THE EFFECTS OF DIANABOL AND ANAEROBIC ENDURANCE EXERCISE ON SELECTED ANATOMICAL AND HISTOCHEMICAL PARAMETERS IN THE ADULT MALE ALBINO RAT

Thesis for the Degree of M. A.
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ABSTRACT

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ADULT MALE ALBINO RAT

By

Robert Charles Hickson

The purpose of this study was to determine the separate and combined effects of an anabolic steroid and an anaerobic program of endurance running on selected anatomical and histochemical parameters in the adult male albino rat. Dianabol, a product of the CIBA Pharmaceutical Co., was the anabolic steroid used. The training program was the high-intensity, short-duration Controlled Running Wheel program developed in this laboratory. Body composition and various organ weights were investigated. Histochemical determinations were made of glycogen storage and phosphorylase activity in ten locations of the gastrocnemous-plantaris-soleus muscle group. The cross-sectional areas of thirty muscle fibers were measured in these same locations.

Forty-two, normal, male albino rats (Sprague-Dawley strain) of three different age levels, were brought

into the laboratory in one shipment. The differences in age were required to accommodate staggered treatment periods set up in conjunction with other concurrent studies using the same facilities. Initiation of treatments began for all animals at 100 days of age.

Fifteen animals were 90 days old (Age-Level 1), twelve animals were 76 days old (Level 2), and fifteen animals were 62 days old (Level 3) at the time of arrival. Each animal was randomly assigned to training-drug treatments within his own age group. All animals were allowed a minimum of 10 days to become acclimated to laboratory conditions before the study began. Since all animals began their training at 100 days of age, the Level 1 animals began the program first and the Level 2 and 3 animals followed at succeeding two-week intervals. Dianabol was administered subcutaneously at a 1-mg/day dose. Treatments were administered Monday through Friday for eight weeks. All animals were supplied with food and water *ad libitum*.

The exercised animals were selected for sacrifice on the basis of having the highest percent of expected revolutions (PER) within their own drug groups. The final sample consisted of 36 animals (six per cell).

At sacrifice, the animals were anesthetized with an intraperitoneal injection of sodium pentobarbitol. Selected organ weights were immediately removed, trimmed,

and weighed while wet. The gastrocnemous, plantaris, and soleus muscles were removed as a unit and frozen in a isopentane-liquid nitrogen system. Fresh-frozen, cross sections were cut at 10 microns using a rotary microtome in a cryostat. Relative localization of glycogen and phosphorylase were quantitatively determined by a histochemical photometer for a sample of thirty fibers in each of ten areas of the muscle unit. Absolute fiber size ($microns^2$), as measured with a polar plainimeter, was also determined for thirty fibers in the same ten muscle areas. The remaining carcass was saved for subsequent body composition analysis.

The results indicated that anaerobic training for eight weeks produced smaller body weights and smaller absolute weights of the liver, spleen, kidneys, and muscle in the exercise group than in the sedentary group. The relative weights of the adrenals, heart, liver, testes, kidneys, and muscle in the exercise group were larger than those in the sedentary group. Relative spleen weight was smaller in the exercise group.

Body weights of the Dianabol and placebo groups were both greater than that of the control group but not different from each other. Both absolute and relative liver weights were higher in the Dianabol group than in the control group, and the relative liver weights were also higher in the Dianabol group than in the placebo group.

Relative spleen weights were less in the Dianabol and placebo groups than in the control group.

Phosphorylase was depleted in all 10 muscle areas as a result of exercise. The Dianabol and placebo groups had less phosphorylase activity than the control group in area 3. An increase of glycogen in areas 4, 5, 6, 7, and 8 occurred with the training program. The Dianabol and placebo groups had less glycogen in area 5 than the control group. Absolute fiber size showed no changes with either the training or drug treatments.

Carcass weight and the percentage of fat were lower while the percentages of water, protein, and ash were higher in the exercise group than in the sedentary group. All of the absolute carcass components in the exercise group were lower than those in the sedentary group.

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Robert Charles Hickson

A THESIS

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DEDICATION

To my Mom, Dad, and Aunt Doris for their confidence and faith in me.

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CHAPTER I

INTRODUCTION

Early investigations led to the conclusion that the use of steroid hormones produced both an androgenic effect and the retention of nitrogen, an anabolic effect (66, 93, 114). Since then, anabolic steroids have been synthesized which stimulate positive protein metabolism as their main function. That is, chemical modifications of testosterone have led to the development of synthetic compounds with largely dichtomized anabolic and androgenic activities.

Krüskemper (73) has summarized the effects of anabolic steroids on protein synthesis. The proposed mechanism of action on protein assimilation is related to an increase in the ribonucleic acid content of cells, and an increase in the activities of enzymes which activate amino acids.

Initially, practical applications of anabolic steroids were limited to clinical medicine where they were used to counteract muscular atrophy, osteoporosis,

and the effects of corticoids. In recent years, steroid application has shifted from the pathological to the exercise end of the pathological-normal-exercise continuum with widespread usage among athletes. With the current emphasis on winning at all costs in sports, steroid use has risen markedly. The type of sports most affected appear to be those which include anaerobic-type events emphasizing strength. As a result of this trend, anabolic steroid usage has become quite common with some athletes consuming the steroids in dosages for surpassing what is recommended clinically.

Anabolic steroid usage by athletes has not had the prior experimentation necessary to determine whether it is useful and without accompanying pathological significance. Information is needed regarding the effects of anabolic steroids on cellular alterations, particularly in skeletal muscle which is most affected by these drugs.

Statement of the Problem

The purpose of this study was to determine the separate and combined effects of an anabolic steroid and an anaerobic program of endurance running on selected anatomical and histochemical parameters in the adult male albino rat. Dianabol, a product of the CIBA Pharmaceutical Co., was the anabolic steroid used. The training regimen was the high-intensity, short-duration Controlled Running

Wheel program previously reported from this laboratory (115). Body composition and various organ weights were investigated. Histochemical determinations were made of glycogen storage and phosphorylase activity in ten locations of the gastrocnemous-plantaris-soleus muscle group. The cross-sectional areas of thirty muscle fibers were measured in these same locations.

Rationale

It was hypothesized that anatomical and histochemical changes are mediated through the metabolic requirements of the animal. Both exercise and anabolic steroids are known to alter metabolic requirements. Thus, it was assumed that the effects of these imposed treatments should be reflected in the parameters selected for investigation.

It was further postulated that cellular responses in skeletal muscle are not only specific to the metabolic requirements of the total muscle but are identifiable by specific muscle areas. Ten pre-selected muscle locations were used to test this hypothesis.

Dianabol has been reported to have moderate or low anabolic activity as compared to other commercially available steroids. It was chosen for this study because it has been the steroid most widely used by athletes.

The rat was selected since it has been shown to provide a reasonably valid biological model for skeletal

muscle. Furthermore, the effects of exercise or organ weights and body composition have been studied chiefly in the rat.

Significance of the Problem

Specific knowledge of the anatomical and histochemical effects of exercise, steroids, and of the exercise-steroid combination are needed. The results of this study can add to that knowledge. Of course, the ultimate questions of whether steroid usage can increase performance and whether steroids can be used in athletes without corresponding pathological changes must await further investigation.

Limitations of This Study

1. The steroid dose of 1 mg/rat/day was chosen after consultation with Dr. J. J. Chart of the department of Endocrinology at CIBA Pharmaceutical Co. This dosage was selected to maximize the anabolic effects of Dianabol. However, there were no known quantitative data to support this judgment. In fact, recent evidence indicates the dosage may have been too low for maximum effects in the rat (14).

2. The exercise program selected for this study represents only one form of "anaerobic" exercise. It was the program judged to be most appropriate

for this study from those currently available at the Human Energy Research Laboratory, Michigan State University. Another program, especially one emphasizing a higher expenditure of strength, might have yielded entirely different results.

3. Due to laboratory facilities and the number of Controlled Running Wheels (CRW) available, the number of animals was limited to forty-two.

4. The experimental period was limited to eight weeks. There was no way of knowing whether this duration was optimal for maximizing the exercise and drug effects.

5. The Cornfield-Tukey argument for statistical inference was applied to the population of all rats similar to those chosen for this study.

6. The results of animal studies cannot be translated directly to humans. However, such studies do provide clues as to the structural and functional changes which may take place in the human.

7. Shock provided the stimulus for the animals to run. However, no control for the shock itself was included in the study.

Definition of Terms

Steroid Hormones.--Those hormones possessing the cyclopentanoperhydrophenanthrene ring system (steroid nucleus) in their molecules. They include the androgens, estrogens, and corticoids.

Androgen.--A generic term for an agent (usually a hormone, e.g., testosterone) that stimulates the activity of the accessory sex organs of the male, encourages the development of the male sex characteristics, or in special cases prevents the latter.

Anabolic Steroid.--A compound which relates to or promotes the process of assimilation of nutritive matter and its conversion into living substance. This includes synthetic processes and requires energy.

Testosterone.--A male steroid hormone with both androgenic and anabolic effects. It is produced by the Leydig cells of the testes under normal conditions.

Dianabol.--A synthetic anabolic steroid derivative of testosterone, produced by CIBA Pharmaceutical Co. The pharmacological name of Dianabol is methandrostenolone while the structural name is 17-methyl-17-hydroxyandrosta-1, 4-dien-3-one.

Myotropic Effect.--The property of an anabolic steroid to increase muscle mass. This term was used, interchangeably with "anabolic effect" in this study.

CHAPTER II

REVIEW OF RELATED LITERATURE

In order to provide a better understanding of the separate effects of exercise and anabolic steroids, these two topics are first reviewed individually. With this background, the work done with exercise plus anabolic steroids is then reviewed.

Effects of Dianabol and Other Anabolic Steroids

Measurement of Myotropic Activity

Pioneer work in this area began with Kochakian (66) who found an increase in nitrogen retention in three castrated dogs which had received a male sex hormone subcutaneously. The positive nitrogen balance was due to changes in urinary urea. Nitrogen retention was greater after the administration of repeated doses (2X daily) than after single large doses. There was a point beyond which increasing the amount of hormone did not increase the amount of nitrogen retained. The weights of the dogs showed significant but not large increases during the

injection period but returned to preinjection levels after cessation of the treatments.

Wainman and Shipounoff (114) initiated research for determining the myotropic effect of steroids. They observed that the perineal muscles (levator ani, bulbocavernosus and ischiocavernosus) were more responsive to castration and treatment with testosterone propionate than were other striated muscles. In normal rats, the administration of testosterone propionate caused an increase in the bulk of these muscles as manifested by an increase in the width of the muscle fibers. This line of investigation eventually led to the development of an appropriate method for determining an anabolic-androgenic index.

Eisenberg and Gordan (73) asked whether the effect on the perineal musculature was due to the anabolic or androgenic component of testosterone. Working with castrated rats, they concluded that any steroid-induced gain in weight of the levator ani muscle was exclusively an expression of the anabolic property of the steroid. Their experimental procedure began with castration of three-week-old rats. Twenty-three days after castration, a steroid was injected daily. The reference compound was testosterone or testosterone propionate. The effects of the reference compound on the levator ani, seminal vesicles, and prostate were compared quantitatively with

those produced by the tested steroid. The ratio of the activities of the two compounds was then calculated as an anabolic-androgenic index.

Papanioclaou and Falk (93) found hypertrophy of the temporal muscles in castrated immature male guinea pigs when treated with testosterone propionate. No quantitative data were given.

In a study examining the same muscle group, Kochakian, Humm, and Bartlett (70) found that castration decreased the weight of the temporal muscles in immature male albino guinea pigs to less than one-third that of normal animals. Subsequent subcutaneous implantation of various steroid pellets increased the weight of the temporal muscles. However, the increase was only to about half of that of normal animals. The myotropic-androgenic ratio (increase in temporal muscle mass divided by the increase in accessory sex organs--seminal vesicles and prostate) was determined for various steroids. The castrated animals' body weight was less than that of the normals. The steroids restored the body weight to normal; but when a maximal response was attained, a further increase in dose either had no further effect or was less effective.

The method of Eisenberg and Gordan was later modified by Hershberger, Shipley, and Meyer (54). They eliminated the twenty-three-day post-castration rest

period for the animals, thus reducing the total assay time from thirty-one to eight days.

In evaluating compounds for myotropic effects, the ratio of the response of the levator ani (LA) to the response of the ventral prostate (VP) was employed. The ratio was calculated as follows:

$$\frac{LA}{VP} = \frac{\text{Levator ani weight (Experimental)} - \text{Levator ani weight (Control)}}{\text{Ventral prostate (Experimental)} - \text{Ventral prostate (Control)}}$$

Their preliminary screening with young male castrated rats showed 19-nortestosterone and other 19-nor analogs of androgens to be effective anabolic and relatively weak androgenic agents. At the same dose levels, 19-nortestosterone showed weak androgenic activity while testosterone showed strong androgenic activity.

Metcalf and Broich (84) measured the anabolic potential of steroids using C^{14} alpha amino-isobutyric acid (AIB). Male rats (150 gm) were castrated and immediately given steroids intramuscularly while under ether anesthesia. Thirty hours later 1.0 ml of AIB solution was administered subcutaneously. Nine hours after AIB injection, the animals were exsanguinated under ether anesthesia by direct cardiac puncture.

The sensitivity of the AIB test was five to sevenfold that of the levator ani weight increment test on the basis of the ratio between percentage increase in uptake

to percentage increase in weight. There was a reduction in urinary AIB excretion even before any nitrogen retention became established. The authors suggested the possibility of using this reduction as an additional or alternate indicator of the anabolic activity of steroids. The major problem was to determine what proportion of the uptake of AIB was attributable to the anabolic effect and what proportion was attributable to the androgenic effect of these steroids.

Overbeck and de Visser (91) compared (a) the phenyl propionates (PP) of testosterone (T) and nandrolone (N), and (b) the decanoates (D) of testosterone and nandrolone after the subcutaneous injection of a single threshold dose into young rats (50-60 gm). The rats were castrated the day before the experiment, and the levator ani test was used as described by Hershberger, Shipley, and Meyer (54).

The anabolic-androgenic ratios were calculated in the following way:

$$Q\ (NPP/TPP) = \frac{R_{Anab.}\ (NPP/TPP)}{R_{Andr.}\ (NPP/TPP)}$$

$$Q\ (ND/TD) \quad \frac{R_{Anab.}\ (ND/TD)}{R_{Andr.}\ (ND/TD)}$$

where:

$R_{Anab.}$ = potency ratio based on effects on levator ani muscle,

$R_{Andr.}$ = potency ratio based on effects on seminal vesicle.

Thus, the principle of this calculation was to establish potency ratios for each activity and then to calculate ratios of these activities. Their results showed that the nandrolone esters were relatively more anabolic and less androgenic than the corresponding testosterone esters. The phenylpropionates were more active than the decanoates but their duration of action was much shorter, especially with regard to the levator ani muscle.

Linearity of the dosage-activity curve which is the essential premise of the Overbeck and de Visser calculation was not met according to Krüskemper (73). He also points out that the reference steroids of the Hershberger method (testosterone propionate, 17 alpha-methyltestosterone and others) are more androgenic than myotropic.

Dianabol as an Anabolic Agent

At a high steroid dose level, the uptake of Dianabol (methandrostenolone), as measured by the C^{14} labeled AIB method (84), was lower than the mean uptake

value of testosterone propionate which served as the standard.

Izzo and Glasser (59) also recorded low anabolic behavior of Dianabol, in that it did not inhibit the course of protein catabolism in fasting rats. Kochakian (68) observed that testosterone propionate (unlike Dianabol) hastened the replenishment of protein in starved rats, although after body weight was restored the nitrogen retention decreased below that of the controls. It also was observed by Lloyd and Anthony (82) that nitrogen retention did not increase in pigs during a six-week period of feeding methandrostenolone at a level of 1 mg/kg of feed starting when the animals were three weeks old. The animals taking methandrostenolone had a larger number of muscle fibers of small diameter than did the controls.

Almqvist, Ikkos, and Luft (3) used graded doses of methandrostenolone (5, 10, and 25 mg/day) and testosterone propionate (25 mg/day) on three metabolically stable subjects all of whom had received steroid therapy before. The results indicated 5 mg/day of methandrostenolone induced nitrogen and calcium retention, while the effects observed with the larger doses were not quantitatively different from the 5-mg/day dose. The nitrogen and calcium retention with the 5-mg/day dose was as great or greater than that induced by testosterone propionate (25 mg/day). Methandrostenolone induced creatinurea but

had no effect on sodium and chloride balances and urinary excretion of 17-ketosteroids.

Arnold, Potts, and Beyler (5) using castrated male rats (200 gm) determined methandrostenolone to be 1.2 ± 0.14 times as effective for nitrogen retention as methyl-testosterone as measured by urinary nitrogen. When androgenic activity was measured by the ventral prostate weight method of Hershberger, methandrostenolone was found to be 0.35 ± 0.045 times as androgenic as methyltestosterone. The resultant relative anabolic-androgenic ratio was 3.4. However, these values were the lowest in nitrogen retention, highest in androgenic activity, and lowest in nitrogen retention-androgenic ratio of the five steroids studied, disregarding methyltestosterone which was the standard.

Other investigators have reported low anabolic effects of Dianabol. Dorfman and Kincl (31) observed decreased seminal vesicles in young castrated rats (21-23 days) treated with Dianabol as compared to those found in similar animals given 17 alpha-methyltestosterone. Levator ani weights were not different. Saarne, Bjerstaf, and Erman (96) administered Dianabol to hospitalized patients and recorded nitrogen retention to be only 1 gm/day. This value corresponds to the generally accepted figure for possible losses through the skin.

However, a positive effect was observed by Sloper and Pegrum (99) who injected 0.2 mg of Dianabol daily in mice (25-40 gm) whose right gastrocnemous was crushed.

The treated mice had an accelration both in phagocytosis and in muscular regrowth. They postulated that the acceleration in myogenesis reflected either an increase in RNA and DNA or the special susceptibility of regenerating tissue to the steroid. The effect on myogenesis was probably maximal on the second and third days after injury.

Further attempts to quantitate the beneficial effects of anabolic steroids on protein metabolism were made by Albanese (2). He developed the Steroid Protein Activity Index (SPAI). The formula is:

$$SPAI = \frac{NSBP}{NISP} = \frac{NBCP}{NICP} \times 100$$

where:

NBSP = nitrogen balance in steroid period

NISP = nitrogen intake in steroid period

NBCP = nitrogen balance in control period

NICP = nitrogen intake in control period

Anabolic agents have a positive SPAI and the magnitude of the value is directly proportional to the metabolic effect. Dianabol's SPAI was determined to be +16 which was the median value of the nine anabolic steroids studied.

Recently Boris, Stevenson, and Trmal (14) injected doses of Dianabol and eleven other steroids for ten consecutive days in rats (60-70 gm) which were 24 to 25 days old at the start of the experiment. Testes weight

decreased significantly at 100 mcg/rat/day. Seminal vesicle weight and ventral prostate weight increased at 1,000 mcg/rat/day. These results showed Dianabol to be neither as active anabolically nor as active androgenically as most of the other steroids. The androgenic values are in disagreement with the high values recorded by Arnold (5).

In a subsequent study Boris, Stevenson, and Trmal (15) administered Dianabol and eleven other steroids for seven consecutive days using the same experimental conditions as before. Potencies were evaluated in terms of the dosages required to double the weights of target organs. Dianabol ranked last in potency rank for the levator ani, ventral prostate, and seminal vesicles.

Role of Anabolic Steroids on Skeletal Muscle Glycogen Concentration

Lewis and McCullagh (81) examined fasted adult male rabbits (2.7-3.5 kg) with administration of methyltestosterone (MT), MT and a high carbohydrate diet, and MT and testosterone propionate TP. The differences observed in the gastrocnemous were not significant although the mean glycogen values (gm %) were: .311 for controls, .338 for MT, .360 for MT and diet, and .351 for MT and TP.

The conclusions drawn by Lewis and McCullagh appear to be in the minority. Leonard (79) observed an increase in the glycogen content of the perineal muscles in normal,

castrated, and hypohysectomized fasting rats that were injected with 1 mg of testosterone propionate for three days. He postulated that the increase in glycogen concentration was an index of renewed growth in these muscles. Supporting this view is Kochakian (68) who reviewed the biochemical evidence on the anabolic property of testosterone, and suggested that the increase in muscle glycogen was indicative of the mechanism by which the hormone exerts its effect.

In order to pursue his theories further, Leonard (80) observed an increase in the glycogen content of the rectus femoris, abdominal, and cremaster muscles, using a 1 mg dose for six days beginning three days after castration in fasting male and female rats. These muscles were used to show that skeletal muscle can be a part of the mechanism by which the hormone exerts an anabolic effect.

The duration of steroid use was studied by Meyer and Hershberger (85) who examined the effects of testosterone propionate (TP) on the glycogen content of the levator and muscle following administration for one, three, five, and seven days in 21- and 54-day-old castrated rats. There was an initial increase in TCA soluble glycogen; however, with continued use of TP (0.1 mg daily) a decrease in glycogen concentration associated with rapid growth of the levator ani was observed. The authors speculated that the early rise in glycogen represented a storage of

potential energy which became depleted in the course of protein synthesis and rapid muscular growth. Supporting this initial rise in glycogen were Adolfson and Ahren (1) who also found glycogen to increase in the levator ani both 10 and 24 hours after injection of testosterone propionate (100 mg/kg) in immature male rats.

The effects of various doses were studied by Talaat and Habib (107), who reported an increase in thigh muscle glycogen in male rabbits with a dose of 5 mg/kg of testosterone propionate for ten days. Twelve days after cessation of treatment with a 10 mg/kg dose an increase in muscle glycogen was recorded, while no residual change was noted with a 5 mg/kg dose. In a later study, Talaat and Habib (108) castrated male rabbits (1 kg) and administered single injections of testosterone propionate at doses of either 1 mg/kg or 5 mg/kg. In animals castrated for 14 days, muscle glycogen levels were elevated both after 12 hours and after 3 days for both doses. In those animals castrated for 30 days, a 1 mg dose increased glycogen levels after 12 hours; however, then values returned to castrated levels after 3 days. The animals receiving 5 mg showed no changes.

The effects of repeated injections of 1 mg for 7 days in the rabbits castrated 14 days earlier resulted in elevated muscle glycogen levels both 12 hours and 3 days after the last injection. With repeated injections of

5 mg doses, glycogen was only temporarily increased after 12 hours. Both the present findings and the results of Almqvist, Ikkos, and Luft (3) indicate that the anabolic activity potential of the steroids is not directly related to increases in dosage.

Effects of Exercise

Response of Skeletal Muscle Glycogen and Phosphorylase

Various studies (10, 57, 58) have shown that a high carbohydrate diet increases the resynthesis of muscle glycogen after exercise to levels far above normal values. It has also been shown that a reciprocal relationship exists between phosphorylase and oxidative enzyme activity (33). However, the specific metabolic relationships between glycogen and phosphorylase in the various skeletal muscle fiber types has not been elucidated conclusively.

Beatty, Peterson, and Bocek (9) determined that glycogen concentrations were higher in the white fibers than in the red fibers of the adductor muscles of rats immediately after sacrifice. After a two-hour incubation period, glycogen concentration in the red fibers was higher. In a later study, the same group of coworkers (13) again found initial glycogen concentrations lower in the red fibers of the rat adductor group. After a two-hour incubation *in vitro*, the decrease of glycogen in red muscle was one-half as great as in white muscle. Ten

times more labeled glucose C^{14} was incorporated into red muscle during sixty minutes of incubation and three times as much after two hours.

Studies also have shown the red area of muscle to have higher glycogen content. Jeffress, Peter, and Lamb (60) found the increase in total glycogen synthetase activity to be greater in the red area than in the white area of the vastus lateralis muscle of guinea pigs. The trained group (treadmill running for 30 minutes at 1.9 km/hr for three weeks) had the highest values, while the exercised group which ran only once had the lowest.

Gillespie, Simpson, and Edgerton (45) showed biochemically that the guinea pig vastus lateralis contained more glycogen in the red region (9.7 mg/g) than in the white region (7.4 mg/g). Histochemically, the red fibers showed more intense staining with PAS than either the white or intermediate fibers. They concluded that the metabolic characteristics of muscles can best be described in terms of their histochemically determined fiber populations.

Other studies have shown no differences in glycogen content by fiber areas. Short, *et al*. (97) trained rats for eight weeks by submaximal running (13.7 m/min) on a motor-driven drum. The animals exercised a total of four hours daily, with five-minute rest periods between each thirty-minute running period, six days a week. Examination of the adductor magnus muscle *in vivo* showed the glycogen

concentration to be higher in red muscle than in white muscle only for the trained animals. The *in vitro* incorporation of glucose C^{14} into glycogen was greatly accelerated in red as compared to white muscle, and glycogen specific activity at the end of the incubation period was higher in the red fibers. However, these results were present in both the control and experimental groups and were not affected by training. The authors concluded that the differences in glycogen concentrations in red and white fibers may be exaggerations of their normal relationships and not unequivocally attributable to training.

Lamb, *et al*. (76) exercised guinea pigs on a motor-driven treadmill (1.9 km/hr). Glycogen concentration was increased 48 hours after exercise; however, both red and white sections of the vastus lateralis muscle exhibited similar patterns of glycogen changes immediately and 48 hours after exercise. Glycogen values of the trained and untrained groups immediately after exercise, when compared with those before exercise, suggested that approximately the same amounts of muscle glycogen were used for the first thirty minutes of exercise by both groups. However, the trained animals with greater glycogen stores could continue to exercise for a longer time. This finding was in agreement with Bergstrom, *et al*. (11) who showed a positive

relationship between muscle glycogen concentration and exercise tolerance in man.

Phosphorylase activity was studied by Rawlinson and Gould (94) who swam rats of three different age groups, for one and two thirty-minute periods daily, for eight weeks. They concluded from biceps femoris homogenates that the total activity of phosphorylase was not affected by either swimming program in any of the groups. Edgerton, et al. (38) exercised guinea pigs on a treadmill at 1.6 km/hr for 5 minutes, 10 minutes, or to exhaustion. Phosphorylase-negative fibers in the plantaris muscle were found with increasing durations of exercise. A higher percentage of fibers in the red region than in the white region became phosphorylase-negative. After exhaustive exercise, white fibers were the most resistent to becoming phosphorylase-negative. No consistent changes were observed in the soleus muscle. This possibly was due to its homogeneous composition of intermediate fibers.

The simultaneous investigation of glycogen and phosphorylase has led to divergent results. Stubbs and Blanchaer (105) demonstrated histochemically in guinea pigs that phosphorylase is more active in white muscle fibers (guadriceps femoris) than in red muscle fibers (adductors), while glycogen synthetase showed no differences. However, quantitative determinations showed glycogen synthetase to be higher in red fibers and total

phosphorylase (A+B) to be higher in white fibers. Stimulation (30 sec. with 1 volt impulses of 20 miliseconds duration at a rate of 20 pulses per sec.) produced a significant conversion of phosphorylase B to A only in white muscle. Stimulation did not alter the synthetase level of red muscle but increased it significantly in white muscle. The quantitative evaluation of glycogen synthetase compared favorably with the results of Jeffress, Peter, and Lamb (60).

Kugelberg and Edstrom (74) induced muscular contration in the anterior tibial muscle of the rat with low frequency shock (5/sec, and 10/sec) and found phosphorylase and glycogen to become negative fastest in A fibers, next in B fibers, and slowest in C fibers. No changes were observed in the soleus. After one and two hours of stimulation (5/sec), glycogen negative fibers were identified but phosphorylase negative fibers were absent. The phosphorylase results were in direct contrast to those of Edgerton, et al. (38), however the methods of inducing changes were not the same. Kugelberg and Edstom concluded that the histochemical method reflects the active form of phosphorylase rather than total phosphorylase, and that histochemical changes in phosphorylase are secondary to changes in glycogen.

In order to investigate the differences further, Edgerton, et al. (39) exercised guinea pigs with wind

sprints and endurance running on a treadmill for twenty weeks. Following the training program, muscular contraction was induced by electrical stimulation (5/sec for 1 hour) of the medial gastrocnemous. Total phosphorylase activity, when examined histochemically, was selectively depleted in the white region. Phosphorylase depletion was paralleled by glycogen depletion when measured histochemically and biochemically. Every phosphorylase-negative fiber was negative for glycogen as determined by the PAS stain. The histochemical depletion of phosphorylase and glycogen was greater in the nontrained than in the trained animals. Phosphorylase activity did not return to prestimulation levels as was reported by Kugelberg and Edstrom.

Quantitative determinations with a histochemical photometer have been done at Michigan State University (unpublished data). Using the same ten selected muscle areas as in the present study (see Figure 3, p. 48) and the same exercise regimen (SHORT), with a duration of eight weeks, phosphorylase was found to decrease in areas 9 and 10 and to increase in areas 4, 5, 6, and 7. Glycogen as measured by the PAS stain showed increased in areas 1, 2, 3, 5, 7, 8, and 9. A slight decrease was observed in area 4.

Body Composition and Organ Weights

The known effects of various types of exercise on the absolute and/or relative values of water, fat, protein, and ash are presented in Table 1. VanHuss, Heusner, and Mickelson (111) have presented data on the residual effects of exercise. No significant differences were observed.

Studies of the effects of various exercise programs on organ weights have been reviewed by Montoye, et al. (87) up to 1960. The results are presented in Table 2. A continuation of the literature since 1960 is presented in Table 3. It has been shown that the residual effects of exercise are not reflected by differences in organ weights (111).

Effects of Exercise and Anabolic Steroids

Human Studies

Johnson and O'Shea (61) recorded significant increases in dynamic strength (bench press and squat) and static strength (cable tensiometry) in twelve matched pairs of subjects, aged 19 to 39, who were on a six-week weight training program. All subjects were fed a high-protein diet throughout the program with the experimental group receiving Dianabol (5 mg) twice daily during the final three weeks of the program. Body weight, biceps and calf size, and oxygen uptake as measured by the Astrand oxygen

TABLE 1.--Effects of Exercise on the Absolute (gm) and Relative (Percent) Body Composition Components.

Reference	Type of Exercise	Subjects	Carcass Wt.	Water		Fat		Protein		Ash	
				Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
Horst (56)	Running	Rats			(+)		(-)		(+)		(+)
Jones (63)	Swimming	Young Rats	-	0	+	-	-	0	+	0	+
Hanson (50)	Swimming	Mature Rats	-	-		-		-		0	
VanHuss (111)	Voluntary	Young Rats	-	-	+	-	-	-	0	0	+
VanHuss (111)	Voluntary and Forced (Swimming)	Young Rats	-	-	+	-	-	-	+	-	+

Note: Within the table + indicates increase, - a decrease, and 0 no change in body composition components due to exercise. Blank spaces indicate the parameter was not investigated. If these symbols are in parenthesis a statistical analysis of the data was not made.

TABLE 2.--Previous Studies of Effects of Forced and Spontaneous Exercise on Organ Weights From Montoye, *et al.* (87).

Reference	Heart		Liver		Kidneys		Spleen		Adrenals		Testes	
	For.	Spont.	For.	Spont.	For.	Spont.	For.	Spont.	For.	Spont.	For.	Spont.
Young Male Rats:												
Donaldson (28)		(+)		(+)		(+)		(+)		(+)		(-)
Donaldson (29)		(+)		(+)		(+)		(+)		(+)		(+)
Hatai (51)		(+)		(+)		(+)		(-)		(0)	0	(+)
Kimeldorf and Baum (65)[r]	+				0		0		+			
Donaldson (28)		(+)		(-)		(+)		(-)		(+)		(+)
McClintock (83)		(0)										
Borovansky (16)	(+)		(-)		(+)				(+)		(+)	
Donaldson (27)		(+)		(-)		(+)		(-)		(+)		(+)
Adult Male Rats:												
Hearn (52)[b]	+								+			
Donaldson (28)		(+)		(+)		(+)		(-)		(+)		(+)
Young Female Rats:												
Donaldson (28)		(+)		(+)		(+)		(-)		(0)		
Donaldson (29)		(+)		(+)		(+)		(+)		(+)		
Donaldson (28)		(+)		(+)		(+)		(+)		(+)		
Borovansky (16)	(+)		(-)		(+)		(+)		(+)			
McClintock (83)		(+)										
Donaldson (27)		(+)		(+)		(+)		(+)		(+)		
Adult Female Rats:												
Donaldson (28)		(+)		(+)		(+)		(+)		(+)		
No Age Given; Rats:												
Van Liere (112)[r] male	+											
Van Liere (112)[r] female	+											
Dogs:												
Bruns (22)[c]		(+)		(+)		(+)						
Grober (47)[c]		(+)		(+)		(+)						
Junkersdorff (64)[c]		(+)		(+)		(+)						
Siebert (98)[c]		(+)		(+)		(+)		(+)				
Steinhaus (103)[r] (run)		(+)		(-)		(+)				(-)		(-)
Steihaus (103)[r] (swim)	(+)		(+)		(+)		(-)		(+)		(-)	
Guinea Pigs:												
Petren[d]		(+)										

[a]In the body of the table + indicates increase, - a decrease, and 0 no change in organ weight due to exercise. If these symbols are in parenthesis a statistical analysis of the data was not made. r indicates relative organ weight.

[b]Animals began exercise before full-grown (100-120 days).

[c]From Donaldson (30).

[d]From Steinhaus (102).

TABLE 3.--Effects of Exercise on the Absolute (gm) and Relative (Percent) Organ Weights Since 1960.

Reference	Type of Exercise	Subjects	Body Weight	Adrenals		Heart		Liver		Spleen		Testes		Kidneys	
				Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
Montoye (87)	Voluntary	Mature Rats	0		0		0		0		0		0		0
Jones (63)	Swimming	Young Rats	-	+				-							
Hanson (50)	Swimming	Mature Rats	-	+		+		+				0			
Lamb (75)	Voluntary	Young Rats	-		+		0		0		0		+		+
Lamb (75)	Voluntary and Forced (Swimming)	Young Rats	-		-		0		0		0		+		-
VanHuss (111)	Voluntary	Young Rats	-	0	+	-	0	-	0	-	0	0	+	-	0
VanHuss (111)	Voluntary and Forced (Swimming)	Young Rats	-	+	+	-	+	-	0	-	0	0	+	-	0

Note: Within the table + indicates increase, - a decrease, and 0 no change in organ weights due to exercise. Blank spaces indicate the parameter was not investigated. If these symbols are in parenthesis a statistical analysis of the data was not made.

uptake test increased in the treated group. The increase in oxygen uptake was not expected.

In an earlier study, Fowler, Gardner, and Egstrom (44) administered 1-methyl-Δ'-androstenolone acetate (Nibal), at a dose of 20 mg/day, alone or in conjunction with a sixteen-week physical condition program. No differences were observed in strength, oxygen uptake ability, serum enzymes, anthropometric measurements, or performance in either the trained or untrained college men who were used as subjects. However, the intensity of the exercise program may have been too low to cause strength increases. In addition, the subjects in the study were not placed on a high-protein diet.

O'Shea and Winkler (90) administered a 10 mg/day dose of oxandrolone (Anavar) to eight competitive swimmers and three weight lifters for six weeks during an eleven-week study. Swimming performance was not improved, however the weight lifters showed considerable improvement in strength during the steroid period. Body weight increased for all subjects during the treatment period. An increase in protein utilization, as measured by Albanese' SPAI index, was found in eight of the eleven subjects. SPAI and body weight of the individuals showed a high correlation. In a subsequent study, Johnson, et al. (62) selected subjects from college physical education activity classes and randomly assigned them to treatment and

placebo groups after four weeks of weight training. A double blind method was used to administer a 10 mg/day dose of Dianabol and a protein supplement for 21 days. There were no significant changes in maximal oxygen uptake, sperm count, or deposition of subcutaneous adipose tissue. Dynamic and static strength and body weight increased in the treatment groups. Similar results were recorded by Bowers and Reardon (17). They administered Dianabol, 10 mg daily for the last 21 days of training, and a protein supplement throughout a six-week weight training program to eighteen experienced weight trainers. The experimental group showed increases in bench press, squat, body weight, and biceps and forearm girths. No changes were recorded in aerobic capacity.

Contrasting results were obtained by Fahey and Broun (43). Young men aged 19 to 32 were matched and the experimental group was given nandrolone decanoate (deca-Durabolin R) intramuscularly at a dosage of 1.0 mg/kg of body weight. The subjects were placed on a ten-week weight training program with steroid injections administered at weeks two, five, and eight of the program. Body weight and oxygen uptake remained unchanged while dynamic and isokinetic strength increased in both groups.

It has been observed that Dianabol increases motor time and decreases latency time in the knee jerk reflex. A greater contractile force, as measured by

maximal weight lifting, also has been attributed to the steroid (4).

Animal Studies

The results of studies of the effects of exercise and anabolic steroids on organ weights are conflicting. Murphy and Eagan (89) administered a "steroid cocktail" of Winstrol (9.3 mg/day), Dianabol (0.3 mg/day), and Durabolin (2.5 mg/wk) and an exercise program of 5 mi/wk of treadmill running to adult male rats for two weeks. Four weeks later, the results of an endurance run were: exercised-steroid group (ES) > exercised (E) > steroid > (S) > control (C). The order of body weights was: C > S = E > ES. Three weeks later, the mean ES weight still remained below that of the other groups. Heart weights did not differ among the groups, while pituitary and testes weights were less for the S and ES groups. Liver weight was 17 percent lower in the ES group than in the C, E, or S groups.

Brown and Pilch (21) administered a low dose (0.5 mg/kg) and a high dose (5 mg/kg) of Dianabol and trained rats both by running (1 ft/sec, 5 bouts, 10 min. duration) and by a progressive high jumping program for six weeks. Adrenals, brain, and heart weights were increased in the high jumpers. Testes, kidney, and levator ani weights were significantly increased in rats injected with low doses of Dianabol. High doses only produced a testes

weight increase, while performance was not affected by either dose.

Measurements of glycogen concentrations have yielded conflicting results. Gillespie and Edgerton (46) determined the role of testosterone in exercise-induced glycogen supercompensation on normal and castrasted guinea pigs. Testosterone propionate (0.833 mg/day) was administered to the castrated guinea pigs. The exercise program consisted of treadmill running (31 m/min) every other day for ten trials. Trials one through five and trials six through ten were 30 and 40 minutes in duration, respectively. The experimental period was twenty-five days and the guinea pigs were sacrificed 48 hours after the final exercise trial. Glycogen values in the vastus teteralis muscle (mg/g) were greater in the normal-trained and castrated-replacement-trained animals than in the castrated-trained and sedentary treatment animals. Exercise was not considered as an independent variable. Taylor and Murray (110) injected Dianabol (.02 mg/kg) daily and exercised rats at 1 mph., 1 hr/day, five days a week. Exercise produced a mobilization of free fatty acids (FFA) from plasma and adipose tissue and glycogen from the biceps and gastrocnemous muscles. Dianabol had no effect upon the storage and utilization of glycogen or upon mobilization of FFA.

CHAPTER III

RESEARCH METHODS

Sampling Procedures

Forty-two, normal, male albino rats (Sprague-Dawley strain) of three different age levels, were brought into the laboratory in one shipment. The differences in age were required to accommodate staggered treatment periods set up in conjunction with other concurrent studies using the same facilities. Initiation of treatments began for all animals at 100 days of age.

Fifteen animals were 90 days old (Age-Level 1); twelve animals were 76 days old (Level 2); and fifteen animals were 62 days old (Level 3) at the time of their arrival. Each animal was randomly assigned to a training-drug treatment group within his own age level. The 90-day-old animals were allowed 10 days to become acclimated to laboratory conditions before the study began. Since all animals began their training at 100 days of age, the Level 1 animals began the program first and the Level 2 and 3 animals followed at succeeding two-week intervals.

See Table 4 for a complete assignment of animals to treatment groups.

TABLE 4.--Random Assignment of Animals To Treatment Groups Within Age Levels.

	Factor A: Training					
	Level 1 (n=15)		Level 2 (n=12)		Level 3 (n=15)	
	Exercise	Sedentary	Exercise	Sedentary	Exercise	Sedentary
Factor B:						
Drug						
Dianabol	4	1	0	4	4	1
Placebo	4	1	0	4	4	1
Control	4	1	0	4	4	1

Research Design

The study was organized into a 2 x 3 factorial design. Factor A, Training, consisted of two treatment groups: (A_1) an exercise group which was subjected to an anaerobic endurance training program, and (A_2) a sedentary group. Factor B, Drug, consisted of three treatment groups: (B_1) a Dianabol group, (B_2) a placebo group, and (B_3) a control group.

For the trained animals, percent of expected revolutions (PER) served as the performance criterion to eliminate one of the four animals within each of the Dianabol, placebo, and control groups of Age Levels 1 and 3. However, due to a possible infectious leg injury,

one animal in the exercise-control group of Level 1 was automatically eliminated from the study. A representation of the experimental design with final cell size can be seen in Table 5.

TABLE 5.--Experimental Design With Final Cell Frequency.

	Factor A: Training	
	Exercise	Sedentary
Factor B: Drug		
Dianabol	n = 6	n = 6
Placebo	n = 6	n = 6
Control	n = 6	n = 6

Training Groups

The two training groups in the study were as follows:

Exercise (E)

The exercise treatment that these animals were subjected to was the SHORT program, which is a high-intensity, short-duration controlled running wheel (CRW) program developed at the Human Energy Research Laboratory, Michigan State University. The CRW apparatus can be described as " . . . a unique animal-powered wheel which is capable of inducing small laboratory animals to participate in highly specific programs of controlled, reproductible exercise" (115). The animals learned to

run by avoidance-response operant conditioning. A low-intensity controlled shock current provided motivation for the animals to run.

Following body weight recordings and drug injections at the start of each treatment period, the animals were placed in individually braked running wheels. A light above the running wheel signaled the start of each work interval. If the animal responded to the light by running at or faster than a preset speed, the light was extinguished and shock was avoided. The time during which the light was on was termed the "acceleration period." If the animal was not running at a predetermined speed by the end of the acceleration period, the light was turned off and a current was applied to the grid running surface of the wheel to induce the animal to run at the prescribed speed. If the animal attained the prescribed speed while being shocked, the shock was immediately discontinued. If the animal slowed down below the prescribed speed, the light and shock sequence was repeated. A typical running program consisted of alternate work and rest periods. During the work periods, the wheel was free to turn; while during the rest periods, the wheel was braked automatically to prevent spontaneous activity. A specified number of alternate work and rest periods (repetitions) constituted one bout of exercise. A single training period would

include several such bouts separated by a relatively long time between bouts.

The exercise program was progessive in nature. That is, the intensity of the program was gradually increased until on the thirty-seventh day of training, and thereafter, the animals are expected to complete eight bouts of exercise with 2.5 minutes of inactivity between bouts. Each bout consisted of six repetitions of 10 seconds of work alternated with 40 seconds of rest. During the work intervals, these animals were required to run at the relatively fast speed of 5.5 ft/sec. For a complete day-by-day description of the training program see Appendix A.

Sedentary (S)

These animals did not receive any type of forced exercise. To compensate for the handling of the exercised animals, the sedentary animals were weighed during each treatment period.

Drug Groups

The three drug groups used in this study were as follows:

Dianabol (D)

The animals were given Dianabol five times a week, prior to each exercise period, throughout the eight-week program. The concentration level was 10 miligrams (mg)

per cubic centimeter (cc) and the dosage level was 1 mg/rat/day or 0.1 cc/day. The Dianabol was dissolved in Mazzola corn oil (the solvent was chosen following a personal communication with Dr. J. J. Chart, CIBA Pharmaceutical Co.).

Placebo (P)

These animals were given Mazzola corn oil, 0.1 cc/day, prior to each exercise period throughout the eight-week program. The corn oil corresponds to the solvent system that was used with the Dianabol group. The placebo was also given to counteract any effects which the injection procedure might have had on the Dianabol rats.

Control (C)

These animals did not receive an injection of any kind.

Experimental Procedures

The animals were given the training and drug treatments once a day, between 6:30 A.M. and 9:30 A.M., Monday through Friday, for eight weeks in the Human Energy Research Laboratory at Michigan State University. Body weights of the trained animals were recorded before and after each exercise period.

Dianabol and the placebo, corn oil, (0.1 cc/day) were injected subcutaneously into the animals following

initial body weight recordings. The area of drug administration was in the lower back (Lumbar) region of the rat.

The performance data for each trained animal was recorded daily. Total revolutions run (TRR) and total expected revolutions (TER) were used to calculate percent of expected revolutions (PER). PER = TRR/TER x 100.

Animal Care

All of the animals were housed in standard, individual, sedentary cages (24 cm. x 18 cm. x 18 cm.) throughout the entire investigation. Since rats are normally more active at night than during daylight hours, the light sequence in the animal quarters was automatically timed to reverse the rat's active period by having the lights off between 1:00 P.M. and 1:00 A.M.

A relatively constant environment was maintained for the animals by daily handling, temperature and humidity control, and regular cage cleaning. Throughout the experiment, all animals had access to food (Wayne Laboratory Blox) and water *ad libitum*.

Sacrifice Procedures

Three sacrifices of twelve animals each were conducted forty-eight to seventy-two hours following the fortieth day of exercise for each age level. After the last treatment period, the animals were placed in metabolism cages. The housing was changed from the sedentary cages

in order that urine volume could be collected for another concurrent study.

On the sacrifice day, final body weights were recorded and then each animal was anesthetized by an intraperitoneal injection, 6 cc of a 6.48 percent Halatal solution (sodium pentobarbitol).

Selected organs were immediately removed, trimmed, and weighed wet. The right hind limb was skinned and the superficial posterior crural muscles were exposed by reflecting the overlying tissues. The right gastrocnemous, soleus and plantaris muscles were removed as a unit. The unit was held by forceps and quick frozen immediately in 2-methylbutane (isopentane). The isopentane had been previously cooled to a viscous fluid (-140 to -185°C) by liquid nitrogen. The frozen muscles were put in aluminum 35 mm film containers and placed in a cryostat at -20°C. Frozen muscle weights were obtained before further processing.

A block of tissue was cut approximately 10 mm long from the bellies of the muscle unit. The "sandwich" blocks were then mounted onto cryostat chucks using 5 percent gum tragacanth. The use of a "sandwich" block was to insure identical freezing, cutting, incubation, fixation, and mounting of tissues from the three muscles of each animal. Fresh-frozen, serial cross sections, 10 micra thick, were cut using a rotary microtome in a

cryostat. Sections were mounted on cover glasses and air dried for at least one hour. The remaining carcass was placed in a plastic bag and frozen for subsequent body composition analysis as described by Mickelson and Anderson (86).

Organ Weights and Body Composition Procedures

Absolute organ weights (gm) were recorded for the adrenals, heart, liver, spleen, testes, and kidneys. Relative organ weights (percent) also were obtained for each animal by dividing the absolute organ weights by body weight.

The body composition parameters studied were the percentages of water, fat, protein, and ash. Absolute values in grams were obtained by multiplying the percentage values by the animal's carcass weight and dividing by 100.

Additional dependent variables in the study included body weight (gm), carcass weight (gm), and absolute (gm) and relative muscle weights (percent).

Histochemical Procedures

Glycogen localization was studied by using the periodic acid-Schiff reaction (PAS) (101). Phosphorylase (Phos) activity was demonstrated by the method of Takeuchi (106).

Histochemical Methods

The metabolic properties of skeletal muscle do not appear to be uniform thorughout a given muscle. Different classifications of skeletal muscle fibers have been developed which are based on cellular constituents and metabolism. In 1962, Stein and Padykula defined A, B, and C fiber types. In 1967, Padykula and Gauthier recommended a change of classification of red, white, and intermediate fibers. Mitochondrial content determined by electron microscopy, was the basis for the change in nomenclature. However, various researchers have continued to use the first method (42, 48, 53, 77, 117).

Recent studies have been conducted to type fibers as red, white, or intermediate, but these have not been entirely consistent with each other (8, 9, 13, 26, 36, 37, 38, 45, 88). Other authors have classified only two types of fibers (I and II), some with subclasses of type II (18, 19, 20, 32, 33, 34, 40, 41).

A standard nomenclature has not been developed due to differences in fiber typing techniques based upon cellular characterisitcs of fibers. Regardless of the system of classification, fiber types and metabolic characteristics have been rated subjectively, usually by visual microscopy. These subjective ratings may have led to the existing differences in fiber typing.

As a result of the fiber typing inconsistencies and a need for a more objective and quantitative method to evaluate histochemically stained tissues, a Histochemical Photometer (HCP) was developed by Wells and Heusner (116) at Michigan State University. The HCP provides an accurate and objective method of determining the percentage of light passing through very small areas of tissue (less than one cell).

The HCP consists of a Prado microprojector, a photocell with associated circuits to measure light intensity, and a digital voltmeter readout. The projecting microscope is mounted so that light passing through a tissue section is projected upwards at an angle, reflected off a flat front-reflecting mirror, and directed onto a white plastic projection surface mounted on a horizontal table top. The intensity of light passing through a 1/16-inch hole in the center of the circle is measured by the photocell and is displaced as a number between 00.0 and 100.0 percent. The readings are independent of incident light level. Zero percentage transmission corresponds to no light received by the photocell. One hundred percent transmission is arbitrarily defined as the amount passing through the glass slide, mounting medium, and cover slip with no intervening tissue.

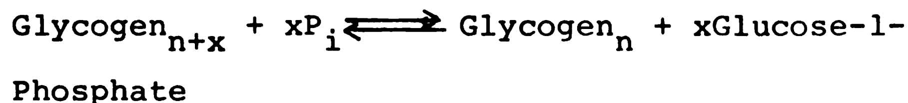
Calibration is required only once for each slide and consists of setting the photometer at zero percent

transmission and then at 100 percent transmission as described above. Recalibration is required when the tissue section is changed because variations in the optical density of glass slides at 100 percent transmission may cause errors of several percent.

The operation of the HCP consists of inserting a slide in the mechanical stage of the microprojector at a magnification of X200. The image of the tissue (muscle cell) to be examined is positioned over the center hole, and following initial calibration, the percentage of light transmission is displayed automatically by the digital voltmeter readout. A model of the histochemical photometer can be seen in Figures 1 and 2.

Histochemical Analysis

The formation of glycogen represents the end product of the synthesis and bonding of glucose units. Phosphorylase is an enzyme responsible for the breakdown of glycogen to glucose-1-phosphate which can then enter the glycolytic sequence of intermediary metabolism.



$$\text{Glycogen}_{n+x} + x\text{P}_i \rightleftharpoons \text{Glycogen}_n + x\text{Glucose-1-Phosphate}$$

Thus, the substrate glycogen and the enzyme phosphorylase are important constituents in anaerobic metabolism although they do not represent the rate limiting steps of metabolic pathways (78).

Fig. I. HISTOCHEMICAL PHOTOMETER, FRONT VIEW

Fig. 2. CLOSE UP OF CONTROL PANEL

To determine glycogen localization and phosphorylase activity, thirty muscle fibers were randomly selected from each of ten areas in the gastrocnemous, plantaris, and soleus muscles. Thirty fibers were selected as being sufficient to represent the metabolic characteristics of an area of muscle. Figure 3 shows the ten areas of the sandwich block selected for study. Values were recorded as percentage of light transmission but analyzed as percentage of light absorption by subtracting the recorded values from 100.

Morphological Analysis

Thirty muscle fibers were randomly selected in each of the same ten areas. These fibers were projected by means of a Prado microporjector X200 on a sheet of white paper and carefully traced. Absolute muscle fiber size was measured with a polar plainimeter for each muscle fiber in all ten areas. The units of area represented square centimeters (cm^2). These values were transformed to square microns and analyzed as such.

Statistical Procedures

Mean values for the thirty fibers per area represented the units of analysis for glycogen, phosphorylase, and absolute fiber size. An arc sine transformation (angular transformation) was applied to normalize

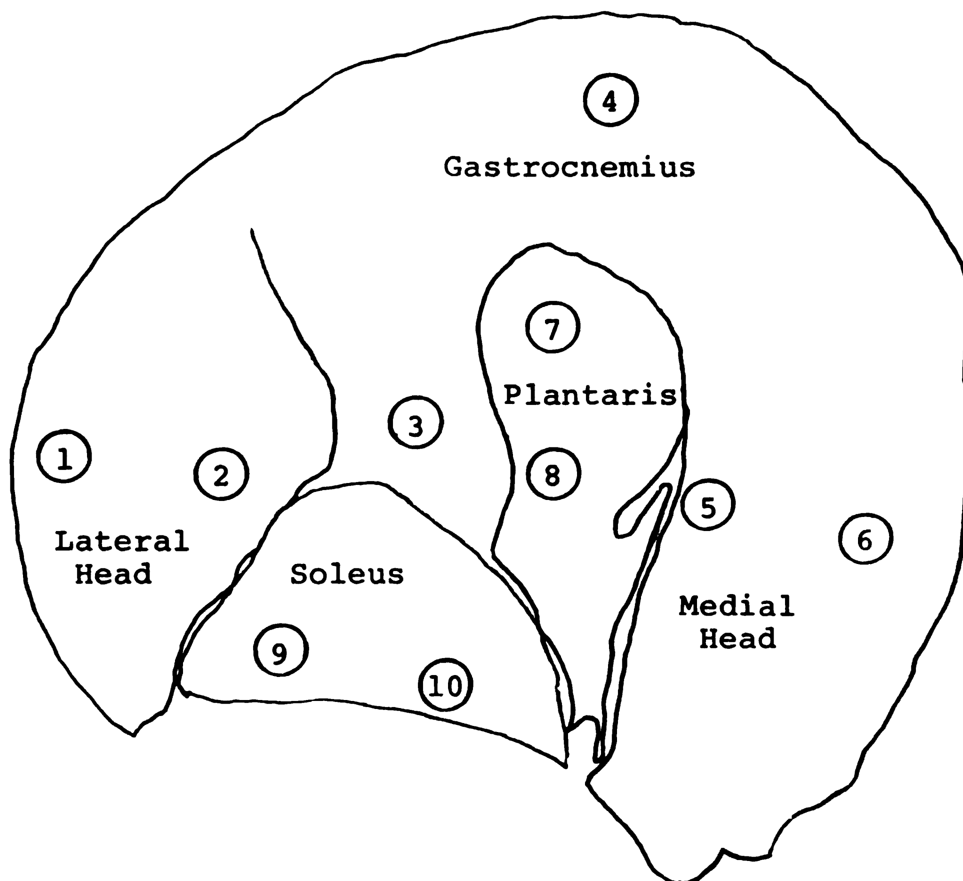


Fig. 3. Diagram of a Typical Cross Section of a "Sandwich" Block of the Gastrocnemius, Plantaris and Soleus Muscles. The Ten Areas Chosen for Study Are Shown as Numbered Circles.

the glycogen and phosphorylase data. This was required to meet the assumption of symmetry neccessary for analysis of variance (100).

As a result of freezing artifacts, the glycogen, phosphorylase, and muscle fiber size values for animal number 25, area 1, were lost. Adjusted cell means, which represented the mean value of the other animals in each respective cell, were substituted for the missing data.

The data were analyzed using the FACREP routine on the Michigan State University Control Data 3600 Computer (CDC 3600). The model for the data represents a two-way, fixed effects ANOVA. The Tukey Test was used to determine the significance of differences between means following significant analysis of variance results for Factor B; Drug, and the Training-Drug interaction. *Post hoc* procedures were not necessary for Factor A; Training, as it contained only two levels. Statistical significance was set at the .05 level for the two-way ANOVA and at the .10 level for the Tukey *post hoc* procedures.

CHAPTER IV

RESULTS AND DISCUSSION

Training Results

The performance of the three drug groups on the CRW SHORT program was determined by the mean daily percent of expected revolutions (PER). Figure 4 shows that the Dianabol and placebo groups responded similarly during the eight-week program, while the control group had slightly lower PER values. This was particularly evident during the last fourteen days of training when the expected running velocity was increased to 5.0 and 5.5 ft/sec.

Histochemical Results

THe raw phosphorylase and glycogen values[1] for the thirty muscle fibers per area are tabulated by muscle area, animal number, training and drug treatments in Tables B-1 and B-2, Appendix B. The mean values, the analysis of variance results, and the appropriate Tukey Test comparisons are presented in Tables 6 and 7.

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[1]Phosphorylase and glycogen values are given in terms of the percent of light absorbed.

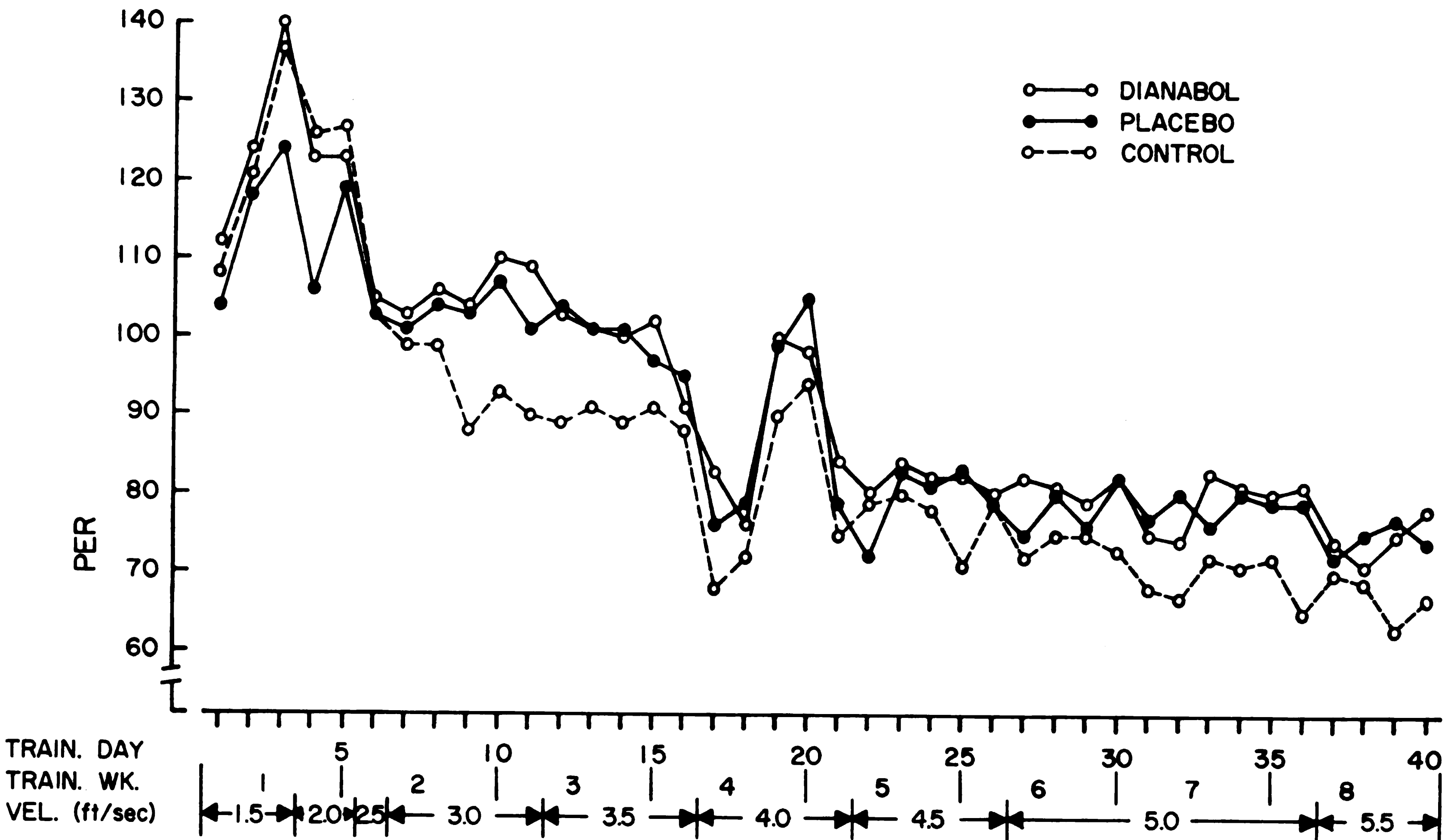

Fig. 4.Mean Daily Percent Expected Revolutions (PER) for CRW SHORT Program

TABLE 6.--Analysis of Variance and Tukey Test Results for Phosphorylase Activity in the Ten Selected Areas of Gastrocnemous, Plantaris, and Soleus Muscles.

	Training		Row Means	ANOVA Results by Rows	Tukey Results by Rows
	Exercise	Sedentary			
Table 6.1. Phosphorylase--Area 1					
Drug					
Dianabol	66.40	70.94	68.67	F = 0.75	
Placebo	64.47	70.32	67.39	P=0.482	
Control	69.08	69.05	69.07		
Column Means	66.65	70.10	68.38*		
ANOVA Results by Columns	F=8.71 P=0.006				
Interaction	F=2.33; P=0.115				
Table 6.2. Phosphorylase--Area 2					
Drug					
Dianabol	64.80	73.39	69.10	F=1.69	
Placebo	64.48	72.83	68.65	P=0.202	
Control	70.14	73.12	71.63		
Column Means	66.47	73.11	69.79*		
ANOVA Results by Columns	F=21.64 P<0.0005				
Interaction	F=1.69; P=0.202				
Table 6.3. Phosphorylase--Area 3					
Drug					
Dianabol	55.34	65.23	60.28	F=4.27	D<C
Placebo	57.45	63.35	60.40	P=0.023	P<C
Control	65.33	65.86	65.60		
Column Means	59.37	64.81	62.09*		
ANOVA Results by Columns	F=10.28 P=0.003				
Interaction	F=2.55; P=0.095				
Table 6.4. Phosphorylase--Area 4					
Drug					
Dianabol	64.54	70.84	67.69	F=1.24	
Placebo	68.92	71.62	70.27	P=0.303	
Control	65.80	73.08	69.44		
Column Means	66.42	71.85	69.13*		
ANOVA Results by Columns	F=15.79 P<0.0005				
Interaction	F=1.04; P=0.365				
Table 6.5. Phosphorylase--Area 5					
Drug					
Dianabol	62.42	71.43	66.92	F=1.49	
Placebo	66.78	74.66	70.72	P=0.242	
Control	70.25	71.62	70.93		
Column Means	66.48	72.57	69.52*		
ANOVA Results by Columns	F=8.12 P = 0.008				
Interaction	F=1.24; P=0.303				

TABLE 6.--Continued.

	Training		Row Means	ANOVA Results by Rows	Tukey Results by Rows
	Exercise	Sedentary			
Table 6.6. Phosphorylase--Area 6					
Drug					
Dianabol	65.72	71.90	68.81	F=3.00	
Placebo	72.61	73.66	73.14	P=0.065	
Control	69.96	71.80	70.88		
Column Means	69.43	72.45	70.94*		
ANOVA Results by Columns	F=4.39 P=0.045				
Interaction	F=1.22; P=0.309				
Table 6.7. Phosphorylase--Area 7					
Drug					
Dianabol	63.72	72.58	68.15	F=0.13	
Placebo	64.14	73.40	68.77	P=0.876	
Control	66.27	72.24	69.25		
Column Means	64.71	72.74	68.72*		
ANOVA Results by Columns	F=2108 P<0.0005				
Interaction	F=0.35; P=0.707				
Table 6.8. Phosphorylase--Area 8					
Drug					
Dianabol	62.11	69.98	66.04	F=1.49	
Placebo	63.87	74.15	69.01	P=0.242	
Control	65.07	71.56	68.32*		
Column Means	65.07	71.56	68.32*		
ANOVA Results by Columns	F=11.54 P=0.002				
Interaction	F=1.98; P=0.156				
Table 6.9. Phosphorylase--Area 9					
Drug					
Dianabol	52.90	60.03	56.47	F=0.38	
Placebo	51.58	59.75	55.67	P=0.687	
Control	57.87	57.02	57.44		
Column Means	54.12	58.93	56.53*		
ANOVA Results by Columns	F=8.33 P=0.007				
Interaction	F=2.91; P=0.070				
Table 6.10. Phosphorylase--Area 10					
Drug					
Dianabol	51.83	61.37	56.60	F=0.11	
Placebo	54.21	61.15	57.68		
Control	57.08	57.11	57.09		
Column Means	54.37	59.88	57.13*		
ANOVA Results by Columns	F=8.73 P=0.006				
Interaction	F=2.32; P=0.116				

*Grand Mean.

TABLE 7.--Analysis of Variance and Tukey Test Results for Glycogen Storage in the Ten Selected Areas of the Gastrocnemous, Plantaris, and Soleus Muscles.

	Training		Row Means	ANOVA Results by Rows	Tukey Results by Rows
	Exercise	Sedentary			
Table 7.1. Glycogen--Area 1					
Drug					
Dianabol	38.34	40.47	39.40	F=2.17	
Placebo	39.81	38.97	39.39	P=0.132	
Control	42.31	40.59	41.45		
Column Means	40.15	40.01	40.08*		
ANOVA Results by Columns	F=0.02 P=0.880				
Interaction	F=1.57; P=0.225				
Table 7.2. Glycogen--Area 2					
Drug					
Dianabol	35.84	35.95	35.89	F=1.61	
Placebo	36.51	34.59	35.55	P=0.216	
Control	38.91	36.25	37.58		
Column Means	37.09	35.60	36.34*		
ANOVA Results by Columns	F=2.27 P=0.142				
Interaction	F=0.70; P=0.505				
Table 7.3. Glycogen--Area 3					
Drug					
Dianabol	39.10	36.93	38.01	F=0.03	
Placebo	38.13	38.41	38.27	P=0.968	
Control	39.80	36.84	38.32		
Column Means	39.01	37.39	38.20*		
ANOVA Results by Columns	F=2.33 P=0.137				
Interaction	F=0.85; P=0.438				
Table 7.4. Glycogen--Area 4					
Drug					
Dianabol	38.11	37.68	37.89	F=1.05	
Placebo	39.02	33.19	36.11	P=0.361	
Control	39.59	35.84	37.72		
Column Means	38.91	35.57	37.24*		
ANOVA Results by Columns	F=9.07 P=0.005				
Interaction	F=2.02; P=0.151				
Table 7.5. Glycogen--Area 5					
Drug					
Dianabol	37.05	35.45	36.25	F=3.91	D<C
Placebo	38.14	33.80	35.97	P=0.031	P<C
Control	39.80	36.46	38.13		
Column Means	38.33	35.23	36.78		
ANOVA Results by Columns	F=20.33 P<0.0005				
Interaction	F=1.36; P=0.271				

TABLE 7.--Continued.

	Training		Row Means	ANOVA Results by Rows	Tukey Results by Rows
	Exercise	Sedentary			
Table 7.6. Glycogen--Area 6					
Drug					
Dianabol	42.70	40.29	41.49	F=1.31	
Placebo	42.22	37.63	39.93	P=0.284	
Control	42.03	39.30	40.67		
Column Means	42.32	39.07	40.70*		
ANOVA Results by Columns	F=16.89 P<0.0005				
Interation	F=0.74; P=0.486				
Table 7.7. Glycogen--Area 7					
Drug					
Dianabol	36.22	34.82	35.52	F=1.81	
Placebo	37.15	31.88	34.52	P=0.181	
Control	37.95	35.03	36.49		
Column Means	37.11	33.91	35.51*		
ANOVA Results by Columns	F=14.25 P=0.001				
Interaction	F=1.77; P=0.188				
Table 7.8. Glycogen--Area 8					
Drug					
Dianabol	37.83	35.86	36.85	F=1.57	
Placebo	38.63	34.07	36.35	P=0.226	
Control	38.83	37.16	37.99		
Column Means	38.43	37.50	37.06*		
ANOVA Results by Columns	F=12.39 P=0.001				
Interaction	F=1.39; P=0.265				
Table 7.9. Glycogen--Area 9					
Drug					
Dianabol	41.05	39.97	40.51	F=0.10	
Placebo	39.97	41.84	40.90	P=0.90[illegible]	
Control	41.83	38.70	49.26		
Column Means	40.95	40.17	40.56*		
ANOVA Results by Columns	F=0.44 P=0.511				
Interaction	F=1.55; P=0.229				
Table 7.10. Glycogen--Area 10					
Drug					
Dianabol	39.56	40.08	39.82	F=0.18	
Placebo	39.17	40.06	39.62	P=0.833	
Control	40.47	40.72	40.60		
Column Means	39.74	40.29	40.01*		
ANOVA Results by Column	F=0.16 P=0.693				
Interaction	F=0.02; P=0.983				

*Grand Mean.

Phosphorylase

Exercise produced a significant depletion of phosphorylase activity in all ten areas of the gastrocnemous, plantaris, and soleus muscles. The drug effect was significant only in area 3 where the Dianabol and placebo groups both had less phosphorylase activity than the control group. The training-drug combination produced no significant changes in any of the areas.

Glycogen

Exercise produced an increase in glycogen storage in areas 4, 5, 6, 7, and 8 of the gastrocnemous and plantaris muscles. No changes were observed in the soleus (areas 9 and 10). The drug effect was significant in area 5 where the control group had a greater glycogen concentration than either the Dianabol or placebo groups. The training-drug combination did not alter glycogen content in any of the areas.

Morphological Results

The raw absolute fiber sizes, in square centimeters, for the thirty muscle fibers per area are tabulated by muscle area, animal number, training, and drug treatments in Appendix C. The mean values, the analysis of variance results, and appropriate Tukey Test comparisons are presented in Table 8.

TABLE 8.--Analysis of Variance and Tukey Test Results for Absolute Fiber Size (Square Microns) in the Ten Selected Areas of the Gastrocnemous, Plantaris, and Soleus Muscles.

	Training		Row Means	ANOVA Results by Rows	Tukey Results by Rows
	Exercise	Sedentary			
Drug					
Dianabol	4958	4708	4833	F=2.32	
Placebo	5250	5625	5438	P=0.115	
Control	4958	5583	5271		
Column Means	5056	5306	5181*		
ANOVA Results by Columns	F=1.12 P=0.299				
Interaction	F=1.21; P=0.312				

Table 8.2--Absolute Fiber Size--Area 2

	Exercise	Sedentary	Row Means	ANOVA Results by Rows	Tukey Results by Rows
Drug					
Dianabol	5083	4333	4708	F=0.51	
Placebo	4333	5000	4667	P=0.605	
Control	4875	5042	4958		
Column Means	4764	4792	4778*		
ANOVA Results by Columns	F=0.01 P=0.914				
Interaction	F=2.65; P=0.087				

Table 8.3. Absolute Fiber Size--Area 3

	Exercise	Sedentary	Row Means	ANOVA Results by Rows	Tukey Results by Rows
Drug					
Dianabol	3417	2750	3083	F=0.56	
Placebo	3208	3375	3292	P=0.576	
Control	3375	3125	3250		
Column Means	3333	3083	3208*		
ANOVA Results by Columns	F=2.17 P=0.152				
Interaction	F=2.01; P=0.152				

Table 8.4. Absolute Fiber Size--Area 4

	Exercise	Sedentary	Row Means	ANOVA Results by Rows	Tukey Results by Rows
Drug					
Dianabol	5625	4333	4979	F=1.88	
Placebo	4458	5750	5105	P=0.169	
Control	5708	6041	5875		
Column Means	5264	5375	5319*		
ANOVA Results by Columns	F=0.07 P=0.787				
Interaction	F=3.41; P=0.046 Disordinal Interaction With Drug, S-D < S-C Disordinal Interaction With Training, E-D > S-D E-P < S-P				

Table 8.5. Absolute Fiber Size--Area 5

	Exercise	Sedentary	Row Means	ANOVA Results by Rows	Tukey Results by Rows
Drug					
Dianabol	4375	3792	4083	F=1.01	
Placebo	4083	3958	4021	P=0.377	
Control	4208	4583	4396		
Column Means	4222	4111	4167*		
ANOVA Results by Columns	F=0.23 P=0.634				
Interaction	F=1.43; P=0.254				

TABLE 8.--Continued.

	Training		Row Means	ANOVE Results by Rows	Tukey Results by Rows
	Exercise	Sedentary			
Table 8.6. Absolute Fiber Size--Area 6					
Drug					
Dianabol	4750	4750	4750	F=1.04	
Placebo	4917	5125	5021	P=0.366	
Control	5250	5125	5188		
Column Means	4972	5000	4986*		
ANOVA Results by Columns	F=0.01 P=0.912				
Interaction	F=0.15; P=0.860				
Table 8.7. Absolute Fiber Size--Area 7					
Drug					
Dianabol	4458	3667	4063	F=0.07	
Placebo	4125	4292	4208	P=0.934	
Control	3750	4542	4146		
Column Means	4111	4167	4139*		
ANOVA Results by Columns	F=0.03 P=0.864				
Interaction	F=2.05; P=0.147				
Table 8.8. Absolute Fiber Size--Area 8					
Drug					
Dianabol	3750	3458	3604	F=0.18	
Placebo	3667	3542	3604	P=0.837	
Control	3625	3833	3729		
Column Means	3681	3611	3646*		
ANOVA Results by Columns	F=0.12 P=0.727				
Interaction	F=0.56; P=0.579				
Table 8.9. Absolute Fiber Size--Area 9					
Drug					
Dianabol	3375	3375	3375	F=0.71	
Placebo	3333	3917	3625	P=0.501	
Control	3708	3250	3479		
Column Means	3472	3514	3493*		
ANOVA Results by Columns	F=0.06 P=0.811				
Interaction	F=3.05; P=0.062				
Table 8.10. Absolute Fiber Size--Area 10					
Drug					
Dianabol	4625	4667	4646	F=0.33	
Placebo	4625	5000	4813	P=0.723	
Control	4583	4417	4500		
Column Means	4611	4694	4653*		
ANOVA Results by Columns	F=0.07 P=0.793				
Interaction	F=0.25; P=0.780				

*Grand Mean.

Neither the training nor the drug treatments used in this study resulted in significant changes in mean fiber size. A significant interaction did occur in area 4. Both the training and drug factors were disordinal. The disordinal drug interaction indicated that the sedentary-Dianabol animals had a smaller mean fiber size than the sedentary-control animals. In the disordinal training interaction, the exercise-Dianabol group showed an increase in mean fiber size over the sedentary-Dianabol group; however, the exercise-placebo group had a smaller mean fiber size than the sedentary-placebo group. Figure 5 illustrates the absolute fiber size interaction effect for area 4.

Organ Weight Results

Body weight and the absolute organ weights are tabulated by animal number, training, and drug treatments in Appendix C. The analysis of variance results and appropriate Tukey Test comparisons are presented in Table 9.

Body weight and the absolute weights of the liver, spleen, kidneys, and muscle were smaller in the exercise group than in the sedentary group. However, the relative weights of the adrenals, heart, liver, testes, kidneys, and muscle in the exercise group were larger than those in the sedentary group. Relative spleen weight was smaller

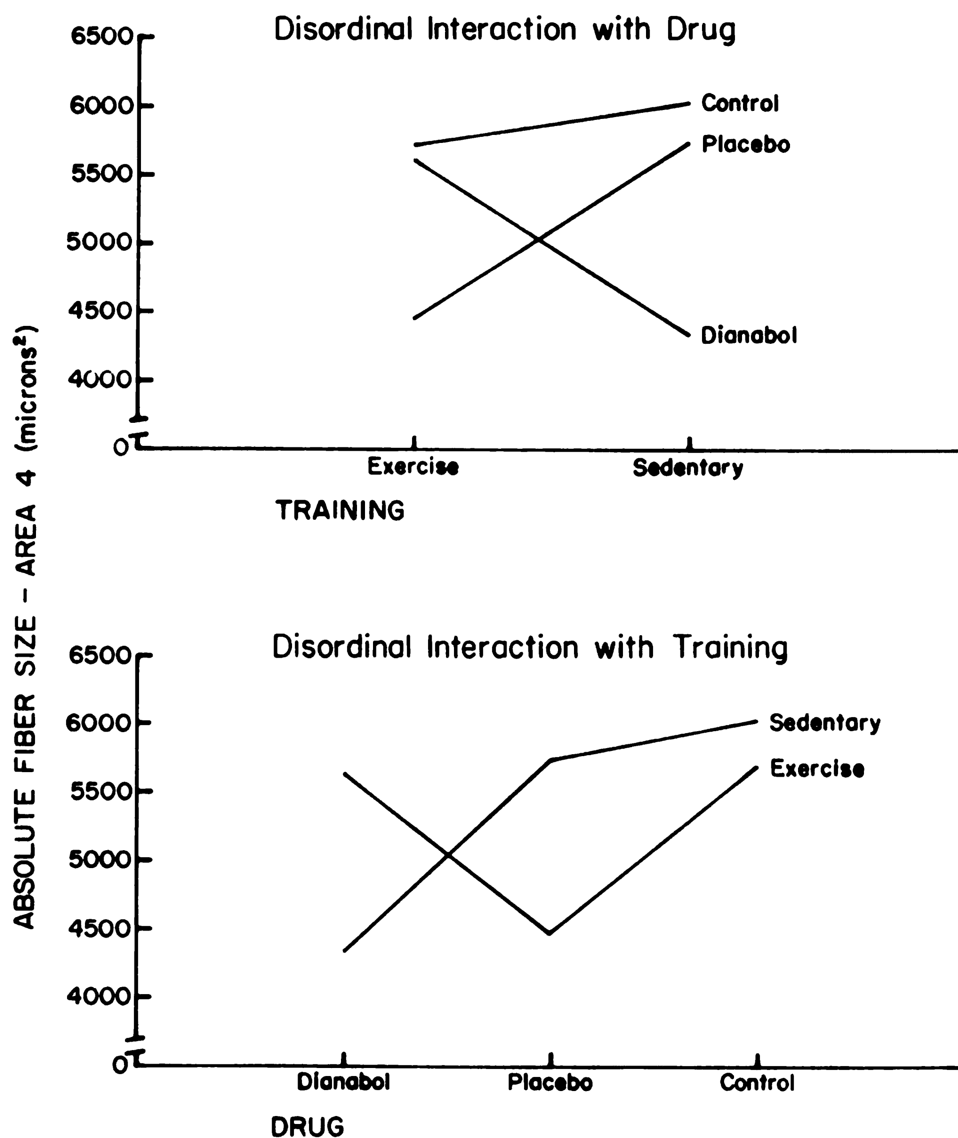

Fig. 5. Significant Interaction Effect: Absolute Fiber Size - Area 4

TABLE 9.--Analysis of Variance and Tukey Test Results for Body Weight and Absolute and Relative Organ Weights.

	Training		Row Means	ANOVA Results by Rows	Tukey Results by Rows
	Exercise	Sedentary			
Table 9.1. Body Weight					
Drug					
Dianabol	436	493	464	F=5.69	D>C
Placebo	430	507	468	P=0.008	P>C
Control	411	483	447		
Column Means	426	494	460*		
ANOVA Results by Columns	F=155.38 P<0.0005				
Interaction	F=1.13; P=0.336				
Table 9.2. Absolute Adrenals Weight ($X10^{-2}$)					
Drug					
Dianabol	5.90	5.35	5.62	F=1.22	
Placebo	5.45	5.08	5.27	P=0.309	
Control	5.49	4.64	5.06		
Column Means	5.61	5.02	5.32*		
ANOVA Results by Columns	F=3.92 P=0.057				
Interaction	F=0.22; P=0.800				
Table 9.3. Absolute Heart Weight					
Drug					
Dianabol	1.35	1.35	1.35	F=2.27	
Placebo	1.38	1.40	1.39	P=0.121	
Control	1.28	1.38	1.33		
Column Means	1.34	1.38	1.36*		
ANOVA Results by Columns	F=3.20 P=0.084				
Interaction	F=1.83; P=0.178				
Table 9.4. Absolute Liver Weight					
Drug					
Dianabol	13.70	13.67	13.68	F=6.56	D>C
Placebo	12.62	13.78	13.20	P=0.004	
Control	11.80	13.33	12.57		
Column Means	12.71	13.60	13.15*		
ANOVA Results by Columns	F=12.51 P=0.001				
Interaction	F=3.48; P=0.044 Disordinal Interaction With Drug, Disordinal Interaction With Training,			E-D > E-P E-D > E-C E-P > S-P E-C > S-C	
Table 9.5. Absolute Testes Weight					
Drug					
Dianabol	3.58	3.73	3.66	F=0.44	
Placebo	3.75	3.80	3.78	P=0.647	
Control	3.68	3.78	3.73		
Column Means	3.67	3.77	3.72*		
ANOVA Results by Columns	F=0.92 P=0.345				
Interaction	F=0.07; P=0.929				

TABLE 9.--Continued.

	Training		Row Means	ANOVA Results by Rows	Tukey Results by Rows
	Exercise	Sedentary			
Table 9.6. Absolute Spleen Weight ($X10^{-1}$)					
Drug					
Dianabol	7.92	10.32	9.12	F=1.60	
Placebo	7.99	9.80	8.90	P=0.218	
Control	7.87	11.66	9.76		
Column Means	7.93	10.60	9.26*		
ANOVA Results by Columns	F=42.44 P<0.0005				
Interaction	F=2.04; P=0.148				
Table 9.7. Absolute Kidneys Weight					
Drug					
Dianabol	2.73	2.92	2.82	F=0.23	
Placebo	2.80	2.93	2.86	P=0.797	
Control	2.82	2.84	2.83		
Column Means	2.79	2.89	2.84*		
ANOVA Results by Columns	F=4.19 P=0.049				
Interaction	F=0.92; P=0.408				
Table 9.8. Absolute Muscle Weight					
Drug					
Dianabol	3.20	3.33	3.26	F=1.31	
Placebo	3.27	3.57	3.42	P=0.284	
Control	3.21	3.47	3.34		
Column Means	3.23	3.46	3.34*		
ANOVA Results by Columns	F=8.92 P=0.006				
Interaction	F=0.50; P=0.613				
Table 9.9. Relative Adrenals Weight ($X10^{-4}$)					
Drug					
Dianabol	1.4	1.1	1.2	F=0.70	
Placebo	1.3	1.0	1.1	P=0.505	
Control	1.3	1.0	1.2		
Column Means	1.3	1.0	1.2*		
ANOVA Results by Columns	F=21.19 P<0.0005				
Interaction	F=0.32; P=0.728				
Table 9.10. Relative Heart Weight ($X10^{-3}$)					
Drug					
Dianabol	3.10	2.74	2.92	F=1.06	
Placebo	3.21	2.77	2.99	P=0.359	
Control	3.12	2.87	3.00		
Column Means	3.15	2.79	2.97*		
ANOVA Results by Columns	F=61.41 P<0.0005				
Interaction	F=1.51; P=0.236				

TABLE 9.--Continued.

	Training		Row Means	ANOVA Results by Rows	Tukey Results by Rows
	Exercise	Sedentary			
Table 9.11. Relative Liver Weight ($X10^{-2}$)					
Drug					
Dianabol	3.14	2.78	2.96	F=3.48	D>P
Placebo	2.94	2.72	2.83	P=0.044	D>C
Control	2.87	2.76	2.82		
Column Means	2.98	2.75	2.87*		
ANOVA Results by Columns	F=22.27 P<0.0005				
Interaction	F=2.41; P=0.107				
Table 9.12. Relative Testes Weight ($X10^{-3}$)					
Drug					
Dianabol	8.20	7.57	7.89	F=2.40	
Placebo	8.70	7.51	8.11	P=0.108	
Control	8.97	7.82	8.40		
Column Means	8.63	7.63	8.13*		
ANOVA Results by Columns	F=27.19 P<0.0005				
Interaction	F=0.90; P=0.417				
Table 9.13. Relative Spleen Weight ($X10^{-3}$)					
Drug					
Dianabol	1.82	2.09	1.96	F=4.38	D<C
Placebo	1.86	1.93	1.90	P=0.021	P<C
Control	1.91	2.41	2.16		
Column Means	1.86	2.15	2.00*		
ANOVA Results by Columns	F=13.80 P=0.001				
Interaction	F=2.58; P=0.092				
Table 9.14. Relative Kidneys Weight ($X10^{-3}$)					
Drug					
Dianabol	6.27	5.92	6.09	F=2.25	
Placebo	6.51	5.78	6.15	P=0.12[illegible]	
Control	6.88	5.88	6.38		
Column Means	6.55	5.86	6.21*		
ANOVA Results by Columns	F=35.59 P<0.0005				
Interaction	F=2.57; P=0.093				
Table 9.15. Relative Muscle Weight ($X10^{-3}$)					
Drug					
Dianabol	7.36	6.75	7.06	F=3.07	
Placebo	7.58	7.05	7.32	P=0.061	
Control	7.82	7.18	7.50		
Column Means	7.59	7.00	7.29*		
ANOVA Results by Columns	F=16.11 P<0.0005				
Interaction	F=0.04; P=0.962				

*Grand Mean.

in the exercise group. Body weights were different as a result of the drug treatment. Both the Dianabol and the placebo groups had higher body weights than the control group. Absolute and relative liver weight differences were significant with the drug treatment. The Dianabol group had greater weights than the control group for both parameters. The Dianabol group also had greater relative liver weights than the placebo group. Relative spleen weight data showed the Dianabol and placebo groups both to have smaller spleens than the control group.

The absolute liver weight interaction effect was significant and disordinal with both training and drug treatments. In the disordinal drug interaction, the exercise-Dianabol group had greater liver weights than both the exercise-placebo and the exercise-control groups. For the disordinal training interaction, both the exercise-placebo and the exercise-control groups had greater values than their sedentary counterparts. Figure 6 illustrates the absolute liver weight interaction effects.

Body Composition Results

Carcass weight and the relative carcass components are tabulated by animal number, training, and drug treatments in Appendix D. The analysis of variance results and appropriate Tukey Test comparisons are presented in Table 10.

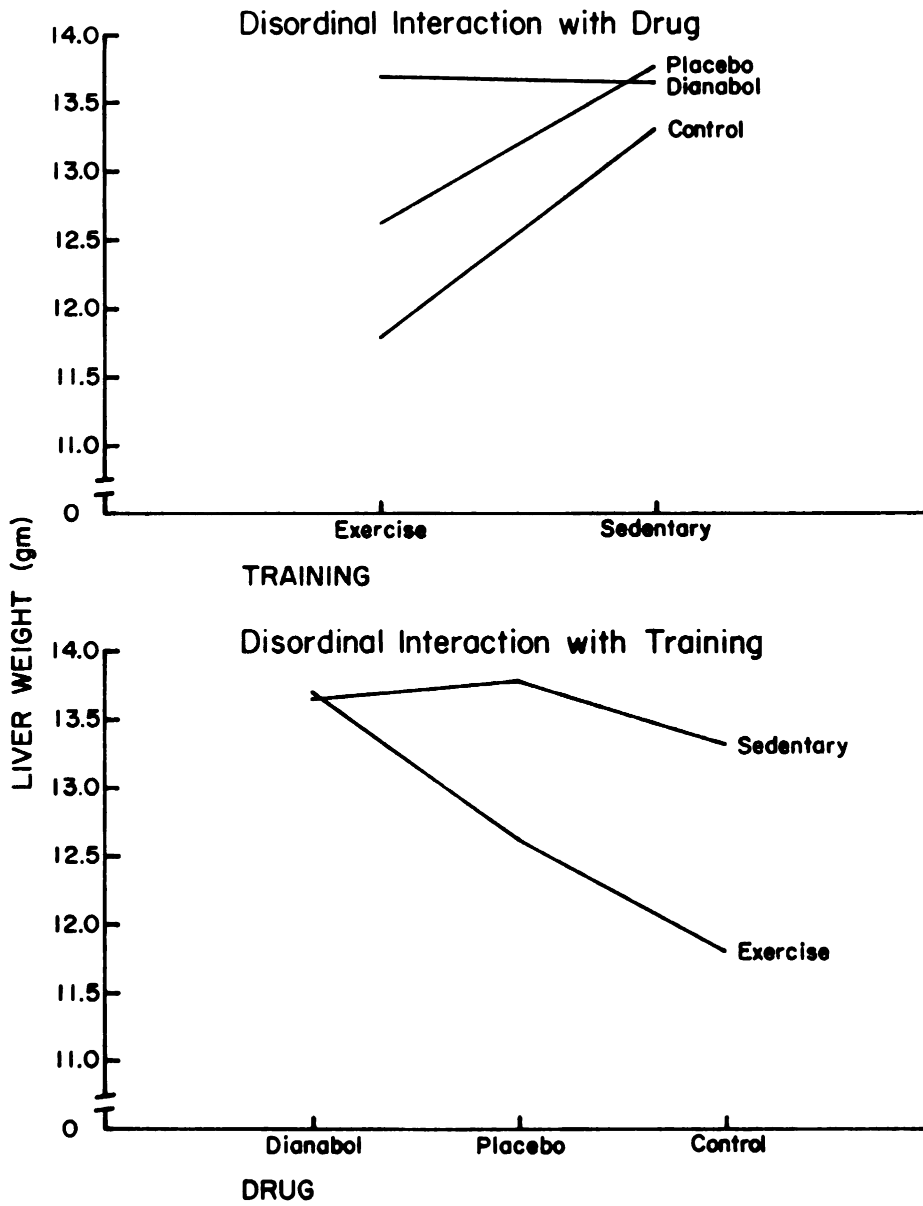

Fig. 6. Significant Interaction Effect: Liver Weight

TABLE 10.--Analysis of Variance and Tukey Test Results for Carcass Weight and the Relative (percent) and Absolute (gm) Carcass Components.

	Training		Row Means	ANOVA Results By Rows	Tukey Results By Rows
	Exercise	Sedentary			
Table 10.1. Carcass Weight					
Drug					
Dianabol	347	405	376	F=3.30	
Placebo	346	415	381	P=0.051	
Control	333	400	367		
Column Means	342	407	374*		
ANOVA Results by Columns	F=197.02 P<0.0005				
Interaction	F=0.53; P=0.594				
Table 10.2. Percent Water					
Drug					
Dianabol	64.94	59.02	61.98	F=2.43	
Placebo	64.71	61.78	63.25	P=0.105	
Control	64.39	61.68	63.04		
Column Means	64.68	60.83	62.75*		
ANOVA Results by Columns	F=58.88 P<0.0005				
Interaction	F=4.24; P=0.024 Disordinal Interaction With Drug, S-D < S-P, S-D < S-C Ordinal Interaction With Training				
Table 10.3. Percent Fat					
Drug					
Dianabol	8.61	15.71	12.16	F=4.03	D>P
Placebo	8.43	12.49	10.46	P=0.028	D>C
Control	8.38	12.28	10.33		
Column Means	8.48	13.50	10.99*		
ANOVA Results by Columns	F=73.28 P<0.0005				
Interaction	F=3.15; P=0.057				
Table 10.4. Percent Protein					
Drug					
Dianabol	21.87	21.17	21.52	F=3.02	
Placebo	22.54	21.33	21.93	P=0.064	
Control	22.58	21.85	22.22		
Column Means	22.33	21.45	21.89*		
ANOVA Results by Columns	F=14.16 P=0.001				
Interaction	F=0.51; P=0.604				
Table 10.5 Percent Ash					
Drug					
Dianabol	3.92	3.73	3.83	F=1.27	
Placebo	4.32	3.83	4.08	P=0.296	
Control	4.21	3.68	3.94		
Column Means	4.15	3.75	3.95*		
ANOVA Results by Columns	F=9.92 P=0.004				
Interaction	F=0.73; P=0.489				

TABLE 10.--Continued.

	Training		Row Means	ANOVA Results by Rows	Tukey Results by Rows
	Exercise	Sedentary			
Table 10.6. Absolute Water					
Drug					
Dianabol	225	239	232	F=2.56	
Placebo	224	256	240	P=0.094	
Control	215	247	231		
Column Means	221	247	234*		
ANOVA Results by Columns	F=49.85 P<0.0005				
Interaction	F=2.82; P=0.076				
Table 10.7. Absolute Fat					
Drug					
Dianabol	29.94	63.64	46.79	F=4.36	D>C
Placebo	27.16	52.00	40.58	P=0.022	
Control	27.97	49.04	38.50		
Column Means	29.02	54.89	41.96*		
ANOVA Results by Columns	F=117.75 P<0.0005				
Interaction	F=2.74; P=0.081				
Table 10.8. Absolute Protein					
Drug					
Dianabol	75.88	85.77	80.83	F=1.53	
Placebo	78.05	88.50	83.27	P=0.233	
Control	75.24	87.33	81.28		
Column Means	76.39	87.20	81.80*		
ANOVA Results by Columns	F=79.26 P<0.0005				
Interaction	F=0.30; P=0.746				
Table 10.9. Absolute Ash					
Drug					
Dianabol	13.58	15.12	14.35	F=2.60	
Placebo	14.98	15.89	15.43	P=0.091	
Control	13.99	14.67	14.33		
Column Means	14.18	15.23	14.71*		
ANOVA Results by Columns	F=5.37 P=0.028				
Interaction	F=0.32; P=0.726				

*Grand Mean.

Carcass weight and the percentage of fat were lower in the exercise group than in the sedentary group. The exercise group, however, had higher percentages of water, protein, and ash than the sedentary group. On an absolute basis, all of the carcass components in the exercise group were lower than those in the sedentary group. The per-percentage and the absolute values of fat were both higher in the Dianabol group than in the control group for the drug treatment. The percentage of fat was also greater in the Dianabol group than in the placebo group.

Data on the percentage of water yielded a significant interaction and was disordinal with the drug treatment. The sedentary-Dianabol group had a lower percentage of water than either the sedentary-placebo or the sedentary-control groups. Figure 7 illustrates the percent water interaction effects.

Discussion

The effectiveness of Dianabol as a performance stimulant was not evident when measured by PER values obtained on the CRW SHORT program. The placebo group had values similar to the Dianabol group during the entire study. The control group had the lowest PER values of all groups during the most vigorous parts of the training program.

The exercise-induced histochemical depletion of phosphorylase in all ten muscle areas, and the accompanying

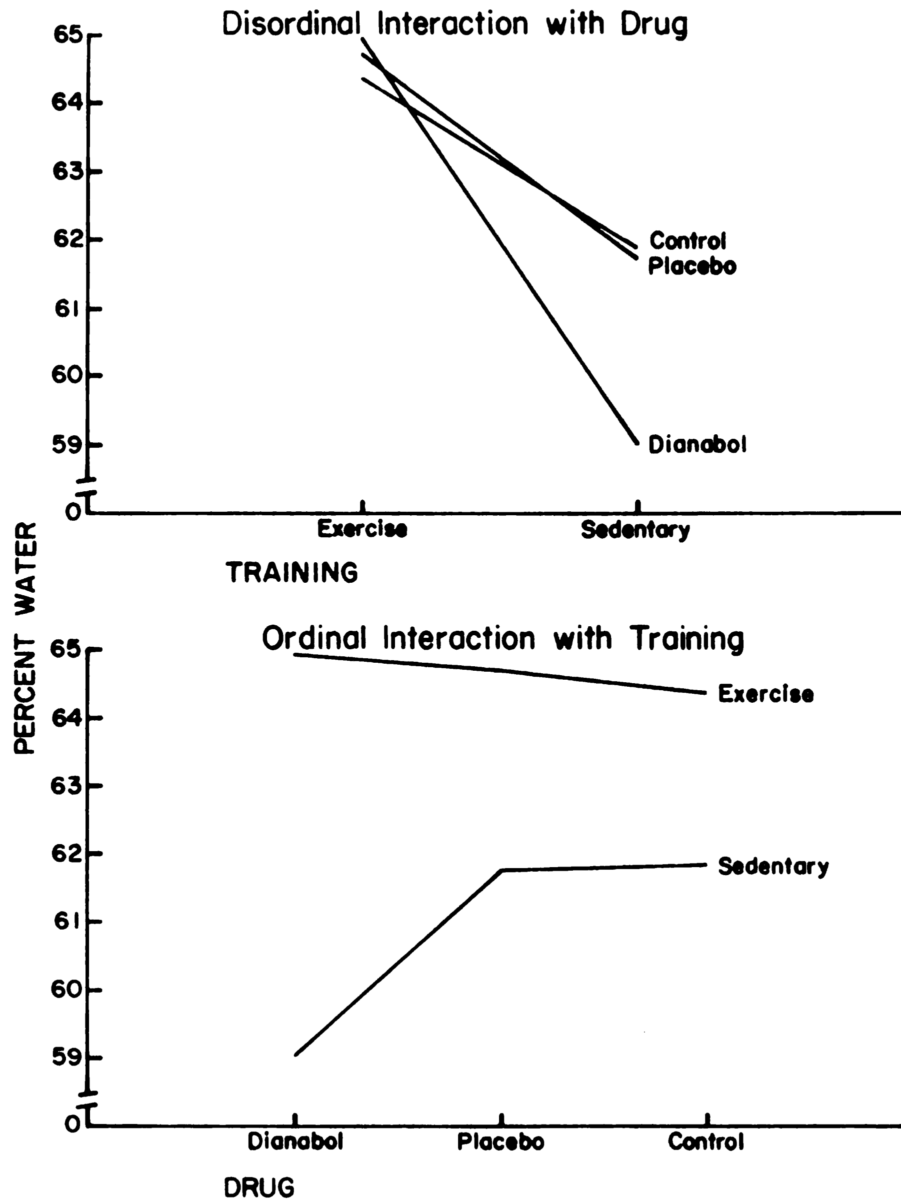

Fig. 7. Significant Interaction Effect: Percent Water

glycogen supercompensation in five muscle areas, lend support to the hypothesis that there are different mechanisms of glycogen breakdown and subsequent resynthesis. Phosphorylase enzymatically degrades glycogen into glucose-1-phosphate which is then acted upon by phosphoglucomutase to form glucose-6-phosphate which enters the glycolytic sequence of anaerobic metabolism. The resynthesis of glycogen, however, is not the reverse of this pathway, and phosphorylase is not a contributing enzyme. The enzyme responsible for the synthesis of glycogen from glucose phosphate units is glycogen synthetase. The presence or absence of phosphorylase does not preclude the presence or absence of glycogen synthetase.

Areas 4, 5, 6, 7, and 8 were the regions that showed an increase in the localization of glycogen. Besides considering the metabolic mechanisms involved, it is important to recognize the possibility that certain muscle areas participated more in the exercise program than other muscle areas. Thus, specific muscle area participation might have influenced the sites of glycogen resynthesis. Referring to Figure 3, it can be seen that the central and medial portions of the gastrocnemous and plantaris had the greatest increases in localization of glycogen.

The training and drug treatments exhibited a reciprocal relationship on glycogen localization in

area 5. Exercise produced an increase in glycogen content while the Dianabol and placebo groups both had lower relative concentrations of glycogen than the control group.

Various studies (17, 61, 62) have shown increases in the total muscle girth of humans as a result of anabolic steroid use with weight training. The present results showed no indication of a change in mean absolute fiber size of rats with anaerobic running exercise or with steroid use.

Muscle weights did change as a result of training. Relative values were larger and absolute values were smaller in the exercise group than in the sedentary group. However, neither the relative nor the absolute muscle weight differences between the groups are attributable to alterations in absolute fiber size. The changes in phosphorylase, glycogen, and absolute fiber size with training and steroid use in the ten selected muscle areas are summarized in Table 11.

The smaller body weights of the exerise group were very evident and confirms the results of previous research (see Table 12). It was difficult to understand why the mean body weights of the Dianabol and placebo groups were both greater than that of the control group but not different from each other. The Dianabol was dissolved in a

TABLE 11.--Effects of Training and Anabolic Steroid Use on Phosphorylase, Glycogen, and Absolute Fiber Size in the Ten Selected Areas of the Gastrocnemous, Plantaris, and Soleus Muscles.

Treatment	Muscle Area									
	1	2	3	4	5	6	7	8	9	10
Training										
Phosphorylase	E<S	E<S	E<S	E<S	E<S	E<S	E<S	E<S	E<S	E<S
Glycogen	N	N	N	E>S	E>S	E>S	E>S	E>S	N	N
Absolute Fiber Size	N	N	N	N	N	N	N	N	N	N
Drug										
Phosphorylase	N	N	D<C P<C	N	N	N	N	N	N	N
Glycogen	N	N	N	N	D<C P<C	N	N	N	N	N
Absolute Fiber Size	N	N	N	N	N	N	N	N	N	N

N = not significant observed.

Note: Absolute Fiber Size--Area 5 produced disordnal interaction effect with training and drug treatments.

TABLE 12.--Effects of Training and Anabolic Steroid Use on Body Weight and on Absolute and Relative Organ Weights.

Treatment	Body Weight	Adrenals		Heart		Liver		Testes		Spleen		Kidneys		Muscle	
		Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
Training	E<S	N	E>S	N	E>S	E<S	E>S	N	E>S	E<S	E<S	E<S	E>S	E<S	E>S
Drug	D>C P>C	N	N	N	N	D>C	D>P D>C	N	N	N	D<C P<C	N	N	N	N

N = not significant.

Note: Absolute liver weight produced a disordinal interaction effect with training and drug treatments.

corn oil solvent and the placebo contained only corn oil. Whether or not the higher body weights in these two groups can be attributed to the corn oil is not known at this time.

While the absolute weights of the liver and kidneys were smaller in the exercise group than in the sedentary group, all of the relative organ weights in the exercise group were larger than those in the sedentary group with the exception of that of the spleen. The spleen showed both smaller absolute and relative weights in the exercise group than in the sedentary group. The exercise program used in this investigation was of a highly anaerobic nature. All of the previous research reviewed on organ weights involved only voluntary or aerobic-type exercise. The relative changes in organ weights resulting from those exercise regimens were considerably less than were observed in the present study. Thus, it can be postulated that the marked changes in relative organ weights are a direct result of the high intensity of the CRW SHORT exercise program.

The relative spleen weight results presented an enigma since this was the only relative weight to be smaller in the exercise group than in the sedentary group. The ability of the spleen to store and release blood is offered as an explanation. The smaller weight may have resulted from the donation of blood by the spleen to help

fulfill an acute or chronic circulatory need in the exercised animals. In man the spleen has been known to decrease in size to release as much as 150 ml of blood to the general circulation (49).

Larger absolute and relative liver weights in the Dianabol group than in the control group and larger relative liver weights in the Dianabol group than in the placebo group suggest a possible functional role of that organ with anabolic steroids. Furthermore, the Dianabol group had higher relative and absolute fat values than the control group, and higher relative fat values than the placebo group. Additional investigation is needed to determine if liver lipids are the cause of the higher liver weights.

The lower carcass weight and absolute and relative fat content as a result of training agree with previous findings. The higher percentages of water, protein, and ash that occurred with training also concur with the literature. The changes in body composition brought about by the exercise regimen and by anabolic steroid use are summarized in Table 13.

TABLE 13.--Effects of Training and Anabolic Steroid Use on Carcass Weight and on Body Composition.

Treatment	Carcass Weight	Water		Fat		Protein		Ash	
		Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
Training	E<S	E<S	E>S	E<S	E<S	E<S	E>S	E<S	E>S
Drug	N	N	N	D>C	D>P D>C	N	N	N	N

N = not significant.

Note: Percent water produced a discrdinal interaction effect with the drug treatment.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

The purpose of this study was to determine the separate and combined effects of an anabolic steroid and an anaerobic program of endurance running on selected anatomical and histochemical parameters in the adult male albino rat. Dianabol, a product of the CIBA Pharmaceutical Co., was the anabolic steroid used. The training program was the high-intensity, short-duration Controlled Running Wheel program developed in this laboratory. Body composition and various organ weights were investigated. Histochemical determinations were made of glycogen storage and phosphorylase activity in ten locations of the gastrocnemous-plantaris-soleus muscle group. The cross-sectional areas of thirty muscle fibers were measured in these same locations.

Forty-two, normal, male albino rats (Sprague-Dawley strain) of three different age levels, were brought into the laboratory in one shipment. The differences in age were required to accommodate staggered treatment

periods set up in conjunction with other concurrent studies using the same facilities. Initiation of treatments began for all animals at 100 days of age.

Fifteen animals were 90 days old (Age-Level 1), twelve animals were 76 days old (Level 2), and fifteen animals were 62 days old (Level 3) at the time of arrival. Each animal was randomly assigned to training-drug treatments within his own age group. All animals were allowed a minimum of 10 days to become acclimated to laboratory conditions before the study began. Since all animals began their training at 100 days of age, the Level 1 animals began the program first and the Level 2 and 3 animals followed at succeeding two-week intervals. Dianabol was administered subcutaneously at a 1-mg/day dose. Treatments were administered Monday through Friday for eight weeks. All animals were supplied with food and water *ad libitum*.

The exercised animals were selected for sacrifice on the basis of having the highest percent of expected revolutions (PER) within their own drug groups. The final sample consisted of 36 animals (six per cell).

At sacrifice, the animals were anesthetized with an intraperitoneal injection of sodium pentobarbitol. Selected organ weights were immediately removed, trimmed, and weighed while wet. The gastrocnemous, plantaris, and

soleus muscles were removed as a unit and frozen in a isopentane-liquid nitrogen system. Fresh-frozen, cross sections were cut at 10 microns using a rotary microtome in a cryostat. Relative localization of glycogen and phosphorylase were quantitatively determined by a histochemical photometer for a sample of thirty fibers in each of ten areas of the muscle unit. Absolute fiber size (microns^2), as measured with a polar plainimeter, was also determined for thirty fibers in the same ten muscle areas. The remaining carcass was saved for subsequent body composition analysis.

The results indicated that anaerobic training for eight weeks produced smaller body weights and smaller absolute weights of the liver, spleen, kidneys, and muscle in the exercise group than in the sedentary group. The relative weights of the adrenals, heart, liver, testes, kidneys, and muscle in the exercise group were larger than those in the sedentary group. Relative spleen weight was smaller in the exercise group.

Body weights of the Dianabol and placebo groups were both greater than that of the control group but not different from each other. Both absolute and relative liver weights were higher in the Dianabol group than in the control group, and the relative liver weights were also higher in the Dianabol group than in the placebo group.

Relative spleen weights were less in the Dianabol and placebo groups than in the control group.

Phosphorylase was depleted in all 10 muscle areas as a result of exercise. The Dianabol and placebo groups had less phosphorylase activity than the control group in area 3. An increase of glycogen in areas 4, 5, 6, 7, and 8 occurred with the training program. The Dianabol and placebo groups had less glycogen in area 5 than the control group. Absolute fiber size showed no changes with either the training or drug treatments.

Carcass weight and the percentage of fat were lower while the percentages of water, protein, and ash were higher in the exercise group than in the sedentary group. All of the absolute carcass components in the exercise group were lower than those in the sedentary group.

Conclusions

The results of the study have led to the following conclusions with regard to the albino rat, to the steroid dosage used, to the CRW SHORT program, and to the duration of the experimental period:

1. Anaerobic endurance running produces a non-selective depletion of phosphorylase in the gastrocnemous, plantaris, and soleus muscles, while glycogen localization is selectively increased in the central and medial portions of these same muscles.

2. Dianabol does not produce as marked change in phosphorylase activity and glycogen localization as does the training program.

3. There are no significant training-drug interaction effects with regard to phosphoylase activity or glycogen storage in any of the muscle areas studied.

4. Absolute fiber size is not affected by either exercise or Dianabol usage.

5. Body weight and the absolute weights of the liver, spleen, kidneys, and muscle are all lower with exercise; however, on a relative basis the adrenals, heart, liver, testes, kidneys, and muscle weights are all higher. Relative spleen weight is lower with exercise.

6. Dianabol increases both absolute and relative liver weights over those found in control animals and also increases relative liver weights over those found in animals injected with a corn oil placebo.

7. Body weights are not different in the Dianabol and corn-oil placebo groups; however, both have greater body weights than control animals.

8. Exercise decreases carcass weight and the percentage of fat while increasing the percentages of water, protein, and ash.

9. Dianabol produces higher percentage and absolute fat values than are found in control animals and a greater percentage of fat than is found in animals receiving a corn oil placebo.

Recommendations

1. A wider variety of enzymes and substrates should be studied. The typing of skeletal muscle fibers is needed.

2. The effects of Dianabol should be studied in conjunction with specific anaerobic and aerobic training programs.

3. The effects of different doses of Dianabol should be studied.

4. The length of the treatment period should be altered to determine if anabolic steroid action is dependent on duration.

5. Biochemical analyses of skeletal muscle are needed to complement the current histochemical research.

6. Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) determinations should be undertaken in conjunction with the Dianabol applications and various exercise regimens.

7. Experimentation with anabolic steroids on human subjects should be withheld until more complete information on the actions of these steroids has been obtained.

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APPENDICES

APPENDIX A

TRAINING PROGRAM

TABLE A-1.--Standard Eight-Week, Short-Duration, High-Intensity Endurance Training Program for Postpubertal and Adult Male Rats in Controlled-Running Wheels.

Wk.	Day of Wk.	Day of Tr.	Acc-eler-ation Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repe-ti-tions per Bout	No. of Bouts	Time Bet-ween Bouts (min)	Shock (ma)	Run Speed (ft/ sec)	Total Time of Prog. (min: sec)	Total Exp. Revo-lu-tions TER	Total Work Time (sec) TWT
0	4=T	-2	3.0	40:00	10	1		5.0	0.0	1.5	40:00	. .	. .
	5=F	-1	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	. .	. .
1	1=M	1	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	2=T	2	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	3=W	3	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	4=T	4	2.5	00:10	10	40	3	5.0	1.2	2.0	49:30	600	1200
	5=F	5	2.0	00:10	10	40	3	5.0	1.2	2.0	49:30	600	1200
2	1=M	6	1.5	00:10	10	28	4	5.0	1.2	2.5	51:40	700	1120
	2=T	7	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	3=W	8	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	4=T	9	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	5=F	10	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
3	1=M	11	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	2=T	12	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	3=W	13	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	4=T	14	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	5=F	15	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
4	1=M	16	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	2=T	17	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	3=W	18	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	4=T	19	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	5=F	20	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
5	1=M	21	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	2=T	22	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	3=W	23	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	4=T	24	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	5=F	25	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
6	1=M	26	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	2=T	27	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
	3=W	28	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
	4=T	29	2.0	00:10	35	10	5	5.0	1.0	5.0	54:34	625	500
	5=F	30	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
7	1=M	31	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
	2=T	32	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	3=W	33	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	4=T	34	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	5=F	35	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
8	1=M	36	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	2=T	37	2.0	00:10	40	6	8	2.5	1.0	5.5	52.10	660	480
	3=W	38	2.0	00:10	40	6	8	2.5	1.0	5.5	52:10	660	480
	4=T	39	2.0	00:10	40	6	8	2.5	1.0	5.5	52:10	660	480
	5=F	40	2.0	00:10	40	6	8	2.5	1.0	5.5	52:10	660	480

APPENDIX B

HISTOCHEMICAL RAW DATA

TABLE B-1.--Mean Phosphorylase Activity Per Animal of 30 Muscle Fibers Per Area in the Ten Selected Areas of the Gastrocnemous, Plantaris, and Soleus Muscles (Percent Light Absorbed = 100 - Percent Light Transmitted).

Animal Number	Treatments		Muscle Area									
	Training	Drug	1	2	3	4	5	6	7	8	9	10
01	E	D	84	82	61	75	66	78	72	66	65	66
03	E	D	84	83	65	81	76	89	78	73	68	57
04	E	D	87	85	66	82	74	82	82	72	59	59
05	E	D	78	73	56	84	74	88	74	72	53	54
06	E	D	83	76	65	83	72	85	72	70	61	61
07	E	D	77	76	63	80	75	86	72	69	65	65
09	E	C	91	92	86	76	91	92	76	91	79	76
10	E	C	85	86	78	86	87	87	86	88	57	67
11	E	C	87	89	83	79	87	85	84	89	66	67
13	S	D	78	82	72	84	76	85	81	79	58	61
14	S	P	80	82	76	86	87	87	86	88	66	79
15	S	C	82	84	79	84	74	80	78	74	64	65
16	S	D	95	96	89	94	93	95	96	93	83	84
17	S	D	92	94	89	94	96	96	94	93	74	87
18	S	D	91	95	88	91	94	87	96	87	82	81
19	S	D	90	91	71	85	91	91	88	91	76	75
20	S	P	90	93	81	90	95	95	94	94	75	74
21	S	P	92	95	71	93	97	94	94	96	72	77
22	S	P	91	96	81	92	95	93	96	92	76	75
23	S	P	92	93	83	93	96	94	94	96	80	81
24	S	C	92	94	91	95	91	89	90	89	71	70
25	S	C	87	94	82	97	96	96	92	89	74	71
26	S	C	85	90	86	91	93	94	93	94	63	62
27	S	C	91	96	77	92	94	92	98	95	81	80
28	E	D	86	80	59	78	77	85	79	78	63	50
29	E	D	76	79	70	84	81	76	82	82	46	57
31	E	D	86	82	83	88	93	87	88	93	79	80
32	E	P	84	84	73	89	90	92	85	86	58	49
34	E	P	81	87	83	90	95	97	89	92	64	78
35	E	P	85	90	83	94	93	95	90	89	67	84
36	E	C	88	89	80	83	91	89	87	86	67	65
38	E	C	88	90	84	88	89	89	85	83	81	78
39	E	C	84	84	84	86	86	87	84	87	78	69
40	S	D	87	90	82	85	84	85	87	84	75	71
41	S	P	85	85	86	85	84	88	84	87	78	74
42	S	C	85	89	83	87	87	87	88	88	68	74

TABLE B-2.--Mean Glycogen Storage Per Animal of 30 Muscle Fibers Per Area in the Ten Selected Areas of the Gastrocnemous, Plantaris, and Soleus Muscles (Percent Light Absorbed = 100 - Percent Light Transmitted).

Animal Number	Treatments		Muscle Area									
	Training	Drug	1	2	3	4	5	6	7	8	9	10
01	E	D	36	31	39	29	37	41	27	33	47	50
03	E	D	34	28	34	36	36	44	31	35	37	33
04	E	D	39	34	35	47	42	52	42	36	49	51
05	E	P	39	27	37	38	38	46	33	36	36	34
06	E	P	37	45	44	48	42	47	34	38	57	55
07	E	P	40	32	32	38	36	40	41	36	42	44
09	E	C	52	37	39	46	39	46	40	38	50	45
10	E	C	51	52	47	49	45	45	43	44	45	50
11	E	C	38	37	37	42	40	40	36	35	42	41
13	S	D	47	39	36	52	35	46	38	33	40	45
14	S	P	44	34	42	32	36	37	33	39	47	50
15	S	C	43	37	33	38	35	41	39	37	44	46
16	S	D	40	32	28	34	29	40	29	34	44	34
17	S	D	35	32	34	33	35	37	34	33	32	37
18	S	D	39	33	42	29	37	43	29	36	47	43
19	S	D	41	31	35	33	30	38	29	31	36	44
20	S	P	42	29	36	33	33	45	26	31	43	45
21	S	P	46	39	39	27	33	33	26	28	45	46
22	S	P	39	28	29	30	28	34	25	28	43	31
23	S	P	27	26	45	26	25	34	23	25	42	37
24	S	C	42	35	35	36	36	37	30	33	32	38
25	S	C	41	29	32	30	39	33	30	36	33	29
26	S	C	43	36	45	38	37	46	36	40	42	51
27	S	C	40	37	35	29	33	38	30	35	46	46
28	E	D	42	38	37	36	30	43	37	35	38	34
29	E	D	40	37	53	39	36	46	36	44	38	41
31	E	D	40	38	41	42	37	50	37	43	50	35
32	E	P	47	35	36	38	34	45	39	38	33	32
34	E	P	42	39	36	39	41	47	34	45	38	40
35	E	P	41	35	44	37	38	46	38	41	42	35
36	E	C	44	36	44	36	46	47	38	41	49	39
38	E	C	40	37	36	34	39	44	35	37	36	34
39	E	C	47	38	46	37	37	47	35	41	45	44
40	S	D	51	40	42	44	36	47	37	39	49	46
41	S	P	40	38	41	32	31	41	35	38	47	40
42	S	C	45	36	36	35	32	46	33	38	38	46

APPENDIX C

MORPHOLOGICAL RAW DATA

TABLE C-1.--Mean Absolute Fiber Size (cm^2) Per Animal of 30 Muscle Fibers Per Area in the Ten Selected Areas of the Gastrocnemous, Plantaris, and Soleus Muscles.

Animal Number	Treatments		Muscle Area									
	Training	Drug	1	2	3	4	5	6	7	8	9	10
01	E	D	2.1	2.7	1.4	2.5	1.6	1.8	1.7	1.5	1.6	2.1
03	E	D	2.0	2.4	1.8	2.3	1.8	2.0	2.4	1.7	1.3	2.2
04	E	D	2.3	1.9	1.5	3.3	1.7	2.0	1.8	1.5	1.5	2.2
05	E	P	2.5	1.8	1.3	1.7	1.5	1.9	1.6	1.5	1.3	2.1
06	E	P	1.7	1.7	0.9	1.4	1.3	1.7	1.5	1.2	1.2	1.8
07	E	P	2.0	1.6	1.3	1.7	1.6	1.9	2.6	1.3	1.3	2.0
09	E	C	1.8	1.8	1.3	2.1	1.7	2.1	1.4	1.4	1.4	2.0
10	E	C	1.7	2.3	1.3	2.5	1.6	1.9	1.2	1.5	1.4	1.6
11	E	C	1.9	1.9	1.4	2.1	1.7	2.2	1.9	1.7	1.5	1.6
13	S	D	1.7	1.8	1.1	1.5	1.4	2.3	1.4	1.5	1.6	2.0
14	S	P	2.8	2.5	1.3	2.0	1.8	2.3	1.4	1.4	1.7	2.1
15	S	C	2.3	2.2	1.2	3.8	1.5	1.8	1.7	1.6	1.1	1.5
16	S	D	1.6	1.4	1.2	1.4	1.3	1.8	1.3	1.1	1.1	1.6
17	S	D	2.6	2.2	1.0	1.7	1.7	2.0	1.4	1.1	1.1	1.3
18	S	D	1.9	1.7	1.2	2.2	1.6	1.9	1.5	2.0	1.4	3.2
19	S	D	1.5	1.6	0.9	1.5	1.5	1.5	1.5	1.1	1.2	1.5
20	S	P	2.1	1.8	1.5	2.0	1.4	1.9	1.8	1.3	1.8	2.1
21	S	P	2.4	2.2	1.6	2.5	1.6	1.7	1.7	1.6	1.6	2.1
22	S	P	1.7	1.5	1.0	2.2	1.4	2.0	1.3	1.1	1.3	1.7
23	S	P	2.3	2.3	1.2	2.2	1.8	2.0	1.8	1.4	1.7	1.8
24	S	C	2.1	2.2	1.3	1.8	2.1	2.3	1.6	1.3	1.5	1.7
25	S	C	2.2	1.8	1.3	2.1	1.9	2.6	2.0	1.7	1.5	2.0
26	S	C	2.4	1.7	1.5	2.3	2.0	2.0	1.3	1.8	1.5	2.2
27	S	C	2.3	2.3	1.1	2.1	1.9	2.1	2.8	1.5	1.1	1.7
28	E	D	2.0	1.8	1.2	2.2	2.0	1.8	1.3	1.4	1.3	1.5
29	E	D	1.8	2.0	1.2	1.9	2.1	2.2	2.1	1.6	1.3	1.7
31	E	D	1.7	1.4	1.1	1.3	1.3	1.6	1.4	1.3	1.1	1.4
32	E	P	2.1	1.7	1.4	1.8	1.5	1.9	1.3	1.9	1.4	1.8
34	E	P	2.2	1.8	1.3	1.7	2.5	2.5	1.5	1.5	1.5	1.6
35	E	P	2.1	1.8	1.5	2.4	1.4	1.7	1.4	1.4	1.3	1.8
36	E	C	2.3	2.1	1.7	2.5	2.0	2.6	1.4	1.5	1.9	2.3
38	E	C	2.3	2.0	1.0	1.7	1.2	1.5	1.4	1.1	1.2	1.5
39	E	C	1.9	1.6	1.4	2.8	1.9	2.3	1.7	1.5	1.5	2.0
40	S	D	2.0	1.7	1.2	2.1	1.6	1.9	1.7	1.5	1.7	1.6
41	S	P	2.2	1.7	1.5	2.9	1.5	2.4	2.3	1.7	1.3	2.2
42	S	C	2.1	1.9	1.1	2.4	1.6	1.5	1.5	1.3	1.1	1.5

APPENDIX D

ORGAN WEIGHT RAW DATA

TABLE D-1.--Body Weight and Organ Weight Results (gm) Presented by Animal Number, Training and Drug Treatments.

Animal Number	Treatments		Body Weight	Adrenals	Heart	Liver	Testes	Spleen	Kidneys	Muscle
	Training	Drug								
01	E	D	435	0.0774	1.3154	14.9227	3.9494	0.7992	2.7664	2.9742
03	E	D	453	0.0496	1.2804	13.7146	3.7946	0.8101	2.7571	3.2956
04	E	D	446	0.0576	1.3970	13.7973	3.5026	0.8289	2.7175	3.0954
05	E	P	426	0.0413	1.3334	12.2499	3.6749	0.6918	2.6670	3.1535
06	E	P	414	0.0334	1.3007	12.5351	3.3929	0.8090	2.9472	2.8682
07	E	P	456	0.0797	1.4260	13.5044	3.9198	0.8629	2.9375	3.4621
09	E	C	415	0.0646	1.3189	13.2426	3.9931	0.7276	3.1373	2.9913
10	E	C	398	0.0592	1.2246	11.8115	3.6359	0.7312	2.6746	3.1042
11	E	C	410	0.0496	1.2759	12.0839	3.3544	0.7800	2.6113	2.9937
13	S	D	485	0.0526	1.3914	14.2140	3.6710	1.0171	3.0307	3.3397
14	S	P	508	0.0500	1.5547	14.6358	3.5619	1.1100	2.9462	3.5358
15	S	C	502	0.0475	1.5257	13.4519	4.2062	1.1809	2.8254	3.3868
16	S	D	488	0.0510	1.3124	13.8906	3.2496	0.8593	3.1193	3.1889
17	S	D	490	0.0575	1.3023	11.9455	3.6099	0.9166	2.9169	3.6033
18	S	D	500	0.0599	1.4270	14.0523	3.6838	1.0034	3.1048	3.4016
19	S	D	487	0.0459	1.3202	13.5571	3.8265	1.1025	2.5021	3.2030
20	S	P	514	0.0578	1.3750	13.6198	3.8173	1.0557	2.9516	3.4564
21	S	P	483	0.0480	1.2760	13.3880	3.6385	0.7778	2.9183	3.7287
22	S	P	502	0.0500	1.4273	13.2878	4.1472	0.9716	2.9953	3.4493
23	S	P	528	0.0523	1.3931	13.7386	3.8738	1.0239	2.9352	3.7961
24	S	C	482	0.0438	1.3073	13.2789	3.4885	1.1696	2.8341	3.4404
25	S	C	494	0.0484	1.4141	13.7450	3.8381	1.4645	2.8286	3.3758
26	S	C	467	0.0555	1.3106	13.3838	3.4420	0.9390	2.7073	3.3548
27	S	C	500	0.0359	1.3410	13.4236	3.9663	1.1207	2.8917	4.0844
28	E	D	419	0.0542	1.3766	12.9060	3.4391	0.8411	2.5778	3.3314
29	E	D	451	0.0586	1.4094	14.6118	3.9617	0.7534	2.9678	3.5409
31	E	D	409	0.0565	1.3213	12.2279	2.8280	0.7196	2.6014	2.9787
32	E	P	451	0.0608	1.4813	12.7851	4.1782	0.7945	3.0099	3.5551
34	E	P	433	0.0559	1.4628	12.5734	3.9467	0.9079	2.5777	3.5015
35	E	P	401	0.0557	1.2879	12.0442	3.3775	0.7302	2.6568	3.0524
36	E	C	435	0.0476	1.3057	12.4489	3.5389	1.0356	2.8642	3.3643
38	E	C	409	0.0572	1.2774	10.5546	3.8675	0.7541	2.8552	3.4768
39	E	C	398	0.0509	1.2915	10.6853	3.7050	0.6947	2.8047	3.3241
40	S	D	506	0.0539	1.3539	14.3664	4.3504	1.2971	2.8163	3.2235
41	S	P	505	0.0469	1.3849	14.0388	3.7759	0.9438	2.8269	3.4487
42	S	C	453	0.0472	1.4100	12.9939	3.7266	1.1194	2.9295	3.1828

APPENDIX E

BODY COMPOSITION RAW DATA

TABLE E-1.--Body Composition Results Presented by Animal Number Training and Drug Treatments.

Animal Number	Treatments		Carcass Weight(gm)	Percent			
	Training	Drug		Water	Fat	Protein	Ash
01	E	D	342	65.04	10.68	20.38	3.21
03	E	D	366	65.66	8.23	21.94	3.49
04	E	D	351	63.52	9.25	21.50	4.41
05	E	P	343	65.54	7.56	21.94	4.19
06	E	P	333	62.93	10.23	21.88	4.39
07	E	P	364	63.49	9.71	21.81	5.27
09	E	C	332	64.01	9.36	22.19	4.07
10	E	C	317	64.74	7.36	22.31	4.56
11	E	C	330	63.64	8.81	22.31	4.50
13	S	D	398	61.43	12.68	21.44	3.68
14	S	P	420	62.88	12.58	20.50	4.06
15	S	C	408	62.72	11.45	21.50	3.68
16	S	D	401	58.52	16.86	21.00	3.70
17	S	D	407	62.26	11.60	22.12	3.62
18	S	D	410	57.74	17.66	20.75	3.58
19	S	D	399	55.24	20.12	20.19	4.05
20	S	P	429	58.43	15.93	20.75	3.99
21	S	P	389	62.29	10.72	22.06	3.96
22	S	P	406	61.61	12.40	21.38	3.86
23	S	P	434	62.71	11.56	21.88	3.75
24	S	C	399	61.33	12.83	21.62	3.75
25	S	C	409	62.56	12.73	21.12	3.17
26	S	C	384	60.78	12.80	22.19	4.08
27	S	C	416	62.82	10.90	22.00	3.39
28	E	D	337	64.40	7.20	23.44	4.56
29	E	D	363	65.14	8.92	21.69	3.82
31	E	D	324	65.89	7.38	22.25	4.02
32	E	P	364	65.44	7.85	23.50	3.69
34	E	P	348	66.25	6.47	23.31	4.26
35	E	P	325	64.63	8.78	22.81	4.13
36	E	C	350	64.76	9.12	22.25	3.67
38	E	C	340	65.23	7.56	23.12	4.01
39	E	C	330	63.98	8.09	23.31	4.44
40	S	D	416	58.92	15.35	21.50	3.77
41	S	P	413	62.75	11.77	21.38	3.35
42	S	C	383	59.89	12.99	22.69	4.00

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