The Effects of Chronic Exercise on Chronotropic and Inotropic Responses of Isolated Rat Atria to Nicotine

A thesis presented to the faculty of the the Department of Biological Sciences of the State University of New York College at Brockport in partial fulfillment of the requirements for the degree Master of Science in Biology.

by

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THESIS DEFENSE

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Abstract

Sprague-Dawley rats were divided into either a control group or one of three training regimens. Rats were conditioned by either motorized wheel running (Group I), a combination of running and forced swimming (Group II) and forced swimming only (Group III). The training consisted of two sixty minute sessions per day, seven days a week for six to eight weeks. At the conclusion of the exercise program the atria were removed and placed in a Krebs-Henseleit solution (pH of 7.2$\pm$.01) continuously oxygenated with 95% O_2 and 5% CO_2 and maintained at a temperature of 34.0$\pm$.05^{o}C. The chronotropic and inotropic responses of the isolated atria to acetylcholine (1×10^{-6}M, 1×10^{-7}M) and nicotine (1×10^{-4}M, 1×10^{-5}M, 1×10^{-6}M) was evaluated. Group II and Group III displayed a significant increase in mean ventricular and mean total heart weights. All exercised rats (Groups I, II and III) were compared to control rats with respect to isolated atrial rates. Exercised rat atria beat at a significantly slower rate. The mean body weight of group I and group II were significantly greater than their control counterparts at the termination of their exercise program. The atria of exercised rats were significantly less sensitive to acetylcholine and nicotine when chronotropic and inotropic responses were evaluated. The results suggest that exercise induced the atria to become less sensitive to acetylcholine both at the muscarinic (effector cell) and the nicotinic (postganglionic) receptor sites, presumably as a result of increased vagal impulse traffic.

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FIGURES

INTRODUCTION

There are many biochemical and physiological adaptations that occur in the cardiovascular system of man and animal as a result of endurance training. Many of these adaptations may play an important role in lowering the susceptibility of the physically active individual to heart diseases (Fox et al., 1971; Paffenbarger and Hale, 1975). In the well trained athlete there is an increase in the efficiency of the cardiovascular system characterized by a relatively large maximal cardiac output, maximum stroke volume, and high maximum oxygen uptake (maximum aerobic power) when compared with untrained individuals (Ekbolm et al., 1968). Codini (1977) and associates showed that rats conditioned by moderate swimming programs displayed similar peak left ventricular systolic pressure and maximum left ventricular dP/dt to sedentary controls. The difference in performance could only be brought out when the hearts were stressed.

Chronic swimming and running has been found to enhance contractile performance of the left ventricle in rats (Thomas and Scheuer, 1979). Shorter relaxation times are focused on the relationship of myocardial performance and Ca^{+2} availablity to the contractile elements (Tibbits et al., 1978; Tibbits et al., 1981). Ashok and Scheuer (1975) have suggested that there is an increase in the myosin ATPase activity. A number of investigators, however, have failed to demonstrate improved cardiac performance as a result of enhanced myocardial contractility due to exercise (Gilbert et al., 1977; Carew and Covell, 1978; Nutter et al., 1981).

Exercise increases the arteriovenous oxygen difference (Ekbolm et al., 1968). Initial cardiovascular fitness and prior to training seems to play an important role in this adaption (Cummingham and Hill, 1975).

Cardiac hypertrophy has been observed as a result of endurance exercise (Leon and Bloor, 1968; Oscai et al., 1971; Dohm et al., 1977, Smith and El-Hage, 1978). Swimming programs have promoted greater cardiac size alterations than running programs (Baldwin et al., 1977). Many other researchers, however have found no changes in heart weight:body weight ratios induced by exercise (Fuller and Nutter 1981; Nutter et al., 1981; Gleason et al., 1983). According to Scheuer and Tipton (1981), the definition of cardiac hypertrophy is controversial in rat heart studies because training is often responsible for a reduction in body weight. This change in body weight could invalidate heart:body weight ratios observed. Less controversial and the most prominent adaptation of a physically fit population is a lower resting heart rate and a slower heart rate upon physical exertion (Frick et al., 1967). Bradycardia adaptations have been elicited in horses (Marsland, 1968), dogs (Tipton et al., 1974; Stone, 1977), and rats (Tipton, 1965). Bradycardia is one of the most commonly observed features of the physically fit individual. However, little is clearly understood about its mechanism(s).

Under normal physiological conditions, it is the two branches of the autonomic nervous system that exert influence over the chronotropy of the heart via the sinoatrial node. The preganglionic neurons of the sympathetic division of the autonomic nervous system exit the central nervous system by way of the thoracic roots. The nerves then synapse outside the central nervous system to form cholinergic ganglia that are located between the spinal cord and the heart. The postganglionic nerves

pass from the ganglia to the heart and release norepinephrine as its transmitter, acting to accelerate the chronotropic response (Vander et al., 1970). Transmitters such as norepinephrine and acetylcholine exert their effects on the heart by altering the permeability of the membrane which results in an alteration in the rate of firing of the active potential. When the cell is at rest the outside of the membrane is ionically positive with respect to the inside and is considerately more permeable to potassium than sodium. In a pacemaker cell, the membrane potential is gradually lost by a decreased K^+ permeability. When a critical threshold is reached there is a change in the permeability to sodium. Sodium ions rush inside the membrane and as a result the membrane polarity is reversed. This marks the first phase of the action potential or depolarization. During the second phase, repolarization, sodium permeability is deactivated and simultaneously potassium permeability increases. This causes the cell to quickly return to resting potential. The action potentials are propagated along the surface of the muscle membrane until tranverse tubules are depolarized. Depolarization of T-tubules results in a release of calcium ions from the sarcoplasmic reticulum which will initiate the sequences leading to myocardial contraction.

In the parasympathetic branch, the preganglionic nerves originate from various areas of the brain stem, (e.g., the nucleus ambiguus and dorsal motor nucleus of the vagus) and synapse on the intrinsic cardiac neurons. These ganglionic neurons are usually associated with the posterior aspect of the atria at the subepicardial level (Loffelholz, 1981). Both synapses are cholinergic and acetylcholine is the transmitter released at the postganglionic terminus that slows the heart rate.

Associated with heart chronotropic regulation are four major types of receptors. Catecholamines act via alpha and beta recetors. Acetylcholine can stimulate either nicotinic and/or muscarinic receptors. Both muscarinic and nicotinic receptors are considered subtypes of cholinergic receptors. Nicotinic receptors are associated with autonomic ganglionic transmission, while muscarinic receptors are located on the plasma membranes of the organs innervated by parasympathetic fibers (Watanabe, 1982).

The effects of acetylcholine have been widely studied in isolated and intact hearts (Hoffman et al., 1954; Schreiner et al., 1957; Denison and Green, 1958). In addition to cardiac vasodilation, acetycholine generally causes a negative chronotropic reaction in the heart. On the other hand, negative and positive inotropic responses have been observed. The release of norepinephrine from the sympathetic nerve endings accounts for the postive inotropy of acetylcholine (Blumenthal et al., 1968). In order to explain the contradictory data concerning acetylcholine and parasympathetic control of the heart, Buccino (1966), and colleagues hypothesized the existence of two distinct cholinergic receptors in the myocardium: a type I muscarinic receptor, which is associated with the vagal nerve endings and is responsive to small concentrations of acetylcholine; it is also blocked by atropine. The type II receptor is a spatially separate receptor, whose stimulation produces responses similar to those produced by nicotine, and is responsive to large concentrations of acetycholine. This hypothesis could explain the different inotropic response of atria and ventricles observed by other investigators.

Acetylcholine sensitivity differs with different structures of the heart. Acetylcholine has been shown to have an inhibitory effect on the hearts of dogs and rabbits (Frautwein, 1963). The inhibitory responses are greater in the sinoatrial node and the atrionodal junction. It is assumed that the differences in sensitivity are related to the density of the receptors in the structures compared (Frautwein, 1963).

Cholinergic innervations produce their action upon the heart by a variety of mechanisms. Acetylcholine and vagal stimulation produces the following changes in the action potential of pacemaker cells of the sinoatrial node recorded by intracellular microelectrodes: (1) a reduction in the slope of the spontaneous diastolic depolarization, (2) elevation of the maximum diastolic potential, (3) a reduction in the duration of the action potential and (4) a shift of the pacemaker to another site in the node (Higgins et al., 1973). Some past investigators have suggested that acetycholine shortens the action potential duration of atrial myocardium by increasing the potassium permeability of the membrane (Burgen and Terruux, 1953). Negative inotropy is caused by the shortening of the plateau by acetylcholine, which diminishes the calcium uptake during the action potential (Grossman and Furchgot, 1964). Investigations by Eick (Eick et al., 1976) indicated that acetylcholine can exert a negative inotropic effect in two ways. By amplifying the outward current, and thereby abbreviating the action potential, acetylcholine has an indirect effect on the slow inward current. When action potential duration is reduced, it is expected that the time in which the slow inward current can flow is also reduced. Negative inotropy results from the shortened time of the slow inward current. In the presence of high concentrations of acetylcholine, a

direct effect is observed; the inhibition of the slow inward current. It is well established that in cardiac tissue, the slow inward current plays a determining role in the force of contraction (Higgins et al., 1973).

In the absence of any external influences, the sinoatrial node fires spontaneously in a rhythmic fashion. The actual discharge frequency of the sinoatrial node at any given time is determined by the action of the sympathetic and parasympathetic nervous systems on the automaticity of the sinoatrial node. The mechanism(s) responsible for altered cardiovascular response to exercise in trained individuals; in particular, training bradycardia, are not well understood. It is possible that they could be a result of altered neural and/or hormonal factors affecting the cardiovascular system during exercise. It is also possible that the bradycardia mechanism(s) could be intrinsic to the heart (Smith and El-Hage, 1977). Several possible mechanisms have been proposed for bradycardia due to endurance training. Truex et al., 1955 studied the relationship between heart rate and body weight in dogs. They concluded that an increase in body weight and heart weight were correlated with a decrease in heart rate. Some investigators associate bradycardia with alterations in the autonomic nervous system. Tipton (1965) reported an increase in the parasympathetic activity. By using atropine sulfate as a parasympathetic blocker and propranolol hydrochoride as a sympathetic blocker, Lin and Horvath (1972) were able to determine the intrinsic heart rate and autonomic tonus on the control of heart rate. From their studies it was concluded that there was a reduction in both parasympathetic and sympathetic activity, with a greater reduction in sympathetic tonus. Training bradycardia has been reported to occur in trained

hypophysectomized rats devoid of pituitary hormones (Tipton et al., 1969). Similar results have been found in unilateral vagotomized, immunologically sympathetomized (Tipton, 1965), and thyroidectomized (Tipton et al., 1968), trained rats. Atropine is a competitive blocker of acetylcholine at muscarinic receptors. When injected into normal and hypophysectomized rats, trained rats show less chronotropic acceleration than their nontrained counterparts (Tipton and Taylor, 1965; Tipton et al., 1969). These results suggest the mechanism(s) underlying bradycardia has a cholinergic basis. It is assumed that animals with more available acetylcholine would be expected to exhibit less of a cardiac response as atropine competes with acetylcholine for receptor sites. This indeed seems to be the case. Neostigmine, an anticholinesterase, when administered to trained and untrained rats resulted in lower heart rates exhibited by the trained rats. This is further evidence for a cholinergic mechanism underlying training bradycardia (Tipton et al., 1966). Choline acetyltransferase catalyzes the reaction: Choline + Acetyl-Co A Acetylcholine + Coenzyme A (Blustajn and Wurtman, 1983). Rats after physical training have shown an increased concentration of choline acetyltransferase in their atria. Increased ventricular concentration of choline acetyltransferase was not observed (Ekstron, 1974).

De Schryver (1959), and collegues observed a reduction in norepinephrine level in the heart after endurance training in rats (23). Other researchers (Leon et al., 1975), however, failed to find any significant difference in heart norepinephrine concentration between untrained rats and rats trained by an intense chronic swimming regimen that produced cardiac hypertropy.

In comparative metabolism studies by Vik and Tucek (1964), it was observed that acetylcholine was higher in the region of the superior vena cava, lower in the remainder of the right atrium and lowest in the left atrium of cats, dogs, guinea pigs, and rats. Increased amounts of acetylcholine have been found in atria of trained rats (Herrlich et al., 1966; Schryver and Strythagen, 1975). It has been observed that isolated atria from exercised rats exhibited lower intrinsic rate than from sedentary rats (Bolter et al., 1973; Smith and El Hage, 1978). Since this bradycardia expresses itself without sympathetic or parasympathetic contribution, it is likely that a training bradycardia mechanism could be intrinsic to the heart.

Smith and El Hage (1978) also observed that when atropine was added to the isolated tissue bath of exercised and sedentary isolated rat atria, the exercised rats showed a significant positive chronotropic response relative to that of the sedentary controls. They concluded that the increased concentration of acetylcholine found in atria has an important involvement in training bradycardia.

METHODS

Thirty-two Sprague-Dawley rats were divided into three groups. Within each group rats were randomly assigned to either a sedentary (control) or exercised (experimental) group. Group I contained six controls and six experimental animals, group II contained six controls, six experimental and group II contained three controls and five experimental members. Initial weights ranged from 167-370 grams. All animals were housed in pairs in environmentally controlled quarters. A pair consisted of a control and a experimental rat. Food and water was given ad libitum.

Two different exercise programs were used; a running and a swimming regimen. There was a 3 week delay from the start of the training program of group I (runners) to the start of the program of group II (run and swim). Likewise there was a 3 week delay from the start of the training of group II to the start of training of group III (swimmers).

The experimental animals used in the running program were exercised in a six compartment motorized activity wheel (Wahmann Manufacturing Co.). Each of the six compartments was separated from the adjoining compartments so that each rat was running in a 10.5 cm. wide, 115 cm. diameter, astroturf lined chamber. The training regimen consisted of running the animal in the activity wheel for two 60 minutes sessions per day. At least 3 hours was allowed between sessions. Training continued seven days a week for six to eight weeks. The rate of the activity wheel was set at 185 meters/min for the entire exercise program. The control group was also placed in the activity wheel twice daily for 5-10 minutes but was not

exercised. All rats were weighed weekly. Heart rates for group I were also taken on a weekly basis. Hearts rates were obtained by permanently placing two wires through the skin on the upper back of the laboratory animal for the purpose of recording the ECG with a model DMP-4B Physiograph (Narco, Houston, Texas).

As a result of a breakdown of the activity wheel, which could not be replaced and was irreparable, an alternate exercise regimen was devised. The breakdown occurred during the fourth week of the training session of group II. All exercise training after that time consisted of swimming the rats in a 20 gal. tank (76 x 35 x 34 c.) twice daily for 60 minutes. At least 3 hours was allowed between the sessions. The temperature of the water was kept constant at $35^{o}C$. The format of the swimming program was similar to that of the running in that it was carried out seven days a week for six to eight weeks. Controls were placed in the tank twice daily for 5-10 minutes but were not allowed to swim.

After the training period the rats were anesthetized with ether and the heart was quickly removed and placed in a dish containing cold modified Krebs-Henseleit solution (0.22 gm KCl, 6.3 gm NaCl, 0.324 gm KH_2PO_4, 0.143 gm $MgSO_4$, 0.282 gm $CaCl_2$, 2.0 gm $NaHCO_3$, 1.8 gm $C_6H_{12}O_6$, dissolved in 1.0 liter of distilled water). The atria were then dissected free from the ventricle and placed in a continuously oxygenated (95% o_2, 5% CO_2) organ bath containing Krebs-Henseleit solution at a pH of 7.2 + 0.1, and maintained at a temperature of $34.0 \pm 0.5^{o}C$. The organ bath chamber was a 30 ml glass cylinder opened at the top and mounted vertically to a metal ring stand (Fig. 1). Along the middle of the tube there were three points to which plastic tubing was attached. The lower tube allowed flow from a

1 liter container of Krebs-Henseleit solution. The middle tube allowed inflow of oxygen and carbon dioxide, and the upper tubing was an overflow drain. Each tube was controlled with a screw-clamp valve. The entire organ bath was immersed in a water bath at $34^{o}C$.

One tip of the atria was attached by means of an S-shaped metal wire to the bottom of a glass rod. The other end of the atria was attached to an S-hook which was connected by means of a thread to a strain gauge transducer mounted above the organ bath. The glass rod was placed inside the organ bath through the top so that the tissue was submerged below the level of the solution and was in close proximity to the inflow of oxygen. Optimal stretch was placed on the muscle by either lowering or raising the height or the transducer. Spontaneous atrial contraction was recorded. A period of stabilization (approx. 30 minutes) was allowed to elapse before beginning recording. The basal rate and contractile force were then determined. Contractile force was measured in centimeters and converted to tension in grams. After a second stabilization period (5-10) minutes the the chronotropic (rate) and the inotropic (force) response of the atria to nicotine (conc. 1×10^{-4} M, 1×10^{-5} M, 1×10^{-6}M) and acetylcholine (conc. 1×10^{-6} M, 1×10^{-7} M) were examined.

The concentrations refer to the concentration of the drug in the organ bath chamber. The responses to the various concentrations of nicotine was determined at 1 hour intervals. Acetylcholine was also administered in the same fashion. After each exposure of the isolated atria to a drug the tissue was washed at least four times before the same drug at a different concentration was added to the organ chamber and at least two times before a different drug was administered to the chamber. Each washing consisted

of completely flushing the organ bath with fresh warmed Krebs-Hensleit solution 6-8 times. An example of an experimental protocol is as follows:

1 hr interval:
1) Nicotine added to bath (conc. 1×10^{-5} M)
2) Wash with Krebs-Hensleit (6-8 times)
3) Wash with Krebs-Hensleit (6-8 times)
4) Acetylcholine added to bath (conc. 1×10^{-6} M)
5) Wash with Krebs-Hensleit (6-8 times)
6) Wash with Krebs-Hensleit (6-8 times)

1 hr interval:
7) Nicotine added to bath (conc. 1×10^{-4} M)
8) Wash with Krebs-Hensleit (6-8 times)
9) Wash with Krebs-Hensleit (6-8 times)
10) Acetylcholine added to bath (conc. 1×10^{-7} M)
11) Wash with Krebs-Hensleit (6-8 times)
12) Wash with Krebs-Hensleit (6-8 times)
13) Nicotine added to bath (conc. 1×10^{-6} M)

The effects of each drug added to the organ bath was determined by measuring the inotropic and chronotropic responses of the tissue.

At the end of the experiment the atria and ventricles were weighed. The order of the administration of drugs was randomized with each experiment. Significance of response was tested using Student's t-test. Drugs used in this study were: Nicotine (Eastman Kodak), Acetylcholine chloride (Sigma).

RESULTS

EFFECTS OF CHRONIC EXERCISE ON ATRIAL WEIGHTS, VENTICULAR WEIGHTS, TOTAL HEART WEIGHTS AND HEART/BODY WEIGHT RATIOS

After the termination of each experiment the atrium and ventricle of each rat was weighed. Table I is a summary of the effects of three different exercise regimens on atrial weights, venticular weights and total heart weights. Group I represents those rats exercised by a motor driven activity wheel. Group II are those animals trained by a combination of motor driven activity wheel running and swimming. Group III's program consisted of swimming only. Mean atrial weights of group I, II and III, when compared to control rats showed no significant difference. Mean venticular weights and total heart weights of those animals exercised by a motor driven activity wheel showed no significant weight change from the control animals. Group II and Group III displayed a significant increase in mean venticular weights ($P \leq .01$., and $P \leq .001$, respectively). Similiar effects were observed with total heart weight. Mean total heart weights of group II and group III were significally increased ($P \leq .02$ and $P \leq .001$) over the controls. Heart weight/body weight ratios were calculated by dividing the mean total by the mean body weight (Table II). The ratio for all control rats was 3.1 mg/g and 3.2 mg/g for all conditoned animals.

EFFECT OF CHRONIC EXERCISE ON BODY WEIGHT

During the exercise program all rats were housed in pairs in environmentally controlled quarters and given food and water ad libitum. Each rat was weighed weekly. At the beginning of the endurance program the

mean body weight of the rats whose exercise program consisted of running was 302 grams. The mean body weight of their control counterparts was 296 grams. These means were not significantly different. It can be seen from figure 2 and table III, that by the conclusion of the endurance training the mean body weight for control rats was 285 gm and 320 gm for exercised animal. This represents a significant increase ($P \leq .001$) in mean body weight. Figure 3 is a graphic representation of those rats trained by a combination of motor driven activity wheel running and swimming and their control counterparts. The endurance program concluded at six weeks. At no time during the six weeks was there a signficant difference between controls and exercised animals (Table IV). Swimming as an exercise regimen caused a significant ($P \leq .02$) increase in body weight (Table V). Figure 4 is the graph of the effects of swimming on rat mean body weight over seven weeks. At week 0 the mean body weights for controls were 235 grams and 201 grams for the animals that were to be conditioned by swimming. At the conclusion, seven weeks, the mean body weight for controls was 396 grams and 448 grams for exercised animals. At week two the exercised animals were significally ($P < .05$) lighter than the control group.

THE EFFECTS OF CHRONIC EXERCISE ON RESTING HEART RATES AND BASAL ISOLATED ATRIAL RATES

Table VI and figure 5 display the mean resting heart rates in beat/min of rats exercised by a motor driven activity wheel for seven consective weeks starting from week two of training. At week two the mean for controls was 423 bpm and 413 bpm for exercised animals. At weeks 3 and 4 the resting heart rates for the rats that had undergone endurance training

was observed to be significally slower ($P \leq .05$ and $P < .01$ respectively). By the termination of the training the mean rate for control rats was 445 ± 7 and 414 ± 21 for exercised animals. This was significant at $P \leq .02$.

At the conclusion of the endurance programs, the atria were isolated and placed in a organ bath containing Krebs-Hensleit solution. When the entire control group (N=13) was compared to the entire excercised group (N=10) the basal isolated atrial rate for conditioned animals was significantly slower ($P < .001$). The effects of three different exercise regimens on basal isolated atria rates was observed. (Table VII). Group I, which were those rats conditioned by a motor driven activity wheel, when compared to the entire control group displayed a significally slower rate ($P < .001$). Rats that were trained by a combintion of activity running and swimming (Group II) also displayed a significant decrease ($P \leq .02$) in basal isolated atria rats. Group III the rats whose regimen consisted of swimming showed no significant decrease in mean basal isolated atria rates. (figure 5).

THE EFFECTS OF CHRONIC EXERCISE ON THE CHRONOTROPIC AND INOTROPIC RESPONSES TO ACETYLCHOLINE AND NICOTINE

Acetylcholine when introduced to the organ bath caused a decrease in the magnitude of the atrial contractile force and a decrease in the frequency of the of the atrial rate. Figure 7a displays a typical response of the isolated atria to acetylcholine when the concentration of acetylcholine in the organ bath was 1 uM. A similar response was observed when the acetylcholine concentration was 0.1 uM except that the decrease in bath contractile force and atrial rate was in most cases not as great.

Both chronotropic and inotropic responses occurred 1-2 seconds after the introduction of the drug to the bath. The duration of the responses varied greatly from atrium to atrium but complete recovery was usually observed after 1 minute.

Acetycholine at both concentrations in some cases caused a complete blockage of spontaneous atrial contractions. This did not last long, about 4 to 8 seconds, then complete recovery was observed as usual.

Nicotine caused a biphasic atrial response when it was added to the organ both. The first phase was similar to that for acetylcholine. Both negative chronotropy and negative inotropy was observed seconds after the introduction of the drug to the bath. The duration averaged about 1 1/2 minutes. The rapid onset of the second phase was characterized by an increase in contractile force and an increase in atrial rate which exceeded the basal level (Fig 7b). Atrial arrest which was sometimes seen with acetycholine was not observed with nicotine in these studies. The mean chronotropic responses of isolated atria of controls and conditioned rats to acetylcholine and nicotine was ascertained. Figure 8 is a graphic illustration of these means. The chronotropic responses to both drugs displayed a dose related effect. Acetycholine (1 uM) caused a much greater reduction in atria rate than did nicotine at the same concentration. Exercised animals were significantly less sensitive to nicotine than their control counterparts. Table VIII shows that the negative chronotropic response to nicotine was greater in control rats. Acetylcholine at 1 uM did not produce a significantly different chronotropic response between control and exercised rats; however, acetylcholine at 0.1 uM did ($P<.001$).

Inotropic responses are tabulated as percent change between pre-drug and post drug treatment with acetylcholine or nicotine.

Inotropic responses to both nicotine and acetylcholine were dose related (fig. 9). Acetylcholine (1 uM) caused a much greater decrease in contractile force than did nicotine at the same concentration. Acetylcholine 1 uM and 0.1 uM had a greater negative inotropic effect in control rats than in exercised animals. This difference was significant at $P \leq .001$ (Table IX). A significant ($P \leq .001$) difference between control and exercised rats with respect to inotropic response was observed with the administration of nicotine at all of the three concentrations used in this study.

DISCUSSION

Chronic exercise elicits a variety of profound adaptions in the body of man and animal. The cardiovascular system is by no mean exempt from physiological adaptions occurring as a result of endurance training. In the conditioned individual the hemodynamic efficiency of the cardiovascular system is improved over that of non conditioned individuals. This improvement is characterized by a large maximum oxygen uptake, maximum stroke volume, and a relatively large maximum cardiac output (Ekbolm et al., 1968).

Experimentally, chronic training has been found to promote cardiac hypertrophy (Dohm et al., 1977; Smith and El-Hage 1978). During exercise there is an increase in the demand of blood needed by the working muscles. The regular sustained increase in work load of the heart causes it to become larger relative to body weight. Some investigators however, found no change in heart weight to body weight resulting from endurance training (Nutter et al., 1981; Gleason et al., 1983). In this study the total heart weight of those rats conditioned by a motor driven activity wheel, running at a rate of 185 meters/min twice a day for eight weeks showed no significant increased in total heart weight over control rats. Those rats whose regimen consisted of swimming in a 20 gal. tank. (76 x 35 x 34 cm) twice a day for 60 min over 7 weeks had heavier total heart weights than their control counterparts. The rats that were trained by a combination of swimming and running also displayed an significant increase in total heart weight over their control group. The level of significance however was less than that of group III's (those animals who swam only). Two important variables that appear to influence heart weight in the absence of cardiovascular abnormalities are physical activity and body weight. (Oscai

et al., 1971). It is the observation of several investigators that rats subjected to a training program gain weight more slowly than sedentary controls (Jones et al., 1964; Smith and El-Hage 1978). It has been suggested that a decrease in food intake and an increase in caloric expenditure is likely to be the cause of the slower growth rates of rat subjected to an exercise training program. It was observed in this study that with the exception of Group II the conditioned animals were significantly heavier at the conclusion of the training program than their control counterparts. It would have been interesting to observe the relationship between caloric intake and the final body weights of these rats. However food consumption measurements was beyond the scope of these experiments. Appetite suppression resulting from exercise is a complex process. It has been suggested that appetite suppression is mediated by an increase in catecholamine levels associated with the stress of exercise, and not only does stress vary with the work load but also with the animal's present physical condition (Oscai et al., 1971). Appetite suppression does not occur in all exercising animals. There seem to be a relationship between appetite suppression and the duration and the severity of the training (Steveson et al., 1966; Oscai et al, 1971). Thomas and Miller (1958) found that in their studies rats conditioned by a regimen consisting of five days training and two days rest displayed a depression in caloric intake on training day but on recovery days displayed an increase in food intake above the control levels. The mechanism of cardiac hypertophy induced by exercise is by no mean simple. If it is true that body weight (and this does seem to be the case) is an important variable in the expression of exercise cardiac hypertrophy, then it is likely that any

mechanism or mechanisms acting on body weight or body composition of the growing exercising animal might effect the development of cardiac hypertrophy.

Some studies suggest that the adaptions to running and swimming might be qualitatively different. Schaible and Scheuer (1979) observed that there was a trend for swimmers to develop greater ventricular pressures and have faster rates of relaxation than runners. Baldwin et al., (1977) found that rats conditioned by a swimming regimen promoted greater cardiac size alterations than did running programs. Results from this study suggest that different regimens of exercise do in fact have different adaptive ramifications on the cardiovascular system of the rat. Swimming produced an increase in total heart weights, but no significant difference was observed between swimmers and their controls with respect to isolated atrial rate. On the other hand, rats subjected to a running regimen displayed the opposite effect; no significant increase in total heart weight, but a significant lowering of basal isolated atrial rate. Both adaptations are beneficial. A larger, healthy heart would be able to compensate for the demand placed upon it by a workload due to exercise. A lower resting heart rate and a lower heart rate upon physical exertion can meet the increased demand of a greater workload by allowing greater ventricular filling. It is very difficult to compare the effect of different exercise regimens. For one, it would be difficult to be sure that the exercise programs have equal intensities. The physiology of an animal supported by land might be different from an animal supported by water. The fact that the animal is placed in water might cause stresses that will interact with either known or unknown mechanism(s) to cause

slight differences in the adaptive response of the cardiovascular system enduced by training. For example, there seem to be a fluid shift from extracellular to vascular when an animal's body is immersed in water (Krasney, 1986).

In addition to cardiac hypertrophy, bradycardia is a commonly observed adaptation induced by training. Its mechanism(s) to date are not clearly understood. Truex et al., (1955) studied the relationship between heart rate and body weight. They concluded that an increase in body weight was correlated with a decrease in heart rate. In the intact animal it is the autonomic nervous system that exerts control over the chronotropy of the heart. Dogs that have undergone cardiac denervation prior to an exercise program do not develop exercise bradycardia (Ordway et al, 1982). Many investigators have therefore attributed exercise bradycardia to alterations in autonomic control. Some authors have suggested a change in both parasympathetic and sympathetic activity (DeSchryver et al., 1969). An increase in parasympathetic (Tipton and Taylor, 1965) has also been cited. The use of pharmacological blocking agents have been very useful in shedding some light on the mechanism(s) of resting bradycardia induced by training. Beta receptor blocking drugs antagonize the effects of catecholamines by competing for receptor sites (Hoffman, 1982). Propranolol is a non-selective member of this class of drug, and has been used to isolate the heart from the influcence of the sympathetic nervous system (Frick, 1967). Atropine, a tertiary ammonium alkaloid ester of tropic acid is found naturally in the plant Atropa belladona (deadly nightshade). Atropine blocks (reversibly) the actions of acetylcholine

at the muscarinic receptor (Katzung, 1982). Because of its actions, atropine has been used to isolate the heart from the influence of the parasympathetic nervous system (Tipton and Taylor, 1965).

The effects of pituitary hormones have been dismissed as having a major role in the development of exercise bradycardia. Tipton (1969) observed that hypophysectomized rats developed a lower resting heart rate as a result of being trained by a motor driven treadmill for 11 weeks. There is strong evidence to suggest that the mechanism(s) involved in exercise bradycardia might be cholinergic in nature. Increased amounts of acetylcholine have been found in the atrial of conditioned rats (Herrlich et al., 1966; Schryer et al., 1925). Also, exercised rats that are given 1 mg/kg intraperitoneal injection of atropine sulfate displayed less cardiac acceleration than non trained rats (Tipton, 1965). This would be the expected results if in fact exercised rats have a greater acetylcholine content than non trained rats, because there would be competition between atropine and acetylcholine for receptor cites.

It could be possible that the mechanism(s) of exercise bradycardia is intrinsic to the heart. Smith and El Hage (1978) observed that exercise bradycardia was established in the isolated atrial of rats trained by a motor driven activity wheel twice a day for one hour, six days a week for seven weeks. They observed that the spontaneous isolated atrial rate of control rats was 235 beats/min. In the present study the isolated atrial rate for control rats was 234. Smith and El-Hage's exercised rat isolated atrial rate was 192 beats/min. In this study the mean of all three exercise regimens was 196 beats/min. When the atria are isolated from the intact rat the atria are no longer under the direct influence of the

parasympathetic or sympathetic nervous system. The study done by Smith and El-Hage suggests that the increased amount of acetylcholine observed by Herrlich et al; (1966) is likely to play an important role in training bradycardia. The effects of acetylcholine have been widely studied in isolated and intact hearts (Hoffman et al., 1954; Schrener et al., 1957; Denison and Green, 1958). Acetylcholine given orally causes a negative chronotropic reaction in the heart. In addition to its chronotropic effect, acetylcholine may also display a negative intropic effect on the atria (Eick, 1976). Similiar effects were observed in the present study. In the parasympathetic nervous system of the intact animal acetylcholine is the neurotransmitter that acts at the postganglionic site (nicotinic receptor) and at the effector cell site (muscarinic receptor). In this study nicotine was used to stimulate the nicotinic receptors on the postganglionic parasympathetic neurons in the isolated atria. Nicotine usually produced a biphasic response in cardiac muscle (Dixon 1924). This is due to a primary release of acetylcholine from postganglionic parasympathetic neurons, which produces inhibition, followed by release of catecholamines with resulting excitation. The inotropic and chronotropic responses of the isolated atria to acetylcholine and nicotine of exercised rats was less than the responses of the control rats. The decrease in sensitivity is likely to be a result of a change in the number of functional receptors. Both nicotine and acetylcholine sensitivity are decreased in the isolated atria of exercise rats which suggest that two different locations are affected, the postganglionic nicotinic receptors and the muscarinic receptor on the surface of the atrial muscle. It has been observed by Katz and Thesleff (1957) that continuous exposure of

acetylcholine or another agonist to the superficial end plates of the frog sartorius muscle resulted in desensitization of the acetylcholine receptor. Katz describe densensitization as "a condition in which the end plate has become refractory to depolarizing agents and from which it recovers only slowly after complete withdrawal of the drug". Katz also suggests that densensitization arises a result of gradual transformation of the drug-receptor compound into an inactive form. There is a relationship between the development of desensitization and the drug concentration (Fatt, 1950). The greater the concentration, the faster the rate of development of densensitization. Desensitization has been observed in a variety of tissues, including intestinal smooth muscle, (Barsoum and Haddum; 1935), frog neuromuscular juction, (Katz and Thesleff; 1957), and arterial smooth muscle. (Khairallah et al, 1969). Rang and Ritter (1970) suggested that the mechanism of densensitization entails the inactivation of specific receptors rather than any non-specific depression of the tissue. To date a number of mechanisms have been preposed to explain densensitization but the phenomenon still remains unclear.

Hypersensitivity to nicotine has been observed in canine hearts as a result of cardiac denervation (Priola et al.; 1983). This observation is consistant with Cannon's law of denervation that states "an effector cell deprived of its normal neurohumoral stimulation will subsequently increase its responsiveness to that same neurohumoral agent when exposed to it after a variable (day to weeks) period of time" (Cannon; 1939). The studies by Priola et al. suggest it is the intracardiac neurons, which are the parasympathetic postganglionic neurons that become hypersensitive to

nicotine and acetylcholine. These intracardiac neurons are left intact after the denervation procedures are implemented. Smith et al (1985) observed that the right atrium is significantly more responsive to nicotine than the left atria and shows a greater hypersensitivity after denervation. It was suggested that the right atrium shows a greater hypersensitivty to nicotine because there are more intracardiac neurons present in the right atrium than the left atrium. It seem logical to assume that any mechanism(s) that alters the spontaneous rate of the heart is likely in some way to interact with the pacemaker cells located in the right atrium.

The response of the right atrium to acetylcholine and nicotine of the chronic denervated dog is one of hypersensitivity. The opposite effect has been observed in this study. Exercise produced a desensitization of the right atria to acetylcholine and nicotine. The hypersensitivity caused by chronic denervation or the desensitivity caused by exercise is likely to be a result of an alteration in parasympathetic nerve traffic. Exercise increases parasympathetic traffic at rest. This would be in line with the observation of Herrlich et al. (1966) where forced exercise increased the amount of acetylcholine in the atria of trained rats. This increase in acetylcholine results in a desensitization of the muscarinic receptors on the effector cells and the nicotine receptors on the postganglionic parasympathetic neurons. (intracardiac neurons). The mechanism of this desensitization of receptors is likely to be functionally similar to that preposed by Katz and Thesleff (1957). An inactivation of portion of the receptor pool (muscarinic and nicotinic) would explain the results observed in this study.

The ability of an organism to adapt to different environmental conditions is remarkable. Usually when one thinks of adaptations in a biological sense one usually thinks of genetic mutations that express themselves in future generations. Equally interesting are the physiological adaptions that occur in cells, tissues, organs and organ system as a result in a change in the external environment. The changes that occur in the cardiovascular system of man and animal as a response to the stress placed upon it by chronic training is a good example. It has been established that many of these cardiovascular adaptations that express themselves in the trained individual act as a deterrant to the development of heart diseases (Fox et al., 1971; Paffenharger and Hale, 1975). A commonly observed feature of training is a lower resting rate (Frick et al., 1967; Tipton et al., 1974). Results from the present study suggest that the mechanism(s) of exercise bradycardia is cholingenic in nature and as Smith and El-Hage suggested, is intrisnic to the heart. The evidence of this study suggest that exercise causes an alteration in the sensitivity of the atria at the receptor level and this alteration changes the characteristic of the entire cardiovascular system.

Table I

Atrial Weights, Venticular Weights and Heart Weights of Exercised Rats Trained by Different Regimens

Group	Atrial Wts			Venticular Wts			Heart Wts		
	N	grams	SEM	P	grams	SEM	P	grams	SEM	P
Controls	15	.16 ±	.03		.92 ±	.04		1.08 ±	.04	
Group I	4	.19 ±	.06	--	.91 ±	.05	--	1.07 ±	.04	--
Group II	4	.13 ±	.00	--	1.05 ±	.10	P≤01	1.18 ±	.11	≤.02
Group III	4	.17 ±	.01	--	1.40 ±	.02	P≤.001	1.45 ±	.06	P≤.001

Group I = running only
Group II = running and swimming
Group III = swimming only

Table II

Heart Weight/Body Weight Ratio
of all Control and Exercised Rats

	Heart Wt/Body Wt mg/gm	N
Control	3.1	15
Exercised	3.2	11

Table III
Body Weights of Group I Members

Weekly Mean body weights (grams) of controls and exercised in group I. All exercised rats were trained by motor driven activity wheel runnung.

Week	Control			Exercised			
	grams	SEM	N	grams	SEM	N	Probability
0	296	± 18	6	302	± 18	6	--
1	296	± 14	6	296	± 10	6	--
2	286	± 14	6	284	± 13	6	--
3	283	± 15	6	285	± 13	6	--
4	285	± 14	6	297	± 12	6	--
5	282	± 16	6	292	± 12	5	--
6	284	± 18	6	291	± 16	5	--
7	277	± 17	6	296	± 5	4	--
(8)Terminal	285	± 16	6	320	± 3	4	$P \leq .001$

Values are mean±standard error. N = number of animals observed

Table IV

Body Weights of Group II Members

Weekly mean body weights (grams) of controls and exercised animals in group II. All exercised rats were trained by an exercise regimen which consisted of running and swimming.

Week	Control			Exercised			
	grams	SEM	N	grams	SEM	N	Probability
0	219	11	6	227	8.0	6	--
1	254	11	6	242	6	6	--
2	266	13	6	270	9	6	--
3	291	15	6	285	12	6	--
4	318	18	6	331	13	5	--
5	372	13	6	374	7	5	--
(6)Terminal	403	22	6	391	8	5	--

Values are mean±standard error. N = number of animals observed

Table V

Body Weights of Group III Members

Weekly mean body weights (grams) controls and exercised animals in group III. All exercised rats were trained by a swimming regimen

Week	Control			Exercised			
	grams	SEM	N	grams	SEM	N	Probability
0	235	± 26	3	201	± 17	5	--
1	276	± 24	3	240	± 16	6	--
2	320	± 10	3	286	± 16	4	$P \leq .05$
3	359	± 22	3	336	± 20	4	--
4	360	± 18	3	344	± 26	3	--
5	374	± 14	6	395	± 45	3	--
6	388	± 6	3	416	± 18	3	--
(7)Terminal	396	± 70	3	448	± 17	3	$P \leq .02$

Values are mean±standard error. N = number of animals observed

Table VI

Resting Heart Rates

Weekly mean resting heart rates (beats per minute) of group I members week two through week eight

Week	Control			Exercised			
	bpm	SEM	N	bpm	SEM	N	Probability
2	423 ±	16	4	413 ±	14	6	--
3	435 ±	16	6	404 ±	25	5	$P \leq .05$
4	427 ±	9	6	400 ±	16	6	$P \leq .01$
5	435 ±	6	6	434 ±	6	6	--
6	443 ±	21	5	436 ±	13	4	--
7	411 ±	13	3	434 ±	12	3	--
8	445 ±	7	6	414 ±	21	4	$P \leq .02$

Values are mean±standard error. N = number of animals observed

Table VII

Basal Isolated Atrial Rates

Mean isolated atrial rates (beats/min.) of all controls and exercised rats.

	bpm	SEM	N	Probability
Control	234 ±	13	13	
Exercised	196 ±	11	10	P≤.001

Basal Isolated Atrial Rates of Exercised Animals

Mean isolated atrial rate (beat/min.) of rats trained by different regimens.

	bpm	SEM	N	Probability
Group I (activity wheel)	169 ±	11	4	P≤.001
Group II (act. wheel & swim)	206 ±	2	3	P≤.02
Group II (swimming)	224 ±	27	3	--

Values are mean±standard error. N = number of animals observed

VIII

Chronotropic Responses

Chronotropic responses of isolated atria to acetylcholine and nicotine. Table shows the mean difference in beats/minutes between pre-drug and post-drug treatment

	Control			Exercised			
	bpm	SEM	N	bpm	SEM	N	Probability
ACH 1×10^{-6}	59	± 20	13	67	± 23	11	--
ACH 1×10^{-7}	48	± 15	13	25	± 9	11	$P \leq .001$
NIC 1×10^{-4}	46	± 12	13	27	± 6	11	$P \leq .001$
NIC 1×10^{-5}	26	± 4	13	21	± 6	11	$P \leq 02$
NIC 1×10^{-6}	11	± 15	13	5	± 2	11	$P \leq .01$

Values are mean±standard error. N = number of animals observed.

Table IX

Inotropic Responses

Inotropic responses of isolated atria to acetylcholine and nicotine. Table shows the mean difference in % change between pre-drug and post-drug treatment.

drug	conc	Control %Change	Sem	N	Exercised %Change	SEM	N	Probability
ACH	1×10^{-6}M	72.4	2.6	13	63.7	5.8	11	$P \leq .001$
ACH	1×10^{-7}M	62.7	5.3	13	38.3	7.4	11	$P \leq .001$
NIC	1×10^{-4}M	45.2	4.3	13	31.1	7.0	11	$P \leq .001$
NIC	1×10^{-5}M	41.8	4.1	13	18.9	5.5	11	$P \leq .001$
NIC	1×10^{-6}M	11.2	4.9	13	2.1	2.1	11	$P \leq .001$

Values are mean$\pm$standard error. N = number of animals observed.

Figure 1

Organ bath.

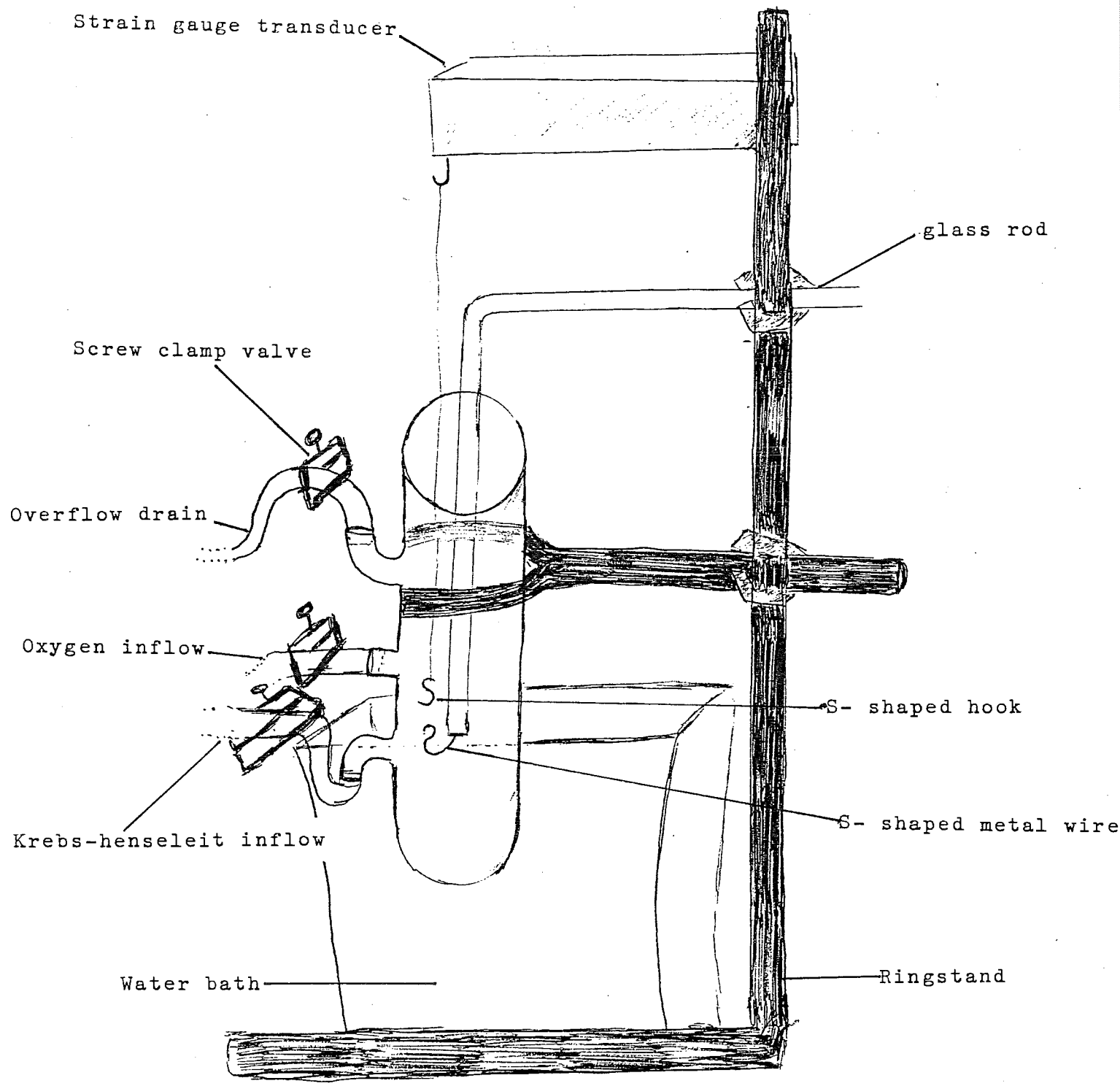

Strain gauge transducer
glass rod
Screw clamp valve
Overflow drain
Oxygen inflow
S
S- shaped hook
S- shaped metal wire
Krebs-henseleit inflow
Water bath
Ringstand

Figure 2

Mean body weights in grams of control rats and rats conditioned by a motor driven activity wheel for eight weeks.

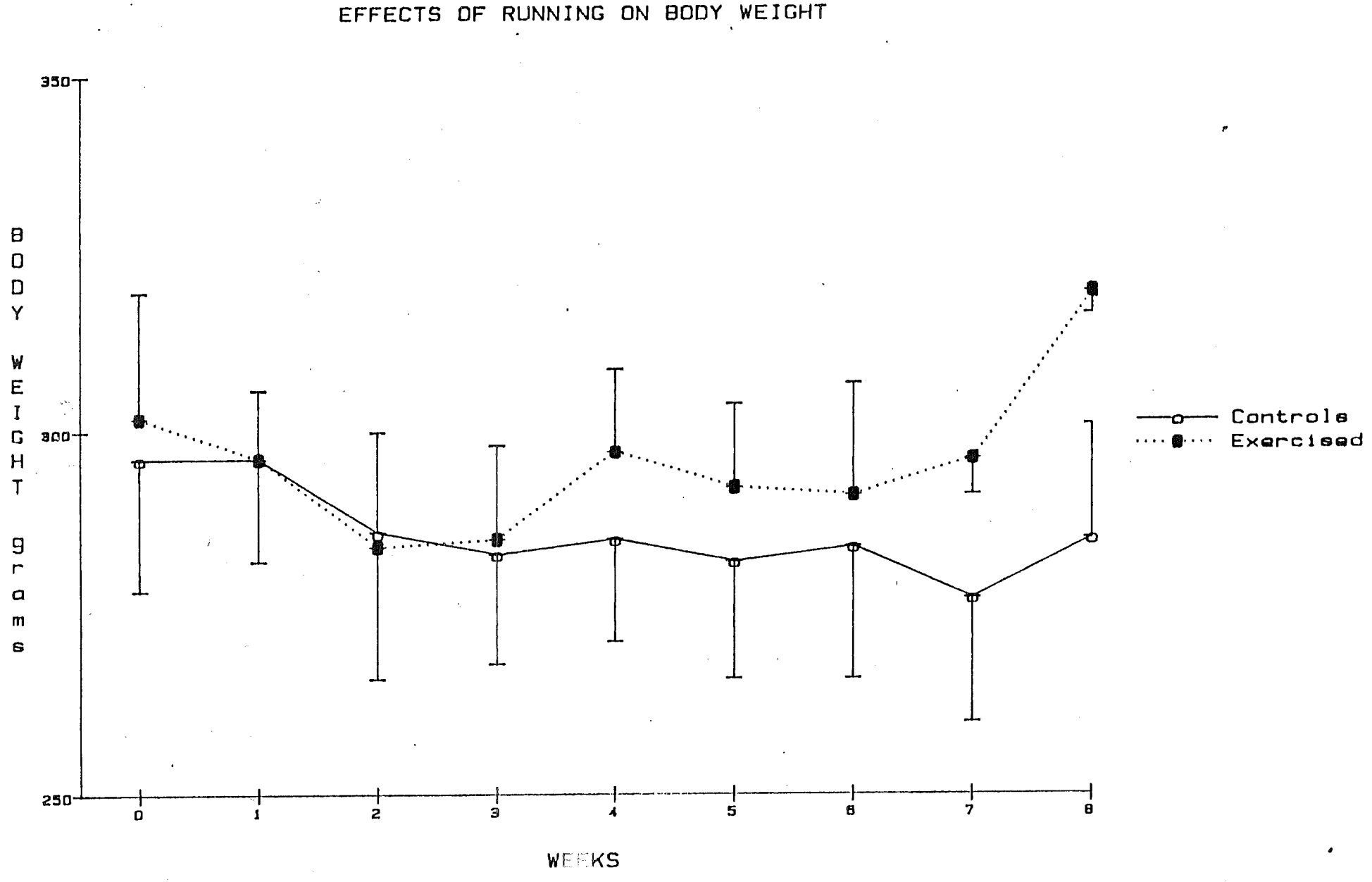

EFFECTS OF RUNNING ON BODY WEIGHT
BODY WEIGHT grams
350
300
250
0
1
2
3
4
5
6
7
8
WEEKS
Controls
Exercised

Figure 3

Mean body weights in grams of control rats and rats conditioned by a combination of running and swimming for six weeks.

EFFECTS OF RUNNING AND SWIMMING IN COMBINATION ON BODY WEIGHT

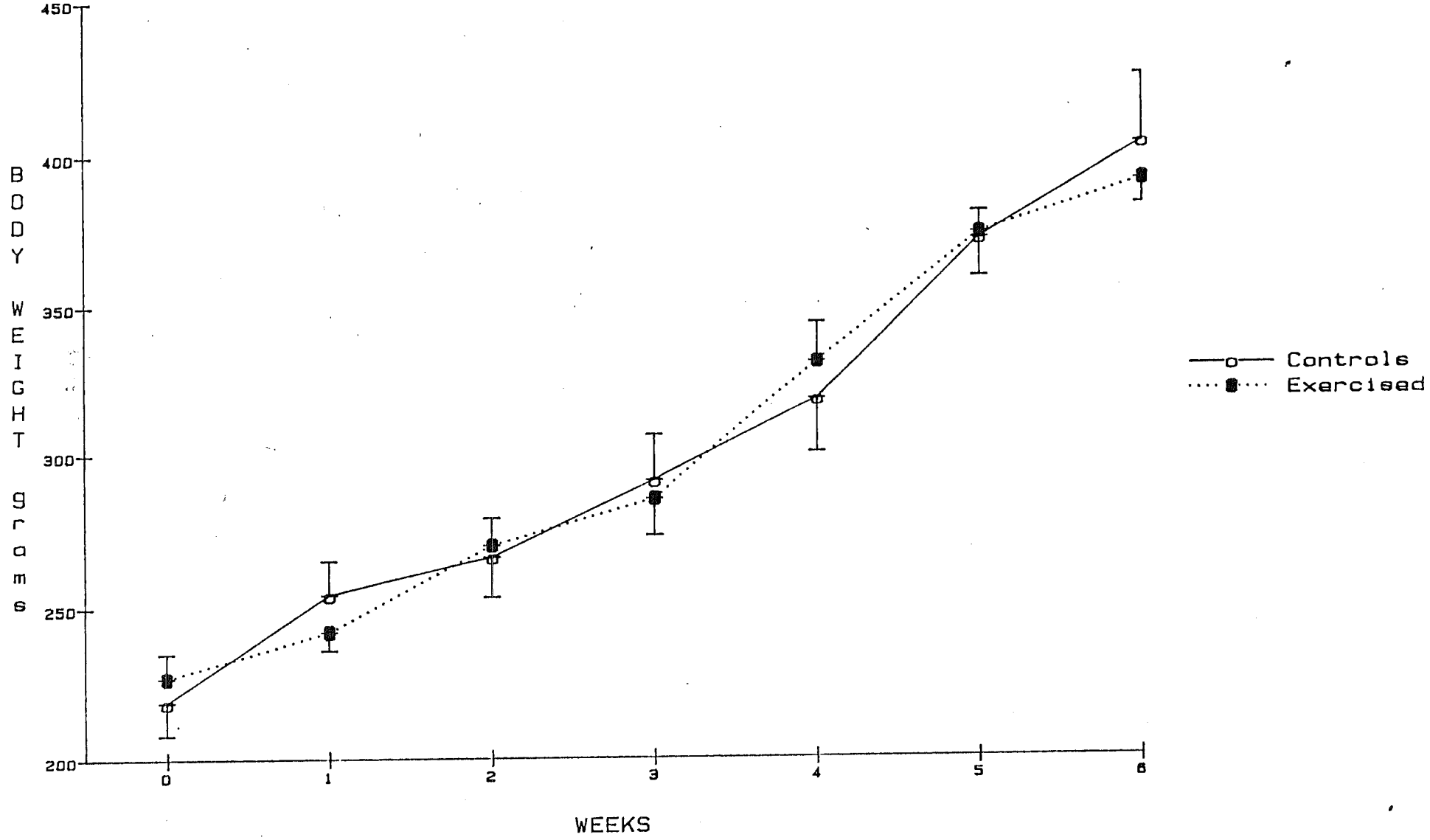


Figure 4

Mean body weights in grams of control rats and rats conditioned by swimming for seven weeks.

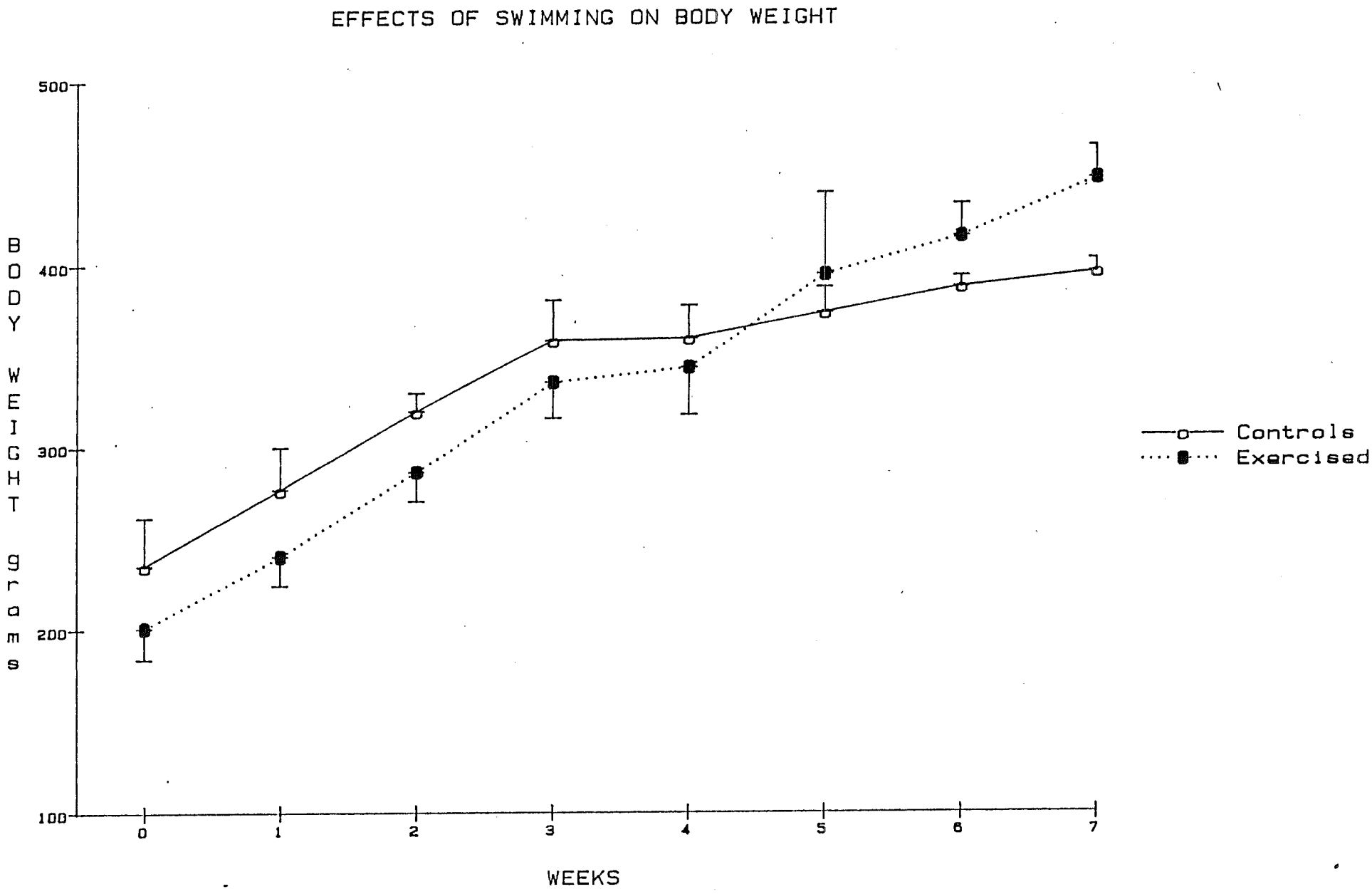

EFFECTS OF SWIMMING ON BODY WEIGHT
BODY WEIGHT grams
500
400
300
200
100
0
1
2
3
4
5
6
7
WEEKS
Controls
Exercised

Figure 5

Mean resting heart rates (beats per minute) of control rats and rats conditioned by motor driven activity running for seven consective weeks.

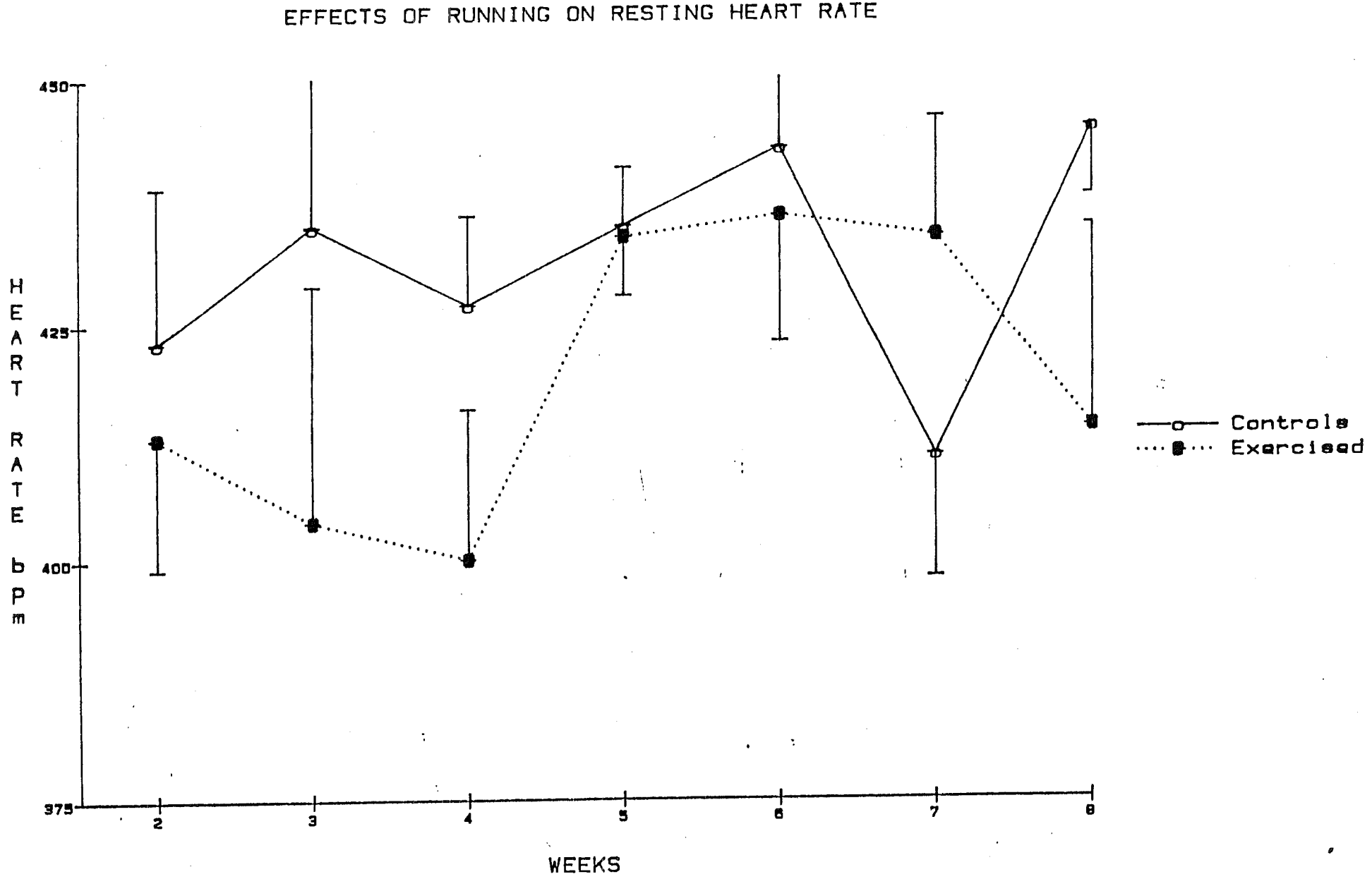

EFFECTS OF RUNNING ON RESTING HEART RATE
HEART RATE bpm
450
425
400
375
2
3
4
5
6
7
8
WEEKS
Controls
Exercised

Figure 6

Spontaneous right atrial frequency (beats per minute) of total control group compared to total conditioned group and three different exercise regimens. Group I = motor driven activity running; Group II - motor driven activity wheel running and swimming; Group III - swimming.

EFFECTS OF EXERCISE ON
SPONTANEOUS RIGHT ATRIAL RATE

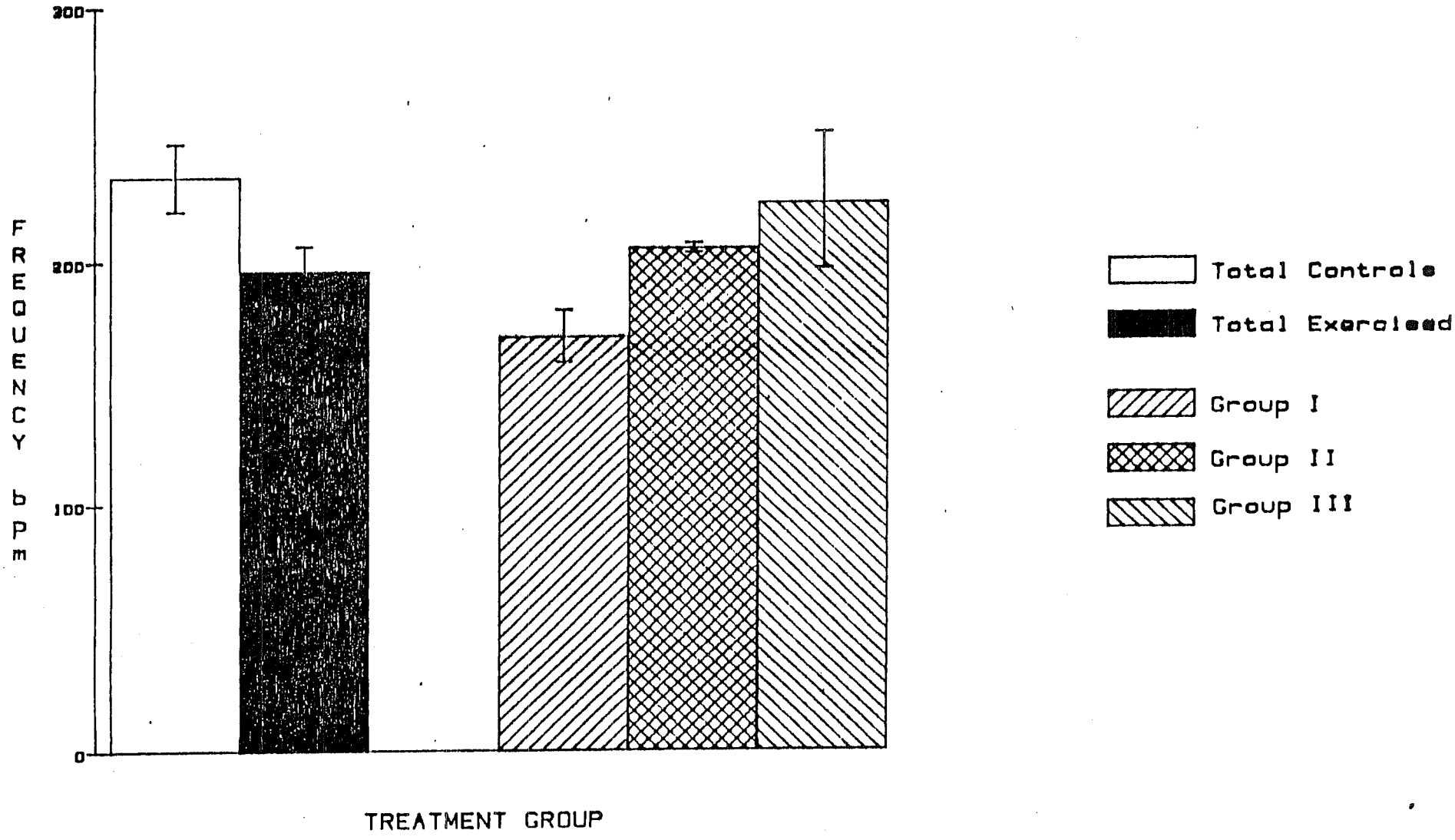


Figure 7

A. Typical response of isolated atria to acetylcholine (conc. = 1 uM; papr speed. 5 cm/sec).

B. Typical response of isolated atria to nicotine. (conc. = 100 uM; paper speed - .5 cm/sec).

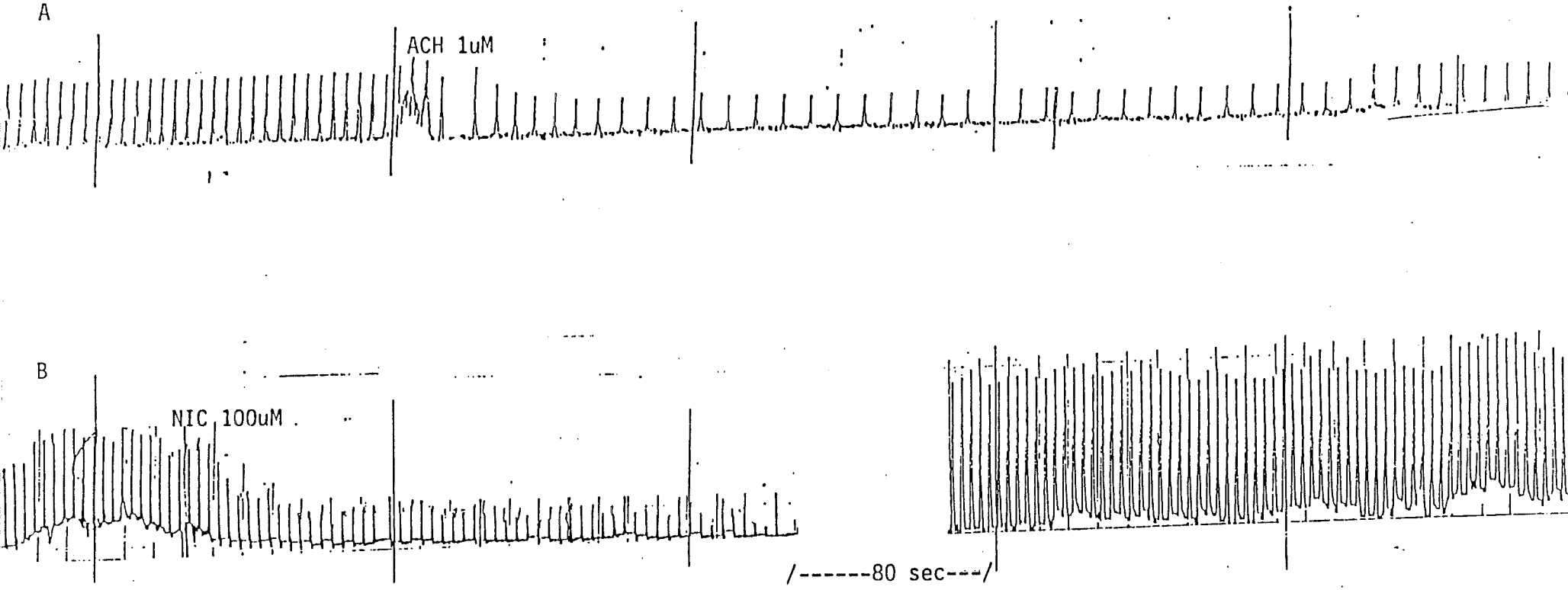

A
ACH 1uM
B
NIC 100uM
/------80 sec---/

Figure 8

The effects of exercise on the chronotropic response of isolated atria to acetylcholine and nicotine. Response are expressed as change in heart rate (beats per minute).

EFFECTS OF EXERCISE ON CHRONOTROPIC RESPONSES
TO ACETYLCHOLINE AND NICOTINE

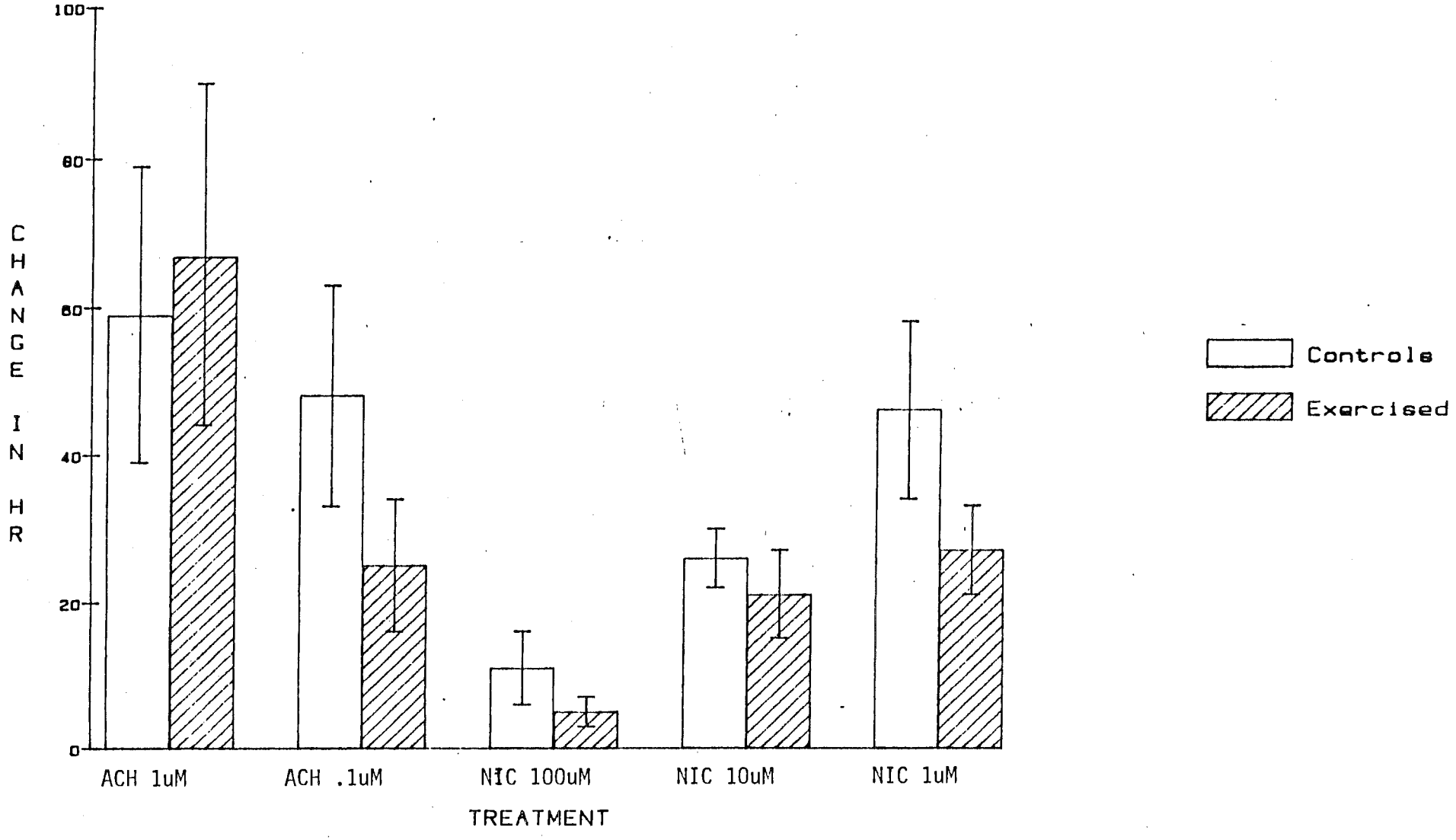
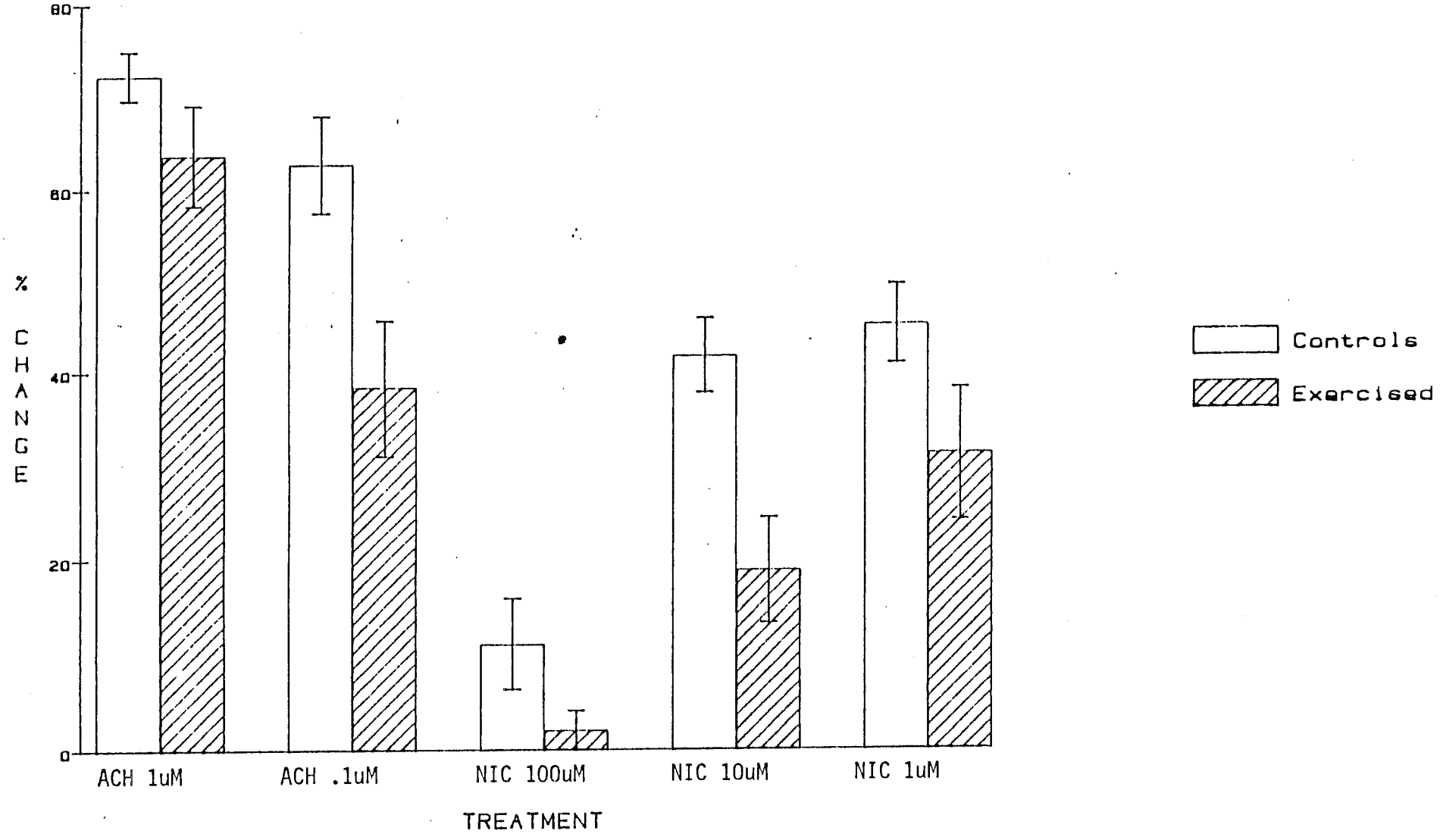


Figure 9

The effect of exercise on the intropic response of isolated atria to acetylcholine and nicotine. Responses are expressed as precent change in contractile force.

EFFECTS OF EXERCISE ON INOTROPIC RESPONSES
TO ACETYLCHOLINE AND NICOTINE



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