CARDIAC Na^{+}, K^{+}, Mg^{+2} and Ca^{+2} CONCENTRATIONS IN CHRONICALLY EXERCISED RATS

A Thesis

Presented to the Faculty of the Department of Biological Sciences

of the State University of New York College at Brockport

in Partial Fulfillment for the Degree of

Master of Science

by

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March 1981

THESIS DEFENSE

FOR

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Master's Degree Candidate

OVED	NOT APPROVED
X	

MASTER'S DEGREE ADVISORY COMMITTEE

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ACKNOWLEDGEMENTS

I am deeply indebted to Dr. Delmont C. Smith for his direction and support in this endeavor and for his critical reading of the text. I would also like to express gratitude to Dr. Larry Kline and Dr. David Branigan for their assistance. Finally, my deepest appreciation to my husband, Jay Tresham, for his constant support and encouragement.

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ABSTRACT

Bradycardia as a result of chronic exercise was induced in a group of male Sprague-Dawley rats. These experimental animals ran two hours daily, 6 days a week for 6 weeks, at a speed of 12.9 meters per minute, in a motorized running cage. Previous studies have indicated that this regimen adequately resulted in exercise bradycardia without corresponding hypertrophy.

Hearts from this experimental group of chronically exercised rats were assayed via atomic absorption spectrophotometry for total tissue content of Na^{+}, K^{+}, Mg^{+2}, and Ca^{+2}. These results were compared with the concentrations obtained from hearts of a group of sedentary control rats. Differences were found in the concentrations of the ions between the two groups, but these differences were all non-significant according to the Student t-test. The following concentration ratios were calculated for both groups: Na^{+}: K^{+}, Mg^{+2}: Ca^{+2}, and Ca^{+2}: Na^{+}. No significant differences were found.

There was considerable variation among individual results; an increased sample size might minimize the effects of this variation on the statistical tests, perhaps indicating significant differences in the ionic concentrations, particularly with regard to calcium concentration.

LITERATURE REVIEW

I.

Exercise-induced bradycardia is one of the most obvious results of endurance training. It has been demonstrated repeatedly that human athletes, such as distance runners and other endurance trained individuals, have lower resting heart rates than do individuals in the general population (Scheuer and Tipton, 1977). Likewise, animal studies have shown that exercised animals have lower resting heart rates than do non-exercised control animals (Frick 1967, Scheur and Tipton, 1977).

Cardiac hypertrophy is also frequently evident as a training effect (Beckner and Winsor, 1954, Scheuer and Tipton, 1977), but many cardiac adaptions as a result of training can occur without, or prior to, corresponding hypertrophy (Bhan, Scheuer 1972, Scheuer and Tipton, 1977). Varying intensity, frequency, and duration of an exercise program will produce a variety of training effects without corresponding hypertrophy. El-Hage (1976) reported that exercise bradycardia, as demonstrated by lower intrinsic heart rates, can be elicited with no corresponding hypertrophy. This result makes it apparent that increased heart mass cannot then be the sole cause of bradycardia.

When one examines the literature for proposed mechanisms in explanation of exercise-induced bradycardia, it becomes

apparent that the causative factors have not been definitely determined. The most widely accepted theory presently is that of enhanced vagal tone or increased cardiac stores of acetylcholine, with a possible decrease in cardiac sympathetic activity (Tipton and Scheuer, 1977, Barnard, Tipton, and Tharp, 1966, Deschyver, Mertens-Strythagen, 1975, Herrlich, Raab, and Gigee, 1960).

Both divisions of the autonomic nervous system can alter heart rate. Adrenergic sympathetic transmitters increase heart rate whereas release of the parasympathetic transmitter, acetylcholine, from nerves supplying the heart, decreases the heart rate. The vagus nerve carries parasympathetic fibers which release acetylcholine in the sino-atrial (SA) and atrioventricular (AV) nodal tissue of the heart. The release of the heart. The release of this transmitter tends to hyperpolarize the pacemaker membrane, thus decreasing the rate of development of prepotentials and ensuing action potentials. Normally the heart is exposed to a steady stream of vagal impulses which inhibit the heart or slow its rate. This constant inhibitory effect is referred to as vagal tone. Thus, enhanced vagal tone, as a cause of exercise bradycardia, implies an increase in conduction of impulses over the vagus nerve and an increased release of acetylcholine which decreases the heart rate (Langley, Telford, and Christensen, 1980). This theory is strongly held by Raab and coworkers. Tipton, likewise, believes exercise bradycardia is due to increased acetylcholine, but he believes this is non-neural acetylcho-

line, produced by atrial myocardium, which augments the effect of vagal acetylcholine. This buildup of atrial non-neural acetylcholine would result from endurance training (Tipton and Taylor, 1965).

It has been demonstrated that atria of trained rats contain significantly more acetylcholine than the atria of non-trained controls (Herrlich, Raab, and Gigee, 1960, Deschyver, and Mertens-Strythagen, 1975). This finding strengthens the foregoing proposed mechanism of exercise bradycardia, but whether this acetylcholine is neural (from the vagus) or non-neural in origin is still indefinite. Increased atrial choline acetyltransferase activity resulting from chronic exercise, as reported by Ekstrum (1974), further supports this hypothesis.

Acetylcholine is inactivated by a group of enzymes known as cholinesterases. Any proposal that acetylcholine is responsible for exercise bradycardia should include a study of activity levels of these enzymes in trained versus non-trained animals. Tipton, Barnard, and Tharp (1966) report such a study done on atrial activity, with the conclusion that training does not alter the activity of such enzymes. This verifies similar results reported by Herrlich, Raab, and Gigee in 1960. These studies indicate that the slowed heart rate cannot be due to longer action of acetylcholine because of lower cholinesterase activity.

Studies in which atropine sulfate (a muscarinic inhibitor)

was administered to trained, experimental animals and non-trained controls, show that the trained animals exhibit less cardiac acceleration than the non-trained controls (Scheuer and Tipton, 1977, Tipton and Taylor, 1964). This result likewise indicates an increased acetylcholine supply in exercised animals, which competes with atropine for receptor sites. These results are based on single injections of atropine into intact animals. When graded doses of atropine are applied to isolated atria (Smith and El-Hage 1978) atropine raised the initially slower atrial rate of exercised rats to that of sedentary animals as it overcame the effects of the resident acetylcholine.

In conjunction with the above studies, work done by Raab et al. (1960) revealed that there is "a linear increase of cardiac sympathetic tone and decline of cardiac neurovegetative counter-regulatory effectiveness in proportion to decreasing degrees of habitual exercise." This study also showed that this adrenergic preponderance, due to lack of chronic exercise, could be reversed by physical training, resulting in the training effects characteristic of cholinergic preponderance. Clinically speaking, Raab points out that this non-exercise cardiac adrenergic preponderance leads to O_2 waste, impairment of cardiac efficiency and myocardial damage. In 1965, Raab published further results in this area which verified the above results.

II.

In addition to autonomic control, there exists a second aspect of cardiac physiology and biochemistry that likely plays a role in exercised-induced changes, this being ionic concentrations and functions. Raab et al., (1969), in an extensive article on myocardial electrolytes and heart disease, indicates the lack of information on the effect of chronic exercise on cardiac ionic concentrations. The need for such a study was suggested by El-Hage (1976) also. The four electrolytes critical to proper cardiac function are calcium, magnesium, potassium, and sodium.

As a result of training one usually observes an increased positive inotropic effect in cardiac function as well as a negative chronotropic effect. This increased contractile strength is most likely effected by calcium ion concentration, since it is essential in excitation-contraction coupling. Calcium interacts with troponin, which changes its configuration and by so doing removes tropomyosin from the actin binding site. Secondly, Ca^{+2} is necessary for the Mg-ATPase hydrolysis of ATP to provide energy for the actin-myosin cross-bridge attachment. The rate of these reactions and the number of filaments coupling or contracting, determine the inotropic effect or the effective force of the contraction. This Ca^{+2} is released to the myofilaments by sarcoplasmic reticuli (SR) located within the muscle fiber. Its release, upon nervous innervation of the fiber, induces contraction and its reabsorp-

tion by the SR, upon cessation of innervation, results in relaxation of the muscle. (Katz, 1966, Langley, Telford, and Christensen, 1980).

Penpargkul et al. (1977) found that sarcoplasmic reticulum storage and transport of Ca^{+2} is greater in the hearts of conditioned versus unconditioned rats. They relate this finding to their previous finding of increased contractile reserve in hearts of conditioned rats (Penpargkul and Scheuer, 1970). Penpargkul states that many investigators have found SR calcium transport to be decreased in depressed hearts where the contractile state had been reduced due to myocardial failure, ischemia or cardiomyopathy.

J. Suko (1973) reports that calcium transport by cardiac SR is increased in hyperthroidism and decreased in hypothyroidism, with observed parallel changes in the cardiac contractile state.

Recently it was reported by Rosenshtraukh et al. (1979) that cardiac muscle function is influenced by creatine phosphate. These same researchers directly demonstrated that the regulation of calcium entry into the cardiac cells' myoplasm, through the surface membrane, is via creatine phosphate. The calcium channels in the membrane are controlled by a protein kinase reaction which utilizes ATP to phosphorylate a membrane protein. Since internal creatine phosphate determines ATP regeneration, it essentially controls calcium entry through the membrane.

Honig and Reddy (1973) report a study which shows that catecholamine analogs inhibit cardiac contraction by increasing the amount of calcium required to activate the ATPase of cardiac myosin B. This catecholamine analog effect can be reversed by increasing calcium concentration. The entire troponin-tropomyosin complex is necessary as the functional receptor for this calcium reversal of the negative inotropic effect.

Catecholamines themselves exert a positive inotropic effect on cardiac contraction. Normally we would suppose that this influence occurs via their effect on subcellular sarcoplasmic reticulum calcium reservoirs, but Morgenstern, Noack, and Kohler (1972) report that in cardiac muscle extracellularly located and membrane bound calcium are involved in regulating and controlling contraction.

> "When isolated atria were incubated in a calcium free Ringer solution, they did not show any contraction although an action potential could be recorded; this indicates that for normal function the heart muscle depends on extracellularly located calcium ions." (Fleckenstein et al., 1967). Reuter and Scholz (1968), too, demonstrated by electrophysiological methods that, in the second step of depolarization, there takes place an influx of calcium ions from the extracellular space into the muscle cell.
>
> Morgenstern et al. (1972)

In this report Morgenstern points out that as extracellular calcium increases, uptake of calcium into the cardiac muscle increases; associated with this increase is an increase

of total tissue calcium content, and an increase in contraction force. If extracellular calcium concentrations are constant, the uptake by the muscle cell can be increased via the use of sympathomimetic amines such as isoprenaline and tyramine, but this uptake is dependent on pH (H^+ ion concentration). Morgenstern suggests that these drugs possibly increase calcium transport via interaction with phospholipids in the membrane which transport ions across an aqueous-lipid solvent interface. Increasing extracellular hydrogen ion concentration decreases the effectiveness of these drugs in increasing calcium ion flux. In this work it was noted that total atrial calcium content did not increase when uptake increased; therefore, it was assumed that there is a corresponding calcium efflux.

In conclusion, Morgenstern suggests that there are "excitable membrane channels which are controlled by gates, their function being regulated by H ions." (Morgenstern et al., 1972). Calcium ions would be bound to negatively charged sites near these channels. If these calcium ions are competitively displaced by hydrogen ions, the channels are then permeable to sodium ions but not to calcium ions. Alternatively, an increased H ion concentration extracellularly might alter the sterical configuration of membrane proteins involved in transmembrane transport of cations (Morgenstern et al., 1972).

An additional interesting approach to the study of electrolyte functions in cardiac physiology is that presented by Raab (1969). In an extensive work, he explores the relation-

ship of myocardial electrolyte derangement to the causation of heart disease. Raab points out that the medical world has known for years that sympathetic neurotransmitters (catecholamines), if experimentally or therapeutically applied to the heart were extremely cardiodestructive, but he states that this information was ignored or at least not thought to be clinically significant. Raab proceeds to present results which show that several types of coronary heart disease, in which there are areas of necrotic destruction in the heart, are directly related to a corresponding potassium, and often magnesium loss in that tissue area, with an increase in sodium. This abnormal distribution of electrolytes is commonly found at most cardiac lesion sites.

As action currents spread over the heart tissue, potassium is extruded from the cardiac cells during systole. Then during the diastolic phase of contraction, extracellular potassium is returned against the K^+ internal/ K^+ external gradient. This movement requires the expenditure of metabolic energy from ATP. As is likewise commonly known, sodium ions move in the opposite direction in the systolic and diastolic phases. As Raab states, the rate at which the ion pump transfers or exchanges sodium and potassium is accelerated via the influence of adrenergic catecholamines. When this basic physiological process is disrupted, it contributes greatly to severe changes in heart muscle, which are normally labeled as degenerative heart disease symptoms.

Potassium and magnesium loss are the most common electrolyte derangement seen in cardiac tissue. Concomitant with K^+ loss is a decrease in the ability of the cardiac tissue to store glycogen. The most common cause of potassium loss is myocardial hypoxia or anoxia. The low O_2 supply to heart tissue reduces the production of ATP which is essential for the active return during diastole of the potassium extruded during systole. Occurring with this potassium and magnesium depleton, is a simultaneous movement of sodium into O_2 depleted cells. It accumulates in much higher than normal concentrations and can be detected in the necrotic tissue areas. Thus, the normal K^+/Na^+ ratio is greatly reduced in areas of myocardial necrosis.

In addition to occlusions and coronary atherosclerosis, a common and potentially dangerous cause of cardiac anoxia or hypoxia, is acute or chronic increase of activity of the cardiac sympathetic nerves and the adrenal medulla, which increase the amount of myocardial epinephrine and norepinephrine. Increased adrenergic catecholamines induce lesions in heart tissue (often called microinfarctions) which are eventually replaced by fibrous scar tissue. This necrotization of myocardial tissue via catecholamines is enhanced by K^+ and Mg^{+2} depletion in the tissue and is suppressed by Na^+ withdrawal from the tissue. Normally the catecholamines increase the rate of K^+ flux in the beating heart, which explains why they increase O_2 consumption to the point where it "exceeds the calculated oxidative energy needed for simultaneously augmented external mechanical work performance." (Raab, 1969).

Raab cites many statistics where cardiac tissue from patients who died of heart disease was found to be low in K^+, and Mg^{+2} and high in Na^+. Lesions or necrosis resulting from K^+ or Mg^{+2} loss can be reversed with administration of potassium and magnesium aspartates plus withdrawal of sodium, probably by increasing ATP formation and activating the ion pump.

In this study, (Raab 1969) it was reported that (in opposition to catecholamine effects) administration of acetylcholine was found to increase myocardial potassium, reduce sodium, and not affect magnesium concentrations. In a perfusion of the heart with acetylcholine, a net gain of K^+ was also observed.

The relationship of all this to exercise-induced training effects is that lack of habitual exercise causes a sustained adrenergic preponderance, whereas, a consistent exercise regimen increases cardiac vagal tone resulting in a parasympathetic preponderance. Physical exertion itself also results in the release of K^+ from skeletal muscle to circulation, where some reaches the cardiac tissue.

Raab devotes a section of his report to prevention of myocardial electrolyte derangements; one of the prophylactic techniques he advises is habitual physical exercise.

> A particularly conspicious and functionally important effect of physical training, namely, a reduction of cardiac sympathetic, and augmentation of cardiac vagal tone at rest and in response to physical effort as well as to sensory and mental stresses, is manifested by a slowing of the heart rate, a prolongation

> of the isometric tension period of the left ventricle, and a reduction of the tension time index.
>
> All of these parameters serve as indirect criteria of myocardial oxygen consumption, and reveal a marked improvement of the heart muscles' oxygen economy, resulting from physical training. An increase in atrial acetylcholine in trained rats points in the same direction.
>
> Acetylcholine increases myocardial K^+ and the K^+/Na^+ ratio. The training-induced autonomic nervous antiadrenergic effects upon myocardial metabolism may be assumed to contribute significantly to the cardioprotective effectiveness of habitual vigorous physical activity by safeguarding the myocardial electrolyte equilibrium from catecholamine-induced hypoxic derangements.
>
> (Raab, 1969)

Prioreschi (1964) reports that potassium chloride salts and forced restraint both are cardioprotective via their ability to increase or restore intracellular potassium in cardiac tissue containing induced lesions.

The role of Mg^{+2} in cardiac physiology is extremely complex. Heart muscle contains large amounts of magnesium intracellularly, some bound and some free. Much of the internal magnesium is complexed as Mg-ATP, with some also found at the myofibrils and in the mitochondria. There is less magnesium extracellularly in comparison but its role is significant.

Magnesium participates in a number of intracellular processes some of which are: tension and relaxation of myo-

fibrils, ATP hydrolysis, calcium binding and release by the sarcoplasmic reticulum, Na^+, K^+ ATP hydrolysis at the membrane (Na^+, K^+ pump), mitochondrial ATPase in oxidative phosphorylation, phosphorylase kinase reaction, and adenyl cyclase activity (Polimeni and Page, 1972).

As previously mentioned, the Mg-ATP complex is hydrolyzed via myosin ATPase active in crossbridge formation, leading to myofibril contraction. If the Mg-ATP complex concentration surpasses a critical point, its excess results in substrate inhibition of the myosin activity. Also involved here is the calcium level, since it must free actin. In a normal muscle cell, Mg-ATP is in excess and Ca^{+2} is low, therefore the cell is relaxed (troponin-tropomyosin bound to actin). But, as a result of an action potential, calcium is released and binds to troponin. This event not only frees actin for binding, but it also serves as a control mechanism supressing excess Mg-ATP inhibition of contraction. Ionic Mg quite likely modulates this Ca^{+2}, Mg-ATP interaction. Increasing free Mg decreases tension development. The mechanism of this Mg^{+2} involvement is unclear. It is known that the heads of the myosin molecule will bind free Mg^{+2} but how this relates to the myosin ATPase activity is unknown. (Polimeni and Page, 1972).

Research reported by Shine and Douglas (1974) revealed that increased concentrations of extracellular magnesium ions resulted in a decrease in contractile force and a decrease in K^+ exchange in the rat heart. Ca^{+2} was found to work as a

hindrance to the Mg^{+2} effect on tension development, but altering calcium ion concentration could not reverse the magnesium ion induced changes in the potassium flux. The net calcium loss from cardiac muscle cells during exposure of the cell to high concentration of Mg^{+2} extracellularly was substantial and is probably explained by Mg^{+2} inhibiting Ca^{+2} influx, or at least displacing Ca^{+2} bound to the sarcolemmal membrane. Interestingly enough, Shine and Douglas found no change in Na^{+} movement and no change in the internal/external Na^{+} concentrations with increased Mg^{+2} externally. It has been suspected that Mg^{+2} stimulates sodium-potassium pump activity and thus a change in Na^{+} concentrations was anticipated but was not apparent in this research. The decrease in K^{+} efflux, which was not altered by increased Ca^{+2}, suggests that Mg^{+2} depressed K^{+} efflux by blocking a K^{+} pathway in the membrane, one which was not an important calcium site. This effect of Mg^{+2} was probably a separate membrane effect, unrelated to magnesium's possible influence of K^{+}-Na^{+} ATPase activity (since Na^{+} showed no change here). (Shine and Douglas, 1974).

Watanabe and Driefus (1972) reported a study of magnesium-potassium interactions in electrophysiology of the heart. They found that altering external ionic magnesium resulted in several electrophysiological changes in the rabbit heart, but that these changes were dependent on or linked to external potassium ion concentrations, and required a normal calcium ion concentration externally. The higher the external K^{+}, the

more effective was increased Mg^{+2} in inducing the particular electrophysiological changes. Also, high Mg^{+2} and low Mg^{+2} concentrations produced opposite effects. High Mg^{+2} prolonged the duration of the effective refractory period, whereas low Mg^{+2} shortened it. In explaining these results, Watanabe and Driefus state that there is antagonism between high K^{+} and high Mg^{+2}, whereas high K^{+} and low Mg^{+2} result in additive action. The same relationship is seen with potassium and calcium. This suggests a similarity of action between calcium and magnesium ions. Increased concentrations of either, externally, tends to stabilize the membrane, but increases the depolarization rate at a particular membrane potential.

In a review written by Shine (1979) in which he reiterates many of the above mentioned effects of alterations in external Mg^{+2}, he concludes that the external processes involving Mg^{+2} can continue uninterrupted when external Mg^{+2} is altered. This is because the internal Mg^{+2} concentration is probably maintained via equilibrium with the internal Mg-ATP complex, and not by transport of Mg^{+2} from outside the cell. Thus, the membrane effects of Mg^{+2} interactions with K^{+}, Na^{+}, and Ca^{+} resulting in changes in ion fluxes, depolarization rate, etc. are relatively separate from Mg^{+2} influence on internal processes.

III.

Bradycardia and increased contractile force are training

effects resulting from a chronic exercise program. They can be induced without concomitant cardiac hypertrophy, thus eliminating it as an explanatory mechanism. The mechanism causing these changes in cardiac function is still under study. In reviewing the importance of ions in cardiac physiology and function, it is quite possible that they may be intricately involved in the mechanism of exercise-induced bradycardia and the corresponding positive inotropy. This research project has investigated that possibility. Phase I consists of induction of bradycardia, without hypertrophy, via an exercise regimen used effectively by El-Hage (1976) for this same purpose. Experimental animals were chronically exercised; in addition, control animals were maintained without exercise. Phase II consists of assaying the hearts of both experimental and control animals via atomic absorption spectrophotometry for total cardiac concentrations of K^{+}, Na^{+}, Mg^{+2}, and Ca^{+2}. The relevant ratios of these ions are calculated, and the results examined for possible relationships of ionic concentrations to the mechanism of exercise bradycardia.

METHODS AND MATERIALS

I. Introduction

Twelve male albino, Sprague-Dawley rats weighing approximately 180 grams were randomly assigned control or experimental labels. Each rat was ear-punched for identification. The rats were initially housed in pairs, one experimental and one control per cage; all the cages were located on a single rack in an animal laboratory where temperature, light, and ventilation were controlled to provide optimal conditions. Growth of the rats caused this housing situation to become too confining for two rats. As a result, the rats were placed in separate cages for the remainder of the experiment; all other conditions were maintained. The rats were given water and Purina rat chow as desired. All animals were given ten days to acclimate to their new surroundings before further procedures were initiated. During this time, all animals were handled at least twice daily by the experimentor.

II. Exercise Program

Following this acclimation period, the experimental animals were started on a training program. They were placed in individual, 4 inch wide, wire compartments of a motorized treadmill made by the Wahman Manufacturing Company. Initially they were exercised 5-10 minutes at a time. This time increment was increased at each exercise period to a maximum of one

hour of exercise at a time. This maximum was achieved in one week. Thereafter, the experimental animals were exercised for one hour, twice daily, at the same time each day, six days a week, for six weeks. The rate of exercise was 12.9 meters per minute or 11-12 cycles of the 14 inch diameter drum per minute. Previous studies (El-Hage, 1976) indicate that this training regimen is adequate to produce significant bradycardia. During this exercise period, the rats were weighed approximately every ten days.

At the conclusion of the six week exercise period final body weights were obtained and all rats were sacrificed via a blow to the back of the head. Hearts were immediately dissected free and rinsed in distilled water. Wet weights were obtained and hearts were placed in plastic vials and frozen.

A second group of 12, male, albino, Sprague-Dawley rats were then randomly assigned to experimental and control groups. The experimental animals were then exercised according to the above regimen. The rats were worked with in two groups, simply because the exercise cage only accomodated six rats. This second group of rats was sacrificed in the same manner; the heart tissue was removed and likewise frozen.

Two problems were encountered in the exercising of the experimental animals. The rats sometimes clung tenaciously to the wire mesh of the compartments as the wheel revolved. Secondly, this wire mesh sometimes cut their feet causing bleeding which made further running difficult. These problems

were eliminated by lining the compartments of the motorized cage with newsprint pads which were changed daily. The cage had been previously modified by removing a central axle. This was done to prevent the rats from clinging to it.

III. Tissue Preparation

Eight of the group I rat hearts and all twelve of the group II rat hearts were prepared for atomic absorption spectrophotometric analysis according to the following procedure, which was adapted from Sturges, Holmes, and Likens (1974), Likens and Bowman (1970), and Chernoff (1975).

1) Obtain wet weight of heart.
2) Dry to constant weight in 40°C incubator in covered porcelain crucibles.
3) Obtain dry weight.
4) Ignite in furnace for 2.5 hours at 500 degrees celcius in porcelain crucibles.
    a) bring temperature slowly to a maximum of 500 degrees ± 20 degrees celcius.
    b) allow temperature to remain at 500 degrees celcius for at least two hours.
    c) crack oven door until temperature drops to 300 degrees celcius.
    d) transfer crucibles to a dessicator.
    e) cool to room temperature.
5) Add about 1 ml. deionized water to crucible to reduce

splattering.

6) Add 10 ml. 6N HCl to crucible.

7) Bring contents slowly to the boiling point on a hot plate.

8) Cool, then filter through Whatman #42 filter paper which has been previously washed with 6N HCl and deionized water.

9) Rinse crucible and paper 3 times with deionized water.

10) Collect filtrate in a 25 ml. volumetric flask. Bring to volume with deionized water.

IV. Atomic Absorption Determination

The 20 samples were read on a Perkin-Elmer Atomic Absorption Spectrophotometer for Na^{+}, Ca^{+2}, Mg^{+2}, and K^{+} ion concentrations. The operating procedure and instrument settings for each ion tested were taken directly from the following Perkin-Elmer operating manual: Analytical Methods for Atomic Absorp-Spectrophotometry, 1966. Perkin-Elmer Co., Norwalk, Conn.

The fuel source and the wavelength at which the samples were read are listed below for each ion. All other procedural information can be obtained from the above source.

Electrolyte	Wavelength Setting	Fuel Source
Na^{+}	295	hydrogen-air
K^{+}	383	hydrogen-air
Mg^{+2}	285	acetylene-air
Ca^{+2}	212	nitrous oxide

The concentrations of the 20 samples were calculated by solving the following proportion using the concentration to absorbance ratio of a known standard which absorbs in the range of the sample. Since the absorbances of all the samples were within a narrow range (they did not show a wide range of absorption values), this eliminated the need of using multiple standards. Samples were read repeatedly until consistent readings were obtained.

$$\frac{\text{conc. sample (x)}}{\text{abs. sample (read)}} = \frac{\text{conc. std. (known)}}{\text{abs. std. (read)}}$$

$$\text{conc. sample} = x = \frac{\text{(abs. sample) (conc. std.)}}{\text{(abs. std.)}}$$

V. Analysis of Data

The data was statistically analyzed via Student's t-test. The formulas and probability tables used in this evaluation were taken from Elementary Probability and Statistics, Gray and Ulm, 1973.

RESULTS

Table I reveals a significant difference ($p<.025$) between the final body weights of the experimental versus the control rats. The unexercised control rats weighed significantly more at the conclusion of the experiment than did the exercised experimental animals. In contrast, the wet and dry weights of the rats' hearts show no significant difference between the control and experimental groups. However, when one examines the heart weight : body weight ratios, it becomes apparent that even though the exercised animals show higher ratios as is expected, there is a difference between the dry weight heart : body weight ratio and the wet weight heart : body weight ratio of the control and experimental groups. The difference in the dry weight heart : body weight between the two groups is greater than the difference in the wet weight heart : body weight ratio between the two groups. This indicates an increase in the solid content of the exercised animals' hearts in comparison to their body weights. The difference between the control and experimental groups in the last ratio of Table II (dry weight heart : wet weight heart) reiterates the above statement. There is no significant difference in the heart weights of the two groups, but there is a small increase in the solid content of the heart of the experimental rats when compared to their body weights.

Tables III-V compare the ionic content of the exercised hearts with the ionic content of the control hearts. Table III and IV reveal no significant difference in K^+ content or Na^+ content between the two groups. Table V, likewise, indicates a non-significant difference in the Ca^{+2} content of the exercised versus the control hearts.

Table VI, again, indicates no significant difference in Mg^{+2} concentrations between experimental and control groups. Examination of the individual data, though, reveals considerable variation in Mg^{+2} content, particularly within the experimental group.

Tables VII-VIII portray concentration ratios of the ions in experimental versus control groups. In interpretation of ionic content data, physiologically, the ratios are often more important than the individual ion concentrations, since it is often the concentration ratios which affect or alter physiological functioning of the tissue.

Table VII reveals a non-significant difference in K^+: Na^+ ratio. The data also reveal variability in the individual results.

Table VIII reveals no significant difference in the Mg^{+2} : Ca^{+2} ratio of the exercised versus control hearts. The individual results do show a range of variation.

Table IX likewise indicates no significant difference between experimental and control hearts with regard to the Ca^{+2} : Na^+ ratio.

TABLE I Body weights and heart weights of control and experimental animals.

Controls				Experimentals			
Rat #	Body wt.(gm)	Heart wet wt.(gm)	Heart dry wt.(gm)	Rat #	Body wt.(gm)	Heart wet wt.(gm)	Heart dry wt.(gm)
1	472.8	1.360	.296	11	386.1	1.272	.283
2	308.0	1.263	.257	12	303.0	1.118	.232
3	417.0	.944	.205	13	308.5	1.185	.244
4	229.0	1.256	.286	14	396.4	1.530	.366
5	453.0	1.453	.293	15	359.2	1.256	.263
6	468.1	1.340	.305	16	301.3	1.201	.264
7	435.0	1.339	.288	17	325.3	1.084	.231
8	527.9	1.410	.255	18	434.1	1.390	.285
9	489.1	1.560	.318	19	450.1	1.690	.349
10	345.0	1.102	.240	20	408.4	1.430	.318
Avg.	434.5	1.303	.274	Avg.	367.2*	1.316"n.s."	.284"n.s."
Std. Error of Mean	28.81	.0556	.0108	Std. Error of Mean	17.63	.0608	.0149

* $p < .025$

TABLE II. Body weight: heart weight ratios of control and experimental animals.

Controls				Experimentals			
Rat #	wet wt.ht. / body wt.	dry wt.ht / body wt.	dry wt.ht. / wet wt.ht.	Rat #	wet wt.ht. / body wt.	dry wt.ht. / body wt.	dry wt.ht. / wet wt.ht.
1	$2.87X10^{-3}$	$6.26X10^{-4}$	$2.18X10^{-1}$	11	$3.29X10^{-3}$	$7.32X10^{-4}$	$2.22X10^{-1}$
2	$4.10X10^{-3}$	$8.34X10^{-4}$	$2.03X10^{-1}$	12	$3.68X10^{-3}$	$7.65X10^{-4}$	$2.07X10^{-1}$
3	$2.26X10^{-3}$	$4.91X10^{-4}$	$2.17X10^{-1}$	13	$3.84X10^{-3}$	$7.91X10^{-4}$	$2.06X10^{-1}$
4	$2.92X10^{-3}$	$6.66X10^{-4}$	$2.27X10^{-1}$	14	$3.85X10^{-3}$	$9.23X10^{-4}$	$2.39X10^{-1}$
5	$3.20X10^{-3}$	$6.46X10^{-4}$	$2.01X10^{-1}$	15	$3.49X10^{-3}$	$7.32X10^{-4}$	$2.09X10^{-1}$
6	$2.86X10^{-3}$	$6.52X10^{-4}$	$2.27X10^{-1}$	16	$3.98X10^{-3}$	$8.76X10^{-4}$	$2.19X10^{-1}$
7	$3.08X10^{-3}$	$6.62X10^{-4}$	$2.15X10^{-1}$	17	$3.33X10^{-3}$	$7.10X10^{-4}$	$2.13X10^{-1}$
8	$2.67X10^{-3}$	$4.83X10^{-4}$	$1.81X10^{-1}$	18	$3.20X10^{-3}$	$6.56X10^{-4}$	$2.05X10^{-1}$
9	$3.18X10^{-3}$	$6.50X10^{-4}$	$2.03X10^{-1}$	19	$3.75X10^{-3}$	$7.75X10^{-4}$	$2.06X10^{-1}$
10	$3.19X10^{-3}$	$6.96X10^{-4}$	$2.17X10^{-1}$	20	$3.50X10^{-3}$	$7.78X10^{-4}$	$2.22X10^{-1}$
Avg.	$2.99X10^{-3}$	$6.30X10^{-4}$	$2.10X10^{-1}$	Avg.	$3.58X10^{-3}$*	$7.73X10^{-4}$**	$2.15X10^{-1}$ "n.s."
Std. Error of Mean	$1.49X10^{-4}$	$3.149X10^{-5}$	$4.35X10^{-3}$	Std. Error of Mean	$8.45X10^{-5}$	$2.46X10^{-5}$	$3.42X10^{-3}$

* $p < .01$
** $p < .005$

TABLE III K^+ content of control hearts and experimental hearts.

Controls				Experimentals			
Rat #	K^+ content (ug)	K^+ (ug)/wet wt.ht.(mg)	K^+(ug)/dry wt.ht.(mg)	Rat #	K^+ content (ug)	K^+(ug)/wet wt.ht.(mg)	K^+(ug)/dry wt.ht.(mg)
1	3028.84	2.227	10.23	11	3183.96	2.503	11.25
2	2764.42	2.189	10.76	12	2716.66	2.430	11.71
3	3028.84	3.209	14.77	13	2972.97	2.509	12.18
4	2980.76	2.373	10.42	14	2961.16	1.935	8.09
5	3300.97	2.272	11.27	15	2938.89	2.340	11.17
6	3689.32	2.753	12.10	16	2500.00	2.082	9.47
7	2956.73	2.208	10.27	17	3413.46	3.149	14.78
8	3533.65	2.506	13.86	18	3519.41	2.532	12.35
9	4158.65	2.666	13.08	19	4399.03	2.603	12.60
10	2740.38	2.487	11.42	20	3557.69	2.488	11.19
Avg.	3218.25	2.489	11.82	Avg.	3216.32"n.s."	2.457"n.s."	11.48"n.s."
Std. Error of Mean	143.118	.00998	.5058	Std. Error of Mean	178.332	.1021	.5695

TABLE IV. Na^+ content of control hearts and experimental hearts.

Controls				Experimentals			
Rat #	Na^+ content (ug)	Na^+(ug)/ wet wt. ht.(mg)	Na^+(ug)/ dry wt. ht.(mg)	Rat #	Na^+ content (ug)	Na^+(ug)/ wet wt. ht.(mg)	Na^+(ug)/ dry wt. ht.(mg)
1	1434.48	1.055	4.846	11	1099.13	.8640	3.883
2	682.76	.5406	2.657	12	976.29	.8732	4.208
3	1034.48	1.095	5.046	13	937.50	.7911	3.842
4	965.52	.7687	3.376	14	1689.65	1.104	4.617
5	1213.79	.8353	4.143	15	1066.66	.8492	4.056
6	1241.37	.9263	4.070	16	1033.33	.8603	3.914
7	1103.44	.8240	3.831	17	966.66	.8917	4.185
8	1275.86	.9048	5.003	18	1324.13	.9526	4.646
9	1068.96	.6852	3.362	19	1379.31	.8161	3.952
10	909.09	.8249	3.788	20	1275.86	.8922	3.956
Avg.	1092.97	.8460	4.012	Avg.	1174.85"n.s."	.8894"n.s."	4.126"n.s."
Std. Error of Mean	67.423	.05185	.2475	Std. Error of Mean	75.710	.02760	.09246

TABLE V. Ca^{+2} content of control hearts and experimental hearts.

Controls				Experimentals			
Rat #	Ca^{+2} content (ug)	Ca^{+2} (ug)/ wet wt. ht.(mg)	Ca^{+2} (ug)/ dry wt. ht.(mg)	Rat #	Ca^{+2} content (ug)	Ca^{+2} (ug)/ wet wt. ht.(mg)	Ca^{+2} (ug)/ dry wt. ht.(mg)
1	147.79	.1086	.4992	11	219.85	.1728	.7768
2	64.10	.0508	.2494	12	121.62	.1087	.5242
3	120.51	.1276	.5878	13	107.14	.0904	.4391
4	137.10	.1090	.4793	14	146.83	.0959	.4011
5	143.49	.0987	.4897	15	124.34	.0989	.4727
6	227.43	.1690	.7456	16	96.71	.0805	.3663
7	128.21	.0957	.4451	17	140.63	.1297	.6087
8	127.54	.0905	.5001	18	151.15	.1087	.5303
9	99.62	.0639	.3132	19	174.49	.1032	.4999
10	126.90	.1151	.5287	20	220.89	.1544	.6946
Avg.	132.27	.1029	.4838	Avg.	150.36"n.s."	.1143"n.s."	.5313"n.s."
Std. Error of Mean	13.07	.0104	.0431	Std. Error of Mean	13.639	.0092	.0408

TABLE VI. Mg^{+2} content of control hearts and experimental hearts.

Controls				Experimentals			
Rat #	Mg^{+2} content (ug)	Mg^{+2} (ug)/ wet wt. ht.(mg)	Mg^{+2} (ug)/ dry wt. ht.(mg)	Rat #	Mg^{+2} content (ug)	Mg^{+2} (ug)/ wet wt. ht.(mg)	Mg^{+2} (ug)/ dry wt. ht.(mg)
1	256.54	.1886	.8666	11	139.08	.1093	.4914
2	192.81	.1526	.7502	12	-	-	-
3	253.36	.2683	1.235	13	335.53	.2831	1.375
4	248.32	.1977	.8682	14	290.32	.1897	.7932
5	250.54	.1724	.8550	15	230.65	.1836	.8769
6	240.07	.1791	.7871	16	206.45	.1718	.7820
7	251.66	.1879	.8738	17	254.90	.2351	1.103
8	228.62	.1621	.8965	18	256.76	.1847	.9008
9	263.42	.1688	.8283	19	287.83	.1703	.8247
10	234.69	.2129	.9778	20	263.42	.1842	.8283
Avg.	242.00	.1890	.8938	Avg.	251.66"n.s."	.1884"n.s."	.8727"n.s."
Std. Error of Mean	6.38	.0104	.0425	Std. Error of Mean	11.28	.0082	.0409

TABLE VII.

Ratios of K^+ concentration (ug/ht.) to Na^+ concentration (ug/ht.) in control and experimental hearts.

Controls		Experimentals	
Rat #	K^+(ug/ht.)/ Na^+(ug/ht.)	Rat #	K^+(ug/ht.)/ Na^+(ug/ht.)
1	2.1114	11	2.8968
2	4.0489	12	2.7826
3	2.9278	13	3.1711
4	3.0872	14	1.7525
5	2.7195	15	2.7552
6	2.9719	16	2.4193
7	2.6795	17	3.5311
8	2.7696	18	2.6579
9	3.8903	19	3.1892
10	3.0144	20	2.7884
Avg.	3.0220	Avg.	2.7944 "n.s."
Std. Error of Mean	.1803	Std. Error of Mean	.1526

TABLE VIII.

Ratios of Mg^{+2} concentration (ug/ht.) to Ca^{+2} concentration (ug/ht.) in control and experimental hearts.

Controls		Experimentals		
Rat #	Mg^{+2}(ug/ht.)/ Ca^{+2}(ug/ht.)	Rat #	Mg^{+2}(ug/ht.)/ Ca^{+2}(ug/ht.)	
1	1.735	11	.6326	
2	3.007	12	-	
3	2.102	13	3.131	
4	1.811	14	1.977	
5	1.745	15	1.854	
6	1.055	16	2.134	
7	1.962	17	1.812	
8	1.792	18	1.698	
9	2.644	19	1.649	
10	1.849	20	1.192	
Avg.	1.970	Avg.	1.759	"n.s."
Std. Error of Mean	.1687	Std. Error of Mean	.1131	

TABLE IX.

Ratios of Ca^{+2} concentration (ug/ht.) to Na^{+} concentration (ug/ht.) in control and experimental hearts.

Controls		Experimentals		
Rat #	Ca^{+2}(ug/ht.)/ Na^{+} (ug/ht.)	Rat #	Ca^{+2}(ug/ht.)/ Na^{+} (ug/ht.)	
1	.1030	11	.2000	
2	.0938	12	.1245	
3	.1165	13	.1142	
4	.1419	14	.0869	
5	.1182	15	.1165	
6	.1832	16	.0936	
7	.1161	17	.1454	
8	.0999	18	.1141	
9	.0931	19	.1265	
10	.1395	20	.1731	
Avg.	.1205	Avg.	.1294	"n.s."
Std. Error of Mean		Std. Error of Mean		

DISCUSSION

I.

Body weight generally is determined by "the balance between energy intake (effective caloric intake) and energy expenditure (basal metabolism, plus activity metabolism plus specific dynamic action of food)." (Thomas and Miller, 1958). The preceding results indicate that the experimental rats weighed significantly less than the control rats at the conclusion of the experiment. This difference in weight must be largely due to the increase in the activity metabolism (energy expenditure) of the experimental rats compared to a very low activity metabolism in the control group. The experimental animals obviously did not increase their caloric intake enough to compensate for this increased energy expenditure, or else the difference between the two groups would not be so great. The animals were allowed to feed ad lib. and the quantity of food intake was not monitored.

El-Hage (1976) reports this same magnitude of difference in body weight between control and exercised animals. Penparkgul et al. (1976), and Scheuer and Stezoski (1972) report similar results. Likewise, the above mentioned researchers all report no significant difference in heart weights between experimental and control animals; results reported herein are in agreement. This result indicates that no cardiac hypertrophy occurred as a result of this training regimen, therefore,

increase in heart size cannot suffice as an explanation of the decrease in chronotopic response of the heart as a result of training as reported by El-Hage (1976).

The significant difference in heart weight to body weight ratios between the two groups is to be expected, due to a significant difference in body weights but a non-significant difference in heart weights. One should notice, though, that the difference is more pronounced in the dry weight ratios as compared to the wet weight ratios. This tends to indicate a slight increase in the solid content of the experimental hearts in relation to their body weights. This increase in solid content can be partially explained by the slight increase in total ionic content as a result of exercise. The results reported herein show a 10 mg. gain in dry weight of the heart in the exercised animals. The total weights of Na^{+1}, K^{+1}, Ca^{+2}, Mg^{+2} equal 4.71 mg. for controls and 4.83 mg. for exercised animals. This is a gain of 0.12 mg. or 1.2% of the total gain in dry weight. The remainder of this 10 mg. gain is likely due, primarily, to an increase in cardiac contractile proteins as a result of exercise.

II.

Raab (1969) suggests that habitual exercise, in addition to increasing arterial dilatability and improving O_2 economy of the heart, probably increases myocardial K^+ content via transfer of K^+ from the exercising muscles and the liver to

the heart tissue. He suggests that this accumulation of K^+ is cardioprotective against K^+ depleting effects of increased sympathetic activity in stress situations. Raab also states that increased cardiac acetylcholine has been shown to increase myocardial K^+ and decrease Na^+. He suggests that cardiac cholinergic preponderance, as a result of habitual exercise, is cardioprotective, by augmenting this K^+ accumulation and Na^+ depletion. On the basis of these proposals, one would predict an increase in K^+ : Na^+ ratio in exercised animals, since there is evidence (Herrlich, Raab and Gigee, 1960, Smith and El-Hage, 1978) that exercise causes an increase in cardiac acetylcholine content.

Increased cardiac acetylcholine decreases the rate of the heart's contraction by action on the sino-atrial nodal membranes. Acetylcholine normally hyperpolarizes membranes by increasing K^+ efflux and decreasing the membranes permeability to Na^+, which increases the resting potential difference across the membrane. This decreases the effective development of an action potential, thus decreasing the frequency of contractions (Langley, Telford, and Christensen, 1980). Therefore, acetylcholine's influence, resulting in bradycardia, is really due to a change in K^+, Na^+ concentrations across the cardiac muscle fiber membranes, and does not necessarily imply any change in total tissue content.

The experimental work reported herein reveals no significant difference in K^+ and Na^+ concentrations between the

experimental (habitually exercised) and the control (non-exercised) groups, and no significant difference in the K^+: Na^+ ratio. Therefore, this data does not support Raab's proposal, but is still consistent with the concept of increased cardiac acetylcholine being responsible for the decreased chronotropic response following training. The acetylcholine exerts its influence by affecting K^+, Na^+ flux, but this does not produce a detectable change in total tissue content of these two ions.

Ca^{+2} ions are related to both the heart's chronotropic and inotropic responses. It is known that Ca^{+2} is necessary for the release of acetylcholine from nerve terminals. (Katz, 1966). The mechanism of this interaction is unknown. If the increased acetylcholine in the exercised rats is neural in origin, then the increased release may be influenced by the calcium concentration at the vagal nerve endings in the heart.

Secondly, Ca^{+2} is necessary for the excitation-contraction coupling process of cardiac muscle. The depolarizing effect of an action potential will not result in a contraction of heart muscle fibers without a sufficient concentration of Ca^{+2} externally (Katz, 1966). Katz states:

> Calcium is an essential "cofactor" in initiating contraction of the heart; sodium is a "competitive inhibitor" and antagonizes the calcium effect. The higher the Ca/Na ratio, the less depolarization is needed to initiate the contractile response; with a sufficiently high value of Ca/Na, the heart-muscle fibers contract even at the normal level of the resting potential.
>
> (Katz, 1966)

In addition, as reported in the literature review, Ca^{+2} affects the inotropic response of the heart. It frees actin from the troponin-tropomyosin complex, and aids in Mg-ATP hydrolysis, which provided energy for the cross-bridge attachments in muscle fibers (Langley, Telford, and Christensen, 1980). The positive inotropy characteristic of training is probably partially due to Ca^{+2} influence on modulation of the contractile force of the cardiac muscle fibers.

Although it is not directly known whether the training regimen used herein results in the characteristic increase in inotropic response, it is entirely possible that these exercised rats would show this increase along with the decrease in chronotropy.

The concentration of Mg^{+2} is involved in cardiac contraction both via exerting effects at the membrane, and by its participation in several intracellular events. It is known that Mg^{+2} acts as an antagonist to Ca^{+2} at the membrane (Shine and Douglas, 1974, Shine, 1979). This is probably because it is in competition with Ca^{+2} for membrane sites. Since Ca^{+2} augments acetylcholine release and influence, Mg^{+2} then would inhibit this or exert an anti-parasympathetic effect on the membrane potential. Thus, a decrease in Mg^{+2} at the membrane, or more accurately, a decreased Mg/Ca ratio would promote increased acetylcholine release and corresponding action.

Internally, ionic magnesium influences force development by the myofibrils via its ratio relationship to the Ca^{+2}

concentration necessary for coupling to occur. It affects the binding and release of Ca^{+2} from the sarcotubules. Specifically increasing Mg^{+2} slows the rate and reduces the magnitude of Ca^{+2} release from the sarcotubules. Internal Mg^{+2} concentration within a very specific range is necessary for Na^{+} and K^{+} stimulated ATP-hydrolysis by the sarcolemma. Mg^{+2} binding to sites on adenyl cyclase increases the velocity of the catalytic conversion of MgATP to cyclic AMP, a reaction necessary for corss-bridge formation (Polimeni and Page, 1973). The comlexity and variety of Mg^{+2} functions as well as its distribution within the cell make it a very difficult ion to study and interpret.

Overall though, if indeed increased acetylcholine is resulting in decreased chronotropy in the exercised animals, then one would expect a decrease in Mg^{+2} and a corresponding increase in Ca^{+2} in their cardiac tissue. With regard to internal Mg^{+2} influences, Shine (1974) concludes that the internal processes involving Mg^{+2} can continue uninterrupted when Mg^{+2} concentration is altered, because internal Mg^{+2} is maintained via internal pools. It is not dependent on transport of external Mg^{+2}. This point tends to suggest that one may not find a change in total tissue Mg^{+2} concentration. Its internal equilibrium and activity may be altered as a result of exercise without any evident change in total content.

The results reported herein show a small, but statistically non-significant (2.4%) decrease in Mg^{+2} in the exercised

hearts. This may reflect the decrease at the membrane related to the Ca^{+2} increase there and the corresponding increase in parasympathetic influence. It is quite likely that the levels and equilibration of internal Mg^{+2} are changing, and are probably affecting the inotropic response of the heart. Since its levels are not dependent on external Mg^{+2} it would not contribute to a change in total tissue content, thus supporting the lack of significant change in Mg^{+2} content with exercise.

The Mg^{+2}/Ca^{+2} ratio in this experiment shows an 11.9% decrease in the exercised animal's ratio versus the control animal's ratio. Although this decrease is statistically non-significant according to the t-test, it is supportive of the prediction that a drop in this ratio would likely occur with increased acetylcholine release and influence in cardiac tissue.

The Ca^{+2}/Na^{+} ratio is reported herein with reference to the previous statement from Katz (1966) on the significance of this ratio on the maintenance of membrane excitability. There is a statistically non-significant 7.3% increase in this ratio in the exercised animals. Presumably an increase in Ca^{+2}/Na^{+} ratio would result in a prolongation of the action potential. This effect could be expected in bradycardia (Mullins, 1981).

III.

The literature review does not include any recent reports on previous work done on rat cardiac total tissue ionic contents

because no such results were found. There was no indication that such measurements have ever been done on habitually exercised rats. In fact, Raab (1969) points out the lack of and need for such data. A limited amount of information was found on cat cardiac ion concentrations. Robertson and Peyser (1951) report Na^+ and K^+ concentration for the myocardium of the cat as follows:

Na^+	19:3 meq./100 gm. (dry)
K^+	39:7 meq./100 gm. (dry)

These values convert to:

Na^+	4.439 ug./mg.
K^+	15.52 ug./mg.

These values compare quite favorably to results reported herein. In addition the Handbook of Biological Data (Spector, 1956) reports concentration values for four electrolytes in fresh cardiac tissue of the rat as follows:

Ca^{+2}	3-20 mg./100 gm. fresh tissue
Mg^{+2}	18-26 mg./100 gm. fresh tissue
K^+	320-340 mg./100 gm. fresh tissue
Na^+	85-95 mg./100 gm. fresh tissue

The values reported herein fall within these reported ranges with the exception of sodium which is slightly higher than the value above. These two comparisons suggest that the method used herein and the data reported are reliable.

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