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Effects of age, training and exercise on plasma lactate dehydrogenase activity in male rats

by

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INTRODUCTION

Variations in plasma enzyme activities have been noted in a number of physiological situations. La Due et al. (1954) first demonstrated elevations in serum glutamic oxalacetic transaminase (GOT) activity following myocardial infarction. Since that time determinations of plasma or serum enzyme activities have been instrumental in the diagnosis of many cardiovascular, hepatic and muscular diseases.

The influence of physical activity on alterations of different enzyme systems has been extensively reviewed (Schmidt and Schmidt, 1969). Significant modifications in plasma or serum enzyme activities as a result of exercise have been reported. However, there has been a considerable amount of variability and contradiction in the published plasma enzyme responses to exercise. Some of the factors responsible for this variability have been demonstrated to be age, sex, diet, environmental temperature, physical training, altitude, hormones and pharmacological agents (Siest and Galteau, 1974). It is also difficult to compare results obtained with different species of animals or different types of exercise.

Significant changes in mammalian enzyme systems have been reported to occur as a result of increasing age (Wilson, 1973). Few investigations have been made to determine the combined effects of age and exercise on plasma enzyme activities. Porter et al. (1971) have described the plasma lactate dehydrogenase (LDH) activities in rats from two age groups following a single bout of swimming exercise. The combined effects of age

and physical training on plasma enzyme responses to exercise has not been thoroughly investigated.

In humans, exercise has resulted in increased plasma enzyme activities, while physical training has reduced the elevation of plasma enzyme activities due to exercise (Cantone and Cerretelli, 1960; Fowler et al., 1962; Hunter and Critz, 1971; and Wolfson et al., 1972). Studies with experimental animals have usually resulted in similar findings (Sangster and Beaton, 1966; Novosadova, 1969; Highman and Altland, 1963; and Papadopoulos et al., 1968). Suggestions have been made to use the determination of certain plasma enzyme activities following exercise as a criterion of physical fitness (Beaton, 1966; and Hunter and Critz, 1971).

This study was designed to investigate the combined effects of age and physical training on the plasma LDH activities of male rats. Plasma LDH activity was also measured following a bout of strenuous physical exercise to determine how rats of different ages and different levels of physical fitness responded to this exercise. Total LDH activity in the plasma as well as the relative percentages of the individual LDH isoenzymes of LDH were determined.

REVIEW OF LITERATURE

Physical exercise has produced alterations in many enzyme systems in both human and animal studies (Schmidt and Schmidt, 1969; and Siest and Galteau, 1974). Several investigators have attempted to characterize the changes in plasma or serum enzyme activities which occur following exercise. Some of the enzyme activities which have been elevated in plasma or serum as a result of exercise include acid phosphatase (Ac. Ph.), alkaline phosphatase (Alk. Ph.), creatine phosphokinase (CPK), aldolase (ALD), glutamate-pyruvate transaminase (GPT), glutamate-oxalacetate transaminase (GOT), lactate dehydrogenase (LDH) and malate dehydrogenase (MDH).

Normally these are intracellular enzymes and are present in plasma in relatively small quantities. Factors which have been implicated in enzyme release from intracellular locations during or after exercise are anoxia or hypoxia (Zierler, 1958; de Leiris et al., 1969; and Butterworth et al., 1970), catecholamines (Raven et al., 1970; and Garbus et al., 1964) and cell damage (Altland and Highman, 1961). It is generally thought that the above factors alter membrane permeability and allow intracellular enzymes to enter the plasma.

Since this study is concerned primarily with the changes which occur in plasma lactate dehydrogenase (LDH) activities following exercise and training, the REVIEW OF LITERATURE will focus on previous investigations concerned with that enzyme.

Lactate Dehydrogenase

Lactate dehydrogenase (LDH) is an intracellular enzyme which catalyzes a reversible biochemical reaction in which pyruvate is reduced to lactate or lactate is oxidized to pyruvate. Nicotinamide adenine dinucleotide (NAD) is a coenzyme in this reaction and acts as a hydrogen acceptor or donor. In cells that are periodically subjected to low oxygen tensions, LDH functions to convert pyruvate to lactate regenerating the oxidized form of NAD which can be utilized in the glycolytic pathway for continued energy production. Lactate formed under such circumstances can be transported to primarily aerobic tissues and oxidized to pyruvate by LDH to be used for energy production in the citric acid cycle (Bhagavan, 1974).

Relatively little LDH activity is normally found in plasma or serum. Appearance of plasma LDH activity has been explained by normal cellular turnover, and erythrocytes, platelets and leukocytes have been implicated as major sources (Hess, 1963 and Mattenheimer, 1971).

LDH activity was first measured in human plasma and serum by Wroblewski and La Due (1955). They established normal values for human serum and also determined serum LDH activities for a number of experimental animals.

Certain pathological and physiological states have been known to elevate the plasma activities of LDH and many other enzymes. Certain diseases of the heart, liver and skeletal muscle can cause release of LDH into the plasma. Physical exercise has also been found to elevate plasma LDH activities.

Isoenzymes of lactate dehydrogenase

The indications that serum LDH activity is present in compounds of different molecular forms was first reported by Vesell and Bearn (1957). By subjecting serum samples from normal subjects and patients with myocardial infarction or leukemia to electrophesis on starch blocks, they were able to demonstrate three peaks of LDH activity in the various protein fractions of human serum. They also determined that significant changes in the relative percentage of each peak of LDH activity occurred in serum samples from patients with myocardial infarction or leukemia. Vesell and Bearn concluded that measuring the LDH activity of electrophoretically separated protein fractions yielded considerably more diagnostic information than the measurement of total serum LDH activity. They also suggested that the three peaks of LDH activity may have originated from different sites.

Within the same year another instance of separation of three distinct LDH activity peaks in human serum was reported (Sayre and Hill, 1957). They postulated that LDH consisted of a single molecular species which could be observed as a number of components in serum due to different binding states resulting from sulfhydryl or other protein interactions. Since that time a large amount of effort has been devoted to characterizing LDH isoenzyme patterns in normal and diseased tissues and this has proven to be a powerful diagnostic tool in the clinical laboratory.

It is now generally accepted that normal human serum or plasma has five components of LDH activity (Wroblewski et al., 1960; Plagemann et al., 1960; and Van Der Helm et al., 1962). These components have been named

isoenzymes by Markert (1959). Each isoenzyme is made up of four subunits and each subunit can be either of the "M" type or "H" type (Markert and Appella, 1961; and Wilson et al., 1963). The M (or A) subunit is more prevalent in skeletal muscle and the H (or B) subunit is more prevalent in cardiac muscle.

Laufer (1961) was the first to suggest that the multiple forms of LDH might function in metabolism in different ways. He noted that different forms of LDH could be found in the blood of a silk worm during various developmental stages. He suggested that certain forms of LDH function more efficiently during anaerobic conditions while other forms are more effective in aerobic conditions.

Kaplan and Goodfriend (1964), Cahn et al.(1962) and Dawson et al. (1964) reported that the properties of M subunits were such that they functioned more efficiently to reduce pyruvate under anaerobic conditions while the properties of H subunits favored lactate oxidation in aerobic conditions.

LDH isoenzyme patterns of many human organs have been determined by a number of researchers (Hill and Meacham, 1961; Vesell, 1961; Wroblewski and Gregory, 1961; and Wieme and Maercke, 1961). Weiland and Pfleiderer (1961) have determined LDH isoenzyme distributions in a number of rat organs. LDH-1 and LDH-2 predominant in heart muscle while LDH-5 is the major isoenzyme in skeletal muscle and liver of most mammals. Pathological changes in these organs have resulted in plasma isoenzyme patterns which reflect the pattern of the affected tissue.

Exercise and Plasma Enzymes

Determination of enzyme activities in serum or plasma before and after physical exercise has produced a variety of results. Enzyme activities have been found to increase following exercise in most studies but enzyme activities have also been found to decrease or remain unchanged. Some of the variable factors responsible for the conflicting results are the intensity and duration of the test exercise, the physical fitness of the subjects or animals and the type of exercise performed (Cunningham and Critz, 1972).

Human studies

The use of treadmill running as a test exercise by Fowler et al. (1962) resulted in increases in serum LDH only when the incline of the treadmill was set at 11 degrees. Other serum enzyme activities increased significantly at less strenuous levels of exercise. The increase in LDH activity following exercise was considerably less in trained individuals than in those leading sedentary lives.

McAllister and Weidner (1975) used treadmill exercise testing on cardiac patients. The treadmill speed and incline were set to provide each individual with both submaximal and maximal levels of exercise. However, in serum sampled before and after the exercise there were no significant changes in LDH activity or a number of other enzyme activities. Cunningham and Critz (1972) also used treadmill exercise in a human study. The treadmill was set at a grade which elicited 95 percent of the maximum heart rate. The subjects also were required to breathe various levels of

oxygen. Increases in serum LDH activities were found after all exercises but varying levels of oxygen had no significant effect on these changes.

A number of investigations have revealed increases in serum LDH activity in well-trained subjects following long distance running (Fowler et al., 1968; Riley et al., 1975; and Rose, Bousser and Cooper, 1970). Running exercise of considerably less distance produced no increase in serum LDH activities in well-conditioned males (Rose et al., 1970; and Sanders and Bloor, 1975).

Halonen and Konttinen (1962) found that a strenuous 16 kilometer march by soldiers undergoing basic military training and accustomed to such exercise resulted in significant increases in serum LDH activities. A stepping exercise lasting four hours produced a significant increase in LDH activity in gold miners only when they were subjected to an environmental temperature of 32.2° C. Exercise at normal room temperature did not produce significant increases in LDH activity (Wyndham et al., 1974).

Bicycle ergometer exercise has produced increased LDH activity in untrained subjects over 40 years of age (Block et al., 1969; and Wolfson et al., 1972). Following a training period of six to twelve weeks the same test exercise produced no increase in serum LDH activity. In younger subjects bicycle ergometer exercise produced an increase in serum LDH (Schwartz et al., 1971). A heavier work load resulted in a greater increase of serum LDH activity.

Ahlborg and Brohult (1967) found a significant increase in serum LDH activities in well-trained men and this elevation remained for one day following exercise on a bicycle ergometer. Training on a bicycle ergometer

for ten weeks caused an increase in the resting levels of plasma LDH activity and did not decrease the amount of elevation of plasma LDH activity following a submaximal exercise (Hunter and Critz, 1971). Recent evidence given by Fojt et al., (1976) has indicated that the increase in serum LDH activity due to bicycle ergometer exercise may be a result of enzyme release from the liver. By inserting a catheter into a hepatic vein of healthy volunteers they were able to measure enzyme activities in hepatic venous blood before, during and immediately following exercise. In a majority of subjects LDH activity in blood from hepatic veins was greater than that of arterial blood during exercise.

Serum creatine phosphokinase (CPK) activity has also been measured in a number of exercise studies. Because CPK is found primarily in muscle tissues it has been used as an index of muscle changes or damage resulting from exercise. Nuttall and Jones (1968) used a weight lifting exercise test to induce changes in serum CPK activity. After only six minutes of repetitive weight lifting (15 percent of body weight) marked increases of serum CPK activity occurred. CPK activity continued to increase in serum until a peak elevation was reached at 10 to 16 hours after exercise which corresponded to the period characterized by muscle stiffness and soreness. Similarly serum CPK activity has been found to reach a maximum value at 18 to 49 hours after submaximal bicycle ergometer exercise (Forssell et al., 1975).

A comparison of inpatients and outpatients has revealed that serum CPK activities of patients confined to bed rest are only half as great as

serum CPK activities of normally active subjects (Griffiths, 1966). This investigator also found that a 53 mile walk by healthy students increased serum CPK activity 24 times.

Submaximal exercise has been found to cause greater increases in serum CPK activities in patients with abnormal electrocardiogram records than in those with normal ECG's (Ledwich, 1973). This suggests that cardiac muscle can also be a source of serum CPK activity following exercise.

Shapiro et al. (1973) have found a striking relationship between a subject's maximum oxygen uptake and his serum CPK activities following a prolonged march. Subjects with low maximum aerobic capacities exhibited the greatest increase in serum CPK activities. In another study this same relationship was noted following a marathon race (Magazanik et al., 1974).

In contrast to the above investigations Galteau, Siest and Poortmans (1976) found no increase in CPK activities in venous blood monitored continually during a submaximal exercise. However, the subjects used were all physically trained athletes. In a number of studies physical training has been found to reduce the increase in CPK activity in serum following exercise (Hunter and Critz, 1971; and Nuttall and Jones, 1968).

Animal studies

Published values of rat serum LDH activities have exhibited large discrepancies and variations. This has resulted primarily from the blood sampling techniques used in early investigations. Blood samples were previously obtained by cardiac puncture and allowed to clot for varying periods of time before the serum was decanted and analyzed for LDH activity.

Papadopoulos et al. (1967) found that the clotting process and cardiac puncture were both responsible for increased LDH activity in rat blood samples. Use of plasma obtained from the vena cava was suggested to overcome the variability in LDH activity in blood.

In most studies with experimental animals, exercise has resulted in an increase in plasma enzyme activities and physical training has reduced the plasma enzyme response to exercise.

Effects of exercise on rat serum enzymes were first demonstrated by Altland and Highman (1961). After 16 hours of strenuous walking exercise, rats exhibited a marked loss in body weight and loss in liver, heart and skeletal muscle glycogen. Concurrent with these changes were marked increases in a number of serum enzyme activities. Serum LDH activities returned to normal within 24 hours after exercise but serum activities of GOT and aldolase remained elevated for up to 144 hours. Histological changes were noted in the liver, kidney, heart and skeletal muscle and 25 percent of the animals had some skeletal muscle necrosis.

In a later study (Highman and Altland, 1963) the same type of exercise was used to train a group of animals for three weeks. The 16 hour test exercise used previously failed to produce any histopathological changes or any significant elevations of serum enzyme activities in trained animals. Serum LDH activity was slightly elevated in trained animals following exercise but because of a large variability its significance was uncertain.

Since that time a number of investigators have attempted to characterize the effects of exercise and training programs on rat serum enzyme

activities. It was generally found that exercise caused an increase in serum enzyme activities and training reduced or obliterated the response (Garbus et al., 1964; Altland et al., 1964; Altland et al., 1968; Novosadova, 1969; and Dieter et al., 1969). It was believed by many investigators that determination of serum enzyme activities would be useful in evaluating exercise training programs and physical fitness of experimental animals.

Papadopoulos et al. (1968) used swimming exercise to physically train rats and found that the intensity and duration of the training program affected the plasma LDH activity response to exercise. A training program consisting of four hours of swimming daily for a least eight weeks was required to prevent the increase in plasma LDH activity following a four hour test swim.

Protection against the increase in plasma LDH activity resulting from ten weeks of training was found to be temporary (Bloor and Papadopoulos, 1969). Trained animals were subjected to a four hour swim at two weeks after the conclusion of the training program and plasma LDH activity increased fivefold. This increase was greater than that exhibited by untrained control animals subjected to the same exercise. This maximum rise in plasma LDH activity corresponded to histological changes in the heart associated with regression of cardiac hypertrophy resulting from training.

Doty, Bloor and Sobel (1971) found a fourfold increase in plasma LDH activities in rats exercised by a three hour swim. However, 90 percent of this increase had disappeared within two hours after exercise. In

a study which determined effects of age and exercise on male rats a comparison of plasma LDH activities was made between four and 18 months of age (Porter et al., 1971). There was no difference in resting plasma LDH activities but a significant difference in the response to exercise was noted. After three hours of swimming exercise plasma LDH activities of four-month-old rats were increased to five times that of control animals while there was only a twofold increase in plasma LDH activities of older rats.

Loegering (1974) compared effects of various modes and intensities of exercise on plasma LDH activities in male rats. Treadmill and swimming exercise both resulted in an increase in plasma LDH activity. Increased intensity of exercise caused greater increases in plasma LDH activity while increased duration of exercise did not result in consistently greater changes. Treadmill exercise was more effective in eliciting greater enzyme activity than swimming.

A number of other enzyme activities in rat serum or plasma have been measured following various types of exercise. Prolonged strenuous exercise has been found to increase serum glutamic-oxalacetic transaminase (GOT) activity (Altland and Highman, 1961; Altland et al., 1968; and Dieter et al., 1969). In other studies changes in serum GOT activities depended on the duration and type of the exercise. Swimming and treadmill exercise of short duration resulted in decreased serum GOT activities while longer bouts of swimming exercise resulted in increased serum GOT activities (Critz and Merrick, 1964; and Critz, 1966).

Creatine phosphokinase (CPK) activity was elevated in rat plasma following both swimming exercise of 30 to 60 minutes duration and treadmill exercise of 30 to 60 minutes duration. Plasma CPK activity increased to a greater extent following exercise of longer durations (Loegering, 1974). An intensive bout of swimming exercise for three hours resulted in a sixfold increase of serum CPK activity which declined rapidly to control levels at seven hours after exercise (Klosak and Penny, 1975).

Malate dehydrogenase (MDH) has been found to increase in plasma samples from rats after treadmill running and swimming, and a training program has been demonstrated to eliminate this plasma MDH activity elevation (Beaton and Oyster, 1969).

A number of other enzyme activities have been measured in rat plasma or serum following exercise. Some of these enzymes are alkaline phosphatase, aldolase, glutamate-oxalacetate transaminase and glutamate-pyruvate transaminase.

Exercise and Muscle Enzymes

Exercise and training have been found to change enzyme activities in skeletal muscle and cardiac muscle. Karlsson et al. (1968) noted an increase in human skeletal muscle LDH activity after three to five hours of heavy muscular exercise. In contrast Gollnick et al. (1967) found no significant changes in skeletal or cardiac muscle LDH activities following 30 minutes of swimming exercise administered to male rats.

A training program of swimming exercise for 35 days administered to rats produced a significant increase in cardiac muscle LDH activity but no

change in skeletal muscle LDH activity occurred (Gollnick and Hearn, 1961). York, Penny and Oscai (1975) have noted a similar increase in rat heart LDH activity following a 16 week training program. Gould and Rawlinson (1959) subjected rats of various ages to a training program of swimming for six weeks and found no differences in skeletal muscle LDH activity due to training at any of the age levels studied.

Recent evidence, however, indicates that long term endurance training may decrease skeletal muscle LDH activity in both men and rats (Karlsson et al., 1975; Suominen and Heikkinen, 1975; Hickson et al., 1976; Baldwin et al., 1973; and Costill et al., 1976).

Exercise and LDH Isoenzymes

In an attempt to determine the origins of increased serum enzyme activities following exercise several investigators have analyzed the LDH isoenzyme profile of serum or plasma following exercise and training. After maximal work on a bicycle ergometer the isoenzyme patterns have remained unchanged (Fowler et al., 1968). LDH-2 and LDH-3 were found to increase slightly while LDH-5 did not appear before or after exercise. However, in another study of healthy humans over 40 years of age, Block et al. (1969) found an increase in activities of all serum LDH isoenzymes except LDH-2 after an exhaustive effort on a bicycle ergometer. In trained subjects the activities of LDH isoenzymes 3, 4 and 5 were found to increase more while the activity of LDH-1 was less elevated compared to untrained subjects.

When patients with atherosclerosis exercised on a bicycle ergometer there was an increase in the relative percentage of LDH-5 in serum samples while LDH-1 remained the same. After a 12 week period of physical training this increase in the percentage of LDH-5 following exercise disappeared (Wolfson, Rose and Bousser, 1972). Schwartz et al. (1971) studied the isoenzyme patterns in serum samples of both trained and untrained males and found an increase in the relative percentages of LDH-4 and LDH-5 following exercise on a bicycle ergometer. The relative percentage of LDH-2 decreased significantly.

Serum samples from well-conditioned long distance runners exhibited significant increases in the relative percentages of LDH-4 and LDH-5 while those of isoenzymes 1, 2, and 3 declined following a marathon run (26.2 miles) (Rose, Bousser and Cooper, 1970). Similar results were obtained from well-conditioned males following a 10,000 meter race (Rose et al., 1970).

LDH isoenzymes have been measured in human skeletal muscle biopsies from endurance and strength trained athletes (Karlsson et al., 1975). Compared to untrained subjects endurance trained athletes have a complete absence of LDH-4 and LDH-5 in arm and leg muscles and higher relative activities of LDH-1 and LDH-2. Strength trained athletes have the same isoenzyme patterns in skeletal muscle as untrained subjects except for a very strong isoenzyme 5 band which corresponds to an increase in total LDH activity.

Altland et al. (1964) found that all LDH isoenzyme bands were intensified in rat serum when exercise was combined with exposure to

altitude. Exercise training during altitude exposure prevented changes in LDH isoenzyme patterns in rat serum following exercise. A later study produced similar results (Altland et al., 1968).

Training eliminated alterations in rat serum LDH isoenzymes following exercise in a study by Garbus et al. (1964) but injection of epinephrine into trained animals eight hours prior to a final exercise test resulted in elevated activities of LDH-1 and LDH-2.

Novosadova (1969) demonstrated a significant increase in LDH-2 activity in heart muscle of rats following a single swimming exercise. In skeletal muscle there was a decrease of LDH-5 activity due to training but blood serum isoenzyme patterns had inconstant changes in all animals and no significant results could be obtained.

A single exercise bout of four hours of swimming caused no changes in isoenzyme patterns of a number of rat organs but significant alterations in plasma isoenzyme patterns could be detected (Papadopoulos et al., 1967). Following exercise LDH-5 increased significantly in rat plasma while slight elevations of LDH-1 and LDH-2 could also be detected.

The maximum rise in plasma LDH activity following exercise of trained rats at two weeks after training had stopped was due almost entirely to the LDH-5 isoenzyme (Bloor and Papadopoulos, 1969). However, a slight increase in LDH-1 could also be detected.

In a study by Raven et al. (1970) trained and untrained rats were forced to swim to exhaustion with five percent body weight attached to their tails. The relative percentage of LDH-5 increased slightly

following exercise but training significantly lowered the relative percentage of LDH-5 in plasma. The percentage of LDH-4 in plasma was elevated significantly after swimming in both trained and untrained animals. Training caused an elevation of plasma LDH-1 but plasma LDH-1 decreased following the exhaustive swim in both trained and untrained rats.

In a recent study Anderson (1976) found an increase in serum LDH-4 and LDH-5 activities following various levels of exercise in horses. The relative percentages of LDH-1, LDH-2 and LDH-3 decreased or remained constant. He concluded that the serum LDH activity rise was due to release of LDH from skeletal muscle and possibly liver.

Although Bolter and Critz (1974) observed significant elevations in plasma LDH activity following treadmill exercise of dogs, no change was found in the H and M subunit composition of plasma.

Alterations in the LDH isoenzyme makeup of rat hearts have been observed as a result of cardiac hypertrophy. York et al. (1975) noted an increase in the percentage of M subunits in heart muscle of rats trained by swimming which corresponded to an overall increase in cardiac LDH activity. An elevation of the percentage of M subunits in left ventricular muscle has more recently been demonstrated in rats subjected to swimming and running training programs, aortic constriction and altitude exposure (York et al., 1976). In contrast Inamdar et al. (1971) found no change in cardiac LDH isoenzyme patterns following aortic constriction in rats.

Age and Lactate Dehydrogenase

In a recent review, Wilson (1973) has listed all of the enzyme changes known to occur with age in a variety of mammalian tissues. It has been determined that LDH activity can decrease, increase or remain constant with age depending on the animal and tissue under investigation.

McQueen et al. (1973) found a significant effect of age on serum LDH activity in a clinical study involving 280 healthy individuals with no history of hepatic or myocardial disease. An overall increase in serum LDH activity was noted in these subjects over an age range of 16 to greater than 55 years. However, in a previous clinical study where the subjects were all ambulatory male residents of a Veteran's Administration center, there was no change in serum LDH activity over an age range of 40 to 89 years (Davis et al., 1966). The subjects in this study were all fed the same diet and were exposed to the same type of environmental conditions while those in McQueen's investigation were not residents of an institution but came from a variety of life styles. Conconi et al. (1963) divided male and female subjects into four age groups and found that the highest serum LDH activities occurred after 65 years of age.

Porter et al. (1971) compared the plasma LDH activities of male rats which were four and 18 months of age. There was no difference in the resting levels of plasma LDH activity between the two ages but there was a significant difference in the plasma LDH response to exercise between the two age groups. After three hours of swimming exercise the plasma LDH activity of the four-month-old rats was increased to five times that of the

control animals while there was only a twofold increase in the plasma LDH activity of the older rats following the same exercise. The resting pre-swim LDH activities in cardiac muscle of the older animals was significantly lower than that of the younger animals. Following the swimming exercise the myocardial LDH activity in the four-month-old rats decreased significantly but the myocardial LDH activity of the older animals remained unchanged.

Changes in LDH activity of most mammalian tissues appears to parallel growth of the animal. Schmukler and Barrows (1966) reported a significant increase in myocardial and skeletal muscle LDH activity during the rapid growth period between one and three months of age in female rats. They found a decrease in skeletal muscle LDH activity between the ages of 12 and 24 months but no decrease in cardiac LDH activity over the same period. Similarly, LDH activity was found to increase significantly in brain, heart and skeletal muscle during the period from one day to 30 weeks of age in female rats (Singh and Kanungo, 1968). In liver tissue, LDH activity increased significantly from one day to four weeks of age and subsequently decreased at 12 weeks of age. Between the ages of 30 and 96 weeks LDH activity was decreased in brain, heart and skeletal muscle while liver LDH activity remained unchanged.

Shukla and Kanungo (1970) measured rates of oxygen uptake in both liver and brain homogenates using lactate as a substrate and reported an increase in the rate of lactate oxidation from birth to 12 weeks of age in liver tissue and an increase in brain lactate oxidation from birth to four

weeks of age. After the peak in lactate oxidation rates for both tissues there was a gradual decrease in the oxidation rates of lactate until 96 weeks of age.

Other reports of liver LDH activity changes with age include a 30 percent increase in liver LDH activity between birth and 65 days of age in male rats (Burch et al., 1963); a 50 percent increase in rat liver LDH activity during the first six months of life (Ross and Ely, 1954); and a ten percent decrease in rat liver LDH activity between birth and maturity (Weber and Cantero, 1959). Bertolini (1962) found no differences in the LDH activity of erthrocytes of various ages nor could he determine differences in LDH activity in erthrocytes from young and old subjects.

The effects of age on the relative percentage of each LDH isoenzyme has also been studied in various tissues. Between the ages of 12 and 24 months no significant changes could be found in the LDH isoenzyme distributions of liver, brain, kidney, skeletal muscle or heart muscle in female rats (Schmukler and Barrows, 1967). However, Kanungo and Singh (1965) reported an increase with age in the activities of LDH-1 and LDH-5 in rat brain tissue until 12 weeks of age. This was followed by a continual increase in LDH-1 but a decrease in LDH-5 activity until 74 weeks of age. Both LDH-1 and LDH-5 activity increased in cardiac muscle until 30 weeks of age followed by a decrease in the activity of both isoenzymes with LDH-5 decreasing more rapidly.

In a later study it was determined that the H to M subunit ratio increased after 30 weeks of age in the brain, heart, skeletal muscle and

liver of female rats. This was not due to an increase in H subunits but to an overall decrease in M subunits (Singh and Kanungo, 1968).

The LDH isoenzyme pattern in rat aortic tissue was also found to be influenced by age (Gerlach and Fegeler, 1972/73). There was a decrease in the relative percentage of LDH-1 and LDH-2 while LDH-3 and LDH-4 increased with age. LDH-5 increased up until the rats weighed 300 grams. The H to M subunit ratio decreased between 50 gram rats and 150 gram rats and then remained relatively constant until the animals weighed 300 grams.

Other Physiological Effects on Enzyme Systems

Many investigations have demonstrated that enzyme systems can be influenced by hypoxia, anoxia, cold exposure, diet and hormones. Some of these studies are summarized here because of the implications which they may lend to the present investigation.

Exposure to reduced oxygen tensions associated with high altitude has been found to affect tissue LDH activity. Penny (1974) exposed rats to a simulated altitude of over 20,000 feet for 57 days. This exposure resulted in a significant increase in total myocardial LDH activity and a twofold increase in the number of M subunits of LDH in heart tissue. In a similar study rats exposed to a simulated altitude of 22,500 feet had a decrease in the myocardial H to M ratio but total myocardial LDH activity remained unchanged (Anderson and Bullard, 1971). More recently Vergnes et al. (1976) found no change in either total myocardial LDH activity or LDH isoenzyme profile of heart muscle from rats exposed to a natural altitude of over 10,000 feet.

Increased skeletal muscle LDH activity has been demonstrated in rats exposed to a simulated altitude of 14,500 feet for 45 days (Mager et al., 1968). In the same study neonatal rats born at this reduced oxygen tension had increased LDH activities in liver, heart and skeletal muscle when compared with control animals born at sea level. The H to M ratio was decreased in myocardial tissue from altitude exposed neonates.

Exposure of dogs to a simulated altitude of 32,000 feet resulted in a continuous increase in serum LDH activity over a seven week exposure period (Highman and Altland, 1961). Increased serum LDH activities following altitude exposure have also been noted in rats (Altland et al., 1964; and Altland et al. 1968).

Penny et al. (1974) induced a state of sideropenic anemia in rats and observed a substantial increase in total LDH activity in heart muscle as well as 54 percent increase in the amount of LDH M subunits in cardiac tissue.

Subjection of isolated rat hearts to an anoxic perfusion medium has caused release of LDH from cardiac muscle (Butterworth et al., 1970; and de Leiris et al., 1969). Appearance of LDH activity did not occur until after the anoxic perfusion had been replaced with oxygenated fluid and normal contractions had begun again. More recently Feuvray et al. (1974) have demonstrated that perfusions of anoxic media for periods up to 30 minutes failed to cause release of LDH activity from isolated rat heart preparations when the heart and perfusion fluids were carefully maintained at 37.5^{o} C.

Another experimental situation which has caused changes in LDH activity in rat plasma or serum is cold exposure. By restraining rats at 2^0 C for two hours, Meltzer (1971) observed a fivefold increase in plasma LDH activity as well as a very significant increase in plasma CPK activity. A period of cold acclimation for 21 days eliminated the increased CPK response to a two hour test exposure.

A period of cold acclimation at 1.7° C for four to six weeks caused a greater elevation of serum LDH following exercise than that of unacclimated rats subjected to the same exercise (Dieter et al., 1969).

Blatt et al. (1965) exposed rats to 5° C for varying lengths of time and noted a decreased amount of M subunits in cardiac muscle but the plasma LDH activities were widely variable and no conclusions could be drawn. Total LDH activity in liver and heart tissue fluctuated during exposure while that of kidney tissue remained unchanged.

An investigation by Marshall et al. (1976) has demonstrated that diet can cause marked changes in serum LDH activities. By feeding high carbohydrate diets to healthy men and women he was able to induce a 30 percent decrease in the serum LDH activity. LDH-1 decreased significantly in the serum of men on the high carbohydrate diet while LDH-3 and LDH-4 increased significantly. In contrast no change in the serum LDH isoenzyme profile was observed in women on the same diet.

Thyroid hormones have been demonstrated to change serum enzyme activities. Elevated levels of CPK in serum from hypothyroid patients have been demonstrated to return to normal after thyroid hormone therapy

(Graig and Ross, 1963). Severe hypothyroidism has caused elevated LDH, CPK and GOT activities in serum while hyperthyroidism resulted in decreased serum CPK and LDH activities. In the same study pregnancy decreased serum CPK, LDH and GOT activities (Fleisher et al., 1965).

Evidence that adrenal hormones may be partly responsible for enzyme activity changes has been presented by a number of authors. Critz and Withrow (1965) pharmacologically blocked or surgically removed the adrenal cortex prior to exercising rats. GOT activity was found to increase in heart and skeletal muscle from intact animals following exercise but no increase in GOT activity was observed in the same organs when the adrenal cortex was blocked or removed.

Hydrocortisone administration potentiated the rise in serum CPK and aldolase activities following fasting in myopathic Syrian hamsters (Solymoss and Jasmin, 1975). In contrast prednisolone administration decreased the elevation in serum CPK activity following exercise in dogs (Wagner and Critz, 1968).

Jacey and Schaefer (1967) exposed guinea pigs to air containing a high concentration of CO_2 and found significant correlations between blood corticosteroid levels and the increase in total plasma LDH activity. After 24 hours of exposure to high levels of CO_2, plasma LDH activities reached a peak as did plasma corticosteroid levels. Increased plasma LDH activities were due to threefold elevations of the relative percentages of LDH-4 and LDH-5.

METHODS AND MATERIALS

Treatment of Animals

Male rats of the Sprague-Dawley strain were maintained in a temperature controlled (25 ± 3° C), artificially lighted room (8:00 A.M. to 10:00 P.M.). Teklad Mouse and Rat Diet and water were provided *ad libitum*.

Animals were of three age groups: 100, 200 and 300 days at the beginning of the experimental period. Within each age group the animals were subdivided into three treatment groups. An exercise (Ex) group was physically trained by treadmill running. A machine control (MC) group was subjected to the same handling and treatment as the exercise group except for the physical exercise. A sedentary control (C) group was maintained in the animal room in cages and was not subjected to any of the handling associated with exercise training. At the end of the experimental period each treatment group was divided into a swim (S) group and a nonswim (NS) group. The swim animals were subjected to an exhaustive swimming exercise immediately prior to sacrifice while the nonswim animals were not.

Exercise Training Technique

Exercise training was administered to the animals on a motor driven treadmill constructed from a modified design of that described by Jette *et al.* (1969). These modifications have been described previously (Auth, 1975). The animals were trained physically five days per week for a period of ten weeks. The first week of training served as a conditioning

period during which the speed of the treadmill and length of the exercise period were gradually increased. At the beginning of the second week of training the animals were running at a speed of 15 meters per minute up an eight degree incline for 30 minutes daily. These conditions were maintained for the remainder of the experimental period.

Machine control animals were placed in the treadmill chambers for 30 minutes daily five days per week for a period of ten weeks. The motor of the treadmill was turned on but disengaged from the treadmill.

Swimming Technique

At the end of the ten week experimental period one half of the animals in each treatment group was subjected to a swimming exercise immediately prior to sacrifice. The animals swam separately in large plastic garbage cans filled with water at 35 $\pm$ 2° C. Weights amounting to four percent of each animal's body weight were attached to the tail. When the animal was unable to reach the surface for a period of ten seconds it was considered to be exhausted (Dawson and Horvath, 1970), removed from the water and towelled dry. The length of time that each animal was in the water was recorded.

Handling of Tissues

At the end of the ten week experimental period the animals were anesthetized with sodium pentobarbital (25 mg/Kg) administered intraperitoneally. All animals were sacrificed between 4:00 P.M. and 12:00 midnight. All animals were weighed and the weights recorded prior to

sacrifice. Animals which were subjected to the swimming exercise were injected with the anesthetic within one minute after removal from the water. Blood samples were removed from the jugular veins using a plastic syringe, placed in heparinized glass centrifuge tubes and centrifuged for ten minutes. The plasma was carefully removed from the centrifuged tubes and stored in sealed plastic tubes overnight at 4° C for subsequent analysis of total LDH activity.

After the blood sample was obtained the heart and liver were rapidly excised, blotted and weighed. The atria and major vessels were trimmed from the heart prior to weighing.

Determination of Total Plasma LDH Activity

Plasma samples were analyzed for total LDH activity within 18 hours after the samples were obtained. LDH-L Stat-Pack (Calbiochem) reagents were used for the analysis as described in Appendix A.

Determination of Plasma LDH Isoenzymes

Plasma samples were stored at -20° C. prior to the isoenzyme analysis. Electrophoretic separation of plasma LDH isoenzymes was carried out on cellulose acetate strips by the method of Preston et al. (1965) as described in Appendix B. The relative percentage of each isoenzyme was determined by densitometric scanning and planimetry. The activity of each isoenzyme was calculated by multiplying the total plasma LDH activity by the relative percentage of each isoenzyme.

Determination of Plasma CPK Activity

Blood samples were sent to Antonik laboratories in Elk Grove Village, Illinois for CPK activity analysis. Only blood samples for 170-day-old and 270-day-old animals were analyzed for total CPK activity

Analysis of Data

Data were analyzed using The Statistical Analysis System (Barr and Goodnight, 1971). The ANOVA procedure was used to calculate the analysis of variance and the MEANS procedure was used to calculate the means of all variables in each experimental group. The REGRESSION procedure was used to adjust the total plasma LDH activities for swim time. Individual experimental groups were compared using t tests. The residual mean squares obtained by analysis of variance for each variable were used as the best estimates of variance.

RESULTS

The results of this study are presented in the following section. Table 1 lists all variables which were studied and also indicates the abbreviations and units used for each variable. In addition, the three treatment groups will be referred to as C for control animals, MC for machine control animals and Ex for treadmill exercise trained animals. Nonswim and swim animals will be designated as NS and S, respectively.

The variables under consideration have been divided into three categories: final body weight and organ weights; plasma enzyme activities and swim time; and plasma LDH isoenzymes.

Final Body Weight and Organ Weights

Final body weight

Means, standard errors and analysis of variance for final body weight are presented in Table 2. Analysis of variance demonstrates that both age and treatment had a highly significant ($p < 0.005$) effect on FBW. There was a significant ($p < 0.001$) increase in FBW in all three treatment groups (C, MC and Ex) between the ages of 170 days and 270 days (Table 3). However, between 270 days and 370 days, no further increase in body weight was observed. FBW values from 370-day-old C animals were slightly less ($p < 0.05$) than those from 270-day-old C animals.

In order to assess the effect of treadmill exercise training on FBW, nonswim and swim animals have been combined within each treatment group. Differences in FBW between NS and S animals resulted from an apparent

Table 1. Variable abbreviations and units used in this study

Variable	Abbreviation	Units
Final body weight	FBW	grams (g)
Heart weight	HW	g
Liver weight	LW	g
Swim time	ST	minutes (min)
Total plasma lactate dehydrogenase activity	TLDH	milliUnits/milliliter (mU/ml)
Total plasma LDH activity adjusted for swim time	A-TLDH	mU/ml
Total plasma creatine phosphokinase activity	TCPK	Antonik Units (AU)
Relative percentage of LDH isoenzyme 1	% LDH-1	percent (%)
Relative percentage of LDH isoenzyme 2	% LDH-2	%
Relative percentage of LDH isoenzyme 3	% LDH-3	%
Relative percentage of LDH isoenzyme 4	% LDH-4	%
Relative percentage of LDH isoenzyme 5	% LDH-5	%
Activity of LDH isoenzyme 1	LDH-1	mU/ml
Activity of LDH isoenzyme 2	LDH-2	mU/ml
Activity of LDH isoenzyme 3	LDH-3	mU/ml
Activity of LDH isoenzyme 4	LDH-4	mU/ml
Activity of LDH isoenzyme 5	LDH-5	mU/ml

Table 2. Means, standard errors and analysis of variance for final body weight (FBW)

	Nonswim	Swim	Nonswim and Swim combined
170 Days	(g)	(g)	(g)
Control	397.3 ± 25.2[a]	403.5 ± 13.0	400.4 ± 13.7
Machine Control	408.0 ± 9.3	421.2 ± 9.9	415.1 ± 6.9
Exercise	406.7 ± 12.0	378.0 ± 7.4	393.1 ± 7.7
270 Days			
Control	487.6 ± 10.3	516.5 ± 10.5	502.8 ± 7.9
Machine Control	517.9 ± 14.9	468.7 ± 17.7	496.8 ± 12.9
Exercise	458.4 ± 9.4	450.7 ± 9.4	454.4 ± 6.6
370 Days			
Control	470.8 ± 18.0	475.2 ± 12.9	473.1 ± 10.5
Machine Control	461.6 ± 12.6	510.6 ± 14.1	490.2 ± 11.9
Exercise	437.4 ± 11.2	460.0 ± 9.6	449.4 ± 7.6
Age Means	170 Days: 402.4	270 Days: 483.3	370 Days: 469.3
Treatment Means	C: 458.5	MC: 464.7	Ex: 431.9

Analysis of Variance

Source	df	F value	Prob>F
Age	2	71.05	0.005
Treatment	2	11.55	0.005
Age * Treatment	4	1.36	ns[b]
Swim	1	0.53	ns

[a]Mean ± standard error of the mean.
[b]ns = not significant at $p < 0.05$.

Table 3. Statistical comparisons made using t test for final body weight (FBW), heart weight (HW) and liver weight (LW)

Groups compared	FBW	HW	LW
170 Days			
C and MC	ns	ns	***
C and Ex	ns	*	***
MC and Ex	*	ns	ns
270 Days			
C and MC	ns	ns	ns
C and Ex	***	ns	***
MC and Ex	**	ns	**
370 Days			
C and MC	ns	**	ns
C and Ex	ns	***	ns
MC and Ex	**	ns	**
Control			
170 Days and 270 Days	***	***	ns
270 Days and 370 Days	*	**	**
Machine Control			
170 Days and 270 Days	***	***	***
270 Days and 370 Days	ns	ns	ns
Exercise			
170 Days and 270 Days	***	***	**
270 Days and 370 Days	ns	ns	ns

ns = not significant at $p < 0.05$.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

failure to distribute animals evenly according to body weight within these two groups.

Exercise training for 10 weeks significantly reduced FBW below that of MC and C animals at 270 days of age ($p<0.01$ and $p<0.001$). At 170 days and 370 days of age, exercise training reduced FBW below that of MC animals ($p<0.05$ and $p<0.01$). No significant difference in FBW was observed between Ex and C animals at these two ages (Table 2 and Table 3).

Organ weights

Heart weights and liver weights were recorded in this study because these two organs represent possible sources of elevated plasma LDH activity following exercise (Fojt et al., 1976; and Papadopoulos et al., 1967). Means, standard errors and analysis of variance for HW are given in Table 4. Both age and treatment affected HW as demonstrated by the analysis of variance. HW increased significantly ($p<0.001$) between 170 days and 270 days of age in all three treatment groups (Table 3). No increase in HW occurred between 270 and 370 days of age. Hearts from 370-day-old C animals were significantly lighter ($p<0.01$) than those from 270-day-old C animals. This was similar to the FBW comparisons between these two ages of C animals. Age affected HW and FBW similarly within all three treatment groups.

Exercise training caused an increase in HW above that of C animals at 170 days ($p<0.05$) and at 370 days of age ($p<0.001$). There were no differences in HW between Ex and MC groups at any of the three age levels. Hearts from MC animals were significantly heavier ($p<0.01$) than those from C animals at 370 days of age (Table 3). At 270 days of age there were no differences in HW between any of the treatment groups.

Table 4. Means, standard errors and analysis of variance for heart weight (HW)

	Nonswim	Swim	Nonswim and Swim combined
170 Days	(g)	(g)	(g)
Control	0.93 ± 0.06[a]	[b]	0.93 ± 0.06
Machine Control	1.05 ± 0.03	1.01 ± 0.05	1.04 ± 0.02
Exercise	1.11 ± 0.04	1.03 ± 0.03	1.07 ± 0.02
270 Days			
Control	1.13 ± 0.04	1.22 ± 0.03	1.18 ± 0.03
Machine Control	1.21 ± 0.04	1.13 ± 0.06	1.18 ± 0.04
Exercise	1.24 ± 0.03	1.17 ± 0.03	1.21 ± 0.02
370 Days			
Control	1.09 ± 0.03	1.10 ± 0.03	1.09 ± 0.02
Machine Control	1.13 ± 0.03	1.26 ± 0.03	1.21 ± 0.03
Exercise	1.18 ± 0.05	1.23 ± 0.04	1.21 ± 0.03
Age Means	170 Days: 1.03	270 Days: 1.18	370 Days: 1.17
Treatment Means	C: 1.09	MC: 1.13	Ex: 1.16
Analysis of variance			
Source	df	F value	Prob>F
Age	2	31.34	0.005
Treatment	2	5.12	0.01
Age * Treatment	4	3.67	0.05
Swim	1	2.57	ns[c]

[a]Mean ± standard error of the mean.
[b]Data not available.
[c]ns = not significant at $p < 0.05$.

Heart weights were corrected for body weight using the REGRESSION procedure. Means, standard errors and statistical comparisons for a adjusted heart weight are given in Table 5. There was a highly significant correlation between FBW and HW ($p<0.0001$). Hearts from Ex animals were significantly heavier than those from C animals at all three age levels ($p<0.001$) when HW was corrected for FBW. Adjusted heart weights from Ex animals were also significantly greater than those from MC animals at 170 and 370 days of age ($p<0.01$) and at 270 days of age ($p<0.001$). It is interesting to note that adjusted heart weights from MC animals were statistically greater than those from C animals at 170 days of age ($p<0.001$) and at 370 days of age ($p<0.01$); however, no difference was observed at 270 days of age.

Table 6 lists the means, standard errors and analysis of variance for LW. Age and treatment both had highly significant effects ($p<0.005$) on LW. Livers from 270-day-old MC and Ex animals were heavier ($p<0.001$ and $p<0.01$) than those from 170-day-old animals in the same treatment groups (Table 3). The mean value for LW of 170-day-old C animals was relatively greater than all other groups but because of a small number of observations and large variance, its significance is doubtful. No differences in LW were observed between 270 days and 370 days of age for MC and Ex animals. Livers from 370-day-old C animals weighed significantly less than those from 270-day-old C animals. This was again similar to the differences in FBW and HW between these two groups.

The effect of exercise training on LW was similar to the effect of training on FBW at 270 and 370 days of age (Table 3). Ex animals had

Table 5. Means, standard errors and statistical comparisons for heart weight adjusted for body weight (AHW)

	AHW	Groups compared	Significance
170 Days	(g)		
Control	0.96 ± 0.04[a]	C and MC	***
Machine Control	1.12 ± 0.02	C and Ex	***
Exercise	1.20 ± 0.02	MC and Ex	**
270 Days			
Control	1.09 ± 0.02	C and MC	ns
Machine Control	1.09 ± 0.02	C and Ex	***
Exercise	1.21 ± 0.02	MC and Ex	***
370 Days			
Control	1.06 ± 0.02	C and MC	**
Machine Control	1.14 ± 0.02	C and Ex	***
Exercise	1.22 ± 0.02	MC and Ex	**

[a]Mean ± standard error of the mean.
** $p < 0.01$.
*** $p < 0.001$.
ns = not significant at $p < 0.05$.

livers that weighed less ($p < 0.01$) than those of MC and C animals at 270 days. At 370 days of age exercise training decreased LW below that of MC animals ($p < 0.01$). The difference in LW between 170-day-old C animals and 170-day-old MC and Ex animals is questionable because of the small number of observations in the C group.

Table 6. Means, standard errors and analysis of variance for liver weight (LW)

	Nonswim	Swim	Nonswim and Swim combined
170 Days	(g)	(g)	(g)
Control	14.23 ± 2.75[a]	[b]	14.23 ± 2.75
Machine Control	11.19 ± 0.48	10.14 ± 0.28	10.90 ± 0.37
Exercise	11.59 ± 0.40	9.31 ± 0.30	10.50 ± 0.35
270 Days			
Control	13.43 ± 0.32	14.25 ± 0.42	13.86 ± 0.28
Machine Control	13.91 ± 0.52	12.78 ± 0.77	13.42 ± 0.45
Exercise	12.45 ± 0.37	11.65 ± 0.35	12.03 ± 0.26
370 Days			
Control	12.13 ± 0.63	12.88 ± 0.57	12.53 ± 0.42
Machine Control	12.49 ± 0.33	14.06 ± 0.70	13.41 ± 0.47
Exercise	11.44 ± 0.44	11.59 ± 0.33	11.52 ± 0.26
Age Means	170 Days: 11.29	270 Days: 13.08	370 Days: 12.43
Treatment Means	C: 13.38	MC: 12.43	Ex: 11.34

Analysis of variance

Source	df	F value	Prob>F
Age	2	16.56	0.005
Treatment	2	21.67	0.005
Age * Treatment	4	5.52	0.005
Swim	1	3.33	ns[c]

[a]Mean ± standard error of the mean.
[b]Data not available.
[c]ns = not significant at $p<0.05$.

Plasma Enzyme Activities and Swim Time

Swim time

Means, standard errors and analysis of variance for ST are listed in Table 7. Age affected the length of time that animals were able to swim ($p<0.05$). By pooling all S animals into three age groups, a significant reduction of swim time can be demonstrated between 170 days and 270 days of age ($p<0.01$). No difference in swim time was observed between 270 and 370 days of age. In most experimental treatment groups one or two animals exhibited extremely long swim times and it is difficult to make comparisons of ST between treatment groups within age groups. It is interesting to note that the Ex groups which had been physically trained for 10 weeks did not have longer swim times than control groups within the same age group. When all S animals were pooled into three treatment groups ignoring age, no differences in ST were found between any treatment groups.

Total plasma LDH activity

Table 8 contains the means, standard errors and analysis of variance for TLDH. Analysis of variance for TLDH demonstrates that TLDH was significantly affected by age ($p<0.005$) and swimming exercise ($p<0.005$). No difference in TLDH occurred between treatment groups within each age group.

There was an increase ($p<0.01$) in TLDH with age between 170 days and 270 days of age when all animals are grouped into three ages ignoring treatment (Table 9). All treatment groups exhibited an increased TLDH with age between 170 days and 270 days of age but the differences were not significant within each treatment group. No increase in TLDH occurred

Table 7. Means, standard errors and analysis of variance for swim time (ST)

	Swim
170 Days	(min)
Control	32.6 ± 18.7[a]
Machine Control	20.6 ± 8.8
Exercise	10.2 ± 4.5
270 Days	
Control	4.2 ± 0.5
Machine Control	5.6 ± 0.3
Exercise	11.5 ± 7.1
370 Days	
Control	9.0 ± 3.0
Machine Control	3.4 ± 0.4
Exercise	3.6 ± 0.6
Age Means	170 Days: 20.4 270 Days: 7.4 370 Days: 5.5
Treatment Means	C: 14.2 MC: 13.1 Ex: 8.7

Analysis of variance

Source	df	F value	Prob > F
Age	2	3.45	0.05
Treatment	2	0.59	ns[b]
Age * Treatment	4	0.86	ns

[a]Mean ± standard error of the mean.
[b]ns = not significant at $p < 0.05$.

Table 8. Means, standard errors and analysis of variance for total plasma LDH activity (TLDH)

	Nonswim	Swim	Nonswim and Swim combined
170 Days	(mU/ml)	(mU/ml)	(mU/ml)
Control	38.6 ± 5.0[a]	58.6 ± 8.7	48.6 ± 5.5
Machine Control	47.3 ± 4.8	64.7 ± 8.7	56.6 ± 5.4
Exercise	32.2 ± 1.1	60.4 ± 7.6	45.6 ± 4.8
270 Days			
Control	52.8 ± 4.3	72.0 ± 7.2	62.9 ± 4.7
Machine Control	59.0 ± 7.7	84.7 ± 9.4	70.0 ± 6.7
Exercise	46.4 ± 1.9	69.9 ± 6.1	58.7 ± 4.2
370 Days			
Control	51.5 ± 7.6	83.0 ± 9.8	68.1 ± 7.3
Machine Control	42.1 ± 5.2	83.2 ± 18.5	66.1 ± 12.3
Exercise	63.9 ± 6.1	66.6 ± 9.1	65.3 ± 5.4
Age Means	170 Days: 50.3	270 Days: 64.1	370 Days: 65.0
Treatment Means	C: 59.4	MC: 63.5	Ex: 56.6
Analysis of variance			
Source	df	F value	Prob > F
Age	2	6.88	0.005
Treatment	2	1.23	ns[b]
Age * Treatment	4	0.73	ns
Swim	1	40.97	0.005

[a]Mean ± standard error of the mean.
[b]ns = not significant at $p < 0.05$.

Table 9. Statistical comparisons made using t test for total plasma LDH activity (TLDH), total plasma LDH activity adjusted for swim time (A-TLDH) and total plasma CPK activity (TCPK)

Groups compared	TLDH	A-TLDH	TCPK
170 Days			
CNS and CS	ns	ns	ns
MCNS and MCS	*	ns	ns
ExNS and ExS	**	*	**
270 Days			
CNS and CS	ns	ns	ns
MCNS and MCS	*	ns	ns
ExNS and ExS	*	*	ns
370 Days			
CNS and CS	**	**	--
MCNS and MCS	**	**	--
ExNS and ExS	ns	ns	--
Control			
170 Days and 270 Days	ns	*	***
270 Days and 370 Days	ns	ns	--
Machine Control			
170 Days and 270 Days	ns	ns	ns
270 Days and 370 Days	ns	ns	--
Exercise			
170 Days and 270 Days	ns	ns	ns
270 Days and 370 Days	ns	ns	--
All Treatments			
170 Days and 270 Days	**	**	***
270 Days and 370 Days	ns	ns	--

ns = not significant at $p<0.05$.
* $p<0.05$.
** $p<0.01$.
*** $p<0.001$.
-- Data not available.

between 270 days and 370 days of age except in the ExNS animals; however, this increase was not significant.

Swimming exercise caused an elevation of TLDH in all treatment groups combined ($p<0.001$). Ex animals exhibited elevations of TLDH after swimming at 170 days ($p<0.01$) and at 270 days ($p<0.05$). No elevation in TLDH was observed in Ex animals following swimming exercise at 370 days.

Swimming exercise caused significant elevations in TLDH in MC animals at 170 days of age ($p<0.05$), 270 days of age ($p<0.05$) and 370 days of age ($p<0.01$). C animals had increased TLDH following swimming at all three age levels but the elevation of TLDH was significant only at 370 days of age ($p<0.01$).

An analysis of covariance using the REGRESSION procedure revealed a significant effect of swim time on TLDH ($p<0.0325$). The adjusted means and standard errors for total plasma LDH activity (A-TLDH) are given in Table 10. After correcting for swim time, Ex animals had significant A-TLDH elevations ($p<0.05$) following swimming exercise at 170 days and 270 days of age (Table 8). MC and C animals exhibited significant elevations in A-TLDH after swimming at 370 days ($p<0.01$) but not at the other two ages.

Total plasma CPK activity

Means, standard errors and analysis of variance for TCPK are given in Table 11. Age, treatment and swimming exercise all affected TCPK as demonstrated by the analysis of variance. Table 8 contains the statistical comparisons between 170-day-old and 270-day-old animals for each treatment

Table 10. Means adjusted for swimtime and standard errors for adjusted total LDH activity (A-TLDH)

	Nonswim	Swim	Nonswim and Swim combined
170 Days	(mU/ml)	(mU/ml)	(mU/ml)
Control	40.0 ± 8.2[a]	52.4 ± 8.2	46.2 ± 5.8
Machine Control	48.7 ± 6.4	61.3 ± 6.0	55.5 ± 4.4
Exercise	33.6 ± 7.0	59.5 ± 7.3	45.9 ± 5.0
270 Days			
Control	54.2 ± 7.7	72.4 ± 7.3	63.8 ± 5.3
Machine Control	60.4 ± 8.2	84.8 ± 9.4	70.9 ± 6.2
Exercise	47.8 ± 7.3	68.7 ± 7.0	58.7 ± 5.0
370 Days			
Control	52.9 ± 8.2	82.3 ± 7.7	68.5 ± 5.6
Machine Control	43.6 ± 10.3	83.8 ± 8.7	67.0 ± 6.7
Exercise	65.3 ± 8.2	67.2 ± 7.7	66.3 ± 5.6
Age Means	170 Days: 50.1	270 Days: 63.7	370 Days: 67.3
Treatment Means	C: 59.9	MC: 62.0	Ex: 56.4

[a]Mean ± standard error of the mean.

group. TCPK was significantly greater at 270 days in both the MC group ($p<0.001$) and C group ($p<0.001$). No difference was observed in TCPK of Ex animals between the two ages.

Swimming exercise caused an elevation of TCPK in Ex animals at 170 days of age ($p<0.01$) but not at 270 days of age. Swimming exercise did not elevate TCPK in MC or C animals at 170 days or 270 days of age

Table 11. Means, standard errors and analysis of variance for total plasma CPK activity (TCPK)

	Nonswim	Swim	Nonswim and Swim combined
170 Days	(AU)	(AU)	(AU)
Control	6.4 ± 0.7[a]	8.8 ± 1.0	7.6 ± 0.7
Machine Control	10.8 ± 0.7	8.1 ± 0.4	9.6 ± 0.5
Exercise	8.6 ± 0.4	18.8 ± 5.1	12.8 ± 2.3
270 Days			
Control	27.3 ± 4.0	33.0 ± 2.3	30.2 ± 2.3
Machine Control	16.7 ± 1.2	21.2 ± 1.9	18.6 ± 1.2
Exercise	14.0 ± 0.8	17.5 ± 2.4	16.0 ± 1.5
Age Means	170 Days: 10.2	270 Days: 21.6	
Treatment Means	C: 18.9	MC: 14.2	Ex: 14.7
Analysis of variance			
Source	df	F value	Prob>F
Age	1	95.78	0.005
Treatment	2	6.45	0.005
Age * Treatment	2	26.85	0.005
Swim	1	11.37	0.005

[a]Mean ± standard error of the mean.

(Table 8). ExS animals had significantly greater TCPK than MCS or CS animals ($p<0.05$) at 170 days of age while C animals had significantly greater TCPK activities than MC or Ex animals at 270 days of age ($p<0.001$).

Plasma LDH Isoenzymes

Means, standard errors and analysis of variance for the relative percentages of all plasma LDH isoenzymes are given in Tables 12 through 16. Comparisons between NS and S animals within each treatment group and between age groups are given in Table 17.

Analysis of variance for the relative percentage of each LDH isoenzyme demonstrates that treatment (MC, Ex and C) had no effect on the pattern of isoenzyme distribution in plasma. Age significantly affected % LDH-1, % LDH-2 and % LDH-5 ($p<0.005$). Swimming exercise caused significant changes in % LDH-1 ($p<0.005$), % LDH-2 ($p<0.05$) and % LDH-4 ($p<0.025$).

The effect of age on % LDH-1, % LDH-2 and % LDH-5 can be demonstrated by combining all treatment groups within each age group. Between 170 days and 270 days of age there was a significant reduction in both % LDH-1 and % LDH-2 ($p<0.001$) and an increase in % LDH-5 ($p<0.001$). This effect was reversed between 270 days and 370 days of age. During this age interval % LDH-1 was increased significantly ($p<0.05$) and % LDH-5 was decreased ($p<0.05$).

When each treatment group is considered separately both Ex and C animals exhibited significant decreases in % LDH-1 between 170 and 270 days of age ($p<0.01$). C animals also had significant decreases in % LDH-2 and % LDH-3 ($p<0.01$) and a significant increase in % LDH-5 ($p<0.001$) between these two ages. MC animals had a greater % LDH-5 ($p<0.05$) and a decreased % LDH-3 ($p<0.05$) at 270 days of age compared to 170-day-old MC animals.

Table 12. Means, standard errors and analysis of variance for relative percentage of LDH isoenzyme 1 (% LDH-1)

	Nonswim	Swim	Nonswim and Swim combined
170 Days	(%)	(%)	(%)
Control	15.6 ± 2.4[a]	10.0 ± 2.6	12.8 ± 1.9
Machine Control	9.6 ± 1.2	11.1 ± 1.5	10.4 ± 1.0
Exercise	18.8 ± 2.1	6.5 ± 2.1	12.6 ± 2.1
270 Days			
Control	7.3 ± 0.8	6.5 ± 0.7	6.9 ± 0.5
Machine Control	8.5 ± 1.6	6.9 ± 0.7	7.8 ± 1.0
Exercise	7.5 ± 1.1	6.7 ± 1.2	7.1 ± 0.8
370 Days			
Control	16.0 ± 3.1	6.1 ± 1.6	11.1 ± 2.1
Machine Control	9.0 ± 1.6	9.6 ± 2.0	9.3 ± 1.3
Exercise	9.5 ± 1.8	10.2 ± 1.9	9.8 ± 1.3
Age Means	170 Days: 11.9	270 Days: 7.2	370 Days: 10.1
Treatment Means	C: 10.3	MC: 9.1	Ex: 9.9

Analysis of variance

Source	df	F value	Prob>F
Age	2	9.89	0.005
Treatment	2	0.59	ns[b]
Age * Treatment	4	0.55	ns
Swim	1	13.11	0.005

[a]Mean ± standard error of the mean.
[b]ns = not significant at $p < 0.05$.

Table 13. Means, standard errors and analysis of variance for relative percentage of LDH isoenzyme 2 (% LDH-2)

	Nonswim	Swim	Nonswim and Swim combined
170 Days	(%)	(%)	(%)
Control	5.5 ± 1.4[a]	1.8 ± 0.3	3.6 ± 0.8
Machine Control	3.5 ± 0.9	3.4 ± 0.6	3.5 ± 0.5
Exercise	4.4 ± 1.3	2.9 ± 0.5	3.7 ± 0.7
270 Days			
Control	1.8 ± 0.6	1.3 ± 0.4	1.6 ± 0.4
Machine Control	2.4 ± 1.0	1.5 ± 0.4	2.0 ± 0.6
Exercise	2.5 ± 0.6	2.1 ± 0.4	2.3 ± 0.3
370 Days			
Control	2.0 ± 0.3	2.5 ± 1.0	2.2 ± 0.5
Machine Control	3.0 ± 1.5	1.3 ± 0.4	2.0 ± 0.7
Exercise	1.9 ± 0.5	3.4 ± 0.6	2.6 ± 0.4
Age Means	170 Days: 3.6	270 Days: 1.9	370 Days: 2.3
Treatment Means	C: 2.5	MC: 2.6	Ex: 2.9
Analysis of variance			
Source	df	F value	Prob>F
Age	2	7.41	0.005
Treatment	2	0.45	ns[b]
Age * Treatment	4	0.14	ns
Swim	1	4.26	0.05

[a]Mean ± standard error of the mean.
[b]ns = not significant at $p<0.05$.

Table 14. Means, standard errors and analysis of variance for relative percentage of LDH isoenzyme 3 (% LDH-3)

	Nonswim	Swim	Nonswim and Swim combined
170 Days	(%)	(%)	(%)
Control	6.2 ± 1.8[a]	2.9 ± 0.5	4.5 ± 1.0
Machine Control	4.2 ± 0.7	4.0 ± 0.5	4.1 ± 0.4
Exercise	1.9 ± 0.7	2.2 ± 0.6	2.1 ± 0.4
270 Days			
Control	2.0 ± 0.4	2.5 ± 0.8	2.2 ± 0.5
Machine Control	1.5 ± 0.5	2.6 ± 0.5	2.0 ± 0.3
Exercise	2.7 ± 0.6	3.5 ± 0.7	3.1 ± 0.4
370 Days			
Control	2.0 ± 0.3	3.5 ± 1.1	2.8 ± 0.6
Machine Control	3.0 ± 1.3	3.2 ± 1.7	3.1 ± 1.1
Exercise	1.8 ± 0.4	2.4 ± 0.5	2.1 ± 0.3
Age Means	170 Days: 3.6	270 Days: 2.5	370 Days: 2.7
Treatment Means	C: 3.2	MC: 3.1	Ex: 2.4
Analysis of variance			
Source	df	F value	Prob >F
Age	2	2.93	ns[b]
Treatment	2	1.51	ns
Age * Treatment	4	2.53	0.05
Swim	1	0.17	ns

[a]Mean ± standard error of the mean.
[b]ns = not significant at $p < 0.05$.

Table 15. Means, standard errors and analysis of variance for relative percentage of LDH isoenzyme 4 (% LDH-4)

	Nonswim	Swim	Nonswim and Swim combined
170 Days	(%)	(%)	(%)
Control	5.8 ± 1.7[a]	6.2 ± 0.9	6.0 ± 0.9
Machine Control	5.3 ± 0.7	9.2 ± 1.4	7.2 ± 0.9
Exercise	3.5 ± 0.8	5.8 ± 1.6	4.7 ± 0.9
270 Days			
Control	4.4 ± 0.7	10.4 ± 2.3	7.2 ± 1.4
Machine Control	6.1 ± 1.5	8.6 ± 1.4	7.2 ± 1.1
Exercise	4.3 ± 0.6	7.6 ± 1.3	5.9 ± 0.8
370 Days			
Control	6.7 ± 1.0	5.8 ± 0.8	6.2 ± 0.6
Machine Control	9.7 ± 4.0	5.9 ± 0.8	7.5 ± 1.7
Exercise	5.9 ± 2.1	8.9 ± 1.2	7.3 ± 1.3
Age Means	170 Days: 6.0	270 Days: 6.9	370 Days: 7.2
Treatment Means	C: 6.6	MC: 7.5	Ex: 6.0
Analysis of variance			
Source	df	F value	Prob>F
Age	2	1.14	ns[b]
Treatment	2	1.60	ns
Age * Treatment	4	0.67	ns
Swim	1	7.52	0.01
Age * Swim	4	3.75	0.025

[a]Mean ± standard error of the mean.
[b]ns = not significant at $p<0.05$.

Table 16. Means, standard errors and analysis of variance for relative percentage of LDH isoenzyme 5 (% LDH-5)

	Nonswim	Swim	Nonswim and Swim combined
170 Days	(%)	(%)	(%)
Control	66.9 ± 4.7[a]	79.0 ± 2.1	73.0 ± 2.9
Machine Control	77.4 ± 1.7	72.3 ± 2.0	74.9 ± 1.4
Exercise	71.4 ± 2.4	82.6 ± 3.0	77.0 ± 2.4
270 Days			
Control	84.6 ± 1.1	79.2 ± 3.3	82.1 ± 1.7
Machine Control	81.5 ± 3.1	80.4 ± 2.0	81.0 ± 1.9
Exercise	83.0 ± 1.8	80.2 ± 2.1	81.6 ± 1.4
370 Days			
Control	73.2 ± 2.9	82.2 ± 2.0	77.7 ± 2.1
Machine Control	75.2 ± 5.9	80.0 ± 2.1	78.0 ± 2.7
Exercise	81.0 ± 2.4	75.1 ± 2.7	78.2 ± 1.9
Age Means	170 Days: 74.9	270 Days: 81.5	370 Days: 77.8
Treatment Means	C: 77.5	MC: 77.8	Ex: 78.9

Analysis of variance

Source	df	F value	Prob>F
Age	2	8.86	0.005
Treatment	2	0.42	ns[b]
Age * Treatment	4	0.39	ns
Swim	1	2.20	ns
Age * Swim	2	4.40	0.025

[a]Mean ± standard error of the mean.
[b]ns = not significant at $p<0.05$.

Table 17. Statistical comparisons made using t test for relative percentages of LDH isoenzymes

Groups compared	% LDH-1	% LDH-2	% LDH-3	% LDH-4	% LDH-5
170 Days					
CNS and CS	*	***	***	ns	**
MCNS and MCS	ns	ns	ns	ns	ns
ExNS and ExS	***	ns	ns	ns	**
270 Days					
CNS and CS	ns	ns	ns	**	ns
MCNS and MCS	ns	ns	ns	ns	ns
ExNS and ExS	ns	ns	ns	ns	ns
370 Days					
CNS and CS	***	ns	ns	ns	*
MCNS and MCS	ns	ns	ns	ns	ns
ExNS and ExS	ns	ns	ns	ns	ns
Control					
170 Days and 270 Days	**	**	**	ns	***
270 Days and 370 Days	*	ns	ns	ns	ns
Machine Control					
170 Days and 270 Days	ns	ns	*	ns	*
270 Days and 370 Days	ns	ns	ns	ns	ns
Exercise					
170 Days and 270 Days	**	ns	ns	ns	ns
270 Days and 370 Days	ns	ns	ns	ns	ns

ns = not significant at $p<0.05$.
* $p<0.05$.
** $p<0.01$.
*** $p<0.001$.

The effect of swimming exercise on % LDH-1, % LDH-2 and % LDH-4 can be demonstrated by combining all experimental animals into nonswim and swim animals. After a swimming exercise % LDH-1 decreased significantly

($p<0.001$) and % LDH-2 decreased slightly ($p<0.05$) in all animals combined. Swimming exercise caused a significant increase in % LDH-4 ($p<0.01$).

Swimming exercise caused a decrease in % LDH-1 in 170-day-old C and Ex animals ($p<0.05$ and $p<0.001$) (Table 17). No change in % LDH-1 was observed after swimming exercise in any of the 270-day-old treatment groups. At 370 days C animals exhibited a decrease in % LDH-1 after swimming ($p<0.001$).

The only change in % LDH-2 following swimming was exhibited by 170-day-old C animals. In this group, swimming exercise resulted in a significant decrease ($p<0.001$) in % LDH-2. There was also a significant decrease in % LDH-3 ($p<0.01$) in 170-day-old C animals.

Although swimming exercise increased % LDH-4 in most treatment groups, the increase was significant only for 270-day-old C animals ($p<0.01$).

Swimming exercise resulted in an increase in % LDH-5 in 170-day-old C animals ($p<0.01$), in 170 day-old Ex animals ($p<0.01$) and in 370-day-old C animals ($p<0.05$).

In order to define more clearly the changes in plasma LDH isoenzyme patterns, the activities of all isoenzymes were calculated by multiplying the relative percentage of each isoenzyme by the total LDH activity measured in the plasma of every animal. The means, standard errors and analysis of variance for LDH isoenzyme activities are presented in Tables 18 through 22. Comparisons between NS and S animals and between age groups are made in Table 23.

Isoenzyme activities were unaffected by treatment (MC, Ex and C). Age caused changes in LDH-4 and LDH-5 while swimming exercise altered

Table 18. Means, standard errors and analysis of variance for plasma activity of LDH isoenzyme 1 (LDH-1)

	Nonswim	Swim	Nonswim and Swim combined
170 Days	(mU/ml)	(mU/ml)	(mU/ml)
Control	5.8 ± 1.0[a]	5.1 ± 1.2	5.4 ± 0.7
Machine Control	3.8 ± 0.6	6.9 ± 0.8	5.3 ± 0.6
Exercise	6.1 ± 0.6	3.3 ± 1.0	4.7 ± 0.7
270 Days			
Control	3.8 ± 0.5	4.7 ± 0.7	4.3 ± 0.4
Machine Control	4.7 ± 1.1	6.0 ± 1.0	5.3 ± 0.7
Exercise	3.4 ± 0.5	5.1 ± 1.2	4.2 ± 0.6
370 Days			
Control	8.0 ± 1.8	4.1 ± 0.8	6.0 ± 1.1
Machine Control	3.5 ± 0.5	6.6 ± 1.0	5.3 ± 0.7
Exercise	5.9 ± 1.0	6.0 ± 1.0	5.9 ± 0.7
Age Means	170 Days: 5.2	270 Days: 4.6	370 Days: 5.7
Treatment Means	C: 5.3	MC: 5.3	Ex: 5.0

Analysis of variance

Source	df	F value	Prob>F
Age	2	1.73	ns[b]
Treatment	2	0.20	ns
Age * Treatment	4	0.83	ns
Swim	1	0.48	ns
Treatment * Swim	2	5.91	0.005

[a]Mean ± standard error of the mean.
[b]ns = not significant at $p<0.05$.

Table 19. Means, standard errors and analysis of variance for plasma activity of LDH isoenzyme 2 (LDH-2)

	Nonswim	Swim	Nonswim and Swim combined
170 Days	(mU/ml)	(mU/ml)	(mU/ml)
Control	2.0 ± 0.5[a]	1.1 ± 0.3	1.6 ± 0.3
Machine Control	1.5 ± 0.5	2.1 ± 0.3	1.8 ± 0.2
Exercise	1.4 ± 0.4	1.7 ± 0.4	1.6 ± 0.3
270 Days			
Control	0.9 ± 0.3	1.1 ± 0.4	1.0 ± 0.2
Machine Control	1.1 ± 0.4	1.3 ± 0.4	1.2 ± 0.3
Exercise	1.2 ± 0.3	1.6 ± 0.4	1.4 ± 0.2
370 Days			
Control	1.0 ± 0.2	2.0 ± 1.0	1.5 ± 0.6
Machine Control	1.0 ± 0.4	1.4 ± 0.7	1.3 ± 0.4
Exercise	1.2 ± 0.3	2.1 ± 0.4	1.6 ± 0.2
Age Means	170 Days: 1.7	270 Days: 1.2	370 Days: 1.4
Treatment Means	C: 1.3	MC: 1.4	Ex: 1.5
Analysis of variance			
Source	df	F value	Prob>F
Age	2	1.65	ns[b]
Treatment	2	0.28	ns
Age * Treatment	4	0.42	ns
Swim	1	2.58	ns

[a]Mean ± standard error of the mean.
[b]ns = not significant at $p < 0.05$.

Table 20. Means, standard errors and analysis of variance for plasma activity of LDH isoenzyme 3 (LDH-3)

	Nonswim	Swim	Nonswim and Swim combined
170 Days	(mU/ml)	(mU/ml)	(mU/ml)
Control	2.0 ± 0.5[a]	1.7 ± 0.3	1.9 ± 0.3
Machine Control	1.7 ± 0.3	2.7 ± 0.6	2.2 ± 0.4
Exercise	0.6 ± 0.2	1.6 ± 0.6	1.1 ± 0.3
270 Days			
Control	1.1 ± 0.2	1.8 ± 0.8	1.4 ± 0.4
Machine Control	0.7 ± 0.2	2.2 ± 0.5	1.4 ± 0.3
Exercise	1.3 ± 0.3	2.5 ± 0.4	1.9 ± 0.3
370 Days			
Control	1.0 ± 0.2	2.6 ± 0.9	1.8 ± 0.4
Machine Control	1.1 ± 0.4	2.4 ± 0.9	1.8 ± 0.6
Exercise	1.1 ± 0.2	1.5 ± 0.4	1.3 ± 0.2
Age Means	170 Days: 1.7	270 Days: 1.6	370 Days: 1.6
Treatment Means	C: 1.7	MC: 1.8	Ex: 1.4
Analysis of variance			
Source	df	F value	Prob>F
Age	2	0.13	ns[b]
Treatment	2	1.00	ns
Age * Treatment	4	1.43	ns
Swim	1	17.54	0.005

[a]Mean ± standard error of the mean.
[b]ns = not significant at $p<0.05$.

Table 21. Means, standard errors and analysis of variance for plasma activity of LDH isoenzyme 4 (LDH-4)

	Nonswim	Swim	Nonswim and Swim combined
170 Days	(mU/ml)	(mU/ml)	(mU/ml)
Control	1.8 ± 0.4[a]	3.5 ± 0.5	2.7 ± 0.4
Machine Control	2.1 ± 0.3	6.0 ± 1.1	4.1 ± 0.8
Exercise	1.1 ± 0.2	4.1 ± 1.5	2.6 ± 0.8
270 Days			
Control	2.3 ± 0.5	6.8 ± 1.1	4.4 ± 0.8
Machine Control	3.1 ± 0.4	7.2 ± 1.6	4.9 ± 0.9
Exercise	2.0 ± 0.3	5.4 ± 1.0	3.7 ± 0.7
370 Days			
Control	3.5 ± 0.8	4.4 ± 0.7	3.9 ± 0.5
Machine Control	4.0 ± 1.7	5.0 ± 1.3	4.6 ± 1.0
Exercise	3.4 ± 1.0	5.3 ± 0.6	4.3 ± 0.6
Age Means	170 Days: 3.1	270 Days: 4.5	370 Days: 4.3
Treatment Means	C: 3.7	MC: 4.6	Ex: 3.5
Analysis of variance			
Source	df	F value	Prob>F
Age	2	4.10	0.025
Treatment	2	2.32	ns[b]
Age * Treatment	4	0.50	ns
Swim	1	42.16	0.005
Age * Swim	2	3.57	0.05

[a]Mean ± standard error of the mean.
[b]ns = not significant at $p<0.05$.

Table 22. Means, standard errors and analysis of variance for plasma activity of LDH isoenzyme 5 (LDH-5)

	Nonswim	Swim	Nonswim and Swim combined
170 Days	(mU/ml)	(mU/ml)	(mU/ml)
Control	26.9 ± 4.5[a]	47.2 ± 8.1	37.1 ± 5.2
Machine Control	31.0 ± 2.5	51.7 ± 12.3	41.4 ± 6.6
Exercise	23.5 ± 1.5	48.7 ± 7.9	36.1 ± 5.1
270 Days			
Control	44.7 ± 4.5	59.9 ± 9.8	51.8 ± 5.3
Machine Control	49.3 ± 7.6	67.9 ± 7.4	57.3 ± 5.8
Exercise	39.0 ± 2.5	57.3 ± 4.0	48.2 ± 3.3
370 Days			
Control	38.1 ± 6.5	62.9 ± 7.0	50.5 ± 5.6
Machine Control	32.5 ± 5.8	67.8 ± 16.1	53.1 ± 10.7
Exercise	52.3 ± 6.3	49.0 ± 7.3	50.8 ± 4.6
Age Means	170 Days: 38.2	270 Days: 53.0	370 Days: 50.4
Treatment Means	C: 46.6	MC: 50.1	Ex: 45.0
Analysis of variance			
Source	df	F value	Prob>F
Age	2	6.77	0.005
Treatment	2	0.72	ns[b]
Age * Treatment	4	0.28	ns
Swim	1	30.64	0.005

[a]Mean ± standard error of the mean.
[b]ns = not significant at $p<0.05$.

Table 23. Statistical comparisons made using t test for activities of LDH isoenzymes

Groups compared	LDH-1	LDH-2	LDH-3	LDH-4	LDH-5
170 Days					
CNS and CS	ns	ns	ns	ns	*
MCNS and MCS	*	ns	ns	**	*
ExNS and ExS	*	ns	ns	*	*
270 Days					
CNS and CS	ns	ns	ns	***	ns
MCNS and MCS	ns	ns	*	**	ns
ExNS and ExS	ns	ns	ns	**	ns
370 Days					
CNS and CS	**	ns	*	ns	*
MCNS and MCS	ns	ns	ns	ns	**
ExNS and ExS	ns	ns	ns	ns	ns
Control					
170 Days and 270 Days	ns	ns	ns	ns	*
270 Days and 370 Days	ns	ns	ns	ns	ns
Machine Control					
170 Days and 270 Days	ns	ns	ns	ns	*
270 Days and 370 Days	ns	ns	ns	ns	ns
Exercise					
170 Days and 270 Days	ns	ns	ns	ns	ns
270 Days and 370 Days	ns	ns	ns	ns	ns

ns = not significant at $p<0.05$.
* $p<0.05$.
** $p<0.01$.
*** $p<0.001$.

LDH-3, LDH-4 and LDH-5 (Tables 18-23).

By combining all animals within each age group, the effect of age on individual isoenzyme activities in plasma can be demonstrated clearly.

Between 170 days and 270 days of age LDH-1, LDH-2 and LDH-3 remained constant. However, LDH-4 and LDH-5 were found to increase significantly between 170 days and 270 days of age ($p<0.01$ and $p<0.001$). This corresponds to the increase in TLDH over this age span. Between 270 days and 370 days of age no changes occurred in any of the LDH isoenzyme activities and no change was observed in TLDH between these two ages.

When individual treatment groups are considered, only MC and C animals had an increase in LDH-5 between 170 and 270 days of age ($p<0.05$). There were no changes in individual isoenzyme activities with age in Ex animals or in C and MC animals between 270 and 370 days (Table 23).

Swimming exercise was found to increase LDH-3, LDH-4 and LDH-5 ($p<0.001$) when all animals were grouped into three ages irrespective of treatment. This corresponds to the increase in TLDH following swimming exercise.

Table 23 demonstrates that swimming exercise resulted in elevated LDH-5 in all treatment groups at 170 days of age ($p<0.05$). At 270 days of age, LDH-5 increased following swimming exercise in all treatment groups but the changes were not statistically significant. At 370 days of age, swimming exercise resulted in an increase in LDH-5 in C ($p<0.05$) and MC ($p<0.01$) animals, but no change was observed in Ex animals.

LDH-4 was elevated in all treatment groups after swimming but the changes were statistically significant only in 170-day-old MC ($p<0.01$) and Ex ($p<0.05$) animals, and in 270-day-old animals (C, $p<0.001$; MC and Ex, $p<0.01$).

LDH-3 was found to increase significantly after swimming in 270-day-old MC animals and 370-day-old C animals ($p<0.05$). However, LDH-3 was generally greater in S animals than in NS animals within all treatment groups.

These results indicate that an increase in age from 170 days to 270 days resulted in an increase in plasma LDH and CPK activity. By determining the relative activities of each LDH isoenzyme, the increase in TLDH appears to be due to elevations of LDH-4 and LDH-5 only. Swimming exercise resulted in an increase in TLDH which was determined to be due mainly to elevations of LDH-3, LDH-4 and LDH-5. Exercise training on a treadmill for 10 weeks had no effect on reducing the elevation of TLDH following the swimming exercise except at 370 days of age.

DISCUSSION

This section will be divided into the same three categories which were used in the RESULTS section: final body weight and organ weights; plasma enzyme activities and swim time; and plasma LDH isoenzymes.

Final Body Weight and Organ Weights

Final body weight

The increase in FBW (Table 2) between 170 days and 270 days of age is similar to that reported by Story (1972), Witte (1974) and Long (1974) for rats of the Sprague-Dawley strain. Both Story and Long reported that the average weekly gain in body weight diminished with increasing age. In this investigation, there was no difference in FBW of rats at 270 days and 370 days of age. This supports the previous work which demonstrated decreased body weight gain with increasing age.

Singh and Kanungo (1968) reported that the peak of growth in rats occurs at approximately 30 weeks of age. After that age, the rate of body weight gain is decreased with increasing age. Story (1972) reported that body weight begins to level off at approximately 220 to 240 days of age. This study demonstrated that body weight does not change to any great extent between 270 and 370 days of age.

The reduction in FBW in exercise trained (Ex) animals is similar to that reported by Story (1972), Long (1974), Witte (1974) and Auth (1975). Auth reported a greater reduction in body weight after ten weeks of exercise training in 100-day-old animals than was observed in this study.

However, Auth used a treadmill training program which was more rigorous (26 meters per minute for one hour daily).

The present study indicates that exercise training caused a greater reduction in body weight at 270 days and 370 days of age than at 170 days of age. This is in agreement with Story (1972) who reported that exercise training had a greater effect on body weight in older animals.

Decreased FBW in exercised animals has been attributed to decreased body fat in epididymal and visceral regions (Hanson et al., 1967; and Jones et al., 1964) and decreased food intake (Mayer et al., 1954). Gross examination of Ex animals at the time of sacrifice in this study revealed that they had less body fat than control animals. No attempt was made to monitor food intake.

As expected there was no difference in FBW between MC and C animals at any of the three ages (Tables 2 and 3). Differences in body weight between NS and S animals within certain treatment groups resulted from an apparent failure to divide these animals evenly according to body weight.

Organ weights

Heart weights and liver weights were demonstrated to increase significantly ($p<0.001$) between 170 days and 270 days of age when all animals are considered irrespective of treatment (Tables 4 and 6). No further increase in HW or LW occurred between 270 days and 370 days of age. Story (1972) and Witte (1974) both found similar increases in HW up to 270 days of age. Changes which were observed in HW and LW apparently coincided with changes in body weight between 170 days and 270 days of age. Control

animals (C) at 370 days of age had significantly smaller hearts ($p<0.01$) and livers ($p<0.01$) than C animals at 270 days of age (Table 3). This was not surprising because the mean FBW of 370-day-old C animals was also less than the mean FBW of 270-day-old C animals ($p<0.05$). No differences in FBW, LW and HW were observed between 270 days and 370 days of age in MC and Ex animals (Table 3).

Analysis of covariance using the REGRESSION procedure demonstrated that FBW had a highly significant effect ($p<0.0001$) on HW. Story (1972) and Witte (1974) have reported similar significant correlations between body weight and heart weight.

Exercise training on a treadmill resulted in a significant increase ($p<0.01$) in HW over that of C animals when these two groups are compared irrespective of age. Ex animals had significantly heavier hearts than C animals at 170 days ($p<0.05$) and 370 days ($p<0.001$) of age even though the FBW of Ex animals was slightly less than that of C animals at these two ages (Tables 2-4). Heart weights from MC and Ex animals did not differ significantly at any of the age levels. At 370 days of age MC hearts were significantly heavier ($p<0.01$) than C hearts even though there was no statistical difference in FBW between these two groups.

Heart weights were corrected for FBW using the REGRESSION procedure. Adjusted heart weights (AHW) reveal that both Ex animals and MC animals had significantly greater AHW than C animals at 170 and 370 days of age (Table 5). At all three ages, Ex animals had significantly greater AHW than MC or C animals. These results are similar to those of Story (1972)

and Oscai et al. (1971). In these studies, animals which were subjected to training programs of either swimming or running had greater heart weights than control animals. Story adjusted heart weights for body weight and Oscai adjusted food intake of control animals so that exercised animals and control animals had comparable body weights. It is apparent that exercise training does result in cardiac hypertrophy.

The differences between AHW of MC animals and that of C animals at both 170 days and 370 days of age suggest that daily handling and confinement in treadmill chambers affected heart weight. Evidence has been provided which demonstrates that endocrinological mechanisms can be involved in cardiac hypertrophy (Beznak, 1962; and Beznak et al., 1969). In these studies both thyroid hormones and desoxycorticosterone were demonstrated to cause significant hypertrophy of heart muscle. The slight cardiac hypertrophy found in 170-day-old and 370-day-old MC animals is probably due to endocrinological changes within these animals. Because this study is not concerned primarily with cardiac hypertrophy mechanisms, further investigation will be necessary in this area.

Differences in liver weight between experimental groups apparently reflect differences in body weight. No correction for body weight was applied to liver weights in this study. The difference in LW between 170-day-old MC and 170-day-old C animals is questionable because of the few number of observations made for LW in these two groups.

Plasma Enzyme Activities and Swim Time

Swim time

The observed decrease in average swim time between 170 and 270 days of age (Table 6) is similar to results reported by Story (1972). He found that young animals (70 to 140 days of age) which swam daily had longer average swim times than older animals (200 to 270 days of age) undergoing the same swimming exercise. His swimming procedure was similar to that used in this study.

Although animals at 370 days of age had average swim times which were less than those of 270-day-old animals, the difference was not significant. It is apparent that increased age resulted in a decreased capacity for physical exercise. This has been demonstrated by a number of other investigations (Edington et al., 1972; Barnard et al., 1974; and Shock, 1961).

Treadmill exercise trained (Ex) animals were not able to swim any longer than MC or C animals. However, they did not demonstrate the decrease in swim time between 170 and 370 days of age which was found in MC and C animals. This suggests that exercise training in older animals maintained a capacity for physical exercise which was similar to that of younger animals. The inability of Ex animals to swim longer than C or MC animals implies that physical training for one type of exercise does not necessarily result in an increased ability for another type.

Total plasma LDH activity

The increase in TLDH between 170 days and 270 days of age (Table 8) occurred in all treatment groups. Between 270 and 370 days of age, TLDH

did not change significantly in any treatment group. Porter et al. (1971) reported that there was no significant difference in rat plasma LDH activity between 4 and 18 months of age. There have been no other investigations made to determine changes with age in plasma LDH activity in rats between these two ages. The results of the present investigation suggest that small, but significant age-related changes in TLDH may occur within the age limits considered in Porter's study.

McQueen et al. (1973) have determined that there is a significant increase in serum LDH activity in humans with age. Serum LDH activities were statistically higher in subjects older than 35 years of age than serum LDH activities in subjects younger than 24 years of age. After 40 years of age, no changes in human serum LDH activity have been observed (Davis et al., 1966).

Total LDH activity has been measured in brain, heart, skeletal muscle, and liver from rats of various ages (Singh and Kanungo, 1968). They reported a significant increase in LDH activity of tissue homogenates from birth to 30 weeks of age in all tissues except liver. After 30 weeks of age there was a significant decrease with age in LDH activity in brain, heart and skeletal muscle homogenates.

Schmukler and Barrows (1966) measured LDH activity in skeletal muscle and heart tissue from rats of different ages. Significantly higher LDH activities were observed at three months of age than at one month of age in both heart and skeletal muscle. Between 12 months of age and 24 months of age, significant decreases in LDH activity occurred in heart and

skeletal muscle. Ross and Ely (1954) reported a 50 percent increase in rat liver LDH activity between birth and 180 days of age. It was suggested by the above investigators that changes in tissue LDH activity with age occurred primarily during the period of most rapid growth.

The peak of the growth period in rats occurs at 30 to 34 weeks of age (Singh and Kanungo, 1968; and Story, 1972). In the present investigation, the experimental period from 200 to 270 days of age corresponds to this peak of growth in rats.

It has been suggested that appearance of LDH activity in plasma is a result of normal cellular turnover (Hess, 1963). It is logical to assume that the increase in LDH activity with age in the tissue homogenates described above may be reflected as plasma or serum LDH activity elevations.

Between 270 days and 370 days of age TLDH did not change significantly in any treatment group or in all animals combined irrespective of treatment (Table 9). Because these same types of changes were observed in FBW with age, an analysis of covariance using regression was carried out in order to determine if there were any effects of FBW on TLDH. No significant correlation was found between FBW and TLDH.

The observed elevations of TLDH which occurred following a swimming exercise are in agreement with results obtained in a number of other studies. Doty et al. (1971) have demonstrated that plasma LDH activity in rats increases fourfold after a swimming exercise of three hours duration. Papadopoulos et al. (1968) observed similar results after rats were forced to swim for four hours. Elevations of TLDH in this study were not as

marked as those described above, but the duration of swimming exercise in this investigation was considerably less than three or four hours.

Loegering (1974) has determined that increased intensity of swimming exercise resulted in consistently greater elevations of plasma LDH activity in rats. Increased duration of swimming exercise was not as effective in causing greater plasma LDH activity elevations. In contrast, the present investigation demonstrated that the duration of swimming exercise influenced TLDH. An analysis of covariance using regression revealed a significant relationship between swim time and TLDH ($p<0.0325$). However, animals in the present study had weights attached to their tails to increase the intensity of swimming exercise. Animals in Loegering's study did not have weights attached to their tails. It is apparent that duration of exercise may be more effective in causing TLDH elevations when the intensity of exercise is greater.

When TLDH was adjusted for swim time (A-TLDH), marked age-related differences in the A-TLDH response to swimming exercise were observed in all treatment groups (Table 10). The percent increase in A-TLDH, as a result of swimming, was reduced with increasing age in Ex animals (77% increase at 170 days, 44% increase at 270 days and 3% increase at 370 days). In contrast, MC and C animals had greater elevations of A-TLDH as a result of swimming exercise with increasing age. In MC animals, A-TLDH increased by 26%, 40% and 92% after swimming exercise at 170 days, 270 days and 370 days, respectively. A similar trend was observed in C animals (31% increase at 170 days, 34% increase at 270 days and 56% increase at 370 days).

This study was designed to investigate the effects of physical training on the plasma LDH activity response to a text exercise and to determine if these effects were influenced by age. The results clearly demonstrate that swimming exercise resulted in consistently greater elevations in older nontrained animals than in younger nontrained animals. In physically trained animals the opposite occurred. Consistently smaller elevations in A-TLDH as a result of swimming were observed in older trained animals when compared to younger trained animals.

Hunter and Critz (1971) and Beaton (1966) have suggested that elevations in plasma enzyme activities following exercise may be used as an indication of the level of physical fitness of an experimental animal or subject. The results of the present investigation strongly suggest that the intensity of treadmill training used in this study was more beneficial for 370-day-old animals than for younger animals. It is possible that increased intensity or duration of treadmill training would have been necessary at 170 days and 270 days of age to prevent an elevation in total plasma LDH activity following a swimming exercise.

Swimming exercise caused greater elevations in total plasma LDH activity with increasing age in nontrained animals. It would appear that the exhaustive swimming exercise became more detrimental to the nontrained rat with increasing age.

Several possible mechanisms have been suggested for the elevation of LDH activity in plasma following exercise. They are muscle cell damage, relative hypoxia in certain tissues and catecholamine release.

Reports of muscle cell damage caused by exercise have been given by Altland and Highman (1961). In order to produce histological changes in skeletal muscle, they subjected rats to a walking exercise at a speed of 6.9 meters/minute for 16 hours. Even with that exercise of excessive duration, slight muscle cell necrosis could be detected in only 25 percent of the animals.

In most investigations concerned with exercise-induced enzyme release into plasma or serum, the duration of the test exercise is much less than 16 hours. Highly significant elevations of plasma LDH activity have been achieved with only 30 minutes of swimming exercise and 30 minutes of treadmill exercise (Loegering, 1974). It is probable that detectable muscle cell damage may cause enzyme release into plasma but is not necessary for the elevation of plasma enzyme activities following exercise.

Certain tissues may be subjected to relative hypoxia or localized oxygen tension changes during exercise. Perfusion of isolated rat hearts with anoxic media has caused release of LDH into the perfusion fluid (Butterworth et al., 1970; and de Leiris et al., 1969). In contrast, Feuvray et al. (1974) could not induce release of LDH during or following perfusion of rat hearts with anoxic media for periods up to 30 minutes.

Zierler (1958) incubated whole rat skeletal muscles in various incubation media and reported an increase in the rate of aldolase efflux with incubation in anoxic media. He suggested that appearance of

glycolytic enzymes in blood plasma represented efflux from muscle as a result of localized changes in oxygen tensions and altered cell membrane permeabilities.

Loegering and Critz (1971) subjected anesthetized dogs to various levels of oxygen ventilation for 30 minutes. It was demonstrated that LDH activity increased significantly in arterial blood following hypoxic exposure. The levels of oxygen ventilation used were such that they produced venous oxygen tensions similar to those reported to occur during maximal exercise. However, the extent of LDH activity elevation following hypoxia was less than that observed following maximal exercise.

Cunningham and Critz (1972) found that exercise-induced elevations in plasma LDH activity were greater when experimental subjects breathed lower levels of oxygen compared to room air. However, the differences were not statistically different. In other studies, exposure of rats to reduced oxygen levels associated with high altitudes has resulted in increased serum LDH activity (Altland et al., 1964; and Altland et al., 1968).

It is apparent that hypoxia can elicit changes in plasma LDH activities and may be a contributing factor in alterations of plasma LDH activities following exercise. However, most investigations suggest that hypoxia is not the primary cause of exercise-related changes in plasma enzyme activities.

Catecholamine release during exercise has also been implicated as a factor in enzyme release into plasma. Raven et al. (1970) have

demonstrated that total plasma catecholamines were increased significantly ($p<0.05$) when rats were forced to swim to exhaustion with five percent of their body weight attached to their tails. The increase in total catecholamines was found to be due primarily to increased norepinephrine levels. Plasma LDH activity was also increased significantly following this exhaustive exercise.

Highman and Altland (1960) were able to block elevations of serum LDH activity in dogs exposed to altitude conditions. They did this by pretreating dogs with pharmacological adrenergic and ganglionic blocking agents. In another study, epinephrine injections prior to exercise resulted in increased activities of serum LDH-1 and LDH-2 (Garbus et al., 1964).

It is apparent that catecholamine release can be one of the factors involved in cellular enzyme release due to exercise. It has been postulated that catecholamines alter cell membrane permeability and allow the efflux of intracellular enzymes (Highman and Altland, 1960).

A number of other hormones have been implicated as factors controlling plasma or serum enzyme activity alterations. These include thyroid hormones (Graig and Ross, 1963; and Fleisher et al., 1965) and glucocorticoids (Critz and Withrow, 1965; Solymoss and Jasmin, 1975; Jacey and Schaefer, 1967; and Wagner and Critz, 1968).

Alterations in plasma enzyme activities following exercise apparently are caused by release of enzymes from intracellular locations into the circulatory system. This may result from cell damage or changes in cell membrane permeability. Any factors which are responsible for membrane

permeability changes may thus be involved in this mechanism of enzyme release. It is reasonable to assume that exercise-induced changes in plasma or serum enzyme activities result from the combined effects of a number of factors and no simple explanation can account for all of the variations which have been observed.

It has been demonstrated that exercise training can reduce the elevations of plasma LDH activity observed following a test exercise (Novosadova, 1969; Papadopoulos et al., 1968; and Garbus et al., 1964). It has been postulated that exercise training increases the number of mitochondria and energy-producing enzyme systems in cardiac and skeletal muscle. During exercise this increased amount of available energy in trained subjects or experimental animals may be responsible for maintaining the normal integrity of cell membranes and prevent enzyme release into serum (Hunter and Critz, 1971).

Total plasma CPK activity

The results obtained concerning TCPK in this study are questionable. The method used by the Antonik laboratories for CPK analysis is geared toward detection of abnormally high CPK activities in blood. Analysis of variance for TCPK reveals a highly significant Age * Treatment interaction (Table 11). This may have been due to the fact that blood samples from different age and treatment groups were sent for analysis at different times. It also may have resulted from different time intervals between sample collection and analysis.

The results of TCPK analysis are included in this study because the same type of age effects and swim effects on TCPK were observed as were

observed on TLDH (Table 11). Klosak and Penny (1975) have observed a sixfold increase in serum CPK activity in rats following a swimming exercise. In another study, Loegering (1974) has observed a threefold increase in TCPK following swimming. The duration of swimming exercise in these two studies was considerably greater than the average swim times observed in the present investigation. It has also been noted that maximum CPK activity elevations occur in serum of humans several hours after exercise (Nutall and Jones, 1968). The time interval between exercise and blood sampling in this study may not have been great enough to detect any marked changes in TCPK.

Plasma LDH Isoenzymes

The relative percentages of LDH isoenzymes present in plasma were determined in this study in order to make some estimates of the origins of increased TLDH between 170 days and 270 days of age, and of increased TLDH as a result of swimming exercise. The LDH isoenzyme distribution patterns of many tissues and organs from rats have been characterized (Wieland and Pfleiderer, 1961; and Papadopoulos et al., 1967). Liver, skeletal muscle, red blood corpuscles and platelets contain relatively high percentages of LDH-5 while heart, kidney and brain have relatively more LDH-1 and LDH-2.

Analysis of variance for the relative percentage of each LDH isoenzyme (Tables 12-16) reveals that age significantly affected LDH isoenzyme patterns in plasma. The relative percentages of LDH-1, LDH-2, and LDH-5 were all affected by age ($p<0.005$). Between 170 days and

270 days of age there was a significant decrease in % LDH-1 and % LDH-2 ($p < 0.001$) and a significant increase in % LDH-5 ($p < 0.001$) in all animals grouped together irrespective of treatment. Between 270 and 370 days of age, there was a slight increase in % LDH-1 ($p < 0.05$) and a slight decrease in % LDH-5 ($p < 0.05$).

The age interval between 170 days and 270 days of age also corresponded to a significant increase in TLDH in all animals combined irrespective of treatment ($p < 0.001$). Changes in relative percentages of LDH isoenzymes are difficult to interpret. In order to clarify changes in the isoenzyme patterns in plasma which occurred in this study, the activities of all five LDH isoenzymes were calculated for each experimental animal (Tables 18-22). By comparing all animals at 170 days of age with all animals at 270 days of age, the increase in TLDH over this age period is apparently due to increased LDH-4 and LDH-5. No change in isoenzyme activities occurred with age between 270 days and 370 days of age (Tables 18-22).

These results are supported by Porter et al. (1971) who found increased plasma LDH-5 activity in 18-month-old animals when compared to four-month-old animals. However, as mentioned earlier, there was no difference in total plasma LDH activity between these two ages. No other data are available on age-related changes in rat plasma LDH isoenzyme patterns.

Many studies concerned with alterations in plasma LDH isoenzyme patterns in rats as a result of exercise and training have been published.

However, in a number of studies, the age of the experimental animals was not defined or animals were selected for study from a certain body weight range. The results of this study indicate that age affects total plasma LDH activity and isoenzyme patterns significantly during the rapid growth period up to approximately 30-36 weeks of age. After this peak in growth there apparently is no change in plasma LDH isoenzyme patterns or TLDH up to 370 days of age. Previous investigations utilizing animals of different ages under 30 weeks of age may have to be reevaluated to account for any possible age effects on plasma enzyme activities.

No significant treatment (Ex, MC and C) effects on plasma LDH isoenzyme patterns were revealed by analysis of variance (Tables 12-16 and 18-22). Raven et al. (1970) found that rats subjected to a training program of swimming exercise for 6 weeks had a significant decrease in % LDH-1 and significant increase in % LDH-5. In contrast, Bloor and Papadopoulos (1969) and Papadopoulos et al. (1968) found that swimming daily for four hours over an experimental period of ten weeks caused no changes in plasma LDH isoenzyme patterns in rats. The animals in Raven's study had initial body weights which ranged from 326 g to 531 g. The changes which were detected in plasma LDH isoenzyme patterns may have been due to age effects rather than training effects.

Swimming exercise prior to sacrifice caused significant changes in TLDH as well as in the isoenzyme distributions in plasma. Analysis of variance (Tables 12-16) reveals that swimming affected % LDH-1 ($p < 0.005$), % LDH-2 ($p < 0.05$), and % LDH-4 ($p < 0.01$). Analysis of variance for

individual isoenzyme activities (Tables 18-22) demonstrates changes in LDH-3 ($p<0.005$), LDH-4 ($p<0.005$) and LDH-5 ($p<0.005$) as a result of swimming exercise. Swimming decreased % LDH-1 and % LDH-2 while increasing % LDH-4 in all animals considered irrespective of age and treatment. However, by determining individual isoenzyme activities LDH-3, LDH-4 and LDH-5 were found to increase significantly as a result of swimming exercise (Tables 20-22). LDH-1 and LDH-2 were not affected by swimming in this study.

These results are in agreement with those of Raven et al. (1970). He used an exhaustive swimming exercise similar to the swimming exercise used in this study. After exercise, rats in that study demonstrated a decreased % LDH-1 ($p<0.01$) and an increased % LDH-4 ($p<0.01$). Similar responses to swimming were observed in both trained and untrained animals.

The results of the present study also agree with those of Anderson (1976) who observed increased serum activities of LDH-4 and LDH-5 following exercise in horses. In contrast, Garbus et al. (1964) reported that individual activities of all five isoenzymes were increased in rat serum following a walking exercise. However, the test exercise employed was one of extremely long duration (16 hours). Others have found increased LDH-1 and LDH-2 activities in serum or plasma from rats as a result of swimming exercise (Papadopoulos et al., 1967; Papadopoulos et al., 1968; and Doty et al., 1971). However, in all of these studies the test exercise was a three or four hour swimming test. It is apparent that the duration of the swimming exercise employed is an important factor in the resulting changes in individual isoenzyme activities.

In the present study the duration of the swimming exercise was relatively less than three or four hours. It is possible that if the animals in this study had been forced to swim for longer periods of time without weights attached to their tails, greater elevations in TLDH and individual isoenzymes would have occurred.

CONCLUSIONS

The results of the present investigation indicate that significant changes occur with age in total plasma LDH activity. This has been determined to be a result of increased activities of LDH-4 and LDH-5. This change occurred primarily during the age period associated with rapid growth of the experimental animals (between 170 days and 270 days of age). After the rate of growth had diminished, no changes occurred in either TLDH or activities of individual isoenzymes (270 days to 370 days of age). These changes occurring with age possibly reflect changes which have been reported to occur with age in a number of organs.

These results strongly suggest that previous investigations concerned with the measurement of plasma or serum enzyme activities, in which the age of the experimental animal is not carefully specified should be reevaluated. Some of the inconsistencies which have been reported may be a direct result of age differences in experimental animals.

Swimming exercise resulted in elevated total plasma LDH activity. This increase in TLDH as a result of swimming exercise was found to be primarily due to increased activities of LDH-3, LDH-4 and LDH-5. This suggests that organs such as skeletal muscle and liver are the primary sites of LDH release during exhaustive exercise of relatively short duration.

In both groups of control animals (MC and C) the elevation of plasma LDH activity (TLDH) following a swimming exercise was augmented by increasing age. In contrast, the elevation of TLDH following exercise in

physically trained animals (Ex) was decreased by increasing age. Ten weeks of treadmill running was apparently more effective in reducing the plasma LDH response to exercise in older animals than in younger animals. It is apparent that the intensity of treadmill training used in this study was more beneficial to older animals (370 days of age) than to younger animals. Increased intensity of training at younger ages may be necessary to prevent elevations in plasma LDH activity as a result of exercise.

SUMMARY

The effects of age, physical training and exhaustive exercise on plasma lactate dehydrogenase (LDH) activity were investigated in male rats. Activities of individual LDH isoenzymes in plasma were also determined. Rats were divided into three age groups: 100 days, 200 days and 300 days of age. Each age group of animals was subdivided into three treatment groups: exercised animals (Ex), machine control animals (MC) and sedentary control animals (C). The Ex animals were physically trained for a period of ten weeks, five days per week, on a treadmill for 30 minutes daily. The treadmill was set at an eight degree incline and a speed of 15 meters per minute. The MC animals were subjected to the same daily handling and confinement within the treadmill chambers as the Ex animals but were not subjected to treadmill running. The C animals were not handled but remained in cages for the ten week period.

At the conclusion of the ten week experimental period, all treatment groups were further divided into nonswim (NS) and swim (S) animals. All S animals were subjected to an exhaustive swimming exercise immediately prior to being anesthetized for blood sampling. All NS animals were anesthetized without having been subjected to a swimming exercise.

Plasma prepared from blood removed from the jugular veins of anesthetized animals was analyzed for total LDH activity and LDH isoenzyme distribution. The duration of the swimming exercise, final body weight, heart weight and liver weight were also determined.

Results of this study were as follows:

1) Final body weight increased with age up until 270 days and remained constant between 270 days and 370 days of age. The exercise trained rats exhibited a reduction in body weight when compared with control animals.

2) The heart weight was increased by exercise training, but also, to a lesser extent, by daily handling and confinement on the treadmill.

3) Animals which had been physically trained were unable to swim longer than control animals at all three age levels but did not exhibit the decrease in duration of swim time with increased age observed in control animals.

4) Total plasma LDH activity increased significantly between 170 days and 270 days of age. This increase corresponded to elevations in the activities of LDH isoenzymes four (M_3H) and five (M_4). No changes in total plasma LDH activity or of individual isoenzyme activities were observed between 270 days and 370 days of age.

5) Total plasma LDH activity increased following an exhaustive swimming exercise. This increase corresponded to elevations in the activities of LDH isoenzymes three (M_2H_2), four (M_3H) and five (M_4).

6) The elevation of total plasma LDH activity following the exhaustive swimming exercise was augmented with increased age in non-trained animals but was diminished with increased age in physically trained animals. This suggests that the exercise training program used in this study was more beneficial at older ages.

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APPENDIX A

Determination of Total Plasma LDH Activity

1. Plasma samples were stored at 4° C prior to analysis and total LDH activity was determined within 18 hours after sample collection. Only samples with no visible hemolysis were used.

2. The following reaction was utilized for total LDH activity determination:

$$\text{L - lactate} + NAD^{+} \xrightarrow{\text{LDH}} \text{NADH} + \text{pyruvate.}$$

NADH production per unit time was measured as the increase in absorbance of light at 340 nm using a Bausch and Lomb spectronic 600 Spectrophotometer.

3. Commercial reagents from Calbiochem (LDH-L Stat-Pack) were reconstituted with double distilled water to yield a substrate solution containing approximately

0.05 M glycine buffer, pH 8.8

0.07 M lactate

0.0035 M NAD

4. Plasma samples and substrate solution were placed in a water bath at 30° C for at least 15 minutes prior to LDH activity determination.

5. 200 microliters of plasma sample were added to 3.0 ml of substrate solution in a clean, dry cuvet and the cuvet was covered with Parafilm and inverted gently 4-5 times.

6. The cuvet was placed in a constant temperature cuvet holder (30° C) within the spectrophotometer and the absorbance was recorded at 30 second intervals for at least 2 minutes.

7. The entire reaction temperature was maintained at 30 + 1° C by circulating water at that temperature through the constant temperature cuvet holder.

8. Total plasma LDH activity was calculated using the following formula:

LDH activity in mU/ml = change in absorbance over 2 minutes x 1290 (Calbiochem, 1974).

9. The reaction temperature was carefully monitored and if it varied from 30° C, the calculated total LDH activity was multiplied by the following temperature factors:

Temperature	Factor
29.0 C	1.070
29.5 C	1.035
30.5 C	0.965
31.0 C	0.930

10. The calculated total LDH activity was reported as the number of milliunits per milliliter of plasma where one milliunit equals one micromole of NADH produced per minute per milliliter of plasma (Elliot and Wilkinson, 1963).

APPENDIX B

Separation of Plasma LDH Isoenzymes

1. Frozen plasma samples were thawed and 15-20 microliters of plasma were applied on the cathode side of a cellulose acetate strip, 2.0 to 2.5 cm from the center (Gelman Sepraphore III, 2.5 x 15.2 cm).

2. Electrophoretic separation of the plasma sample was carried out on the cellulose acetate strips using a Gelman Rapid Electrophoresis Chamber and Gelman High Resolution Buffer (Tris-Barbital-Sodium Barbital; pH 8.8; ionic strength, 0.075). A current of 1.0 to 1.5 ma per strip was applied for 90 minutes using a Gelman DC power supply.

3. During the electrophoretic separation a working solution was prepared from the following reagents:

a. 1.0 ml of 1.0 M sodium lactate (60 percent syrup diluted 1:4 with 0.1 M phosphate buffer, pH 7.5)

b. 3.0 ml of nitro blue tetrazolium (1.0 mg/ml in double distilled water)

c. 0.3 ml of phenazine methosulfate (1.0 mg/ml in double distilled water)

d. 1.0 ml of 0.1 M phosophate buffer (13.6 g KH_2PO_4 plus 3.3 g NaOH per liter of double distilled water)

e. 10 mg of nicotinamide adenine dinucleotide (NAD, stored desiccated in 10 mg aliquots in the freezer and added to the final working solution in the dry state)

4. All reagents were stored at 5° C. Phenazine methosulfate was prepared fresh weekly.

5. During the electrophoretic separation, clean cellulose acetate strips (2.5 x 7.5 cm) were floated on the working solution in a petri dish until fully saturated and then placed on glass plates in a covered plastic box lined with moist, surgical gauze squares.

6. After the electrophoretic separation, the cellulose acetate strips carrying the plasma sample LDH isoenzymes were inverted and placed on top of the reagent-soaked strips. The plastic box was closed and incubated in an oven maintained at 37° C for 30 minutes.

7. After incubation both the reagent strip and sample strip were removed from the plastic box, fixed in a methanol-acetic acid solution (50% methanol; 40% double distilled water; 10% glacial acetic acid) for 10 minutes, and allowed to dry.

8. The strips were scanned on a Photovolt Densicord 542 densitometer.

9. Estimation of the relative percentage of each LDH isoenzyme was determined by planimetry of the areas beneath the curves of the densitometric tracings.