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EXERCISE, ANABOLIC STEROIDS AND CASTRATION: THE EFFECT ON ENERGY MOBILIZATION

by

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A THESIS

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DEDICATION

To those who made it all possible

John and Edith Murray

ABSTRACT

Seventy-two male Wistar rats were used in this study to determine the effects of prolonged exercise, an anabolic steroid and castration on energy storage and mobilization in trained and untrained rats.

The animals were randomly assigned into a control group of 20 animals and an exercise group of 52 animals, respectively. The exercised rats were trained to run on a motor driven treadmill and after four weeks were capable of running continuously for one hour at one mph, 5 days per week. Each animal continued to run at this intensity for an additional six weeks. Twenty-six of the trained rats were run to exhaustion immediately prior to sacrifice and the time to fatigue was recorded. All remaining animals were sacrificed at rest. During the course of the experiment one-half of the exercised rats and one-half of the sedentary rats received an anabolic steroid, "Winstrol" (17β-hydroxy-17α-methylandrostano (3,2-c) pyrazol) (I.P. 0.80 mg/ kg), once a day, for ten weeks. Prior to the training regime, bilateral castration was performed upon 16 of the exercise animals and 8 of the control rats. Unilateral castration was performed upon an equal number in each group. No surgical operation was performed on the other animals. The weights of the testicles were recorded, and at the conclusion of the experiment, they were compared to the weights of the testicles of the non-castrated animals.

Analysis of the results indicated that training had no significant effect on the parameters measured, with the exception that the increase in the weight of the body and the liver was retarded with training. Prolonged exercise to fatigue resulted in a decrease in gastrocnemius glycogen and an increase in plasma FFA levels and lipid

FFA mobilization. The anabolic steroid "Winstrol", had no effect on the body weight, liver, spleen, heart, or testicular weights in the trained rats. However, in the untrained animals the body and the liver weights were lighter. Both the trained and sedentary animals had smaller adrenals, with steroid administration. The resting blood glucose levels, FFA levels, and the glycogen stores were not altered by the drug. "Winstrol" had no effect on the mobilization or the utilization of glycogen or blood glucose with exhaustive exercise.

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ABBREVIATIONS

FFA - free fatty acids

ATP - adenosine triphosphate

ACTH - adrenocorticotrophin

SGOT - serum glutamic - oxaloacetic transaminase

RNA - ribonucleic acid

B-DPN - B-diphosphopyridine nucleotide

B-DPNH - B reduced diphosphopyridine mucleotide

GL-6-P - glucose-6-phosphate

K - potassium

P - phosphorous

Ca - calcium

ICSH - interstitial cell stimulating hormone

DPN - diphosphopyridine nucleotide

IP - intra-peritoneal

PCA - percholric acid

H_2SO_4 - sulphuric acid

IN - one normal

CHAPTER I

INTRODUCTION

Since Kochakian's (79, 80) classic demonstration of the nitrogen-retaining properties of testosterone, many attempts have been made to find synthetic steroids that would produce the anabolic or growth-promoting effects characteristic of testosterone without causing the androgenic or secondary sex-stimulating effects. Among the compounds purported to have preferential anabolic activity is 17β-hydroxy-17α-methylandrostano (3, 2-c) pyrazole (Winstrol). "Winstrol" is one of the steroid drugs most widely used for weight gain, since it exerts a positive effect on nitrogen retention (110). However, it is most often utilized by athletes because of its low androgenic properties.

In the recent past, the use of anabolic steroids has been largely limited to athletes participating in events in which muscular strength is required. As a result, most of the researchers studying the effects of anabolic steroids on trained individuals have utilized a weight training regime (28, 69, 72, 140, 146). At the present time, however, use of these drugs appears to be more widespread among athletes in various other sports requiring endurance type training (125).

Johnson and O'Shea (69) observed a significant increase in the oxygen uptake of individuals subjected to anabolic steroids and a weight training regime. However, these findings (69) have not been substantiated by other studies on endurance athletes (126) or on athletes engaged in strength activities (28, 72). Endurance training clearly induces significant adaptations in the histochemical and biochemical characteristics of muscular fibres (26). The oxidative capacity of the skeletal muscles of animals trained to run on forced or voluntary exercise wheels or motor-driven treadmills, for from 6 to 23 weeks, has been shown to increase (56). An increase in the cross-sectional area of the skeletal muscle fibre has also been demonstrated (26, 46). By examining the effects of androgen deprivation, the physiological function of androgen can best be demonstrated. Such effects are apparent in the primary and secondary sex organs, and skeletal muscles; in the general metabolic effects on skeleton, skeletal muscle, body organs and water balance (155).

Statement of the Problem

The purpose of this study was to determine the changes that occur in the storage and mobilization of energy sources, as a result of an anabolic steriod, exercise, and castration to male rats. Blood and muscle samples and the body and organ weights were to be obtained from control animals, trained and untrained rats, and bicastrated, unicastrated and non-castrated animals.

Changes in the following variables were to be considered for each of the above groups, at rest and at fatigue:

(1) the concentrations of glucose, lactate and free fatty acids in the blood;

(2) skeletal and heart muscle glycogen concentrations;

(3) the concentration of free fatty acids in a tissue sample from the epidydimal fat pads;

(4) the animal's body weight and the weights of the liver, spleen, adrenals, heart, and testicles.

Rationale Behind the Study

It is generally accepted that glycogen and free fatty acids are used as fuels by working muscle (43). During prolonged exercise to fatigue the limited quantity of substrate in muscle is supplemented by mobilization of energy reserves from the liver and adipose tissue, respectively. Many physiological substances are known to stimulate mobilization of stored energy reserves, however, the effects induced by the androgens are not clear.

It would appear that the mechanism of action of the androgens must vary in accordance with the ability of the tissue to respond to the presence of a particular androgen (97). The factors of age, sex, and steroid dosage also have a profound influence on the characteristics and location of the myotropic effect. A great many studies investigating the effects of anabolic steroids on carbohydrate, fat and protein metabolism have been carried out, however, the mechanisms controlling these metabolic processes, during rest and exercise, are not fully understood.

Most research into the effects of anabolic steroids on trained individual has dealt with the areas of muscular strength (28, 53, 69, 72, 126, 140, 146), power (69), and oxygen uptake (69, 72, 126, 146). In addition, a few studies (28, 58, 69, 126, 150) have considered the blood chemistry.

In spite of the widespread use of anabolic steriods by athletes to improve their performance, the knowledge in this area is minimal. Very little consideration has been given to the effects of anabolic steroids on energy storage and mobilization, for endurance type events or training.

CHAPTER II

REVIEW OF THE LITERATURE

Anabolic Steriods

Nearly every tissue in the body is influenced to a certain degree in its development and functions by androgens, depending on the chemical nature and proportion of the various androgens present (81, 82, 95), and the animal species (81, 86, 95). The mechanism of action of the androgen varies in accordance with the ability of the tissue to respond to the presence of a particular androgen (97). The factors of age, sex, steroid dosage, and type of steroid (97) also have a profound influence on the characteristics and location of the myotrophic effects.

Early investigators (31, 101-106, 116) attempted to demonstrate the presence of a hormone (hormones) in the testes, which was capable of producing a decrease in urinary nitrogen excretion. Kochakian and Murlin (79) and Kochakian (80) reported that "male hormone" extracts prepared from the urine of male medical students, produced a marked decrease in the urinary nitrogen excretion of "thin" and "fat" castrated dogs, fed a constant diet. This was due to a decrease in urea. A similar reduction in urinary nitrogen was produced by small frequent injections or by a single large injection (81).

Kochakian (89) and Stafford et al (148) later described the nitrogen-retaining effect produced by the administration of an anabolic steriod. No later than 2 - 3 days after the initial steroid administration to the castrated animals, nitrogen excretion decreases. After a few days, it reaches a minimum, remains at this level for several days, and then in spite of continued steroid administration, it rises again and soon reaches the original level. This decline of the effect cannot be delayed simply by a larger dosage (90). The maximum rate of retention was found to be directly proportional to the mass of the dog at .05 to .06gm nitrogen/ kg body weight/ day (81).

The normally functioning testes, on the other hand, not only decrease the maximum attainable response but also further delays its appearance by about two weeks. If the individual is producing an excess amount of protein anabolic steroids, as in the adreno-genital syndrome, then the parenterally administered substances are ineffective (81). Therefore, it appears that the animal will respond to a protein anabolic stimulus from a steroid, only if it is receptive to that particular stimulus (81). Cessations of the androgens, always resulted in the loss of some of the nitrogen by the "fat" dog, but only in one experiment in the "thin" dog. This presumably is a rebound phenomenon. The time at which the effect declines is variable and depends on the physiological state of the experimental animals. The decreasing effect is delayed considerably in animals deficient in either androgen or protein, indicating that the stimulation of protein synthesis by anabolic steroids apparently ceases as soon as any protein deficiencies have been overcome (110).

The advance from simple description of the nitrogen-retaining effect to the interpretation of the androgen effect as a stimulation of the formation of cellular protein resulted when numerous authors reported that nitrogen-retention is paralled by a lowered excretion of K, P, Ca, creatine, creatinine, and water, and that this retention could occur without an increase in extracellular concentrations of these substances. Specifically, concentrations of K and P follow those of nitrogen, i.e. are in proportions consistant with protein anabolism. The amount of water retained is determined by the binding capacity of the newly synthesized proteins (110).

Stimulation of protein synthesis by anabolic steroids seems to be qualitatively independent of the functional condition of the endocrine glands (110). The nitrogen-retaining property of androgens takes place in the absence of the testes, anterior pituitary (44, 81, 83, 137), the adrenals (83, 90), and the thyroid gland (92). It is not yet certain whether anabolic steroids can act at all in the complete absence of insulin (33, 98, 147).

The amount of nitrogen retained under the influence of an androgen is dependent, within limits, upon the composition of the diet before and during the balance studies (110). Most critical is the protein content. With a protein-free diet (57) and with fasting (8), the nitrogen-retaining activity of anabolic steroids is decreased appreciably or is completely absent (164). The protein-sparing effect of carbohydrate is too short in duration (112) to allow an increase in nitrogen retention. With a minimal protein diet (0.2 gm nitrogen/ kg body weight/ day), it was shown that a measurable nitrogen-retention appears only when the amount of dietary protein rises above the minimum. Similarly, an increase in the protein content of the diet, above an optimal level, did not result in additional nitrogen retention with testosterone propionate (91).

Androgens show definite effects on carbohydrate metabolism that persist throughout administration even when the effects on protein metabolism are no longer apparent (98). This increase in body weight over that accounted for by extra protein anabolism and the decrease in blood and urine sugar, could be explained by conversion of the extra carbohydrate to fat under androgen stimulation (98). It is of interest that the normal animal, without surplus available glucose, responds to androgen treatment with an increased utilization of endogenous fat (98).

A small portion of the carbohydrate utilized under androgen stimulation may be deposited as glycogen along with the increase in muscle mass (12, 114, 118, 141), and some may be oxidized to compensate for the loss of energy due to the decrease in protein catabolism (98). Meyer and Hershberger (118) concluded that the effect of testosterone propionate on the musculature is mediated by a primary effect on energy-producing processes in the musculature and that protein synthesis is accelerated when additional energy in the form of glycogen becomes available. Bergamini et al (12) state that RNA and protein synthesis are essential to hormonal influence and that increased glycogen synthesis depends upon protein synthesis. These authors (12) suggest that increased glycogen synthesis can be attributed to the greater penetration and more rapid phosphorylation of blood glucose, and also to increased GL - 6 - P, independent of glycogen-synthetase activity. These two phenomena would explain the increased glycogen synthesis demonstrated by the increased incorporation of both glucose and pyruvate (12).

The reports concerning changes in liver glycogen, after the administration of anabolic steroids, are conflicting (111, 160). Weisenfeld and Goldner (160) have suggested that the liver glycogen stores are reduced, due to either inhibition of gluconeogenesis or an alteration in liver function. Landon et al (111), on the other hand, observed that there were still adequate supplies of glycogen in the liver, and that the glycolytic pathway was normal.

In recent animal studies, Gillespie and Edgerton (36) showed that the ability of muscle tissue to synthesize and store glycogen is partially dependent on adequate testosterone levels. They (36) stated that approximately 33% of the enhanced glycogen storage resulting from training appears to be attributable directly or indirectly to the presence of normal testoterone production or adequate testosterone propionate replacement. The observed rise in testosterone in exercise may therefore, be important in utilization and replenishment of muscle glycogen (150).

Testoterone may in addition influence carbohydrate metabolism in muscle by increasing the availability of creatine to ATP to form creatine-phosphate (128). Administration of synthetic androgens that are methylated at the 17-position, such as "Winstrol", causes an increase in creatinuria, which is probably due to increased creatine synthesis in the liver or kidneys (140). If muscle phosphocreatine had been significantly changed, current theories would indicate that shifts in rate of phosphate exchange, and therefore of lactate and pyruvate production, would have resulted. Since this did not occur, it would appear that the creatine changes after methyl testosterone are not due to any shifts within the muscles themselves (140).

Aside from their influence on the synthesis of lipoproteins (32, 59), androgens and anabolic steroids have only a minimal significant effect on lipid metabolism (110). The response of the serum total cholesterol and phospholipid concentrations appear to be determined by the nature of the responses of the major lipoprotein fractions, since virtually all of the serum cholesterol and phospholipids circulate as lipoproteins, rather than as individual molecules of lipid (32). It should also be noted that no decreased activity was noticed in respect to lipoprotein effects with the steroidal pyrazoles or other testosterone derivatives studies (59). The demonstration of this relationship between chemically determined lipids and the serum does not determine whether the primary effects of gonadal steroids with respect to lipids is on the metabolism of the lipid molecule as such, or on some aspect of lipid synthesis or degradation (32).

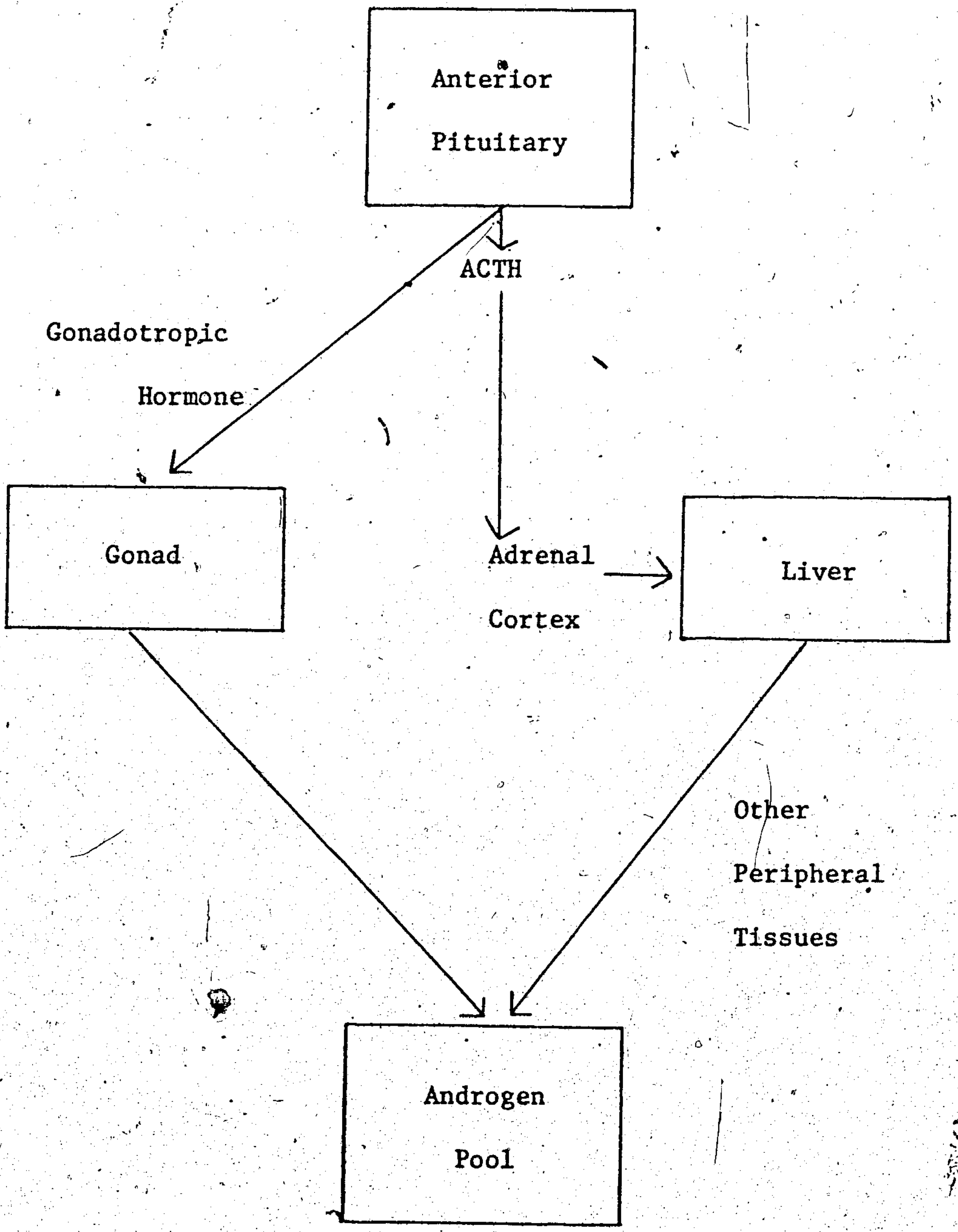
The decrease in effect of an androgen on nitrogen retention was a concomittant decrease in body weight is attributed to a loss of body fat accompanied by a redistribution of carcass protein to the continued synthesis of new tissue at other sites, especially the accessory sex organs and to a smaller degree the kidneys. The depletion of both carcass protein and fat is influenced by the intensity of the dosage and the duration of the androgen treatment (88).

The loss of carcass fat on prolonged treatment may be due to an increase in energy metabolism due either to increased activity of the animal, therefore, a secondary phenomenon, or it may represent the cost of redistributing the protein from the carcass to the internal organs. The nature of the demand, in any event, is such that it is not compensated for by an increase in appetite (88). The ability of the androgen to stimulate a utilization of the carcass fat occurs readily only in those animals that had or would have had a higher than normal deposit of carcass fat as a result of prolonged time after castration. In those animals that had a normal or subnormal amount of fat the utilization of the carcass fat did not occur until the nitrogen retention effect of the androgen had decreased (88).

Very few studies have reported data on the influence and androgens and anabolic steroids on oxygen uptake ability (28, 69, 72, 126). Johnson and O'Shea (69) observed a statistically significant increase in oxygen uptake in male subjects given "Danabol" for three weeks, and a weight training programme for six weeks. However, a later examination of the effects of "Danabol" on competitive swimmers produced no changes in the oxygen uptake ability (126). At this time, no evidence has been found that athletes whose success depends upon cardiovascular endurance have used steroid treatment (69).

Figure 1

Control of the Production of Testosterone



Johnson and O'Shea (69) also reported that some of their subjects indicated a near absence of normal muscle soreness and stiffness following the training sessions. It may be that by increasing the SGOT levels a negative nitrogen balance is prevented from developing, and therefore, an athlete could recover physiologically to a greater extent between exercising periods permitting training at, or near capacity with greater frequency (69). Other authors (59) observed an increased sense of well-being, an increase in energy, and a decrease in fatiguability, after androgen administration.

The chemical data obtained, in the analysis for protein, non-protein and water in the temporal and hindleg muscles of the guinea pig (97, 142) correlates highly with histologic observations (97). The diameter of the muscle fibrils has been shown to decrease after castration and to increase after testosterone administration. There was no indication of a loss or gain in the number of fibrils, therefore, the changes in striated muscle following castration and androgen administration are a matter of atrophy and hypertrophy, respectively.

Castration

The levels of testoterone are maintained by a negative -feedback control mechanism operating through the hypothalamus and the pituitary (figure 1). Ninety-five per cent of the androgen secretion in the male is of testicular origin. However, several moderately active male sex hormones called adrenal androgens are continually secreted by adrenal cortex. In the normal physiology of the human being these adrenal androgens have relatively insignificant effects.

The physiological function of androgen can be seen best by examining the effects of androgen deprivation. Such effects are apparent in the primary and secondary sex organs and skeletal muscles; in the general metabolic effects on skeleton, skeletal muscle, body organs and water balance (154). In all cases, a distinction should be made between pre-pubertal and post-pubertal results of castration, for in general pre-pubertal testicular androgen deprivation results in failure of development of both morphological and behavioural male characteristics. Post-pubertal castration, on the other hand, does not necessarily cause complete regression of androgen-dependent tissues (155). It may be that the adrenals are capable of partially maintaining these androgen-dependent tissues following post-pubertal castration (155).

The primary sex structures are the ducts associated with the transport of spermatozoa, the seminal vesicles and prostrate, levator ani and bulbocarvernosus (and all other muscles of the perineal complex), and the external genitalia. All of these are completely dependent on androgen both for their morophological and functional intergrity. Pre-pubertal castration results in an immediate cessation of the growth of some of these tissues (97). Several other organs - lacrimal glands, salivary glands, kidneys and urinary bladder - show a considerable decrease in their rates of growth and maximum size attained after castration (97).

The adrenals, spleen and thymus, in contrast with the other organs, increased in weight after castration. The spleen and the thymus attained a maximum size about two weeks after castration and then decreased, but remained larger than in the normal animal. The decrease in size of these organs coincided with the increase in size of the adrenals (97).

In adult male rats, the reaction of the adrenals depends very much on the steroid dosage employed. Only intermediate dosages (up to 5 mg of testosterone daily) cause atrophy of the gland (11, 14). The observed weight responses, of course, do not allow any conclusions about the function of the adrenal cortex. Whether these phenomena are due to an androgen-dependent alteration of the ACTH secretion or to a direct effect of the androgens on the adrenals, cannot be answered at this time (110). However, a major consideration is that gonadal function was significantly affected by the injected hormone and the results thus represent a combination of both exogenous hormone and altered endogenous gonadal secretion (77), since the secretion of gonadotropin (ICSH) is inhibited by anabolic steroids (110).

Both the kidney and the liver are affected to some degree by castration and androgen replacement. Kochakian et al (88) observed a marked increase in kidney size only after injection for 125 days. A small increase in the weight of the liver resulted, but extensions of the injections did not enhance the effect (88). The percentage of water and nitrogen in the liver and kidney, with administration of androgens, depends on the dosage of the steroid (113, 143). The weight of the liver decreases, as does nitrogen content with relatively high dosages of testosterone propionate and with a protein deficiency (110). The protein and water content of the kidney (87) and the activity of the renal enzymes (97) change in direct proportion to the weight changes. Both the liver and the kidney possess the potential to oxidize far in excess of the amount of androgen that might be circulated in the blood stream or required by the animal, however, the amount of co-enzyme (DPN) present is relatively small compared with the amount needed to give a maximum effect (95).

The changes in weight that occur in all of the tissues after castration and androgen administration are accompanied by changes in the nucleic acids (123). It is now established that growth (protein synthesis) is mediated through the synchronized action of several specific RNA's (100). The nature of the androgen influence varies among the different categories of nucleic acids and also the various tissues, however, it seems that the protein and amino acid content of the tissues varied in direct proportion with the changes in weight (99). It appears that the primary site of action of androgens is in the area of synthesis of RNA for incorporation into the microsomal material, so that synthesis of protein and enzymes (99) may be accomplished. Some changes occur in the nuclear RNA (99).

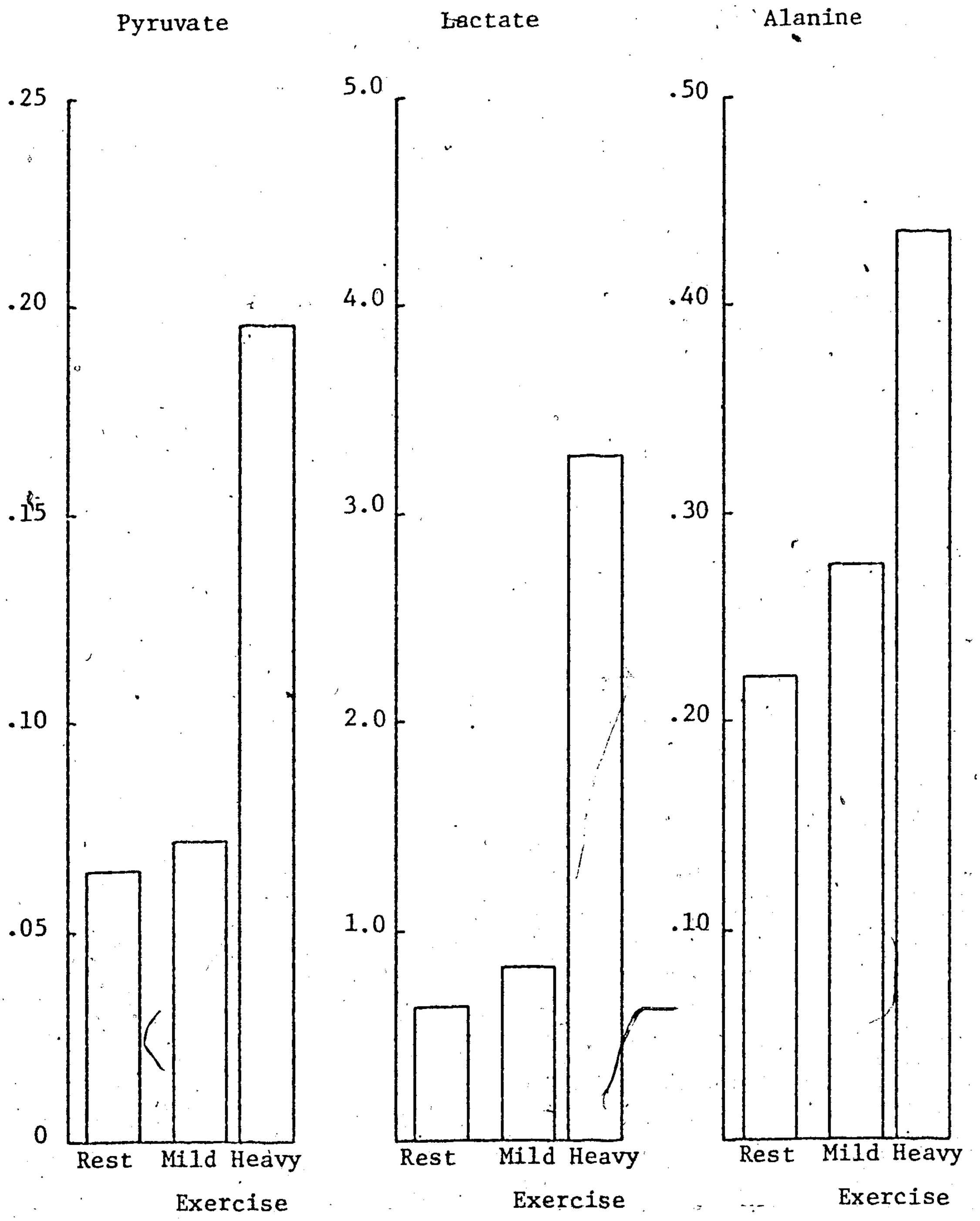
Exercise

It has been demonstrated that physical training can produce a significant increase in the oxidative capacity, enzyme activity, and total protein of the mitochondrial fraction of the rat skeletal muscle (56). These changes however, occur only when the exercise is of a low tension - high repetition nature (40). It is also a well established fact that certain forms of exercise cause an increase in muscle size. The diameters of the individual muscle fibres increase, and the fibres gain in total quantity of contractile protein, as well as various nutrient and intermediary metabolic substances, such as ATP phospho-creatine and glycogen (46).

Protein Metabolism and Catobolism

The skeletal muscles of animals trained to run on forced or voluntary exercise wheels or on motor-driven treadmills for from 6 to 23 weeks have in increase in the concentration of sarcoplasmic protein and a decrease in the concentration of myofibrillar protein (45). However, the optimal duration and intensity of the exercise needed to induce the increase in mitochondrial protein concentration is unknown (39, 56, 108).

Figure 2



Arterial Pyruvate, Lactate and Alanine During Exercise

(Mean, mMole / litre)

It has been suggested that intensity may be as important as duration in producing these changes (108).

The influence of exercise on amino acid metabolism is also of interest. Felig and Wahren (27) observed a highly significant correlation at rest and during mild and heavy exercise. The effect of exercise on the arterial concentrations of the glycolytic end-products pyruvate and lactate and on arterial alanine levels are shown in figure 2. During both mild and heavy exercise the anticipated increases in arterial pyruvate and lactate were observed (27). There was also a notable increase in arterial alanine. The arterial concentration of all other amino acids was unchanged from resting levels or varied less than 10 per cent (27).

It would seem that alanine formation and release are not solely dependent on protein dissolution, but are related to peripheral glucose utilization and pyruvate formation. Accordingly, it appears that alanine is synthesized by transamination of glucose-derived pyruvate (27). In as much as certain amino acids, notably leucine, isoleucine, and valine are preferentially catabolized in muscle, a steady flow of amino groups is available for this transamination (119). Supporting this theory is the recent observation that a significant proportion of the muscle glycogen utilization in exercise cannot be accounted for by lactate formation or carbon dioxide production (61). Alanine may serve as a key endogenous substrate for hepatic glucose production. It has been estimated that production of glucose-derived alanine by muscle occurs at 35-60% of the rate at which lactate is produced, and that alanine and that alanine release may account for 12-18% of the glucose extracted by muscle (27).

The studies on the acute effect of exercise on protein catabolsim are contradictory (17, 18, 21, 35). Carbohydrate and fatty acids are the major energy sources for muscular work in the isocaloric state (5, 19, 49, 65, 127). According to Benedict et al (10), in the hypocaloric state protein can supply a significant proportion of the calories during exercise. Mole and Johnson (120), on the other hand, stated that the enhanced protein catabolism produced by exercise is not mediated by a hypocaloric state or a caloric deficit. These authors (120) observed that protein catabolism occurred following exercise, when the subjects were in a hypocaloric state. It may be that a greater quantity of amino acids and proteins accumulate in the muscles with hypercaloric feeding (120).

The increased excretion of nitrogen and sulphur after exercise may reflect the catabolism of proteins that have leaked out of the muscles (120). An increase in circulating amino acids could also occur from a re-distribution of the free amino acid pools of the tissues (129). Prolonged exercise appears to inhibit the secretion of insulin (131, 166). Moreover, strenuous exercise results in an increase in circulating corticosteroids (132). Both of these hormonal effects of exercise would favour a re-distribution of the free amino acids from muscle to blood and the catabolism of amino acids in the liver and kidneys (122). However, this only occurs in the hypocaloric state.

Glucose Mobilization and Utilization

Evidence seems to indicate that the liver has the capacity to increase its glucose production drastically during physical exertion (13, 136). It would seem that the increased arterial glucose concentration during heavy work is not caused by a reduction in muscle glucose utilization, but a marked increase in splanchnic glucose production, related to the work performed. Although hepatic gluconeogenesis increases during exercise it can supply no more than 10-20% of the total glucose production, the remainder presumably being derived from hepatic glycogenolysis (158). During androgen administration, there may also be an increase in hepatic gluconeogenesis, due to an increase in corticosterone, which is a glucocorticoid (29).

Blood glucose assumes an increasingly important role as a substrate for muscle oxidation as exercise increases. According to Hermansen et al (51), during exercise of moderate to heavy intensity (50 - 70%), there is a significant decrease in blood sugar and in plasma insulin. In severe exercise, it was found that there was little or no fall in blood glucose (75 - 80% maximal oxygen consumption), while in work at 85 - 90% of the maximal oxygen consumption, which lasts for 20 minutes or more, there was a small but significant increase in circulating glucose concentration.

Muscle Glycogen Mobilization and Utilization

During submaximal exercise requiring 60 - 80% of aerobic power, glycogen consumption is very high and the local stores are gradually depleted, at fatigue (13, 52, 61). The rate of glycogen depletion is determined by the relative rather than the absolute intensity of the muscle (61). During prolonged exercise the muscle glycogen content falls as a triphasic curve (13). The greatest glycogen depletion occurred in the first 20 minutes of exercise, followed by plateau effect over a 40 - 60 minute period and then a final decline to the point of exhaustion (153).

The resting glycogen content of skeletal muscle can be elevated through training (13, 52, 61). An increase in glycogen in the heart muscle has also been noted, after training (25). It has been shown that the activity of several individual aerobic and glycolytic enzymes is increased by muscular exercise and training (56). The increased activity rate of the glycolytic enzymes implies that glycogenolysis increases with muscular work (153). According to Taylor et al (153), the rate of glycogen depletion was much faster and the total amount of glycogen used was greater following training. However, this finding does not agree with Hultman's (61) or Holloszy's (55) hypothesis that glycogen breakdown is slower in well-trained than in untrained subjects. Recent studies, however, on the effect of training on the levels of phosphorylase and glycogen synthetase would tend to substantiate Taylor's hypothesis (154).

Free Fatty Acid Mobilization and Utilization

Lipids are the most concentrated source of energy utilized by the organism, yielding per gram, over twice as many calories as carbohydrate or proteins (162). The depot lipid consists chiefly of triglycerides and the more nearly saturated it is, the larger the energy yield from oxidation. In man, for example, the adipose tissue is from 90 - 99% triglyceride, with small amounts of diglycerides, phospholipids, and cholesterol (54). The lipids in the tissues consist both of neutral fat and phospholipids (139).

Prior to being used these triglycerides must be reduced to the metabolized form of free fatty acids (162). The FFA formed in this manner rapidly diffuse out of the adipose tissue into the blood, since they are lipid soluable and therefore, can diffuse through the cell membrane. The ionized fatty acids combine, almost immediately, with the albumin of the plasma proteins and this complex is then transported to the other parts of the body in the blood. Anywhere from 3 to 30 molecules of fatty acid can combine with a single albumin molecule (46).

The triglycerides can be rapidly mobilized with only a small loss of energy in the conversion. The free energy change on hydrolysis amounts to about 1.4% of the total energy yielded by oxidation of the molecule (22). The plasma FFA level, for instance may decrease (30) or increase (9) during work, or it may remain unaltered (49), depending on the intensity and duration of the exercise (133).

Fat, in the form of FFA, serves as a major fuel for muscular work in man (152). It has been estimated that fat may contribute up to 90% of the total substrate oxidized during submaximal work (15, 49, 64, 152). There is also an extremely rapid increase in the FFA concentration after exercise with the level being about doubled in 5 minutes (13), indicating that exercise is a stimulus for lipolysis during muscular work (152). Gollnick et al (43) have indicated that more than one hormonal system may be involved activating lipolysis in the rat during exercise. Basu et al (9) arrived at similar conclusions for exercising humans.

Johnson et al (71) observed that trained and untrained subjects showed definite differences in the concentration of metabolities, in their blood, related to energy supply. The FFA levels rose steadily in the trained and untrained during exercise, but while it almost doubled in the athletes it trebled in the untrained group after one and one-half hours. These authors (71) hypothesized that athletes can oxidize fatty acids more effectively than can untrained subjects, due to their increased ability to oxidize metabolic fuels aerobically (66). In the untrained dog, during moderate exercise, the oxygen supply to the adipose tissue is inadequate, and therefore re-esterification prevails and the turnover rate of FFA decreases (66). Morgan et al (121) found that after 4 - 6 weeks of quadriceps training there was a wide range of values for intracellular triglycerides in both the control and trained muscles. However, there was a net increase in triglycerides in the trained muscles as compared to the control.

Blood Lactate

If the exercise is of short duration and high intensity (greater than 60% of the maximal oxygen consumption) lactic acid is more likely to be produced (55). Karlsson and Saltin (74) observed that high values for blood lactate were obtained when the type of exercise was such that the oxygen supply was generally inadequate. This means there is a greater utilization of carbohydrate, since only carbohydrate can take part in the anaerobic energy yield (5), and lactic acid is produced.

Increased quantities of lactate found in the blood are observed to decrease during sedentary recovery or in recovery involving submaximal exercise (78). The locations of removal and the fate of the lactate and the various removal sites are but partially understood. The amounts found in urine (68, 70) or excreted in sweat appear to be negligible. The kidneys might, however, remove a significant quantity by gluconeogenesis. Removal has been observed in the heart (16), in the liver (134), and in the resting limbs with resting skeletal muscle tissue presumably playing the dominent role in the latter (78).

Use of lactate as a substrate would appear to be its fate in the heart and skeletal muscle. It is generally held that gluconeogenesis is impossible in mammalian skeletal muscle due to the lack of appropriate enzymes for conversion of pyruvate to phosphopyruvate (109). The fate of the lactate in the liver would presumably be via gluconeogenesis (78).

A lower lactate production has been observed in the muscles of trained individuals during submaximal exercise (55). One factor which probably contributes to, and acts synergistically with, the decreased rate of glycolysis, in the trained individual, is the relatively greater utilization of fatty acid oxidation to fulfill the energy requirements of submaximal exercise (19, 48, 65, 66). Another possibility, which has been suggested by Holloszy et al (55), is that glycolysis and glycogenolysis occur at a slower rate in the muscles of trained individuals during submaximal exercise. As a result, pyruvate and DPNH should be formed at a slower rate at a given submaximal work load, accounting for a lower lactate production in trained as compared to sedentary muscles (55).

Body and Organ Weights

Training and exercise appear to affect the body weight and the weights of several organs in the body. Kimeldorf and Baun (76) observed that daily exhaustive swimming exercise of 15 to 30 minutes duration is capable of significantly depressing the rate of body weight gain. This reduction in body weight is probably the result of an increased energy expenditure through exercise, accompanied by a concurrent decrease in food consumption (76, 144, 160). The absolute weight of the gluteus maximus (76) and the leg musculature (149) was reduced but, relative to the body weight, the musculature was larger in the exercised animals. The heart may constitute a much greater portion of the body weight in exercised animals, since daily exhaustive exercise was found to produce a significant increase in its size (76, 149). Gollnick et al (41), however, did not find this increase in weight in their trained animals.

The responses of the kidney, testes, thyroid and spleen to exercise depends on the work intensity, duration of the exercise and the degree of repetition of the exercise experience imposed. Kimeldorf and Baum (76) observed that the weights of the testes and the pituitary were not appreciably altered by the number of exercise trails used in their study, despite the decrease in body weight. However, it is generally concluded that training produces hypertrophy of the adrenals (37, 42, 47, 50, 76) and that a decrease in spleen weight occurs during exercise (7, 42, 144, 149, 157). Exhaustive exercise results in a significantly smaller kidney size. However, the per cent change in kidney weight was nearly identical to the per cent change in body weight, therefore the proportion of kidney to body weight was undisturbed by exercise. The weight of the thyroid gland decreased at the beginning of the exercise regime but, this trend was subs[illegible]ersed, so that the thyroid gland of the exercised animals was [illegible]y larger than that of the control animals (76).

CHAPTER III

PROCEDURES

Seventy-two male Wistar rats, with initial body weights ranging from 175 to 225 grams, were used in this experiment. At the initiation of the study, the animals were six to eight weeks old. All animals were housed in individual 7x10x7 inch selfcleaning cages in a temperature-controlled room maintained at $24^{o} \pm 1^{o}C$. The humidity in the room was relatively constant. The day length (8 a.m. - 8 p.m.) was artificially controlled by an automatic timer. The animals were fed a complete diet (Rockland Complete Diet for Rats). Each animal received daily food and water, ad libitum. Each animal was observed daily for stress signs and was weighed each Monday, so that any sudden weight changes could be observed.

The rats were randomly assigned into a control group of 20 animals and an exercise group of 52 animals, respectively. The exercised rats were trained to run on a motor driven treadmill (Collins Company) and after four weeks were capable of running continuously for one hour at one mph, (See Appendix A). Each animal continued to run at this intensity for an additional six weeks. Initially, mild electrical stimulation was used to train the rats to run.

Prior to the exercise programme, bilateral castration was performed, under light ether anaesthesia, upon sixteen of the exercised animals and eight of the control rats. Unilateral castration was performed upon an equal number in each group. No surgical operation was performed on the other animals. The weights of the testicles were recorded, and at the conclusion of the experiment, they were compared to the weights of the testicles of the non-castrated and uni-castrated animals.

During the course of the experiment one half of the exercised rats and one half of the non-exercised rats received an anabolic steroid, 17 β - hydroxy - 17α - methyl androstano (3, 2-c) pyrazol, (Winstrol - Registered brand of Stanazolol - 50 mg/ml Anabolic), once a day, for ten weeks. The accepted dosage of 5.0 mg for each 65 kg of body weight, per day, ~~for humans~~ was used. The proportional dosage per rat was 0.16 mg of "Winstrol." One ml of "Winstrol" was diluted in 300 ml of distilled water and each animal was injected I.P., with a dose of 1 cc daily. The solution was shaken each time it was used to ensure that the concentration was homogeneous.

After a period of ten weeks, sacrificing was begun. Thirty-two to thirty-four animals were sacrificed each week for three weeks. The exercised rats continued to run daily, until they were sacrificed. The body weights of the animals were recorded just prior to death. Twenty-six of the trained rats were run to exhaustion immediately prior to death and the time to fatigue was recorded. All remaining animals were sacrificed at rest.

The animals were sacrificed, by insanguination, under light ether anaesthesia. A mid-line incision was made and eight to ten ml of blood were withdrawn from the bifurcation of the abdominal aorta into heparinized syringes. Two ml of the blood were quickly pipetted into a centrifuge tube, containing 4.0 ml of cold Perchloric Acid (PCA), to be used for the determination of the lactate concentration (145). One ml of the blood was pipetted into an Erlenmeyer flask and was then assayed for glucose concentration (124). The remainder of the blood was centrifuged at 3000 rpm to obtain the blood plasma. Duplicate one ml samples of fresh plasma were removed to be analyzed for plasma free fatty acid concentration.

The "true glucose" levels were measured by a modification of the enzymatic technique of Nelson and Somogyi (124). The alkaline solution is heated with the protein-free filtrate, so that the blood sugar reduces the cupric hydroxide to cuprous oxide. Arsenomolydate is then added. This reoxidizes the copper and is itself reduced to give a complex of deep greenish-blue colour, the intensity of which is proportional to the amount of reducing sugar present (124).

The absorbences were read on a Beckman DU-2 spectrophotometer at 540 mu. Distilled water was used in the blank and the reading of the blank was adjusted for 100 per cent transmittance or zero optical density, on the spectrophotometer. Standard glucose solutions were made by diluting the standard glucose stock solution with 0.25 per cent benzoic acid. The concentrations of these standard solutions were plotted against their absorbence readings to produce a standard curve. (Sample in Appendix A).

The left gastrocnemius and the left biceps brachii muscles, as well as the heart were removed, and all visible fat, connective tissue and blood were removed with a probe, forceps, and gauze. The samples were weighed immediately on a Roller-Smith precision torsion balance and then transferred with forceps to the bottom of capped test tubes. Immediately the samples and test tubes were frozen in a mixture of Dry Ice and 95 per cent ethanol. The pan, with traces of blood still remaining, was again weighed, and the weight of the blood subtracted from the previous weight to give the weight of the sample in the test tube. Samples were maintained deep-frozen (60°K) until assay.

Glycogen was determined, in the samples of skeletal and heart muscle, by the technique of Lo, Russel and Taylor (115). This modified phenol-sulphuric acid colorimeter technique has been shown to compare favourably with previously substantiated methods for glycogen recovery, colour reaction and reliability (115).

All chemicals used were reagent grade or certified A.C.S. For the standard glycogen solutions, glycogen powder (25 mg, Fisher No. G-47, reagent chemical) was dissolved in distilled water to five ml. This gave a glycogen concentration of five mg/ ml. Less concentrated standard glycogen solutions were prepared by volumetric dilution of this stock solution. Water distilled in pyrex was used throughout.

After the samples were removed from the deep freeze, they were kept in ice until assayed. An appropriate dilution of a prepared glycogen solution was made by the addition of distilled water. The weight of the original muscle sample determined the degree of dilution used. A one ml sample of the above glycogen solution was hydrolyzed using sulphuric acid. The acid was added to yield glucose, which was then dehydrated to form 5-hydrozymethyl furfural. This substance combines with two molecules of phenol to give a yellow-orange colour. Blanks were prepared by using ml of distilled water instead of the glycogen solution.

The absorbence was read on a Beckman DU-2 spectrophotometer at 490 mu. All tests were carried out in triplicate to minimize errors resulting from accidental contamination with cellulose lint. Triplicate one ml samples of standard glycogen solutions, containing from five ug to 100 ug glycogen, were made. The average readings of their absorbences were plotted against the amount of glycogen used to produce a standard curve. (Sample in Appendix A).

The method used for the determination of long-chain free fatty acids was developed by Dole and Meinertz (24). This method of extraction has been used mainly for studies of changes in the concentration of fatty acids in blood plasma (23), and for the output of fatty acids from the isolated samples of adipose tissue (162).

The ternary mixture, heptane-isopropyl alcohol-water, provides a convenient two-phase system for extraction of long-chain fatty acids (23). When the solvent components are taken in suitable proportions, the phases separate rapidly, without centrifugation. The long-chain fatty acids distribute predominantly into the upper, nonpolar phase, whereas the more polar acids remain below (24).

The single extraction method of Dole and Meinertz (24) was used wit the following modifications: One ml of fresh plasma was analyzed rather than 2 ml and therefore, the amount of each reagent used for the analysis was also halved. As well, Nile Blue A was used as the titration indicator, in place of thymol Blue, as it gave a more reproducible end-point.

Recrystallized palmitic acid was weighed and dissolved in heptane to make a reference solution with known concentration. The quantity of sodium hydroxide used to titrate the standard solutions was plotted against the concentration of the palmitic acid solution, to produce a standard curve. (Sample in Appendix A). The blank extract contained 1.0 cc of water instead of plasma; the standard, 3.0 cc of heptane. A new blank and standard were made each day. The acid was titrated with a base, sodium hudroxide, in the presence of one ml of indicator. Nitrogen was bubbled through the alkali, while titrating to eliminate carbon dioxide.

Fat was removed from the epididymal fat pads and weighed on a Roller-Smith precision torsion balance. A fat sample, weighing approximate 800 mg was transferred with forceps into a 25 ml Erlenmeyer flask containin 20 ml of fat extraction mixture: IN H_2S04 (0.1 volume), heptane (1.0 volume), isopropyl alcohol (4.0 volume). The fat sample was then homogenized at medium speed in a Virtis "45" homogenizer for five minutes.

Five ml of the above solution were placed in a screw cap test tube and were shaken, and then left to stand for five minutes. The procedure for the analysis of plasma free fatty acids was then followed (24). A second fat sample was removed from the fat pad and weighed. Approximately 300 mg of fatty tissue was added to 4.0 ml of Krebs Ringer solution containing 5 per cent bovine albumin (PH 7.4). One ml aliquots were taken before and after three hours of incubation at 37°C in a metabolic shaker. The procedure of Dole and Meinertz (24) for the analysis of plasma free fatty acids was then followed.

The blood lactic acid was determined by the Sigma Kit Method (145). In the presence of lactic dehydrogenase, lactic acid and B-Diphosphopyridine Nucleotide (B-DPN) are converted to pyruvic acid and reduced B-Diphosphopyridine Nucleotide (B-DPNH) respectively. Since B-DPNH has a high optical density at 340 mu and B-DPN a low optical density at 340 mu, the concentration of lactic acid converting excess B-DPN to B-DPNH may be determined by measuring the optical density of the reaftion mixture at 340 mu. As this reaction will not go to completion unless the pyruvic acid is removed from the mixture, a glycerine-hydrazine buffer is added in order that the hydrazine will complex with the pyruvic acid.

A series of working standard lactic acid solutions were made using distilled water. The samples and standards were read at 340 mu on the Beckman DU-2 spectrophotometer. The blank was adjusted for 100 per cent transmittance. A standard curve was obtained by plotting the lactic acid standard solutions in mg per cent against the absorbence readings for these solutions (Sample in Appendix A).

Several of the testicles and the adrenals were preserved in formaldehyde so that histological studies could be carried out at a later date. Serial sections of the adrenals and the testicles were embedded in parafin wax and fixed in 10 per cent formalin and stained with hematoxylin and eosin.

Statistical Analysis

The experiment was conducted using a completely random design with four rats per treatment group. Initially each rat was randomly assigned to one of eighteen treatment groups. Each of the eighteen treatment groups were tested individually as to their effect on the variable being measured, i.e. glycogen, glucose, body weight, etc. Fifteen different one-way analyses of variance were run. Only the values obtained at the conclusion of the experiment were used in these analyses.

In addition to the analyses of variance tests, multiple comparisons were made between cell means using the Newman-Keuls procedure (164). This modified statistic is particularly useful in probing the nature of the differences between treatment means following an overall F. The Newman-Keuls procedure is more stringent than the Tukey test, i.e. a larger difference is required for statistical significance. The 0.05 level of alpha was considered the point of significance for all F ratios and multiple comparisons.

CHAPTER IV

RESULTS

The summary tables of the cell means, and the results from the Analysis of Variance and the Newman-Keuls procedures are found in Appendices B and C.

Sources of Energy for Metabolism

Graphical representations of the means contained in Appendix B, for glycogen in the gastrocnemius, biceps and heart; free fatty acids in the plasma and the adipose tissue; and glucose and lactic acid in the blood, are found in Figures 3 to 10.

Blood glucose levels were not altered by training, castration or anabolic steroids. An exception to this finding was evident in the group that was run to fatigue, after receiving the anabolic steroid ($P<0.05$) (Figure 6).

Significant treatment effects for training for glycogen in the gastrocnemius were found (Figure 3). A comparison between pairs of cell means yield significant differences in glycogen in the gastrocnemius, for the trained groups sacrificed at rest and at fatigue ($P<0.01$). There were no significant differences in glycogen in the bicep or heart muscle for these trained groups. (Figures 4 to 5). Neither training nor the anabolic steroid had any significant effect on the amount of glycogen stored in the heart or skeletal muscles. Castration produced no significant changes in the levels of glycogen in the gastrocnemius, Biceps or heart muscle, in any of the animals.

Exercise to fatigue resulted in increased mobilization or an increased concentration of FFA in all of the trained groups, except for the unicastrated, anabolic steroid group. Training was found to produce

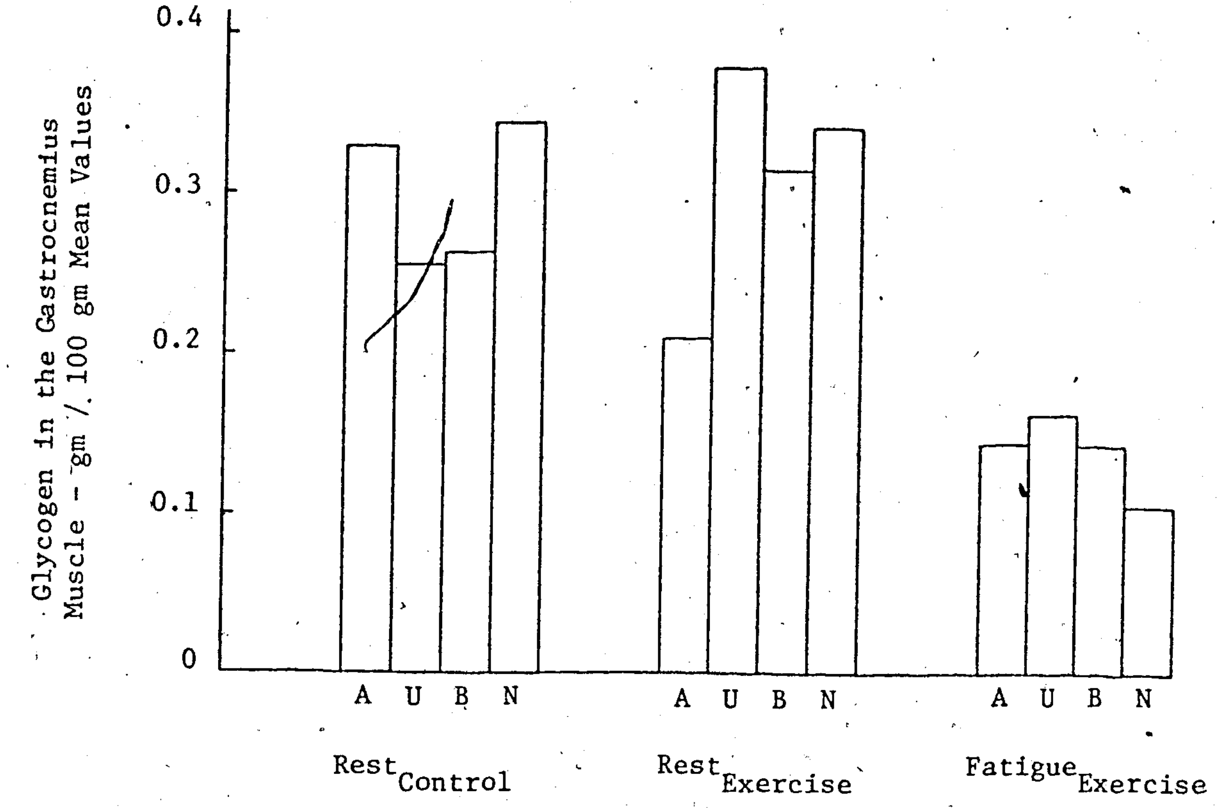


Figure 3

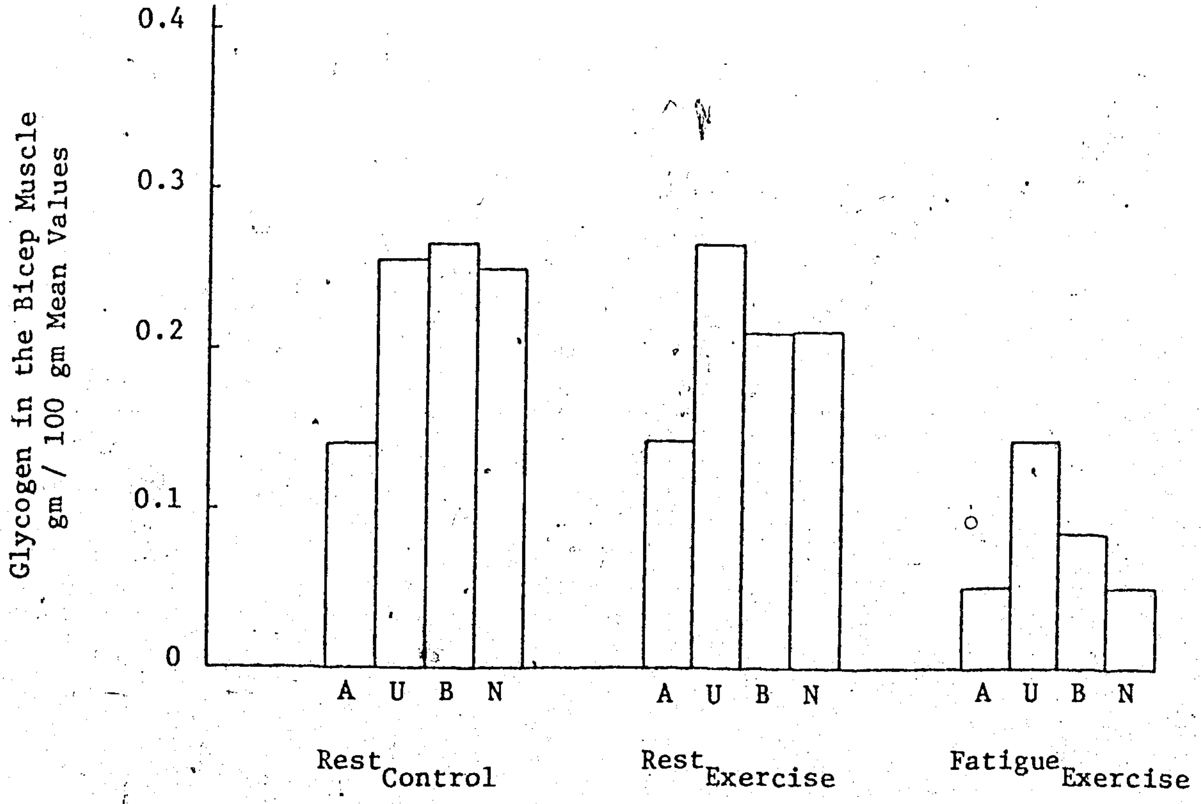
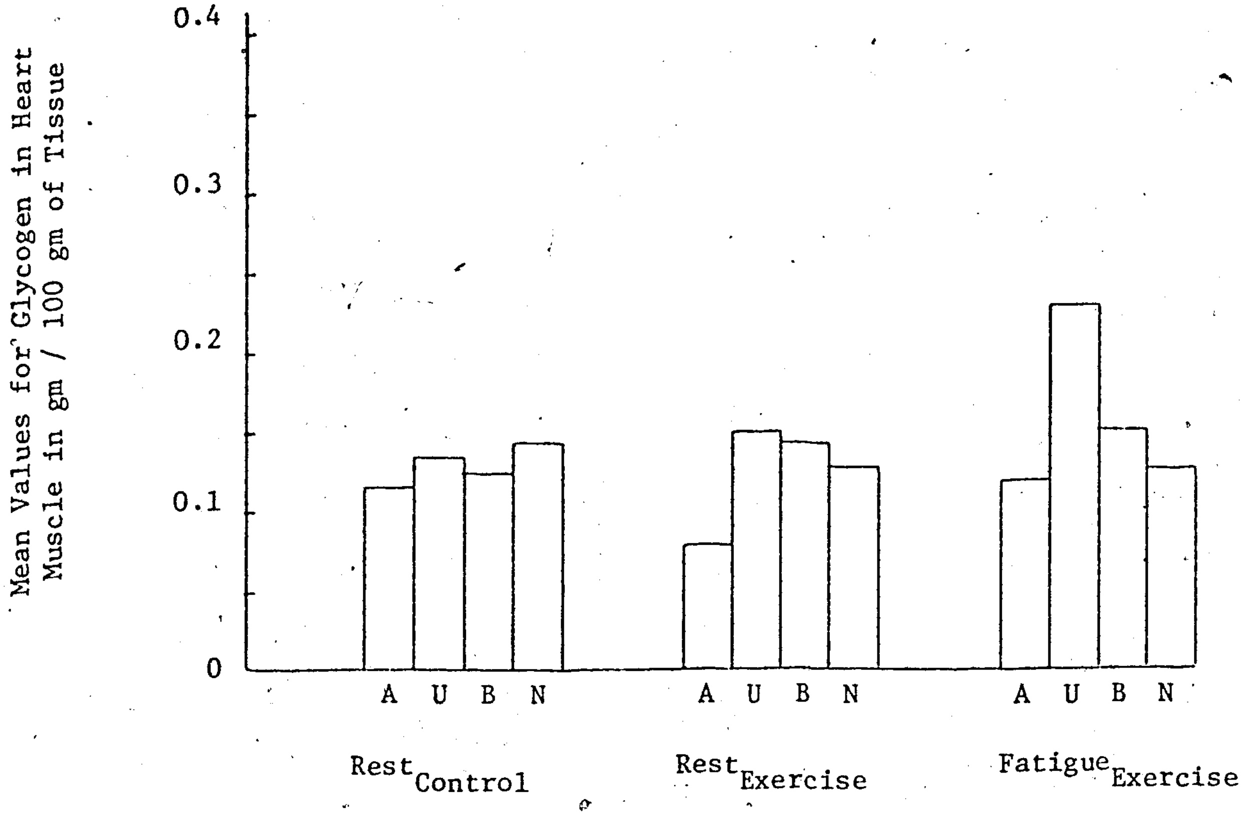


Figure 4



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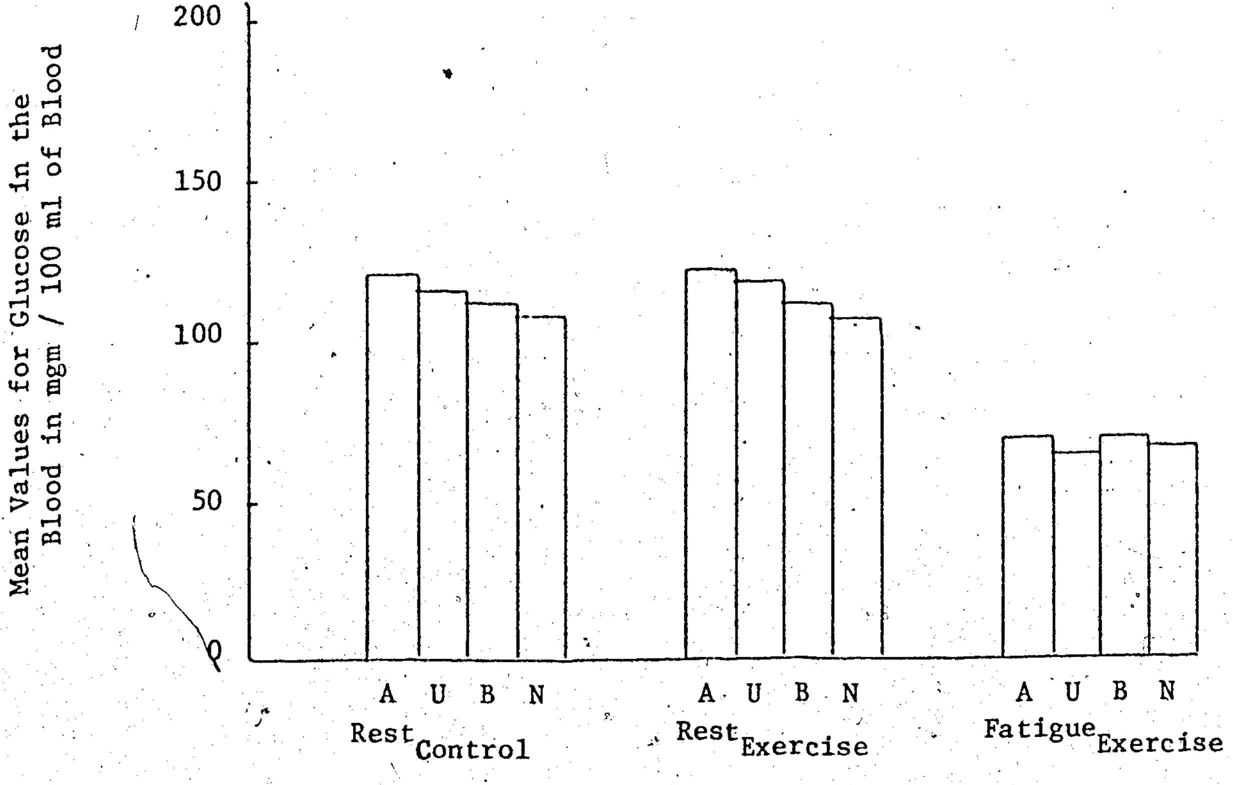
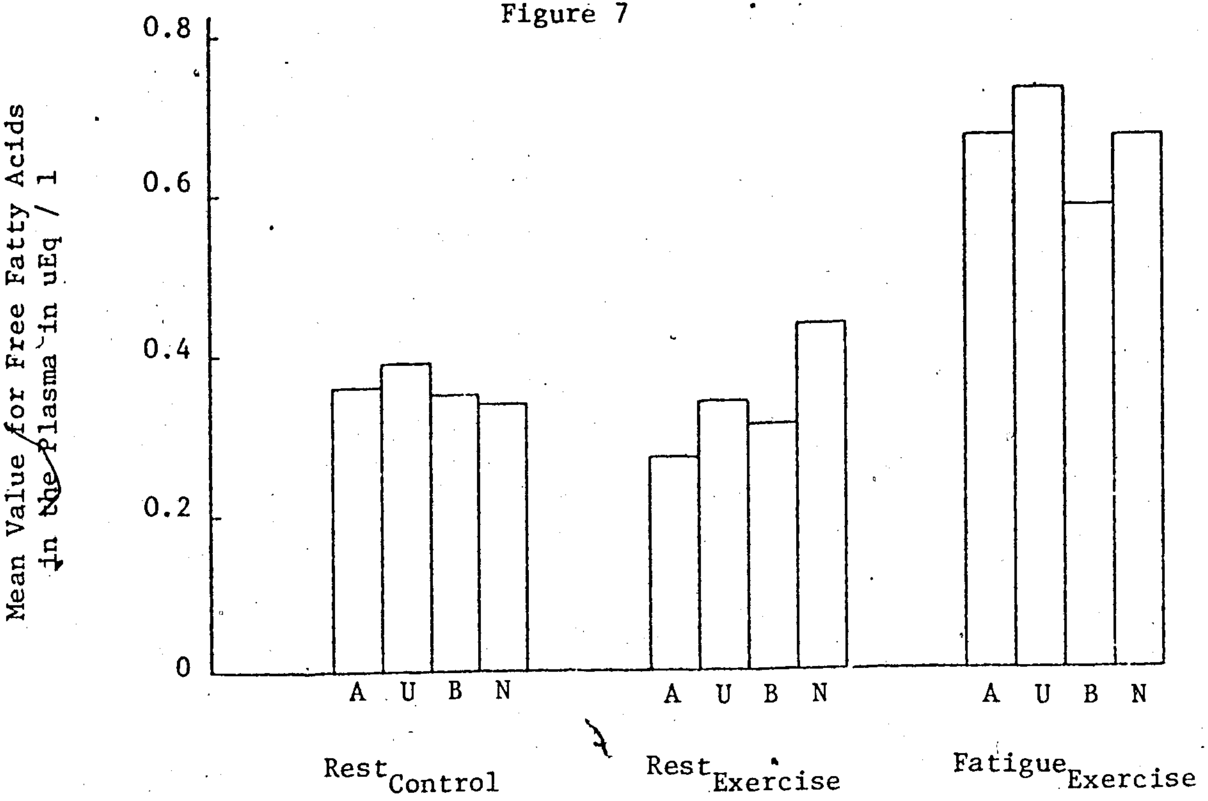


Figure 6

Figure 7



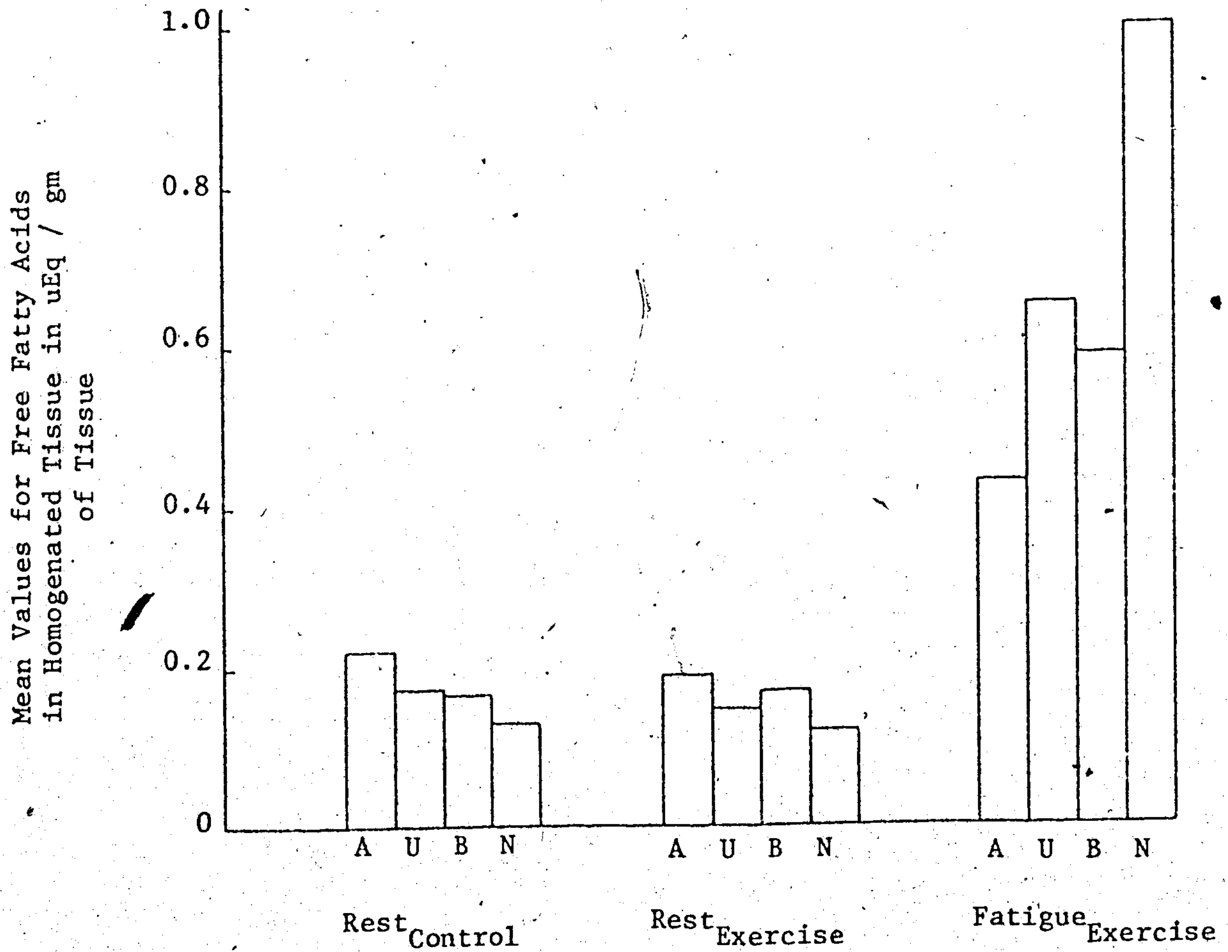
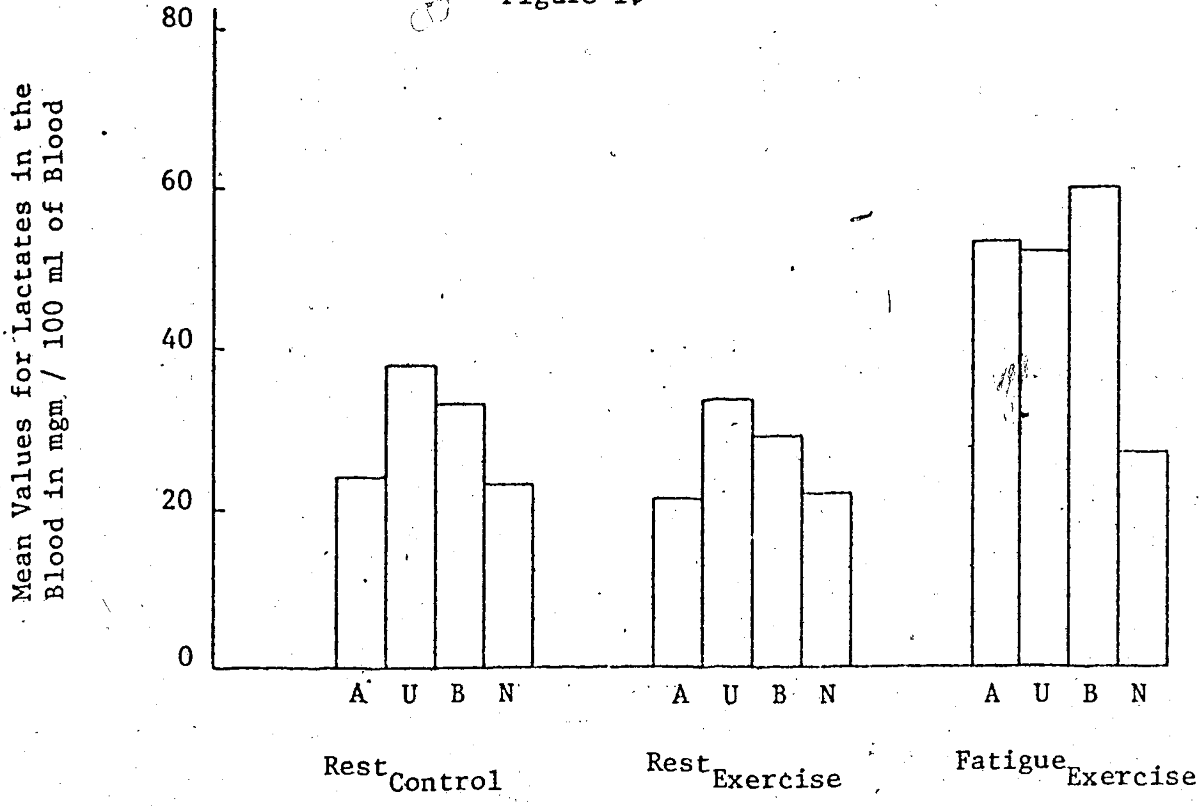


Figure 8

Figure 10



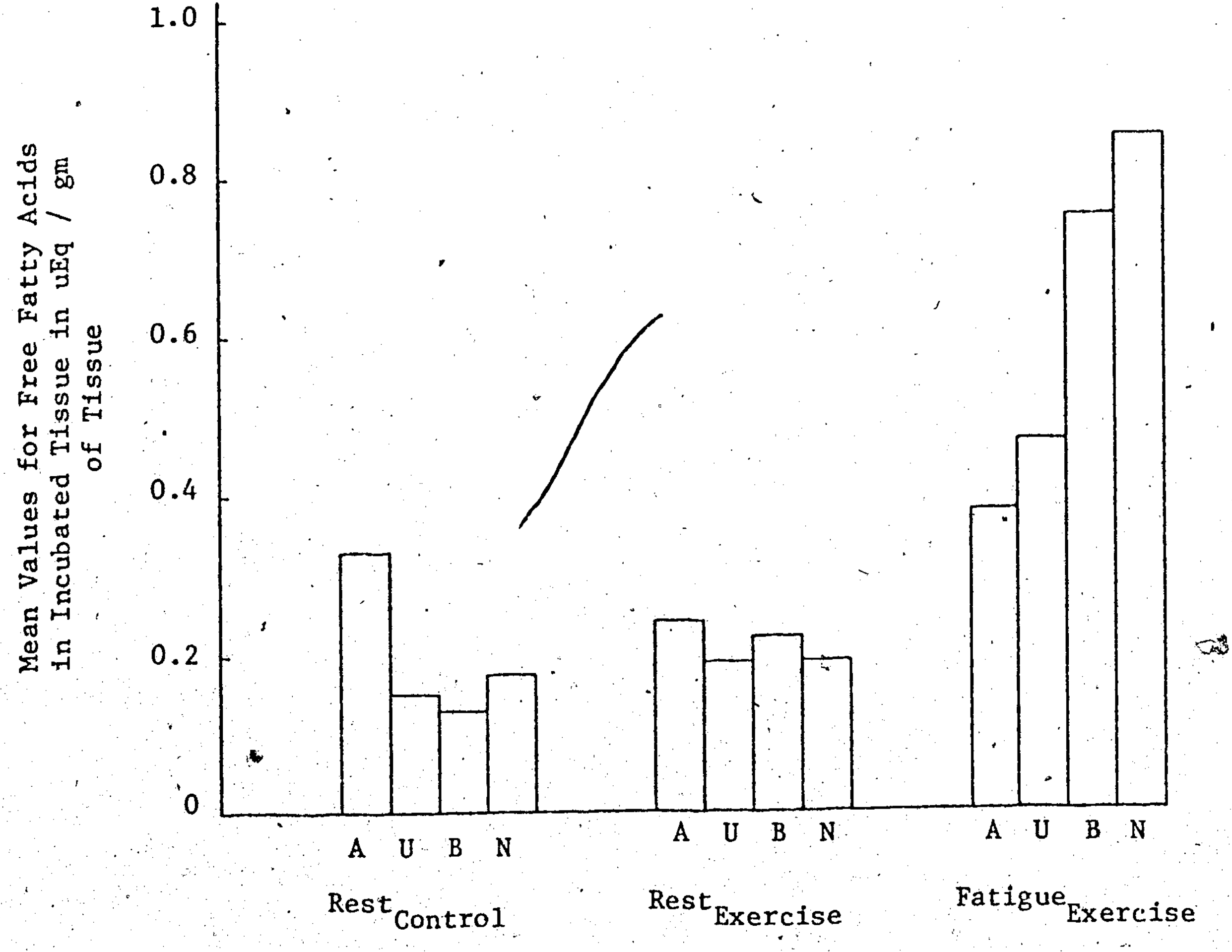
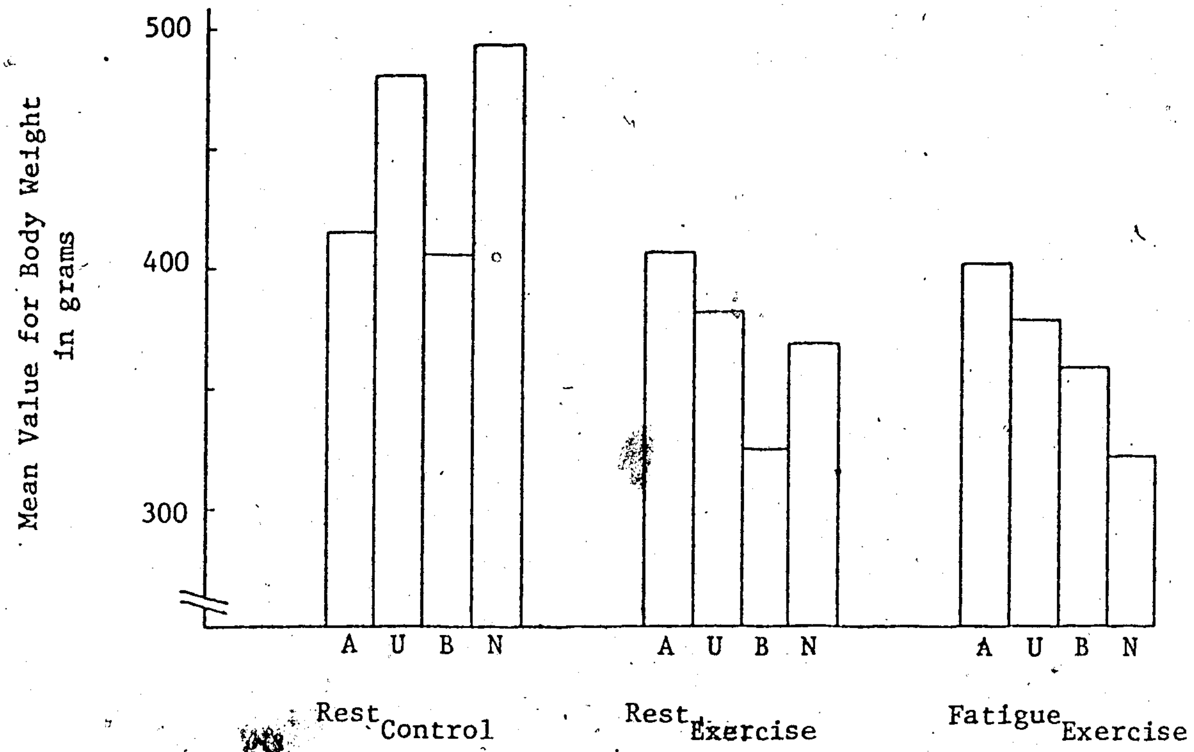


Figure 9

Figure 11



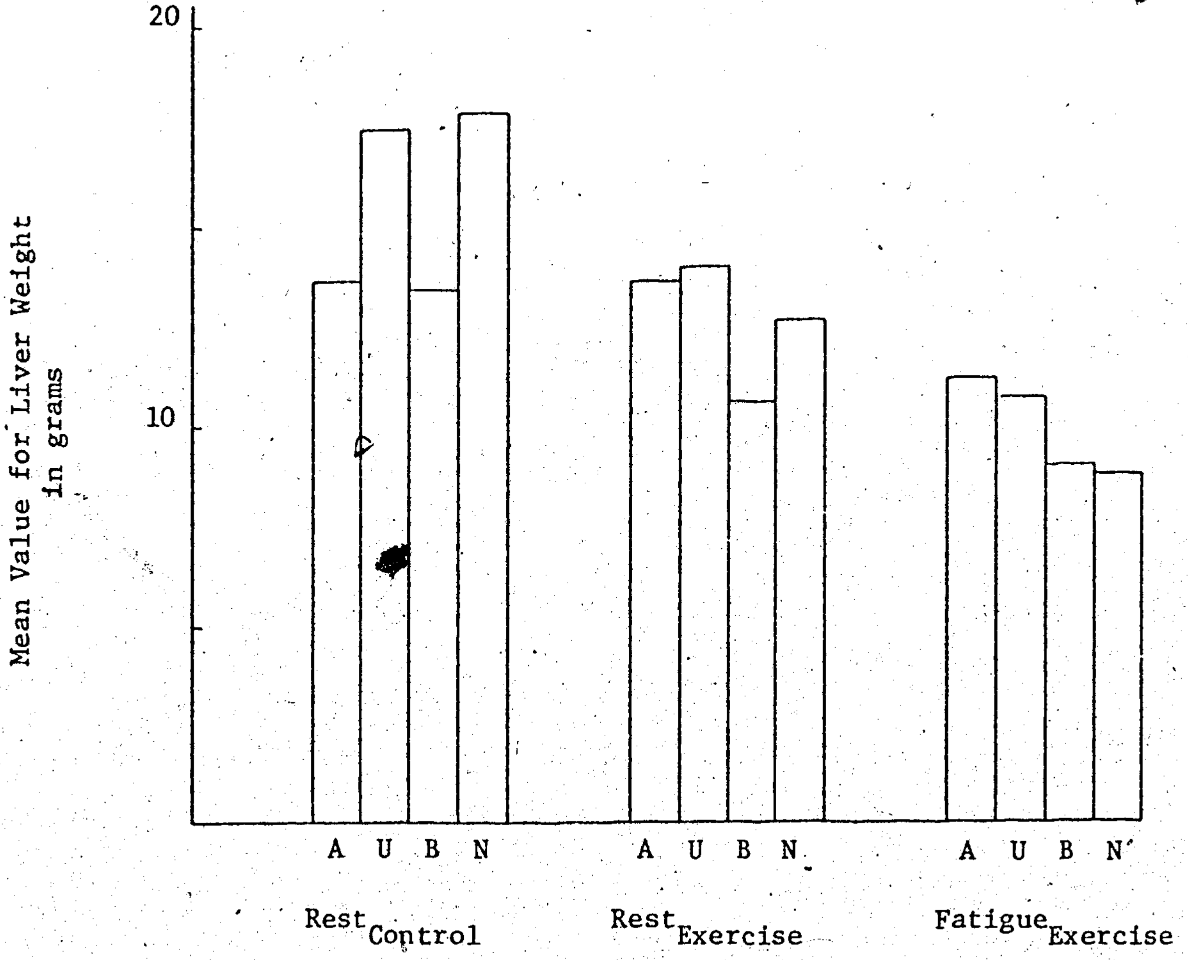


Figure 12

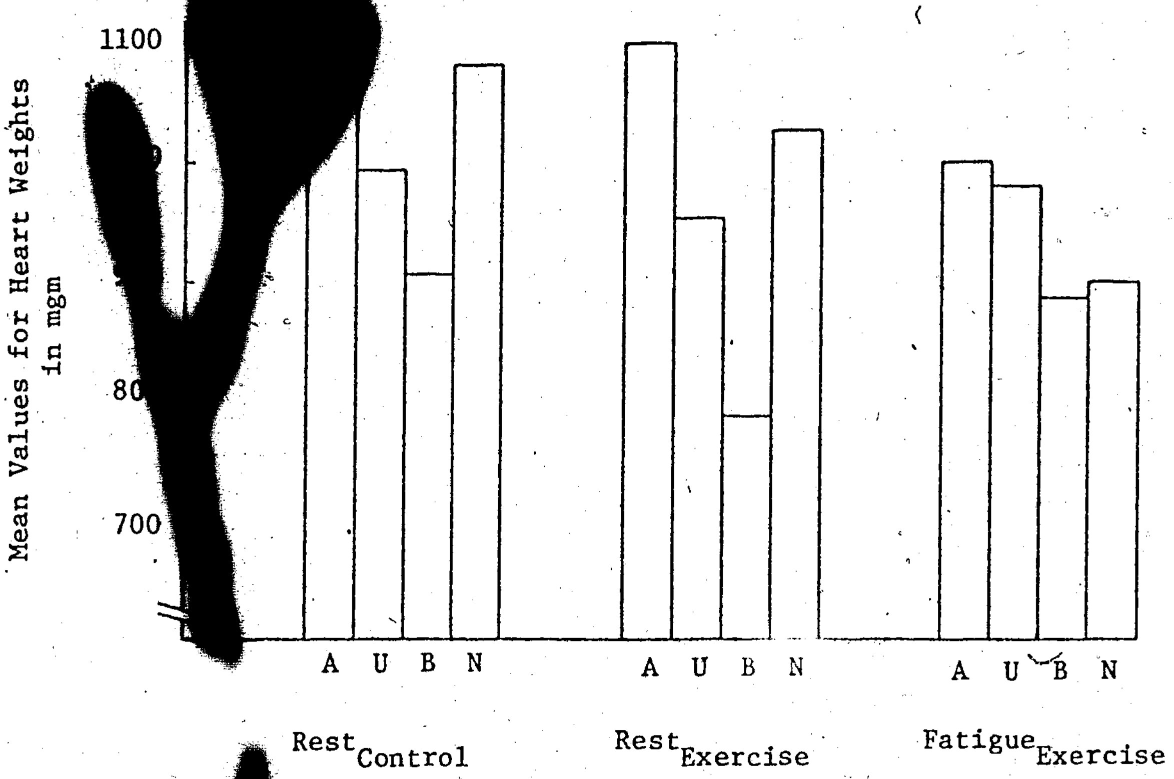


Figure 13

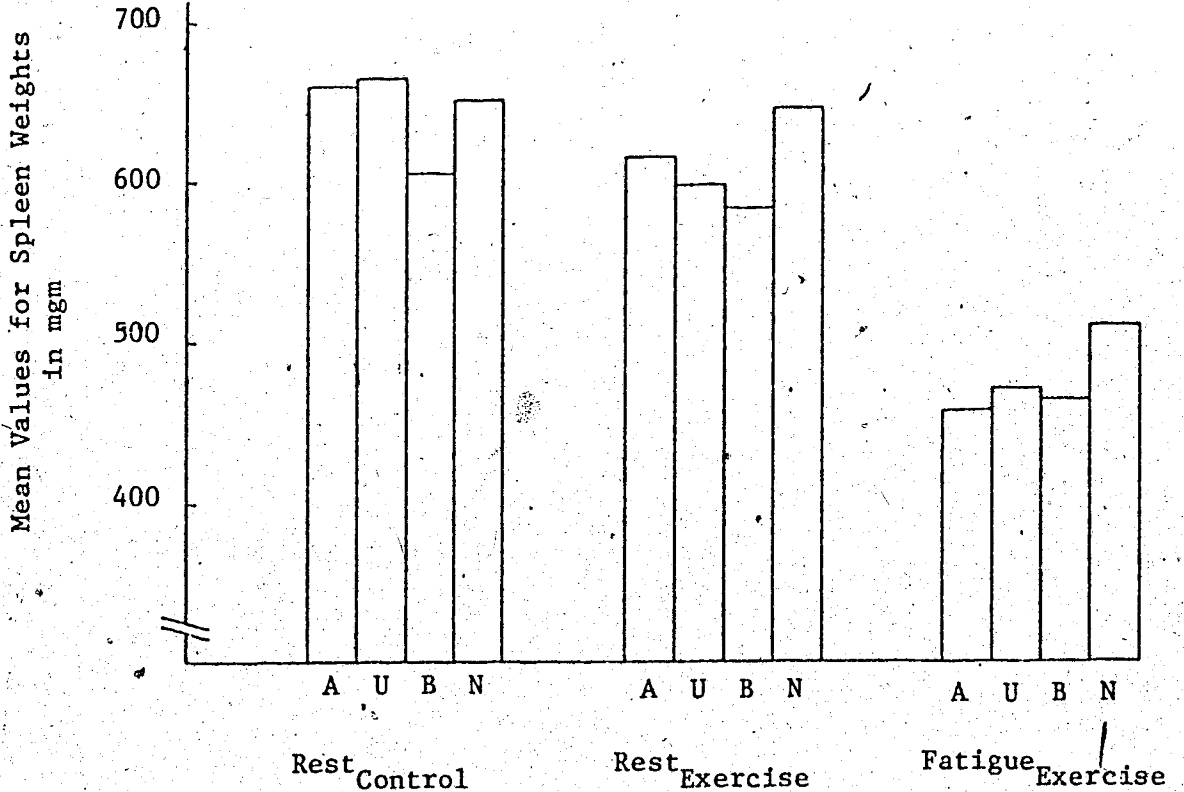


Figure 14

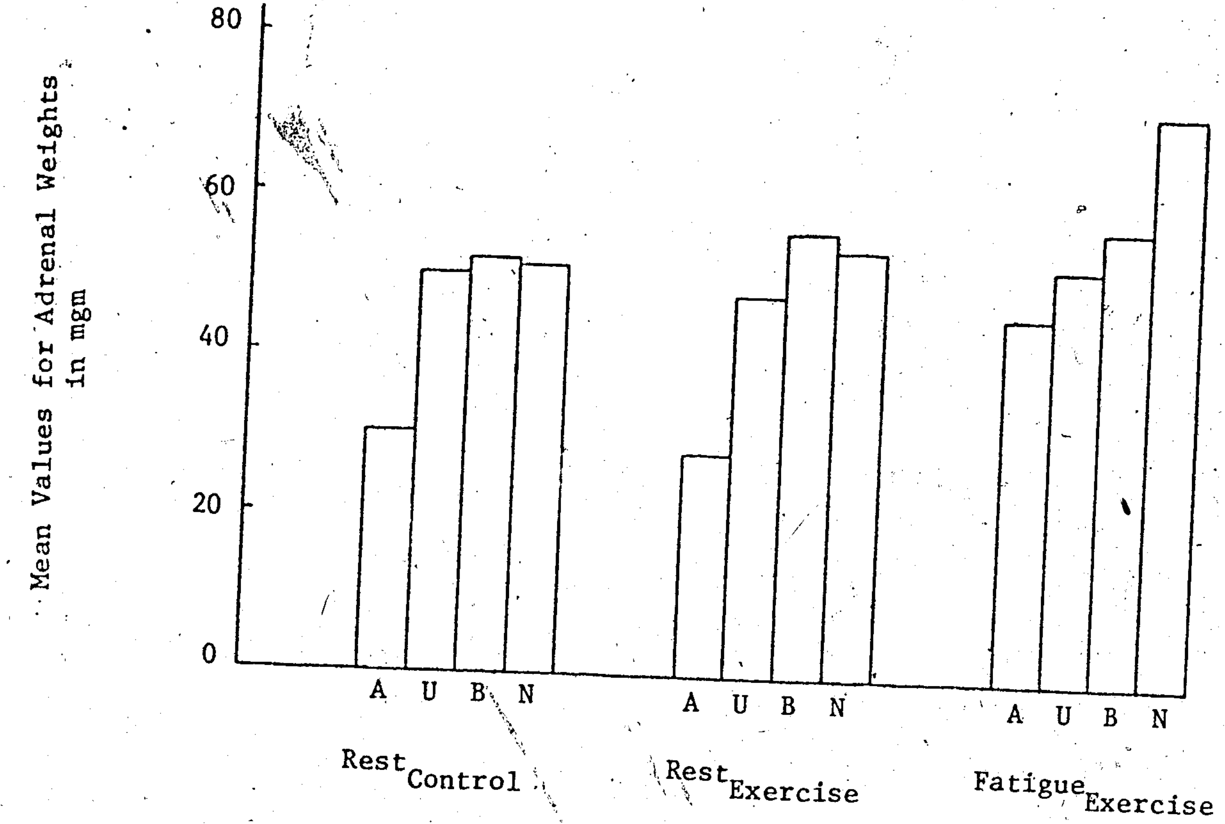


Figure 15

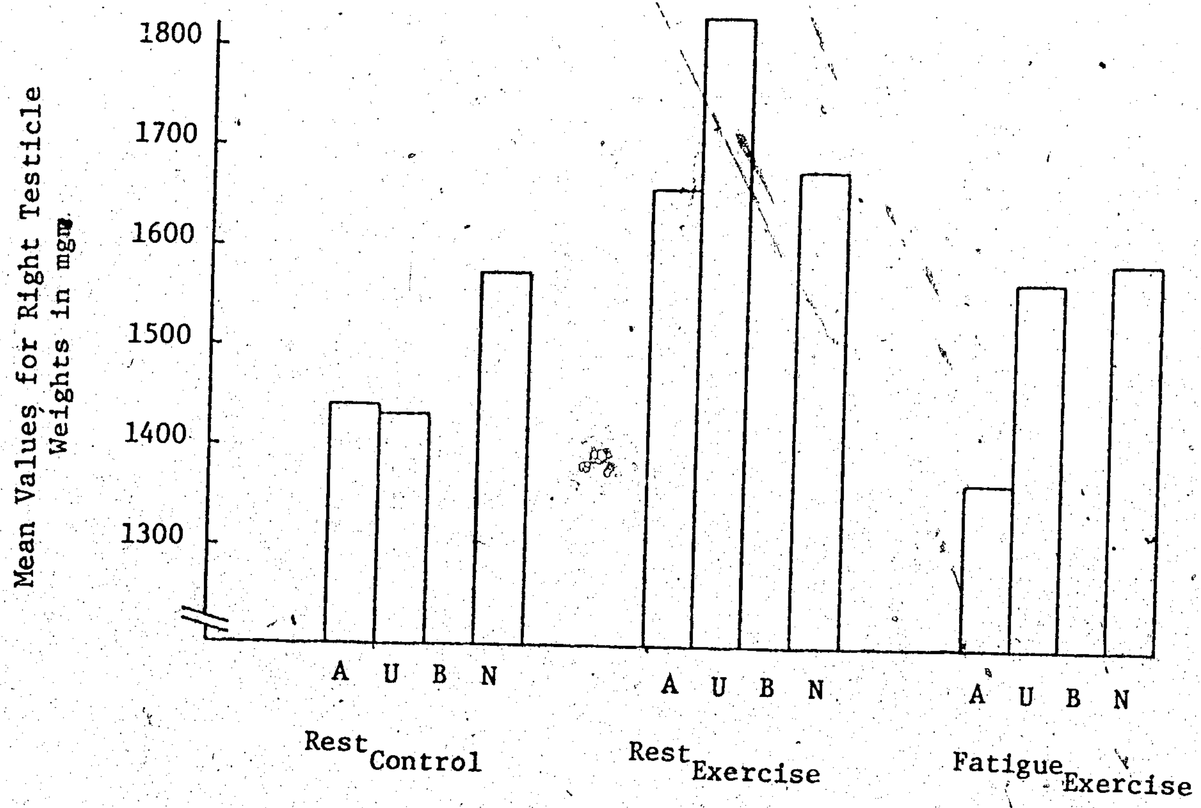


Figure 16

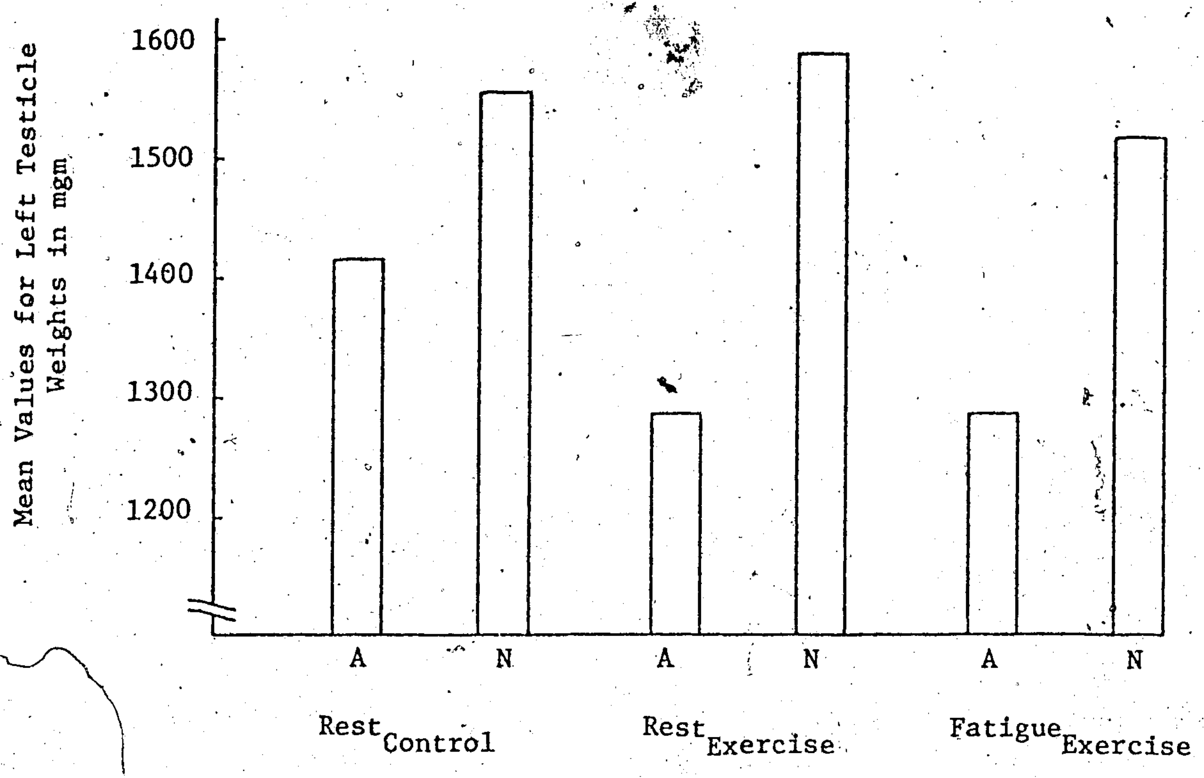

Mean Values for Left Testicle Weights in mgm
1600
1500
1400
1300
1200
A
N
A
N
A
N
Rest Control
Rest Exercise
Fatigue Exercise

Figure 17

little change in the resting FFA concentrations of any group.

There was a significant difference, in the concentration of lactate, between the cell means of the non-castrated fatigue group and the bilaterally castrated fatigue group. ($P<0.05$) (Figure 10).

Changes in Organ or Body Weights

The data, representative of the body weights for the eighteen treatment groups, at the time of sacrifice, is found in Figure 11 and Appendices B and C. All animals were of the same relative weight at the initiation of the study. At the termination of the experiment, the weights of the trained animals were less than the weights of the control groups ($P<0.01$). The animals subjected to bicastration alone, were lighter than the animals which received no castration, training, or anabolic steroids, ($P<0.01$) (Figure 11). A similar finding was evident for the non-trained rats given "Winstrol," when they were compared with their respective control group, which did not receive any "Winstrol," ($P<0.01$) (Figure 11). The weights of thr trained animals receiving the "Winstrol" did not differ significantly from the weights of the trained rats not receiving the anabolic steroid, or from the weights of the non-trained animals receiving the drug, (Figure 11).

The changes in the liver weight that occurred parallel the changes in the body weights, (Figure 12). There was a significant difference between the weights of the livers of the trained animals and the non-trained animals, ($P<0.01$) (Figure 12). The livers of the non-trained rats, which received the anabolic steroid, were significantly smaller than the livers of their respective non-trained control animals, which did not receive the drug, ($P<0.01$) (Figure 12).

There were no significant changes in the weights of the spleen or the heart for any of the treatment groups, (Figures 13 and 14 respectively). However, both the trained and untrained groups, which received the "Winstrol," had smaller adrenals than their respective control groups, ($P<0.01$) (Figure 15). On the other hand, the weight of the adrenals did not differ between the trained and untrained groups.

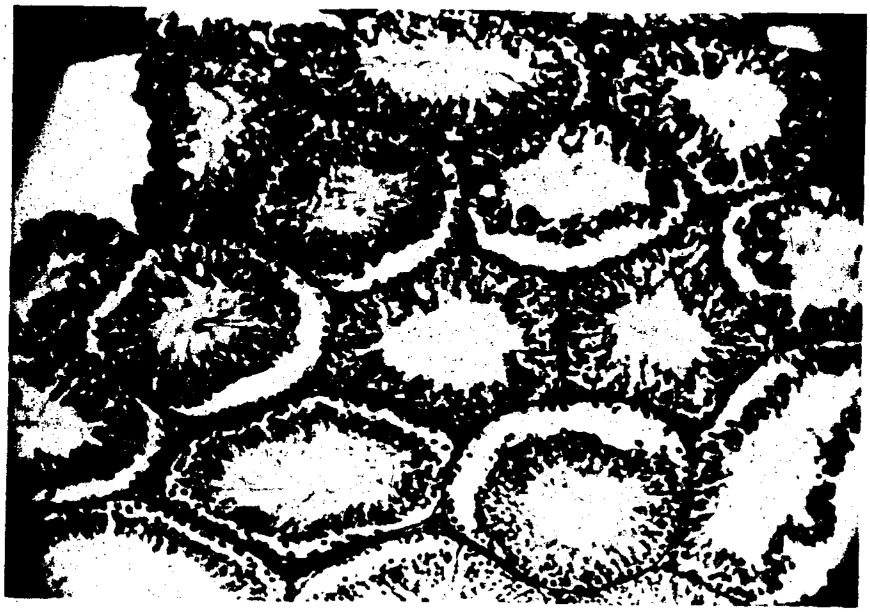
The weights of the testicles did not change significantly under any of the experimental conditions, (Figures 16 and 17). However, when the weights of the left testicles in the unicastrated animals, which were obtained prior to the start of the experient, were compared to the weights of the right testicles, which were obtained at the end of the study, there was a great deal of variability. The percentage increase in weight was anywhere from less than 1 per cent to greater than 100 per cent. No differences sere observed in the testicles or the adrenals, when they were examined histologically. (Figure 18 to 23).

Figure 18A

Serial Section of the Testicle of a Control Rat, Given the Anabolic Steroid "Winstrol". Total Magnification is 100 times.

Figure 18B

Serial Section of the Testicle of a Control Rat. Total Magnification is 100 times



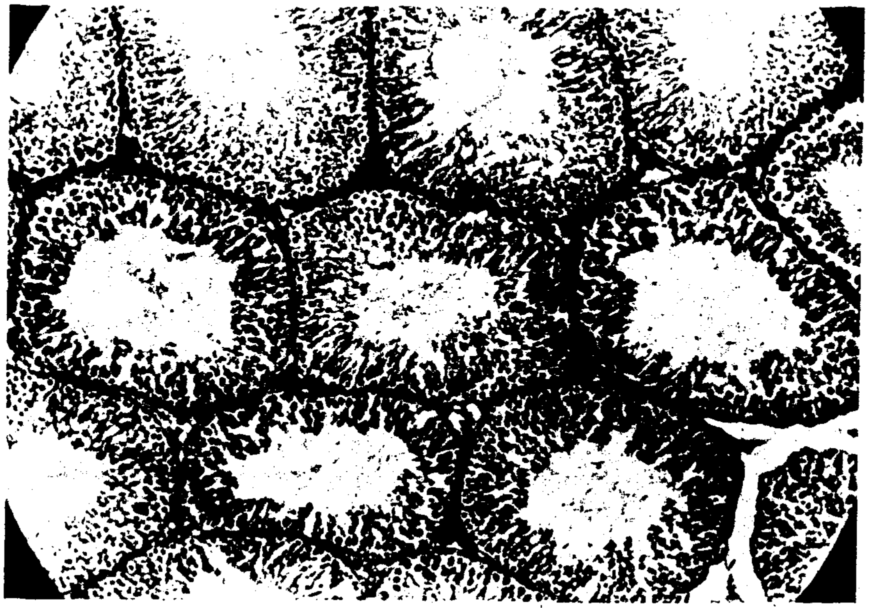


Figure 19

Magnification of the Serial Sections of the Testicles is 100 times.

Scale is from 0.1 to 1.2 mm.

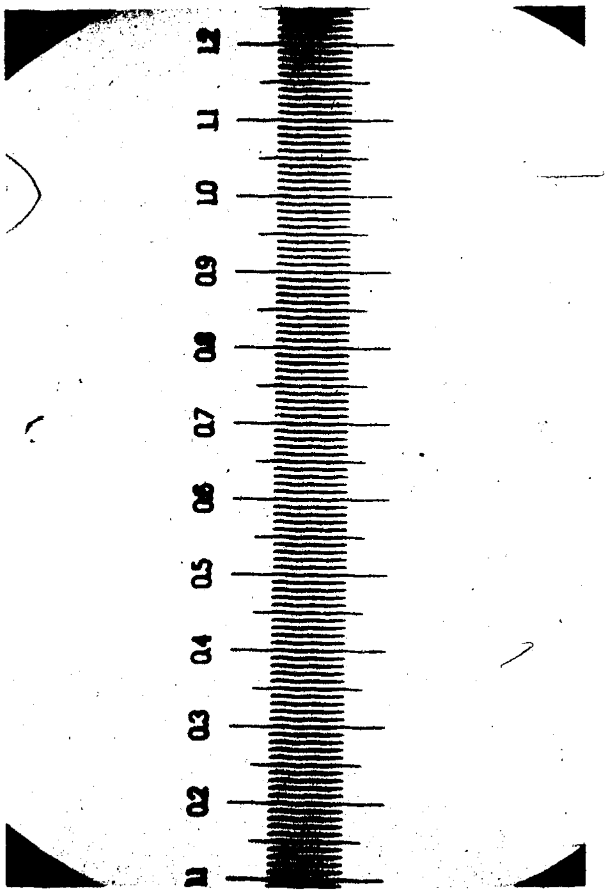

0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2

Figure 20

Serial Section of the Adrenal of a Control Rat.
Total Magnification is 400 times.

Figure 21

Serial Section of the Adrenal of a Trained Rat, Sacrificed at Rest. Total Magnification is 400 times.

Figure 22

Serial Section of the Adrenal of a Trained Rat, Sacrificed at Fatigue. Total Magnification is 400 times.

CHAPTER V

DISCUSSION

Body and Organ Weights

Kimeldorf and Baum (76) state that daily exhaustive swimming exercise of 15 to 30 minutes duration results in a significant depression of the rate of body weight gain. This reduction in body weight is probably the result of an increased energy expenditure through exercise, accompanied by a concurrent decrease in food consumption (76). In the present study, the trained animals weighed less than the control animals. These results are in agreement with the findings of Kimeldorf and Baum (76) and Gollnick et al (41).

Castration leads to a gradual retardation of the increase in weight of growing male rats and guinea pigs, however, in the rat, all skeletal muscles and the skin decrease in weight and size, respectively, in proportion (110), whereas, in the guinea pig some muscles decrease in size more than others (110). The animals which were subjected to bicastration alone were lighter than the animals which received no castration, training, or anabolic steroid, in the present study. These results are supported by the findings of Kochakian and others (81, 85, 86, 88, 96, 110). The bicastrated, trained group did not differ significantly in weight from the trained control group, therefore, it would appear that the effects of bicastration and exercise are not additive, i.e. the training somehow alters the effects of bicastration.

In 1935 Kochakian (80) reported that the energy lost due to a decrease in protein catabolism was compensated by an increased catabolsim of fat. It is known from animal experiments that anabolic steroids in high dosages and with prolonged administration results in a decrease in total fat content (20, 87,107). In the present study, the body weights of the non-trained group, given "Winstrol," differed significantly from the body weights of the non-trained group, not given the drug. The difference between these two groups may be due to a loss of body fat in the steroid group. On the other hand, it may be that the daily injection of the drug, I.P., acted as a stress for the animal, resulting in a decrease in body weight. Selye (144) states that exposure to a stressful situation can cause a reduction in body weight, regardless of the nature of the stressing agent.

There is a small decrease in the weight of the liver, with castration (97). This is probably due to a decrease in the water and fat content (84, 87). Kruskemper (110) observed that no fat accumulated in the liver of normally fed rats, when they were given testosterone propionate over a long period of time. The response of the weight and nitrogen content of the liver, with the administration of androgens, depends on the dosage of the steroid (84, 113). The changes in liver weight that occurred paralled the changes in the body weight. There was a significant difference between the weights of the livers of the trained animals and the non-trained animals. Also, the livers of the non-trained rats, which received the anabolic steroid, were significantly smaller than the livers of their respective non-trained control animals, which did not receive the drug. The decrease in weight of the liver, with training, may be due to a loss of liver fat and/or water. This may also be the case with the administration of the anabolic steroid.

There were no significant changes in the weights of the spleen or the heart for any of the treatment groups. These findings are in agreement with those of Gollnick et al (42). These authors (42) and Tipton et al

(157), both observed that training had no effect on spleen weight, when adjusted for final body weight. Kimeldorf and Baum (75), on the other hand, observed that the spleen of exercised animals was significantly larger than that of the controls, toward the beginning of the study. However, a trend toward recovery in weight was evident at the time of sacrifice (76). The weight of the spleen is reduced during exercise, when it is adjusted for final body weight (42).

Neither training, exercise, nor their interaction produced a significant effect on the heart weight, after adjusting for final body weight, according to Gollnick et al (41). On the other hand, Kimeldorf and Baum (76) observed that the heart constituted a much greater portion of the body weight in exercised animals than in controls. However, this increase was not adjusted for final body weight. With castration the weight of the heart decreases slightly, while the weight of the spleen increases. This loss of weight in the heart indicates that it is slightly dependent on the internal secretions of the testes (97). According to Tipton et al (157), the spleen weight is influenced by the presence of the growth hormone (STH). The relationship that exists between the growth hormone and the effects of the androgens is still uncertain, however, Martin et al (117) have suggested that the growth promoting effects of the androgens may be mediated through enhanced growth hormone release by the pituitary.

It is generally concluded that training produces hpyertrophy of the adrenals (37, 42, 47, 50, 76). Castration also results in an increase in adrenal weight due to hypertrophy of each of the cortical zones, with the exception of the glomerulosa (97). Intermediate doses of androgens (up to 5 mg/ day), on the other hand, cause moderate atrophy (11, 14).

The observed weight response, of course, does not allow any conclusions about the function of the adrenal cortex. Whether these phenomena are due to an androgen-dependent alteration of ACTH secretion or to a direct effect of the androgens on the adrenals cannot be answered completely at this time (110). There was no significant difference in the adrenal weights between the trained and untrained groups, which is contrary to most of the research (37, 42, 47, 50, 76). A wide variation within the trained group was observed, which could account for the difference. However, both the trained and untrained groups, which received the "Winstrol," had smaller adrenals than their respective control groups. Therefore, it would appear that the results of the steroid treatment, in the present study, are in agreement with the literature (11, 14).

The weight of the testicles did not change significantly under any of the experimental conditions. The results, which indicated that training had no significant effect on the weight of the testes are in agreement with the findings of Kimeldorf and Baum (76) who observed that the absolute weight of the testes was not appreciably altered, despite the decrease in body weight. When the weights of the left testicle in the unicastrated animals, which were obtained prior to the start of the experiment, were obtained at the end of the study, there was a great deal of variability. The percentage increase in weight was anywhere from less than 1 per cent to greater than 100 per cent. One or two testicles from each of the experimental and control groups were examined histologically to determine if they had been altered in any way. No differences were observed. (Figures 18 to 20). The adrenals were also examined, and no effects were observed. (Figures 21 to 23).

Sources for Energy for Metabolism

Glucose

According to Tipton and Taylor (156) it takes approximately 8 weeks to train an animal, therefore, it is assumed that the animals used in this study were trained, when they were sacrificed. The trained animal has a decreased rate of glycolysis, which acts synergistically with a relatively greater utilization of fatty acids (55).

According to Hermansen et al (51) during exercise of moderate to heavy intensity (50 - 70% maximal oxygen consumtpion), there is a significant decrease in blood sugar and in plasma insulin. In severe exercise, it was found that there was little or no fall in blood glucose (75 - 80% maximal oxygen consumption), while in work at 85 - 90% of the maximal oxygen consumption, which lasts for 20 minutes or more, there was a small but significant increase in circulating glucose concentration.

Rodahl et al (133) observed that when the work load is such that it can be continued for several hours, there is a rise in the FFA/glucose ratio. These findings are in accordance with the earlier observation of Christensen and Hansen (19) that fatigue during prolonged work can be characterized by a decrease in blood sugar and a decrease in RQ. The FFA/glucose ratio may rise during the last effort due to a slight increase of lactate production accompanied by hyperventilation (19). This increased uptake of glucose partially compensates for relative lack of glycogen consumption (60).

Hultman and Nilsson (60) observed that at the end of prolonged muscular work appreciable amounts of glucose are produced by glycogenolysis in the liver, but the blood sugar content still tends to fall. Rowell

(135) and others (13) also observed that glucose production by the liver can contribute substantially to the substrates utilized during moderate to heavy exercise.

Androgens show definite effects on carbohydrate metabolism that persist throughout their administration, even when the effects on protein metabolism are no longer apparent (98). An explanation as to the extra increase in body weight may be found in the decrease in blood and urine sugar (98). Bergamini et al (12) have suggested that there is a more rapid phosphorylation of blood glucose, and also an increase in glucose - 6 - phosphate independent of glycogen - sythetase activity. These two phenomena would explain the increased glycogen sythesis demonstrated by the incorporation of both glucose and pyruvate (12).

In the present study, the fatigue animals did not have a significant decrease in blood glucose. During prolonged exercise of this type, a relatively greater utilization of fatty acid oxidation will be used to fulfill the energy requirements (55). Also, the increased production of glucose by the liver (13, 61, 135) may have been similar in quantity to the increased uptake by the muscles, during the exercise. The observation that a significant difference in blood glucose levels existed between the trained group receiving the anabolic steroid, when they were sacrificed at rest and at fatigue, should be regarded with some reserve. A wide variation within each group was observed, which could account for this difference. However, it may be that the anabolic steroid and the exercise were additive in their effects on the blood glucose levels, since each tends to produce a decrease in blood sugar, depending on the work load.

Glycogen

During submaximal exercise requiring 60 - 80% of aerobic energy, glycogen consumption is very high and the local stores are gradually depleted, at fatigue (61, 62, 153). The rate of glycogen depletion is determined by the relative rather than the absolute work intensity of the muscle (61). Bergstrom and Hultman (13) observed that only working muscle groups utilized glycogen during the work period. Thus, no fall was noted in the glycogen content of the resting muscle groups. It was also shown that the decrease in glycogen content varied from muscle to muscle. For example, the main energy-supplying substrates for the energy metabolism of the heart muscle are glucose, lactate and FFA (75).

The observations made in the present study are in agreement with those of Hultman et al (62) and others (138), in that the glycogen levels were significantly lower in the animals sacrificed at fatigue than they were in the trained animals sacrificed at rest. Also, there were no significant decreases in the glycogen content of the heart and biceps muscles, which is in agreement with the findings of Bergstrom and Hultman (13).

The resting glycogen content of the muscle can be elevated through training (41, 61, 153). It has been shown that the activity of several individual aerobic and glycolytic enzymes is increased by muscular exercise and training (56). The increased activity rate of the glycolytic enzymes implies that glycogenolysis increases with muscular work (153). Proctor and Best (130) observed that the glycogen content of the exercising muscles increases appreciably and often strikingly in dogs. However, ~~this~~ difference between the trained and the untrained tended to disappear, if training was continued after 15 - 16 days. They (130) observed that after 5 - 6 weeks the glycogen contents of the muscles were in some cases as similar as they were before training commenced. Since the animals in the present study were trained for approximately 10 weeks, the results obtained would tend to support the findings of Proctor and Best (130). No significant difference was found between the trained and the non-trained groups, in the glycogen content of the gastrocnemius, biceps or heart muscle. However, there was a wide variation within the trained, rest group, for glycogen in the gastrocnemius, which could account for this failure to produce an increased glycogen content.

It has been shown that castration is followed by a decrease in glycogen levels in the rat perineal muscles (114) and also in the levator ani muscle (2, 12). Castration also had a similar effect on the liver glycogen level (151). The influence of androgens on liver and muscle glycogen are not conclusive. Leonard (114) observed that the administration of testosterone increased glycogen synthesis in the rat perineal muscles in both normal and castrated animals, quite apart from pituitary influence. Meyer and Hershberger (118) found a pronounced rise in the glycogen content of the levator ani muscle of castrated male rats, within 24 to 72 hours after treatment with testosterone propionate. After several days the glycogen content decreased; that is about the time at which the androgen dependent growth of the muscle begins with an increase in protein content. Meyer and Hershberger (118) concluded that the effect of testosterone propionate on the musculature is mediated by a primary effect of testosterone on the musculature and that protein synthesis is accelerated, when additional energy in the form of glycogen becomes available. Bergamini et al (12) suggest that increased glycogen synthesis can be attributed to the greater penetration and more rapid phosphorylation of blood glucose, and also to increase Gl - 6 - P, independent of glycogen -

synthetase activity. In the present study, the "Winstrol" did not have a significant effect on the glycogen content of the gastrocnemius, biceps or heart muscle. It may be that the anabolic steroids influence only the metabolic processes of the androgen-dependent muscles.

The possibility that active anabolic steroids lead to the depletion of liver glycogen has also been discussed. Weisenfeld and Goldner (160) hypothesized that the liver glycogen was exhausted, due to an interference with the function of the liver by the anabolic steroid. Weisenfeld (159) suggested the possibility that the active anabolic steroids act via an inhibition of ACTH, and consequently of glucocorticoid secretion, leading to depletion of liver glycogen. Landon et al (111), on the other hand, found that there are still adequate supplies of glycogen in the liver and that the glycolytic pathway is normal.

Gillespie and Edgerton (36) observed that approximately 33% of the enhanced glycogen storage resulting from training appears to be attributable directly or indirectly to the presence of normal testosterone production or adequate testosterone propionate replacement. Whether or not the administration of androgens to normal animals would increase the glycogen storage to an even greater degree, was not discussed. "Winstrol" had no significant effect on the glycogen levels in the gastrocnemius, biceps or heart muscle, in the present study, nor did castration.

During heavy exercise (1 hour), and especially at the end of prolonged muscular work, appreciable amounts of glucose are produced by glycogenolysis in the liver (62). If the anabolic steroid does deplete the liver glycogen, then the animals ability to perform prolonged exercise could be limited, depending on the energy sources available, since the liver glycogen is assumed to be the main source of carbohydrate for muscular exercise in fully aerobic conditions (62). If, however, the anabolic steroid increases the glycogen level in the skeletal muscles, then the exercise would be prolonged, depending on the work load. The role of muscle glycogen was assumed to be that of a depot for anaerobic work only, leading to the formation of lactic acid, so that the work load would have to be quite heavy (62). The present study does not support the hypothesis that anabolic steroids increase glycogen in skeletal muscles (Figure 3).

Free Fatty Acids

Fat, in the form of free fatty acid, serves as a major fuel for muscular work in man (15). It has been estimated that fat may contribute up to 90% of the total substrate oxidized during submaximal work (15, 49, 66, 152). There is also an extremely rapid increase in the FFA concentration after exercise with the level being about doubled in 5 minutes (65) indicating that exercise is a stimulus for lipolysis during muscular work (152). Gollnick et al (43) have indicated that more than one hormonal system may be involved in activating lipolysis in the rat during exercise. Basu et al (9) arrived at similar conclusions for exercising humans. The rate of uptake of plasma FFA, either at rest or in exercising dogs is directly and linearly dependent upon the levels of FFA in the plasma (i.e. a simple mass action effect) (67). However, the plasma FFA levels are not solely regulated by the rate of uptake. According to Armstrong et al (3), all mechanisms which operate (in dogs) to regulate the FFA (and hence the rate of fat utilization) appear to do so by regulating the level of release from adipose tissue.

In the present study, the animals had an increased concentration of FFA in the plasma and in the incubated and homogenated fat tissues, after a prolonged exercise period. This would support the findings of Issekutz et al (67), Pruett (131) and Holloszy (55). However, the concentration of FFA in the resting fat tissue was not altered with training (Figure 8 and 9). Johnson et al (71) observed that trained and untrained subjects showed definite differences in the concentration of metabolites, in their blood, related to energy supply. The FFA levels rose steadily in the trained and untrained during exercise, but while it almost doubled in the athletes it trebled in the untrained group after one and one-half hours. Johnson et al (71) suggest that athletes can oxidize fatty acids more effectively than can untrained subjects, due to their increased ability to oxidize metabolic fuels aerobically (64). In the untrained dog, during moderate exercise, the oxygen supply to the adipose tissue is inadequate, and therefore re-esterification prevails and the turnover rate of FFA decreases (64). Morgan et al (121) observed that after 4 - 6 weeks of quadriceps training there was a wide range of values for intracellular triglycerides in both the control and trained muscles. However, there was a net increase in triglycerides in the trained muscle as compared to the control.

Castration did not alter the mobilization or concentration of FFA in the plasma or the fat tissue, of the trained or untrained animals. Kochakian and Murlin (79) observed that castration resulted in a decrease in energy and nitrogen metabolism, with a decrease in protein, water and ash content in proportion to the decrease in the weight loss of the muscle (86, 93). Only with androgen support was there an increased utilization of carcass fat.

The decreasing effect of an androgen on nitrogen retention with a concomitant decrease in body weight is attributed to a loss of body fat accompanied by a redistribution of carcass protein (88). The loss of carcass fat on prolonged treatment may be due to an increase in energy metabolism due to either increased activity of the animal, therefore a secondary effect, or it may represent the cost of redistribution of the protein from the carcass to the internal organs (88). In those animals that had a normal or subnormal amount of carcass fat, the utilization of the fat was delayed and did not occur until the nitrogen-retention effect of the androgen had disappeared (88).

Blood Lactate

The concentration of lactic acid produced during exercise depends to a large extent on the intensity of the work, and its duration. There appears to be no marked increase in lactic acid concentration, until the work load is above 50 - 60% of the maximal oxygen consumption. Above this work oad, there is a rapid accumulation of lactate, until a maximum value is reached.

It has been observed by several authors (1, 5, 73) that during prolonged moderate aerobic exercise (less than 60% of the maximal oxygen consumption), the lactic acid concentration peaks between 5 and 15 minutes, and then there is a gradual decline for the remainder of the exercise period. It appears that, during moderate to relatively heavy work which is prolonged, the total metabolism within the liver, kidney and other related organs is sufficient to consume all lactate produced (134).

Astrand (4) interprets the decrease in blood lactate concentration as being an expression of a more effective oxygen transport during the beginning of the work, leading to a diminished anaerobic energy yield. Holloszy et al (55) do not agree with this hypothesis and they have postulated several biochemical adaptions, which occur in skeletal muscles, as being the cause of this adaption to exercise. It has been suggested that glycolysis and glycogenolysis occur at a slower rate in the muscles of trained individuals during submaximal exercise (55). As a result, pyruvate and DPNH should be formed at a slower rate at a given submaximal work load, accounting for a lower lactate production in trained as compared to sedentary muscles (55). The other factor which probably contributes to, and acts synergistically with, the decreased rate of glycolysis, in the trained individual, is the relatively greater utilization of fatty acid oxidation to fulfill the energy requirements of submaximal exercise (19, 48, 64, 65).

In the present study, the animals which were exercised to fatigue did not have significantly greater plasma lactate concentrations than the trained animals, which were sacrificed at rest. These observations may have resulted from the fact that the animals were trained (4, 55, 138), or from the fact that the prolonged exercise was primarily aerobic in nature (37, 152).

Neither castration nor the anabolic steroid had any significant effect on the lactate concentration. The significant difference between the bicastrated, fatigue group and the control fatigue group is probably due to the fact that the control, fatigue group had a lower lactate concentration than would be expected.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Seventy-two male Wistar rats were used in this study to determine the effects of prolonged exercise, an anabolic steroid and castration on energy storage and mobilization in trained and untrained rats.

The animals were randomly assigned into a control group of 20 animals and an exercise group of 52 animals, respectively. The exercised rats were trained to run on a motor driven treadmill and after four weeks were capable of running continuously for one hour at one mph, 5 days per week. Each animal continued to run at this intensiry for an additional six weeks. Twenty-six of the trained rats were run to exhaustion immediately prior to sacrifice and the time to fatigue was recorded. All remaining animals were sacrificed at rest. During the course of the experiment one-half of the exercised rats and one-half of the non-exercised rats received an anabolic steroid, "Winstrol" (17β-hydroxy-17α-methylandrostano (3, 2-c) pyrazol) (I.P. 0.80 mg/ kg), once a day, for ten weeks.

Prior to the exercise programme, bilateral castration was performed upon 16 of the exercise animals and 8 of the control rats. Unilateral castration was performed upon an equal number in each group. No surgical operation was performed on the other animals. The weights of the testicles were recorded, and at the conclusion of the experiment, they were compared to the weights of the testicles of the non-castrated and unilaterally castrated animals.

Analysis of the results indicated that training had no significant effect on the parameters measured, with the exception that the growth rate of the body and the liver was retarded with training. Prolonged exercise to fatigue resulted in a decrease in gastrocnemius glycogen and an increase in plasma FFA levels and lipid FFA mobilization.

The anabolic steroid, "Winstrol," had no effect on the body weight, liver, spleen, heart or testicular weights in the trained rats. However, in the untrained animals the body and liver weights were less. Both the trained and the untrained animals had smaller adrenals, with steroid administration.

The resting blood glucose levels, FFA levels, and the glycogen stores were not altered by the drug. "Winstrol" had no effect on the mobilization of FFA or the utilization of glycogen or blood glucose with exhaustive exercise.

Conclusion

The anabolic steroid, "Winstrol" had no significant effect on the storage or mobilization of energy sources, in the trained and sedentary rat.

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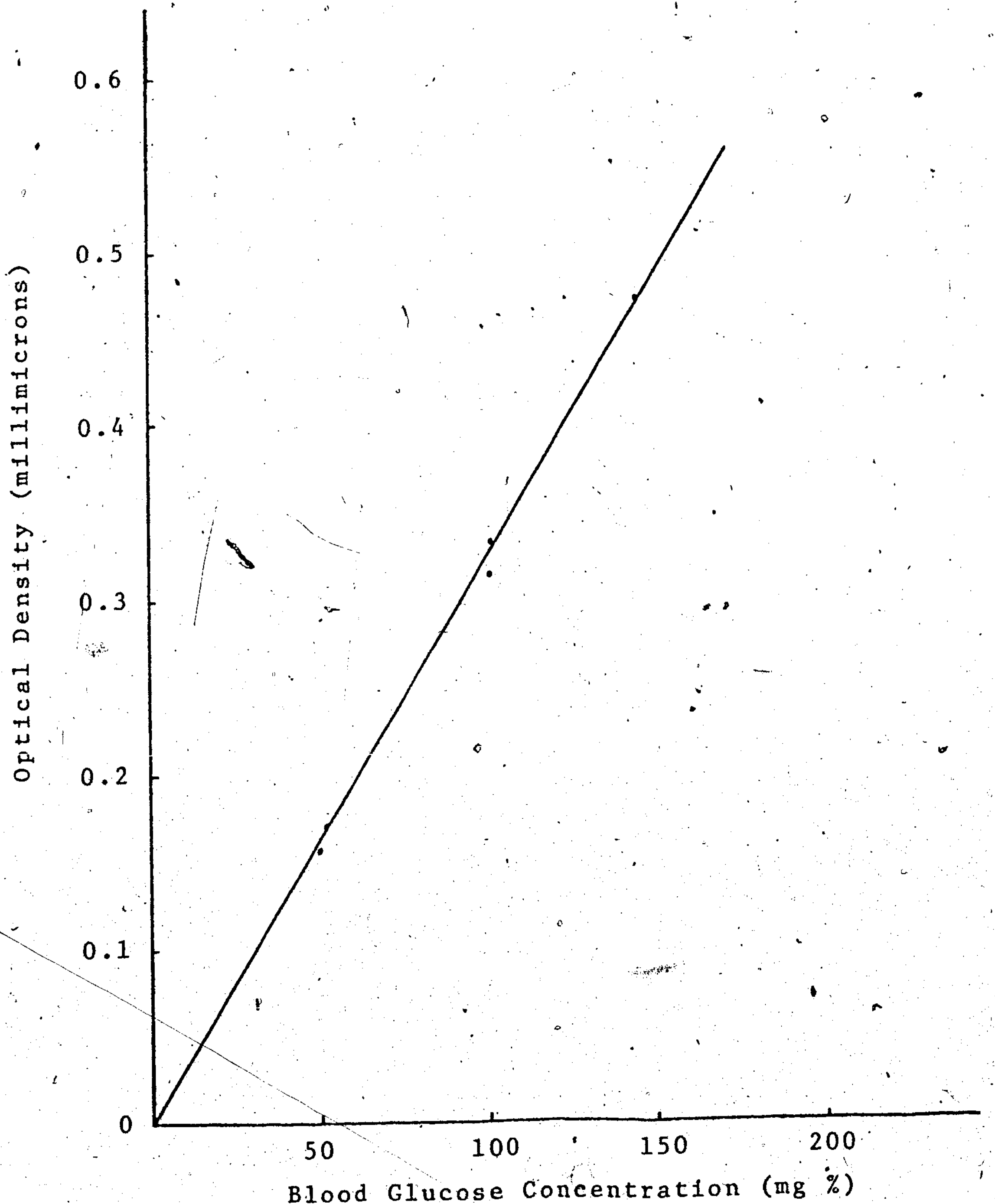
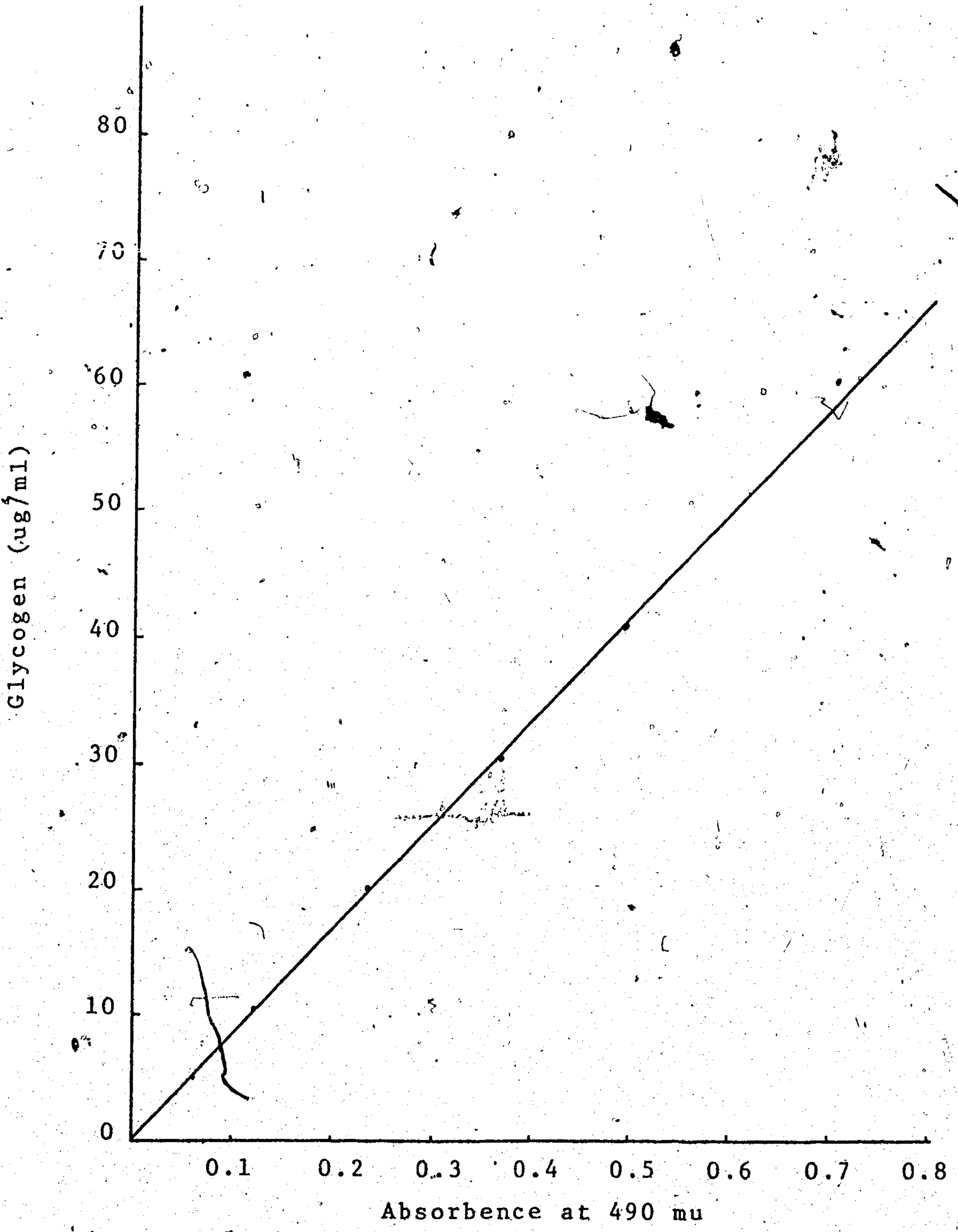
APPENDIX A

STANDARD CURVES, TRAINING PROGRAMME

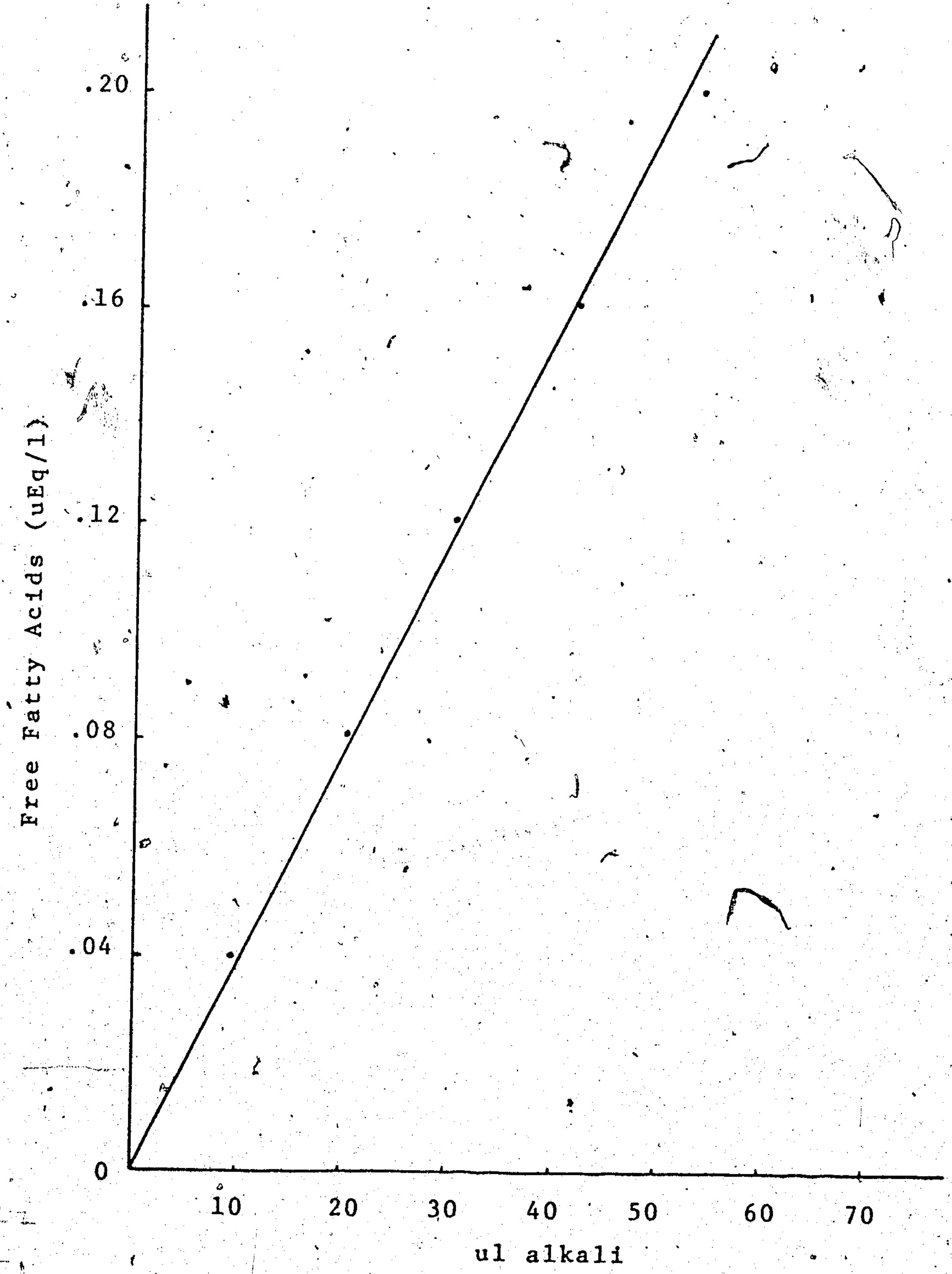
Standard Curve for Blood Glucose



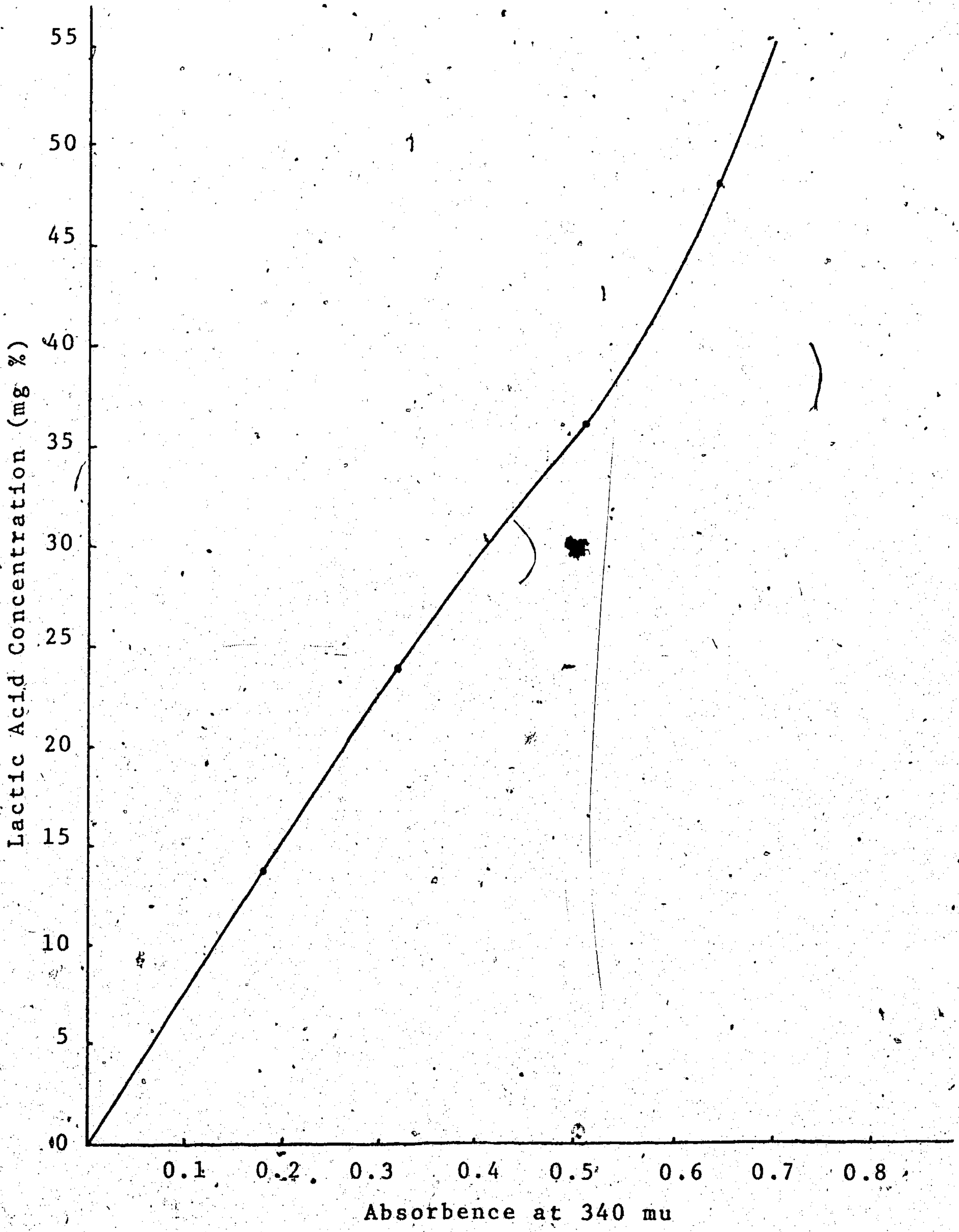
Standard Curve for Glycogen



Standard Curve for Free Fatty Acids



Standard Curve for Lactic Acid



TRAINING PROGRAMME

Day 1 and 2 - 5 minutes at 0.5 mph

Day 3 and 4 - 10 minutes at 0.5 mph

Day 5 and 6 - 15 minutes at 0.5 mph

Day 7 and 8 - 20 minutes at 0.5 mph

Day 9 and 10 - 30 minutes at 0.5 mph

Day 11 - 35 minutes at 0.5 mph

Day 12 - 40 minutes at 0.5 mph

Day 13 and 14 - 45 minutes at 0.5 mph

Day 15 and 16 - 50 minutes at 0.5 mph

Day 17 to 20 - 55 minutes at 0.5 mph

Day 21 - 60 minutes at 0.5 mph

Day 22 to 25 - 60 minutes at 0.75 mph

Day 26 - 60 minutes at 1.0 mph

APPENDIX B

SUMMARY OF ANALYSIS OF VARIANCE

Table 1

Summary of Analysis of Variance

Glycogen in the Gastrocnemius Muscle

Source of Variance	SS	df	MS	F
Treatments	0.1112	25	0.04	10.29
Experimental Error	0.3373	78	0.00	**
Total	0.4485	103		

Glycogen in the Biceps Brachii

Source of Variance	SS	df	MS	F
Treatments	0.5751	24	0.02	8.50
Experimental Error	0.2114	75	0.00	**
Total	0.7865	99		

* Significant at the 0.05 level of confidence

** Significant at the 0.01 level of confidence

Table 2
Summary of Analysis of Variance

Glycogen in the Heart Muscle

Source of Variance	SS	df	MS	F
Treatments	0.1193	25	0.00	2.61
Experimental Error	0.1426	78	0.00	
Total	0.2619	103		

Glucose in the Blood

Source of Variance	SS	df	MS	F
Treatments	0.7470	25	2987.8	7.08
Experimental Error	0.3292	78	422.00	**
Total	1.0762	103		

* Significant at the 0.05 level of confidence
** Significant at the 0.01 level of confidence

Table 3

Summary of Analysis of Variance

Free Fatty Acids in Plasma

Source of Variance	SS	df	MS	F
Treatments	0.3000	25	0.120	9.77
Experimental Error	0.9583	78	0.100	**
Total	1.2583	103		

Free Fatty Acids in Homogenated Tissue

Source of Variance	SS	df	MS	F
Treatments	0.5833	25	23.33	24.17
Experimental Error	0.7530	78	0.970	**
Total	1.3363	103		

* Significant at the 0.05 level of confidence

** Significant at the 0.01 level of confidence

Table 4

Summary of Analysis of Variance

Free Fatty Acids in Incubated Tissue

Source of Variance	SS	df	MS	F
Treatments	0.3674	25	14.69	16.10
Experimental Error	0.7119	78	0.910	**
Total	1.0793	103		

Lactates in the Blood

Source of Variance	SS	df	MS	F
Treatments	0.1636	25	654.3	4.15
Experimental Error	0.1231	78	157.8	**
Total	0.2867	103		

* Significant at the 0.05 level of confidence

** Significant at the 0.01 level of confidence

Table 5

Summary of Analysis of Variance

Body Weight

Source of Variance	SS	df	MS	F
Treatments	0.1884	25	7535.2	7.26
Experimental Error	0.8101	78	1038.5	**
Total	0.9985	103		

Liver Weight

Source of Variance	SS	df	MS	F
Treatments	0.6444	26	24.79	12.82
Experimental Error	0.1566	81	1.93	
Total				

* Significant at the 0.05 level of confidence

** Significant at the 0.01 level of confidence

Table 6
Summary of Analysis of Variance

Spleen Weights

Source of Variance	SS	df	MS	F
Treatments	0.8295	26	31,904.6	4.19
Experimental Error	0.6162	81	7,607.9	**
Total	1.4457	107		

Heart Weights

Source of Variance	SS	df	MS	F
Treatments	0.7459	25	29,834.9	3.41
Experimental Error	0.6822	78	8,746.5	**
Total	1.4281	103		

* Significant at the 0.05 level of confidence

** Significant at the 0.01 level of confidence

Table 7

Summary of Analysis of Variance

Adrenal Weights

Source of Variance	SS	df	MS	F
Treatments	0.1035	25	414.0	6.35
Experimental Error	0.4942	78	63.36	**
Total	0.5977	103		

Right Testicle Weights

Source of Variance	SS	df	Ms	F
Treatments	0.7886	17	463,874.8	12.49
Experimental Error	0.2005	54	37,137.8	**
Total	0.9891	71		

* Significant at the 0.05 level of confidence

** Significant at the 0.01 level of confidence

Table 8

Summary of Analysis of Variance

Left Testicle Weights

Source of Variance	SS	df	MS	F
Treatments	0.3483	8	435,330.0	8.75
Experimental Error	0.1344	27	49,769.5	**
Total	0.4827	35		

* Significant at the 0.05 level of confidence

** Significant at the 0.01 level of confidence

APPENDIX C

RESULTS: NEWMAN-KEULS PROCEDURE FOR MULTIPLE COMPARISONS

Table 9

Significance of Multiple Comparisons Using Newman-Keuls Procedure for Glycogen in Gastrocnemius

	BEAF	CEF	UEAF	BEF	EAF	UEF	UCAR	EAR	UCR	BCR	BEAR	BER	CAR	BCAR	CR	ER	UER	UEAR
BEAF											++	++	++	++	++	++	++	++
CEF												++	++	++	++	++	++	++
UEAF												++	++	++	++	++	++	++
BEF												++	++	++	++	++	++	++
EAF												++	++	++	++	++	++	++
UEF												+	++	++	++	++	++	++
UCAR														++	++	++	++	++
EAR																		++
UCR																		
BCR																		
BEAR																		
BER																		
CAR																		
BCAR																		
CR																		
ER																		
UER																		
UEAR																		

+ $P < .05$

++ $P < .01$

Table 10

Significance of Multiple Comparisons
Using the Newman-Keuls Procedure
for Glycogen in the Biceps

	BEAF	CEF	EAF	BEF	UEAF	CAR	BEAR	UEF	EAR	UEAR	BER	ER	BCAR	UCAR	CR	UCR	UER	BCR
BEAF										++	++	++	++	++	++	++	++	++
CEF										++	++	++	++	++	++	++	++	++
EAF										++	++	++	++	++	++	++	++	++
BEF														++	++	++	++	++
UEAF														++	++	++	++	++
CAR															+	++	++	++
BEAR																	+	+
UEF																		
EAR																		
UEAR																		
BER																		
ER																		
BCAR																		
UCAR																		
CR																		
UCR																		
UER																		
BCR																		

+	P < .05
++	P < .01

Table 11

Significance of Multiple Comparisons Using the Newman-Keuls Procedure for Glycogen in the Heart

	EAR	UEAF	CAR	BEAR	EAF	UEAR	BCR	CEF	UCAR	CER	UCR	CR	BER	BEAF	BEF	UER	BCAR	UEF
EAR																		++
UEAF																		+
CAR																		+
BEAR																		+
EAF																		
UEAR																		
BCR																		
CEF																		
UCAR																		
CER																		
UCR																		
CR																		
BER																		
BEAF																		
BEF																		
UER																		
BCAR																		
UEF																		
+	$P < .05$																	
++	$P < .01$																	

Table 12

Significance of Multiple Comparisons Using the Newman-Keuls' Procedure for Glucose in the Blood

	BEAF	UEF	CEF	EAF	BEF	BEAR	UEAF	CER	CR	UEAR	BCR	BER	UCAR	UCR	UER	BCAR	CAR	EAR
BEAF												+	++	++	++	++	++	++
UEF													+	+	++	++	++	++
CEF														+	+	+	++	++
EAF																	+	+
BEF																	+	+
BEAR																		
UEAF																		
CER																		
CR																		
UEAR																		
BCR																		
BER																		
UCAR																		
UCR																		
VER																		
BCAR																		
CAR																		
EAR																		

+ $P < .05$

++ $P < .01$

Table 13

Significance of Multiple Comparisons
Using the Newman-Keuls Procedure
for Plasma Free Fatty Acids

	UCAR	EAR	BER	BEAR	UER	CR	BCAR	BCR	CAR	UCR	CER	UEAR	UEAF	BEF	CEF	EAF	BEAF	UEF
UCAR														++	++	++	++	++
EAR														+	++	++	++	++
BER															++	++	++	++
BEAR															++	++	++	++
UER															++	++	++	++
CR															++	++	++	++
BCAR															++	++	++	++
BCR															++	++	++	++
CAR															++	++	++	++
UCR																++	++	++
CER																		++
UEAR																		++
UEAF																		
BEF																		
CEF																		
EAF																		
BEAF																		
UEF																		

+	$P < .05$
++	$P < .01$

Table 14

Significance of Multiple Comparisons Using the Newman-Keuls Procedure for Free Fatty Acids in Incubated Tissue

	BCAR	UCR	UCAR	BEAF	BCR	CR	UER	CER	UEAR	BEAR	BER	EAR	CAR	EAF	UEAF	UEF	BEF	CEF
BCAR														++	++	++	++	++
UCR														+	++	++	++	++
UCAR														+	++	++	++	++
BEAF														+	++	++	++	++
BCR															++	++	++	++
CR															+	++	++	++
UER																++	++	++
CER																++	++	++
UEAR																++	++	++
BEAR																++	++	++
BER																++	++	++
EAR																++	++	++
CAR																	++	++
EAF																	++	++
UEAF																	++	++
UEF																	++	++
BEF																	+	++
CEF																		
+	$P < 0.05$																	
++	$P < 0.01$																	

Table 15

Significance of Multiple Comparisons Using the Newman-Keuls Procedure for Free Fatty Acid in Homogenated Tissue

	CER	CR	UEAR	UER	UCAR	BCR	BCAR	BER	UCR	EAR	BEAR	CAR	UEAF	EAF	BEAF	BEF	UEF	CEF
CER													++	++	++	++	++	++
CR													++	++	++	++	++	++
UEAR													++	++	++	++	++	++
UER													+	++	++	++	++	++
UCAR													++	++	++	++	++	++
BCR														++	++	++	++	++
BCAR													++	++	++	++	++	++
BER													++	++	++	++	++	++
UCR													++	++	++	++	++	++
EAR													++	++	++	++	++	++
BEAR													++	++	++	++	++	++
CAR														++	++	++	++	++
UEAF																	++	++
EAF																	++	++
BEAF																		++
BEF																		++
UEF																		++
CEF																		

+	$P < 0.05$
++	$P < 0.01$

Table 16

Significance of Multiple Comparisons Using the Newman-Keuls Procedure for Lactate in the Blood

	EAR	CER	CR	CAR	CEF	UEAR	BEAR	BER	UCAR	UER	BCR	BCAR	UCR	BEAF	UEAF	UEF	EAF	BEF
EAR																		++
CER																		++
CR																		++
CAR																		++
CEF																		+
UEAR																		
BEAR																		
BER																		
UCAR																		
UER																		
BCR																		
BCAR																		
UCR																		
BEAF																		
UEAF																		
UEF																		
EAF																		
BEF																		
+	$P < .05$																	
++	$P < .01$																	

Table 17

Significance of Multiple Comparisons
Using the Newman-Keuls Procedure
for Body Weights

	CEF	BER	BEAF	BEF	BEAR	CER	UEAF	UEF	UER	UEAR	EAF	EAR	BCR	BCAR	CAR	UCAR	UCR	CR
CEF															+	++	++	++
BER																++	++	++
BEAF																++	++	++
BEF																++	++	++
BEAR																++	++	++
CER																++	++	++
UEAF																	++	++
UEF																	++	++
UER																		++
UEAR																		++
EAF																		++
EAR																		++
BCR																		++
BCAR																		++
CAR																		++
UCAR																		
UCR																		
CR																		

+ $P < .05$
++ $P < .01$

Table 18

Significance of Multiple Comparisons Using the Newman-Keuls Procedure for Liver Weights

	CEF	BEF	BEAF	BER	UEF	BEAR	EAF	UEAF	CER	BCR	CAR	EAR	BCAR	UER	UEAR	UCAR	UCR	CR
CEF										++	++	++	++	++	++	++	++	++
BEF										++	++	++	++	++	++	++	++	++
BEAF												++	++	++	++	++	++	++
BER																++	++	++
UEF																++	++	++
BEAR																++	++	++
EAF																++	++	++
UEAF																++	++	++
CER																	++	++
BCR																	++	++
CAR																	++	++
EAR																	++	++
BCAR																	++	++
UER																	++	++
UEAR																		
UCAR																		
UCR																		
CR																		

+	$P < .05$
++	$P < .01$

Table 19

Significance of Multiple Comparisons Using the Newman-Keuls Procedure for Heart Weights

	BER	BEF	BEAF	CEF	BCR	UEAR	BEAR	BCAR	UER	UCAR	UEF	UCR	UEAF	EAF	CER	CAR	CR	EAR
BER															+	+	++	++
BEF																		
BEAF																		
CEF																		
BCR																		
UEAR																		
BEAR																		
BCAR																		
UER																		
UCAR																		
UEF																		
UCR																		
UEAF																		
EAF																		
CER																		
CAR																		
CR																		
EAR																		
+	$P < .05$																	
++	$P < .01$																	

Table 20

Significance of Multiple Comparisons Using the Newman-Keuls Procedure for Spleen Weights

	BEAF	EAF	UEAF	BEF	UEF	CEF	BEAR	BER	UER	UEAR	BCR	EAR	CER	CR	CAR	UCR	UCAR	BCAR
BEAF																	+	++
EAF																	+	++
UEAF																		++
BEF																		++
UEF																		++
CEF																		+
BEAR																		
BER																		
UER																		
UEAR																		
BCR																		
EAR																		
CER																		
CR																		
CAR																		
UCR																		
UCAR																		
BCAR																		
+	P < .05																	
++	P < .01																	

Table 21

Significance of Multiple Comparisons Using the Newman-Keuls Procedure for Adrenal Weights

	EAR	CAR	BEAF	EAF	UEAF	BCAR	UEAR	UER	UCR	UCAR	UEF	CR	BCR	CER	BEAR	BER	BEF	CEF
EAR									++	++	++	++	++	++	++	++	++	++
CAR												++	++	++	++	++	++	++
BEAF																		++
EAF																		++
UEAF																		++
BCAR																		++
UEAR																		++
UER																		++
UCR																		++
UCAR																		++
UEF																		++
CR																		++
BCR																		++
CER																		
BEAR																		
BER																		
BEF																		
CEF																		

+	$P < .05$
++	$P < .01$

Table 22

Significance of Multiple Comparisons
Using the Newman-Keuls Procedure
for Right Testicle Weights

	UEAF	EAF	UCR	CAR	CR	UEF	CEF	UCAR	EAR	ER	UEAR	UER
UEAF												
EAF												
UCR												
CAR												
CR												
UEF												
CEF												
UCAR												
EAR												
ER												
UEAR												
UER												

\+ P < .05

++ P < .01

Table 23

Significance of Multiple Comparisons Using the Newman-Keuls Procedure for Left Testicle Weights

	EAR	EAF	CAR	CEF	CR	ER
EAR						
EAF						
CAR						
CEF						
CR						
ER						

+ P 0.05

++ P 0.01

APPENDIX D

RAW DATA

TABLE 24

GASTROCNEMIUS MUSCLE GLYCOGEN FOR EXPERIMENTAL ANIMALS

No.	NON-TRAINED	No.	TRAINED REST	No.	TRAINED FATIGUE
NORMAL RATS					
78	0.363	66	0.406	82	0.103
79	0.422	67	0.328	83	0.105
80	0.338	68	0.231	84	0.101
81	0.268	69	0.448	85	0.110
ANABOLIC STEROID TREATED RATS					
Mohan	0.446	Bert	0.287	74	0.142
Bob	0.273	Steve	0.186	75	0.104
Max	0.276	Herb	0.104	76	0.093
Jack	0.320	Robert	0.320	77	0.176
UNICASTRATED RATS					
62	0.244	49	0.433	53	0.193
63	0.246	50	0.394	54	0.159
64	0.267	51	0.356	55	0.092
65	0.251	52	0.345	56	0.149
BICASTRATED RATS					
58	0.282	41	0.288	45	0.163
59	0.256	42	0.322	46	0.097
60	0.260	43	0.387	47	0.040
61	0.236	44	0.291	48	0.205
UNICASTRATED AND ANABOLIC STEROID TREATED RATS					
37	0.269	25	0.536	29	0.099
38	0.226	26	0.322	30	0.020
39	0.110	27	0.539	31	0.108
40	0.077	28	0.225	32	0.224
BICASTRATED AND ANABOLIC STEROID TREATED RATS					
17	0.332	5	0.277	9	0.076
18	0.346	6	0.277	10	0.135
19	0.333	7	0.277	11	0.059
20	0.316	8	0.277	12	0.094

TABLE 25

BICEPS BRACHII MUSCLE GLYCOGEN FOR EXPERIMENTAL ANIMALS

No.	NON-TRAINED	No.	TRAINED REST	No.	TRAINED FATIGUE
NORMAL RATS					
78	0.271	66	0.406	82	0.103
79	0.261	67	0.328	83	0.105
80	0.230	68	0.231	84	0.101
81	0.221	69	0.448	85	0.110
ANABOLIC STEROID TREATED RATS					
Mohan	0.201	Bert	0.143	74	0.051
Bob	0.054	Steve	0.142	75	0.072
Max	0.113	Herb	0.113	76	0.030
Jack	0.051	Robert	0.173	77	0.061
UNICASTRATED RATS					
62	0.283	49	0.357	53	0.154
63	0.246	50	0.229	54	0.252
64	0.246	51	0.202	55	0.045
65	0.253	52	0.263	56	0.088
BICASTRATED RATS					
58	0.243	41	0.115	45	0.116
59	0.324	42	0.193	46	0.015
60	0.245	43	0.271	47	0.076
61	0.245	44	0.245	48	0.100
UNICASTRATED AND ANABOLIC STEROID TREATED RATS					
37	0.210	25	0.198	29	0.079
38	0.229	26	0.170	30	0.074
39	0.303	27	0.243	31	0.108
40	0.200	28	0.198	32	0.100
BICASTRATED AND ANABOLIC STEROID TREATED RATS					
17	0.167	5	0.125	9	0.035
18	0.187	6	0.125	10	0.054
19	0.283	7	0.125	11	0.038
20	0.208	8	0.125	12	0.048

TABLE 26

HEART MUSCLE GLYCOGEN FOR EXPERIMENTAL ANIMALS

No.	NON-TRAINED	No.	TRAINED REST	No.	TRAINED FATIGUE
			NORMAL RATS		
78	0.170	66	0.149	82	0.126
79	0.101	67	0.099	83	0.134
80	0.134	68	0.126	84	0.122
81	0.160	69	0.151	85	0.126
			ANABOLIC STEROID TREATED RATS		
Mohan	0.127	Bert	0.053	74	0.122
Bob	0.191	Steve	0.115	75	0.118
Max	0.061	Herb	0.067	76	0.137
Jack	0.081	Robert	0.079	77	0.111
			UNICASTRATED RATS		
62	0.129	49	0.186	53	0.305
63	0.139	50	0.090	54	0.303
64	0.119	51	0.155	55	0.130
65	0.158	52	0.170	56	0.182
			BICASTRATED RATS		
58	0.134	41	0.169	45	0.106
59	0.103	42	0.064	46	0.164
60	0.157	43	0.205	47	0.079
61	0.105	44	0.131	48	0.245
			UNICASTRATED AND ANABOLIC STEROID TREATED RATS		
37	0.146	25	0.131	29	0.143
38	0.126	26	0.115	30	0.089
39	0.146	27	0.131	31	0.056
40	0.104	28	0.111	32	0.142
			BICASTRATED AND ANABOLIC STEROID TREATED RATS		
17	0.154	5	0.119	9	0.166
18	0.174	6	0.119	10	0.046
19	0.204	7	0.119	11	0.111
20	0.139	8	0.119	12	0.251

TABLE 27

BLOOD GLUCOSE FOR EXPERIMENTAL ANIMALS

No.	NON-TRAINED	No.	TRAINED REST	No.	TRAINED FATIGUE
		NORMAL RATS			
78	125	66	124	82	81
79	101	67	100	83	79
80	125	68	121	84	83
81	120	69	125	85	81
		ANABOLIC STEROID TREATED RATS			
Mohan	150	Bert	121	74	86
Bob	144	Steve	137	75	70
Max	148	Herb	198	76	89
Jack	131	Robert	118	77	102
		UNICASTRATED RATS			
62	142	49	123	53	106
63	135	50	141	54	60
64	117	51	161	55	67
65	143	52	117	56	75
		BICASTRATED RATS			
58	122	41	111	45	86
59	125	42	121	46	93
60	115	43	138	47	53
61	132	44	142	48	119
		UNICASTRATED AND ANABOLIC STEROID TREATED RATS			
37	127	25	119	29	82
38	126	26.	126.	30	52
39	146	27	122	31	149
40	135	28	116	32	174
		BICASTRATED AND ANABOLIC STEROID TREATED RATS			
17	141	5	94	9	64
18	126	6	94	10	76
19	142	7	94	11	79
20	134	8	94	12	70

TABLE 28

FREE FATTY ACIDS IN PLASMA FOR EXPERIMENTAL ANIMALS

No.	NON-TRAINED	No.	TRAINED REST	No.	TRAINED FATIGUE
		NORMAL RATS			
78	0.32	66	0.45	82	0.55
79	0.31	67	0.47	83	0.67
80	0.37	68	0.45	84	0.81
81	0.34	69	0.40	85	0.68
		ANABOLIC STEROID TREATED RATS			
Mohan	0.37	Bert	0.26	74	0.67
Bob	0.31	Steve	0.32	75	0.72
Max	0.45	Herb	0.27	76	0.51
Jack	0.31	Robert	0.23	77	0.77
		UNICASTRATED RATS			
62	0.34	49	0.37	53	0.77
63	0.28	50	0.36	54	0.72
64	0.47	51	0.29	55	0.58
65	0.47	52	0.31	56	1.02
		BICASTRATED RATS			
58	0.34	41	0.30	45	0.47
59	0.42	42	0.27	46	0.83
60	0.32	43	0.21	47	0.56
61	0.32	44	0.40	48	0.45
		UNICASTRATED AND ANABOLIC STEROID TREATED RATS			
37	0.19	25	0.32	29	0.56
38	0.21	26	0.27	30	0.41
39	0.24	27	0.73	31	0.48
40	0.33	28	0.48	32	0.64
		BICASTRATED AND ANABOLIC STEROID TREATED RATS			
17	0.36	5	0.32	9	0.90
18	0.33	6	0.32	10	0.98
19	0.29	7	0.32	11	0.48
20	0.40	8	0.32	12	0.38

TABLE 29

FREE FATTY ACIDS IN HOMOGENATED TISSUE FOR EXPERIMENTAL ANIMALS

No.	NON-TRAINED	No.	TRAINED REST	No.	TRAINED FATIGUE
			NORMAL RATS		
78	1.16	66	1.11	82	11.77
79	1.59	67	1.03	83	10.63
80	1.14	68	1.38	84	9.84
81	1.19	69	1.44	85	10.81
			ANABOLIC STEROID TREATED RATS		
Mohan	2.29	Bert	1.45	74	4.39
Bob	2.14	Steve	2.49	75	6.78
Max	1.66	Herb	1.98	76	3.18
Jack	2.72	Robert	1.52	77	2.96
			UNICASTRATED RATS		
62	[illegible]	49	1.46	53	4.02
63	[illegible]	50	1.59	54	7.69
64	[illegible]	51	1.39	55	5.88
65	[illegible]	52	1.58	56	8.29
			BICASTRATED RATS		
58	[illegible]3	41	2.33	45	4.90
59	[illegible]9	42	1.49	46	9.37
60	[illegible]3	43	1.68	47	5.10
61	[illegible]6	44	1.34	48	4.34
			UNICASTRATED AND ANABOLIC STEROID TREATED RATS		
37	1.67	25	1.53	29	3.70
38	1.04	26	1.45	30	4.93
39	1.89	27	1.78	31	4.02
40	1.44	28	1.06	32	3.88
			BICASTRATED AND ANABOLIC STEROID TREATED RATS		
17	1.55	5	1.92	9	6.13
18	1.26	6	1.92	10	6.31
19	2.11	7	1.92	11	5.11
20	1.78	8	1.92	12	3.85

TABLE 30

FREE FATTY ACIDS IN INCUBATED TISSUE FOR EXPERIMENTAL ANIMALS

No.	NON-TRAINED	No.	TRAINED REST	No.	TRAINED FATIGUE
NORMAL RATS					
78	0.76	66	1.56	82	9.09
79	2.21	67	1.61	83	8.76
80	2.91	68	2.57	84	7.99
81	0.82	69	1.87	85	8.31
ANABOLIC STEROID TREATED RATS					
Mohan	1.82	Bert	2.38	74	4.04
Bob	4.31	Steve	1.71	75	4.78
Max	2.50	Herb	2.86	76	3.04
Jack	4.36	Robert	2.43	77	3.51
UNICASTRATED RATS					
62	1.37	49	2.12	53	3.03
63	1.21	50	0.99	54	4.80
64	1.26	51	3.32	55	6.93
65	1.37	52	1.16	56	3.93
BICASTRATED RATS					
58	1.21	41	2.91	45	10.34
59	2.49	42	1.95	46	8.16
60	0.60	43	2,55	47	4.77
61	1.49	44	1.18	48	6.96
UNICASTRATED AND ANABOLIC STEROID TREATED RATS					
37	1.12	25	2.07	29	4.12
38	0.77	26	1.48	30	3.61
39	1.93	27	2.06	31	4.71
40	1.39	28	1.80	32	4.44
BICASTRATED AND ANABOLIC STEROID TREATED RATS					
17	0.60	5	2.00	9	1.87
18	1.46	6	2.00	10	1.42
19	0.94	7	2.00	11	0.00
20	0.87	8	2.00	12	1.93

TABLE 31

BLOOD LACTATES FOR EXPERIMENTAL ANIMALS

No.	NON-TRAINED	No.	TRAINED REST	No.	TRAINED FATIGUE
NORMAL RATS					
78	30	66	15	82	22
79	23	67	22	83	31
80	19	68	29	84	26
81	18	69	21	85	29
ANABOLIC STEROID TREATED RATS					
Mohan	23	Bert	17	74	53
Bob	31	Steve	20	75	47
Max	26	Herb	22	76	62
Jack	16	Robert	24	77	48
UNICASTRATED RATS					
62	35	49	37	53	44
63	38	50	29	54	41
64	41	51	37	55	86
65	37	52	27	56	36
BICASTRATED RATS					
58	35	41	31	45	70
59	35	42	25	46	58
60	27	43	37	47	60
61	34	44	23	48	53
UNICASTRATED AND ANABOLIC STEROID TREATED RATS					
37	24	25	18	29	51
38	29	26	27	30	44
39	37	27	29	31	46
40	35	28	39	32	35
BICASTRATED AND ANABOLIC STEROID TREATED RATS					
17	42	5	29	9	35
18	24	6	29	10	39
19	29	7	29	11	41
20	47	8	29	12	55

TABLE 32

BODY WEIGHTS FOR EXPERIMENTAL ANIMALS

No.	NON-TRAINED	No.	TRAINED REST	No.	TRAINED FATIGUE
			NORMAL RATS		
78	512	66	403	82	304
79	524	67	326	83	316
80	466	68	304	84	322
81	410	69	379	85	318
			ANABOLIC STEROID TREATED RATS		
Mohan	380	Bert	377	74	328
Bob	389	Steve	402	75	342
Max	427	Herb	426	76	431
Jack	397	Robert	357	77	401
			UNICASTRATED RATS		
62	462	49	310	53	410
63	469	50	392	54	386
64	441	51	385	55	341
65	489	52	378	56	313
			BICASTRATED RATS		
58	385	41	282	45	356
59	415	42	326	46	328
60	381	43	345	47	337
61	382	44	320	48	353
			UNICASTRATED AND ANABOLIC STEROID TREATED RATS		
37	439	25	422	29	385
38	422	26	390	30	341
39	453	27	304	31	364
40	460	28	417	32	331
			BICASTRATED AND ANABOLIC STEROID TREATED RATS		
17	373	5	349	9	334
18	340	6	349	10	340
19	425	7	349	11	344
20	432	8	349	12	328

TABLE 33

LIVER WEIGHTS FOR EXPERIMENTAL ANIMALS

No.	NON-TRAINED	No.	TRAINED REST	No.	TRAINED FATIGUE
NORMAL RATS					
78	19.60	66	14.80	82	8.52
79	19.40	67	11.40	83	9.31
80	17.60	68	9.60	84	8.73
81	14.00	69	13.40	85	8.46
ANABOLIC STEROID TREATED RATS					
Mohan	12.97	Bert	11.57	74	11.32
Bob	13.77	Steve	13,77	75	10.70
Max	15.17	Herb	15.87	76	12.00
Jack	11.97	Robert	13.17	77	10.60
UNICASTRATED RATS					
62	16.80	49	11.60	53	12.20
63	17.00	50	14.60	54	11.40
64	17.40	51	15.20	55	10.40
65	18.60	52	14.40	56	8.60
BICASTRATED RATS					
58	12.20	41	8.39	45	9.60
59	15.00	42	11.79	46	8.20
60	13.20	43	12.39	47	9.20
61	13.20	44	9.79	48	9.40
UNICASTRATED AND ANABOLIC STEROID TREATED RATS					
37	16.20	25	15.19	29	11.40
38	15.60	26	13.79	30	11.20
39	16.70	27	11.59	31	12.80
40	15.40	28	15.59	32	12.20
BICASTRATED AND ANABOLIC STEROID TREATED RATS					
17	12.20	5	10.99	9	8.59
18	13.40	6	10.99	10	9.79
19	14.00	7	10.99	11	10.20
20	15.20	8	10.99	12	11.00

TABLE 34

HEART WEIGHTS FOR EXPERIMENTAL ANIMALS

No.	NON-TRAINED	No.	TRAINED REST	No.	TRAINED FATIGUE
NORMAL RATS					
78	1160.6	66	1101.4	82	890.1
79	1207.2	67	960.4	83	920.4
80	1011.0	68	1006.5	84	910.6
81	924.8	69	1061.4	85	911.8
ANABOLIC STEROID TREATED RATS					
Mohan	1092.2	Bert	1157.0	74	1008.1
Bob	941.8	Steve	1078.2	75	1059.6
Max	1096.4	Herb	1141.2	76	1006.0
Jack	1076.8	Robert	1019.8	77	955.4
UNICASTRATED RATS					
62	985.6	49	838.0	53	1051.4
63	1029.8	50	976.4	54	1049.0
64	955.8	51	973.6	55	944.6
65	1009.2	52	1029.2	56	867.8
BICASTRATED RATS					
58	914.8	41	744.4	45	1028.4
59	927.6	42	832.4	46	793.6
60	910.2	43	779.1	47	728.6
61	912.6	44	784.8	48	960.8
UNICASTRATED AND ANABOLIC STEROID TREATED RATS					
37	1014.6	25	890.4	29	988.4
38	915.0	26	1090.8	30	941.4
39	1004.4	27	766.0	31	989.8
40	966.2	28	933.6	32	1078.8
BICASTRATED AND ANABOLIC STEROID TREATED RATS					
17	806.8	5	943.8	9	924.6
18	995.8	6	943.8	10	929.2
19	923.4	7	943.8	11	911.2
20	1089.2	8	943.8	12	836.2

TABLE 35

SPLEEN WEIGHTS FOR EXPERIMENTAL ANIMALS

No.	NON-TRAINED	No.	TRAINED REST	No.	TRAINED FATIGUE
NORMAL RATS					
78	665.2	66	817.0	82	484.1
79	664.2	67	652.8	83	480.2
80	665.2	68	656.4	84	476.3
81	656.8	69	743.0	85	491.6
ANABOLIC STEROID TREATED RATS					
Mohan	647.2	Bert	524.0	74	491.6
Bob	659.8	Steve	565.8	75	442.2
Max	718.2	Herb	700.0	76	553.6
Jack	623.8	Robert	576.2	77	428.4
UNICASTRATED RATS					
62	638.8	49	542.0	53	473.2
63	623.0	50	640.4	54	453.6
64	675.0	51	688.0	55	558.8
65	720.8	52	677.4	56	346.8
BICASTRATED RATS					
58	545.0	41	432.4	45	570.0
59	682.0	42	670.6	46	437.3
60	599.2	43	490.6	47	378.6
61	625.0	44	522.6	48	433.2
UNICASTRATED AND ANABOLIC STEROID TREATED RATS					
37	676.4	25	783.2	29	430.6
38	687.6	26	616.8	30	336.4
39	771.6	27	473.8	31	611.2
40	613.4	28	795.6	32	468.2
BICASTRATED AND ANABOLIC STEROID TREATED RATS					
17	612.4	5	560.2	9	444.4
18	783.2	6	560.2	10	454.6
19	806.6	7	560.2	11	438.4
20	846.6	8	560.2	12	494.0

TABLE 36

ADRENAL WEIGHTS FOR EXPERIMENTAL ANIMALS

NORMAL RATS

78	50.8	66	56.4	82	72.2
79	56.2	67	49.0	83	76.3
80	51.0	68	52.0	84	69.4
81	52.0	69	59.4	85	70.4

ANABOLIC STEROID TREATED RATS

Mohan	20.2	Bert	36.4	74	46.1
Bob	17.8	Steve	22.0	75	44.2
Max	34.0	Herb	20.4	76	46.6
Jack	16.2	Robert	38.4	77	46.0

UNICASTRATED RATS

62	49.0	49	35.8	53	51.2
63	51.2	50	49.6	54	54.8
64	47.0	51	56.4	55	52.2
65	50.1	52	49.6	56	49.0

BICASTRATED RATS

58	47.2	41	54.4	45	64.6
59	70.6	42	58.4	46	60.4
60	46.6	43	62.6	47	43.2
61	47.4	44	47.6	48	57.8

UNICASTRATED AND ANABOLIC STEROID TREATED RATS

37	41.4	25	47.2	29	50.4
38	49.8	26	46.2	30	44.6
39	51.2	27	32.4	31	41.8
40	56.2	28	60.2	32	46.2

BICASTRATED AND ANABOLIC STEROID TREATED RATS

17	50.0	5	55.6	9	35.8
18	46.8	6	55.6	10	43.2
19	55.0	7	55.6	11	58.2
20	33.2	8	55.6	12	44.4

TABLE 37

RIGHT TESTICLE WEIGHTS FOR EXPERIMENTAL ANIMALS

No.	NON-TRAINED	No.	TRAINED REST	No.	TRAINED FATIGUE
			NORMAL RATS		
78	1567.8	66	1613.2	82	1723.6
79	1712.8	67	1573.2	83	1623.8
80	1636.0	68	1495.0	84	1529.3
81	1142.4	69	1707.0	85	1542.6
			ANABOLIC STEROID TREATED RATS		
Mohan	1282.6	Bert	1719.8	74	1326.4
Bob	1583.6	Steve	1433.0	75	1393.4
Max	1644.8	Herb	1577.0	76	1348.0
Jack	1094.8	Robert	1586.4	77	1296.8
			UNICASTRATED RATS		
62	1421.8	49	1708.2	53	1555.4
63	827.0	50	1544.8	54	1774.6
64	1664.2	51	1963.8	55	1470.4
65	1665.8	52	1695.6	56	1272.8
			UNICASTRATED AND ANABOLIC STEROID TREATED RATS		
37	1566.6	25	1597.4	29	1426.8
38	1575.2	26	1779.0	30	1447.4
39	1535.0	27	1363.6	31	992.2
40	1561.4	28	1716.2	32	1369.4

TABLE 38

LEFT TESTICLE WEIGHTS FOR EXPERIMENTAL ANIMALS

No.	NON-TRAINED	No.	TRAINED REST	No.	TRAINED FATIGUE
			NORMAL RATS		
78	1484.8	66	1637.2	82	1400.6
79	1741.4	67	1515.0	83	1486.3
80	1616.8	68	1515.0	84	1626.9
81	1371.4	69	1671.0	85	1583.4
			ANABOLIC STEROID TREATED RATS		
Mohan	1595.2	Bert	791.4	74	1371.8
Bob	1583.6	Steve	1131.8	75	1393.4
Max	1706.0	Herb	1640.2	76	1393.4
Jack	814.2	Robert	1532.2	77	1387.8

APPENDIX E

RESULTS: TABLES OF MEANS

Table 39

Means for Glycogen in the Gastrocnemius in grams/100 gm Tissue

	A	B	U	N	A+B	A+U
Rest control	.329* ±.081	.259 ±.019	.252 ±.010	.348 ±.064	.332 ±.012	.171 ±.092
Rest exercise	.224 ±.098	.327 ±.047	.382 ±.040	.353 ±.096	.227 ±.000	.406 ±.158
Fatigue exercise	.129 ±.038	.126 ±.073	.148 ±.042	.105 ±.004	.091 ±.033	.113 ±.084

Means for Glycogen in the Biceps Brachii in grams/100 gm Tissue

	A	B	U	N	A+B	A+U
Rest control	.105 ±.070	.264 ±.040	.257 ±.018	.246 ±.024	.211 ±.051	.236 ±.047
Rest exercise	.143 ±.025	.206 ±.069	.263 ±.068	.210 ±.043	.125 ±.000	.202 ±.030
Fatigue exercise	.054 ±.018	.077 ±.044	.135 ±.090	.048 ±.003	.044 ±.009	.090 ±.016

* Means ± Standard Deviation of the Mean

Table 40

Means for Glycogen in the Heart Muscle in grams/100 gm Tissue

	A	B	U	N	A+B	A+U
Rest control	.115* ±.058	.125 ±.026	.136 ±.017	.141 ±.031	.168 ±.028	.131 ±.020
Rest exercise	.079 ±.027	.142 ±.060	.150 ±.042	.131 ±.024	.119 ±.000	.122 ±.011
Fatigue exercise	.122 ±.011	.149 ±.074	.230 ±.088	.127 ±.005	.144 ±.087	.108 ±.043

Means for Glucose in the Blood in mgm/100 ml Blood

	A	B	U	N	A+B	A+U
Rest control	143.3 ±8.54	123.5 ±7.05	134.3 ±12.0	117.8 ±11.4	135.8 ±7.41	133.5 ±9.26
Rest exercise	143.5 ±37.3	128.0 ±14.5	135.5 ±19.8	117.5 ±11.8	94.0 ±.000	120.8 ±4.27
Fatigue exercise	86.8 ±13.2	87.8 ±27.2	77.0 ±20.3	81.0 ±1.63	72.3 ±6.65	114.3 ±56.8

* Means ± Standard Deviation of the Mean

Table 41

Means for Free Fatty Acids in Plasma in uEq/l

	A	B	U	N	A+B	A+U
Rest control	.360* ±.066	.350 ±.048	.390 ±.096	.335 ±.027	.345 ±.047	.243 ±.062
Rest exercise	.270 ±.037	.308 ±.084	.333 ±.039	.443 ±.030	.320 ±.000	.450 ±.207
Fatigue exercise	.668 ±.113	.578 ±.175	.733 ±.117	.668 ±.109	.685 ±.299	.523 ±.100

Means for Free Fatty Acids in Homogenated Tissue in uEq/gm Tissue

	A	B	U	N	A+B	A+U
Rest control	2.20 ±.437	1.65 ±.244	1.74 ±.471	1.27 ±.214	1.68 ±.360	1.51 ±.363
Rest exercise	1.86 ±.481	1.71 ±.436	1.51 ±.097	1.24 ±.201	1.92 ±.000	1.46 ±.299
Fatigue exercise	4.33 ±1.75	5.90 ±2.27	6.97 ±1.93	9.95 ±.072	5.35 ±1.13	4.13 ±.548

* Means ± Standard Deviations of the Mean

Table 42

[illegible] Fatty Acids in Incubated
[illegible] in uEq/gm Tissue

	[illegible]	B	U	N	A+B	A+U
Rest control	[illegible]* [illegible]	1.45 ±.788	1.28 ±.067	1.68 ±1.06	.968 ±.360	1.30 ±.489
Rest exercise	[illegible]35 [illegible]475	2.15 ±.757	1.90 ±1.07	1.90 ±.465	2.00 ±.000	1.98 ±.125
Fatigue exercise	[illegible].84 [illegible]47	7.47 ±2.19	4.67 ±1.67	8.54 ±.485	1.31 ±.899	4.22 ±.473

[illegible] for Lactates in the Blood
in mgm/100 ml Blood

	A	B	U	N	A+B	A+U
Rest control	24.0 ±6.27	32.8 ±3.86	37.8 ±2.50	22.5 ±5.45	35.5 ±10.8	31.3 ±5.91
Rest exercise	20.8 ±2.99	29.0 ±6.32	32.5 ±5.26	21.8 ±5.74	29.0 ±.000	28.3 ±8.62
Fatigue exercise	52.5 ±6.86	60.3 ±7.14	51.8 ±23.1	27.0 ±3.92	42.5 ±8.70	44.0 ±6.68

* Mean ± Standard Deviation of the Mean

Table 43

Means for Body Weight in Grams

	A	B	U	N	A+B	A+U
Rest control	398.3* ± 20.4	390.8 ± 16.8	465.3 ± 19.8	478.0 ± 51.8	392.5 ± 43.8	443.5 ± 16.8
Rest exercise	390.5 ± 30.0	318.3 ± 26.4	366.3 ± 37.9	353.0 ± 45.1	348.0 ± .000	383.3 ± 54.7
Fatigue exercise	388.0 ± 37.6	343.5 ± 13.3	362.5 ± 43.7	315.0 ± 7.75	336.5 ± 7.00	355.3 ± 24.2

Means for Liver Weights in Grams

	A	B	U	N	A+B	A+U
Rest control	13.5 ±1.35	13.4 ±1.17	17.5 ±.807	17.7 ±2.59	13.7 ±1.25	16.0 ±.591
Rest exercise	13.6 ±1.78	10.6 ±1.84	14.0 ±1.60	12.3 ±1.94	11.0 ±.009	14.0 ±1.81
Fatigue exercise	11.2 ±.647	9.10 ±.622	10.7 ±1.55	8.76 ±.388	9.90 ±1.01	11.9 ±.740

* Means ± Standard Deviations of the Mean

Table 44

Means for Heart Weights in mgm

	A	B	U	N	A+B	A+U
Rest control	1051.8 ± 73.8	916.3 ±7.75	995.1 ±31.8	1075.9 ±130.9	953.7 ±119.2	975.1 ±45.1
Rest exercise	1099.1 ± 62.4	787.7 ±36.0	954.3 ±81.6	1032.4 ± 61.8	943.8 ±.000	920.2 ±134.1
Fatigue exercise	1007.4 ± 42.5	877.8 ±140.0	978.2 ±88.9	903.7 ±9.98	900.3 ±43.4	999.6 ±57.4

Means for Spleen Weights in mgm

	A	B	U	N	A+B	A+U
Rest control	662.3 ± 40.2	612.8 ± 56.9	664.4 ± 43.5	654.3 ± 16.1	762.2 ±103.2	687.3 ± 65.0
Rest exercise	629.9 ± 68.7	584.2 ±146.6	598.3 ± 79.0	648.2 ±143.3	[illegible]51.2 ± 18.1	608.5 ±130.5
Fatigue exercise	458.5 ± 87.3	469.4 ± 81.4	479.7 ± 55.2	521.5 ± 82.7	44[illegible]5 ± 11.0	468.1 [illegible]15.3

* Means ± Standard Deviation of the Mean

Table 45

Means for Adrenal Weights in mgm

	A	B	U	N	A+B	A+U
Rest control	29.7* ±23.3	53.0 ±11.8	49.3 ±1.79	52.5 ±2.52	46.3 ±9.33	49.7 ±6.15
Rest exercise	27.8 ±8.32	55.8 ±6.38	47.9 ±8.65	54.3 ±4.51	55.6 ±.000	46.5 ±11.4
Fatigue exercise	45.7 ±1.05	56.5 ±9.30	51.8 ±2.41	72.1 ±3.05	45.4 ±9.34	45.8 ±3.59

Means for Right Testicle Weight in mgm

	A	B	U	N	A+B	A+U
Rest control	1401.5 ±258.6		1394.7 ±395.5	1514.7 ±255.1		1559.6 ± 17.5
Rest exercise	1579.1 ±117.2		1728.1 ±173.8	1597.2 ± 88.3		1614.1 ±183.2
Fatigue exercise	1341.2 ± 40.7		1518.3 ±207.9	1529.8 ± 82.2		1309.0 ±213.7

* Means ± Standard Deviation of the Mean

Table 46

Means for Left Testicle Weight in mgm

	A	B	U	N	A+B	A+U
Rest control	1424.8 ±410.8			1553.6 ±160.4		
Rest exercise	1273.9 ±389.0			1584.6 ± 81.5		
Fatigue exercise	1385.5 ± 9.71			1524.3 ±101.3		

* Means ± Standard Deviation of the Mean