1970

The Effect Of Physical Activity On Coronary Cast Weight And On Creatine Phosphokinase And Glutamic-oxalacetic Transaminase Levels In Plasma, Heart And Skeletal Muscles Of Rats

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THE EFFECT OF PHYSICAL ACTIVITY ON CORONARY CAST WEIGHT AND ON CREATINE PHOSPHOKINASE AND GLUTAMIC-OXALACETIC TRANSAMINASE LEVELS IN PLASMA, HEART AND SKELETAL MUSCLES OF RATS

by

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Submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

Faculty of Graduate Studies

The University of Western Ontario

London, Canada

September, 1969

This investigation was supported by a grant from the Defence Research Board of Canada. Personal support for the investigator was also provided by this organization. The investigator wishes to express his appreciation to this organization.

ACKNOWLEDGEMENTS

The author wishes to express his deepest appreciation to Dr. Jerry B. Critz for his guidance and assistance throughout this study. The author is very grateful for the advice and constructive criticism offered by the members of his advisory committee: Dr. P.G. Dellow, Dr. P.F. Mercer and Dr. J.J. Seguin, and by the Head of the Department of Physiology, Professor J.A.F. Stevenson. Special thanks are also extended to Mrs. V. Feleki for her valuable technical assistance, to Dr. J.M. Wanklin for advice on the statistical analysis of data, to Miss P. Baker for the preparation of illustrations, to Mrs. E. Rennie for the typing of this dissertation, and to Dr. G.W. Stavraky and Dr. A. Novakova for aid in the translation of foreign publications.

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ABSTRACT

Experiments were performed on rats to investigate the effect of exercise and physical training on levels of the enzymes creatine phosphokinase (CPK) and glutamic-oxalacetic transaminase (GOT) in plasma and in cardiac, soleus and gastrocnemius muscles. The capacity of the coronary circulation was estimated from the weights of vinyl-acetate casts prepared from the coronary arterial trees of resting rats. Rats were subjected to six different levels of physical conditioning: restricted rats (living in $\frac{1}{2}$ size cage; 3 weeks), control rats (standard cages; 3 weeks), spontaneously active rats (standard activity cage; 3 weeks) and physically trained rats (enforced swimming on alternate days for two, four or six weeks). At the end of each conditioning period the appropriate rats were sacrificed in either the resting condition or immediately following a single sixty minute swim.

Six weeks was the minimum training duration necessary to produce elevated enzyme levels in all three types of muscle. The resting myocardial CPK level increased from 31.1 International milliunits per microgram nitrogen (mU/ugN_2) in control rats to 35.9 mU/ugN_2 in rats trained for six weeks, and the resting soleus CPK level increased from 68.0 to 80.5 mU/ugN_2. Training rats for six weeks resulted in an elevation in the resting myocardial GOT level from 22.6 to 27.6 mU/ugN_2, soleus GOT level from 12.0 to

15.5 mU/ugN_2 and gastrocnemius GOT level from 4.07 to 5.68 mU/ugN_2. Rats trained for six weeks had post-exercise muscle enzyme levels that were similarly elevated above the post-exercise levels of controls. Cardiac muscle displayed an earlier enzyme adaptation to repeated exercise than did either type of skeletal muscle. The post-exercise enzyme levels of cardiac muscle became elevated after as little daily exercise as had occurred in the spontaneously active group. The post-exercise GOT levels in soleus (12.8 mU/ugN_2) and gastrocnemius muscles (5.12 mU/ugN_2) of restricted rats were also elevated above the post-exercise levels of controls (11.0 and 3.83 mU/ugN_2, respectively). Post-exercise muscle enzyme levels displayed training adaptation more readily than did the resting enzyme levels.

The resting level of plasma GOT (PGOT) in rats trained for two (85.8 International Units per liter), four (83.7 U/l) or six weeks (95.0 U/l) was higher than the PGOT level of controls (76.8 U/l). The PGOT level appears to be dependent upon a tissue to plasma gradient. The resting plasma CPK level was not elevated in trained rats. These data demonstrate that training produces a specific effect on the plasma level of each particular type of enzyme.

The coronary cast to heart weight ratio in restricted, spontaneously active or rats trained for periods of four or six weeks was higher than in corresponding controls. Three weeks

appeared to be the minimum duration of increased physical activity that is necessary to elicit an increased coronary cast to heart weight ratio.

The results obtained in restricted rats suggested a non-specific stress reaction had occurred and was responsible for the changes.

I. INTRODUCTION

Exercise and certain pathological conditions are normally followed by an elevation in the activity of various enzymes in blood plasma. The elevations that occur in pathological conditions are believed to be due to efflux of enzymes from necrotic cells. Reversible cellular membrane permeability changes are thought to be responsible for the efflux of cellular enzymes that occurs during exercise. It is currently believed that ischemia may be responsible for cellular necrosis, while hypoxia may produce the reversible changes associated with exercise. The effect of exercise or physical training on plasma or tissue enzyme levels has been studied in the past in an attempt to understand the mechanisms involved in the alterations of enzyme levels. The present investigation studied the effects of physical training on creatine phosphokinase (CPK) and glutamic-oxalacetic transaminase (GOT) levels in the heart and skeletal muscles and the blood plasma of the rat.

During the last twenty years several investigators have demonstrated that repeated exercise (physical training) elevates the activity of certain enzymes in the muscle cells of the rat. A thirty day physical training program of fifteen minutes swimming exercise per day was sufficient to elicit these changes (184). In most of these investigations, however, the training program consisted of daily swimming or running exercise for a period of five to eight weeks.

The activity of cellular enzymes in the blood serum is normally very low. A single bout of exercise results in an elevation of serum enzyme activity but this elevation can be reduced by physical training. The resting level of the plasma enzyme activity in trained rats is higher than that in control rats (18, 21).

Alterations in serum enzyme levels occurring under physiological circumstances have been explained on the basis of two principal theories. Most investigators believe that the permeability of cellular membranes is altered during exercise, resulting in a greater efflux of enzymes into the blood. Others maintain that a change in the serum enzyme levels reflects an altered tissue to serum enzyme gradient.

A recent study demonstrated that animals exhibiting low serum transaminase activity possessed low tissue activity and, conversely, animals possessing high serum activity displayed high transaminase activity in various body tissues (186). These observations support the theory that serum enzyme levels are dependent upon a tissue to serum enzyme gradient.

Much information was available concerning the effect of exercise and physical training on the blood levels of CPK and GOT. However, few investigators had studied the effect of physical exertion on the CPK and GOT activities in cardiac and skeletal muscle.

One of the earliest beneficial effects of physical training appears to be an improvement in the coronary circulation (115, 164, 166). This effect has been demonstrated in rats with the aid of a coronary vinyl-acetate cast technique to estimate the size of the coronary arterial tree (166). An improvement in the coronary circulation of experimental rats appeared before any signs of cardiac hypertrophy.

In the present investigation the simultaneous measurement of coronary cast size and the CPK and GOT activities of the blood plasma, cardiac muscle, slow (red) soleus muscle and fast (white) gastrocnemius muscle of the rat were employed to study the adaptation of body tissues to exercise. CPK represented enzymes involved in anaerobic metabolism and GOT represented enzymes involved in aerobic metabolism. The present experiments were designed to determine whether or not the muscle and plasma CPK and GOT levels are affected by single or repeated exercise episodes and, if they are, to establish the time of onset of measurable adaptation to training. Three different types of muscle were studied in order to determine which types of muscle adapt most readily to exercise. The plasma and muscle enzyme activity levels were compared in an attempt to establish whether or not plasma enzyme levels are dependent upon a muscle to plasma enzyme gradient. If a muscle to plasma gradient does exist, it may be possible to employ plasma

enzyme levels as indices of physical training. The present investigation provided a more complete spectrum of exercise levels than had been used by previous investigators, and attempted to demonstrate the effect of various types of exercise, and lack of exercise, on plasma and muscle enzyme levels and coronary cast size. It also provided data on the effect of physical training on plasma and muscle enzyme activities in rats in either the resting or the post-exercise condition. Physical training programs of two to six weeks duration were chosen to determine the time of onset of any changes.

II. HISTORICAL REVIEW

1. Description and Diagnostic Use of Serum Enzymes

The significance and function of intracellular enzymes have been investigated by physiologists and biochemists for many years. However, it was the discovery of intracellular enzymes in blood serum, and the association of elevated serum enzyme levels with various diseases that provided the major impetus in this field of study.

Creatine phosphokinase catalyzes the reversible transfer of a high energy phosphate group from creatine phosphate to adenosine diphosphate (ADP), resulting in the production of adenosine triphosphate (ATP) and creatine:

$$\text{Creatine Phosphate} + \text{ADP} \overset{\text{CPK}}{\rightleftharpoons} \text{Creatine} + \text{ATP}$$

This enzyme is found predominantly in skeletal muscle. Comparatively smaller amounts, in terms of enzyme activity, are found in cardiac muscle and brain tissue (88, 112). There is evidence that a substantial amount of this enzyme also occurs in human semen (79,112). Other tissues possess little or no CPK (88,112). Creatine phosphokinase is essential for the rapid regeneration of ATP. In the presence of this enzyme ADP can be anaerobically phosphorylated to ATP (182). The tissue distribution of CPK appears to reflect the importance of this enzyme as a catalyst in a chemical reaction that releases a reserve source of energy during muscular contraction. CPK appears to be most beneficial to skeletal muscle, which is capable of operating

anaerobically for long periods of time. The heart and brain tissues, which have a low tolerance for hypoxia, would also benefit from a readily available reserve source of energy.

The enzyme, glutamic-oxalacetic transaminase catalyzes the reversible transfer of an amino group from aspartic acid to alpha-ketoglutaric acid, with the production of oxalacetic acid and glutamic acid:

$$\underset{\text{acid}}{\text{aspartic}} + \alpha\text{-}\underset{\text{acid}}{\text{ketoglutaric}} \overset{\text{GOT}}{\rightleftharpoons} \underset{\text{acid}}{\text{oxalacetic}} + \underset{\text{acid}}{\text{glutamic}}$$

GOT is present in most of the body tissues but it occurs in greatest concentration in the heart, liver, skeletal muscle, brain and kidney (8,106,186). In addition to the interconversion of amino acids illustrated above, GOT may also play a role in intermediary metabolism by providing a common pathway between amino acids and keto acids (31). The appropriate keto acids which are formed in this reaction could enter the Krebs cycle and result in an elevated production of ATP. In view of the dependence of the Krebs cycle upon an aerobic environment, it is reasonable to assume a greater potential value of GOT in the aerobically functioning myocardium, than in skeletal muscle.

CPK and GOT are normally present in the blood serum in very small amounts (88,106) and it is believed that their existence in serum is the result of slow efflux from the various tissues (16,40,120). The function of CPK and GOT in the blood serum is unknown (40).

Due to the tissue distribution of CPK and GOT, elevations of their serum levels have been used to indicate disease or damage

to specific body organs. Serum creatine phosphokinase (SCPK) has been used extensively in the detection of myocardial infarction, muscular dystrophy and various other muscle and brain diseases (88,118). Serum glutamic-oxalacetic transaminase (SGOT) has been used for the detection of both heart and liver diseases (38,40,41). The serum enzyme elevations resulting from the above conditions are considered to be due to tissue necrosis (1,2,87,141). Tissue necrosis has been isolated as the causative mechanism on the basis of the magnitude and durations of the enzyme elevations observed (2,15,88,127,141).

Alterations in serum enzyme activities have been observed under certain physiological circumstances, including parturition, age, sex, physical exertion and other stresses. Parturition is associated with an increase in both SCPK (54) and SGOT (111) activities. The CPK activity in human serum is high at birth, falls to below adult levels immediately after birth, and then gradually increases to reach the adult level between the ages of one month and five years (77,78,140,183). The human adult level of either SCPK or SGOT activity remains constant throughout life (140,175). However, the SGOT activity of sheep has been shown to increase with age (107). Human males possess higher serum CPK activity levels than females, both in young individuals and in adults (97,140,175,183). SGOT activity is higher in male subjects than it is in females (175).

2. Exercise and Physical Training

(i) Serum Enzymes

Many investigators have studied the effects of exercise and physical training on tissue and serum enzyme activities. Decreases in the serum activities of certain enzymes have been demonstrated following exercise in men (42,111,132) and in rats (5,43,91). The post-exercise serum activity of a few enzymes have remained unchanged in human subjects (17,78,102,109,131,169) and in rats (21,43,91,155). However, muscular activity has usually resulted in an elevation of serum enzyme activity in man (4,3,17,58,59,65,78,82,86,102,108,109, 125,131-3,150,156,157). Elevations also have been demonstrated in various lower species including: monkeys (124), rabbits (80), horses (30,35), dogs (22,114,171,179) and rats (5-7,18,21,27,39,62,91,92, 121,122,138,155,159). The serum enzyme alterations that follow exertion appear to be related to the intensity and duration of the exercise load (7,39,58,59). Many investigators have demonstrated that these alterations can be reduced or prevented by physical training (4,6,18, 27,30,58,59,62,65,91,122,133,138,159).

Altland and Highman (1961) studied the effects of exercise on serum enzyme levels and on the morphology of various tissues of rats (5). These investigators forced rats to run in a rotating cage at a rate of 6.9 meters per minute for sixteen hours. Exercise of this nature resulted in a fall in the level of serum alkaline phosphatase (SAkP) and a marked elevation in the activities of serum glutamic-oxalacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), serum

lactic dehydrogenase (SLDH) and serum aldolase (SAld). The magnitude and duration of the post-exercise response varied with each enzyme. SGOT activity displayed a five-fold increase and returned to the control level by 72 hours post-exercise. Almost all of the rats in this study showed severe fatty changes in the liver, kidney and thigh muscles; mild fatty changes in heart muscle, marked depletion of lipid in the adrenal cortex and occasional necrotic muscle fibers. Except for the necrotic muscle fibers, all of these changes were absent 72 hours after exercise. The authors suggested that there may be a causal relationship between pathologic changes and serum enzyme alterations after severe, prolonged exercise. They also discussed the possibility of an alteration in the permeability of mitochondria or cellular membranes contributing to the enzyme elevations observed after exercise.

In 1963 these investigators conducted another study in which they compared the effects of training on the post-exercise elevations of serum enzymes (91). Rats were exercised as described in their previous publication for periods of either six, twelve or sixteen hours. Another group of rats was physically trained by forcing the animals to run for six hours each day for periods of one to twenty days. These rats were then subjected to a sixteen hour exercise episode. Untrained rats and rats in the first four days of training displayed a post-exercise decrease in SAkP activity and post-exercise elevations in the activities of SGOT, SGPT, SLDH and SAld. In the untrained rats these changes were progressively greater with the increasing work loads. In trained animals the alterations were progressively reduced throughout

the training program. After twenty days of physical conditioning, a sixteen hour run still caused a decrease in SAkP activity but produced no significant change in any of the other enzyme activities. When the tissues of the trained and the untrained rats were examined for morphological changes, no correlation was seen between necrosis and the post-exercise enzyme alterations. In view of the results of these experiments, the authors favored the concept that the permeability of mitochondria or cellular membranes was altered during exercise, permitting enzymes to leak out of the cells and accumulate in the blood. No mechanism was offered to explain the post-exercise fall in SAkP activity.

Cardinet, Fowler and Tyler reported that race horses in training have an increased resting SGOT activity (30). This elevation progressively decreased as training proceeded and then increased again in the latter stages of training. Although these investigators collected blood samples from resting horses, the pattern of the SGOT activity changes appears similar to the pattern of post-exercise elevations observed by other investigators. In the early stages of physical training, the exercised horses displayed SGOT levels that were fifty percent higher than those of non-exercised horses. The 68th and the 84th days of training found the SGOT levels 33 and 13 percent, respectively, higher than those of control horses. On the 100th (final) day of physical training, the trained horses again had SGOT levels that were fifty percent higher than those of control horses. The authors did not explain the elevated SGOT level that was observed at the end of the training period. However, they noted that it occurred at a time when the

intensity of training was reduced but feed intake maintained.

In 1964 Garbus, Highman and Altland investigated the effects of exercise and training on various serum enzymes and lactic dehydrogenase isoenzymes of the rat (62). The electrophoretic pattern of LDH isoenzymes and the SGOT/SGPT ratio revealed that the heart contributed greatly to the observed post-exercise elevations of serum enzymes. (The SGOT/SGPT ratio is used clinically to differentiate between heart and liver diseases. A high ratio indicates a myocardial origin for the elevated serum enzymes and a low ratio indicates that the enzymes have originated from the liver.) Garbus et al. were of the opinion that a general and diffuse change of cellular membrane permeability was occurring, not only in the heart, but in many other organs throughout the body. These permeability changes may have been due to hypoxia or to catecholamines released from the adrenal medullae. These investigators underlined the fact that elevations in serum enzyme levels can result from non-specific stresses which cause no myocardial or other specific organ lesions. The authors further suggested that the permeability changes may have been due to a decreased cellular metabolism that would occur when glycogen stores were depleted and metabolism supported by poorly mobilized lipid stores. This suggestion implies that, while enough energy is available to maintain the increased metabolic activity that is associated with exercise, there is not enough energy available to maintain the functional integrity of cellular membranes.

After short, maximal exertion in human subjects, Nerdrum and Berg observed a transient rise in SGOT activity but no change in SLDH activity (131). However, SGOT activity remained unchanged after prolonged,

strenuous exercise, while SLDH activity was elevated. It appeared from these observations that different enzymes will respond independently to different degrees of exertion. The authors offered several explanations for the serum enzyme changes seen after exercise. The alterations may have been due to: increased permeability of the cellular membrane, accelerated enzyme synthesis, enhanced enzyme activation or decreased enzyme inactivation.

Several investigators demonstrated that the post-exercise elevation of serum creatine phosphokinase could be reduced by physical training (4,59,122,133). In one of these investigations men were subjected to various degrees of exercise on either the treadmill or the bicycle ergometer (59). The SLDH isoenzyme pattern and total SCPK activity were compared in order to detect the tissue of origin of the serum enzymes. This comparison demonstrated that the post-exercise elevations of serum enzymes were due predominantly to efflux from skeletal muscle.

(ii) Tissue Enzymes

The tissue distribution of enzymes reflects not only the energy requirements of various tissues but also the energy requirements of different parts of the same tissue. The GOT activity in each of the four chambers of the heart appears to be related to the work load imposed on each chamber. GOT activity is greatest in the left ventricle, with decreasing amounts being present in the right ventricle, left auricle and right auricle (13). The appearance of GOT and CPK in the tissues of the fetus and the new-born infant corresponds with the development of contractility (146,149,170). Fast, white skeletal muscles possess higher glycolytic and lower oxidative enzyme activities than the slow, red muscles (25,50,64,134).

Several investigators employed experimental manipulations to demonstrate that various cardiac and skeletal muscle enzymes become elevated as an adaptation to increased work loads. Myocardial GOT and GPT activities increased in both the early and late stages of experimental cardiac hypertrophy produced by aortic valve lesions in the dog (176). Chronic experimental systemic hypertension produced by desoxycorticosterone acetate administration (37) or coarctation of the abdominal aorta (44) resulted in an elevation of GOT activity in the left ventricle, with no change occurring in the right ventricle. One to twelve hours of isotonic twitch stimuli (one stimulus every eight seconds) resulted in an elevated CPK activity of the isolated sartorius muscle from Rana temporaria (105).

Research conducted in the early 1950's demonstrated that physical training could produce an increase in the activities of hexokinase, lactic dehydrogenase and succinic dehydrogenase in skeletal muscle (184). Hearn and Wainio investigated the effect of training on the aldolase activity of rat heart and skeletal muscle (85). The training regimen consisted of swimming for one-half hour daily in 32°C water for five, six, seven or eight weeks. When compared to the enzyme levels of pair-fed control animals the aldolase activities of heart and gastrocnemius muscle were elevated after five, six, and seven weeks of training but only the gastrocnemius aldolase activity was elevated after eight weeks of training. The authors considered a training program of this nature to be moderate and suggested that a more severe

regimen would produce greater elevations in tissue aldolase.

Using similar training programs to study the enzyme response in the skeletal muscle of the hind leg of the rat, Gould and Rawlinson failed to show changes in the activity levels of lactic dehydrogenase (LDH), malic dehydrogenase (MDH), phosphorylase, adenosine triphosphatase (ATPase) and creatine phosphokinase (71,148). They postulated that the adaptation of metabolic processes to physical training could occur: 1) with no change in enzyme levels (increased performance could be due to hypertrophy of muscle tissue), 2) by greater activity of existing enzymes or, 3) by new enzyme synthesis. Gould and Rawlinson favored the second of these possibilities, that enzymes operate minimally at rest but increase their efficiency upon exercise.

In 1961 Gollnick and Hearn investigated the effects of physical training on the LDH and ATPase activities in rat cardiac and gastrocnemius muscles (67,84). The training program involved a thirty minute swimming stress (37°C) each day for 35 consecutive days. Confirming the observations of Gould and Rawlinson, Gollnick and Hearn found that training had no effect on the LDH and the ATPase activity levels of gastrocnemius muscle. However, physical training resulted in an increased level of these enzymes in myocardial tissue. Cardiac and adrenal hypertrophy and a decreased kidney weight were evident in trained rats. Gastrocnemius muscle weights of trained animals were similar to those of control rats. The authors suggested that swimming exercise caused a greater stress on the heart than on skeletal muscle. Subsequent experimentation has demonstrated that while the myocardial

LDH activity is elevated in trained rats, the LDH activity of gastrocnemius muscle is lower in these animals (68). A single exercise episode did not alter the LDH activity of rat tissues (68), however, muscular exercise resulted in an elevation of LDH activity in the quadriceps muscle of man (104).

Single bouts of treadmill exercise and physical training resulted in elevated hexokinase activity in both red and white skeletal muscles of guinea pigs (144). The skeletal muscle activities of succinate dehydrogenase, reduced diphosphopyridine nucleotide (NADH) dehydrogenase, NADH cytochrome c reductase, succinate oxidase and cytochrome oxidase doubled as a result of a vigorous six week training program in rats (96).

Alterations in tissue GOT and CPK activities have occurred after single exercise episodes in trained and untrained rats. The effects of muscular exercise on serum and tissue GOT activities were studied by Critz and Merrick (43). Rats were exercised by treadmill running (34m/min) for seven or fifteen minutes or, swimming (23°C) for fifteen or sixty minutes. The animals that were subjected to the seven minute running episode showed no alteration of either serum or tissue GOT. Each of the other three groups of rats demonstrated a fall in serum GOT activity and variable tissue enzyme responses following exercise. Gastrocnemius muscle GOT activity was increased in both swimming groups while heart and liver GOT activities were elevated in rats that either ran for fifteen minutes or swam for sixty minutes. With increasing exercise loads, adrenal ascorbic acid was progressively depleted.

The authors suggested that moderate to severe exercise or psychological stress, causing the release of adrenocortical hormones, may be responsible for the observed elevations of tissue enzymes. Serum CPK elevations of 80, 50, 30 and 160 percent have been demonstrated in rats after swimming exercise of fifteen minutes, one hour, five hours and ten hours, respectively (121). These changes were accompanied by an increased skeletal muscle CPK activity after only the fifteen minute and five hour swimming episodes. In rats that were subjected to a two month training period, a single swimming session (15 min., 5 hrs. or 10 hrs.) resulted in an elevation of skeletal muscle CPK activity but the serum level was elevated after only the ten hour swim (122). The creatine phosphokinase activity of cardiac muscle remained unchanged after all swimming episodes in both studies. Maksimova contended that during a single exercise episode at any of the three work loads, skeletal muscle CPK activity increased in response to a greater energy requirement (122).

Sangster and Beaton demonstrated an elevated malic dehydrogenase (MDH) activity in the liver and blood plasma of rats subjected to a two hour swim (155). Physically training rats for a period of four weeks resulted in an elevation of the resting liver MDH activity. Glutamic-pyruvic transaminase activities of liver and blood plasma were neither altered by the single exercise episode nor by physical training. The authors suggested that exercise promotes enzyme synthesis in the liver, resulting in larger quantities of enzymes diffusing into the blood.

Beaton (18) and Beaton and Oyster (21) utilizing treadmill and swimming exercise, respectively, demonstrated an elevation of the resting

MDH activity of blood plasma in trained rats. Beaton suggested that an increased cellular membrane permeability was the cause of plasma enzyme elevations after exercise. He believed that the elevated tissue enzyme levels could have resulted from an increased synthesis during physical training, but not during a single exercise episode.

Zimmerman, Dujovne and Levy, studying six vertebrate species, demonstrated that there was a high correlation between serum transaminase levels and the enzyme activities present in body tissues (186). Species possessing high transaminase activity in the blood serum generally had higher tissue activities than species exhibiting low serum activity. An exception was found in the horse which possessed a high serum GOT activity and low enzyme activity in the various body tissues. The report of these authors strongly suggested that the physiological occurrence of transaminase activity in blood serum is dependent upon a tissue to serum enzyme gradient.

The effects of restricted activity on tissue and serum enzymes appeared to complement the observations made during exercise and physical training. Out-patients had serum creatine phosphokinase levels that were twice as great as those found in in-patients (78). The act of arising from bed after a prolonged bed rest, resulted in elevations of SCPK activity that were similar to those observed following myocardial infarction (157). Muscular dystrophy patients exhibited low levels of SCPK activity during bed rest (59).

Kendrick-Jones and Perry demonstrated that skeletal muscle CPK activity was lower in rats allowed only restricted movement than it was in control rats (105). After exercise both groups displayed similar net increases in CPK activity, but showed no changes in muscle protein nitrogen content. These authors concluded that contractile activity causes an increase in the activity of enzymes that are necessary for muscle metabolism. They suggested that this increase might be due to activation of an inactive precursor or synthesis of new enzyme. In animals with restricted movement the muscles would have low levels of enzyme activity which could markedly increase upon exertion, while non-restricted animals would have adequate enzyme activity available for normal levels of muscular activity.

(iii) Mechanisms for Alterations of Enzyme Levels

Although the serum enzyme changes that are seen during pathological conditions have been attributed to tissue necrosis, few investigators have considered it likely that similar cell damage is responsible for the changes that are seen in physiological states (4,5,90,108,150). Several investigators have reported that physical exercise caused little or no demonstrable damage to the cells of various body tissues (62,82,86).

The physiological efflux of intracellular enzymes has been explained on the basis of cellular membrane permeability changes. These permeability changes might be caused by any combination of a number of factors including: adrenal medullary (39,59,62,82,131) or cortical (59,87) hormones; changes in hydrogen or potassium ion concentrations (59);

changes in body temperature (59) or hypoxia (6,18,21,58,59,62,131,159, 185). Sustained muscular work may lead to glycogen depletion, anaerobic glycolysis and a metabolic state in which enough ATP is synthesized to maintain muscular contraction, but not enough to maintain the functional integrity of cellular membranes (59,62,87). Although many causative factors have been proposed to explain changes in the permeability of cellular membranes, few investigations have studied the problem directly.

Using in vitro preparations of rat diaphragm muscle, Zierler observed that cellular membrane permeability was affected by many factors that alter muscle metabolism: glucose depletion, hypoxia, muscle contraction, hereditary muscular dystrophy, insulin, iodoacetate, dinitrophenol and cyanide (185). The factors increased membrane permeability to the extent that a two- to five-fold increase in the rate of diffusion of aldolase occurred. The author stated that cellular membrane pores should not be thought of as fixed anatomical entities; instead, they should be thought of as transient structures. He further suggested that sites sufficiently large for diffusion opened up randomly throughout the entire membrane. In a related study, isoncotic plasma volume expansion caused a change in capillary permeability that was great enough to accelerate the passage of radioactive macromolecules (molecular weight: 51,000-255,000) from the blood to lymphatic vessels (160). The authors of this report suggested that the concept of capillary permeability should include the fact that pore size is subject to alteration with changes in plasma volume as well as other factors.

If membrane permeability changes are responsible for the elevated serum enzyme levels observed after exercise, these changes appear to be reduced in trained individuals. Membrane permeability changes resulting from hypoxia might be mitigated in trained individuals due to their greater muscle vascularity than untrained individuals (58,62). An improved circulation to the working muscles would allow greater diffusion of oxygen to the active muscle fibers.

Although the membrane permeability theory has now been widely accepted, another theory has received substantial support. Post-exercise serum enzyme elevations may merely reflect a tissue to serum enzyme gradient that had become elevated during exercise due to an accelerated synthesis, activation or turnover of intracellular enzymes (18,39,58, 59,131,155).

The onset, magnitude and duration of serum enzyme elevations appears to differ with each particular enzyme. Batsakis and Briere outlined the enzymatic pattern that was seen in the serum of patients with myocardial infarction (15). Serum CPK activity was elevated three to four hours after infarction: elevations of SGOT and SLDH activities appeared at twelve and eighteen hours, respectively, post-infarction. SCPK activity had returned to the normal level by three days after infarction, while SGOT and SLDH levels remained elevated for four and seven days, respectively. SCPK, SGOT and SLDH activities had risen to peak values of 11, 4.2 and 3.6 times their respective normal values. From these observations it appeared that the SCPK activity level was a better indicator of myocardial injury than was either the SGOT or SLDH level.

Several investigators have presented evidence that CPK, GOT and LDH are released to the serum in a similar pattern after exercise (3,5,7,58,59,65,133). This, however, has not been a consistent observation (6,17,82,91,131). Since the intensity, duration and types of exercise were markedly different in these investigations, the results cannot be directly compared. The pattern of enzyme release in response to various types, intensities and durations of exercise remains to be determined.

(iv) General Physiological and Morphological Changes during Exercise

Bloor, Leon and Pasyk subjected rats to a one hour swim (32-35°C) either daily or twice each week for a period of ten weeks (26). In both of these groups the final body weights were ten percent less than the body weight of pair-fed control rats. Training animals by either intermittent exercise or by daily exercise resulted in cardiac hypertrophy of seventeen and twenty percent, respectively. The observed cardiac hypertrophy was due to an increased number of myocardial fibers. The cytoplasmic mass of liver and adrenal cortical cells was increased in the trained animals of both groups but the number of cells was less than in control rats. Neither liver nor adrenal gland weight was affected by the physical conditioning programs used in this study. Kidney weights and the number of renal glomeruli were reduced in trained animals.

Bloor et al. compared the above observations with the results of another investigation in which the effects of hypoxia on the cellular development in mice were studied (129). The cellular structure of the

liver, kidney and adrenal glands of exercised animals was similar to that observed in hypoxic mice. The similarity suggested that a state of relative hypoxia was responsible for the changes associated with exercise. However, the hearts of hypoxic mice not only weighed less than those of control animals but they possessed a reduced number of myocardial fibers. Bloor et al. concluded that factors other than hypoxia had produced the cardiac changes that were seen in physically trained animals.

In another investigation rats were subjected to either vigorous physical training (treadmill running: 1 mph for 1 hr.; twice daily; 6 d/wk for 5 wks.) or intermittent hypoxia (simulated altitude of 24,000 ft.; 8 hrs/d for 4 wks.) (116). Both exercise and hypoxia resulted in cardiac hypertrophy. Exercise produced hypertrophy of both ventricles, while hypoxia caused a greater hypertrophy in the right ventricle than in the left ventricle. Although histological examination was not included in this study, the authors stated that the hypertrophy was due to an enlargement of myocardial fibers. In support of this statement, myocardial fiber enlargement rather than proliferation is currently believed to be responsible for the cardiac hypertrophy that results from exercise (181).

Physically trained rats (1 hr. swim; 35°C; 42 consecutive days) displayed adrenal hypertrophy, cardiac hypertrophy and reduced spleen and liver weights when compared to control animals (69). Neither physical training nor splenectomy had an effect on hemoglobin concentration or hematocrit. The authors concluded that the spleen does not

significantly alter these blood components in the exercising rat.

Critz and Merrick observed that seven or fifteen minutes of treadmill exercise had no effect on the hematocrit of rats (43), however, hemoconcentration was observed after all durations of swimming exercise (39,43). Splenectomy did not prevent the hemoconcentration produced by swimming exercise (39).

A vigorous, graded treadmill training program over a period of fifteen weeks had no effect on the weights of the quadriceps, gastrocnemius, soleus, gluteus and hamstring muscles of the rat (139). The myoglobin concentration in the quadriceps and hamstring muscles was increased approximately eighty percent above that found in the muscles of control rats. Since the myoglobin concentration was not altered in the abdominal muscles of trained rats, the authors suggested that local factors were responsible for the increases observed in exercising muscles.

The effect of training on the coronary circulation has been studied by several investigators. Employing three different staining methods for capillary counting, Hakkila found a decreased capillary concentration in the hypertrophic hearts of physically trained guinea pigs (81). He maintained that the capillary to myocardial fiber ratio never deviated from the proportion of one to one. In trained rats the myocardial capillary concentration decreased because they were pushed farther apart by the larger muscle fibers.

Eckstein subjected dogs to chronic narrowing of the circumflex coronary artery and studied the development of the coronary collateral

circulation (51). He found that mild coronary constriction did not augment the myocardial vascularity unless the dogs were simultaneously subjected to a moderate physical training program.

Tepperman and Pearlman estimated the size of the coronary arterial tree from the weight of a vinyl acetate cast (173). Using this technique the authors demonstrated an increase in the coronary cast weight to heart weight ratio in female rats that were physically trained by daily treadmill exercise and in male rats that were trained by daily swimming exercise. This effect was not observed in male rats that were subjected to daily treadmill exercise. The results of this study indicated that true enlargement of the coronary tree capacity, increased distensibility of coronary vessels or a combination of these two factors had occurred in the trained animals.

Stevenson, Feleki, Rechnitzer and Beaton employed the coronary cast technique to investigate the effects of frequency, duration and mode of exercise on coronary tree size (166). Rats that were subjected to treadmill or swimming exercise twice per week for four weeks developed a significantly increased coronary cast to heart weight ratio. Frequent (treadmill exercise, five days per week) or strenuous exercise (swimming four hours per day, four days per week) failed to produce a significant increase in the cast to heart weight ratio. The exercised rats showed no evidence of cardiac hypertrophy and generally failed to gain as much weight as their corresponding controls. The heavier and the more frequent exercise loads resulted in poorer body weight increases than

did the lighter and less frequent work loads. There was a positive correlation ($r = 0.81$) between weight gain and the cast to heart weight ratio of exercising rats. Stevenson suggested that an increased capacity of the myocardial vasculature occurring in the absence of hypertrophy may be one of the earliest beneficial effects of exercise (164). He stated that the coronary tree size is dependent not only on total heart size but it is also dependent on the anabolic state of the exercising animal. The results of the coronary cast study indicated that an improvement in an organism's functional reserve capacity does not necessitate a strenuous physical training program. The results indicated that rest periods are important for the anabolic processes that accompany an improving physical condition.

Leon and Bloor, studying the coronary circulation of rats, demonstrated that swimming exercise, either daily or twice per week, resulted in an increase in the extra-coronary artery collateral circulation and an increase in the myocardial capillary to fiber ratio (115). (The extra-coronary arterial circulation is derived from branches of the internal mammary and subclavian arteries which anastomose with the coronary arteries at the level of the atria.) When compared to control rats, the cross sectional area of the main coronary arteries was unchanged in both groups and cardiac hypertrophy occurred in only the daily swimmers. In support of the observations of Stevenson et al. (166), the improvement in both the extra-coronary collateral circulation and the capillary to fiber ratio occurred early in training, before the onset of cardiac hypertrophy.

In addition to the structural changes that have been demonstrated in physically trained animals, an improvement has been observed in their capacity for exercise. In an exhaustive run, physically trained rats (daily treadmill exercise for fifteen weeks) were able to run six times longer than control rats (139). Physically trained rats living on a high carbohydrate diet were able to endure a swimming stress for a longer period than control rats (19).

3. Stress

An animal or human responds to any of a variety of stressful situations with an increased output of hormones from the adrenal gland. Many investigators have suggested that the adrenal hormones may be responsible for the serum and tissue enzyme changes that occurred during exercise.

Adrenal medullary hormones were thought to have enhanced the cellular membrane permeability in exercising animals, resulting in the release of intracellular enzymes. Proponents of this concept believed that permeability changes were due to a relative hypoxia occurring in the muscle fibers as a result of the vasoconstricter effects of catecholamines. Several investigators have demonstrated that intravenous or subcutaneous administration of large doses of adrenaline or noradrenaline resulted in the elevations of several serum enzymes in dogs, rats, rabbits and guinea pigs (63,93,94,123). However, SGOT activity was not elevated in dogs that were administered physiological doses of noradrenaline (117). It appeared from these results that factors other than the catecholamines

were involved in the serum enzyme changes that occurred during exercise.

Adrenocortical hormones were also believed to play a role in changing the permeability of the cellular membranes during exercise (59,87). However, prednisolone, a synthetic glucocorticoid, has been shown capable of inhibiting cellular membrane permeability changes (70,76,126,158). The post-exercise elevation of serum CPK activity was reduced in dogs as a result of prednisolone administration (179).

Adrenocortical hormones were thought to be responsible for the alterations of tissue enzyme activity that were observed in exercising animals. Several investigators have demonstrated that natural or synthetic glucocorticoid administration, in vivo or in vitro, resulted in an increased synthesis of enzymes (53,76,99,126,154,158). Critz and Withrow found that surgical or pharmacological (Dilantin administration) adrenalectomy prevented the elevation of tissue GOT activity that had occurred in exercised rats (45).

In view of the possible influence that the adrenal gland may have on the activity levels of tissue and serum enzymes, the remainder of this review will be devoted to a consideration of the effect of exercise and other stresses on adrenal function.

An elevation of the venous blood level of 17-hydroxycorticosterone has been observed following exercise in dogs (167,168). The circulating levels of cortisol and corticosterone increased in humans during the first two to three minutes after the onset of heavy exercise (150 watts) and returned to normal within the next five minutes (113). Other

investigators have found a decrease in the blood level of cortisol and in the urinary excretion rate of 17-ketosteroids (33) after exercise in humans. The latter investigators found an increased excretion rate of 17-ketosteroids in humans during the emotional stress of an examination (33). They suggested that any emotional stress might cause an increased formation of adrenocortical hormones in preparation for the physical stress that usually followed. The decreased levels of circulating adrenocortical hormones that are observed during exercise may reflect an increased utilization by the various tissues (33,36,95).

Another indication that the adrenocortical response to exercise differs from the response to emotional stress was obtained from an investigation performed on the crew members of rowing teams (95). Urinary 17-hydroxycorticosteroids were elevated during the four hour period preceding a race or time trial. On practice days, when the same amount of work was performed, there was no increase in the urinary 17-hydroxycorticosteroids.

Frenkle and Csalay studied the adrenal cortex of rats subjected to daily swimming exercise over a six week period (60). Adrenal gland hypertrophy occurred in rats that were physically trained for either three weeks or six weeks. The levels of both glucocorticoids and mineralocorticoids increased during the first three weeks of daily exercise but had returned to control levels by the end of six weeks of physical training. The authors noted a similarity between the results of their experiment and Selye's general adaptation syndrome.

(Selye contends that normal blood glucocorticoid levels occur during the resistant stage of the general adaptation syndrome.)

A positive correlation was demonstrated between adrenal gland enlargement and the cardiac hypertrophy that occurred during physical training (147). The increase in adrenal weight was not proportional to the work load but occurred predominantly during two stages of exertion; at work loads that involved less than eighteen percent of the organism's maximal capacity and at work loads approximating two-thirds the maximal capacity. Prokop distinguished between three phases of physical training, analagous to the alarm phase, phase of resistance and phase of exhaustion of Selye's general adaptation syndrome (147). He referred to the first phase as the "phase of adaptation', during which adaptation to exercise was initiated and a slow improvement in performance occurred. The second phase was called the "phase of reached and completed adaptation", and in this phase the individual displayed his best performance. The third phase, the "readaptation phase", resulted in a decreased performance and overtraining.

Adrenocortical hyperactivity, which is a sign of non-specific stress (150), resulted from a variety of stressful situations, including: heat (22,23), cold (33,120), electric shock (61), trauma (33), restraint (14,110,145) and handling (61).

Friedman, Ader, Grota and Larson compared the plasma corticosterone levels of control, electrically shocked and experimentally handled rats (61). Five minutes after the respective stress,

the electrically shocked and the handled rats displayed elevated plasma corticosterone levels. Fifteen minutes after the stress, the plasma corticosterone levels were still rising in the shocked animals but were falling in the handled rats. At sixty minutes post-stress the handled rats displayed normal plasma corticosterone levels but the corticosterone levels were still elevated in the plasma of the electrically shocked animals. The authors maintained that pain and physical stress were not as important in producing an elevation of plasma corticosterone levels as was the stress of a novel situation.

The results of several investigations indicate that the adrenal gland may be a common link between the enzyme changes observed after exercise and the changes that occurred during other stresses. Serum enzyme elevations have been observed in various species following heat stress (22,23), cold stress (120,143), acceleration (34,83), vibration (34), restraint (34,142) and noise (34). Elevation of the basal activity levels of several serum enzymes was observed in cold-acclimatized men (120) and heat-acclimatized dogs (22,23).

III. METHODS AND MATERIALS

1. Enzyme Experiments

(i) Experimental Design

Three hundred male albino rats of the Wistar strain, weighing between 200-250 grams, were divided into six groups each subjected to a different level of physical conditioning. A control group consisted of rats housed individually for three weeks in standard laboratory cages (8 in. x 9 in. x 7 in.). A second group of rats was subjected to a restricted environment by housing the animals individually for three weeks in cages that were one-half the size of standard cages. (4 in. x 9 in. x 7 in.). In a third group the rats were allowed complete freedom of movement. They were housed individually for three weeks in cages (6 in. x 10 in. x 5 in.) that gave access to a freely rotating drum (diameter: 14 in. x width: 4.5 in.). The number of revolutions travelled by each rat was recorded by mechanical counters. The fourth, fifth and sixth groups of rats were housed individually in standard laboratory cages for a period of two, four or six weeks, respectively. These animals were forced to swim for one hour, three times per week, in individual plastic tanks (diameter: 12 in. x depth: 12 in.) containing water at a temperature of 28-30°C. The rats in the above six groups, and in all of the following experiments, lived in an environment with a temperature of 22.5-23.5°C and twelve hours of daily light exposure. They were provided a standard

laboratory diet and water ad libitum.

Rats that were subjected to the above procedures were analyzed for enzyme activity in the plasma and for the enzyme activity and total protein nitrogen content of soleus, gastrocnemius or cardiac muscle. Some of the rats in each of the six groups were sacrificed and analyzed on the last day of the experimental conditioning period. These animals had been at rest for at least 24 hours prior to sacrifice. (Appendix A demonstrates the results of a pilot study in which the plasma GOT and CPK activities returned to the resting levels within 24 hours after exercise.) The remaining rats were sacrificed immediately following a sixty minute swim and the various tissues were subsequently analyzed for enzyme activity and total protein nitrogen content.

(ii) Experimental Procedure

On the day of sacrifice the animals were anesthetized by intraperitoneal administration of a lethal dose of pentobarbital sodium (200 mg/kg). When the animal had reached a surgical level of anesthesia, a five milliliter blood sample was withdrawn by cardiac puncture, transferred to a heparinized centrifuge tube and centrifuged for five minutes at 1600 g. Non-hemolyzed plasma was separated by aspiration, refrigerated at 5°C and analyzed for enzyme activity within six hours.

Immediately after withdrawal of the blood sample, twenty to thirty milligrams of the left soleus, left gastrocnemius or left cardiac ventricular muscle were removed and diluted 1:1000 with

physiological saline (5°C). The muscle samples were immediately homogenized, separated in the cold by centrifugation (10,000 g at 2°C for ten minutes), decanted and the supernate was refrigerated (5°C) until analyzed. Assay for muscle CPK activity was performed approximately three hours after sacrifice. Pilot studies indicated that the preparation and storage temperature and the timing of the assay procedure were important factors affecting the stability of CPK in the supernate and the reproducibility of the results. Since the GOT activity of the muscle supernate was shown to be stable for only four to six hours, assay for muscle GOT was performed as soon as possible after sacrifice. The relatively stable proteins were analyzed for nitrogen within six hours.

Assay for CPK activity was performed after the method of Rosalki (152) using the procedure and reagents supplied by Calbiochem (Box 54282, Terminal Annex, Los Angeles 54, California). This method has been evaluated and extensively discussed elsewhere in the literature (89,153). A modification of the original method of Oliver was used for the analysis of the creatine phosphokinase activity in muscle tissue (135). Oliver recommended that a potassium chloride solution be used to dilute tissue samples since this diluent reduced the adenosine triphosphatase contaminants of tissue homogenates. In the present investigation a preliminary study showed that substitution of a 0.9 percent sodium chloride diluent did not alter the measured CPK activity. Oliver also cautioned that myokinase may interfere with the CPK assay when using tissue homogenates. A preliminary

investigation revealed that interference from myokinase was negligible in solutions as dilute as those analyzed in the present study (special reagents given in Appendix B). This observation confirmed a previous report that the high adenosine monophosphate content of the commercial reagent capsule inhibits myokinase activity (153). In order to reduce the muscle CPK activity to a level that could be measured using Rosalki's assay, the final gastrocnemius muscle dilution ratio became 1:15,000 (one milligram wet muscle weight diluted with fifteen milliliters of physiological saline), the dilution ratio for soleus muscle became 1:7000 and the cardiac muscle dilution ratio became 1:3000. Since the timing of the CPK analysis was critical, dilution from the refrigerated supernate (1:1000) to the final ratios was performed within two minutes immediately preceding insertion of the cuvette into the spectrophotometer. The assay procedure of Calbiochem was then followed in the same manner as with plasma samples. Previous investigators had observed erroneously high (73) or low (88) CPK values resulting from the dilution of samples containing high CPK activity. Since the high CPK activity of muscle samples necessitated large dilution ratios, a constant dilution ratio was maintained for each particular muscle studied in the present investigation.

GOT activity was determined according to the method and subsequent modifications of Babson, Shapiro, Williams and Phillips (9-11). The control buffer, which was used in the original method (11) to correct for the interference produced by hemolytic, lipemic and

icteric serum samples, was not used in the present investigation: plasma samples of this nature were discarded. GOT activity was measured in cardiac, soleus or gastrocnemius muscle using 0.1, 0.1 or 0.2 milliliter, respectively, of the supernate from the original 1:1000 diluted homogenate. The method of Babson et al. required the preparation of a standard curve relating light absorbance to the known GOT activity of standard solutions (11). Versatol-E, a commercial serum enzyme standard, was used to prepare standard solutions (10).

Total protein nitrogen content of the muscle supernate was measured using a modification of the procedure described by Natelson (130). At a wavelength of 390 mμ, as used in Natelson's spectrophotometric procedure, the sensitivity of the method was so great that only small quantities of nitrogen could be accurately determined. To measure quantities of total protein nitrogen which were present in muscle tissues, it was necessary to read the optical density of the reaction mixture at a wavelength of 490 mμ (136). Analysis was performed on 2.0 milliliter aliquants of the supernatant solution (1:1000) and the final volume of the nesslerized reaction mixture was diluted to twenty milliliters. Commercial Nessler's reagent was used in the present investigation (Nessler Reagent, Item No. 2634, Hartman-Leddon Company, Philadelphia, Pa.).

One International unit of any enzyme is that amount which will catalyze the transformation of one micromole of substrate per minute under standard conditions (100). In the present investigation CPK or GOT activity was determined using a reaction temperature of 30°C or

37°C, respectively. Plasma enzyme activity was expressed in International units per liter (IU/l) and muscle enzyme activity was expressed in International milliunits per microgram nitrogen (mU/ug N_2).

In the rats that were analyzed for gastrocnemius enzyme activity, additional parameters were measured. Immediately upon withdrawal of the blood samples in this group, hematocrit readings were taken by a micro method using the Adams Autocrit CT-2905 microhematocrit centrifuge. Wet soleus muscle weight and final body weight were measured in this group and expressed as the ratio of soleus weight to body weight (mg/g).

(iii) Statistical Analysis

Analysis of covariance was used in the present investigation to adjust the mean enzyme activities of the cardiac and soleus muscles from control, spontaneously active and physically trained rats (162,180). This statistical method was employed in order to segregate the the variable under investigation (physical conditioning) from the interference of an unforeseen and uncontrollable variable. Over a period of $1\frac{1}{2}$ years the interfering variable resulted in an increase in the soleus and cardiac enzyme levels in successively purchased groups of rats. The mean enzyme levels of cardiac and soleus muscle were adjusted by employing regression equations relating enzyme activity to the time of analysis (in months). Determinations of the enzyme levels in restricted rats, and in the series of rats involving gastrocnemius analysis, were not affected by the unknown variable since these series of experiments were each completed during a two month period. A change in either the reagents or the assay techniques did not appear to have introduced the variable since similar results were obtained by two investigators using two different assays. Although the causative factor remains unknown to

the author, he suggests two possibilities: a) the variable originated in the laboratories from which the rats were purchased, or b) the variable was introduced as a result of non-specific stress induced by gastrointestinal or respiratory infections which were prevalent among rats during the past year.

Analysis of variance was employed to test the effect of physical training on both resting and post-exercise hematocrit levels, soleus to body weight ratios and enzyme levels. The effect of physical training on cardiac and soleus enzyme levels was statistically tested using both the adjusted and the unadjusted means. Following the analysis of variance individual means were compared using the Student-Newman-Keuls range test for samples with unequal numbers of animals (163). Either Student's "t" test or the modified "t" test of Welsh (for the comparison of samples with unequal variances) (57) was used to compare the mean parameters of restricted rats with those of control rats and the mean parameters of resting animals with those of exercised animals. The null hypothesis was rejected at the five percent level.

2. Coronary Artery Cast Experiments

(i) Experimental Design and Procedure

The following section of the present investigation was separated into two experimental blocks of animals. In the first block restricted rats and spontaneously active rats were compared to a three week control group and, in the second block, animals that were physically trained for two, four or six weeks were each compared to a corresponding control group.

Within the first block, thirty male Wistar rats weighing between 190-250 grams were divided into three equal groups with a mean initial body weight of 215 grams for each group. These three groups were subjected to the same physical conditioning regimens as the first three groups of rats in the enzyme experiments. They consisted of a control group, a group allowed restricted activity and a group of spontaneously active rats, all housed for three weeks in the appropriate cages and controlled environments.

The second experimental block of animals comprised 48 male Wistar rats weighing between 220-300 grams. These rats were divided into six equal groups with a mean initial body weight of 265 grams for each group. Three of these groups were subjected to the same physical training programs of two, four and six weeks duration as were the last three series of rats in the enzyme experiments. The remaining three groups of rats in this experimental block served as two, four and six week control groups.

On the last day of the experimental conditioning period, or in the swimming groups, 24 hours after the last swim, the animals were weighed and anesthetized by intraperitoneal administration of pentobarbital sodium (45 mg/kg). Vinyl acetate casts of the coronary arterial trees were prepared following the method of Tepperman and Pearlman (173). A Harvard Apparatus Co. 600-900-S infusion/withdrawal pump was incorporated for the perfusion of the vinyl acetate solution. Wet cardiac ventricular weight and coronary cast weight were recorded

for each animal.

Inherent to this technique is the possibility of errors resulting from the difficulty to control the vinyl-acetate perfusion pressure (166,173). The difficulty arises from the leakage of vinyl-acetate through the ends of blood vessels that were severed while opening the thoracic cage (173). The inherent errors are randomized by performing blind experiments.

(ii) Statistical Analysis

Student's "t" test was used as the statistical test of significance in these experiments and the null hypothesis was rejected at the five percent level.

3. Accessory Experiments

Consideration of the data obtained from restricted rats made it necessary to design experiments which would test these animals from different approaches.

(i) Effect of Time in Restrictive Cages on Coronary Cast Size

The first experiment was designed to determine the minimal duration of restricted activity that would produce an increase in the coronary cast weight to heart weight ratio.

Thirty male Wistar rats weighing between 250-310 grams were divided into six equal groups with a mean initial body weight of 273 grams for each group. Three of these groups were housed in restrictive cages and the other three groups were housed in standard laboratory cages. At the end of three days, one week or two weeks,

rats from a restricted and a control group were weighed and anesthetized by intraperitoneal injection of pentobarbital sodium (45 mg/kg). When the animals had reached a surgical level of anesthesia, coronary vinyl-acetate casts were prepared as described in the preceding experiments and wet cardiac ventricular and coronary cast weights were recorded for each animal.

(ii) Total Amount (Time) of Daily Activity of Restricted and Control Rats

The second and third experiments were designed to test whether or not the results observed in restricted animals might be due to increased activity, even though the space available for movement was reduced.

Eighteen male rats of the Wistar strain weighing between 200-250 grams were divided into two equal groups. The rats of one group were housed in standard laboratory cages and the rats of the other group lived in restrictive cages for a period of three weeks. Each cage was placed individually on a platform that was supported by foam rubber cushions. Two permanent magnet, induction transducers, extending between the platform and its base, were sensitive to vibrations produced by the animal's activity. The electrical signals generated in the transducers were recorded by means of a Grass Model 7 polygraph. The total activity time of each rat was recorded for 24 hours on the first, eighth, fifteenth and twenty-second day of the experimental period. Activity time was expressed as a percent of the 24 hours.

(iii) Swimming Endurance of Spontaneously Active, Control and Restricted Rats

Previous investigators have demonstrated that physically conditioned rats, maintained on a high carbohydrate diet, were able to swim for a significantly longer period of time than untrained rats (19). If the restricted animals in the present study were more active than control rats, they should be able to swim for a longer time.

Thirty male rats of the Wistar strain weighing between 190-230 grams were divided into three equal groups. The rats were housed in standard laboratory cages, restrictive cages or cages allowing spontaneous exercise and were provided a high carbohydrate diet (19) and water, ad libitum, for a period of three weeks. Initial and final body weights were recorded for each animal. At the end of the experimental period, the animals were forced to swim to exhaustion in water at 22°C. Several investigators had demonstrated the importance of water temperature on the length of time required to swim to exhaustion (20,46,172). In order to shorten the swimming duration, the rats in the present study were forced to swim in 22°C water. In addition, the work load was increased by taping lead weights, equal to six percent of the animal's body weight, to the base of the tail. The criterion for exhaustion was established as the time when the rat sank to the bottom of the tank and remained there for a period of sixty seconds (19).

(iv) Adrenal Ascorbic Acid Levels in Control and Restricted Rats

Although the restricted rats were only mildly limited in their activity, it was possible that a stress response was occurring. The following experiment employed adrenal ascorbic acid concentration as an index of adrenocorticoid secretion.

Fifty-five male rats of the Wistar strain weighing between 200-250 grams were divided into five equal groups. These groups served as either a control group, housed in standard laboratory cages for three weeks, or one of four experimental groups, housed in restrictive cages for periods of three days, one week, two weeks or three weeks. At the termination of each experimental period, the animals were sacrificed by decapitation. The adrenal glands were removed, trimmed free of connective tissue, weighed and homogenized in thirty milliliters of cold 2.5 percent meta-phosphoric acid (5°C). The homogenate was filtered through Munktell 00 filter paper and analyzed for ascorbic acid according to the method of Briggs and Munson (29). Adrenal ascorbic acid levels were expressed as milligrams per hundred grams of wet adrenal weight.

(v) Effect of Adrenalectomy on the Coronary Cast Weight Changes in Restricted Rats

In the final experiment the role of the adrenal gland was further investigated by studying the effect of restricted activity on the coronary cast to heart weight ratio of adrenalectomized rats.

Sixteen normal male rats of the Charles River strain, with a mean initial body weight of 218 grams, were divided equally into a control group and a group of restricted rats. Sixteen, commercially adrenalectomized, male Charles River rats, with a mean initial body weight of 191 grams, were divided similarly into two equal groups. All adrenalectomized rats were provided physiological saline while normal rats were provided water to drink, ad libitum. Standard laboratory chow was provided to all rats, ad libitum.

At the end of a three week conditioning period the coronary cast was prepared and final body weight, wet cardiac ventricular weight and coronary cast weight were recorded for each animal.

(vi) Statistical Analysis

Student's "t" test was used to statistically compare the means in all the experiments of this section. The null hypothesis was rejected at the five percent level.

IV. RESULTS

1. Enzyme Experiments

(i) Muscle Enzyme Levels in Control, Spontaneously Active and Physically Trained Rats

Tables I through VIII show the mean enzyme levels of myocardial and soleus muscles, both before and after adjustment by analysis of covariance. These tables demonstrate that the adjustment did not statistically alter the results. Removal of the extraneous variable allowed the adjusted means to portray more accurately the effect of spontaneous activity and physical training on the myocardial and soleus CPK and GOT levels. Adjustment of the enzyme levels resulted in a consistent decrease in the mean CPK and GOT levels of the groups of rats trained for six weeks and a decrease in the mean myocardial CPK levels of the four week training groups. The adjustment consistently raised the mean CPK and GOT levels of the spontaneously active groups and also the mean myocardial CPK levels of control rats. These effects parallel the relative time periods in which the animals in the various groups were purchased, subjected to experimental treatment and analyzed for enzyme activity. A greater proportion of the rats in the four and six week training programs received experimental treatment in the latter months of this investigation, a greater proportion of the control animals were studied in the early months and the spontaneously active rats were studied within the first four months of this investigation.

The adjusted means indicate that enzyme elevations, occurring in cardiac and soleus muscles after a mild six week training program, are not as great as would appear from the unadjusted means. In the following sections of this dissertation, myocardial and soleus CPK and GOT levels will be discussed in terms of the adjusted means. The mean enzyme levels that are displayed in Figures 1-4 are the adjusted values. Unadjusted means of myocardial and soleus enzyme levels are shown along with the standard errors of the mean in Appendix C and D. The mean total protein nitrogen content of cardiac and soleus muscle are contained, along with the standard errors of the mean, in Appendix G.

Tables I and II and Figure 1 demonstrate that the experimental treatments had a significant effect on the resting and post-exercise, myocardial CPK levels. Although the resting myocardial CPK level did not become elevated until after six weeks of intermittent swimming exercise (Table I, Figure 1), the post-exercise levels in spontaneously active rats and in all three groups of trained rats were higher than the post-exercise level in the control group (Table II, Figure 1).

The rats in the latter series had been subjected to a sixty minute swim immediately prior to sacrifice but in all other respects the two series of rats had received similar experimental treatment. All of the rats in the swimming groups were subjected to a one hour swim, three times per week: the spontaneously active rats that were sacrificed in the resting state ran a total of

1112 ± 227 revolutions per day while the spontaneously active rats in the post-exercise series ran 1206 ± 187 revolutions per day.

Figure 1 illustrates that a single exercise episode did not alter the mean myocardial CPK level in any of the groups, i.e. in any particular group of rats, the resting CPK level was never different from the post-exercise CPK level.

Physical training resulted in elevations in the resting and post-exercise myocardial GOT levels that were similar to the elevations observed in the CPK levels (Tables III and IV, Figure 2). By the end of the six week training program, resting rats displayed a greater mean myocardial GOT level than resting control rats (Table III, Figure 2). An elevation was not observed in the resting, myocardial GOT levels of spontaneously active rats or rats physically trained for two or four weeks. After two, four or six weeks of physical training the post-exercise myocardial GOT level was elevated above the post-exercise level in control rats (Table IV, Figure 2). Spontaneously active rats displayed a mean post-exercise, myocardial GOT level that was similar to the post-exercise control level. A sixty minute swim immediately prior to sacrifice did not affect the myocardial GOT level in any of the five groups of rats (Figure 2).

CPK activity in the soleus muscles of resting rats was increased after six weeks of physical training (Table V, Figure 3). Neither spontaneous activity for three weeks nor the shorter swimming training programs had any effect on the resting, soleus muscle CPK activity. The effect of training on post-exercise, soleus CPK

levels is shown in Table VI and Figure 3. Rats that were physically trained for a period of six weeks displayed a higher post-exercise, soleus CPK level than the post-exercise level in control rats. Spontaneously active rats and rats trained for two and four week periods had mean post-exercise, soleus CPK levels that were comparable to that of the control group. A single exercise episode did not alter the CPK level in the soleus muscles of control, spontaneously active or physically trained rats (Figure 3).

Analysis of variance showed that physically trained rats possessed higher resting, soleus GOT levels than control rats (Table VII and Figure 4). A comparison of the various group means revealed that an elevation did not occur until rats had been physically trained for six weeks. Rats subjected to intermittent swimming exercise for two or six weeks demonstrated higher post-exercise, soleus GOT levels than control rats (Table VIII and Figure 4). There was no difference among the post-exercise, soleus GOT levels in control rats, spontaneously active rats and rats that were physically trained for four weeks. Figure 4 shows that the soleus GOT levels were not affected by a single exercise episode. (The post-exercise soleus GOT levels were not statistically different from the resting levels).

Tables IX and X and Figures 5 and 6 demonstrate the effect of exercise and physical training on the mean levels of CPK and GOT in gastrocnemius muscle. Standard errors of the mean are shown in Appendix E. These means were not adjusted by analysis of covariance. Total protein nitrogen content of gastrocnemius muscle can be seen in Appendix G.

TABLE I

Resting Myocardial Creatine Phosphokinase Levels in Control, Spontaneously Active and Physically Trained Rats

	Control	Spontaneously active	Physically trained		
			two weeks	four weeks	six weeks
Unadjusted mean	28.1*	25.9	31.0	31.3	39.3
Adjusted mean	31.1	31.0	31.8	29.2	35.9**
Number of animals	12	10	13	21	16

*International mU/ug N2

**P <0.05, compared to control level

Analysis of Variance

Source	Sum of squares	Degrees of freedom	Variance estimate	F ratio	Significance
Unadjusted:					
Between groups	1419.74	4	354.94	10.7	P <0.01
Within groups	2230.76	67	33.29		
Adjusted:					
Between groups	410.34	4	102.58	6.5	P <0.01
Within groups	1036.23	66	15.70		

TABLE II

Post-exercise Myocardial Creatine Phosphokinase Levels in Control, Spontaneously Active and Physically Trained Rats

	Control	Spontaneously active	Physically trained		
			two weeks	four weeks	six weeks
Unadjusted mean	25.7*	29.7	31.0	34.3	37.2
Adjusted mean	26.7	31.9**	33.0**	32.7**	34.4**
Number of animals	16	14	17	21	17

*International mU/ug N2 **P<0.05, compared to control level

Analysis of Variance

Source	Sum of squares	Degrees of freedom	Variance estimate	F ratio	Significance
Unadjusted:					
Between groups	1286.51	4	321.63	7.4	P <0.01
Within groups	3493.16	80	43.66		
Adjusted:					
Between groups	544.27	4	136.07	6.4	P <0.01
Within groups	1692.40	79	21.42		

Figure 1

Myocardial creatine phosphokinase (CPK) activity, before and after exercise, in rats subjected to different types and durations of physical conditioning.

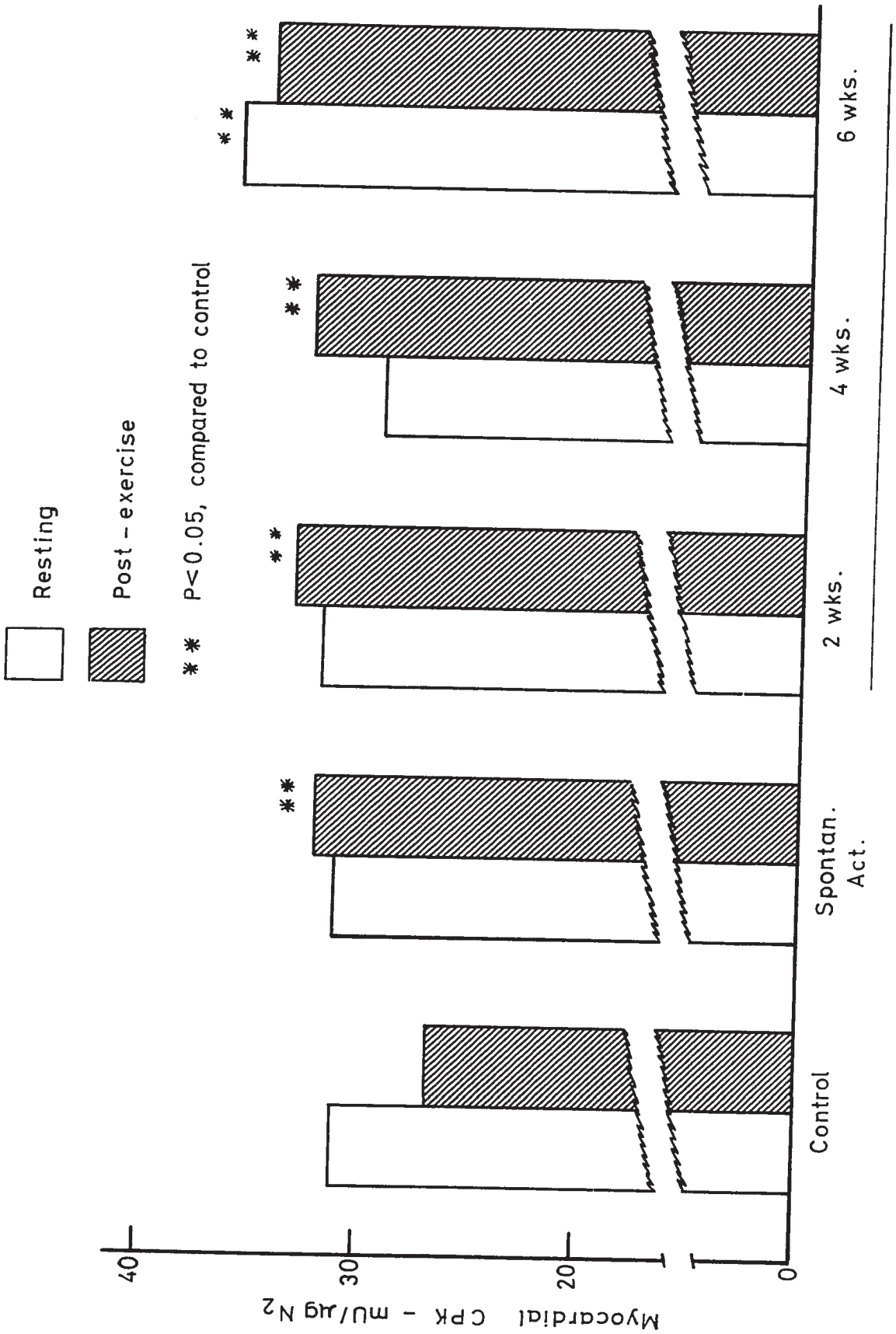

Resting
Post - exercise
** P<0.05, compared to control
Myocardial CPK - mU/mg N2
40
30
20
0
Control
Spontan. Act.
2 wks.
4 wks.
6 wks.
Physical Training

TABLE III

Resting Myocardial Glutamic-oxalacetic Transaminase Levels in Control, Spontaneously Active and Physically Trained Rats

	Control	Spontaneously active	Physically trained		
			two weeks	four weeks	six weeks
Unadjusted mean	22.6*	19.1	23.1	23.8	28.8
Adjusted mean	22.6	21.2	23.3	23.6	27.6**
Number of animals	17	10	13	14	18

*International mU/ug N_2 **P <0.05, compared to control level

Analysis of Variance

Source	Sum of squares	Degrees of freedom	Variance estimate	F ratio	Significance
Unadjusted:					
Between groups	704.56	4	176.14	21.5	P <0.01
Within groups	547.82	67	8.18		
Adjusted:					
Between groups	294.41	4	73.60	16.5	P <0.01
Within groups	294.75	66	4.47		

TABLE IV

Post-exercise Myocardial Glutamic-oxalacetic Transaminase Levels in Control, Spontaneously Active and Physically Trained Rats

	Control	Spontaneously active	Physically trained		
			two weeks	four weeks	six weeks
Unadjusted mean	22.4*	20.7	23.9	24.7	27.1
Adjusted mean	22.2	22.6	24.1**	24.6**	26.0**
Number of animals	18	10	17	16	18

*International mU/ug N_2

**P <0.05, compared to control level

Analysis of Variance

Source	Sum of squares	Degrees of freedom	Variance estimate	F ratio	Significance
Unadjusted:					
Between groups	335.49	4	83.87	9.2	P <0.01
Within groups	671.84	74	9.08		
Adjusted:					
Between groups	146.95	4	36.74	7.5	P <0.01
Within groups	356.74	73	4.89		

Figure 2

Myocardial glutamic-oxalacetic transaminase (GOT) activity, before and after exercise, in rats subjected to different types and durations of physical conditioning.

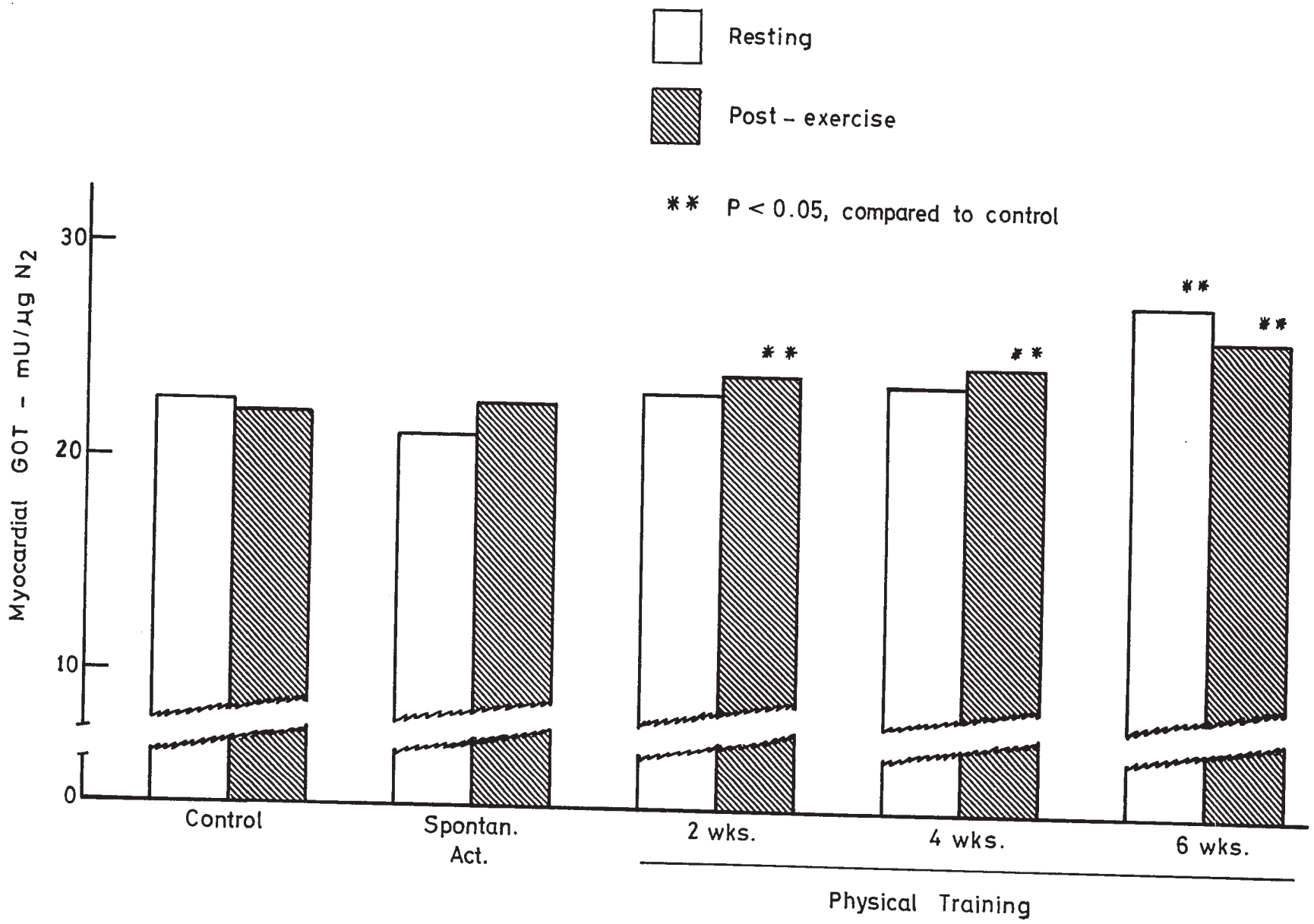

Resting
Post – exercise
** P < 0.05, compared to control
Myocardial GOT – mU/µg N2
30
20
10
0
**
**
**
**
Control
Spontan. Act.
2 wks.
4 wks.
6 wks.
Physical Training

TABLE V

Resting Soleus Creatine Phosphokinase Levels in Control, Spontaneously Active and Physically Trained Rats

	Control	Spontaneously active	Physically trained		
			two weeks	four weeks	six weeks
Unadjusted mean	63.7*	63.1	68.1	71.0	85.9
Adjusted mean	68.0	71.9	70.4	68.0	80.5**
Number of Animals	8	10	14	21	17

*International mU/ug N_2

**P <0.05, compared to control level

Analysis of Variance

Source	Sum of squares	Degrees of freedom	Variance estimate	F ratio	Significance
Unadjusted:					
Between groups	4850.35	4	1212.59	8.5	P <0.01
Within groups	9278.38	65	142.74		
Adjusted:					
Between groups	1605.21	4	401.30	4.3	P<0.01
Within groups	5992.35	64	93.63		

TABLE VI

Post-exercise Soleus Creatine Phosphokinase Levels in Control, Spontaneously Active and Physically Trained Rats

	Control	Spontaneously active	Physically trained		
			two weeks	four weeks	six weeks
Unadjusted mean	61.5*	60.2	70.5	68.6	80.2
Adjusted mean	63.3	62.2	71.6	67.3	78.2**
Number of animals	13	14	13	20	18

*International mU/ug N_2 **P <0.05, compared to control level.

Analysis of Variance

Source	Sum of squares	Degrees of freedom	Variance estimate	F ratio	Significance
Unadjusted:					
Between groups	4120.67	4	1030.17	9.5	P <0.01
Within groups	7878.04	73	107.92		
Adjusted:					
Between groups	2473.61	4	618.40	6.5	P <0.01
Within groups	6829.32	72	94.85		

Figure 3

Soleus creatine phosphokinase (CPK) activity, before and after exercise, in rats subjected to different types and durations of physical conditioning.

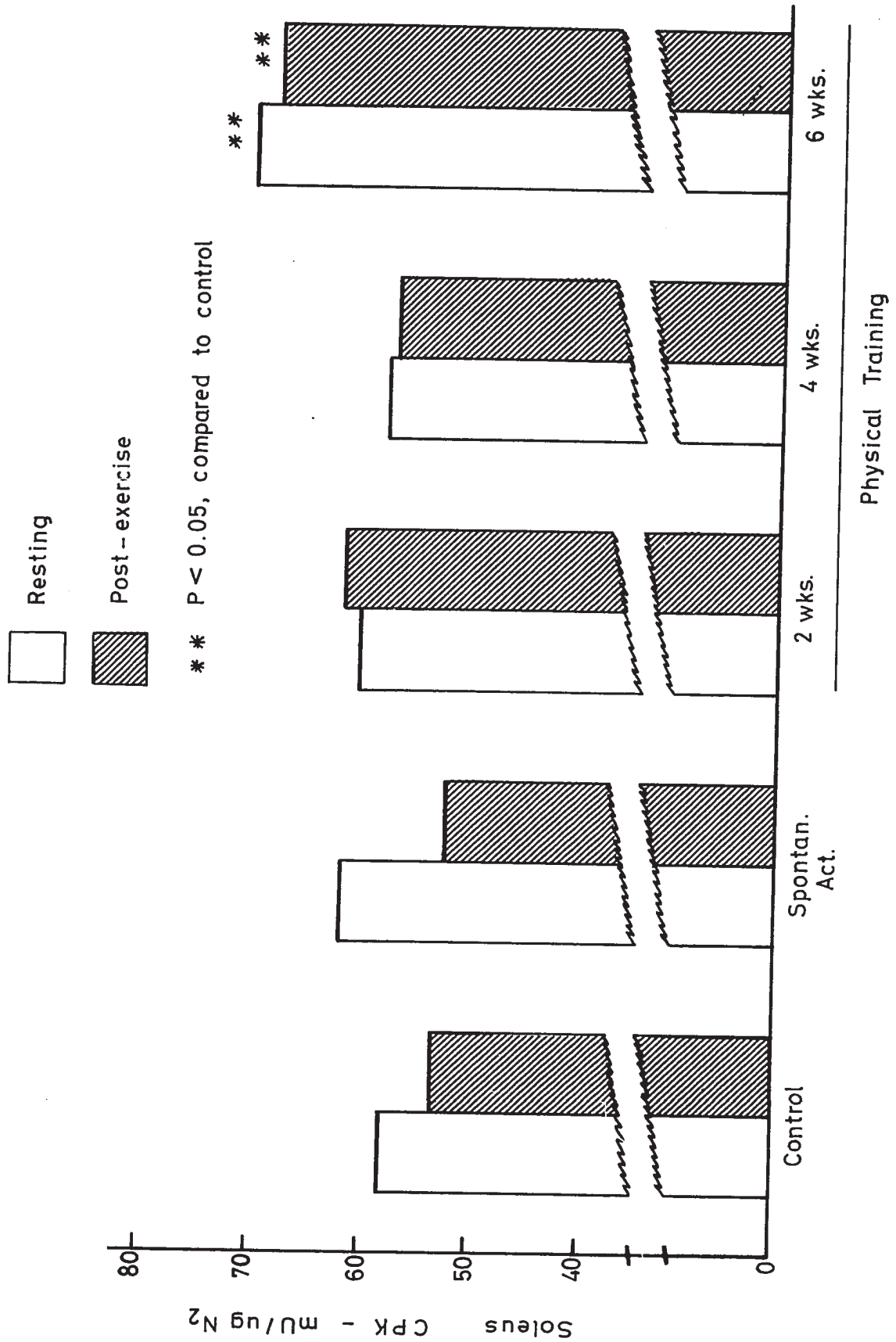

Resting
Post-exercise
** P < 0.05, compared to control
Soleus CPK - mU/ug N2
80
70
60
50
40
0
Control
Spontan. Act.
2 wks.
4 wks.
6 wks.
Physical Training

TABLE VII

Resting Soleus Glutamic-oxalacetic Transaminase Levels in Control, Spontaneously Active and Physically Trained Rats

	Control	Spontaneously active	Physically trained two weeks	four weeks	six weeks
Unadjusted mean	11.9*	10.5	12.4	13.1	16.2
Adjusted mean	12.0	12.2	12.6	13.0	15.1**
Number of animals	17	10	13	14	19

*International mU/ug N_2 **P <0.05, compared to control level

Analysis of Variance

Source	Sum of squares	Degrees of freedom	Variance estimate	F ratio	Significance
Unadjusted:					
Between groups	281.96	4	70.49	12.5	P <0.01
Within groups	384.22	68	5.65		
Adjusted:					
Between groups	94.49	4	23.62	7.8	P <0.01
Within groups	202.23	67	3.02		

TABLE VIII

Post-exercise Soleus Glutamic-oxalacetic Transaminase Levels in Control, Spontaneously Active and Physically Trained Rats

	Control	Spontaneously active	Physically trained		
			two weeks	four weeks	six weeks
Unadjusted mean	11.8*	10.9	13.2	13.0	15.6
Adjusted mean	11.7	12.1	13.6**	12.9	14.8**
Number of animals	17	10	16	16	18

*International mU/ug N_2

**P <0.05, compared to control level

Analysis of Variance

Source	Sum of squares	Degrees of freedom	Variance estimate	F ratio	Significance
Unadjusted:					
Between groups	189.81	4	47.45	9.3	P <0.01
Within groups	368.89	72	5.12		
Adjusted:					
Between groups	92.64	4	23.16	9.0	P <0.01
Within groups	182.84	71	2.58		

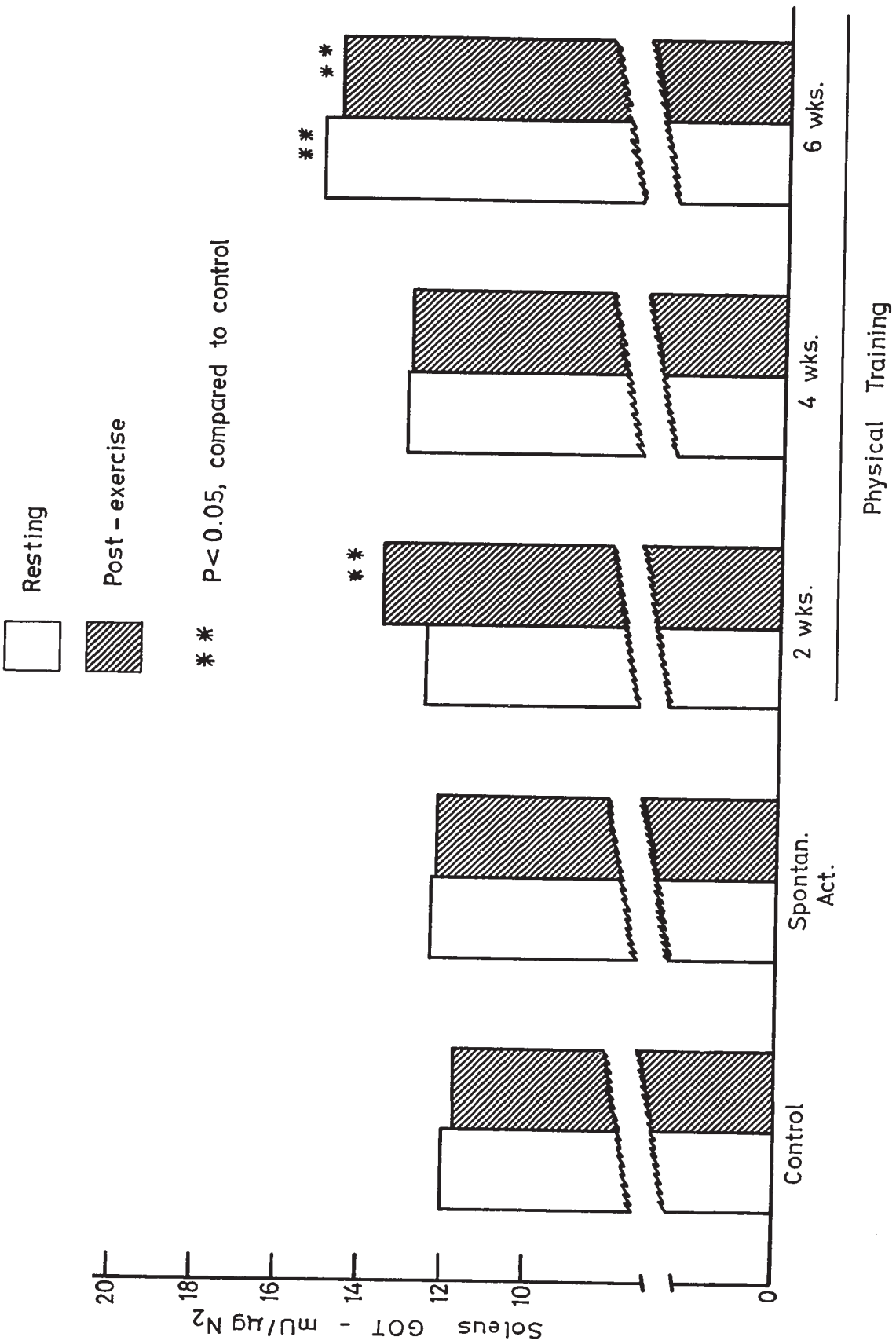

Resting
Post - exercise
** P< 0.05, compared to control
Soleus GOT - mU/µg N2
20
18
16
14
12
10
0
Control
Spontan. Act.
2 wks.
4 wks.
6 wks.
Physical Training

TABLE IX

Resting and Post-exercise Gastrocnemius Creatine Phosphokinase Levels in Control, Spontaneously Active and Physically Trained Rats

	Control	Spontaneously active	Physical training		
			two weeks	four weeks	six weeks
Resting	132.1† (9)	122.2 (8)	130.5 (8)	133.7 (8)	122.6 (8)
Post-exercise	114.9** (8)	116.8 (7)	144.1*,** (8)	135.7 (7)	125.5 (7)

†International mU/ugN2
( ) Number of animals

*P <0.05, compared to control level
**P <0.05, compared to resting level

Analysis of Variance

Source	Sum of squares	Degrees of freedom	Variance estimate	F ratio	Significance
Resting:					
Between groups	971.22	4	242.81	1.1	-
Within groups	7849.05	36	218.03		
Post-exercise:					
Between groups	4763.62	4	1190.01	4.2	P <0.01
Within groups	8540.76	32	266.90		

Figure 5

Gastrocnemius creatine phosphokinase (CPK) activity, before and after exercise, in rats subjected to different types and durations of physical conditioning.

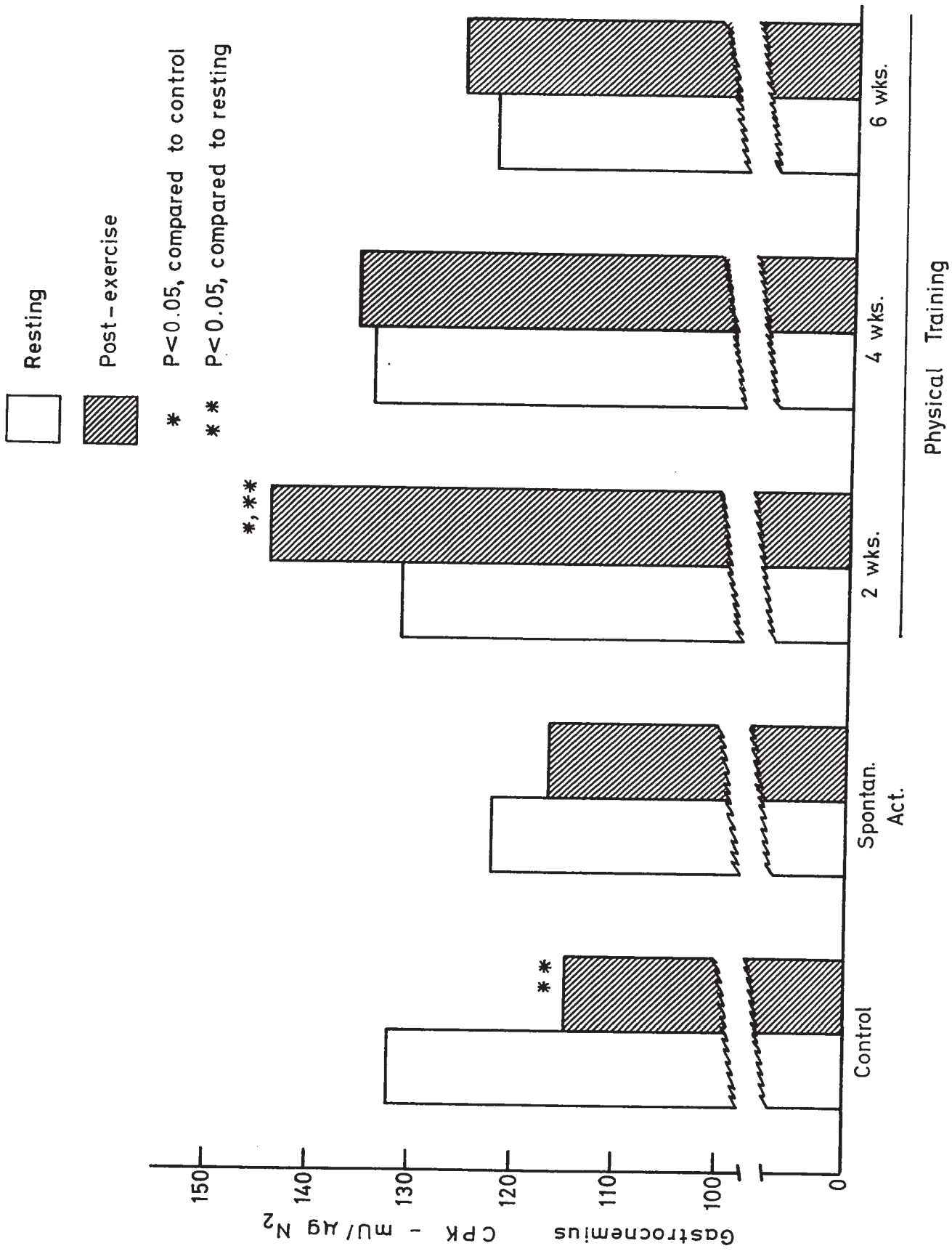

Resting
Post-exercise
* P<0.05, compared to control
** P<0.05, compared to resting
Gastrocnemius CPK - mU/mg N2
150
140
130
120
110
100
0
Control
Spontan. Act.
2 wks.
4 wks.
6 wks.
Physical Training
**
*,**

TABLE X

Resting and Post-exercise Gastrocnemius Glutamic-oxalacetic Transaminase Levels in Control, Spontaneously Active and Physically Trained Rats

	Control	Spontaneously active	Physical training		
			two weeks	four weeks	six weeks
Resting	4.07 † (9)	3.97 (8)	4.22 (8)	4.85 (8)	5.68* (8)
Post-exercise	3.83 (8)	4.86 (7)	4.84 (8)	5.30* (7)	5.42* (7)

†International mU/ug N_2
( ) Number of animals

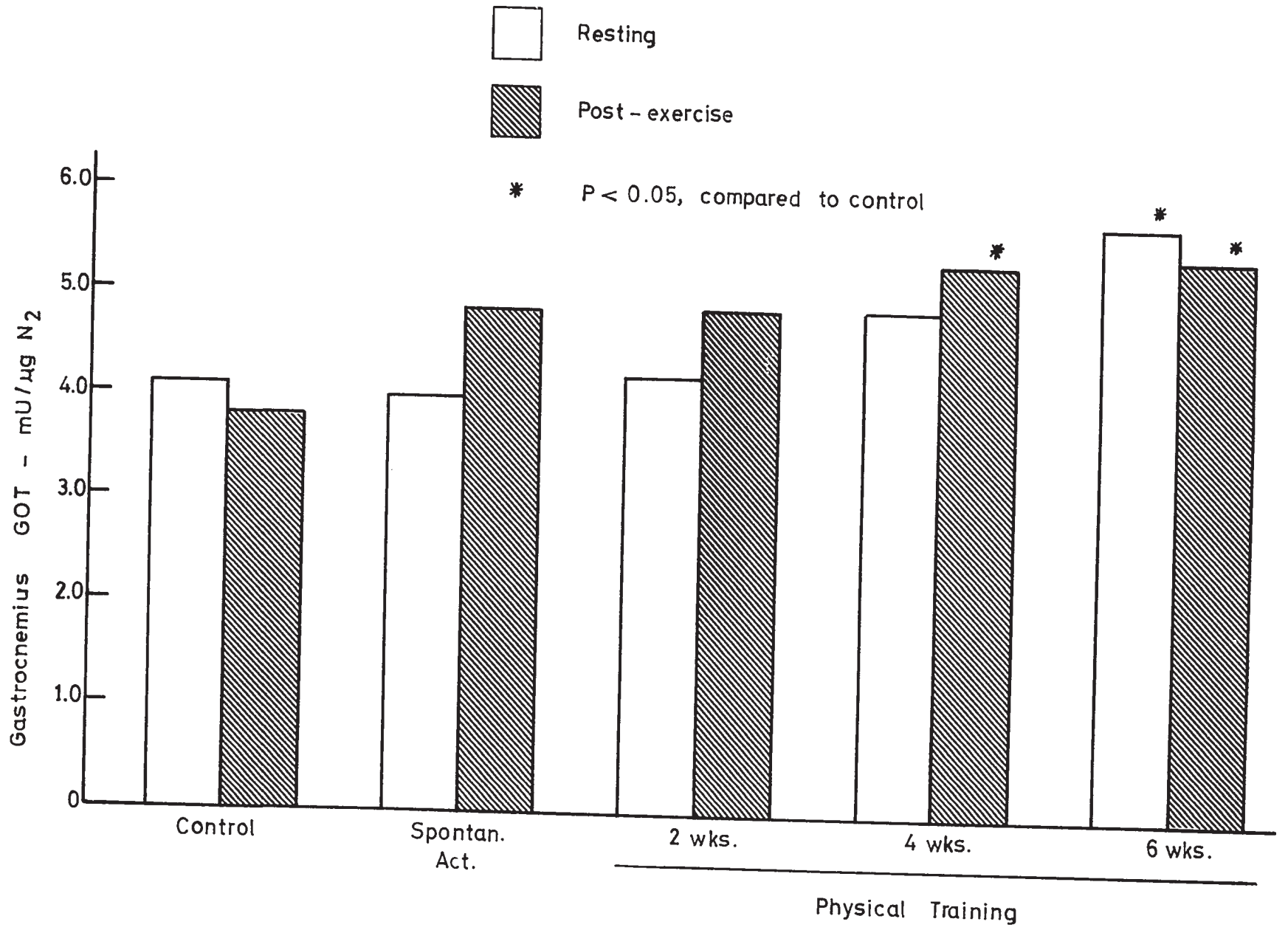
*P <0.05, compared to control level

Analysis of Variance

Source	Sum of squares	Degree of freedom	Variance estimate	F ratio	Significance
Resting:					
Between groups	16.68	4	4.17	6.2	P <0.01
Within groups	24.40	36	0.68		
Post-exercise:					
Between groups	11.93	4	2.98	2.8	P <0.05
Within groups	34.10	32	1.07		

Figure 6

Gastrocnemius glutamic-oxalacetic transaminase (GOT) activity, before and after exercise, in rats subjected to different types and durations of physical conditioning.


Resting
Post - exercise
* P < 0.05, compared to control
Gastrocnemius GOT - mU/µg N2
6.0
5.0
4.0
3.0
2.0
1.0
0
Control
Spontan. Act.
2 wks.
4 wks.
6 wks.
Physical Training

Physical training had no effect on the resting, gastrocnemius CPK level but it did have an effect on the post-exercise CPK level (Table IX, Figure 5). Rats that were physically trained for two weeks displayed a higher mean post-exercise, gastrocnemius CPK level than the post-exercise level of control animals. There was no difference among the post-exercise gastrocnemius CPK levels of control rats, spontaneously active rats and rats that were physically trained for four or six weeks. The mean gastrocnemius CPK level in resting, control rats decreased after a single sixty minute swim. A single sixty minute swim resulted in a net increase (from resting level to post-exercise level) in the CPK activity in gastrocnemius muscles of rats that were physically trained for two weeks. A single exercise episode did not alter the gastrocnemius CPK levels in spontaneously active rats or in rats that had been trained for periods of four or six weeks. Spontaneously active rats that were sacrificed in the resting state had run 1394 ± 347 revolutions per day and the rats that were sacrificed after a sixty minute swim had run 1856 ± 430 revolutions per day.

Table X and Figure 6 show the effect of single and repeated exercise on the gastrocnemius GOT levels. The mean GOT level of the gastrocnemius muscles of resting rats did not become elevated until after six weeks of intermittent swimming exercise. The post-exercise, gastrocnemius GOT levels, however, became elevated earlier during training than the resting levels. Rats that had been subjected to either the four or the six week training program displayed higher mean

post-exercise GOT levels than the mean post-exercise levels in control animals. The post-exercise GOT levels in the gastrocnemius muscles of spontaneously active rats or rats that had been trained for two weeks were comparable to the post-exercise control level. Gastrocnemius GOT levels were not altered after rats were subjected to a single exercise episode, i.e. there was no difference between the resting level and the post-exercise level in any of the five groups of rats.

(ii) Plasma Enzyme Levels in Control, Spontaneously Active and Physically Trained Rats

Tables XI and XII and Figures 7 and 8 demonstrate the effect of exercise on the mean CPK and GOT levels in the plasma of control, spontaneously active and physically trained rats. Standard errors of the mean are shown in Appendix F.

The resting plasma creatine phosphokinase (PCPK) levels in spontaneously active and physically trained rats were unchanged from the resting control level (Table XI and Figure 7). After two or four weeks of physical training the absolute post-exercise PCPK level was less than the post-exercise level in control rats. The absolute post-exercise PCPK levels in spontaneously active rats and rats that had been trained for six weeks were comparable to the absolute post-exercise PCPK level in control animals. A single sixty minute swim resulted in a net elevation in the PCPK activity in control rats, spontaneously active rats and rats that were trained for four or six weeks. A post-exercise elevation in PCPK activity was not observed in rats subjected to intermittent swimming exercise for two weeks.

The resting level of plasma glutamic-oxalacetic transaminase (PGOT) was increased by physical training (Table XII and Figure 8). A comparison of the means with the range test revealed that the mean resting PGOT levels in only the two and six week swimming groups were greater than the resting level in the control group. Applying Student's "t" test to the same means showed that the resting PGOT level in the four week swimming group was also greater than the control level, $P<0.05$. The "t" test was used here in order to reveal a marginally significant value (180). Table XII and Figure 8 show only the statistical results obtained when the control group was compared to the experimental groups by means of the range test. The range test also revealed that the mean resting PGOT level in the six week swimming group was higher than the resting level in any of the other groups, $P<0.05$. Spontaneously active rats had a resting PGOT level that was the same as the control group. A single exercise episode resulted in a net elevation of the PGOT level in each of the five groups of rats. The net elevations observed in spontaneously active and physically trained rats appeared to be less, but were not statistically smaller, than the elevation observed in the control group. Neither spontaneous activity nor physical training altered the absolute post-exercise PGOT level.

(iii) Effect of Restricted Activity on Plasma and Muscle Enzyme Levels

Tables XIII and XIV and Figures 9 and 10 demonstrate the effect of restricted activity on plasma and muscle CPK and GOT levels. Figures 9 and 10 readily illustrate the distribution of CPK and GOT in

TABLE XI

Resting and Post-exercise Plasma Creatine Phosphokinase Levels in Control, Spontaneously Active and Physically Trained Rats

	Control	Spontaneously active	Physical training		
			two weeks	four weeks	six weeks
Resting	35.0 † (19)	42.1 (15)	40.2 (20)	41.9 (24)	39.4 (18)
Post-exercise	70.7** (24)	72.4** (19)	47.2* (22)	53.4*,** (23)	66.2** (16)

†International units per liter of plasma
( ) Number of animals

*P <0.05, compared to control level
**P <0.05, compared to resting level

Analysis of Variance

Source	Sum of squares	Degrees of Freedom	Variance estimate	F ratio	Significance
Resting:					
Between groups	641.00	4	160.25	0.9	-
Within groups	17040.00	91	187.25		
Post-exercise:					
Between groups	10622.73	4	2655.68	6.27	P <0.01
Within groups	41925.27	99	423.49		

Figure 7

Plasma creatine phosphokinase (CPK) activity, before and after exercise, in rats subjected to different types and durations of physical conditioning.

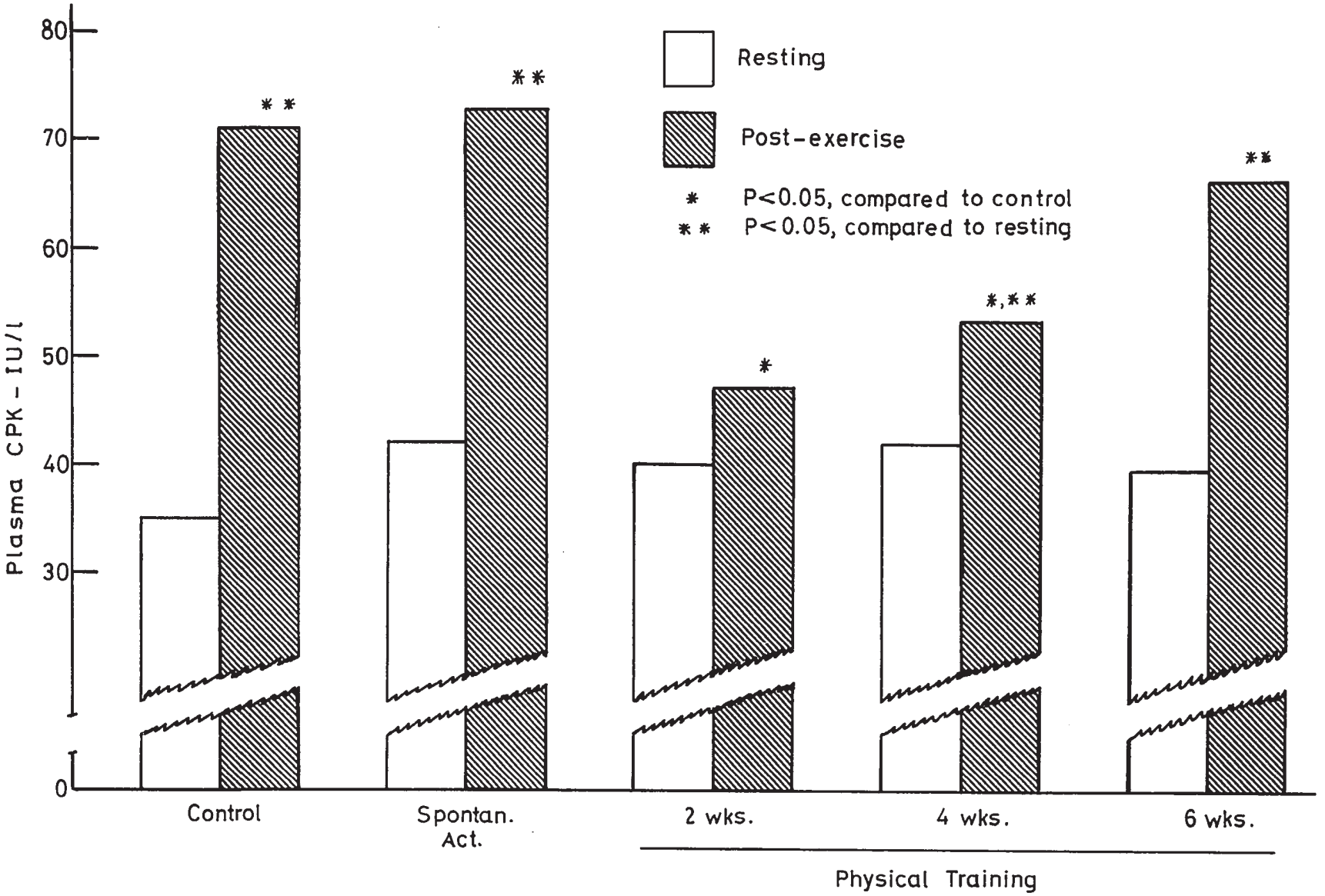

80
70
60
50
40
30
0
Plasma CPK - IU/l
Resting
Post-exercise
* P<0.05, compared to control
** P<0.05, compared to resting
* *
* *
*
*,**
**
Control
Spontan. Act.
2 wks.
4 wks.
6 wks.
Physical Training

TABLE XII

Resting and Post-exercise Plasma Glutamic-oxalacetic Transaminase Levels in Control, Spontaneously Active and Physically Trained Rats

	Control	Spontaneously active	Physical training		
			two weeks	four weeks	six weeks
Resting	76.8† (23)	81.5 (14)	85.8* (17)	83.7 (14)	95.0* (19)
Post-exercise	106.5** (24)	105.0** (17)	101.8** (24)	100.0** (22)	116.8** (18)

†International units per liter of plasma
( ) Number of animals

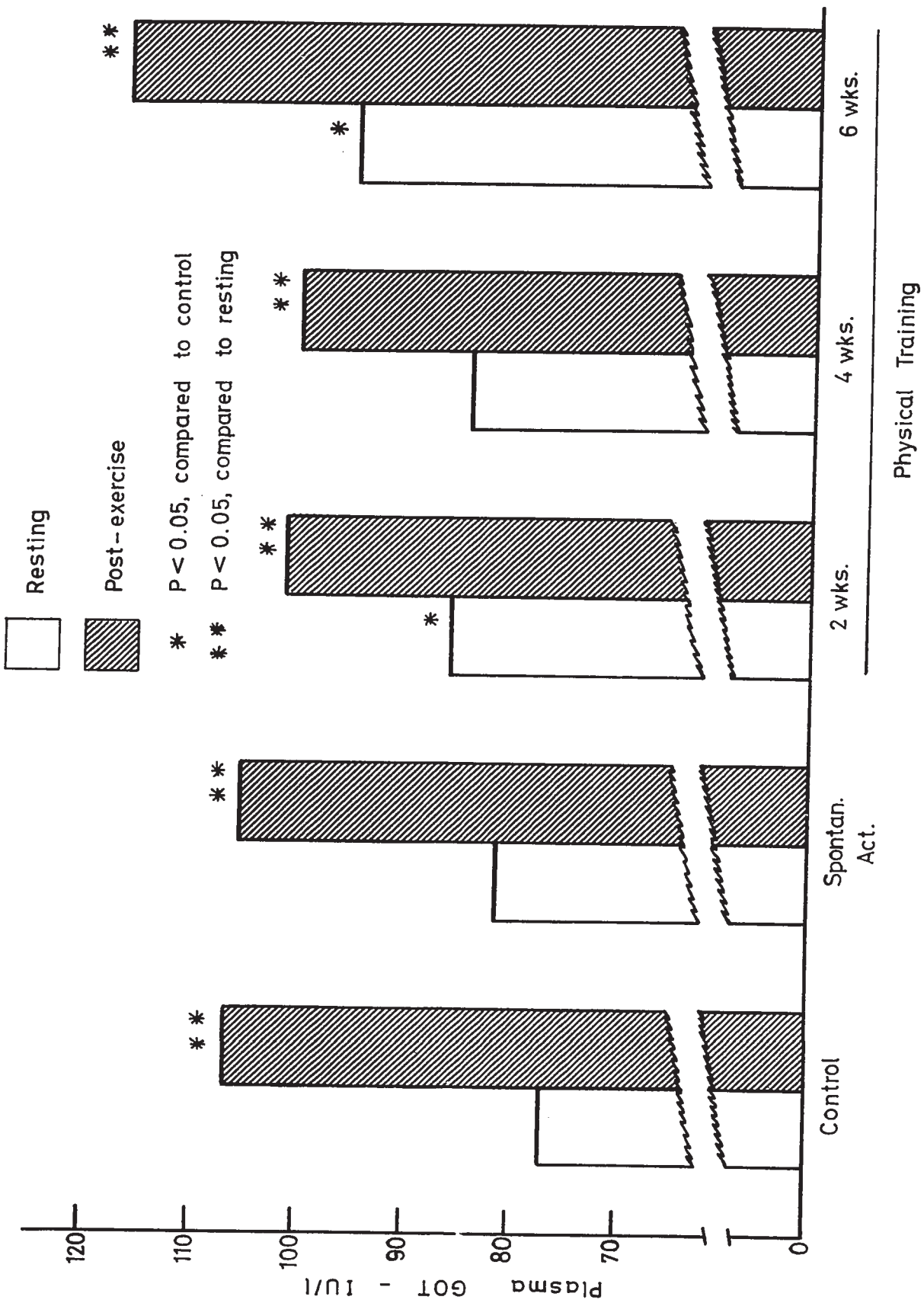
*P <0.05, compared to control level
**P <0.05, compared to resting level

Analysis of Variance

Source	Sum of squares	Degrees of freedom	Variance estimate	F ratio	Significance
Resting:					
Between groups	3628.52	4	907.13	9.8	P <0.01
Within groups	7566.79	82	92.28		
Post-exercise:					
Between groups	3322.93	4	830.73	2.4	-
Within groups	34008.23	100	340.08		

Figure 8

Plasma glutamic-oxalacetic transaminase (GOT) activity, before and after exercise, in rats subjected to different types and durations of physical conditioning.


Plasma GOT - IU/l
120
110
100
90
80
70
0
Control
Spontan. Act.
2 wks.
4 wks.
6 wks.
Physical Training
Resting
Post-exercise
* P < 0.05, compared to control
** P < 0.05, compared to resting

the three types of muscle that were investigated. Figure 9 shows that CPK activity is highest in gastrocnemius (white) muscle, with progressively lesser amounts being present in soleus (red) muscle and cardiac muscle. GOT activity is highest in cardiac muscle, with progressively lesser amounts being present in soleus and gastrocnemius muscle (Figure 10). Total protein nitrogen content in the muscles of these rats is shown in Appendix G.

A single exercise episode resulted in a marked elevation in PCPK activity in both control and restricted rats (Table XIII and Figure 9). The resting PCPK level, post-exercise PCPK level and net post-exercise PCPK elevation occurring in restricted rats were similar to those observed in control rats. CPK levels in the muscles of restricted rats were comparable to those observed in control rats. Exercise (sixty minute swim) resulted in an increased myocardial CPK level in restricted rats and, as previously mentioned, a decrease in the gastrocnemius CPK level of control rats. There was no post-exercise alteration in the soleus or gastrocnemius CPK level in restricted rats and no change in the cardiac or soleus CPK levels in control rats.

Restricted and control rats displayed similar resting PGOT levels, post-exercise PGOT levels and net post-exercise PGOT elevations (Table XIV and Figure 10). A sixty minute swim resulted in an elevation in the soleus and gastrocnemius GOT levels in restricted rats. Exercise did not result in a change in the myocardial GOT level of restricted rats nor did it alter the GOT levels in the muscles of control rats. The resting myocardial GOT level in restricted rats was

TABLE XIII

The Effect of Restricted Activity on Plasma and Muscle Creatine Phosphokinase Levels in Rats

Experimental series	Plasma (U/l)	Heart (mU/ug N_2)	Soleus (mU/ug N_2)	Gastrocnemius (mU/ug N_2)
Control:				
resting	35.0 ± 2.4† (19)	28.1 ± 1.8 (12)	63.8 ± 0.9 (8)	132.1 ± 4.2 (9)
post-exercise	70.7 ± 5.2* (24)	25.7 ± 2.2 (16)	61.5 ± 1.9 (13)	114.9 ± 6.3* (8)
Restricted activity:				
resting	43.1 ± 3.6 (15)	25.1 ± 1.4 (9)	64.8 ± 1.7 (11)	113.6 ± 9.0 (8)
post-exercise	64.4 ± 5.5* (16)	30.4 ± 0.9* (8)	70.6 ± 4.4 (9)	109.4 ± 5.4 (8)

†Mean ± S.E.M.
( ) Number of animals

*P <0.05, compared to resting level

Figure 9

Creatine phosphokinase (CPK) activity, before and after exercise, in plasma and in myocardial, soleus and gastrocnemius muscles of control and restricted rats.

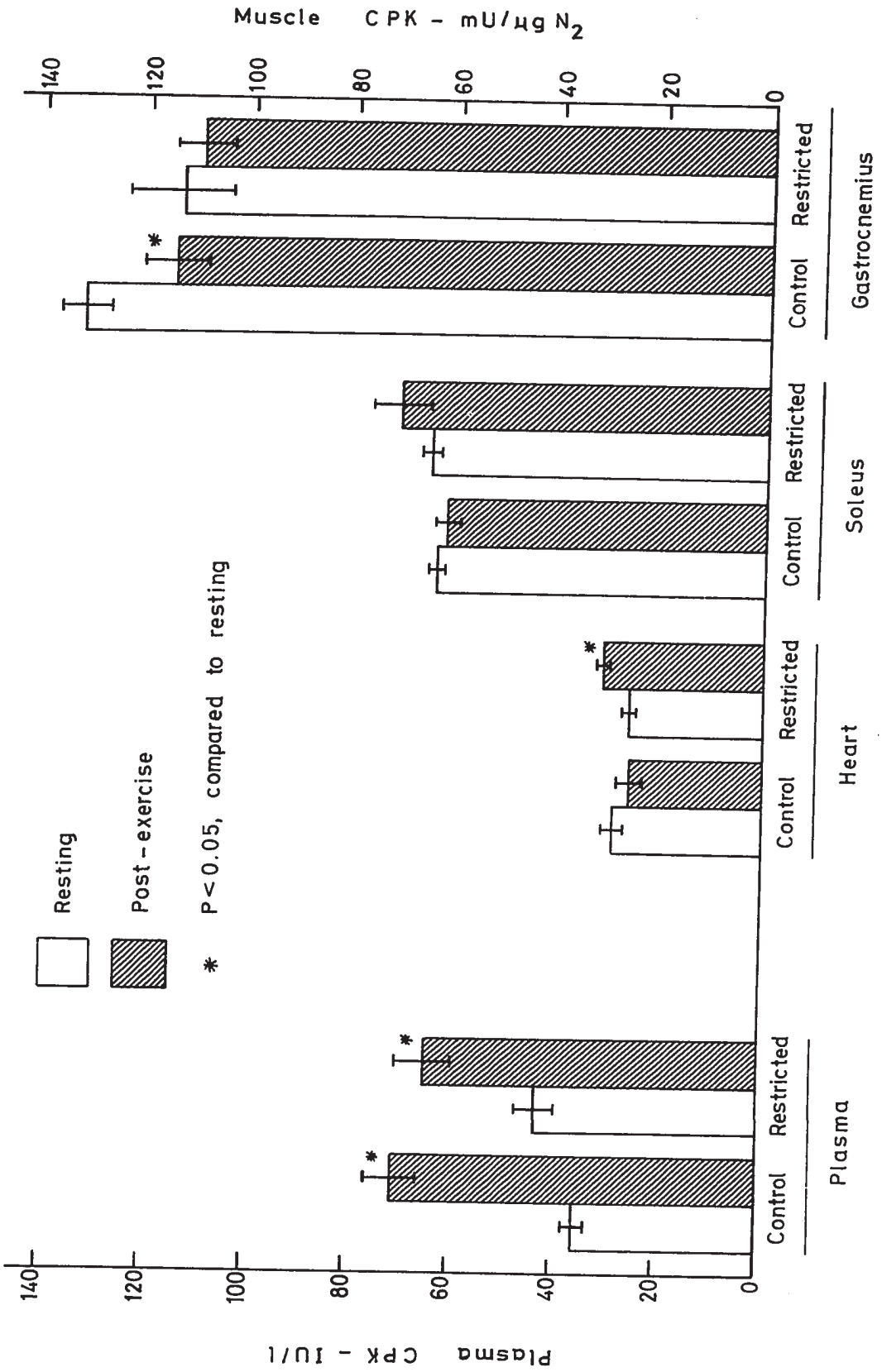

Muscle CPK - mU/µg N2
140
120
100
80
60
40
20
0
Control
Restricted
Gastrocnemius
Soleus
Heart
Plasma
Resting
Post-exercise
* P<0.05, compared to resting
Plasma CPK - IU/l

TABLE XIV

The Effect of Restricted Activity on Plasma and Muscle

Glutamic-oxalacetic Transaminase Levels in Rats

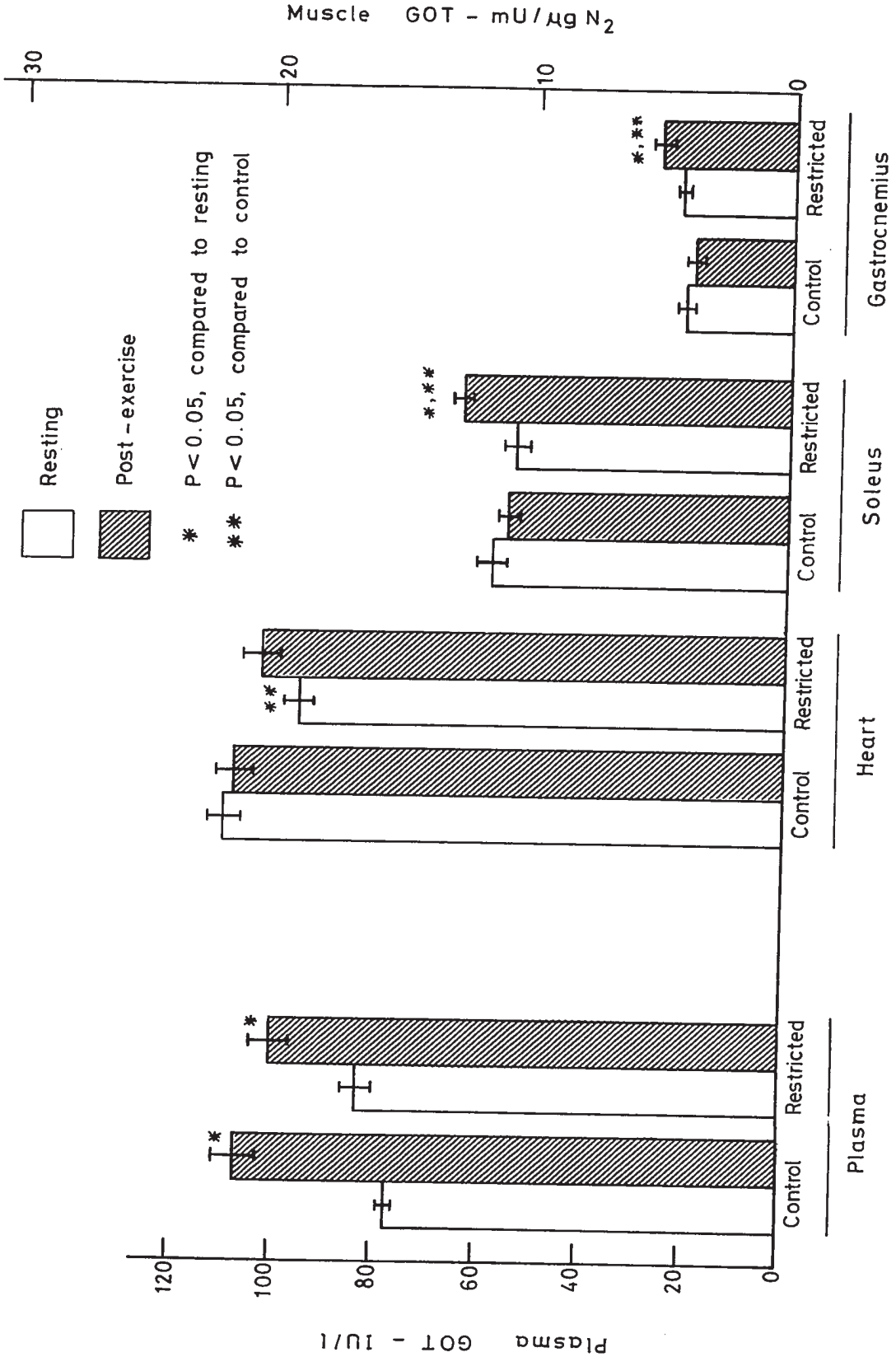
Experimental series	Plasma (U/l)	Heart (mU/ug N_2)	Soleus (mU/ug N_2)	Gastrocnemius (mU/ug N_2)
Control:				
resting	76.8 ± 1.9 † (23)	21.9 ± 0.6 (12)	11.6 ± 0.5 (12)	4.07 ± 0.16 (9)
post-exercise	106.5 ± 4.3* (24)	21.5 ± 0.6 (12)	11.0 ± 0.4 (12)	3.83 ± 0.36 (8)
Restricted activity:				
resting	82.8 ± 2.9 (17)	19.0 ± 0.6** (11)	10.7 ± 0.6 (11)	4.17 ± 0.21 (8)
post-exercise	100.0 ± 3.5* (19)	20.5 ± 0.6 (9)	12.8 ± 0.4*,** (9)	5.12 ± 0.38*,** (8)

†Mean ± S.E.M.
( ) Number of animals

*P <0.05, compared to resting level
**P <0.05, compared to control level

Figure 10

Glutamic-oxalacetic transaminase (GOT) activity, before and after exercise, in plasma and in myocardial, soleus and gastrocnemius muscles of control and restricted rats.


Muscle GOT - mU/µg N2
30
20
10
0
Resting
Post-exercise
* P < 0.05, compared to resting
** P < 0.05, compared to control
*,**
Control
Restricted
Gastrocnemius
Soleus
Heart
Plasma
Plasma GOT - IU/l
120
100
80
60
40
20
0

lower than the resting level in control rats. Soleus and gastrocnemius GOT levels in resting restricted rats were comparable to those in resting control rats. Restricted rats possessed higher post-exercise, soleus and gastrocnemius GOT levels than the post-exercise levels in control rats. Control and restricted rats had comparable post-exercise myocardial GOT levels.

(iv) Hematocrit and Soleus Muscle Weight to Body Weight Ratio

A single exercise episode resulted in hemoconcentration in restricted, control and spontaneously active rats, and in rats that had been subjected to intermittent swimming exercise for six weeks (Table XV). Hemoconcentration did not occur in rats that had been physically trained for two or four week periods. Each of the experimental groups displayed resting and post-exercise hematocrit levels that were similar to those of control animals.

The soleus muscle weight to body weight ratio of the spontaneously running group was greater than it was in control animals (Table XV). Restricted rats and rats that were physically trained by swimming exercise possessed mean soleus to body weight ratios that were comparable to the mean of the control rats. The soleus to body weight ratio was not affected by a single sixty minute swim immediately prior to sacrifice.

2. Coronary Cast Weight to Heart Weight Ratio

Table XVI shows the coronary cast to heart weight ratio in resting control rats and in resting rats that had been physically trained for periods of two, four or six weeks. The mean initial body weights

TABLE XV

Effect of Exercise, Physical Training and Restricted Activity on the Hematocrit and Soleus/Body Weight Ratio of the Rat

Experimental series	Hematocrit		Soleus/body weight	
	resting (%)	post-exercise (%)	resting (mg/g)	post-exercise (mg/g)
Restricted activity	44.4 ± 0.6† (7)	48.7 ± 0.7** (8)	0.353 ± 0.017 (8)	0.361 ± 0.019 (8)
Control	44.9 ± 1.0 (9)	48.3 ± 0.7** (8)	0.357 ± 0.013 (9)	0.366 ± 0.010 (8)
Spontaneous activity	46.0 ± 0.6 (8)	50.4 ± 1.1** (7)	0.433 ± 0.021* (8)	0.482 ± 0.026* (7)
Physically trained:				
two weeks	44.8 ± 0.7 (8)	46.1 ± 0.5 (8)	0.369 ± 0.013 (7)	0.355 ± 0.018 (8)
four weeks	46.6 ± 0.7 (8)	48.7 ± 0.7 (7)	0.404 ± 0.020 (8)	0.389 ± 0.012 (7)
six weeks	45.8 ± 0.4 (8)	49.2 ± 1.1** (7)	0.377 ± 0.013 (8)	0.379 ± 0.019 (7)

†Mean ± S.E.M.
( ) Number of animals

*P <0.05, compared to control level
**P <0.05, compared to resting level

TABLE XVI

Effect of Physical Training on Coronary Cast Weight in Resting Rats

Experimental series	Final body weight (g)	Heart weight (g)	Heart/body weight (%)	Cast weight (mg)	Cast/heart weight (mg/g)
Control (2 week)	334.3 ± 10.5† (8)	1.24 ± 0.08	0.37 ± 0.01	4.80 ± 0.27	3.96 ± 0.28
Physically trained	334.5 ± 14.9 (6)	1.23 ± 0.08	0.37 ± 0.03	5.53 ± 0.39	4.66 ± 0.60
Control (4 week)	393.8 ± 12.2 (8)	1.33 ± 0.07	0.34 ± 0.02	4.98 ± 0.24	3.80 ± 0.20
Physically trained	372.5 ± 22.8 (8)	1.34 ± 0.09	0.36 ± 0.01	6.01 ± 0.28*	4.56 ± 0.19*
Control (6 week)	411.1 ± 23.3 (7)	1.35 ± 0.06	0.33 ± 0.01	5.03 ± 0.31	3.74 ± 0.18
Physically trained	405.7 ± 5.0 (7)	1.41 ± 0.05	0.35 ± 0.01	6.33 ± 0.24*	4.53 ± 0.22*

†Mean ± S.E.M.
( ) Number of animals

*P <0.05, compared to control animals

were the same for all groups. Rats in the two, four and six week control groups weighed 262.8 ± 4.0, 263.0 ± 8.7 and 260.1 ± 11.8 grams, respectively, at the beginning of the conditioning period. Rats in the two, four and six week swimming groups had mean initial body weights of 267.0 ± 6.8, 262.8 ± 8.1 and 272.3 ± 4.9 grams, respectively. The final mean body weight, heart weight and heart to body weight ratio were similar for the experimental and control groups after each duration of treatment. Compared to the means of the four or six week control group, the four or six week swimming groups displayed an increase in both the coronary cast weight and the coronary cast to body weight ratio. These responses did not occur after two weeks of intermittent swimming exercise.

The results of the coronary cast measurements in control, spontaneously active and restricted rats are displayed in Table XVII. Mean initial body weights of 215.1 ± 6.3, 217.9 ± 6.4 and 212.2 ± 4.0 grams were recorded for control, spontaneously active and restricted rats, respectively. Spontaneously active rats ran a total of 1083 ± 230 revolutions per day. Control and restricted rats had comparable final mean body weights but the final mean body weight of the running group was significantly lower than either of the other groups. Although the mean heart weight did not differ among the groups, the heart to body weight ratio was smaller in restricted rats than it was in either the control or spontaneously active rats. There was no difference between the mean heart to body weight ratio of spontaneously active and control rats. The mean coronary cast weight of restricted rats was greater than

the control mean but there was no difference between the mean cast weights of spontaneously active rats and control animals. Both the restricted and the spontaneous running groups had a greater coronary cast to heart weight ratio than control rats.

3. Accessory Experiments on Restricted Rats

Table XVIII demonstrates the effect of the duration of restricted activity on the coronary cast to heart weight ratio. Rats in the three day, one week and two week control groups had mean initial body weights of 276.8 ± 8.9, 272.8 ± 7.6 and 272.2 ± 9.0 grams, respectively. The mean initial body weights of the three day, one week and two week restricted groups were 273 ± 3.2, 272.6 ± 7.0 and 272.4 ± 10.0 grams, respectively. Each restricted group demonstrated a final mean body weight, heart weight and heart to body weight ratio that was comparable to its corresponding control group. Restricted activity for a duration of two weeks resulted in a mean coronary cast weight and a coronary cast to heart weight ratio that were greater than the mean of the two week control group. Shorter durations of restricted activity had no effect on these parameters.

Restricted rats were active for the same proportion of time per day as control rats (Table XIX). Spontaneously active rats were able to swim longer in an exhaustive swim than either control or restricted rats (Table XX and Figure 11). The spontaneously active rats in this experiment had run a total of 936 ± 307 revolutions per day. Control rats were able to swim longer than restricted rats. The initial body

TABLE XVII

Effect of Spontaneous Activity and Restricted Activity on Coronary Cast Weight in Resting Rats

Experimental series	Final body weight (g)	Heart weight (g)	Heart/body weight (%)	Cast weight (mg)	Cast/heart weight (mg/g)
Control	301.3 ± 7.7 † (10)	1.26 ± 0.05	0.41± 0.01	4.20 ± 0.28	3.33 ± 0.15
Spontaneous activity	273.8 ± 9.6* (9)	1.13 ± 0.03	0.41 ± 0.02	4.64 ± 0.41	4.10 ± 0.31*
Restricted activity	298.3 ± 10.3 (8)	1.14 ± 0.04	0.37 ± 0.01*	5.37 ± 0.15*	4.77 ± 0.20*

†Mean ± S.E.M.

( ) Number of animals

*P <0.05, compared to control animals

TABLE XVIII

Effect of Various Periods of Restricted Activity on Coronary Cast Weight in Resting Rats

Experimental series	Final body weight (g)	Heart weight (g)	Heart/body weight (%)	Cast weight (mg)	Cast/heart weight (mg/g)
Control (3 days)	260.8 ± 8.1† (4)	0.95 ± 0.06	0.37 ± 0.02	4.16 ± 0.55	4.44 ± 0.60
Restricted activity	268.4 ± 4.2 (5)	1.01 ± 0.04	0.38 ± 0.02	5.02 ± 0.23	4.98 ± 0.18
Control (1 week)	260.0 ± 10.5 (5)	0.93 ± 0.05	0.36 ± 0.01	3.74 ± 0.16	4.09 ± 0.36
Restricted activity	267.2 ± 9.9 (5)	0.93 ± 0.07	0.35 ± 0.02	4.03 ± 0.22	4.37 ± 0.22
Control (2 weeks)	306.2 ± 10.1 (5)	1.07 ± 0.06	0.35 ± 0.02	3.75 ± 0.31	3.51 ± 0.24
Restricted activity	298.2 ± 12.8 (5)	1.11 ± 0.09	0.37 ± 0.03	5.61 ± 0.37*	5.16 ± 0.45*

†Mean ± S.E.M.
( ) Number of animals

*P <0.05, compared to control animals

TABLE XIX

Effect of Cage Size on the Daily Activity of the Rat

Experimental series	Length of time in cage environment			
	day 1	day 7	day 14	day 21
Standard cages (8 in. x 9 in. x 7 in.)	42.9 ± 2.8* (7)	38.8 ± 0.4 (8)	42.3 ± 1.4 (9)	41.3 ± 0.7 (7)
Restrictive cages (4 in. x 9 in. x 7 in.)	41.6 ± 0.8 (7)	39.2 ± 0.9 (9)	38.9 ± 1.1 (9)	41.6 ± 0.2 (7)

*Total daily activity expressed as a percent of 24 hours ± S.E.M.

( ) Number of animals

XX

TABLE XX

Effect of Spontaneous Activity and Restricted Activity on the Length of Swimming Time to Exhaustion of Rats

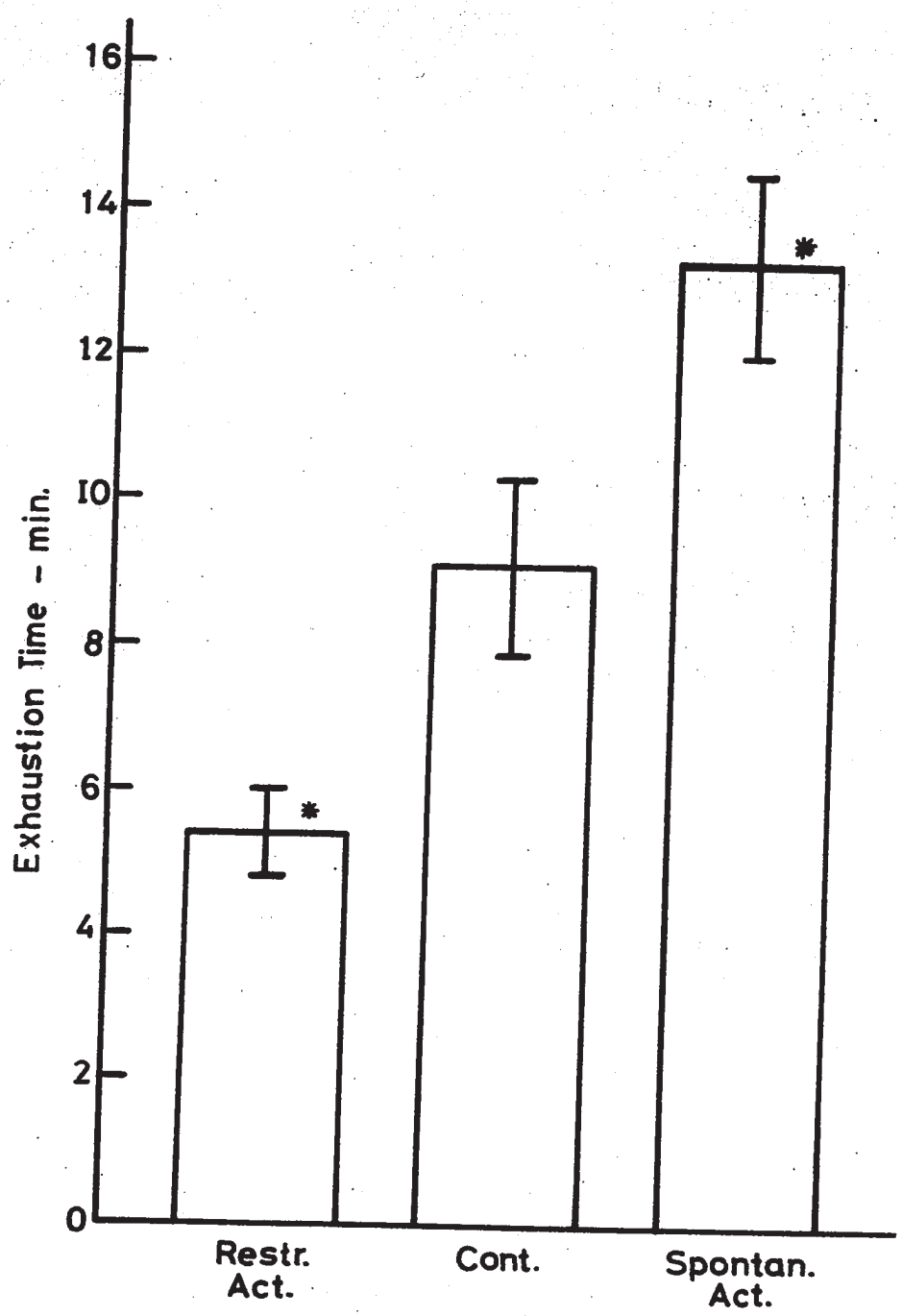
Experimental series	Initial body weight (g)	Final body weight (g)	Exhaustion time (min)
Restricted activity	205.0 ± 3.8† (8)	295.5 ± 8.6	5.40 ± 0.64*
Control	201.3 ± 3.5 (10)	290.1 ± 7.1	9.08 ± 1.20
Spontaneous activity	203.9 ± 4.5 (8)	263.5 ± 9.9*	13.29 ± 1.21*

†Mean ± S.E.M.
( ) Number of animals

*P <0.05, compared to control animals

Figure 11

Swimming exhaustion time for control rats, restricted rats and spontaneously active rats.



* $P < 0.05$, compared to control

weights were comparable for the three groups but spontaneously active rats weighed less than either of the other groups by the end of the experimental conditioning period.

Rats that had been subjected to a restricted environment for one, two or three weeks demonstrated mean adrenal ascorbic acid levels that were lower than those in the control groups (Table XXI and Figure 12). The mean adrenal weight for each of these groups was comparable to that of the control group. Rats that had lived in a restricted environment for three days demonstrated a mean adrenal weight that was greater than that of the control group. The adrenal ascorbic acid level of this group was comparable to the control level.

Table XXII illustrates the effect of adrenalectomy on the elevated coronary cast to heart weight ratio that occurs in restricted animals. The initial and final body weights, heart weights and heart to body weight ratios of restricted rats were similar to those of corresponding control animals. Normal animals that had been subjected to a restricted environment demonstrated a greater coronary cast weight and coronary cast to heart weight ratio than the normal control animals. A similar response was not observed in the adrenalectomized restricted animals, although the mean cast weight and cast to heart weight ratio of this group were greater than those of the intact control animals. The mean initial and final body weights were greater and the heart to body weight ratios were lower in the normal groups than they were in the adrenalectomized groups of animals.

TABLE XXI

Effect of Time in Restricted Activity on Adrenal Ascorbic Acid Levels

Experimental Series	Adrenal Weight (mg)	Adrenal ascorbic acid (mg/100g adrenal tissue)
Control (3 weeks)	36.9 ± 1.8† (11)	552.1 ± 7.5
Restricted activity (3 days)	44.6 ± 2.1* (11)	542.5 ± 8.6
Restricted activity (1 week)	33.6 ± 1.7 (11)	527.5 ± 7.1*
Restricted activity (2 weeks)	38.6 ± 1.5 (11)	524.5 ± 8.4*
Restricted activity	37.9 ± 1.7 (11)	525.2 ± 8.3*

†Mean ± S.E.M.

( ) Number of animals

*$p<0.05$, compared to control animals

Figure 12

Adrenal ascorbic acid levels in control rats and rats subjected to various durations of restricted activity.

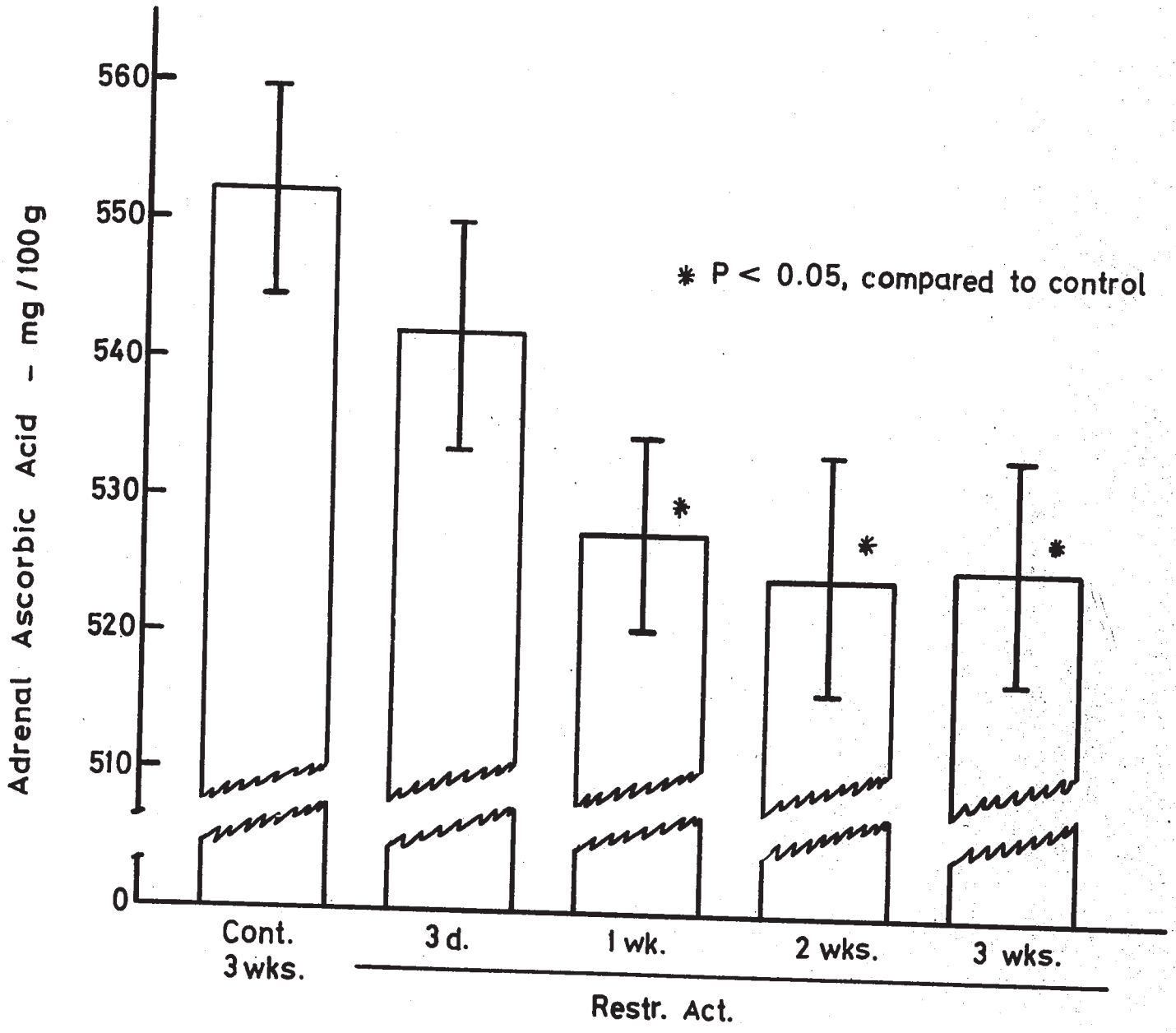

Adrenal Ascorbic Acid - mg/100g
560
550
540
530
520
510
0
* P < 0.05, compared to control
*
*
*
Cont. 3 wks.
3 d.
1 wk.
2 wks.
3 wks.
Restr. Act.

TABLE XXII

Effect of Adrenalectomy on Coronary Cast/Heart Weight Ratio of Rats subjected to Restricted Activity

Experimental series	Initial body weight (g)	Final body weight (g)	Heart weight (g)	Heart/body weight (%)	Cast weight (mg)	Cast/heart weight (mg/g)
Intact control	217.5 ± 2.4† (8)	357.5 ± 4.6	1.37 ± 0.04	0.36 ± 0.01	4.07 ± 0.19	2.99 ± 0.15
Intact restricted	217.8 ± 4.8 (8)	349.4 ± 7.4	1.38 ± 0.04	0.38 ± 0.01	5.40 ± 0.42*	3.89 ± 0.25*
Adrenalectomized control	187.8 ± 5.5* (8)	291.5 ± 12.2*	1.27 ± 0.06	0.42 ± 0.01*	4.45 ± 0.28	3.53 ± 0.24
Adrenalectomized restricted	194.3 ± 7.0* (6)	298.0 ± 17.0*	1.34 ± 0.11	0.42 ± 0.02*	4.95 ± 0.21*	3.81 ± 0.32*

† Mean ± S.E.M.

( ) Number of animals

*P <0.05, compared to intact control animals

V. DISCUSSION

A review of the effect of exercise and physical training on muscle enzyme levels revealed three general trends in the elevation of enzyme levels. First, six weeks of intermittent swimming exercise was the minimum amount of training necessary to produce elevated enzyme levels in all three types of muscle. Second, cardiac muscle displayed elevated enzyme levels earlier during training than did soleus or gastrocnemius muscles. Third, post-exercise muscle enzyme levels were elevated earlier during training than were the enzyme levels of resting rats.

In the present investigation, intermittent swimming exercise over a six week period resulted in an elevation of the resting and post-exercise CPK and GOT activities in the cardiac and soleus muscles, and an elevation of the resting and post-exercise GOT activity in the gastrocnemius muscles of rats. The type of swimming exercise used in the present study is known to cause a three-fold increase in aerobic metabolism (119). During aerobic metabolism, an augmentation of GOT activity would appear to be more beneficial to the working muscles than an elevation of CPK activity. Rawlinson and Gould had previously demonstrated that CPK activity was not elevated in the hind leg muscles of physically trained rats (148).

The muscles that were analyzed were not named, but they were taken from the 'biceps region of the hind leg'. In view of the results of the present study, it appears probable that Rawlinson and Gould analyzed white skeletal muscle.

Neither spontaneous running nor two or four weeks of intermittent swimming exercise produced sufficient training to cause an elevation in the resting CPK or GOT activity of cardiac, soleus, or gastrocnemius muscle. Previous studies had shown that the levels of other enzymes became elevated in the skeletal muscle (184) and liver (155) of rats subjected to similar degrees of physical training. A consideration of these studies indicates that each type of enzyme and each type of body tissue will respond differently to physical training.

The physical training programs employed in the present investigation were comparable to the most moderate programs used by other investigators. Beaton subjected rats to treadmill exercise for one hour (1044 meters per hour) on eleven occasions in sixteen days (18). In the present investigation rats that were allowed access to freely-rotating drums travelled approximately 1400 meters per day. Although Yakovlev and Yampolyskaya (184) subjected rats to daily swimming exercise for a period of thirty days, total swimming time for these rats was $1\frac{1}{2}$ hours longer than the shortest swimming time imposed upon rats in the currently employed training

programs. Beaton and Oyster (21) and Sangster and Beaton (155) subjected rats to swimming exercise for one hour, three times per week, for periods of 24 days and 28 days, respectively. The latter training program was identical to the four week training program employed in the present study. In terms of total swimming time, the six week program of the present study was three hours longer than the six week programs (71,148), and thirty minutes longer than the five week programs (67,84,85), employed by previous investigators.

The post-exercise CPK and GOT activity levels in the present study, appeared to be more sensitive indices of training than the resting enzyme levels. Isolated values of statistical significance occurred in the post-exercise, soleus GOT level and in the post-exercise, gastrocnemius CPK level after two weeks of training. Considering that the post-exercise, soleus GOT level markedly fell in the fourth week of training, and the post-exercise, gastrocnemius CPK level had markedly declined by the sixth week of training, the biological significance of the elevations that occurred in the two week swimmers is not readily apparent. In gastrocnemius muscle, however, the elevated GOT level in the four week swimmers appeared to represent an early adaptation to exercise.

An increase was observed in response to repeated exercise more frequently in skeletal muscle GOT than CPK. The soleus muscle

of GOT level was elevated after four or six weeks of repeated exercise while the soleus CPK level was not elevated until after six weeks of exercise. Six weeks of training resulted in an elevated gastrocnemius GOT level but had no effect on gastrocnemius CPK activity. Rat soleus muscle is characterized by red "slow" fibers and gastrocnemius muscle is characterized by white "fast" fibers. Skeletal muscles, in general, contain a heterogeneous rather than a homogeneous muscle fiber type. Red muscle, such as soleus muscle, contains a predominance of fibers having high oxidative and low glycolytic enzyme activity, with a lesser number of fibers having high glycolytic and low oxidative enzyme activity (52). White muscle, such as gastrocnemius muscle, contains a predominance of fibers possessing high glycolytic and low oxidative enzyme activity, with a lesser number of fibers possessing high oxidative and low glycolytic enzyme activity. On the basis of histochemical enzyme analysis Edgerton, Gerchman and Carrow have shown that repeated swimming exercise resulted in an increased proportion of red to white fibers in rat plantaris (fast) muscles (52). They were not able to show a similar change in soleus muscles since soleus muscles already possessed a high number of red type fibers. An increased red to white fiber ratio, however, may provide one explanation for the observation that skeletal muscle GOT activity became elevated

more readily and earlier during repeated exercise than CPK activity. GOT activity is higher in red than in white muscles and CPK activity is higher in white than in red muscles. A shift in fiber type toward the red fiber type would result in a higher level of total muscle GOT activity, especially in gastrocnemius muscle. Since the CPK activity of soleus muscle was also elevated as a result of repeated exercise the rate of enzyme synthesis or other factors may have been altered as a result of training.

Short, Cobb, Kawabori and Goodner demonstrated lower lactate concentrations in the skeletal muscles of trained rats than in untrained rats (161). This particular training effect was more pronounced in white muscle fibers than it was in red fibers. Low lactate accumulation in trained muscle might be due to a decreased rate of glycolysis, increased oxidation of alpha-glycerol phosphate, increased pyruvate oxidation, increased utilization of phosphogens or removal of lactate by an increased blood flow to the muscles of trained individuals. Holloszy demonstrated an elevation in the activity of various respiratory enzymes in the gastrocnemius muscles of physically trained rats (96). He discussed that physically trained rats attain higher maximum rates of oxygen consumption during strenuous exercise than untrained individuals. This indicates that trained individuals have a greater ability to utilize aerobic metabolic pathways than untrained

individuals, and would rely less on the glycolytic pathway. The present study demonstrated that the enzyme GOT is elevated in the muscles of physically trained rats. This adaptation may be one mechanism by which trained animals are capable of increasing energy production by way of oxidative metabolic pathways. GOT may provide some of the keto-acids that are involved in oxidative metabolism thereby increasing the energy producing capacity of the Kreb's cycle. Since the CPK level in rat soleus muscle became elevated after six weeks of repeated exercise it appears that an increased utilization of phosphogens for energy production may also be responsible for the low, post-exercise, muscle lactate concentrations found in trained individuals. It seems doubtful that the relatively lower post-exercise muscle lactate levels of trained animals would be due to an increased muscle blood flow and an increased removal of lactate. Cardiac output and, consequently peripheral blood flow has been shown to be lower during submaximal exercise in the trained than in the untrained condition (177). The evidence presented in the current study, together with complimentary evidence of previous studies, indicates that trained individuals are more capable than untrained individuals to utilize oxidative pathways and phosphogens for the production of energy in skeletal muscles during exercise.

During physical training cardiac muscle displayed a more sensitive enzyme adaptation than did either type of skeletal muscle. The post-exercise, myocardial CPK activity in spontaneously active rats and in each of the three groups of trained rats was higher than

the post-exercise control level. The post-exercise, myocardial GOT activity was elevated after all durations of intermittent swimming exercise. Gollnick and Hearn demonstrated in the rat that swimming exercise imposed a greater work load on the heart than on skeletal muscles (67). These investigators found an elevation of myocardial lactic dehydrogenase (LDH) activity in trained rats but no change in the LDH activity of gastrocnemius muscle. They also observed cardiac hypertrophy and an increased myocardial protein content but similar changes were not apparent in gastrocnemius muscle. In the present investigation, however, there was no cardiac hypertrophy in any of the six groups of rats and the soleus to body weight ratio increased in only the spontaneous running rats. The myocardial enzyme elevations, occurring in the absence of hypertrophy, indicate that enzymatic systems in cardiac muscle can readily adapt to the energy requirements of repeated exercise. Since soleus hypertrophy did not occur in the swimming groups, and elevated skeletal muscle enzyme levels did not occur until after six weeks of repeated swimming exercise, it appeared that swimming exercise did not impose a great work load on leg muscles.

Spontaneous running activity may not have imposed as great a work load on the heart as did swimming activity. The elevated myocardial CPK level observed in these animals may have provided a greater reserve source of energy for short bursts of running activity. Since myocardial GOT activity was not elevated in these animals it appears that normal GOT levels are sufficiently high to sustain spontaneous running activity.

Several investigators had previously demonstrated that a single exercise episode resulted in an elevated enzyme activity in the heart, skeletal muscle or liver (43,104,105,121,122,144,155). An elevation in the GOT activity of heart and skeletal muscles (43), and an elevated CPK activity in skeletal muscle (121, 122) had been observed after just fifteen minutes of moderate exercise. However, these investigators did not find the same response in all of the tissues studied, nor did they consistently observe the response at higher work loads. In the present study a single exercise episode did not result in a significant elevation in the muscle CPK or GOT levels of control, spontaneously active or physically trained rats.

Intracellular enzyme levels are dependent upon the processes of synthesis, inactivation and catabolism (16). The degree of activation of intracellular enzymes is dependent upon many metabolic and structural activators and inhibitors including: concentration of hydrogen ions, temperature, availability of substrate, metal ions (cofactors) and special organic molecules (coenzymes), structural configuration and orientation of enzymes within the cell (49, 182). A change in one or any combination of these factors could be responsible for the elevated intracellular enzyme activity that occurs during exercise or physical training.

The processes of synthesis and degradation of intracellular enzymes are apparently regulated by various hormones. Administration of growth hormone (187) or testosterone (151) to the rat resulted in a decreased muscle transminase activity. The administration of

thyrotropic hormone (72), thyroxine (32), adrenocorticotrophic hormone (72) and glucocorticoids (53, 154) to rats or mice resulted in an increased GOT or CPK activity in various body tissues. Adrenocortical hormones were thought to be involved in the tissue enzyme elevations that occurred in exercising animals (43). This hypothesis was strengthened by the demonstration of Critz and Withrow that adrenalectomy prevented the elevation of tissue GOT levels that they had observed in normal exercising rats (45).

The results of the present investigation do not establish which of the two processes, synthesis or activation, is occurring during exercise or physical training. Since the enzyme elevations occurred in excess of total muscle protein content, the response cannot be attributed to the normal growth process. Since glucocorticoids have been shown by others to be capable of inducing intracellular enzyme synthesis, and since an increased secretion of glucocorticoids normally occurs at the onset of an exercise episode, it is reasonable that the recurrent exercise episodes in the present investigation may have resulted in repeated stimulation of glucocorticoid secretion which ultimately was responsible for an increased synthesis of intracellular CPK and GOT.

Although significant elevations of muscle CPK or GOT levels did not occur after a single sixty minute swim, apparent increases were sometimes observed. Small increases in muscle enzyme levels resulting from a sixty minute swim may have been additive to elevations

occurring during physical training. The combined effect may explain why post-exercise, muscle enzyme levels were elevated earlier during training than were the resting levels. The observation that post-exercise, muscle enzyme levels were elevated earlier than were the resting enzyme levels indicates that the effect of physical training on muscle enzyme levels is more readily observed in animals in the post-exercise condition.

The plasma enzyme elevations that were observed after a single exercise episode confirm the observations made by the majority of previous investigators. Previous investigators had shown that post-exercise serum enzyme elevations were small or did not occur in trained animals. In the present study there was no post-exercise PCPK elevation in rats trained for two weeks, and the increase observed in the rats trained for four weeks was less than that seen in control animals. In rats that were trained for six weeks, spontaneously active rats and restricted rats, a sixty minute swim resulted in PCPK elevations that were similar to those of the control rats. Physical training did not affect the PGOT response to exercise. The observation that physical training did not prevent the post-exercise serum enzyme elevation indicates that the training programs used in the present study were not severe. Physical training resulted in an elevated PGOT level but had no effect on the resting PCPK activity.

The occurrence of enzymes in the blood plasma is believed to result from the normal breakdown and turnover of body tissues (16,40,120). The concentration gradient that exists between the

intracellular and extracellular fluid favors the diffusion of enzymes from the cell to the extracellular fluid. Under normal conditions diffusion is limited by the relative impermeability of the cell membrane. Alterations in permeability result in an increased efflux of intracellular enzymes.

At the present time there is no rational explanation for the differential release of intracellular enzymes. The rate of efflux is apparently not dependent on the molecular weight of the enzymes (6,17,47,58,59,65,82,91,131). However, recent evidence indicates that the previously known enzyme molecular weights may be erroneous. In the past the established molecular weight of GOT has been 110,000 (49), however, independent investigations conducted in 1968 have shown that the molecular weight of GOT is considerably less (12,24,55,56). One of these studies (12) reported the molecular weight of GOT to be 79,000. The known molecular weight of CPK is approximately 81,000 (49). If a relationship does exist between molecular weight and the egress of intracellular enzymes, the differential efflux of CPK and GOT probably depends upon other factors.

The differential pattern of enzyme release may be related to the strength and degree of binding to the particulate components of the cell (74). Intracellular CPK and/or GOT activity is found in two fractions: a bound mitochondrial fraction and a soluble cytoplasmic fraction (38,40,137). The soluble fraction appears to be more readily released from the cells than the bound form. The major component that is lost from cells during pathological states is the

soluble form (98,101,103). Ottaway has demonstrated that CPK is not only bound in mitochondria but is also bound to myofibrillar structures within the muscle cells (137). It is possible that a fraction of the bound enzymes becomes detached from intracellular binding sites as a result of ionic or intracellular pH changes that occur during muscle contraction. If this does occur, it is likely that a myofibrillar component of intracellular enzymes would be lost more easily from cells than a mitochondrial component since mitochondrial enzymes would have to pass through both a mitochondrial membrane and a cellular membrane before reaching the extracellular space. Under the proposed circumstances both soluble and myofibrillar CPK fractions could be readily lost from muscles during exercise whereas GOT would be lost mainly from the soluble component. The ease with which GOT or CPK would be lost from cells during exercise would then depend on the proportion of the enzyme that exists in non-mitochondrial fractions of the cell. A comparison of the percentages of non-mitochondrial CPK and GOT existing in rat muscle cells has not yet been made. However, <u>in vitro</u> studies on chicken muscle indicate that a relatively greater proportion of intracellular GOT exists in bound form than does CPK (47). Under all experimental conditions CPK had a greater efflux rate than GOT. The relative influence of molecular weight, strength and locus of intracellular binding or other factors on the pattern of enzyme release remains to be elucidated.

The type of exercise that was used in the present study resulted in a relatively greater efflux of CPK than GOT. The post-exercise PCPK and PGOT elevations observed in control rats were 100 and 40 percent respectively. A response such as this may have been related to a relatively greater muscle to plasma concentration gradient for CPK than for GOT. Figures 9 and 10 illustrate that the activity of CPK in each type of muscle was relatively greater than the activity of GOT.

The muscle cell membrane undergoes an increased permeability to various inorganic ions during the action potential preceding muscular contraction. Due to this permeability change there is rapid influx of sodium and calcium ions followed by a rapid efflux of potassium ions. These ion fluxes are believed to result from an increase in the size of pores or channels in the cellular membrane (66). It is difficult to conceive that the efflux of large enzyme molecules which occurs during exercise could result from a similar increase in the size of pores in cellular membranes. Hemoglobin (molecular weight: 64,000) escapes from the erythrocyte during hemolysis, leaving the stroma, or "ghost cell", with an intact cell membrane (66). However, the osmotic changes responsible for hemolysis and/or the extreme swelling of the erythrocyte that occurs during hemolysis are not likely to occur in muscle cells during contraction. A more likely mechanism for the release of intracellular enzymes during exercise would be by a process of reverse pinocytosis. Large macromolecules could get through a membrane without actually piercing it by becoming attached to the internal

surface of the membrane which would then surround it and open up outwardly to liberate the macromolecule to the exterior of the cell (28).

The effect of physical training on post-exercise PCPK levels was paralleled by an apparently similar trend in the post-exercise PGOT levels. Cardinet et al. observed a similar pattern for serum GOT levels in race horses during training (30). Frenkle and Csalay may have provided an explanation for the relatively smaller post-exercise plasma elevations that occurred in the early stages of training. These investigations observed increased blood glucocorticoid levels after three weeks of daily swimming exercise but normal levels by the sixth week of training (60). In view of the attenuating effect of the glucocorticoids on membrane permeability changes (70,76,126,158,179), the smaller post-exercise elevations in PCPK and PGOT activities may have been a reflection of an elevated circulating glucocorticoid level. The large elevations in PCPK and PGOT activity that were observed after six weeks of training may have occurred as a result of a normalization of glucocorticoid levels.

Hemoconcentration is a known result of exercise (39,179,128). During exercise fluid is shifted from the blood into the interstitial space as a result of increased capillary blood pressure associated with the elevated systolic pressure that occurs with exercise (128). There is also evidence that increased metabolism in cells during exercise, resulting in the catabolism of large

molecules into a number of smaller ones, may cause an increased intracellular osmotic pressure which draws water into the cells at the expense of the interstitial and intravascular fluids. The net result of a reduction in the amount of intravascular fluid is an increased concentration of erythrocytes, hemoglobin and plasma proteins.

In the present investigation a post-exercise hemoconcentration was not observed in rats that were physically trained for two or four weeks. Since elevations in the post-exercise plasma enzyme levels were minimal after two or four weeks of training, it may appear that hemoconcentration was responsible for the enzyme elevations that occurred during exercise in the other groups of rats. Hematocrit measurements showed that the concentration of red blood cells in control animals was increased by less than ten percent after exercise. This increase cannot be totally responsible for the post-exercise plasma CPK elevation of 100 percent or the GOT elevation of 40 percent.

Beaton (18) and Beaton and Oyster (155) had previously observed an increase in the resting malic dehydrogenase activity in the blood plasma of physically trained rats. In the present investigation the GOT levels of resting rats were elevated above the control level after two and four weeks of intermittent swimming exercise. Moreover, the resting PGOT level of rats trained for six weeks was greater than the resting levels of the rats trained for

two or four weeks. These data indicate that the PGOT activity in resting rats progressively increases with physical training. Since the muscle GOT levels were also elevated in trained rats, PGOT activity may be dependent upon a tissue to plasma enzyme gradient. Cardiac muscle and possibly, gastrocnemius muscle may have contributed to the elevated PGOT activity that occurred early in training. The highly elevated PGOT activity observed after six weeks of training appeared to relate to the highly elevated GOT levels observed in all three types of muscle. The complimentary increase that was observed in plasma and muscle GOT levels, however, was not apparent in the CPK levels or rats subjected to the same training programs.

An increased capacity of the coronary vasculature is thought to be one of the earliest beneficial effects of physical training (115,164). The coronary cast to heart weight ratio, measured from vinyl-acetate casts of the coronary arterial trees of rats, had been found to increase after three, four (166) and five (173) weeks of physical training. In the present investigation the cast to heart weight ratio was increased after four and six weeks of physical training and after three weeks of spontaneous running activity but not after two weeks of swimming training. From these various observations it appears that, in order to observe an increase in the cast to heart weight ratio, an elevated level of physical activity must be maintained for a minimum of three weeks. Whether or not the coronary cast size is related to

the physiological capacity of the coronary vasculature remains to be investigated.

The two types of exercise that were employed in the present investigation (intermittent swimming or spontaneous daily running activity) had different effects on the final body weights of rats. Rats subjected to intermittent swimming exercise gained as much weight as the corresponding control animals, while rats subjected to spontaneous daily running activity gained less weight than control animals. Similar observations have been made by other investigators (166). Food intake and body weight have been found to decrease in rats on exercise days, with a compensatory increase occurring on the following (rest) days (165, 174).

Although control, spontaneously active and physically trained rats did not demonstrate elevated muscle enzyme levels after a single sixty minute swim, elevations resulting from a single exercise episode were observed in the myocardial CPK level and in the soleus and gastrocnemius GOT levels of restricted rats. Such a response may be due to the activation of an inactive precursor or to the synthesis of new enzyme (105). Beaton stated that synthesis may be responsible for the enzyme elevation observed in physically trained rats but it was probably not involved in the changes that occurred during a single exercise episode (18).

Kendrick-Jones and Perry demonstrated that skeletal muscle CPK levels were lower in resting restricted rats than in resting control rats (105). These investigators inferred that the

lower muscle enzyme levels in resting restricted rats were due to the disuse caused by inactivity. In the present study restricted activity had no effect on the muscle CPK levels of resting rats; however, restricted activity resulted in a low level of myocardial GOT. Kendrick-Jones and Perry did not reveal the degree to which they restricted the environment of rats in their investigation. It is possible that the difference between the results of their experiments and those of the present study merely reflect a different degree of activity restriction.

The restricted rats in the present investigation yielded certain results that were similar to the results obtained from physically trained rats. Firstly, the post-exercise soleus and gastrocnemius GOT levels in restricted rats were higher than the post-exercise GOT levels in control animals. Secondly, restricted rats demonstrated a higher mean coronary cast to heart weight ratio than that found in control rats. Further investigation revealed that the cast to heart weight ratio in these rats was increased after they had been subjected to restricted activity for only two weeks. In terms of the coronary cast measurements, the restricted rats not only had a greater coronary arterial capacity than control animals, but the increase occurred earlier in restricted animals than in physically active rats.

Although the space available for movement was reduced in the restricted cages, the restricted animals may have been hyperactive in these cages. Two additional experiments were performed

in an attempt to reveal the level of physical activity and the degree of physical conditioning of restricted rats. The results of the first of these investigations indicated that restricted animals were as active as control rats. The second experiment demonstrated that the length of time required to swim to exhaustion was progressively greater in restricted, control and spontaneously active rats. These results indicated that the restricted animals were not being physically conditioned as a result of hyperactivity in their cages.

The author of the present dissertation believes that the adrenal cortex may be involved in the plasma and muscle enzyme changes that occurred with physical training. If the adrenal glands are involved in these responses, it is possible that they are also involved in the changes that occurred in the coronary cast to heart weight ratio. Non-specific stress may have been responsible for the increases in the muscle enzyme levels and coronary cast to heart weight ratio in restricted animals.

Stressful situations, regardless of the type of stress, bring about various physiological responses such as: adrenal enlargement, thymus and lymphatic involution and bleeding ulcers in the digestive tract (178). These responses characterize what Hans Selye has called the "general adaptation syndrome" (GAS). The GAS develops and subsides over three stages in time: 1) the alarm reaction or period in which the above symptoms become manifest, 2) stage of resistance, during which the stress symptoms seem to

disappear but the resistance of the organism to stress is greater than normal and 3) stage of exhaustion, during which the resistance of the previous stage is lost, the original symptoms reappear and death eventually follows.

The observations that adrenalocortical hyperactivity occurs in restrained animals (14, 110, 145) indicates that the non-specific stress reactions are elicited in immobilized animals. Pfeiffer subjected rats to either a restricted or a control environment for a five week period. The restraint coefficient (rat volume to cage volume) was .338 to .475 for restricted rats and 0.021 to 0.033 for control animals (145). Compared to control animals, the restricted rats showed no increase in mortality rate or gastric ulceration, however, restricted activity resulted in an increased adrenal weight and thymus involution. Pfeiffer reported that the rats in his study were not severely stressed by the experimental treatment. In the present study the restraint coefficient was approximately 0.048 to 0.085 and 0.024 to 0.042 for restricted and control rats, respectively. Compared to the rats in Pfeiffer's study, the rats in the present study were only mildly restricted. If adrenal hormone hypersecretion did occur in the restricted rats of the present study, it would appear that minimal reduction of cage size is sufficient to elicit a non-specific stress reaction.

The following experiments, designed to test the possibility that adrenal hormone hypersecretion might have been occurring in

restricted animals, failed to provide sufficient evidence to conclude that non-specific stress was occurring in these animals. Either adrenal hypertrophy or a decrease in the adrenal ascorbic acid level is indicative of hypersecretion of adrenal hormones. After subjecting rats to a restricted environment for three days adrenal hypertrophy was observed while the adrenal ascorbic acid level remained unchanged. After living in a restricted environment for one week, two weeks or three weeks, rats displayed normal adrenal gland weights while the adrenal ascorbic acid levels were lower than the control level. Due to the inconsistency of these observations it is difficult to establish any biological significance. Both of these parameters indicated adrenal gland hyperactivity but the two parameters showed contradictory results as to the time period in which hyperactivity was occurring. Although the adrenal ascorbic acid level decreased below the control level in rats subjected to a small cage environment for one, two or three weeks, the decrease was only marginally significant ($p < 0.05$) and represented only five percent of the control adrenal ascorbic acid level.

The observations that were made on adrenalectomized rats indicated that the adrenal gland may be involved in the increase in the coronary cast to heart weight ratio that occurred in restricted rats. While normal restricted rats displayed a greater mean coronary cast to heart weight ratio than normal control rats, adrenalectomized restricted rats had a mean coronary cast to heart weight ratio that

was comparable to that of adrenalectomized control rats. However, since the mean coronary cast weight and mean cast to heart weight ratio of the adrenalectomized control group were somewhat higher than they were in the normal control group, the results obtained from the adrenalectomized rats should be interpreted with caution.

Although adrenocorticoid determinations were not included in the present study, the results of previous investigations indicate that adrenocortical hormones may be involved in the plasma and tissue enzyme changes and possibly the coronary cast weight changes that were observed in both the physically trained and restricted animals. The effects of emotional factors and physical exercise on the adrenal cortex, however, are not identical (33,95). Emotional factors are usually associated with an increased secretion of hormones from the adrenal cortex. Emotional factors are thought to initiate this response in order to prepare the organism for the physical activity that usually follows (33). The decreased urinary and blood corticoid levels that accompany exercise are believed to be the result of an increased utilization of these hormones by the body tissues (33,36,95). Repeated exercise is believed to increase the size and lower the secretion threshold of the adrenal gland, resulting in a greater reserve of adrenocorticoids and a faster adrenal response to stress (128). The small plasma enzyme elevations that are observed after exercise in physically trained animals may possibly be due to the stabilizing effect of glucocorticoids on cell membranes. Elevations of muscle enzyme levels may be the result of the ability of glucocorticoids to induce intracellular enzyme synthesis.

VI. SUMMARY AND CONCLUSIONS

Intermittent swimming exercise over a period of six weeks resulted in a significant increase in creatine phosphokinase activity in the heart and soleus muscle of the rat. CPK activity was elevated in the heart after only two weeks of repeated swimming exercise and after three weeks of spontaneous running activity. A large increase in the plasma CPK level of control rats resulted from a single exercise episode. After two weeks of physical training the PCPK level was not altered by a single exercise episode. Physical training for longer periods (four and six weeks) resulted in progressively greater post-exercise PCPK elevations. Rats that were physically trained for six weeks displayed post-exercise PCPK elevations that were similar to those of control animals.

Subjecting rats to repeated exercise for a period of six weeks resulted in the elevation of GOT levels in cardiac, soleus and gastrocnemius muscles. Myocardial GOT activity was elevated after only two weeks of intermittent swimming exercise. Both control and physically conditioned rats displayed elevated PGOT levels after a single exercise episode, however, physically conditioned rats appeared to have smaller post-exercise PGOT elevations than control animals. Physically trained rats had higher resulting PGOT levels than resting control rats. The resting PGOT level appears to be dependent upon a muscle to plasma enzyme gradient.

Resting restricted rats possessed a lower myocardial CPK level than resting control rats. The post-exercise soleus and gastrocnemius muscle GOT levels in restricted rats were higher than the post-exercise levels in control rats. The PCPK, PGOT, myocardial CPK, soleus GOT and gastrocnemius GOT levels of restricted rats were higher after a sixty minute swim than they were before the swim.

The coronary cast to heart weight ratio was increased in spontaneously active rats, rats that had been subjected to restricted activity for two or three weeks and rats that had been physically trained for four or six weeks.

The unusual observations made on restricted rats were apparently not the result of hyperactivity but may have been due to a mild non-specific stress reaction occurring in these animals.

The results of the present investigation clearly demonstrated the following phenomena:

1) The activities of the enzymes creatine phosphokinase and glutamic-oxalacetic transaminase were elevated in the heart and skeletal muscles of rats subjected to repeated bouts of exercise.

2) Six weeks of intermittent swimming exercise was the minimum amount of physical training necessary to produce elevated enzyme levels in all three types of muscles.

3) In terms of enzyme activity levels, cardiac muscle displayed an earlier adaptation to repeated exercise than did either soleus or gastrocnemius muscle.

4) Post-exercise muscle enzyme levels were levated earlier during physical training than were the enzyme levels of resting rats. It is therefore suggested that post-exercise rather than resting muscle enzyme levels be measured in studies involving the effects of physical training on muscle enzyme levels.

5) The PGOT level in resting rats increased progressively with physical training while the PCPK level remained unchanged. This observation demonstrated that training produces a specific effect on the plasma level of each particular type of enzyme. It may be possible to utilize the plasma GOT level as an indicator of the state of physical conditioning of an animal or human.

6) An elevation in the coronary cast to heart weight ratio was not observed until rats had been subjected to repeated exercise over a period of three weeks.

7) The effects of cage size restriction on rat enzyme levels and coronary cast weight were similar in many ways to the effects of increased physical activity.

An increased secretion of hormones by the adrenal gland was discussed as a possible causative factor for the enzyme and coronary cast weight changes that were observed. Hypersecretion by the adrenal gland may have been caused by a sustained non-specific stress reaction occurring in restricted rats. In spontaneously active and physically trained rats a brief increase in the adrenal secretion rate could have occurred at the onset of each

exercise episode. A sustained or a recurrent increase in the adrenal secretion rate may have produced similar effects on both the muscle enzyme levels and the coronary cast weights of rats.

BIBLIOGRAPHY

(1) Agress, C.M. and Estrin, H.M. The biochemical diagnosis of heart disease. Springfield: Charles C. Thomas, 1963.

(2) Agress, C.M., Jacobs, H.I., Glassner, H.F., Lederer, M.A., Clark, W.G., Wroblewski, F., Karmen, A. and La Due, J.S. Serum transaminase levels in experimental myocardial infarction. Circulation 11: 711-713, 1955.

(3) Ahlborg, B. and Brohult, J. Metabolic changes after long-term physical exercise. Försvarsmedicin 2: 35-49, 1966.

(4) Ahlborg, B. and Brohult, J. Immediate and delayed metabolic reactions in well trained subjects after prolonged physical exercise. Acta Med. Scand. 182: 41-54, 1967.

(5) Altland, P.D. and Highman, B. Effects of exercise on serum enzyme values and tissues of rats. Amer. J. Physiol. 201: 393-395, 1961.

(6) Altland, P.D., Highman, B. and Garbus, J. Exercise training and altitude tolerance in rats: blood, tissue, enzyme and isoenzyme changes. Aerospace Med. 35: 1034-1039, 1964.

(7) Altland, P.D., Highman, B. and Nelson, B.D. Serum enzyme and tissue changes in rats exercised repeatedly at altitude: effects of training. Amer. J. Physiol. 214: 28-32, 1968.

(8) Awapara, J. and Seale, B. Distribution of transaminases in rat organs. J. Biol. Chem. 194: 497-502, 1952.

(9) Babson, A.L. and Phillips, G.E. An improved colorimetric transaminase assay. Clin. Chem. 11: 533-534, 1965.

(10) Babson, A.L. and Shapiro, P.O. A note on the colorimetric assay for transaminase in serum. Clin. Chim. Acta 8: 326-327, 1963.

(11) Babson, A.L., Shapiro, P.O., Williams, P.A.R. and Phillips, G.E. The use of a diazonium salt for the determination of glutamic-oxalacetic transaminase in serum. Clin. Chim. Acta 7: 199-205, 1962.

(12) Banks, B.E.C., Doonan, S., Lawrence, A.J. and Vernon, C.A. Molecular weight and other properties of aspartate aminotransferase from pig heart muscle. Europ. J. Biochem. 5: 528-529, 1968.

(13) Barbieri, E. (1957). Cited from: Critz, J.B. and Withrow, T.J. Adrenocortical blockade and the transaminase response to exercise. Steroids 5: 719-728, 1965.

(14) Bartlett, R.G. Jr. Stress adaptation and inhibition of restraint-induced (emotional) hypothermia. J. Appl. Physiol. 8: 661-663, 1956.

(15) Batsakis, J.G. and Briere, R.O. Enzymatic profile of myocardial infarct. Amer. Heart J. 72: 274-279, 1966.

(16) Batsakis, J.G. and Briere, R.O. Interpretive enzymology. Springfield: Charles C. Thomas, 1967.

(17) Baumann, P., Escher, J. and Richterich, R. The behavior of serum enzymes during exercise. Schweiz. Z. Sportmed. 10: 33-51, 1962. Cited from: Chem. Abst. 62: 2084d, 1965.

(18) Beaton, J.R. Liver and plasma malic dehydrogenase activities in the exercised rat. Proc. Soc. Exp. Biol. Med. 123: 598-600, 1966.

(19) Beaton, J.R. and Feleki, V. The effect of nutritional state on ability of the rat to swim to exhaustion. Canad. J. Physiol. Pharmacol. 44: 597-603, 1966.

(20) Beaton, J.R. and Feleki, V. Effect of diet and water temperature on exhaustion time of swimming rats. Canad. J. Physiol. Pharmacol. 45: 360-363, 1967.

(21) Beaton, J.R. and Oyster, B. A note on the combined effects of exercise and food restriction on plasma enzyme activities in the rat. Canad. J. Physiol. Pharmacol. 47: 396-398, 1969.

(22) Bedrak, E. Blood serum enzyme activity of dogs exposed to heat stress and muscular exercise. J. Appl. Physiol. 20: 587-590, 1965.

(23) Bedrak, E., Hammer, R. and Goldberg, S. Plasma enzymes in relation to aldosterone administration and heat acclimatization in rats. J. Appl. Physiol. 22: 297-300, 1967.

(24) Bingol, G. Determination of molecular weights of serum glutamic-oxalacetic transaminase and serum glutamic-pyruvic transaminase by filtration with a Sephadex G-200 dextran gel column. Saglik Dergisi 42: 67-94, 1968. Cited from: Chem. Abst. 69: 103255r, 1968.

(25) Blanchaer, M.C. and van Wijhe, M. Isozymes of lactic dehydrogenase in skeletal muscle. Amer. J. Physiol. 202: 827-829, 1962.

(26) Bloor, C.M., Leon, A.S. and Pasyk, S. The effects of exercise on organ and cellular development in rats. Lab. Invest. 19: 675-681, 1968.

(27) Bloor, C.M. and Papadopoulos, N.M. Plasma lactic dehydrogenase activity and myocardial cellular changes after cessation of training. J.Appl. Physiol. 26: 371-374, 1969.

(28) Bourne, G.H. Division of labor in cells. 1st ed. New York: Academic Press, 1964.

(29) Briggs, F. and Munson, P. Studies on the mechanism of stimulation of ACTH secretion with the aid of morphine as a blocking agent. Endocrinology 57: 205-219, 1955.

(30) Cardinet, G.A., Fowler, M.E. and Tyler, W.S. The effects of training, exercise and tying-up on serum transaminase activities in horses. Amer. J. Vet. Res. 24: 980-984, 1963.

(31) Cohen, P. Transamination with purified enzyme preparations (transaminase), J. Biol. Chem. 136: 565-601, 1940.

(32) Comunale, A. Influence of adenosine triphosphate on transamination in cerebral tissue after thyroxine treatment. Gazz. Int. Med. Chir. 72: 434-438, 1967. Cited from: Chem. Abst. 67: 29572g, 1967.

(33) Connell, A.M., Cooper, J. and Redfearn, J.W. The contrasting effect of emotional tension and physical exercise on the excretion of 17-ketogenic steroids and 17-ketosteroids. Acta Endocr. 27: 179-194, 1958.

(34) Cope, F.W. and Polis, B.D. Increased plasma glutamic-oxalacetic transaminase activity in monkeys due to nonspecific stress effect. Aerospace Med. 30: 90-96, 1959.

(35) Cornelius, C.E., Burnham, L.G. and Hill, H.E. Serum transaminase activities of thoroughbred horses in training. J. Amer. Vet. Med. Ass. 142: 639-642, 1963.

(36) Cornil, A., De Coster, A., Copinschi, G. and Franckson, J.R.M. Effect of muscular exercise of the plasma level of cortisol in man. Acta Endocr. 48: 163-168, 1965.

(37) Critz, J.B. Myocardial transaminase response to elevated blood pressure. Steroids 1: 445-449, 1963.

(38) Critz, J.B. Glutamic-oxalacetic transaminase: origin and fate. Minn. Med. 48: 1309-1313, 1965.

(39) Critz, J.B. Effect of swimming exercise on serum glutamic-oxalacetic transaminase and hematocrit of rats. Proc. Soc. Exp. Biol. Med. 121: 101-104, 1966.

(40) Critz, J.B. Heart disease, exercise and serum glutamic-oxalacetic transaminase. S. Dakota J. Med. 20: 27-30, 1967.

(41) Critz, J.B. Transaminase in liver disease. Med. Times 96: 268-273, 1968.

(42) Critz, J.B. and Merrick, A.W. Serum glutamic-oxalacetic transaminase levels after exercise in men. Proc. Soc. Exp. Biol. Med. 109: 608-610, 1962.

(43) Critz, J.B. and Merrick, A.W. Transaminase changes in rats after exercise. Proc. Soc. Exp. Biol. Med. 115: 11-14, 1964.

(44) Critz, J.B. and Withrow, T.J. Myocardial transaminase following coarctation of the abdominal aorta. Proc. Soc. Exp. Biol. Med. 116: 38-40, 1964.

(45) Critz, J.B. and Withrow, T.J. Adrenocortical blockade and the transaminase response to exercise. Steroids 5: 719-728,1965.

(46) Dawson, C.A., Nadel, E.R. and Horvath, S.M. Cardiac output in the cold-stressed swimming rat. Amer. J. Physiol. 214: 320-325, 1968.

(47) Dawson, D.M. Efflux of enzymes from chicken muscle. Biochim. Biophys. Acta 113: 144-157, 1966.

(48) Demos, G.T., Hale, H.B. and Williams, E.W. Anticipatory stress and flight stress in F-102 pilots. Aerospace Med. 40: 385-388, 1969.

(49) Dixon, M. and Webb, E.C. Enzymes. 2nd ed. New York: Academic Press, 1964.

(50) Dubowitz, V. and Pearse, A.G.E. Enzymic activity of normal and dystrophic human muscle: a histochemical study. J. Path. Bact. 81: 365-378, 1961.

(51) Eckstein, R.W. Effect of exercise and coronary artery narrowing on coronary collateral circulation. Circ. Res. 5: 230-235, 1957.

(52) Edgerton, V.R., Gerchman, L. and R. Carrow. Histochemical changes in rat skeletal muscle after exercise. Exp. Neurol. 24: 110-123, 1969.

(53) Eischeid, A. and Kochakian, C. Effect of cortisone acetate on aspartic-glutamic transaminase activity of mouse tissue. Proc. Soc. Exp. Biol. Med. 85: 339-341, 1954.

(54) Emery, A.E. and Pascasio, F.M. The effects of pregnancy on the concentration of creatine kinase in serum, skeletal muscle and myometrium. Amer. J. Obstet. Gynecol. 91: 18-22, 1965.

(55) Esipova, N.G., Dembo, A.T., Tumanyan, V.G. and Polyanovskii, O.L. Molecular morphology of aspartate transaminase. Molec. Biol. 2: 527-535, 1968. Cited from: Chem. Abst. 69: 74088f, 1968.

(56) Farrelly, J.G. and Churchich, J.E. Reconstituted aspartate amino-transferase, physical studies. Biochim. Biophys. Acta 167: 28-90, 1968.

(57) Ferguson, G.A. Statistical analysis in psychology and education. New York: McGraw-Hill, Chap. 10: 131-156, 1959.

(58) Fowler, W.M. Jr., Chowdhury, S.R., Pearson, C.M., Gardner, G. and Bratton, R. Changes in serum enzyme levels after exercise in trained and untrained subjects. J. Appl. Physiol. 17: 943-946, 1962.

(59) Fowler, W.M., Gardner, G.W., Kazerunian, H.H., and Lauvstad, W.A. The effect of exercise on serum enzymes. Arch. Phys. Med. 49: 554-565, 1968.

(60) Frenkle, R. and Csalay, L. Effect of regular muscular activity on adrenalcortical function in rats. J. Sports Med. 2: 207-211, 1962.

(61) Friedman, S., Ader, R., Grota, J. and Larson, T. Plasma corticosterone response to parameters of electric shock stimulation in the rat. Psychosom. Med. 29: 323-328, 1967.

(62) Garbus, J., Highman, B. and Altland, P.D. Serum enzymes and LDH isoenzymes after exercise and training in rats. Amer. J. Physiol. 207: 467-472, 1964.

(63) Garbus, J., Highman, B. and Altland, P.D. Alterations in serum enzymes and isoenzymes in various species induced by epinephrine. Comp. Biochem. Physiol. 22: 507-516, 1967.

(64) Garcia-Bunuel, L., Garcia-Bunuel, V.M., Green, L. and Subin, D.K. Lactate dehydrogenase forms in denervation and disuse atrophy of red and white muscle. Neurology 16: 491-495, 1966.

(65) Gardner, G.W., Bratton, R., Chowdhury, S.R., Fowler, W.M. and Pearson, C.M. Effect of exercise on serum enzyme levels in trained subjects. J. Sport. Med. 4: 103-110, 1964.

(66) Giese, A.C. Cell physiology. 3rd ed. Philadelphia: W. B. Saunders, Co., 1968.

(67) Gollnick, P.D. and Hearn, G.R. Lactic dehydrogenase activities of heart and skeletal muscle of exercised rats. Amer. J. Physiol. 201: 694-696, 1961.

(68) Gollnick, P.D., Struck, P.J. and Bogyo, T.P. Lactic dehydrogenase activities of rat heart and skeletal muscle after exercise and training. J. Appl. Physiol. 22: 623-627, 1967.

(69) Gollnick, P.D., Struck, P.J. Soule, R.G. and Heinrick, J.R. Effect of exercise and training on the blood of normal and splenectomized rats. Int. Z. Angew. Physiol. 211: 169-178, 1965.

(70) Gordis, L. and Nitowsky, H.M. Lysosomes in human cell cultures. Kinetics of enzyme release from injured particles. Exp. Cell. Res. 38: 556-569, 1965.

(71) Gould, M.K. and Rawlinson, W.A. Biochemical adaptation as a response to exercise. I. Effect of swimming on the levels of lactic dehydrogenase, malic dehydrogenase and phosphorylase in muscles of 8, 11 and 15 week old rats. Biochem. J. 73: 41-44, 1959.

(72) Graig, F.A. and Alter, S. Effect of ACTH and dibutyryl cyclic AMP (DCAMP) on adrenal creatine phosphokinase (CPK). Endocrine Society Meeting Program: June 27-29, 1969.

(73) Graig, F.A., Smith, J.C. and Foldes, F.F. Effect of dilution on the activity of SCPK. Clin. Chim. Acta 15: 107-111, 1967.

(74) Green, D.E., Murer, E., Hultin, H.O., Richardson, S.H., Salmon, B., Brierley, G.P. and Baum, H. Association of integrated metabolic pathways with membranes. I. Glycolytic enzymes of the red blood corpuscle and yeast. Arch. Biochem. Biophys. 112: 635-647, 1965.

(75) Green, S.E. Personal communication, 1967.

(76) Griffin, M.J. and Cox, R.P. The mechanism of hormonal induction of alkaline phosphatase in human cell cultures. I. Effect of puromycin and actinomycin D. J. Cell. Biol. 29: 1-9, 1966.

(77) Griffiths, P.D. Serum levels of creatine phosphokinase. J. Clin. Path. 17: 56-57, 1964.

(78) Griffiths, P.D. Serum levels of ATP: creatine phosphotransferase (creatine kinase). The normal range and effects of muscular activity. Clin. Chim. Acta 13: 413-420, 1966.

(79) Griffiths, P.D. and Lehmann, H. Estimation of creatine phosphokinase as an additional method for identification of seminal strains. Med. Sci. Law 4: 32-34, 1964.

(80) Guglielmetti, P., Tominz, L. and Andreuzgi, P. Pathology of electrocution. Behaviour of creatine phosphokinase activity in the experimental electrocution. First results. Boll. Soc. Ital. Biol. Sper. 41: 1491-1494, 1965. Cited from: Chem. Abst. 65: 4417f, 1966.

(81) Hakkila, J. Studies on the myocardial capillary concentration in cardiac hypertrophy due to training. Ann. Med. Exp. Biol. Fenn. 33: 1-82, 1955.

(82) Halonen, P.I. and Konttinen, A. Effect of physical exercise on some enzymes in the serum. Nature 193: 942-944, 1962.

(83) Hawrylewiez, E.J. and Blair, W.H. Biochemical measure of impact stress in chimpanzees. Aerospace Med. 36: 369-371, 1965.

(84) Hearn, G.R. and Gollnick, P.D. Effect of exercise on the adenosinetriphosphatase activity in skeletal and heart muscle in rats. Int. Z. Angew. Physiol. 19: 23-26, 1961.

(85) Hearn, G.R. and Wainio, W.W. Aldolase activity of the heart and skeletal muscle of exercised rats. Amer. J. Physiol. 190: 206-208, 1957.

(86) Henley, K.S., Schmidt, E. and Schmidt, F.W. Serum enzymes. J. Amer. Med. Assoc. 174: 977-981, 1960.

(87) Hess, B. Enzymes in blood plasma. New York: Academic Press, 1963.

(88) Hess, J.W., MacDonald, R.P., Frederick, R.J., Jones, R.N., Neely, J. and Gross, D. Serum creatine phosphokinase (CPK) activity in disorders of heart and skeletal muscle. Ann. Intern. Med. 61: 1015-1028, 1964.

(89) Hess, J.W., MacDonald, R.P., Matho, G.J.W. and Murdock, K.J. Serum creatine phosphokinase: evaluation of a commercial spectrophotometric method. Clin. Chem. 13: 994-1005, 1967.

(90) Highman, B. and Altland, P.D. Serum enzyme rise after hypoxia and effect of autonomic blockade. Amer. J. Physiol. 199: 981-986, 1960.

(91) Highman, B. and Altland, P.D. Effects of exercise and training on serum enzyme and tissue changes in rats. Amer. J. Physiol. 205: 162-166, 1963.

(92) Highman, B. and Altland, P. Effect of dimethyl sulfoxide, exercise and training on rat serum enzymes and tissues. Amer. J. Physiol. 213: 770-782, 1967.

(93) Highman, B., Altland, P.D. and Garbus, J. Pathological and serum-enzyme changes after epinephrine in oil and adrenergic blocking agent. Arch. Path. 80: 332-344, 1965.

(94) Highman, B., Maling, H.M. and Thompson, E.C. Serum transaminase and alkaline phosphatase levels after large doses of norepinephrine and epinephrine in dogs. Amer. J. Physiol. 196: 436-440, 1959.

(95) Hill, S.R., Goetz, F.C., Fox, H.M., Murawski, B.J. Krakauer, L.J., Reifenstein, R.W., Gray, S.J., Reddy, W.J., Hedberg, S.E., St. Marc, J.R. and Thorn, G.W. Studies on adrenocortical and psychological response to stress in man. Arch Intern. Med. 97: 269-298, 1956.

(96) Holloszy, J.O. Biochemical adaptations in muscle: effect of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. J. Biol. Chem. 242: 2278-2282, 1967.

(97) Hughes, B.P. Serum enzyme changes in muscle disease and their relation to tissue changes. Proc. Roy. Soc. Med. 56: 178-182, 1963.

(98) Huzino, A., Kimura, H., Aburaya, T. and Katunuma, N. Leakage of aspartate transaminase from dog heart muscle after experimental myocardial infarction. J. Biochem. 54: 452-454, 1963.

(99) Il'in, V.S. and Polikarpova, L.I. The effect of hydrocortisone and insulin on the alanine-α-ketoglutarate and aspartate-α-ketoglutarate transaminase in rat liver. Vop. Med. Khim. 13: 278-282, 1967. Cited from: Chem. Abst. 67: 29612v, 1967.

(100) International union of biochemistry: enzyme nomenclature, Amsterdam: Elsevier Publish., 1965.

(101) Izumi, K. Changes of glutamate transaminase isozyme in experimental myocardial infarction. Jap. Circ. J. 31: 501-503, 1967.

(102) Jirka, Z. and Novosadova, J. Changes in glutamic-oxalacetic transaminase levels after physical work of a sporting manner. Acta Univ. Palocki. Olomuc. Fac. Med. 1966: 241-247, 1966. Cited from: Chem. Abst. 67: 19422, 1967.

(103) Kar, N.C. and Pearson, C.M. Glutamic-oxalacetic transaminase isoenzymes in human myopathies. Proc. Soc. Exp. Biol. Med. 116: 733-735, 1964.

(104) Karlsson, J., Diamant, B. and Saltin, B. Lactate dehydrogenase activity in muscle after prolonged severe exercise in man. J. Appl. Physiol. 25: 88-91, 1968.

(105) Kendrick-Jones, J. and Perry, S.V. Enzymic adaptation to contractile muscle activity in skeletal muscle. Nature 208: 1068-1070, 1965.

(106) King, J. Practical Clinical Enzymology. London: D.Van Nostrand, 1965.

(107) Kovac, J. and Machycek, V. Activity of glutamic-oxalacetic transaminase in the blood serum of healthy sheep. Folia Vet. 10: 23-30, 1966. Cited from: Chem. Abst. 68: 20161k, 1968.

(108) Kreisle, J., Queen, D. and Bowman, B. Myoglobinuria following exhaustive muscular effort. Report of a case with determination of serum enzyme activities. Texas J. Med. 56: 421-425, 1960.

(109) Krestner, W., Paetzel, A. and Weinreich, J. Changes in creatine-phosphokinase activity in serum during physical exertion. Med. Klin. 61: 1858-1862, 1966. Cited from: Chem. Abst. 66: 17479y, 1967.

(110) Lang, C.M. Effects of psychic stress on atherosclerosis in the squirrel monkey (Saimiri sciureus). Proc. Soc. Exp. Biol. Med. 126: 30-34, 1967.

(111) Leats (1961). Cited from: Critz, J.B. Effect of swimming exercise on serum glutamic-oxalacetic transaminase and hematocrit of rats. Proc. Soc. Exp. Biol. Med. 121: 101-104, 1966.

(112) Lehmann, H. and Griffiths, P.D. Creatinephosphokinase activity in semen. Lancet 2: 498, 1963.

(113) Lehnert, V.G., Leiber, H. and Schaller, K.H. Plasmacortisol und Plasmacorticosteron im Anpassungsstadium der dosierten körperlichen Arbeit. Endokrinologie 52: 402-405, 1968.

(114) Lending, M., Slobody, L.B. and Mestern, J. Effects of convulsions on cerebrospinal fluid and plasma activity of glutamic-oxalacetic transaminase and lactic dehydrogenase. Neurology 9: 672-677, 1959.

(115) Leon, A.S. and Bloor, C.M. Effects of exercise and its cessation on the heart and its blood supply. J. Appl. Physiol. 24: 485-490, 1968.

(116) Van Liere, E.J., Krames, B.B. and Northup, D.W. Differences in cardiac hypertrophy in exercise and hypoxia. Circ. Res. 16: 244-247, 1965.

(117) Loegering, D.J. and Critz, J.B. The effect of noradrenaline infusions and adrenergic blocking agents on serum glutamic-oxalacetic transaminase in dogs. Canad. J. Physiol. Pharmacol. 46: 627-633, 1968.

(118) Loegering, D.J., Critz, J.B. and Wagner, J.A. Serum creatine phosphokinase as a diagnostic aid. Minn. Med. 50: 1751-1755, 1967.

(119) McArdle, W.D. Metabolic stress of endurance swimming in the laboratory rat. J. Appl. Physiol. 22: 50-54, 1967.

(120) Mager, M., Blatt, W.F. and Newman, R.W. Lactic dehydrogenase isozymes variations in the plasma of men exposed to cold. J. Appl. Physiol. 24: 616-618, 1968.

(121) Maksimova, L.V. Activity of ATP-creatine phosphotransferase in the muscles and blood after muscular effort. Ukr. Biokhim. Zh. 37: 131-136, 1965.

(122) Maksimova, L.V. The rise in creatine kinase activity in the blood during muscular effort. Ukr. Biokhim. Zh. 38: 425-429, 1966.

(123) Maling, H.M., Highman, B. and Thompson, E.C. Some similar effects after large doses of catecholamines and myocardial infarction in dogs. Amer. J. Cardiol. 5: 628-633, 1960.

(124) Mandil, M.J., Robinson, F.R. and Luce, E.A. SGOT levels in man and the monkey following physical and emotional exertion. Aerospace Med. 33: 1216-1223, 1962.

(125) Massarrat, S. Daily fluctuations of the enzyme activities of glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase and lactic dehydrogenase in the serums of patients with liver disease and healthy subjects. Klin. Wschr. 42: 91-94, 1964. Cited from: Chem. Abst. 60: 14973, 1964.

(126) Melnykovych, G. Glucocorticoid-induced resistance to deoxycholate lysis in He La cells. Science 152: 1086-1087, 1966.

(127) Menache, R. and Gaist, L. Colorimetric microdetermination of blood serum creatine kinase activity and its clinical importance. Boll. Soc. Ital. Biol. Sper. 43:39-41,1967. Cited from: Chem. Abst. 67: 71548d, 1967.

(128) Morehouse, L.E. and Miller, A.T. Physiology of exercise. Saint Louis: 5th ed. C.V. Mosby, 1967.

(129) Naeye (1966). Cited from: Bloor, C.M., Leon, A.S. and Pasyk, S. The effects of exercise on organ and cellular development in rats. Lab. Invest. 19: 675-681, 1968.

(130) Natelson, S. Microtechniques of clinical chemistry for the routine laboratory. Springfield: Charles C. Thomas, 1957.

(131) Nerdrum, H.J. and Berg, K.J. Changes of SGOT and SLDH on physical exertion. Scand. J. Clin. and Lab. Invest. 16: 624-629, 1964.

(132) Nerdrum, H.J. and Nordøy, S. Changes of serum glutamic-oxalacetic transaminase following exercise in patients with and without coronary disease. Scand. J. Clin. Lab. Invest. 16: 617-623, 1964.

(133) Nuttall, F.Q. and Jones, B. Creatine kinase and glutamic-oxalacetic transaminase activity in serum: kinetics of change with exercise and effect of physical conditioning. J. Lab. Clin. Med. 71: 847-854, 1968.

(134) Ogata (1960). Cited from: Garcia-Buñuel, L., Garcia-Buñuel, V.M., Green, L. and Subin, D.K. Lactate dehydrogenase forms in denervation and disuse atrophy of red and white muscle. Neurology 16: 491-495, 1966.

(135) Oliver, I.T. A spectrophotometric method for the determination of CPK and myokinase. Biochem. J. 61: 116-122, 1955.

(136) Oser, B.L. Hawk's physiological chemistry. 14th ed. New York: McGraw-Hill, 1965.

(137) Ottaway, J.H. Evidence for binding of cytoplasmic creatine kinase to structural elements in heart muscle. Nature 215: 521-522, 1967.

(138) Papadopoulos, N.M., Leon, A.S., Bloor, C.M. Effects of exercise on plasma lactate dehydrogenase and isoenzyme activities in trained and untrained rats. Proc. Soc. Exp. Biol. Med. 129: 232-234, 1968.

(139) Pattengale, P.K. and Holloszy, J.O. Augmentation of skeletal muscle myoglobin by a program of treadmill running. Am. J. Physiol. 213: 783-785, 1967.

(140) Pearce, J.M., Pennington, R.J. and Walton, J.N. Serum enzyme studies in muscle disease. Part I. Variations in serum creatine kinase activity in normal individuals. J. Neurol. Neurosurg. Psychiat. 27: 1-4, 1964.

(141) Pearce, J.M., Pennington, R.J. and Walton, J.N. Serum enzymes studies in muscle disease. Part II. Serum creatine kinase in muscular dystrophy and in other myopathies and neuropathic disorders. J. Neurol. Neurosurg. Psychiat. 27: 96-99, 1964.

(142) Pearl, W., Balazs, T. and Buyske, D. The effect of stress on serum transaminase activity in the rat. Life Sci. 5: 67-74, 1966.

(143) Penneys, R., Sayen, A., Montgomery, H. and Goldberg, B. Glutamic-oxalacetic transaminase, lactic dehydrogenase, and creatine changes with local cold injury of the rabbit limb. Life Sci. 4: 1685-1691, 1965.

(144) Peter, J.B., Jeffress, R.N. and Lamb, D.R. Exercise: effects on hexokinase activity in red and white skeletal muscle. Science 160: 200-201, 1968.

(145) Pfeiffer, C.J. The physiologic effects of restricted activity in the rat: stress effects of chronic restraint. Exp. Med. and Surg. 25: 201-217, 1967.

(146) Ponomareva, T. and Drel, K. (1964). Cited from: Critz, J.B. Heart disease, exercise and serum glutamic-oxalacetic transaminase. S. Dakota J. Med. 20: 27-30, 1967.

(147) Prokop, L. Adrenals and sport. J. Sports Med. 3: 115-121, 1966.

(148) Rawlinson, W.A. and Gould, M.K. Adenosinetriphosphatase and creatine phosphokinase activity in muscles of exercised rats. Biochem. J. 73: 44-48, 1959.

(149) Read, W.O. and Johnson, D.C. Creatine phosphokinase activity in heart and skeletal muscle of fetal rabbits. Proc. Soc. Exp. Biol. Med. 102: 740-741, 1959.

(150) Remmers, A.R.Jr. and Kaljot, V. Serum transaminase levels. Effect of strenuous and prolonged physical exercise on healthy young subjects. J.A.M.A. 185: 968-970, 1963.

(151) Rindi, G. and Perri, V. Effects of castration and testosterone treatment on the action of various transaminase in rat tissue. Arch. Sci. Biol. 39: 343-351. 1955.

(152) Rosalki, S.B. A capsule test for creatine phosphokinase. Proc. Assoc. Clin. Biochem. 4: 23-25, 1966.

(153) Rosalki, S.B. An improved procedure for serum creatine phosphokinase determination. J. Lab. and Clin. Med. 69: 696-705, 1967.

(154) Rosen, F., Roberts, N.R., Budnick, L.E. and Nichol, C.A. Corticosteroids on transaminase activity: the specificity of the GPT response. Endocr. 65: 256-264, 1959.

(155) Sangster, J.F. and Beaton, J.R. Alternations in enzyme activities as a consequence of exercise (swimming) in the rat. Proc. Soc. Exp. Biol. Med. 122: 542-544, 1966.

(156) Schlang, H.A. and Kirkpatrick, G.A. The effect of physical exercise on serum transaminase. Amer. J. Med. Sci. 242: 338-341, 1961.

(157) Schneider, K.W. and Heise, E.R. The diagnostic significance of an increased CPK in serum. German Med. Mthly. 8: 397-402, 1963.

(158) Schreiber, G. and Lesch, R. Behavior of GOT, GPT, LDH and SDH in serum on administration of prednisolone. Med. Klin. 60: 1123-1125, 1965.

(159) Sentenace-Roumanou, H., Joly, R., Cheftel, C. and Reynier, M. Influence of walking on glutamic-oxalacetic transaminase activity of rat serum. C. R. Soc. Biol. 160: 1276-1280, 1966.

(160) Shirley, H.H., Wolfram, C.G., Wasserman, K. and Mayerson, H.S. Capillary permeability to macromolecules: stretched pore phenomenon. Amer. J. Physiol. 190: 189-193, 1957.

(161) Short, F.A., Cobb, L.A., Kawabori, I. and Goodner, C.J. Influence of exercise training on red and white rat skeletal muscle. Amer. J. Physiol. 217: 327-331, 1969.

(162) Snedecor, G.W. and Cochran, W.G. Statistical methods. 6th ed. Ames, Iowa: Iowa State Univ. Press, 1967.

(163) Sokal, R.R. and Rohlf, F.J. Biometry: the principles and practice of statistics in biological research. San Francisco: W.H. Freeman and Co., 1969.

(164) Stevenson, J.A.F. Exercise, food intake, and health in experimental animals. Canad. Med. Ass. J. 96: 862-867, 1967.

(165) Stevenson, J.A.F., Box, B.M., Feleki, V. and Beaton, J.R. Bouts of exercise and food intake in the rat. J. Appl. Physiol. 21: 118-122, 1966.

(166) Stevenson, J.A.F., Feleki, V., Rechnitzer, P. and Beaton, J.R. Effect of exercise on coronary tree size in the rat. Circ. Res. 15: 265-269, 1964.

(167) Suzuki, T. Effect of physical exercise on adrenal 17-hydroxycorticosteroid secretion rate in the dog. In: Raab, W. ed. Prevention of ischemic heart disease. Springfield: Charles C. Thomas, Chap. 19: 169-171, 1966.

(168) Suzuki, T., Yamashita, K. and Mitamura, T. Muscular exercise and adrenal 17-hydroxycorticosteroid secretion in dogs. Nature 181: 715, 1958.

(169) Swaiman, K.F. and Awad, E.A. Creatine phosphokinase and other serum enzyme activity after controlled exercise. Neurology 14: 977-980, 1964.

(170) Swaiman, K.F. and Bradley, W.W. Maturational biochemical changes in rabbit bladder muscle. Alterations in the composition and enzyme activity of the urinary bladder smooth muscle of rabbits during ontogeny. Invest. Urol. 5: 115-118, 1967.

(171) Swaiman, K.F. and Bradley, W.E. Creatine phosphokinase in detection of visceral muscle injury. Proc. Soc. Exp. Biol. Med. 130: 612-614, 1969.

(172) Tan, E.M., Hanson, M.E. and Richter, C.P. Swimming time in rats with relation to water temperature. Fed. Proc. 13: 150-151, 1954.

(173) Tepperman, J. and Pearlman, D. Effects of exercise and anemia on coronary arteries of small animals as revealed by the corrosion-cast technique. Circ. Res. 9: 576-584, 1961.

(174) Thomas, B.M. and Miller, A.T. Jr. Adaptation to forced exercise in the rat. Amer. J. Physiol. 193: 350-354, 1958.

(175) Thomson, W.H.S. Determination and statistical analyses of the normal ranges for five serum enzymes. Clin. Chim. Acta 21: 469-478, 1968.

(176) Tomita, K. Studies on myocardial protein metabolism in cardiac hypertrophy. Jap. Heart. J. 7: 566-589, 1966.

(177) Varnauskas, E., Bergman, H., Honk, P. and Bjorntorp, P. Haemodynamic effects of physical training in coronary patients. Lancet 2: 8-12, 1966.

(178) De Vries, H.A. Physiology of exercise for physical education and athletics. Dubuque: Wm. C. Brown Company Publishers, 1966.

(179) Wagner, J.A. and Critz, J.B. The effect of prednisolone on the serum creatine phosphokinase response to exercise. Proc. Soc. Exp. Biol. Med. 128: 716-720, 1968.

(180) Wanklin, J. Personal communication, 1969.

(181) Wannemacher, R.W. Jr. and McCoy, J.R. Regulation of protein synthesis in the ventricular myocardium of hypertrophic hearts. Amer. J. Physiol. 216: 781-787, 1969.

(182) White, A., Handler, P. and Smith, E.L. Principles of biochemistry. 3rd ed. New York: McGraw-Hill, 1964.

(183) Wiesmann, U., Colombo, J.P., Adam, A. and Richterich, R. Determination of cysteine-activated creatine kinase in serum. Enzym. Biol. Clin. 7: 266-284, 1966. Cited from: Chem. Abst. 67: 40367q, 1967.

(184) Yakovlev, (1950). Cited from: Gould, M.K. and Rawlinson, W.A. Biochemical adaptation as a response to exercise. I. Effect of swimming on the levels of lactic dehydrogenase, malic dehydrogenase and phosphorylase in muscles of 8, 11, and 15 week old rats. Biochem. J. 73: 41-44, 1959.

(185) Zierler, K.L. Muscle membrane as a dynamic structure and its permeability to aldolase. Ann. N.Y. Acad. Sci. 75: 227-234, 1958.

(186) Zimmerman, H.J., Dujovne, C.A. and Levy, R. The correlation of serum levels of two transaminases with tissue levels in six vertebrate species. Comp. Biochem. and Physiol. 25: 1081-1089, 1968.

(187) Zuchlewski, A. and Gaebler, O. Changes in the activity of transaminases and L-glutamic acid dehydrogenases induced by growth hormone. Arch. Biochem. 66: 463-473, 1957.

APPENDICES

APPENDIX A

Durations of the Post-exercise Elevations of Creatine Phosphokinase and Glutamic-oxalacetic Transaminase in the Blood Plasma of the Rat

Enzyme	Post-exercise sampling time			
	5 min. (IU/l)	12 hrs. (IU/l)	24 hrs. (IU/l)	48 hrs. (IU/l)
Creatine phosphokinase	71.8 ± 8.8† (6)	47.7 ± 8.1 (7)	42.3 ± 3.2 (7)	40.7 ± 4.2 (6)
Glutamic-oxalecetic transaminase	118.3 ± 1.5 (7)	122.0 ± 4.5 (7)	96.6 ± 5.1 (7)	95.6 ± 7.5 (7)

†Mean ± S.E.M.
( ) Number of animals

APPENDIX B

The interference of myokinase in the CPK assay was tested by the following procedures. Optical density change(Δ O.D.) measured during a ten minute "no CPK" control reaction was subtracted from the Δ O.D. occurring when a sample reacted for ten minutes with the normal CPK reagents. The control reaction mixture contained all the constituents of the CPK assay except creatine phosphate. The control reaction mixture was prepared by dissolving an ATP Calsul (Cat. #869006-containing glucose, glucose-6-phosphate dehydrogenase, hexokinase, nicotinamide adenine dinucleotide phosphate, and magnesium chloride) in three milliliters of the following solution:

Cysteine HCl (Cat.#2430)	25 mg.
ADP (Cat.#117325)	27 mg.
AMP (Cat.#1181)	210 mg.
Water	30 ml.

Dissolve and adjust to pH 7

and finally adding 0.1 milliliter of the muscle supernate in its final dilution ratio. After a six minute incubation period at 30°C, the Δ O.D. occurring in this reaction mixture was measured over a ten minute period.

All reagents and suggestions were supplied by Calbiochem (75).

APPENDIX C

Unadjusted Levels of Myocardial Creatine Phosphokinase and Glutamic-oxalacetic Transaminase

Experimental series	Creatine phosphokinase		Glutamic-oxalacetic transaminase	
	resting (mU/ug N2)	post-exercise (mU/ug N2)	resting (mU/ug N2)	post-exercise (mU/ug N2)
Control	28.1 ± 1.8† (12)	25.7 ± 2.2 (16)	22.6 ± 0.5 (17)	22.4 ± 0.5 (18)
Spontaneous activity	25.9 ± 1.6 (10)	29.7 ± 2.0* (14)	19.1 ± 0.7 (10)	20.7 ± 0.9 (10)
Physical training:				
two weeks	31.0 ± 0.6 (13)	31.0 ± 1.6* (17)	23.1 ± 1.1 (13)	23.9 ± 0.8* (17)
four weeks	31.3 ± 1.8 (21)	34.3 ± 1.6* (21)	23.8 ± 1.0 (14)	24.7 ± 1.0* (16)
six weeks	39.3 ± 1.0* (16)	37.2 ± 0.7* (17)	28.8 ± 0.6* (18)	27.1 ± 0.7* (18)

†Mean ± S.E.M.
( ) Number of animals

*P <0.05, compared to control level; statistical significance obtained on covariance adjusted means

APPENDIX D

Unadjusted Levels of Soleus Muscle Creatine Phosphokinase and Glutamic-oxalacetic Transaminase

Experimental series	Creatine phosphokinase		Glutamic-oxalacetic transaminase	
	resting (mU/ug N_2)	post-exercise (mU/ug N_2)	resting (mU/ug N_2)	post-exercise (mU/ug N_2)
Control	63.7 ± 0.9† (8)	61.5 ± 1.9 (13)	11.9 ± 0.4 (17)	11.7 ± 0.5 (17)
Spontaneous activity	63.1 ± 3.6 (10)	60.2 ± 3.6 (14)	10.5 ± 0.4 (10)	10.9 ± 0.6 (10)
Physical training:				
two weeks	68.1 ± 2.0 (14)	70.5 ± 1.8 (13)	12.4 ± 0.9 (13)	13.2 ± 0.6* (16)
four weeks	71.0 ± 3.2 (21)	68.6 ± 3.2 (20)	13.1 ± 0.7 (14)	13.0 ± 0.7 (16)
six weeks	85.9 ± 3.7* (17)	80.2 ± 2.0* (18)	16.2 ± 0.7* (19)	15.6 ± 0.5* (18)

†Mean ± S.E.M.
( ) Number of animals

*P <0.05, compared to control level; statistical significance obtained on covariance adjusted means

APPENDIX E

Gastrocnemius Muscle Creatine Phosphokinase and Glutamic-oxalacetic Transaminase Levels

Experimental series	Creatine phosphokinase		Glutamic-oxalacetic transaminase	
	resting (mU/ug N_2)	post-exercise (mU/ug N_2)	resting (mU/ug N_2)	post-exercise (mU/ug N_2)
Control	132.1 ± 4.2† (9)	114.9 ± 6.3** (8)	4.07 ± 0.16 (9)	3.83 ± 0.36 (8)
Spontaneous activity	122.2 ± 9.5 (8)	116.8 ± 11.3 (7)	3.97 ± 0.28 (8)	4.86 ± 0.52 (7)
Physical training:				
two weeks	130.5 ± 4.4 (8)	144.1 ± 3.1*,** (8)	4.22 ± 0.35 (8)	4.84 ± 0.39 (8)
four weeks	133.7 ± 2.7 (8)	135.7 ± 5.6 (7)	4.85 ± 0.22 (8)	5.30 ± 0.20* (7)
six weeks	122.6 ± 4.4 (8)	125.5 ± 3.3 (7)	5.68 ± 0.46* (8)	5.42 ± 0.51* (7)

† Mean ± S.E.M.
( ) Number of animals

*P <0.05, compared to control level
**P <0.05, compared to resting level

APPENDIX F

Plasma Creatine Phosphokinase and Glutamic-oxalacetic Transaminase Levels

Experimental series	Creatine phosphokinase		Glutamic-oxalacetic transaminase	
	Resting (IU/1)	post-exercise (IU/1)	resting (IU/1)	post-exercise (IU/1)
Control	35.0 ± 2.4† (19)	70.7 ± 5.2** (24)	76.8 ± 1.9 (23)	106.5 ± 4.3** (24)
Spontaneous activity	42.1 ± 5.5 (15)	72.4 ± 5.4** (19)	81.5 ± 2.2 (14)	105.0 ± 5.0** (17)
Physical training:				
two weeks	40.2 ± 2.9 (20)	47.2 ± 2.9* (22)	85.8 ± 2.6* (17)	101.8 ± 3.4** (24)
four weeks	41.9 ± 2.9 (24)	53.4 ± 4.5*,** (23)	83.7 ± 1.9 (14)	100.0 ± 3.1** (22)
six weeks	39.4 ± 2.5 (18)	66.2 ± 4.5** (16)	95.0 ± 2.9* (19)	116.8 ± 5.0** (18)

†Mean ± S.E.M.
( ) Number of animals

*P <0.05, compared to control level
**P <0.05, compared to resting level

APPENDIX G

Total Protein Nitrogen Content of Cardiac, Soleus and Gastrocnemius Muscles in the Rat

Experimental series	Cardiac		Soleus		Gastrocnemius	
	resting	post-exercise	resting	post-exercise	resting	post-exercise
Restricted activity	12.8 ± 0.5†'** (9)	12.8 ± 0.4 (9)	11.4 ± 0.2 (11)	11.5 ± 0.3 (9)	14.2 ± 0.3** (8)	12.4 ± 0.5* (8)
Control	11.2 ± 0.4 (12)	11.9 ± 0.2 (16)	11.1 ± 0.2 (8)	11.1 ± 0.2 (13)	13.0 ± 0.3 (9)	13.3 ± 0.2 (8)
Spontaneous activity	12.6 ± 0.2** (10)	12.3 ± 0.1 (14)	10.9 ± 0.4 (10)	11.2 ± 0.2 (14)	13.5 ± 0.2 (8)	12.6 ± 0.3* (7)
Physical training:						
two weeks	12.0 ± 0.3 (13)	12.0 ± 0.2 (17)	11.4 ± 0.3 (14)	11.6 ± 0.2 (13)	13.9 ± 0.1** (8)	13.6 ± 0.3 (8)
four weeks	12.4 ± 0.3** (21)	12.1 ± 0.1 (21)	11.5 ± 0.3 (21)	11.5 ± 0.1 (20)	13.6 ± 0.2 (8)	14.3 ± 0.3 (7)
six weeks	13.0 ± 0.2** (16)	12.8 ± 0.2** (17)	11.8 ± 0.2 (17)	11.7 ± 0.4 (18)	15.2 ± 0.2** (8)	15.0 ± 0.4** (7)

†Nitrogen content expressed as ug/mg of wet muscle weight ± S.E.M.
( ) Number of animals

*P <0.05, compared to resting level
**P <0.05, compared to control level