ABSTRACT

Stanley Barone Jr. THE EFFECTS OF VASECTOMY AND EXERCISE ON TESTOSTERONE LEVELS IN MICE. (Under the direction of Everett C. Simpson) Department of Biology, March 1986.

Forty male CD-1 mice were used in this study in an attempt to find an acceptable animal model for subsequent studies of vasectomy and exercise. This thesis was concerned with the following treatments; execise non-vasectomized, exercised vasectomized, sedentary vasectomized, and sedentary non-vasectomized. The parameters examined in this study were; body weight changes over time, body densities, wet and dry weights of accessory sex glands, wet and dry weights of testes, and endogenous testosterone levels.

This study was conducted with a two-factor random complete block design. Ten litters of mice with four male littermates randomly assigned to each treatment combination constituted the ten blocks. All parameters were examined at the 0.05 significance level and signifcance was tested using Tukey's test for contrasts of means. The F_{max} test was used with the data of all parameters and they were determined to not be heterogeneous at the 0.05 level.

One significant result of this study was there were no deaths of the mice until the designated time at the end of the study (12 weeks). There was no significance treatment or block effect apparent for body weights or body densities.

Wet weights of accessory sex glands showed a significant treatment effect. The exercised non-vasectomized treatment group was significantly different from the vasectomized sedentary and the non-vasectomized sedentary treatment groups. The vasectomized exercised treatment was not significantly different from any of the other treatment groups. There was no significant treatment or block effect for dry weights of accessory sex glands.

Wet and dry weights of right and left testes did not show a significant treatment or block effect and the testes were not significantly different among individuals.

The analysis of variance of testosterone done with the entire data set and without regard to the time sampled did not have a significant treatment or block effect. When the data for the first 90 minutes samples (N=12) were examined there was a significant treatment effect and block effect. All treatment combinations' means were determined significantly different with Tukey's test except the vasectomized sedentary treatment compared with the sedentary control.

Exercise stimulated increased secretion of the accessory sex glands which is beleived to be an anabolic effect of testosterone. The data for the radioimmunoassay of testosterone were inconclusive because of variation within treatments. Vasectomy did not appear to have any significant effects on testosterone or its androgenic effect as determined with the bioassay of accessory sex glands.

THE EFFECTS OF VASECTOMY AND EXERCISE ON TESTOSTERONE LEVELS IN MICE

A Thesis

Presented to

the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Biology

by

Stanley Barone Jr.

February 1986

THE EFFECTS OF VASECTOMY AND EXERCISE

ON TESTOSTERONE LEVELS OF MICE

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ACKNOWLEDGEMENTS

I would like to express my most sincere appreciation to the following people for their invaluable contributions to this study and to my education:

To Dr. Everett Simpson for his assistance throughout this study as my advisor and in particular for his patience in editing this manuscript.

To Dr. Thomas M. Louis for his guidance and the priviliged use of his laboratory facilities.

To Dr. Patricia Daugherty and Dr. John Laurie for graciously serving on my committee.

To Dr. Francis S. Bridges for the use of his treadmill and his support in this project.

To Tim Charles for his photographic assistance.

To James Crosier and Stephen Bunnell for their invaluable technical assistance and moral support.

To Dr. O'Brien for his assistance in statistical design and analysis of this study.

To Chet Johns for his assistance on the wordprocessor.

To the Departments of Biology and Anatomy for the use of their facilities and financial support throughout this study.

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INTRODUCTION

The widespread use of anabolic steroids in the field of sports has resulted in closer scrutiny and monitoring of the use of these steroids. Many sports competitions now monitor the levels of anabolic steroids in the blood stream or urine of competitors subsequent to competition and even traces of these may disqualify a competitor. Anabolic steroids are being used to enhance athletic performance. However, there are undesirable physiological side effects. Goldman (1985) reported the findings of preliminary epidemiological studies that pharmacologically prepared anabolic steroids are indicated in pathophysiological effects. Androgens are known to greatly alter liver metabolism (Galbo, H., 1983). It appears the use of anabolic steroids may lead to an increased incidence of otherwise rare hepatic cancers (Goldman, W., 1985).

A plausible relationship between anabolic steroids and coronary risk factors may exist. It has been noted in several studies that differences occur in the incidence of coronary heart disease between men and women. This difference is significantly reduced after menopause in women. Male survivors of myocardial infarctions appear to have higher levels of plasma estradiol than their age-matched controls (Nordoy, A., et al., 1979) (Gutai, J., et al., 1981).

It appears the coronary risk factor of men is related to lipoprotein levels. High density lipoproteins (HDL) are a protective factor against heart disease and are found in higher levels in women than men (Gutai, J., et al., 1981). Many studies of the effects of androgens on lipoprotein levels have been done but as yet no casaul relationship has been elucidated.

Testosterone is one of several known androgens, and androgens have long been known to stimulate muscle growth in males. The thick and crested neck of the bull is one example. It has become obvious that androgens are intrinsically related to the sexual dimorphism of muscle mass in mammals (Turner, D. et al., 1976). This relationship has been applied to athletics where strength and endurance are a necessity. Androgens have been used in a wide array of sports for their anabolic effects. These anabolic steroids are being used to increase muscle mass, aerobic capacity, and muscle strength (Wilkerson, J.E. et al., 1980).

Testosterone has also been shown to influence sexual behavior in males and to influence aggression and social behavior. Many stressful stimuli of both animals and humans have been shown to alter testosterone secretions. Some of these stress factors include surgical stress, climate, shock avoidance, psychological stress (Ismail, A.H. et al., 1977) and altitude (Humpeler, E. et al., 1980). The adrenal cortical production of steroids would be of intrinsic importance in these stress related phenomena (Ismail, A.H. et al., 1977). Initially the study of testosterone (17-B-hydroxy-4-androsten-3-one) was confined to the physiology of reproduction, but androgens have also been shown to be intrinsic in the expression of secondary sex characteristics including hair patterns, voice changes, skeletal configurations and the regulation of sebaceous gland activity (Turner, D. et al., 1976).

In plasma approximately 99% of the testosterone is bound to plasma proteins which limits the amount of free testosterone available to exert a physiological effect (Barnhart, E.R., 1985). In the rodent model

there is not a specific sex hormone-binding globulin as in man, but testosterone is predominantly bound to serum albumen (Hadley, M.E., 1984).

METABOLISM

Steroid hormones, of which testosterone is an example, are derived from the acetate pool and the absorption of extracellular lipoproteins. The specific enzyme systems for the production of testosterone from cholesterol (Figure 1) are found in the interstitial cells of Leydig and to a lesser degree in the adrenal cortex (Hadley, M.E., 1984). Some minor steroid conversion takes place peripherally in skeletal muscle (Greenspan, F.S. et al., 1983). There are two other enzyme systems. They reside in the accessory sex glands and certain hypothalamic nuclei. The first, 5-a-reductase, converts testosterone to 5-a-dihydrotestosterone and the second, aromatase, aromatizes testosterone to estradiol (Figure 2) (Hadley, M.E., 1984).

Inactivation of testosterone occurs primarily in the liver and involves the oxidation of the 17-OH group, the reduction of the A ring, and the reduction of the 3 keto group (Figure 3 and Figure 4) (Hadley, M.E., 1984). Testosterone's metabolic half-life varies from 10 minutes to 100 minutes (Barnhart, E.R., 1985). Steroids administered orally or intraperitoneally are inactivated readily but those administered subcutaneously or intramuscularly absorb slowly into the systemic circulation and bypass hepatic inactivation (Turner, D. 1976) (Table 1). The primary urinary byproducts of testosterone are androsterone and

FIGURE 1

A diagramatic representation of the desmolase system in the biosynthesis of sex steroids. The numbers denote the following enzymes: 1 = 20,22-desmolase; 2 = 3-beta-hydroxysteroid dehydrogenase and 5,4-isomerase; 3 = 17-hydroxylase; 4 = 17,20-desmolase; 5 = 17-ketoreductase; 6 = 5-alpha-reductase; 7 = aromatase (Greenspan, F.S. et al., 1883. p. 338).

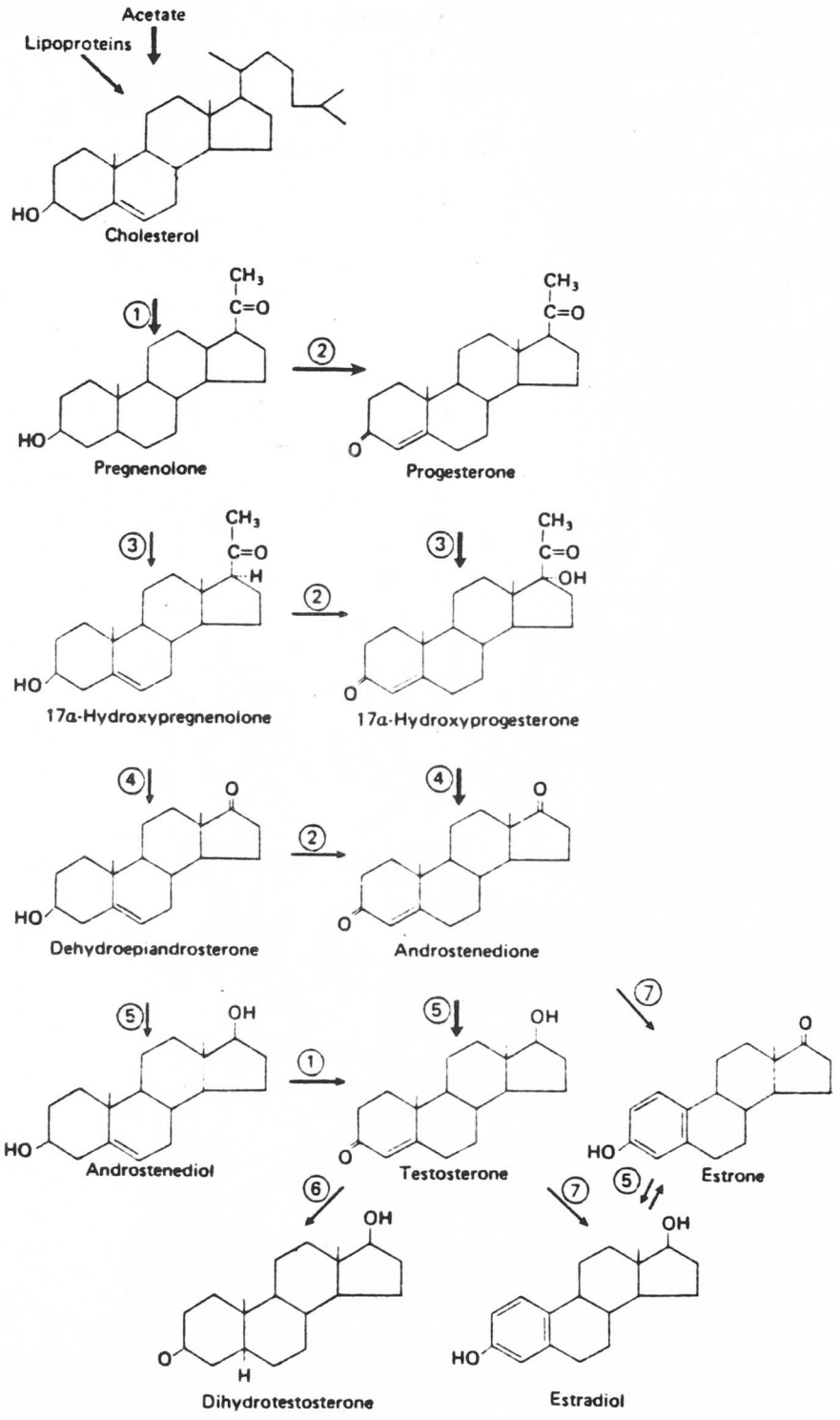

Acetate
Lipoproteins
HO
Cholesterol
CH3
C=O
Pregnenolone
Progesterone
H
OH
17α-Hydroxypregnenolone
17α-Hydroxyprogesterone
O
Dehydroepiandrosterone
Androstenedione
Androstenediol
Testosterone
Estrone
Dihydrotestosterone
Estradiol

FIGURE 2

A representation of the mediation of testosterone's androgenic effects on certain target organs (Hadley, M.E., 1984. p. 390).

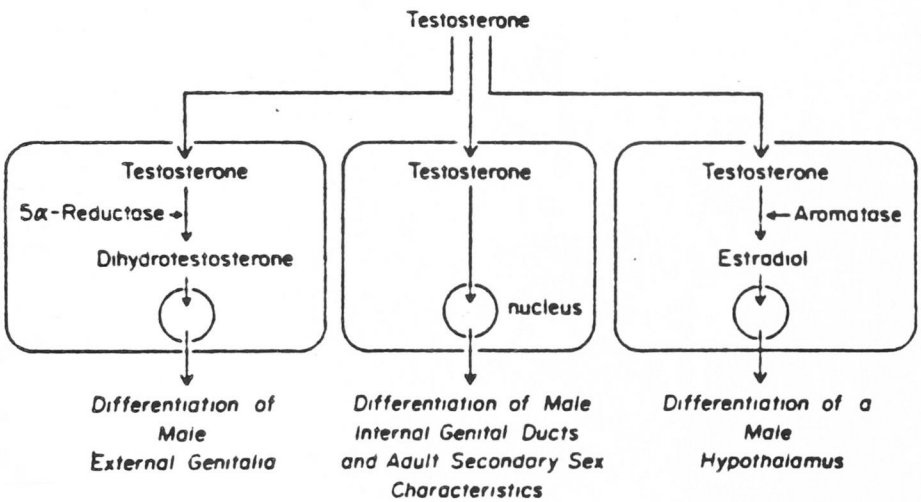
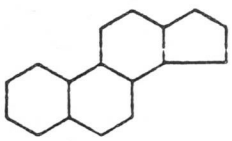
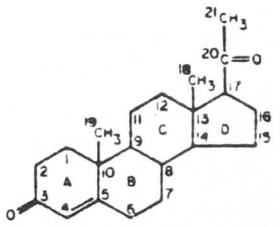

Testosterone
Testosterone
5α-Reductase
Dihydrotestosterone
Differentiation of Male External Genitalia
Testosterone
nucleus
Differentiation of Male Internal Genital Ducts and Adult Secondary Sex Characteristics
Testosterone
Aromatase
Estradiol
Differentiation of a Male Hypothalamus

FIGURE 3

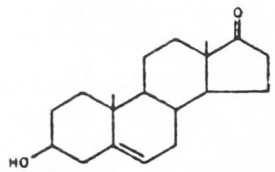
A diagramatic representation of the structure and nomenclature of steroids (Hadley, M.E., 1984. p. 350)



Cyclopentanoperhydrophenanthrene Nucleus



"C 21" Steroid (Progesterone)



"C 19" Steroid (Dehydroepiandrosterone)

Table 1

Representative androgen preparations: Dosages and routes of administration.

Oral administration	
Fluoxymesterone (Halotestin, Ora-Testryl)	5–10 mg daily
Mesterolone (Mestoranum, Pro-viron)*	30–60 mg daily
Sublingual (buccal) administration	
Methyltestosterone (Android, Metandren, Oreton Methyl, Testred)	25–50 mg daily
Subcutaneous administration	
Testosterone pellets	450 mg (6 pellets) every 4–6 months
Intramuscular administration	
Testosterone cypionate (Depo-Testosterone)	100–200 mg every 2–3 weeks
Testosterone enanthate (Delatestryl)	100–200 mg every 2–3 weeks
Testosterone propionate	50 mg 2–3 times a week

*Not available in the USA.

etiocholanolone which are excreted as glucuronide and sulfate conjugates (Figure 4) (Hadley, M.E., 1984).

All androgens, including testosterone, mediate their anabolic effects by binding to intracellular receptors. These complexes bind to non-histone proteins thus deregulating specific segments of the genome for transciption (Figure 5). The transcribed mRNA is translated into proteins with the end result being an overall increase in the retention of dietary nitrogen (Hadley, M.E. 1984). Anabolic steroids cascade over a broad metabolic range increasing the synthesis of protein, DNA, RNA, and glycogen stores (Lamb, D.R., 1975) (Galbo, H., 1983). Anabolic steroids are used clinically when a deficiency of endogenous testosterone exists or to counter the demineralization of bone due to osteoporosis or adrenal corticoid therapy in both men and women (Barnhart E.R. et al., 1985) (Table 2). Contraindications exist because of the androgenization of females, the premature ossification of the epiphyseal plates of long bones in prepubescents and the increase in growth of prostatic tumors (Barnhart, E.R. 1985). For these reasons it is preferable to use an agent with a lower androgenic to anabolic ratio but it has not been possible to separate the androgenic effects from the anabolic effects even in the many synthetic androgens (Weast, R.C. 1979).

VASECTOMY

Vasectomy has been positively correlated with heart disease in several animal studies (Tung, S.K., 1975). The proposed mechanism is triggered by persistent antibody production after vasectomy. This is

FIGURE 4

A diagramatic representation of testicular biosynthesis of steroids and hepatic clearance of testosterone (Hadley, M.E., 1984. p. 404).

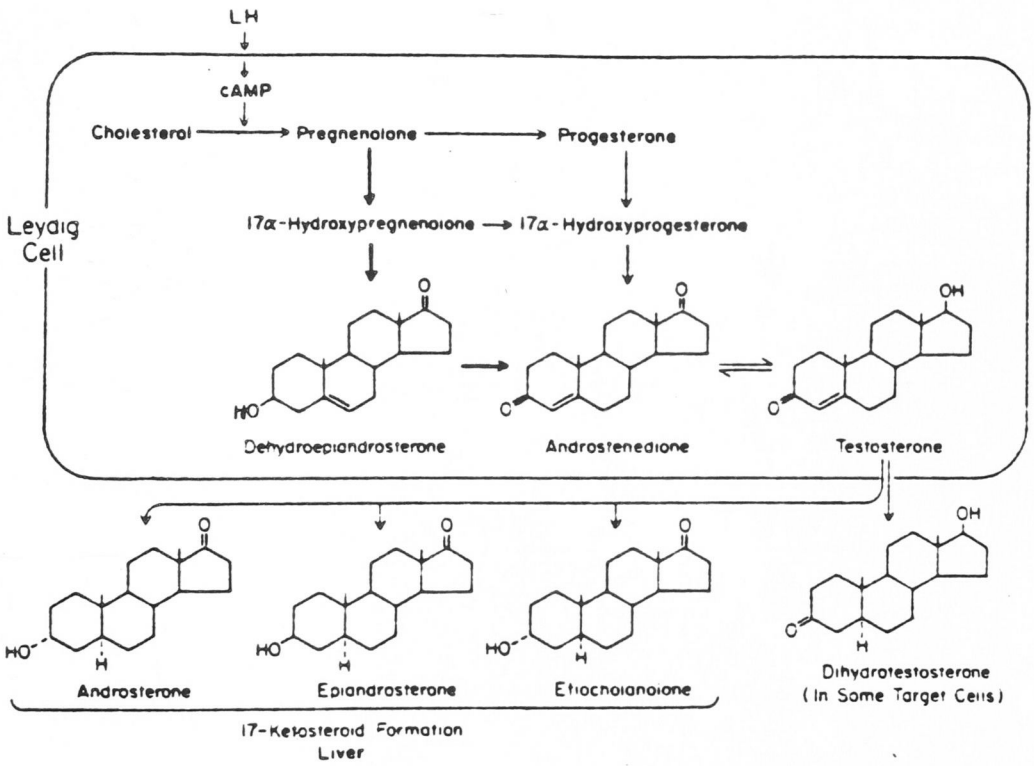

LH
cAMP
Cholesterol
Pregnenolone
Progesterone
Leydig Cell
17α-Hydroxypregnenolone
17α-Hydroxyprogesterone
Dehydroepiandrosterone
Androstenedione
Testosterone
Androsterone
Epiandrosterone
Etiocholanolone
Dihydrotestosterone
(In Some Target Cells)
17-Ketosteroid Formation
Liver

FIGURE 5

A representation of the general scheme of steroid hormones' translational and transcriptional effects. S = steroid; R_A, R_B = cytosolic receptor protein; DNA = deoxyribonucleic acid; mRNA = messenger ribonucleic acid (Greenspan, E.C. et al., 1983).

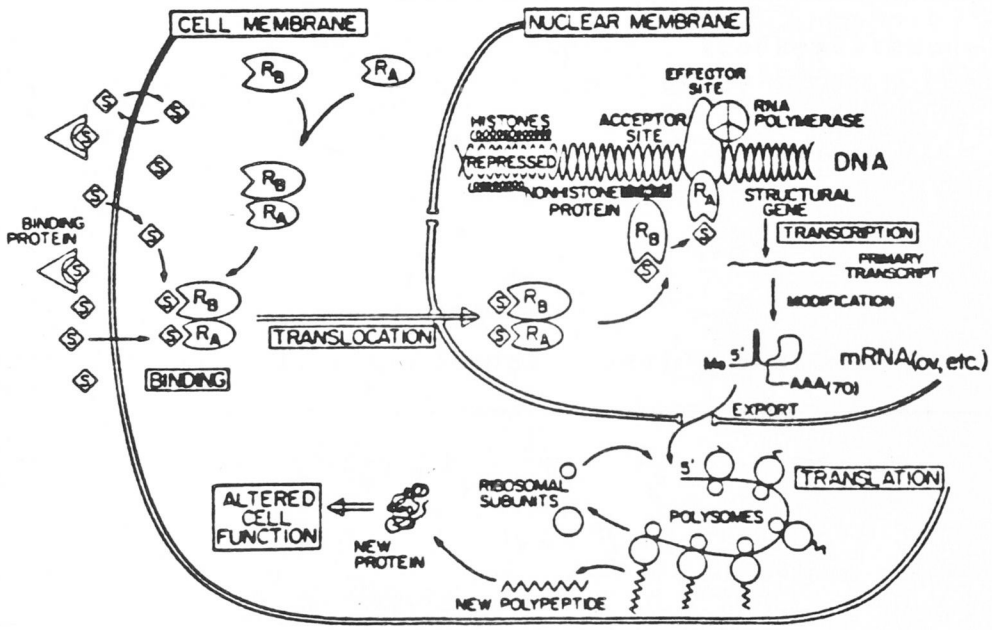

CELL MEMBRANE
NUCLEAR MEMBRANE
EFFECTOR SITE
RNA POLYMERASE
HISTONES
ACCEPTOR SITE
REPRESSED
DNA
NONHISTONE PROTEIN
STRUCTURAL GENE
TRANSCRIPTION
PRIMARY TRANSCRIPT
MODIFICATION
BINDING PROTEIN
TRANSLOCATION
BINDING
mRNA(ov, etc.)
AAA(70)
EXPORT
TRANSLATION
RIBOSOMAL SUBUNITS
POLYSOMES
ALTERED CELL FUNCTION
NEW PROTEIN
NEW POLYPEPTIDE

Table 2

Adverse Effects of Androgen Therapy

Prostatic hypertrophy
Cholestatic jaundice (methyltestosterone, fluoxymesterone)
Hepatoma (methyltestosterone, fluoxymesterone)
Premature epiphyseal fusion
Water retention with hypertension
Erythrocytosis
Inhibition of spermatogenesis
Gynecomastia
Priaprism
Acne
Aggressive behavior
Alterations of thyroid and adrenal function tests

thought to be a response to sperm antigens which leak into the circulatory system and thus trigger the production of circulating immune complexes. These sperm antigens are believed to be acrosomal in origin (Isahakia, M. et al., 1984). These circulating immune complexes appear to intensify both the development of atherosclerosis and pathological changes in the testes, similar to experimental allergic orchitis (Bigazzi, P.E., 1976). Sperm antigens do not normally elicit this autoimmune response because of the blood testes barrier (Hadley, M.E., 1984). The indication of histologic change in the testes warrants study to determine if the interstitial cells of Leydig undergo functional alterations. This autoimmune response has been documented in several animal models (primates, rodents, rabbits) including many murine strains (Anderson, D.J., 1983; Isahakia, M. et al., 1984; Kojima, A. et al., 1983; Tung, K.S.K. et al., 1979).

These circulating immune complexes also appear to be related to tumorogenesis (Anderson, D.J. et al., 1983). In a long-term study of mice which were vasectomized a significant number had spontaneous tumors and the predominant type of tumor was hepatic tumors (Anderson, D.J. et al., 1983). Apparently both vasectomy and pharmacologic preparations of androgens may influence histopathologic changes in the liver.

Some researchers suggest that vasectomy leads to an elevation of testosterone levels (Smith, K.D. et al., 1979) but inconsistencies do exist in results reported by different authors (Goebelsman, U.G.S. et al., 1979). The effects of vasectomy on testosterone levels are of central concern in this research project.

EXERCISE

The physiologic effects of exercise have been studied in several animal models and the resulting physiological alterations are not simple to outline. It is believed that several aspects of an exercise regimen are important and these aspects are: if the regimen is submaximal or maximal, aerobic or anaerobic, and the duration of the exercise regimen (Galbo, H., 1983). This research project is concerned primarily with a submaximal, aerobic exercise regimen. It has been shown that generally submaximal exercise or nonexhaustive aerobic exercise regimens result in an increase in testosterone levels (Galbo, H., 1983). Maximal or exhaustive aerobic regimens have been shown to lead to a significant decrease in testosterone levels subsequent to exercise (Kuopasalmi, K. et al., 1980; Kuusi, T. et al., 1984; Guezennec, C.Y. et al., 1982; Opstad, P.K. et al., 1982). The amount of time elapsing after the exercise regimen is significant because a plateau two hours post-exercise appears when measuring testosterone levels b (Dohm, G.L. et al., 1978; Koupasalmi, K. et al., 1980).

Physically active individuals have been shown to have lower low density lipoprotein cholesterol (LDL/C) ratios and greater high density lipoprotein cholesterol ratios (HDL/C) (Kuusi, T. et al., 1984), therefore exercise in moderation is believed to be beneficial to cardiovascular health.

Exercise regimens in female also may lead to an increase in androgen levels which are probably of adrenal cortical origin or systemic sex steroid conversion. Androstenedione is the main androgen of physiological importance in females since its biosynthesis in the

adrenal cortex is greater than that of testosterone (Hadley, M.E., 1984). These increases in androgen levels coincide with significant decreases in estradiol levels indicating steroid conversion (Weiss, L.W. et al., 1983). These significant decreases in estradiol result in menstrual abnormalities, especially oligomenorrehea or a prolonged menstrual cycle (Boyden, T.W. et al., 1983; Carli, G. et al., 1983).

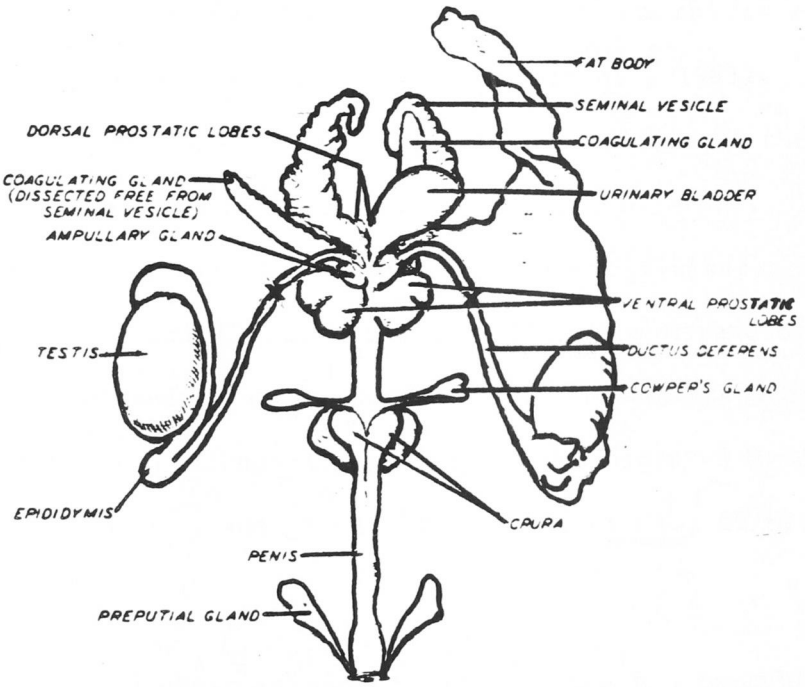
ACCESSORY SEX GLANDS

The accessory sex glands studied were the seminal vesicles and the coagulating glands. The other important accessory sex glands are the epididymus, the prostate glands, the bulbourethral gland (Cowper's gland), preputial gland, and the penis (Figure 6).

The body of the seminal vesicle is flattened dorsoventrally and is hookshaped with transversely oriented ridges in the surface (Hebel, R. et al., 1976). Histologically the seminal vesicles consist of alveolar glands, connective tissue, and a uniformly continuous layer of smooth muscle (Greenspan, F.S. et al., 1983). The seminal vesicles are the source of fructose in the semen which provides the spermatozoa with nutrition. The vesicles also secrete phosphorylcholine, ergothioneine, ascorbic acid, flavins, and prostaglandins, according to Greenspan et al. (1983). Approximately 60% of the total seminal fluid volume is derived from the seminal vesicles (Greenspan, F.S. et al., 1983). Along the inner curve of the seminal vesicle and within the same connective tissue sheath is the coagulating gland (Greene, E.C., 1963). When removed from the sheath of connective tissue the coagulating glands are quite villous. They serve to coagulate the semen and form a copulation

FIGURE 6

The rodent reproductive tract dissected out in toto. This drawing is of the ventral perspective with the urinary bladder pulled slightly to the side. The X's denote the location of ligation of the vas deferens. The fat body was removed on the animal's right side and the coagulating gland dissected away from the seminal vesicle. The coagulating glands are regarded by some as anterior prostatic lobes. (Turner, D. et al., 1976. p. 422).


FAT BODY
SEMINAL VESICLE
DORSAL PROSTATIC LOBES
COAGULATING GLAND
COAGULATING GLAND (DISSECTED FREE FROM SEMINAL VESICLE)
URINARY BLADDER
AMPULLARY GLAND
VENTRAL PROSTATIC LOBES
TESTIS
DUCTUS DEFERENS
COWPER'S GLAND
EPIDIDYMIS
CRURA
PENIS
PREPUTIAL GLAND

plug that prevents insemination of the female by any subsequent coitus (Greene, E.C., 1963).

The chemistry of the accessory sex glands are greatly affected by androgenic action. Androgens form a complex with androgen-binding protein that is secreted by the Sertoli cells of the seminiferous tubules (Turner, D. et al., 1976). The androgen complex is carried throughout the reproductive tract by ciliary action and is absorbed by its different target organs (Greenspan, F.S. et al., 1983). The result is a cascade of anabolic effects leading to hyperplasia of the accessory sex glands and increased fluid secretion (Turner, D. et al., 1976). The accessory sex glands have been studied in other bioassays using many androgenic steroids and it was noted that they are morphologically and physiologically dependent upon androgens for the maintenance of their integrity (Table 3). Androgenic activity can be assessed by determining changes in the accessory sex glands (Turner, D. et al., 1976).

PURPOSE

The current interest in sports and exercise has brought attention to the use of steroids that we normally think of as sex steroids. Their use, and sometimes abuse, has been the subject of many popular articles. However, the rapid increase in their use in sports as an anabolic drug has left many questions unanswered and conflicting findings seem to compound the problem. It was assumed that some of the confusion existing in the previously mentioned studies might be due to a lack of controlled conditions. This is a major problem in human studies. It was my intention, that if an acceptable animal model could be found,

Table 3 RELATIVE ANDROGENIC AND ANABOLIC ACTIVITIES OF SOME REPRESENTATIVE ANDROSTANES AND ANDROSTENES*

EXPERIMENTAL ANIMALS	CHICK COMB‡	VENTRAL PROSTATE	RAT SEMINAL VESICLE	LEVATOR ANI MUSCLE	RAT EXORBITAL LACRIMAL GLAND
Testosterone†	100	100	100	100	100
5α-Dihydrotestosterone	228	268	158	152	74
17α-Methyltestosterone	300 (231)	103	100	108	162
17α-Methyl-5α-dihydrotestosterone	480	254	78	107	–
Androst-4-ene-3, 17-dione	121 (262)	39	17	22	14
5α-Androstane-3, 17-dione	115 (182)	33	13	11	–
5α-Androstane-3α, 17β-diol	75	34	24	30	238
Androst-4-ene-3β, 17β-diol	(76)	124	133	95	–
5α-Androstane-3β, 17β-diol	2	–	10	–	5
Androst-4-en-3-on-17α-ol	–	8	2	3	–
5α-Androstan-3α-ol-17-one	115 (238)	53	8	10	46
19-Nortestosterone	(86)	–	10	180	52
19-Nordihydrotestosterone	118	–	–	–	–
17α-Methyl-19-nortestosterone	–	25	25	60	81
Testosterone propionate	(380)	161	146	187	195
5α-Androstan-17β-ol	128 (227)	–	–	–	5

*From Liao, S., and Fang, S. Vitamins Hormones 27:17, 1969.

†Testosterone as 100. Rat tests are by injection; comb test by inunction.

‡For comb test, relative activity numbers without parentheses are from Dorfman *et al.*; with parentheses are from Ofner *et al.*

that conditions could be more uniformly controlled than in most typical human studies. An appropriate animal model would allow for less genetic diversity, a standardized diet, a standardized photoperiod, and a controlled exercise regimen. The vasectomies performed in this study were similar to the same procedures performed on humans. Vasectomy is performed as a irreversible means of birth control in humans and is not done routinely for scientific investigative purposes. This is why most studies on the effects of vasectomies in humans are from an epidemiological perspective which offers limitations in certain controlled conditions. The maturational life time of the animal in the experimental model is also of great concern in relation to humans. The size of the animal used is of great significance because a small animal model is less expensive and allows for greater control of the parameters being studied. CD-1 mice were used in this study in an attempt to find an acceptable model for subsequent studies.

This thesis is concerned with endogenous testosterone in mice under the following conditions: exercise only, exercise and vasectomy, and vasectomy only. These treatment combinations are compared to their respective control groups. There are several important questions that will be addressed by this thesis. Does exercise cause a significant increase in testosterone levels? Does vasectomy influence testosterone levels? Does vasectomy and / or exercise affect the accessory sex glands? Will changes in testosterone levels influence tissue density, which are an indirect indication of anabolic effects?

MATERIALS AND METHODS

ANIMALS

Mice of the CD-1 strain were used because of their hardiness and relatively large size at maturity (30 to 40 grams). A breeding colony of 5 male littermates and 15 females were obtained from the ECU vivarium. They were maintained on a 12 hour light-dark photoperiod. Food (Animal Lab Blocks, Wayne Labs) and water were available ad libitum for the full term of the study. The forty male mice for this study were obtained from ten litters of this colony. All ten litters were born within a four day period. At weaning (28 days) all males were separated from their female littermates, ear tagged, and caged individually in standard cages (13x28x15 cm). During the course of the study animal cages were changed weekly and the bedding used was Bed-o-cobs manufactuered by Davison Division. The males reached sexual maturity at approximately 50 to 55 days and weighed a minimum of 25 grams.

VASECTOMY

Twenty mice, age 55 to 59 days were chosen at random to be vasectomized. The vasectomies were done by making a one centimeter midsagital incision into the lower abdominal wall. The vas deferens were located immediately posterior to the bladder and before they descended into the scrotum. Once located each vas deferen was brought to the surface of the incision and ligated with a double knot of silk suture midway between the epididymus and the prostatic lobes (Figure 6). Immediately after bilateral ligation each vas deferen was returned to its original site, the abdominal muscles were sutured with a chromic

suture (absorbable) and the skin was sutured with silk or nylon. All operations were performed under general anesthesia by injection of sodium pentobarbital at a dilution of 6 mg/cc and at a dosage of approximately 0.1 cc per 10 grams of body weight. An ether cone was used as needed to maintain anesthesia. After much practice operating times were reduced to 15 minutes or less for the animals in this study. This method was proven to be the most practical means of vasectomizing mice and facilitated the quickest recovery with minimum trauma to testicular tissue.

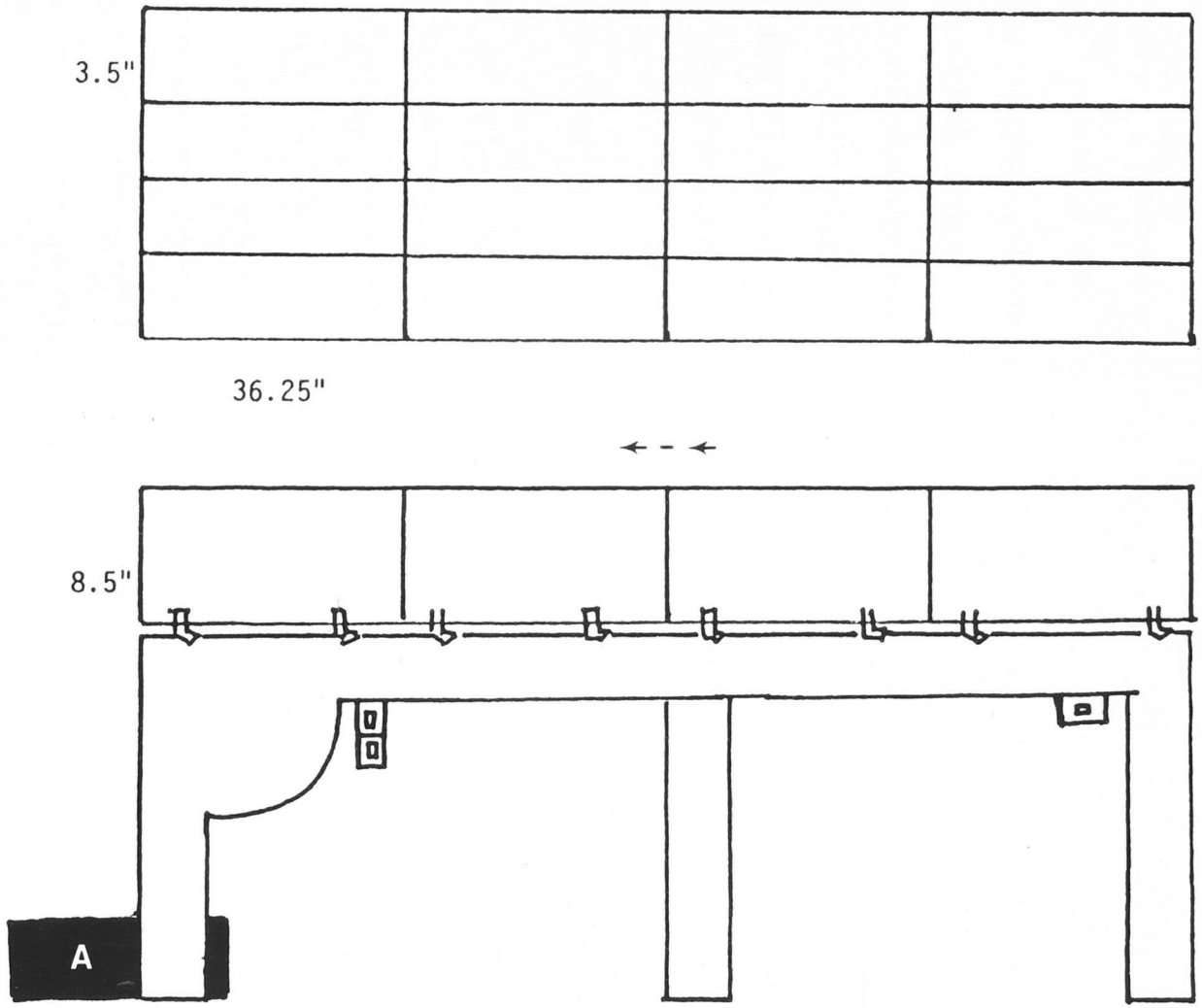
Sham operations were performed in a preliminary study under the same prescribed conditions and methods except that no bilateral occlusion was affected. The six sham vasectomized animals were compared to six litter mate controls. No statistical significance was found in the preliminary study between the controls and sham vasectomized mice with respect to the same parameters examined in this study; thus the number of animals required was lowered since sham operations were discontinued.

EXERCISE REGIMEN

All vasectomized mice were allowed ten days of recovery from surgery before the exercise regimen began. Mice were run on a motor drive animal treadmill in the Biology Department's Animal Facilities at a rate of 0.48 km/hr. The custom built treadmill was divided into separate lanes for each mouse to run in (Figure 7). The lanes of the treadmill were covered with a polyethelene cover which confined the mice to their respective runways. The separation of animals in both their

FIGURE 7

A schematic representation of the treadmill used in this study. The compartments were covered with a polyethelene cover to assure mice were contained to their respective compartments. The treadmill was operated in the direction of the arrow at the rate of 0.48km/hr. during the period of exercise. Structure A was a waste collector. The arrows denote the direction of movement of the treadmill belt.



2.5" foam blocks are at ends of runways to protect mice tails from injury.

cages and on the treadmill prevented fighting. The exercised animals were divided into two groups of ten to facilitate monitoring during exercise. The first week of the exercise regimen consisted of a 15 minute daily training regimen, performed each day of the week at the prescribed treadmill speed. The second week's regimen consisted of 30 minutes of continuous exercise a day, seven days a week. The subsequent 10 weeks of exercise consisted of a regimen of one hour of continuous running a day at the prescribed speed, seven days a week. All exercise was conducted between 7:00 AM and 7:00 PM on any given day. After each day of use the treadmill was cleaned to eliminate animal excrements. The total man-hours required for care, feeding and exercise was approximately 20 hours a week.

BLOOD SAMPLING

All animals were exercised at 7:00 AM on the day they were to be sampled. Each mouse's weight was recorded and then it was anesthetized by the previously described method before blood sampling. Blood samples were obtained between 8:00 AM and 11:00 AM to limit diurinal variation (Dohm, G.L. et al., 1978; Kuoppasalmi, K. et al., 1980). Samples were taken successively from different mice at 5 minute intervals for a period ranging from 35 minutes to 175 minutes post exercise. Blood samples were obtained by cardiac puncture with heparinized 1.5 inch, 20 gauge needles attached to 5cc syringes. After blood was collected the animals were sacrificed. This method of blood sampling provided approximately 2.0 mls of blood which was cooled in an ice bath to $4^{o}C$ until centrifugation. Plasma was decanted after centrifugation and

frozen until assayed. After blood samples were taken, body densities were determined by Archimedes' Principle (Appendix A). The animals were weighed in air and then weighed in water (Roche, A.F. et al., 1981). Animals were soaked in water in order to allow body temperatures to equilibrate with that of the water bath in which they were weighed. After body density determinations were done the animals accessory sex glands and testes were removed.

SEX ACCESSORY GLANDS AND TESTES

The accessory sex glands were examined as a biological index of change in testosterone levels. The specific accessory sex glands studied were the coagulating glands and the seminal vesicles (Figure 6).

These glands were excised from the mice at postmortem and placed in individually labeled vials. In order to remove the accessory sex glands without loss of their contents. Forceps were used to clamp the neck region of the glands. After locating this region the glands were separated from the rest of the reproductive tract with a quick snip of the scissors. The coagulating gland and the seminal vesicles were placed in the same vial since they were encapsulated within connective tissue and to separate them would lead to possible rupture of the glands and loss of fluid. The glands were weighed on a Mettler AE 163 digital balance to the nearest tenth of a milligram. The dry weights were obtained by lyophylizing the tissue in a Labconco freeze dryer for 36 hours. After lyophilization the tissue was weighed on the same balance as before but to the nearest one hundreth of a milligram. After the wet and dry weights of these glands were determined, the respective

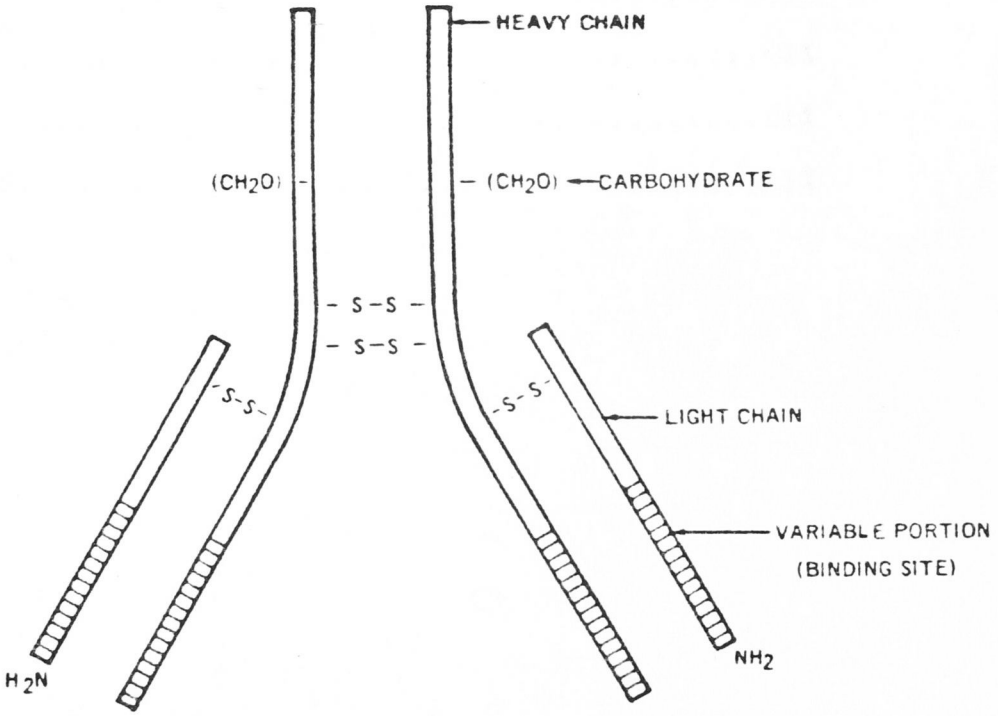
testicular dry and wet weights were determined. These weights were then expressed as a percentage of total body weight for the appropriate animal. These percentages were then transformed for statistical analysis using the arc sin transformation.

RADIOIMMUNOASSAY

Testosterone levels were determined by radioimmunoassay of plasma samples from male CD-1 mice. The underlying principle of the radioimmunoassay employed in this steroid work was the competitive binding of an unknown amount of endogenous testosterone with a defined amount of radioactively labeled testosterone at the binding sites of the specific antibody (Figure 8). The antibody used in this assay was obtained from the Pasteur Institute and was used at a dilution of 1/14,000 providing an antibody binding coefficient of approximately 32%. This antibody provided a great deal of specificity when crossreacted with other sex steroids and cholesterol, but not with 5-a-dihydrotestosterone which had a 50% crossreactivity (Table 4). The tritiated testosterone used was obtained from New England Nuclear (Figure 9) and was diluted 1/12,000 to achieve an activity of 10,000 counts per minute. Preparation of standard stock testosterone (SIGMA) was weighed on a Cahn electrobalance to the nearest ug, and dissolved in absolute ethanol and subsequently stored at $4^{o}C$. All reagents for each assay were made in sufficent quantity to use in all the assays (Table 5). Recovery percentages were calculated by counting 10% of the extract, correcting for the percentage of sample used and dividing by the total counts per minute of added tracer (100% tube). The recovery

FIGURE 8

A diagramatic representation of the immunoglobulin of RIA antibodies (Miller, W.W. et al., 1976. p. 3).



STRUCTURE OF IgG

TABLE 4

CROSSREACTIVITY OF TESTOSTERONE ANTIBODY WITH STEROIDS.

TESTOSTERONE	100%
5-a-DIHYDROTESTOSTERONE	50%
ANDROSTENEDIONE	1%
ESTRONE	<1%
ESTRADIOL	<1%
ESTRIOL	<1%
PROGESTERONE	<1%
CHOLESTEROL	<1%

TABLE 5

FLOW CHART OF RADIOIMMUNOASSAY

200 ul of plasma + 400 ul of PBS

10 ul of "hot testosterone" + 50 ul of PBS

Incubate 10 minutes at room temperature

Extract steroids with 2 mls of petroleum ether

Vortex for 15 minutes

Freeze in dry ice methanol bath and decant supernatant & repeat extraction 4 times vortexing between each extraction

Flash evaporate tubes under nitrogen

Resuspend in 200 ul of PBS

Aliquot for recovery (40 ul + 160 ul of PBS)	Aliquot for RIA (200 ul)
	Add 100 ul of antibody + 50 ul PBS to all tubes (except TC and NSB)
100% tube for recovery (10 ul + 190 ul of PBS)	
Add to scintillation vials	Incubate for 20 min.
Add 5 ml of scintillation fluid	Add 100 ul "hot" testosterone + 50 ul of PBS to all tubes
Count in liquid scintillation counter.	
	Incubate overnight at $4^{o}C$
calculate results	Add 0.5 ml of DCC to all tubes except TC which gets 0.5 ml of PBS
	Incubate 10 min. in centrifuge at $4^{o}C$ (stripping)
	Spin samples for 10 min. at 2800 rpm
	Decant samples into vials and add 5.0 ml
	Scint-Verse II to vials and count
	Calculate results by logit transformation

percentages for all assays was greater than 90%. The antibody and radioactively labelled testosterone in the samples and standards were incubated overnight at $4^{o}C$. Separation of the antibody-bound testosterone and the free (unbound) testosterone was accomplised by "stripping" with dextran-coated charcoal (DCC) and centrifugation. The unbound smaller testosterone molecules tend to stick to the charcoal mixture. Dextran-coated charcoal (0.5 mls) was added to all tubes except the total count tubes. The total count tubes received 0.5 mls of phosphate buffer solution (PBS) instead of DCC. After addition of DCC all the tubes were incubated in the centrifuge at $4^{o}C$ for ten minutes and then spun for ten minutes at 28,000 rpm. After centrifugation the supernatant was decanted into scintillation vials, 5.0 mls of Scint-Verse II cocktail were added and the samples and standards were counted in a Beckman Liquid Scintillation Counter. The concentration of the unlabeled bound hormone was determined by calculations from standard curves that were linearized by logit transformation (Figure 9). The intra-assay and inter-assay coefficent of variation was less than 20% in replicates that were not repeated.

STATISTICS

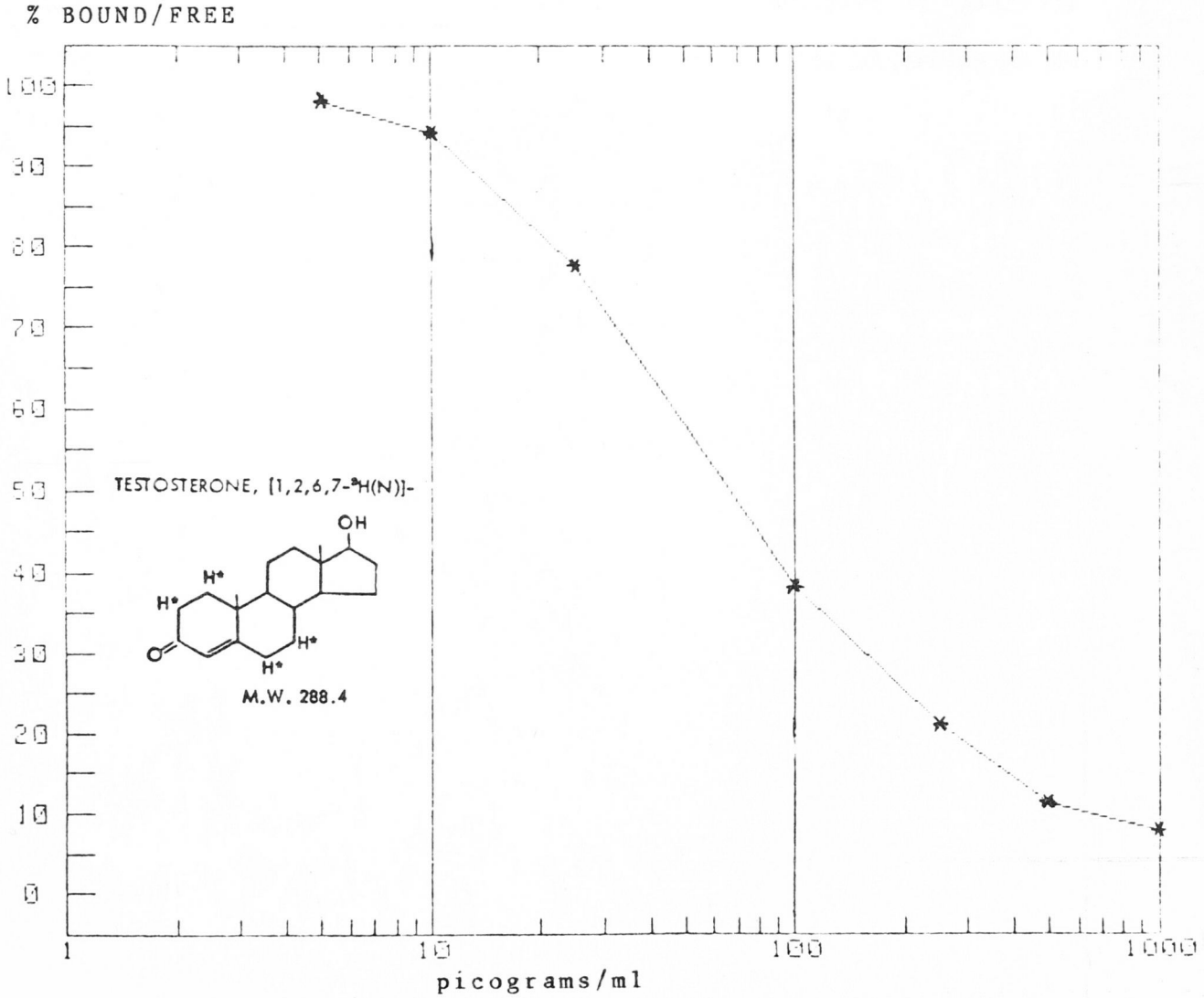
This study was conducted with a two-factor random complete block design (Gill, J.L., 1978). Ten litters of mice with four littermates being randomly assigned to each treatment combination constituted the ten blocks. Litters were blocked in order to account for genetic variability. The forty mice used were between 55-59 days old and weighed at least 25 grams to assure that they were reproductively

FIGURE 9

A typical standard curve for testosterone radioimmunoassay and an inset of the tritiated testosterone used in the RIA's of this study (New England Nuclear). The standard curve was generated with the aid of the Hewlett Packard 9845B.

THE SLOPE OF THE CURVE = -.192402693247
THE Y INTERCEPT = 1.77565453242
THE CORRELATION COEFFICENT (R) = -.987923607667
THE 'T' VALUE FOR R = -14. 2573861825 D.F. = 6

CONC. OF STANDARD	AVERAGE CPM	% BOUND	ACTUAL CONC. OF STD. CALCULATED FROM CURVE	# OF SAMPLES
0.00	2587	100.0	5.634	5
5.00	2538	98.0	6.247	5
10.00	2443	94.1	7.653	5
25.00	2041	77.7	17.961	5
100.00	1078	38.4	138.786	5
250.00	656	21.1	340.711	5
500.00	415	11.2	567.753	5
1000.00	332	7.8	677.793	5



mature. The treatment combinations in this study were: exercised vasectomized, exercised non-vasectomized, sedentary vasectomized, and sedentary non-vasectomized (controls). The parameters analyzed were: testosterone concentrations in plasma, wet weight ratios and dry weight ratios of the accessory sex glands, testicular wet weight and dry weight ratios, body fat percentages, and gross weight changes. The variance was tested for heterogenity utilizing the F_{max} test. The data were analyzed by a two-way analysis of variance with random complete blocks at a 95% significance level. In addition to the analysis of variance, Tukey's test was used for multiple comparison of the significance of treatment effects.

RESULTS

One significant result of this study was that there were no deaths of the mice until the designated time at the end of the study. Also no training problem was observed in the daily exercise regimen during the study as compared to a preliminary study (unpublished) performed with rats under the same exercise regimen. The rats tended to allow themselves to be carried by the treadmill rather than run when they came in contact with the rear of the runway. This was not a problem in this study using mice but care was taken to eliminate any injury to the mice while running by placing foam blocks at the end of the runway.

Body weights were determined at day one of the study (initial weight) and before anesthesia was administered on the final day of the study (final weight). The difference between initial and final weight changes of animals were analyzed and no significant differences were apparent among the treatment combinations and there was no block effect attributed to litters at the 0.05 level (Appendix BI and Table 6).

Wet weights of animals were determined for experimental subjects after blood samples were collected in order to calculate the body fat percentages by Archimedes' principle. These percentages were transformed for statistical analysis using an arc sin conversion (Appendix BII). It is important to note that the smaller the value for body fat the greater the lean body mass. There was no statistical significance between the treatments for body fat percentages at the 0.05 level (Table 6). It appears that neither exercise nor vasectomy had a significant influence on lean body mass composition.

TABLE 6 ANALYSIS OF VARIANCE OF BODY WEIGHT CHANGES

SOURCE	DEGREES FREEDOM	SUM OF SQUARES	MEAN SQUARES	F VALUES
MODEL	12	98.403	8.200	1.62
ERROR	27	136.693	5.062	
TOTAL	39	235.096		
BLOCK	9	61.321		1.35
TREATMENT	3	37.082		2.44

ANALYSIS OF VARIANCE OF TRANSFORMATIONS OF BODY FAT PERCENTAGES

SOURCE	DEGREES FREEDOM	SUM OF SQUARES	MEAN SQUARES	F VALUES
MODEL	12	41020.772	3418.3977	0.4735
ERROR	24	173259.38	7219.141	
TOTAL	36	214280.15		
BLOCK	9	29484.648		0.4538
TREATMENT	3	11438.061		0.5281

$F_{0.05(2)}$

Wet weights of accessory sex glands were measured and divided by total body weight to determine ratios. These ratios were also transformed using the arc sin transformation so the data could be analyzed (Appendix CI). The data were analyzed by two-way analysis of variance with complete blocks. There was a significant treatment effect but no block effect at the 0.05 significance level. The variance was examined with the F_{max} test and was not heterogeneous at the 0.05 level (Table 7). Tukey's test for multiple comparison of means was used to compare treatments and distinguish which treatments, if any, were significantly different. The exercised non-vasectomized treatment group's mean was significantly different from the vasectomized sedentary and the control groups' means. The vasectomized exercised treatment group's mean was not significantly different from any of the other treatment groups at the 0.05 significance level (Table 7 and Figure 10).

The dry weights of the accessory sex glands were determined in toto after lyophilization. The data were again examined as a percentage of total body weight and were transformed for statistical analysis using the arc sin transformation (Appendix CII). The dry weights of accessory sex glands were not significantly different at the 0.05 level for treatments or blocks (Table 7).

Wet weights of the right and left testes were determined individually and assessed as a percentage of total body weight and transformed for statistical analysis as before (Appendix D). The wet weights of right and left testes were not significantly different and there was no significant block effect or treatment effect at the 0.05 level (Table 8).

TABLE 7 ANALYSIS OF VARIANCE OF WET WEIGHTS OF ACCESSORY SEX GLANDS

SOURCE	DEGREES FREEDOM	SUM OF SQUARES	MEAN SQUARES	F VALUES
MODEL	12	3709.78	309.15	2.01
ERROR	24	3692.23	153.84	
CORRECTED TOTAL	36	7402.02		
BLOCK	9	1455.55		1.05
TREATMENT	3	2254.23		4.88 *

$F_{0.05(2)}$

* significant at the 0.05 level.

TUKEY'S TEST OF CONTRAST OF THE MEANS

CONTRAST	$X_B - X_A$	SE	q	q_a	CONCLUSION
VASECTOMY & CONTROL	4.12	8.78	1.047	3.63	Means are equal.
EXERCISE & CONTROL	19.82	8.78	5.05	3.63	Means are * not equal.
VAS/EXER. & CONTROL	13.22	8.78	3.37	3.63	Means are equal.
VASECTOMY & EXERCISE	15.72	8.78	4.01	3.63	Means are * not equal.
VASECTOMY & VAS/EXER.	9.11	8.78	2.325	3.63	Means are equal.

q(0.05) * significant

ANALYSIS OF VARIANCE OF DRY WEIGHTS OF ACCESSORY SEX GLANDS

SOURCE	DEGREES FREEDOM	SUMS OF SQUARES	MEAN SQUARES	F VALUES
MODEL	12	2.288	0.1907	1.4292
ERROR	24	3.202	0.1334	
TOTAL	36	5.491		
BLOCK	9	1.409		1.1738
TREATMENT	3	0.830		2.0742

$F_{0.05(2)}$ No significance.

FIGURE 10

A comparison of the mean transformed wet weight/ body weight percentages of the accessory sex glands of exercised and vasectomized mice.

TRANSFORMED WET WGHT./ BODY WGHT. % OF ACCESSORY SEX GLANDS

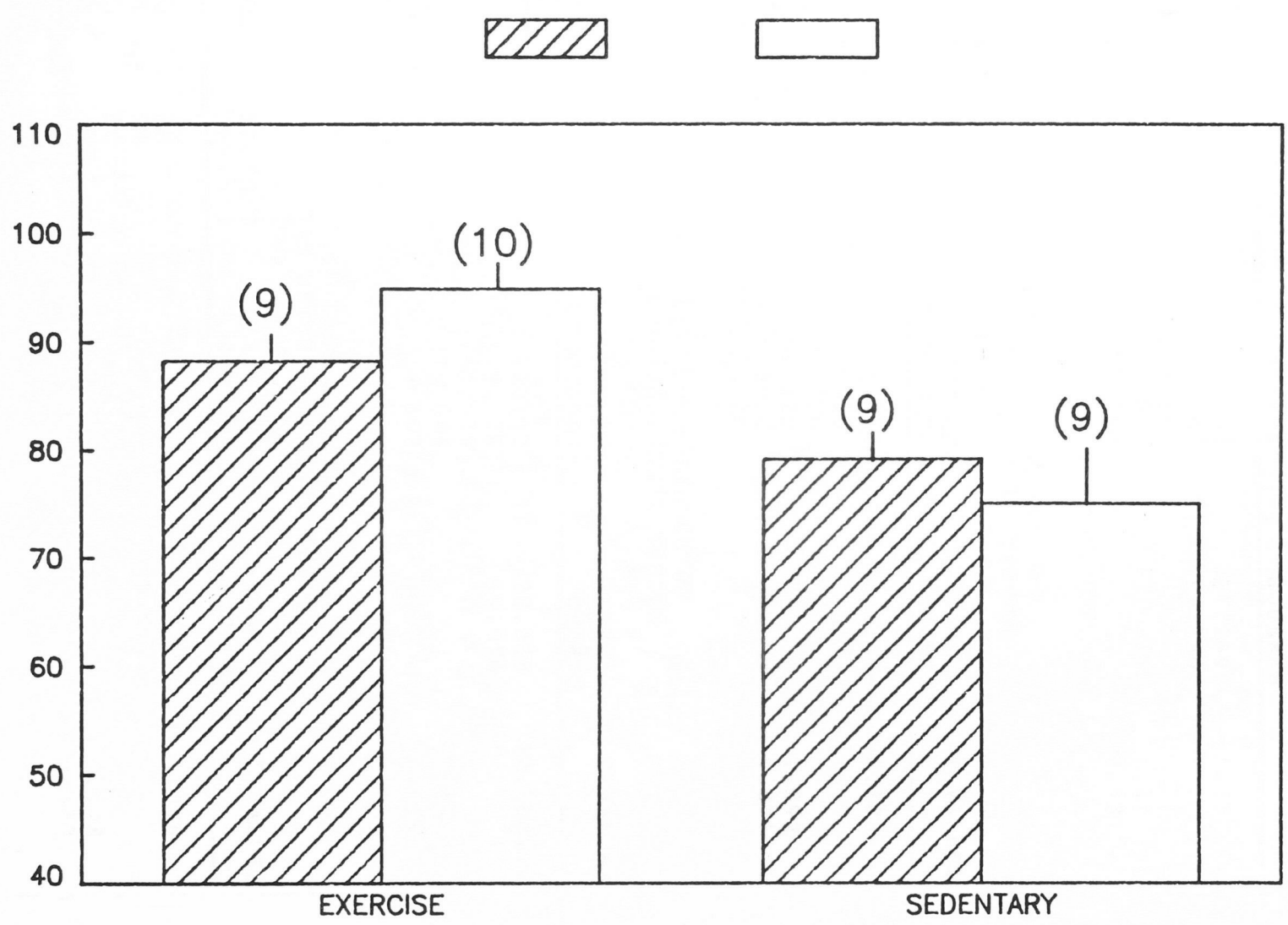


TABLE 8 ANALYSIS OF VARIANCE OF
WET WEIGHTS OF RIGHT TESTES

SOURCE	DEGREES FREEDOM	SUMS OF SQUARES	MEAN SQUARES	F VALUES
MODEL	12	255.232	21.269	1.23
ERROR	24	413.665	17.236	
TOTAL	36	668.898		
BLOCK	9	228.072		1.47
TREATMENT	3	27.160		0.53

ANALYSIS OF VARIANCE OF
DRY WEIGHTS OF RIGHT TESTES

SOURCE	DEGREES FREEDOM	SUMS OF SQUARES	MEAN SQUARES	F VALUES
MODEL	12	0.1788	0.0149	1.188
ERROR	24	0.3010	0.0125	
TOTAL	36	0.4798		
BLOCK	9	0.1459		1.292
TREATMENT	3	0.0328		0.874

ANALYSIS OF VARIANCE OF
WET WEIGHTS OF LEFT TESTES

SOURCE	DEGREES FREEDOM	SUMS OF SQUARES	MEAN SQUARES	F VALUES
MODEL	12	342.702	28.558	2.8632
ERROR	24	239.376	9.974	
TOTAL	36	582.079		
BLOCK	9	321.686		3.583
TREATMENT	3	14.682		0.490

ANALYSIS OF VARIANCE OF
DRY WEIGHTS OF LEFT TESTES

SOURCE	DEGREES FREEDOM	SUMS OF SQUARES	MEAN SQUARES	F VALUES
MODEL	12	0.2643	0.0220	1.998
ERROR	24	0.2645	0.0110	
TOTAL	36	0.5288		
BLOCK	9	0.2458		2.478
TREATMENT	3	0.0136		0.4131

$F_{0.05(2)}$

The dry weights of testes were determined individually after lyophilization. The data were assessed as percentages of total body weight and transformed for statistical analysis as stated previously (Appendix E). The dry weights of the testes were not significantly different when comparisons were made between right and left testes of the same individual and there was no significant block effect or treatment effect at the 0.05 level (Table 8).

The data from the radioimmunoassay of testosterone were examined by a two-way analysis of variance with complete blocks (Appendix F). The variation between treatments was not significant at the 0.05 level. The F_{max} test was employed to determine if the variance was heterogeneous at the 0.05 significance level. The variance was determined to not be heterogeneous at the 0.05 level using the F_{max} test (Table 9 and Figure 11).

The statistical design of this model was examined with respect to testosterone concentrations and it was determined that time was not considered in the model. This appeared to contribute to the error term when the individual animal testosterone concentrations were plotted against sampling time. When the distribution of the scattergram (Figure 12) was examined it was noted that the animals sampled at later times showed greater variation within the treatments. This may be attributable to metabolism of androgens after the cessation of exercise or to diurnal fluctuations. Since the variation within treatments also increased dramatically in the controls 90 minutes post exercise, diurinal fluctuations appeared to be suspect. For this reason it was proposed that examination of the first 90 minutes' samples might reduce

FIGURE 11

A comparison of the means of the different treatment combinations after RIA determined concentrations of testosterone following vasectomy and exercise.

TESTOSTERONE LEVELS FOLLOWING VASECTOMY & EXERCISE

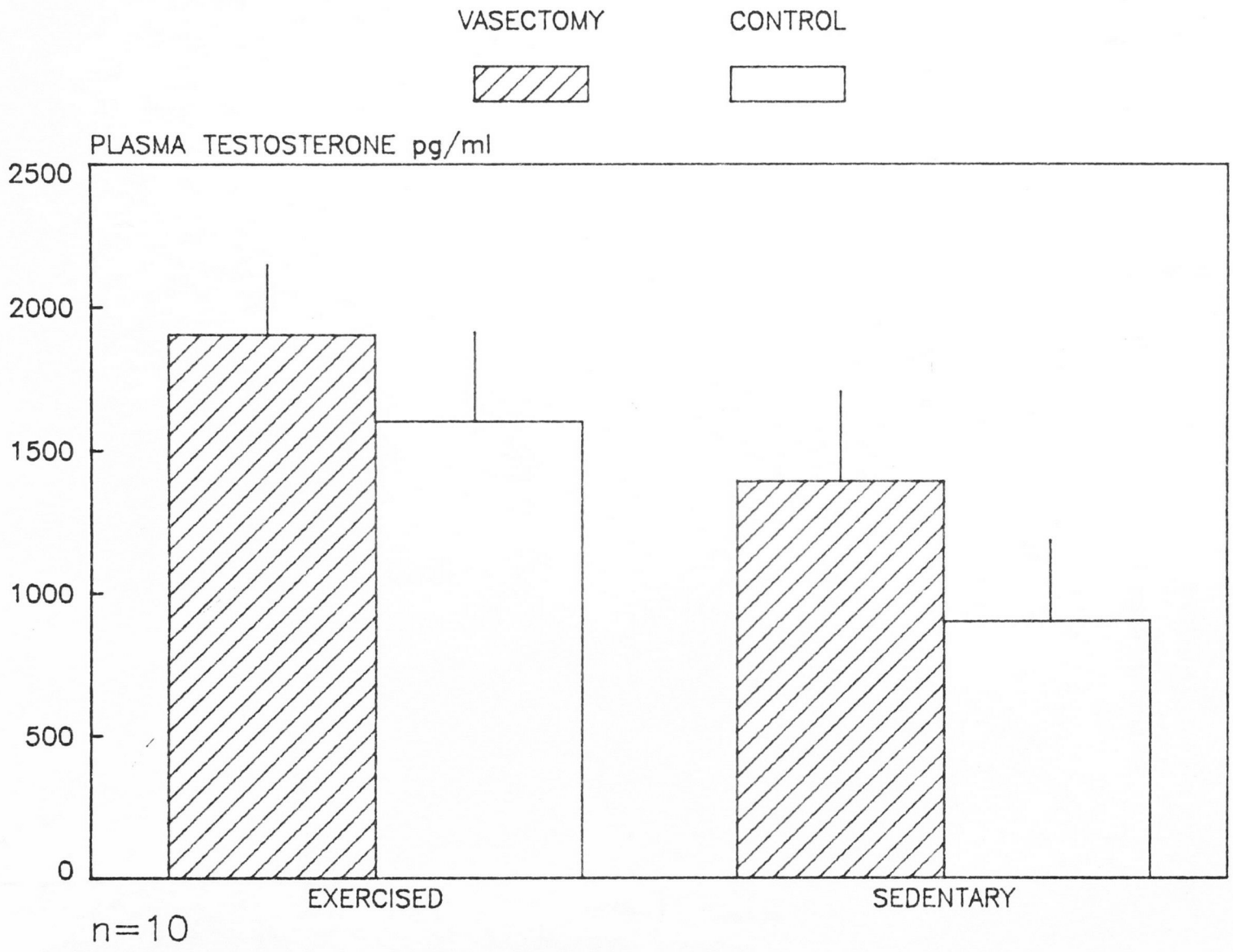
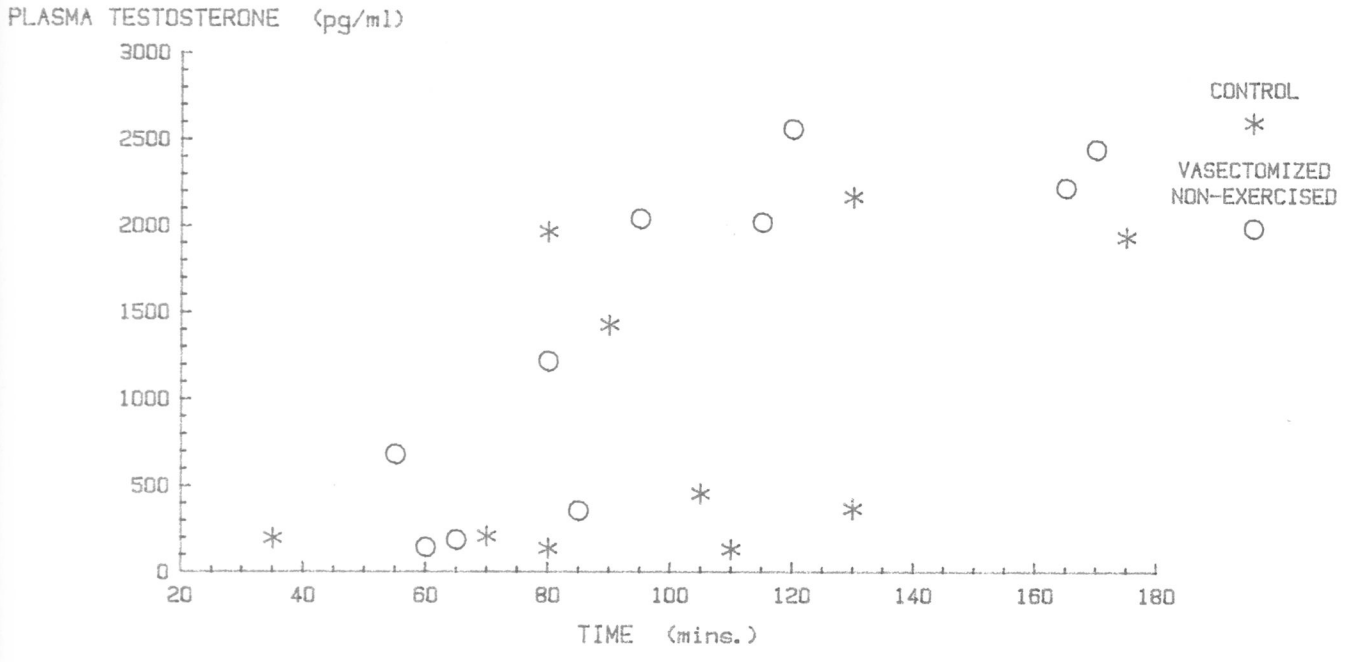


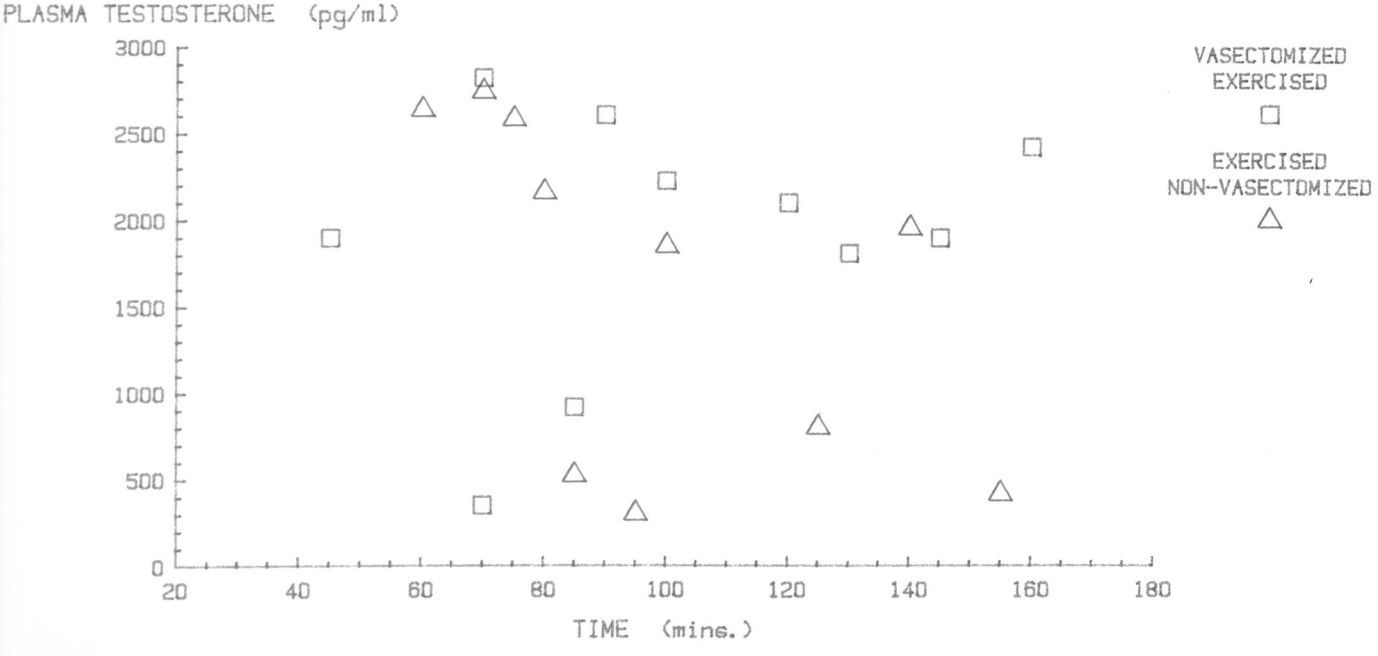
FIGURE 12

Scattergrams of the testosterone concentrations of individuals graphed with respect to time. Specific treatments are designated on scattergrams.

PLASMA TESTOSTERONE & TIME



PLASMA TESTOSTERONE & TIME



the unexplained variation. Both rodent and human studies have indicated that the release of testosterone is somewhat episodic but noted that the lowest diurnal variation takes place between 8:00 AM and 11:00 AM (Dohm, G.L. et al., 1978; Kuoppasalmi, K. et al., 1980). There was an attempt to sample in this time frame but not all animals were sampled within the prescribed time period. This accounted for some of the variation and so the data were examined in their entirety and with respect to time. This accounts for the two different analysis of variances of the testosterone levels in this study.

The analysis of variance of testosterone levels done with the entire data set regardless of the sampling time did not have a significant block effect or treatment effect at the 0.05 level (Table 9).

When considering the data with repect to the specified time interval for sampling only the data for the animals within complete blocks for the 90 minute period post-exercise were used in the subsequent analysis of variance for complete blocks (Table 9). There was a significant treatment effect and a significant block effect. Tukey's test was again used (Table 9) and all treatment combinations' means were shown to be significantly different, except for the vasectomized sedentary treatment group's mean compared with the sedentary control group's mean.

TABLE 9 ANALYSIS OF VARIANCE OF TESTOSTERONE CONCENTRATIONS IN PLASMA (N = 40)

SOURCE	DEGREES FREEDOM	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	12	10534455.26	877871.27	.98
ERROR	27	24093951.96	892368.59	
CORRECTED TOTAL	39	34628407.23		
BLOCK	9	5226744.61		.65
TREATMENT	3	5307710.65		1.98

$F_{0.05(2)}$

ANALYSIS OF VARIANCE OF TESTOSTERONE CONCENTRATIONS FOR FIRST 90 MINUTES, COMPLETE BLOCKS (N=12)

SOURCE	DEGREES FREEDOM	SUM OF SQUARES	MEAN SQUARES	F VALUE	
EXERCISE	1	828416.01	828416.01	7.52	*
VASECTOMY	1	1351161.2	1351161.2	12.28	*
EXERCISED/ VASECTOMIZED	1	1397677.1	1397677.1	12.70	*
BLOCKS	12	12796008.0	1066334.0	9.69	*
ERROR (ANIMALS)	240	26408964.0	110037.35		

* significant at the 0.05 level.

TUKEY'S TEST OF CONTRAST OF THE MEANS

CONTRAST	$\bar{X}_B - \bar{X}_A$	SE	q	q_a	CONCLUSION
VASECTOMY & CONTROL	70.35	165.85	.424	3.633	Means are equal.
EXERCISE & CONTROLS	1492.29	165.85	8.99	3.633	Means are not equal.
VAS/EXER. & CONTROLS	867.99	165.85	5.23	3.633	Means are not equal.
VASECTOMY & EXERCISE	1562.64	165.85	9.42	3.633	Means are not equal.
VASECTOMY & VAS/EXER.	938.35	165.85	5.66	3.633	Means are not equal.

$q_{(0.05)4,k} = 3.633$

VAS/EXER.= vasectomized exercised treatment.

DISCUSSION

After examination of the results it was most interesting to note the differences of the accessory sex glands wet weight ratios in the exercised and vasectomized groups. These weights were significantly higher in the exercised animals as a group than in the non-exercised animals as a group. This seems to indicate that the glands were under hormonal influences to increase their secretion thereby increasing their size. The dry weight ratios of the accessory sex glands were examined to determine if this increase in size was a result of glandular hypertrophy. The dry weight ratios were not significantly different and this indicated that there was no appreciable hypertrophy of the accessory sex glands. Thus the increase in glandular weight was due to increased secretion of the seminal fluid. There is a parallel phenomenon in females where estradiol causes increases in endometrial weights.

The effects with regard to exercise show that this regimen may be used in future studies and provide researchers with another model depicting the anabolic effects of testosterone. It can not be ascertained from this study whether there are any directly related health risks resulting from vasectomy and / or exercise but we have established a small animal model for future study. Future studies should examine the specific mechanisms by which testosterone might be affected by vasectomy and exercise. There is substantial evidence associated with the anabolic effects of testosterone resulting from exercise but there is still uncertainty as to why vasectomy might affect testosterone levels. When examining the mechanisms of testosterone

regulation one notes that lutenizing hormone (LH) is reponsible for stimulating the interstitial cells of Leydig's steroidogenesis.

Release of LH should also be studied in this model because of its stimulatory effect on sex steroid synthesis. It was easier to measure changes in titers of testosterone and determine if they were significant because LH is released even more episodically than the steroids that it eventually stimulates. For this reason repeated blood samples will need to be collected from the same animal in a study involving LH plasma levels. This is somewhat difficult to do with mice because of the small total blood volume and because the protocol of this experiment involved sacrifice of the animals to obtain maximal blood sample volumes for the radioimmunoassays.

Hepatic clearance of testosterone may complicate the experimental model and further studies of the biochemical alterations associated with exercise should be undertaken with this animal model. What immunological effects vasectomy may have on hepatic function is an interesting research problem but it requires an extensive longitudinal study surpassing this investigation. Exercise can alter testosterone levels as a result of increased stress. Stress increases corticoid levels and corticoids, particularly cortisone, inhibit sex steroid synthesis. This may be one of the chief reasons for lower testosterone levels found in studies involving exhaustive exercise regimens.

A possibility that may partially explain the androgenic effects of testosterone is in the amount of free testosterone available to the cells. The resulting physiological changes may be effected without

changes in testosterone titers because it is the free testosterone that is available for diffusion into target cells. The results reported from studies of sex hormone binding globulins (SHBG) are contradictory. Some authors state that the affinity of SHBG for testosterone remains unchanged while others disagree. One point of interest is that a specific binding affinity for testosterone is not present in rodents because rodents lack a specific SHBG. The physiological effects of androgens on secondary sex characteristics in man and rodents may indicate that specific plasma protein binding may not be as important as some believe.

Other important points from an endocrinological perspective are what changes may transpire in receptor proteins for both LH and testosterone. The number of receptor proteins for LH in the interstitial cells seem to be enhanced by FSH. FSH may be secreted in higher titers as a result of reabsorption of sperm after vasectomy but significantly higher titers of FSH have not been reported in the literature examined by this researcher. Vasectomy may affect testosterone levels because of pathologic changes resulting from circulating autoimmune complexes. No changes in testosterone levels were concretely apparent in this study when it was examined in its entirety. Further research would pursue histological studies of related to testosterone's synthesis, secretion, or clearance to discern the sinificanct effects of circulating autoimmune complexes.

In conclusion, one limitation of this study was the animal model's small total blood volume and its availablity for sampling. Hormones are

released episodically as homeostatic mechanisms require and when only one blood sampling of an animal is done it may be indicative of sporadic change. This was the chief reason for examining the biological indices and the determinations of hormone levels in conjunction. This provided an estimate of the more long term androgenic effects of testosterone. The significance of the bioassay of the accessory sex glands warrants further study. The data for the radioimmunoassays were misleading because of the great variation within treatment groups, but general trends can be surmised when outliers are disregarded. It appears that vasectomy had little or no effect on testosterone levels while exercise indeed had a stimulatory effect on testosterone. One problem to pursue in future studies would be the sampling of animals simultaneously. The sampling of one animal randomly from each of the four treatment combinations may be more effective in reducing the variation attributable to the error term. Blocking the animals according to litter showed little or no effect in this study and may be disregarded in future studies.

APPENDIX A

ARCHIMEDES' PRINCIPLE OF BODY DENSITY DETERMINATION

Wa = WEIGHT IN AIR
Db = BODY DENSITY
RV = RESIDUAL VOLUME

Residual volume for mice was undeterminable; so lungs were removed before body densities were determined.

$$K = \frac{\text{(WEIGHT IN AIR) - (WEIGHT IN WATER)}}{\text{WATER DENSITY}}$$

$$Db = \frac{\text{WEIGHT IN AIR}}{K - RV}$$

$$\frac{4.95}{Db} - 4.5 \times 100 = \text{percent body fat}$$

DATA CHART BI

GROSS WEIGHT CHANGES: INITIAL, FINAL, & DIFFERENCE IN GRAMS

COLUMNS = LITTERS

VASECTOMY SEDENTARY	25.0 35.9 10.3	26.5 33.0 6.5	24.2 32.8 8.6	28.0 39.1 11.1	30.9 42.2 11.3	29.0 40.4 11.4	29.8 38.2 8.4	30.7 38.5 7.8	30.5 40.4 9.9	29.9 38.0 8.1
EXERCISED NON–VAS.	36.0 36.5 6.5	28.5 36.6 8.1	27.6 39.8 12.2	27.8 36.2 8.4	31.7 42.0 10.3	32.1 38.8 6.7	29.7 40.6 10.9	32.2 38.2 6.0	29.6 35.5 5.9	33.5 43.8 10.3
VASECTOMY EXERCISED	23.7 33.6 9.9	29.6 36.0 6.4	25.2 36.0 10.8	29.8 38.8 9.0	27.7 34.0 6.3	29.3 43.4 14.1	33.8 43.5 9.7	30.6 37.0 6.4	28.4 44.2 15.8	35.0 41.1 6.1
SEDENTARY NON–VAS.	29.0 34.5 5.5	27.8 34.0 6.2	27.0 32.1 5.1	28.8 35.8 7.0	32.7 40.8 8.1	27.8 37.2 9.4	31.3 39.1 7.8	31.0 38.2 7.2	31.7 37.8 6.1	32.7 37.0 4.3

DATA CHART BII

TRANSFORMATION OF BODY FAT PERCENTAGES

VASECTOMY SEDENTARY	490.9	472.4	476.4	468.6	514.2	503.8	M.V.	505.1	425.8	501.1
EXERCISED NON–VAS.	113.7	481.9	514.2	556.4	376.1	M.V.	631.6	426.8	492.5	476.1
VASECTOMY EXERCISED	450.7	483.4	530.1	409.3	476.1	503.5	398.9	427.4	492.5	469.7
SEDENTARY NON–VAS.	557.0	483.9	477.4	540.7	460.6	499.3	450.9	514.2	501.1	M.V.

MISSING VALUE = M.V.

DATA CHART CI

TRANSFORMATIONS OF WET WEIGHT/BODY WEIGHT PERCENTAGES OF ACCESSORY SEX GLANDS

COLUMNS = LITTERS

VASECTOMY SEDENTARY	64.75	76.95	99.31	67.61	72.24	108.31	M.V.	72.44	75.34	75.46
EXERCISED NON-VAS.	97.01	98.66	90.84	93.82	99.03	91.30	96.78	94.87	90.31	96.07
VASECTOMY EXERCISED	96.20	91.69	93.34	74.77	95.68	M.V.	91.05	64.82	78.76	108.10
SEDENTARY NON-VAS.	93.77	62.09	46.72	70.53	74.77	96.72	71.44	73.46	85.94	M.V.

DATA CHART CII

TRANSFORMATIONS OF FREEZE DRIED WEIGHT/BODY WEIGHT PERCENTAGES OF ACCESSORY SEX GLANDS

VASECTOMY SEDENTARY	2.766	3.082	3.561	2.737	2.989	4.287	M.V.	2.965	2.958	2.972
EXERCISED NON-VAS.	3.529	3.552	3.054	3.283	3.635	3.085	3.444	3.294	3.129	3.435
VASECTOMY EXERCISED	3.501	3.113	3.276	2.984	3.372	M.V.	3.139	2.790	2.935	4.062
SEDENTARY NON-VAS.	3.381	2.531	2.234	2.905	2.980	3.449	2.932	2.922	3.068	M.V.

MISSING VALUE = M.V.

DATA CHART DI

TRANSFORMATIONS OF WET WEIGHT/BODY WEIGHT PERCENTAGES OF LEFT TESTIS

COLUMNS = LITTERS

VASECTOMY SEDENTARY	35.24	28.52	35.48	34.26	29.66	37.93	M.V.	38.99	32.52	38.23
EXERCISED NON–VAS.	33.95	32.01	27.41	28.86	30.13	38.29	33.83	31.17	37.52	33.83
VASECTOMY EXERCISED	36.51	47.00	20.09	35.36	30.06	M.V.	35.24	35.18	35.36	35.72
SEDENTARY NON–VAS.	32.07	33.02	33.52	33.58	29.80	35.06	32.52	36.03	36.15	M.V.

DATA CHART DII

TRANSFORMATIONS OF WET WEIGHT/BODY WEIGHT PERCENTAGES OF RIGHT TESTIS

VASECTOMY SEDENTARY	38.07	27.48	34.38	32.64	28.59	37.40	M.V.	36.92	34.45	36.93
EXERCISED NON–VAS.	34.69	32.33	27.34	28.79	29.66	37.76	32.13	36.20	36.63	32.83
VASECTOMY EXERCISED	36.03	36.45	21.97	34.14	19.55	M.V.	34.08	33.83	34.20	34.45
SEDENTARY NON–VAS.	31.82	32.08	31.94	34.14	29.19	34.63	32.58	35.42	35.36	M.V.

MISSING VALUE = M.V.

DATA CHART EI

TRANSFORMATIONS OF FREEZE DRIED WEIGHT/BODY WEIGHT PERCENTAGES OF LEFT TESTIS COLUMNS = LITTERS

VASECTOMY SEDENTARY	1.383	1.222	1.359	1.211	1.163	1.381	M.V.	1.384	1.247	1.378
EXERCISED NON--VAS.	1.360	1.262	1.093	1.250	1.178	1.418	1.239	1.468	1.365	1.236
VASECTOMY EXERCISED	1.344	1.456	.9449	1.281	.8913	M.V.	1.345	1.270	1.309	1.337
SEDENTARY NON--VAS.	1.210	1.262	1.268	1.357	1.144	1.327	1.311	1.256	1.300	M.V.

DATA CHART EII

TRANSFORMATIONS OF FREEZE DRIED WEIGHT/BODY WEIGHT PERCENTAGES OF RIGHT TESTIS

VASECTOMY SEDENTARY	1.373	1.122	1.408	1.270	1.241	1.388	M.V.	1.433	1.249	1.406
EXERCISED NON--VAS.	1.347	1.263	1.101	1.258	1.196	1.409	1.278	1.002	1.437	1.230
VASECTOMY EXERCISED	1.403	1.416	.8802	1.306	1.225	M.V.	1.338	1.340	1.362	1.295
SEDENTARY NON--VAS.	1.281	1.309	1.272	1.327	1.184	1.363	1.311	1.332	1.337	M.V.

MISSING VALUE = M.V.

DATA CHART F

MEAN TESTOSTERONE CONCENTRATIONS DETERMINED BY RIA
COLUMNS = LITTERS

VASECTOMY SEDENTARY	142.37	355.45	2045.9	2231.6	2456.5	679.5	186.3	1218.8	2026.4	2570.8
EXERCISED NON--VAS.	2580.6	1852.4	800.6	416.9	2160.9	2635.2	2739.2	522.7	301.8	1954.3
VASECTOMY EXERCISED	348.55	2604.3	2096.8	1896.8	2420.7	1895.1	2819.8	917.1	2225.8	1805.7
SEDENTARY NON--VAS.	136.64	455.07	132.6	2176.8	1944.8	195.9	207.6	1968.4	1429.2	370.2

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