in the fiber density. The first is an increase in the interstitial compartment of the myocardium, and the second is an increase in the cross-sectional area of the myocytes. The stereologic analyses of the ventricular myocardium in this study indicated an increase in the relative volume of the interstitium. Whether myocyte hypertrophy occurred is unknown since absolute measurement of myocyte diameters was not undertaken. However, it cannot be determined from the available data whether the increase in the relative volume of the interstitium would account wholly for the nonsignificantly larger ventricular weights found in both groups of active animals. It is therefore possible that the decrease in the density of the myocytes was a result of slight fiber hypertrophy and an increase in the interstitial compartment. If the decreased myocyte density resulted entirely

from one of the above factors, or from a combination of both, and no new capillaries were formed as a result of exercise, one would expect to observe a decrease in the capillary density that paralleled the decrease in fiber density. However, no changes were observed in the capillary density in either age group of active animals. In light of the autoradiographic findings that indicated endothelial cell proliferation in the ventricular myocardium of exercised animals (Mandache et al., 1972; Ljungquist and Unge, 1973, 1977; Carlsson et al., 1978; Unge et al., 1979), the possibility exists that capillary neoformation occurred in the present study sufficient to maintain the capillary density and to increase the capillary/fiber ratio.

It should be noted that most studies that employed enforced exercise have reported significant increases in the capillary density and much larger increases in the capillary/fiber ratio than were observed in the present study (Leon and Bloor, 1968, 1976; Bloor and Leon, 1970; Tomanek, 1970; Bell and Rasmussen, 1974). This difference in magnitude may well be the result of differences in the intensity and duration of the conditioning stimulus. It has been suggested by Hudlicka (1982) that hypoxia and/or increased coronary blood flow are major factors involved in capillary neoformation. If the amount of capillary growth is proportional to the degree of hypoxia or

enhanced coronary blood flow, then voluntary running would, in all probability, result in lower changes in capillarization than forced exercise since voluntary running presumably is conducted at a lesser intensity than enforced activity.

In adult animals neocapillarization occurs in response to certain physiologic and pathologic stimuli. This subject has been extensively reviewed by Folkman and Cotran (1976). Endothelial proliferation has been demonstrated in the endometrium during the ovulatory cycle and around hair follicles during the hair growth cycle. In addition, endothelial proliferation occurs during wound healing, inflammatory granulation tissue formation, and tumor growth. Although the mechanisms of endothelial proliferation are uncertain, factors such as oxygen tension, local hypoxia, metabolite accumulation, and secretions from various cells have been linked with the process in wound healing. The neovascularization associated with tumor growth appears to be mediated by a secretion from the tumor cells. This tumor angiogenesis factor (TAF) is capable of diffusing through tissues to act on existing vasculature that lies 2-5mm from the boundary of the tumor. In view of these findings, normal tissues have been tested for angiogenic properties. Only cells from the salivary gland and the kidney of the adult mouse have demonstrated such properties at this time. However, the

possibility that striated muscle synthesizes and secretes an angiogenic factor cannot be completely disregarded.

Chronic endurance exercise has been shown to increase capillary density and the capillary/fiber ratio in skeletal muscle from young rats (Carrow et al., 1967; Adolfsson et al., 1981) and from humans (Andersen, 1975; Andersen and Henriksson, 1977). Muscle fiber transformation from fast twitch glycolytic (type IIb) and fast twitch oxidative-glycolytic (type IIa) to slow twitch oxidative (type I) fibers has been observed in endurance-trained skeletal muscle (Faulkner et al., 1971; Maxwell et al., 1973). The number of capillaries around these transformed fibers increases towards the values demonstrated for existing type I fibers, although the mechanism for this neocapillarization is not understood.

Although statistical comparisons were not made between age groups, the two groups of prepubertal animals in the present study demonstrated slightly greater absolute values for capillary and myocyte densities than the young adult animals. This concurs with the results reported by Tomanek (1970) which indicated an age-related reduction in both densities and a slight decrease in the capillary/fiber ratio.

The stereologic analyses of the myocytes revealed a significant increase in the mitochondrial/myofibrillar ratio in both groups of active animals. This resulted primarily from a significant decrease in the percent

volume of the myofibrils. The percent volume of the mitochondria was increased significantly in the young adult animals and nonsignficantly in the prepubertal animals. Greater mitochondrial/myofibrillar ratios have been reported for rats trained by swimming (Kleitke et al., 1966; Bozner and Meessen, 1969; Guski, 1980; Guski et al., 1980b, 1981). However, stereologic analyses in other studies revealed no changes in the relative volumes of mitochondria and myofibrils in rats (Paniagua et al., 1977) and mice (Kainulainen et al., 1979) subjected to programs of enforced running. It should be noted that the latter study utilized the apical region of the left ventricle for stereologic analysis. Whether this region is subjected to the same wall stresses as the mid-wall region of the ventricle is uncertain.

Chronic endurance exercise has been shown to induce changes in the activity levels of selected oxidative enzymes (Morgan et al., 1971; Kiessling et al., 1971; Gollnick et al., 1973) and in the ultrastructure (Morgan et al., 1971; Kiessling et al., 1973) of human skeletal muscle. The vastus lateralis of young men conditioned for 14 weeks demonstrated similar changes in the relative volume composition of the myocytes to those observed in cardiac muscle. However, the changes would appear to be age-related since similar alterations were not seen in two older

groups of men despite demonstrated increases in maximum oxygen uptake and cytochrome oxidase activity (Kiessling et al., 1973, 1974).

Similar morphologic and biochemical changes to those induced by endurance conditioning have been observed when fast twitch fibers (type II) were subjected to low frequency electrical stimulation (Salmons and Henriksson, 1981; Eisenberg and Salmons, 1981). The stimulated type II fibers gradually assumed the morphologic characteristics of type I fibers. The myocytes became smaller in diameter, the percent volume and the numerical density of the mitochondria increased, the sarcotubular system was reduced, and the myosin in these fibers was found to be the slow form rather than the fast form that is normally found in type II fibers.

According to Guski et al. (1980b), since the studies of Wollenberger and Schulze (1962) and Poche et al. (1968) the mitochondrial/myofibrillar ratio has been accepted as a structural standard for the metabolic capacity and the energy condition of the cardiac myocyte. Thus, it may be inferred from this that the increased ratios reported for the active animals in the present study indicate an enhanced metabolic and energy state, whereas the decreased values observed in chronic pressure overload are indicative of a lesser state.

The increase in the mitochondrial/myofibrillar ratio that was observed in the present study was caused by changes in the relative volume composition of the myocytes that are similar to, but of lesser magnitude than, those reported by Bozner and Meessen (1969) for rats conditioned by swimming. These authors observed increases in the relative volumes of the mitochondria and the matrix and a concomitant decrease in the percent volume of the myofibrils. Only one other study has been found that reported the relative volumes of mitochondria, myofibrils and matrix in conjunction with an exercise-induced increase in the mitochondrial/myofibrillar ratio. Guski et al. (1981) observed a slight decrease in the percent volume of myofibrils, a moderate decrease in the percent volume of the matrix which was due to a decrease in the free sarcoplasmic space, and a large increase in the relative volume of the mitochondria.

The increase in the relative volume of mitochondria, although not significant in the prepubertal animals of the present study, is reflected in a significant increase in the numerical density of the organelles per unit area of sarcoplasm in both age groups of the active animals. No significant differences were observed in the size of the mitochondrial profiles, although absolute values were less for the active animals. Smaller, more numerous mitochondria have been reported in previous studies (Bozner and Meessen, 1969; Guski et al., 1980a, 1980b,

1981) and may be indicative of mitochondrial biogenesis in the conditioned myocardium by some mechanism such as mitochondrial division (Banister et al., 1971). Guski et al. (1980b) also reported increases in the ratio of mitochondrial inner membrane to myofibrils in the conditioned rats. Since the inner membrane functions in oxidative phosphorylation and active transport mechanisms, the investigators suggested that an increase in the ratio of mitochondrial inner membrane to myofibrils was indicative of an increased "phosphorylation potential". According to Meerson and Breger (1977), the "phosphorylation potential" is an essential component of the rapid adaptation of the myocardium to an increased workload and indirectly affects long-term adaptation processes by influencing protein synthesis. This, in turn, affects the structural and enzymatic properties of the myocyte and thus determines the performance of the heart.

The study of Kruglova et al. (1982) provided some support for this hypothesis. These authors subjected rats to the swimming protocols of Guski et al. (1980a, 1980b, 1981), and attempted to correlate increases in the mitochondrial/myofibrillar ratio, numerical density of mitochondria, and the surface density of the mitochondrial inner membrane with alterations in the activity levels of selected oxidative enzymes. The results demonstrated fluctuations of the enzyme activity levels that approximated

the observed changes in the morphometric parameters. However, care should be taken when attempting to compare and correlate the histochemical and morphometric data.

The percent volume of the matrix did not differ significantly between the treatment groups in the present study, possibly due to the anticipated low power of the statistical comparison for this component. However, the slight increases observed in the active animals are similar to the results of previous studies that demonstrated dilatation of the sarcoplasmic reticulum and, to a lesser extent, the T-system and the Golgi apparatus in chronically exercised rats (Arcos et al., 1968; Bozner and Meessen, 1969; Sarkisov et al., 1969; Guski et al., 1981). An increase in the ratio of SR membrane surface area to contractile elements, which Page (1978) postulated is an extremely important physiological relationship, results in a relatively larger area of membrane to function in Ca^{++} release and uptake. Guski et al. (1981) hypothesized that this may be one of the reasons, in addition to enhanced ATP synthesis and hydrolysis, why an exercise-conditioned myocardium demonstrates greater velocities of shortening and relaxation than the heart of a sedentary animal.

In comparison to the studies that reported the effects of exercise on the myocardial ultrastructure, Wassilew et al. (1982) examined the effects of prolonged

immobilization on the rat. The results show that this treatment has opposite effects from those of endurance exercise. Decreases were observed in the mitochondrial/ myofibrillar ratio and the percent volume of the sarcoplasmic reticulum.

The relative volume composition of the left ventricular myocardium would thus appear to be directly dependant on the workload imposed upon the heart by chronic physical activity, and to undergo changes in response to alterations in the amount of chronic activity performed.

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The purpose of this study was to determine the effects of voluntary running on selected ultrastructural parameters in the hearts of prepubertal and young adult male albino rats.

Animals were randomly assigned to a sedentary control (SED) group or to a voluntary running (VOL) group. The treatment was initiated when the prepubertal animals were 30 days of age and the young adult animals were 70 days of age. All of the animals were sacrificed after 12 weeks of the experimental period. The final sample comprised 6 animals in each of the older groups and 4 animals in each of the prepubertal groups.

Absolute and relative values for heart weight and ventricular weight were determined. Tissue samples from the middle portion of the left ventricular free wall were routinely fixed and prepared for transmission electron microscopy. Thick sections were examined by light microscopy to determine the capillary density, fiber density, and the capillary/fiber ratio. Electron micrographs, printed at a final magnification of X8250, were obtained

from thin sections of the tissue. Stereologic analyses of the electron micrographs were performed, using the point counting method (Weibel, 1979), to determine the relative volumes of various components of the myocardium, the interstitium, and the myocytes. Comparative statistical analyses were performed using the Student t-test. All histometric and stereologic analyses were carried out on tissue that was cut in cross-section.

The results showed that there were significant increases in the relative heart weights and relative left ventricle weights of both of the VOL groups. However, these increases were due to significantly lower body weights in the active groups rather than to greater absolute heart weights in these animals.

The histometric data suggest that increased capillarization occurred in both groups of VOL animals. Evidence for this is provided by the significant increase in the capillary/fiber ratio, which was caused by a reduction in the myocyte density.

The results of the stereologic analyses demonstrated alterations in the ultrastructure of the myocardium in the VOL animals. Both of the active groups had a greater relative volume of interstitium than the control animals. This resulted from an increase in the percent volume of

the extravascular space and a slight increase in the percent volume of the capillaries.

The relative volume composition of the myocytes was altered in the active animals. These animals had a decrease in the percent volume of myofibrils, an increase in the percent volume of mitochondria, and a slight increase in the matrix, which comprised the sarcoplasmic reticulum, the T-system and free sarcoplasmic space. The active animals had a higher mitochondrial/myofibrillar ratio than the sedentary animals with an increased areal density of mitochondria. The size of the mitochondrial transverse profiles did not differ between the VOL and SED groups at either age.

Conclusions

The following conclusions may be drawn from the results of this study:

1. Voluntary running can reduce body weight gains in male albino rats, thus producing greater relative heart weights than in sedentary animals.

2. The microcirculation of the left ventricle would not appear to be compromised by chronic voluntary activity, and may be enhanced.

3. The ultrastructure of the myocardium is affected by chronic voluntary running. The ratio of energy producing structures to contractile elements is increased, as is the ratio of interstitium to myocytes.

4. Voluntary running appears to have similar effects in both prepubertal and young adult male rats.

Recommendations

1. In order to gain more insight into the effects of different intensities of voluntary activity upon myocardial ultrastructure, a further study should be performed with a large number of animals. The active animals may then be divided into groups based upon the amount of activity undertaken during the experimental period.

2. In order to more fully evaluate the effects of voluntary exercise on the myocardium, electron micrographs of higher magnification should be used for examination of the sarcoplasmic reticulum, the T-system, and the Golgi complex.

3. Physiological and biochemical studies should be carried out in conjunction with future investigations.

4. An animal model that has greater similarities to man in terms of the cardiovascular system, and with regard to responses to exercise, should be employed in future investigations.

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APPENDICES

APPENDIX A - Morphologic Data

Animal Number	Treat-ment	Initial Body Wt. (gm)	Final Body Wt. (gm)	Heart Wt. (gm)	Ventricle Wt. (gm)	Lt. Vent. Wt. (gm)	Heart Wt. Body Wt. ($X10^3$)	Vent. Wt. Body Wt. ($X10^3$)	LV Wt. Body Wt. ($X10^3$)
K1	VOL	63	309	1.276	1.119	0.904	4.129	3.621	2.926
K2	VOL	72	432	1.522	1.341	1.038	3.523	3.104	2.403
K4	VOL	71	330	1.366	1.233	0.934	4.139	3.736	2.830
K5	VOL	63	278	1.190	1.069	0.859	4.281	3.845	3.090
K6	SED	68	427	1.371	1.225	0.915	3.211	2.869	2.143
K7	SED	63	376	1.188	1.070	0.807	3.160	2.846	2.146
K8	SED	63	423	1.370	1.232	1.030	3.239	2.913	2.435
K9	SED	68	421	1.271	1.142	0.939	3.019	2.713	2.230
B1	VOL	308	590	2.059	1.924	1.263	3.490	3.261	2.141
B2	VOL	315	511	2.187	2.034	1.157	4.280	3.980	2.264
B3	VOL	269	509	1.817	1.696	1.089	3.570	3.332	2.139
B4	VOL	323	547	2.237	2.099	1.297	4.090	3.837	2.371
B5	VOL	314	609	2.058	1.918	1.271	3.379	3.149	2.087
B6	VOL	296	448	1.886	1.760	1.098	4.210	3.929	2.451
B7	SED	294	614	2.048	1.907	1.209	3.336	3.106	1.969
B8	SED	305	572	1.955	1.805	1.189	3.418	3.156	2.079
B9	SED	289	606	1.956	1.812	1.201	3.228	2.990	1.982
B10	SED	298	621	1.923	1.766	1.198	3.097	2.844	1.929
B11	SED	307	601	2.161	2.099	1.135	3.596	3.493	1.889
B12	SED	299	545	1.859	1.721	1.175	3.411	3.158	2.156

Appendix B - Morphometric Data

Animal Number	Myocardium				Interstitium				Myocytes			Pmit / Pmf	Mit. Profile (sq.μ)	N_{Amit}
	V_{Vcel}	V_{Vint}	V_{Vcap}	V_{Vevs}	V_{Vcap}	V_{Vevs}	V_{Vend}	V_{Vlum}	V_{Vmf}	V_{Vmit}	V_{Vmat}			
K1	85.70	14.30	7.43	6.87	51.88	48.12	18.89	32.99	56.16	33.41	10.43	0.595	0.366	0.950
K2	82.78	17.22	8.02	9.19	46.61	53.39	12.98	33.63	54.05	36.93	9.03	0.683	0.462	0.808
K4	84.37	15.63	7.41	8.22	47.41	52.59	14.27	33.14	53.90	35.83	10.27	0.665	0.440	0.826
K5	84.60	15.40	6.39	9.01	43.45	56.55	14.27	29.09	52.59	38.30	9.10	0.728	0.403	0.911
K6	84.68	15.33	7.26	8.06	47.39	52.61	15.33	32.06	59.95	31.63	8.43	0.528	0.421	0.776
K7	88.88	11.13	4.89	6.24	44.93	55.07	14.99	29.94	57.51	34.25	8.24	0.596	0.531	0.645
K8	86.12	13.88	6.55	7.33	48.95	51.05	16.60	32.35	56.36	34.65	8.99	0.615	0.426	0.789
K9	86.43	13.58	6.99	6.59	51.49	48.52	15.25	36.24	55.17	35.30	9.54	0.640	0.390	0.855
B1	80.85	19.15	8.03	11.12	41.93	58.08	11.71	30.22	53.45	37.69	8.86	0.706	0.473	0.796
B2	81.39	18.61	7.95	10.66	42.71	57.30	12.25	30.45	53.44	38.09	8.47	0.713	0.428	0.891
B3	79.69	20.31	9.59	10.73	47.20	52.80	14.14	33.06	52.08	38.49	9.43	0.739	0.482	0.799
B4	86.06	13.94	5.74	8.20	41.17	58.83	10.22	30.94	55.53	34.32	10.15	0.618	0.397	0.864
B5	81.14	18.86	9.14	9.71	48.49	51.51	13.72	34.77	55.13	35.64	9.23	0.647	0.470	0.759
B6	83.98	16.03	7.31	8.71	45.54	54.46	15.53	30.01	54.61	36.27	9.13	0.664	0.484	0.763
B7	86.04	13.96	6.28	7.69	44.94	55.06	13.07	31.87	56.71	34.00	9.30	0.600	0.436	0.781
B8	84.84	15.16	8.10	7.06	53.42	46.58	16.74	36.69	58.23	33.65	8.12	0.578	0.487	0.692
B9	84.50	15.50	6.94	8.54	44.77	55.23	15.28	29.49	59.68	32.02	8.30	0.537	0.465	0.689
B10	86.21	13.79	6.21	7.58	45.06	54.94	11.88	33.18	56.94	34.19	8.87	0.601	0.463	0.749
B11	82.59	17.41	7.77	9.64	44.65	55.35	16.11	28.54	59.12	32.61	8.27	0.552	0.440	0.742
B12	85.93	14.08	6.58	7.50	46.71	53.29	15.81	30.91	59.49	32.33	8.19	0.543	0.429	0.753

Appendix C - Histometric Data

Animal Number	Capillaries Counted	Caps/mm^2	Muscle Fibers Counted	Fibers/mm^2	Capillary/Fiber Ratio
K1	1192	3895	1096	3582	1.0876
K2	1151	3761	1065	3480	1.0808
K4	1101	3467	1019	3330	1.0805
K5	1137	3716	1054	3444	1.0787
K6	1087	3552	1025	3350	1.0605
K7	1182	3863	1108	3621	1.0668
K8	1174	3837	1106	3614	1.0615
K9	1095	3578	1029	3363	1.0641
B1	994	3248	936	3059	1.0620
B2	1033	3376	957	3128	1.0794
B3	984	3216	913	2984	1.0778
B4	1025	3350	961	3141	1.0666
B5	1004	3281	933	3049	1.0729
B6	1062	3471	993	3245	1.0695
B7	1028	3360	974	3183	1.0554
B8	1018	3327	961	3141	1.0593
B9	1051	3435	985	3219	1.0670
B10	1006	3288	957	3128	1.0512
B11	994	3248	942	3078	1.0552
B12	979	3199	919	3003	1.0653

Appendix D - Procedures for Selecting a Suitable Lattice Test System

Approximate relative volume of structure	V_V
Required test points - $\frac{t\alpha^2}{SEM^2} \cdot \frac{(1-V_V)}{V_V}$	P_T*
Micrograph magnification	M_1
Micrograph area (cm^2)	A_{T_1}
Largest profile area at M_1 (cm^2)	a_m
Point spacing (cm) - >1.1 ($\sqrt{a_m}$)	d_1
Points per micrograph - A_{T_1}/d_1^2	P_{T_1}
Micrographs required - P_T^*/P_{T_1}	n

The above procedures are from Weibel (1979, p. 116)