METABOLISM OF TESTOSTERONE FOLLOWING CONTINUOUS INFUSION OF [^{3}H] TESTOSTERONE: EFFECT OF ORCHIECTOMY AND TESTICULAR ATROPHY

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CHAPTER I

INTRODUCTION

Every civilization has manifested a desire to avoid excessive sterility and fertility, but there has always been a gap between the desire to control conception and its effective prevention. Dwindling energy reserves, food shortages, and rising prices make people well aware of the demands placed on our technical and natural resources by an ever increasing world population. Although the birth rate is declining in many nations, substantial advances in medical care and in the prevention and treatment of disease has increased the life expectancy of each individual. The limited resources and unchecked population growth make the future look bleak and demonstrate the need to develop birth control measures to ensure adequate food and energy for everyone.

Recent developments in the use of orally active hormonal steroids point to the acceptability of this mode of contraception. However, this procedure has only proven acceptable in females and thus excludes about half of the world's population. At the present time there is no acceptable chemical contraceptive for men because the dose of hormone needed can not be achieved by oral administration of steroids. The birth control method developed for men must be safe, relatively inexpensive, effective, reversible, not change libido or nonsexual behavior, and not interfere with coitus.

The administration of steroid hormones via subcutaneous

polydimethysiloxane implants offers promise in fulfilling some of the above requirements for a method of birth control and also could be available to a majority of the world's population. Use of the proper size testosterone-filled implant could cause azospermia, via a decrease in the levels of plasma gonadotropins while maintaining the plasma testosterone levels near normal (Frick, 1973). The normal level of testosterone could maintain libido while the individual would be essentially sterile. The method could be reversible by simply removing the implant or not replacing an empty implant by a new one. While the use of subdermal Silastic implants for birth control in humans appears to satisfy the requirements for a birth control procedure, additional studies are needed to understand and explain the inhibitory action of testosterone on the male reproductive system.

In normal animals and man, pituitary gonadotropin release is regulated, in part, by circulating testosterone titers by a so called negative feedback loop (Davidson, 1969). However, recent observations in laboratory animals indicate that testosterone-filled Silastic capsules can suppress pituitary gonadotropin secretion even though plasma testosterone concentration remains with the normal range (Berndtson, et al., 1974). A number of explanations for this adjustment of the normal feedback response in the testosterone treated animal are possible. First, testosterone could be converted under conditions of greater mass movement of testosterone to a metabolite that suppresses pituitary gonadotropin secretion. A correlate to this explanation could be a change in the localization or binding of testosterone or one of its metabolites in certain tissues. Secondly, a change in the amount of free versus bound testosterone in the plasma of the testosterone treated animal could be

important since the free steroid is thought to be the active form of testosterone (Vermeulen and Verdonck, 1969). Thirdly, the sustained release of testosterone from the Silastic capsule could interrupt the episodic pattern of testosterone secretion and be important since the episodic bursts of plasma testosterone are believed to be correlated with the release of gonadotropins (Bartke, *et al.*, 1973).

One explanation for the action of a subdermal testosterone-filled Silastic implant is that there is a change in the uptake and/or metabolism of testosterone. This explanation is easiest to investigate due to the availability of the method of constant infusion. Continuous infusion rather than a single injection of radiolabeled testosterone may more closely mimic the metabolism of endogenous testosterone. Since castration of male rats and the concomitantly lower plasma androgen levels cause a significant increase in the uptake of radiolabeled testosterone in various tissues (Bruchovsky and Wilson, 1968), castrate animals will be used in the present study in addition to normal and testosterone treated animals to ascertain the effects of low androgen exposure on testosterone metabolism. Comparison of the uptake and metabolism of testosterone following constant infusion with that found previously after single injection will aid in evaluation of the infusion procedure.

Thus, the purposes of the present investigation were: (1) to determine changes in the metabolism of infused testosterone in male rats exposed to different levels of testosterone (castrate, normal, and testosterone treated), and (2) to determine any changes in the uptake and retention of infused testosterone or its tritiated metabolites in various tissues.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Androgens have been used since the middle ages (Needham, 1968). In 1100 A.D., the Chinese administered large quantities of testicular tissues orally and developed a procedure to fractionate hundreds of gallons of human or animal urine. Precipitations and sublimations of urine yielded a white, crystalline material probably containing mixtures of androgens, estrogens and cholesterol. The Chinese were aware of a number of stimulating actions of these tissue or urinary extracts on the male reproductive organs and secondary sexual characteristics. Today, it is known that androgens affect almost every vertebrate tissue regardless of the genotypic sex.

Androgens exert two fundamental actions (Young, _et al._, 1964): (1) they promote irreversible morphogenetic or organizational effects during a restricted period of fetal or early neonatal life, and (2) they exert reversible "activational" effects throughout the life span of the individual. The first category includes the initial embryonic differentiation of male accessory primordia such as the mesonephric ducts and urogenital sinus as well as the organizational action of androgens on structures in the central nervous system to cause acyclic production of pituitary gonadotropins and male patterns of copulatory behavior. The second category includes excitatory or inhibitory effects on the

hypothalamo-hypophyseal-gonadal axis, differentiation of various male secondary sexual tissues and numerous effects on nonsexual tissues such as an increased vascularity of tissues, skeletal growth, and promotion of protein anabolism (Wilson, 1962).

This review is concerned primarily with the uptake and metabolism of testosterone in various tissues and the experimental techniques used to investigate this retention. It will be divided into subcategories reflecting the approach to the following questions: (1) how does a normal circulating titer of testosterone provoke a reduced secretion of pituitary luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and (2) what effect do circulating androgen titers have on the uptake and metabolism of testosterone in tissues known to depend on this hormone.

Background information will be provided demonstrating that the uptake and metabolism of testosterone in the pituitary gland, hypothalamus, and cerebral cortex may be important since a metabolite of testosterone could regulate circulating levels of LH and FSH in animals receiving subdermal testosterone capsules. In addition to the uptake and metabolism of testosterone in neural tissues, information will be presented on the fate of testosterone in hepatic (the homeostatic elimination organ), prostatic (the "classical" organ of androgen action), and testicular (the production organ) tissue in order to develop a comparative understanding of testosterone metabolism in animals receiving contraceptive doses of exogenous testosterone.

A brief review of the history and use of polydimethylsiloxane capsules for the sustained release of hormonal steroids will be included and the rationale and assumptions underlying the use of continuous infusions and steady state will be discussed.

The Uptake and Metabolism of Labeled Testosterone by Different Tissues

Early investigations identifying the sites of testosterone uptake in animals relied upon ^{14}C-labeled testosterone of low specific activity. Barry, et al. (1952) were able to localize [4-^{14}C] testosterone only in the liver and kidney (the organs concerned with the excretion of the hormone) in laboratory mice and rats. Moreover, Dirscherl and Mosebach (1961) detected no retention of ^{14}C in any organ or tissue of castrate mice after injecting [4-^{14}C] testosterone intraperitoneally. However, Greer (1959) and Harding and Samuels (1962) found about twice as much radioactivity in the ventral prostate as in muscle, but the radioactivity in the prostate and blood was similar.

All the investigators mentioned above used doses of testosterone greatly in excess of the physiological values expected in laboratory rodents and undoubtedly prevented the specific retention of this steroid by its "target" tissues (Robel, et al., 1973). The smallest dose of testosterone administered was 40 μg and many of the doses exceeded 100 μg. The availability of tritium labeled testosterone with a higher specific activity permitted Chatfield and Wilson (1966) to demonstrate that [^{3}H] testosterone preferentially accumulated in the prostate, seminal vesicles and preputial gland of castrate rats. In addition, Tveter and Attramadal (1968) injected approximately 1 μg of [1,2-^{3}H] testosterone intramuscularly into adult rats 3 days after castration and noted that tritium was concentrated in the prostate and liver with a maximum concentration 1-2 hr after injection. The amount of radioactivity incorporated was diminished by the simultaneous administration of unlabeled testosterone, demonstrating the specificity of the process involved in concentrating

testosterone within the prostate.

In addition to the specific activity, the method used to administer radioactive testosterone to animals is also very important. Meli (1963) showed that subcutaneous (SC) or intramuscular (IM) injections of testosterone proprionate were more effective in preventing regression of the prostate and seminal vesicles than intraperitoneal (IP) injections. He hypothesized that SC or IM injections permitted testosterone to be absorbed more slowly and thus led to a more prolonged and sustained hormonal response. In comparison to SC or IM injections, IP injections were much less effective since most of the absorbed hormone passed into the portal system and was inactivated by the liver. Intravenous injections deliver the hormone directly into the circulatory system; the fate of the injected radioactive hormone thus mimics that of unlabeled endogenous hormone.

A brief discussion of the rationale behind choosing each of the tissues used in the present investigation along with recent findings pertaining to the uptake and metabolism of testosterone in these tissues is presented below. A diagram depicting the enzymes involved in testosterone metabolism is shown in Figure 1.

Liver

Since the liver is the most important organ in the control system regulating plasma steroid hormone levels (Schriefers, 1967), hepatic testosterone metabolism was investigated to assess the role of this homeostatic tissue in animals receiving exogenous testosterone. Numerous factors are known to affect steroid metabolism in the liver: the sexual state of the animal, stage of development, state of nourishment,

Figure 1. Some of the Metabolites of Testosterone With the Enzymes Believed to be Involved in Their Transformations. (Compiled and redrawn from Dorfman and Ungar, 1965).

(1) = 17β-hydroxysteroid dehydrogenase
(2) = 19-hydroxylase and aromatizing enzymes
(3) = 17α-hydroxysteroid dehydrogenase
(4) = Δ^4-5α reductase
(5) = Δ^4-5β reductase
(6) = 3α-hydroxysteroid dehydrogenase
(7) = 3β-hydroxysteroid dehydrogenase

Estradiol − 17β

Estrone

(2)

(2)

Testosterone

(1)

Androst-4-ene-3,17-dione

(3)

Epitestosterone

(4)

(4)

(5)

17β − Hydroxy-5α androstan − 3 − one

5α − Androstane − 3,17 − dione

5β − Androstane − 3,17 − dione

(6)

(7)

(6)

(7)

(6)

Androsterone

Epiandrosterone

Etiocholanolone

3β − Hydroxy − 5β androstan − 17 − one

(1)

(1)

(1)

5α − Androstane − 3α, 17β − diol

5α − Androstane − 3β, 17β − diol

5β − Androstane − 3α, 17β − diol

conditions of stress and hormone treatment (Schriefers, 1967). Thus, alterations in hepatic testosterone metabolism could be expected by changing the amount of testosterone administered.

The enzymes involved in hepatic steroid metabolism can be grouped into hydrogenases (or reductases), dehydrogenases, hydroxylases and transferases. The major changes in the steroid molecule are reductive, particularly in ring A. Δ^4-hydrogenases form 5α- and 5β-isomers of various steroid by reducing the Δ^4 double bond. The hydrogenases or reductases have a high degree of steric specificity. The Δ^4-5α-reductases have been localized in microsomes whereas the Δ^4-5β-reductases appear in highest concentration in the cytosol. The reductases require NADPH as a cofactor, and the systems are apparently irreversible (Forchielli and Dorfman, 1956).

The majority of the secondary alcohols formed upon reduction of the 3-ketone group of the steroid molecule have the 3α-configuration. The 3α-hydroxysteroid dehydrogenases appear to be relatively nonspecific as far as structure outside of ring A is concerned, and these reversible enzymes can use either NAD or NADP (Tomkins, 1956). Small amounts of 3β-, 17β-, 11β-, 20β-, 16β-, and 16α-hydrosteroid dehydrogenases have been found in the liver (Sweat, et al., 1950; Repke and Samuels, 1964). Thus, dehydrogenases seem to be present for all positions in the steroid molecule where ketone-secondary alcohol interconversion could occur. However, the dehydrogenases do not seem as selective with respect to the general structure of the steroid substrate as the Δ^4 reductases. Most of the dehydrogenases utilize either NAD or NADP as cofactors, and the direction of conversion depends on the relative concentrations of both substrates and cofactors at any one time.

Hepatic steroid hydroxylases are not as important in steroid metabolism as are the dehydrogenases and reductases. However, hydroxylations at the 2, 6β, 7α, 15α, and 16α positions have been described (Starke and Kutova, 1962; Colas, 1962). Such hydroxylations require NADPH and oxygen and are essentially irreversible. A 17β-dehydrogenase may be important in the conversion of estrone to estradiol (Brown and Marrian, 1957).

The above three enzyme groups produce products that are more polar than testosterone but still not very hydrophilic. Therefore, they would not be readily displaced from plasma binding proteins and thus retained during the process of glomerular filtration by the kidneys if they were not converted largely to glucuronisides and sulfates. Conjugation of hormonal steroids to glucuronide and sulfate takes place primarily in the liver. Glucuronidase transfers glucuronic acid from uridine diphosphoglucuronic acid to steroids with the formation of uridine diphosphate (Isselbacher, 1956). The sulfate is transferred from adenosine 3'-phosphate-5'-phosphosulfate by enzymes in the liver supernatant (Robbins and Lipmann, 1956). Most androgens are excreted largely as the glucuronides. The glucuronide derivatives of steroid hormones readily pass the glomerulus, but the kidney clears sulfates much more slowly (Kellie and Smith, 1957).

Although testosterone is conjugated to glucuronic acid by the liver, the action of hepatic reductases, dehydrogenases and hydroxylases cannot be ignored. The activity of these enzymes is extremely important since the ability of the liver to form conjugates is apparently dependent on the steroid structure of the glucuronic acid acceptor (Rao and Taylor, 1965).

Qualitative and quantitative aspects of androgen metabolism have

been shown to depend on genotypic sex (Schrieffers, 1967). Forchielli and Dorfman (1956) found that the microsomal 5α-reductase of rat liver homogenates was higher in females than males. The administration of estradiol benzoate and testosterone proprionate in both normal and castrate male rats, respectively, increased and decreased hepatic 5α-reductase activity (Schrieffers, 1967). Breuer, et al. (1968) found that the 5α-reductase activity present in the cytosol was partly inhibited by microsomal 5α-reductases. Although the total 5α-reductase activity depends on the substrate used, tissue preparation (slices, microsomal fraction or homogenate) and experimental conditions, it is clearly higher in the liver of female than male rats (Schriefers, 1967). Hydroxylation of steroids by the liver, particularly 3α-hydroxysteroid dehydrogenase, was much higher in male than female rats (Oto, et al., 1972). In addition, Baulieu and Mauvais-Jarvis (1964) have found that androgen metabolism in the liver of male rats proceeds via the 17β-hydroxy pathway and the 17-keto pathway predominates in the liver of female animals. Another sex-dependent enzyme system involves the formation of steroid glucuronides which was higher in male than female rats because of the yet unexplained higher formation of glucuronic acid in males (Rao and Taylor, 1965).

Thus, hepatic androgen metabolism is mainly reductive in females and oxidative in males with hydroxylation about 40 times higher in liver from males than females. Castration or estradiol-treatment in male rats have a tendency to feminize their metabolisms, whereas androgen treatment causes a masculinization of metabolism.

Besides sex-dependent changes in enzyme activities from livers of adult animals, a number of investigations have found age-dependent changes in enzyme activity related to the presence or absence of

testosterone during pre- or post-natal development. The ability of androgens to permanently direct or program the subsequent capacity of the liver to metabolize steroids has been reviewed by Einarsson, et al. (1973). Einarsson has recognized the following categories of enzymes: (1) enzymes with a basal level regulated by nongonadal factors yet reversibly inducible by androgens including 2β-, 6β-, and 18-hydroxylases and 5β-reductase; (2) enzyme systems reversibly imprinted by androgens neonatally and reversibly stimulated by androgens post-pubertally, including 2α-hydroxylase, the 3β- and 17β-hydroxysteroid dehydrogenases, and 5β-reductase; and (3) enzyme systems primarily regulated by nongonadal factors and only slightly affected by androgens including 7α- and 16α-hydroxylases. These systems imprinted at birth required increasing amounts of testosterone proprionate and longer times to be imprinted as the category number increased (Gustafsson and Stenberg, 1974).

Thus, exposure to testosterone in neonatal life opens the possibility for permanent activation or depression of certain enzyme systems in the liver at puberty. In marked contrast, the effect of exogenous testosterone on the activity of these hepatic enzyme systems in adulthood should be temporary since the process of hepatic differentiation has been completed (Denuf and DeMoor, 1968).

The above discussion demonstrates that the liver is concerned primarily with the elimination of circulating steroids. If active steroids remained in the circulation for long periods of time, their circulating concentrations could not influence the moment-to-moment control of pituitary LH and FSH secretion. However, through the action of the liver, active steroids have a relatively short half-life and rapid turnover rate, and thus testosterone or its metabolites can exert a high degree of

control over gonadotropin secretion.

Brain and Anterior Pituitary

The relationship between the anterior pituitary gland and the gonads has been investigated intensively since 1917 when Addison showed that basophilic cells in the pituitary gland increased in size and number with formation of granules in response to castration. By 1927, Smith demonstrated that hypophysectomy caused testicular atrophy and that hypohysectomized males receiving pituitary transplants exhibited a striking increase in testis weight as well as the ability to mate and to sire normal litters. These and other investigations permitted Moore and Price (1932) to propose the concept that the gonads and the pituitary gland play upon each other toward the achievement of a balance of function. This hypophyseal-gonadal relationship has been referred to as "negative feedback" or "push-pull".

Soon after the demonstration of this reciprocal control of the pituitary-testicular axis, the first successful attempt to activate the release of pituitary ovulating hormone by applying electrical stimuli to the brain was made by Marshall and Verney (1936). They applied a 50-cycle, 30 volt shock across the rabbit head and noted ruptured follicles 24 hr later. Subsequently, a variety of experimental techniques and end-points established the role of specific hypothalamic nuclei in the regulation of the synthesis and release of gonadotropic hormones from the anterior pituitary. For example, electrical stimulation of the hypothalamic tuber and preoptic region provoked ovulation in rabbits (Harris, 1937). In addition, bilateral lesions immediately anterior and ventral to the paraventricular nucleus enhanced gonadotropin release in rats

(Hillarp, 1949). Subsequently, the dependence of the pituitary gland on the medial basal hypothalamus was shown by grafting pituitary glands in hypophysectomized animals (Harris and Jacobsohn, 1952). Grafts placed under the median eminence, under the temporal lobe, or in the empty pituitary capsule became well vacularized, but only grafts under the median eminence acquired vascular connections with hypophyseal portal vessels. Importantly, only the animals with grafts under the median eminence exhibited normal pituitary histology and gonadotropin secretion as judged by regular estrous cycles, pregnancy, and the production of live young. Harris and Jacobsohn concluded that the secretion of anterior pituitary hormones was under hypothalamic control, mediated by the hypophyseal portal circulation.

Halasz, _et al._ (1965) termed the region in which grafts showed normal histology the "hypophysiotrophic area" of the hypothalamus. This area includes the entire arcuate nucleus, the ventral part of the anterior periventricular nucleus, and the medial part of the retrochiasmatic area. Halasz, _et al._ concluded that neurons throughout the hypophysiotrophic area contain substances necessary for the secretion of gonadotropins, although these substances are normally transported to the median eminence and released into the hypophyseal portal circulation.

Using a different technique, Lisk (1962) implanted crystalline steroids packed into barrels of hypodermic tubing and found that testosterone-filled implants in the arcuate nucleus of male rats led to a decrease in gonadal and accessory sex organ weight. The importance of the median eminence region in the male rat was demonstrated by using implants of cyproterone, an antiandrogen (Bloch and Davidson, 1967). This synthetic steroid blocks the retention of testosterone in androgen target

cells (Fang, *et al.*, 1969). Based on the assumption that this was the principal action of cyprotereone in the brain, Bloch and Davidson reasoned that intracerebral cyproterone implants would decrease androgen accumulation in the hypophysiotropic region and thus stimulate pituitary gonadotropin release. They found that implants of cyproterone in the median eminence of immature male rats resulted in hypertrophy of the testes, seminal vesicles and prostate when compared to control rats implanted with cholesterol.

In contrast to the above findings with hypothalamic steroid implants Bogdanove (1963) was able to inhibit castration cell formation by placing estrogen pellets in the pituitary gland of an ovariectomized female rat. The effects of pituitary estradiol implants were restricted to tissue around the pellet and the size of the affected area depended upon the amount of estrogen implanted. Bogdanove concluded that steroids from small implants in the pituitary would not reach a sufficient number of gonadotropin cells to affect significantly the function of the entire gland. However, implants placed in the median eminence are directly above the pituitary portal circulation and might diffuse into the portal circulation, thus perfusing the entire pituitary with steroids. Thus, implants in the hypothalamus may operate by release of the hormone into the hypophyseal portal vessels thereby acting at the pituitary level rather than the hypothalamic level.

Smith and Davidson (1967) apparently solved the controversy of the inhibitory site of action for gonadal steroids by using animals with transplanted pituitaries. When they transplanted four pituitary glands to the kidney capsule of a male rat and several weeks later removed the animal's own pituitary, they found that in a small percentage of cases

the animals showed testicular maintenance indicating that some releasing factor reached the transplanted pituitary. In these animals, testosterone implants in the median eminence region resulted in a decrease of testicular weight, number of mature spermatozoa, and diameter of the seminiferous tubules. Smith and Davidson maintained that, in the case of testosterone, a direct effect of the hormone on the hypothalamus had been demonstrated.

The controversy continued as Kingsley and Bogdanove (1973) used unilateral intrapituitary implants of micropellets containing testosterone propionate, dihydrotestosterone benzoate, and a synthetic androgen 7α-methyl-19-nortestosterone acetate in castrated males to show a reverse of castration cell development and an increased intrapituitary FSH:LH ratio (an action of systemically administered androgens). The unilateral implants did not affect the entire pituitary. Thus, Kingsley and Bogdanove concluded that their results showed a direct action of androgens on the pituitary, but they also stated that the question of where "physiological" amounts of gonadal steroids act to influence the gonadotrophic activity of the hypothalamic-pituitary unit remains unanswered.

Since testosterone is the principal circulating androgen in males (Hall, 1970), evidence demonstrating that testosterone could be concentrated by cells in the brain and pituitary would provide evidence that gonadal steroids act at both sites. Dry-mount autoradiographs revealed that radioactive testosterone was selectively concentrated and retained in the hypothalamus, other limbic structures, and in the pituitary (Phaff, 1968). Similarly, Sar and Stumpf (1973) found reduced silver grains, attributed to the uptake of radiolabeled testosterone over cell bodies of neurons in the preoptic area, arcuate and ventromedial nucleus,

the hippocampus, the amygdala as well as cells in the anterior pituitary gland. As a result of these studies, the consensus emerged that the brain and pituitary gland were able to concentrate radioactivity following the injection of radiolabeled testosterone, but autoradiographic procedures do not permit the identification of localized radioactive molecules. An additional criticism of some studies utilizing autoradiography is the possibility of diffusion of radioactivity during fixation thus giving false localization of radioactivity. Therefore, following extraction and scintillation counting, radioactivity in the pituitary was found to be higher following estradiol injection than when testosterone was injected (Tveter, 1970). This demonstrates a differential uptake of these two steroids in the pituitary. Similar techniques were used to investigate the factors influencing testosterone uptake by a number of brain regions (McEwen, *et al.*, 1970a, 1970b). The highest uptake was recorded in the pituitary gland followed by the hypothalamus, preoptic area, and septum. Hypophysectomy and cyproterone decreased, and castration increased uptake in the brain. More recently, Noess and Attramadal (1974) found radioactivity bound to the macromolecules in the pituitary, hypothalamus, preoptic area, and the cerebral cortex after IM testosterone injection. All of the above authors did not characterize the radioactivity in any of the tissues.

The demonstration that the conversion of testosterone to dihydrotestosterone (DHT) in the prostate as a necessary step in the action of testosterone (Bruchovsky and Wilson, 1968) caused a number of investigators to hypothesize testosterone metabolism could be a prerequisite for androgen action in regulating gonadotropin secretion by the pituitary gland. For example, Sholiton and Werk (1969) used *in vitro* incubations

of whole rat brains with [^{14}C] testosterone to show metabolism to androstenedione, DHT, and a small amount of 5α- and 5β-androsterone. Similarly, in a more definitive study, Jaffe (1969) found that minces of rat pituitary, hypothalamus, and cerebral cortex were capable of converting [7-^{3}H] testosterone to androstenedione and DHT. The greatest conversion of testosterone to androstenedione and DHT, per mg of tissue, occurred in the pituitary. Jaffe concluded that pituitary luteinizing hormone secretion may be regulated by DHT acting alone or in combination with testosterone.

Another *in vitro* investigation studied the localization of 5α-reductase, 3α- and 3β-hydroxysteroid dehydrogenases in the brain and pituitary of the male rat (Rommerts and van der Molen, 1971). They found that the hydroxysteroid dehydrogenases were present in the soluble fraction while the 5α-reductase activity was localized in the microsomal fraction. The 5α-reductase activity, relative to the whole brain, was highest in the cerebellum followed by the hypothalamus and then the pituitary. The function of this apparently higher 5α-reductase activity in the cerebellum was not determined. A study of some of the hormonal factors influencing the conversion of [^{14}C] testosterone to DHT showed that testosterone decreased and castration increased the 5α-reductase activity in the pituitary glands of rats (Massa, *et al.*, 1972). This report also implicated a role for FSH in modulating 5α-reductase activity but failed to show any effect of LH and ACTH. The conclusion that emerged from these studies is that the conversion of testosterone to DHT was probably necessary for initiating androgen-induced feedback in the pituitary gland. Jouan, *et al.* (1973) studied the association of previously injected [^{3}H] testosterone to macromolecules in nuclei isolated

from rat pituitary gland. Characterization of the radioactivity revealed that testosterone (66%) and a small amount of DHT (7%) were associated with the macromolecules.

Another hypothesis explaining how androgens may influence the central nervous system is aromatization of androgens to estrogens. Androstenedione is readily aromatized to estrone by homogenates of anterior hypothalamus but not posterior hypothalamus, pituitary gland or cerebral cortex (Naftolin, et al., 1972). In addition evidence has been presented to show that the hypothalamus of males can aromatize twice as much androstenedione to estrone than that of females (Reddy, et al., 1974). Despite the evidence for aromatization developed in vitro, injection of dihydrotestosterone (a reduced ring A compound which cannot be converted to estradiol (Ito and Horton, 1971) decreased LH in castrate male rats (Swerdloff, et al., 1972). In view of the capacity of DHT to suppress pituitary LH secretion, aromatization of androgens to estrogens may not be the mechanism regulating gonadotropin secretion in males. However, comparisons of the efficacy of testosterone, DHT and estradiol to suppress pituitary LH release in castrate male rats revealed that estradiol was the most and testosterone the least potent of the three steroids tested (Verjans, et al., 1974). Estradiol was more than 60 times and DHT 4 times as potent as testosterone in inhibiting gonadotropin secretion in this model system.

In summary, the above review points to the need to understand: (1) the site or sites (in the hypothalamus or pituitary gland or both) androgens act to inhibit gonadotropin secretion, and (2) in what form androgens act in these tissues. These two problems need to be resolved in order to understand the mechanism by which circulating testosterone

regulates pituitary gonadotropin secretion.

Ventral Prostate

Greer (1959) injected 100 μg of [4-^{14}C] testosterone and found a significant accumulation of radioactivity in the ventral prostate and seminal vesicles of male rats. The accessory sex glands retained appreciably more radioactivity than muscle, adrenal or salivary tissue, indicating a selective retention of androgen by these target tissues. In addition, Harding and Samuels (1962) studied the uptake and intracellular distribution of radioactivity in various tissues after injecting [4-^{14}C] testosterone IP into rats. The total radioactivity present in the ventral prostate was only slightly higher than that in blood, but the concentration of radioactivity present in chloroform extracts of the prostate contained the majority of the radioactivity which was identified as androstenedione. These authors also found that blood and liver contained a large amount of conjugated metabolites which were absent from the ventral prostate, suggesting that the prostate selectively accumulated free but not conjugated metabolites of testosterone.

In 1968, Bruchovsky and Wilson described the metabolism of [1,2-^{3}H] testosterone following intravenous injection to normal and functionally hepatectomized male rats. These investigators obtained evidence, in both types of animals, that testosterone was taken up by the prostate within 1 min after its administration and that at least 90% was converted to 3α-androstanediol, 5α-dihydrotestosterone (DHT) and androsterone. However, only tritiated testosterone and DHT were recovered from prostatic nuclei 2 hr after testosterone injection. Moreover, the conversion of testosterone to DHT in various tissues of 11 species has been studied and the

highest rate of formation was found in the rat prostate (Gloyna and Wilson, 1969). These authors concluded that the formation of DHT is not an obligatory reaction for all of the effects of testosterone but is probably responsible for its cell proliferative effects in the prostate.

Dihydrotestosterone, androstenedione and 6β-hydroxytestosterone were present in prostatic tissue incubated with ^{3}H-testosterone, demonstrating the presence of an oxidative pathway (Plasse, et al., 1971). Comparison of the extent to which the prostate gland of castrate rats metabolized seven different androgens in vitro provided evidence for Δ^{4}-5α-reductase, 3α-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase in prostatic tissue (Bruckovsky, 1971). Similarly, incubation of testosterone in organ cultures of prostatic tissue permitted the isolation of DHT, both 3α- and 3β-androstanediol and androsterone (Robel, 1971). No DHT was found after incubation of 3β-androstanediol, suggesting the irreversibility of 3β-hydroxysteroid dehydrogenase. Robel's studies suggested the presence of two categories of testosterone metabolities: DHT and 3α-androstanediol being representative of one series, and 3β-androstanediol typical of the second category. He concluded that these categories sustained the hypothesis that testosterone action is mediated by two kinds of metabolites, DHT controlling tissue growth and 3β-androstanediol controlling secretion.

Studies on the retention of ^{3}H-DHT by a protein in prostate cell nuclei demonstrated that if prostate cell nuclei were first isolated from castrated rats and then incubated with testosterone or DHT, there was no retention of DHT in a protein-bound form (Fang, et al., 1969). However, upon addition of a cytosol fraction, the nuclear fraction was found to retain DHT. Along similar lines, Fang and Liao (1971) described two

cytoplasmic proteins capable of binding DHT, but only one of these DHT-protein complexes was accepted by the nucleus. Thus, Liao and his associates, as well as numerous other investigators, proposed a scheme for the action of androgens on the prostate. In this scheme, testosterone or other androgens are accumulated by the prostate, converted to DHT which in turn is bound to one or more cytoplasmic proteins. The DHT-protein complex is then available to bind to the nucleus and thus initiate part of the androgen action on the prostate.

The prostate gland was included in the present investigation because testosterone metabolism has been studied extensively in this tissue and it was believed that the prostate gland could serve as a model for studies aimed at elucidating the uptake, retention and metabolism of testosterone following *in vivo* infusion of [^{3}H] testosterone.

Testis

The rat testis metabolizes testosterone *in vitro* to 5α-androstane-3α, 17β-diol; 5β-androstane-3α, 17β-diol; 5α-androstane-3β, 17β-diol; 16α-hydroxytestosterone; Δ^{4}, 16-androstadiene-3-one, and Δ^{16}-androsten-3β-ol (Stylianou, *et al.*, 1961). Detection of these metabolites suggests the presence of 5α- and 5β-reductase, and 16α-hydroxylase. Moreover, Nayfeh and Baggett (1966) found considerable (8-40%) conversion of ^{14}C progesterone to 3α-androstanediol implying that testicular tissue can synthesize testosterone from progesterone and then metabolize this androgen to 5α- reduced products.

To assess the localization of testosterone metabolites within the testis, incubation of isolated segments of interstitial tissue and seminiferous tubules with [^{14}C] testosterone demonstrated that the tubules

formed 3α-androstanediol with a small amount of dihydrotestosterone (DHT) and androstenedione. On the other hand, androstenedione was the predominant metabolite in the interstitial tissue (Rivarola and Podesta, 1972). The physiological role for the conversion of testosterone to androstenedione in the interstitial tissue was not known. Similar findings were reported by Dorrington and Fritz (1973) with 3α-androstanediol being the major metabolite of [^{14}C] testosterone in the seminiferous tubules and DHT the major metabolite in a cellular suspension of spermatocytes. The implication inherent in these results is that spermatocytes may resemble other androgen responsive tissues from the standpoint of metabolizing testosterone to one or more 5α-reduced products.

In an extensive study of androgen metabolism in the rat testis, Sowell, *et al*. (1974) used both *in vivo* and *in vitro* techniques. Following the subcutaneous injection of 100 μCi of [^{3}H] testosterone, the concentration of radiolabeled testosterone was always higher in blood than testis whereas [^{3}H] DHT and [^{3}H] 3α-androstanediol concentrations were always higher in the testis than blood. In addition, testes of immature animals formed significantly more 5α-reduced androgens including 3α- and 3β-androstanediol and androsterone than testicular tissue from mature animals after *in vitro* incubation with [^{3}H] testosterone. Localization of the hydroxysteroid dehydrogenases was shown when incubation of [^{3}H] DHT with seminiferous tubules produced primarily 3α-androstanediol while interstitial tissue produced 3β-androstanediol. When [^{3}H] 3α-androstanediol was incubated with either seminiferous tubules or interstitial tissue the principal metabolite was androsterone, indicating that 17β-hydroxysteroid dehydrogenase was present in both testicular compartments. Evidence indicating that testosterone was converted to DHT

by seminiferous tubules was confirmed, and extended to show that the quantities of DHT and 3α-androstanediol produced by the seminiferous tubules increase in proportion to the concentration of testosterone in the incubation media (Lloret and Weisz, 1974). This result suggests that the intratubular concentration of testosterone may play a role in modulating the intratubular level of DHT.

Recently, Dorrington and Fritz (1975) have localized two of these enzymes to two cellular preparations from the testis. The spermatocyte-enriched preparations contain 5α-reductase while the Sertoli cell-enriched preparations contain some 5α-reductase and a high level of 3α-hydroxysteroid dehydrogenase. Testosterone conversion to 5α-reduced metabolites in the testis may be of paramount importance since receptors for androgens (androgen binding protein and a "cytoplasmic receptor") have been postulated to be required for the mediation of the androgen stimulus to the germ cells (Hansson, *et al.*, 1974; Means, *et al.*, 1975).

Aromatization of androgens to estrogens may be an important mechanism regulating testis function since estradiol was shown to be produced in seminiferous tubules and localized in the interstitial tissue (deJong, *et al.*, 1974). Confirmation of this localization and the demonstration that estradiol binds to a so called "receptor" in interstitial tissue implies that the cells of the seminiferous tubules might communicate with interstitial cells by secreting estradiol and thus provide a regulatory signal for interstitial cell function.

The review of testicular testosterone metabolism illustrates two important reasons for assessing in the present study the extent to which the testis metabolizes testosterone. First, the *in vitro* data demonstrates that the testis contains 5α-reductase and aromatizing enzymes

that can lead to the production of two metabolites, DHT and estradiol, respectively, that are believed to play regulatory roles in sperm production. Secondly, since testosterone increased DHT and 3α-androstanediol formation *in vitro*, the effect of testosterone-filled Silastic implants on testosterone metabolism needs to be studied following the *in vivo* administration of [^{3}H] testosterone because of the effects this treatment can have on the testis.

Steroid Diffusion Through Silicone Rubber

Dzuik and Cook (1966) were the first to demonstrate that hormonal steroids diffuse through polydimethylsiloxane (Silastic) capsules at relatively constant rates. Since their initial observation numerous steroids, including testosterone, have been shown to pass through silastic tubes either *in vivo* or *in vitro* at a fairly constant rate for extended periods of time (Kincl, *et al.*, 1968; Moon and Bunge, 1968; Reddy and Piasal, 1973; Berndtson, *et al.*, 1974).

Kincl, *et al.* (1968) proposed that steroid diffusion through Silastic capsules was inversely proportional to wall thickness and directly proportional to the surface area of the implant. Comparisons of the efficacy of administering hormonal steroids via subcutaneous injection, oral administration, and subdermal Silastic implants revealed that 6 to 25 times less megestrol acetate was needed to produce comparable biological effects when administered via a Silastic implant (Chang and Kincl, 1968). A subsequent investigation showed testosterone-filled Silastic capsules were 15 to 30 times more effective than a daily injection of an oily solution of testosterone (Chang and Kincl, 1970). Enough testosterone was released through the wall of the Silastic capsules

during a 16 wk period to maintain the accessory sex glands of mature castrate rats (Moon and Bunge, 1968). More recently, Reddy and Prasal (1973) found a significant decrease in testicular weight and a slight increase in seminal vesicle and prostate weight when male rats received 130 μg testosterone per day via a subdermal Silastic capsule.

Comparison of the capacity of various androgens to inhibit or maintain spermatogenesis in rats established that a 200 mm^2 testosterone-filled Silastic capsule decreased testicular weight by 40 to 50% during a 56 day interval (Berndtson, et al., 1974). The decline in testis weight was accompanied by an eight-fold drop in serum LH and FSH concentrations even though plasma testosterone titers and the dry weight of the accessory sex glands were not affected by the testosterone released from 200 mm^2 capsules. Two possible mechanisms were suggested to explain how apparently normal blood levels of testosterone could inhibit pituitary gonadotropin release. First, androgens released from Silastic capsules could be converted to another hormonal steroid which inhibits gonadotropin secretion. Secondly, episodic increase in testosterone secretion may have been attenuated and replaced by the steady release of testosterone from the Silastic capsule which could be more efficient for inhibiting gonadotropin release than the normal fluctuating plasma testosterone levels.

Thus, testosterone is released from Silastic capsules at a constant rate, and a 200 mm^2 implant is capable of maintaining testosterone titers near normal yet cause a decrease in testis weights and serum LH and FSH levels. Although a variety of studies are still needed to establish the efficacy and safety of the use of Silastic capsules in man, this approach shows sufficient promise as a possible male contraceptive to warrant

investigation into the metabolism of testosterone in the testosterone treated rat.

Steady State and Infusion Techniques

Radioactive molecules are assumed to behave chemically and physiologically exactly like their natural counterparts except for slight effects due to differences in mass. When radioactive molecules are administered to animals the mass of the radioactive molecule can be very small in comparison with the mass of the endogenous molecule. As a result radioactive materials have several potential uses in the intact animal. One of these is to identify metabolic pathways by which a radioactive precursor is converted to a radioactive product. In such experiments, the animal body may be viewed as an assortment of pools or compartments, defined by both the species (compound, ion, etc.) and the space in which it is distributed. Thus, two different compounds distributed in the same space or one compound distributed in two spaces are considered to be two different pools (Gurpide, *et al.*, 1963).

One method of introducing a radioactive molecule into an animal is via constant intravenous infusion. Immediately after starting the infusion of a tracer, the concentration of the infused hormone in the blood increases until a steady state is reached. As the specific activity of the infused material increases, the amount of radioactivity removed from the circulation also increases since the total rate of removal of the compound is assumed to be constant. After some length of infusion, the rate of exit of the labeled compound approximately equals its rate of entry into the circulation, and an isotopic steady state is established (Gurpide, 1971). Thus, by studying the distribution of tritiated

testosterone and its labeled metabolites while the animal is in an isotopic steady state, the results obtained will more closely mimic the metabolism of endogenous testosterone.

Several assumptions underly a steady-state condition: (1) stable incorporation of isotopic atoms in the tracer; (2) complete mixing of labeled and unlabeled material in each metabolic pool, and that this mixing is rapid in comparison with the rates of transfer of material between the pools; (3) the negligible amount of labeled material added to any pool in comparison to the size of the compartment; (4) all rates of metabolism and production of a hormone remain constant during the experiment; (5) no changes in the concentration of the compound either in the blood or in the tissues; and (6) identical metabolism of the administered tracer and endogenously produced compound (Gurpide, et al., 1963; Gurpide, 1971).

With these assumptions in mind, the infusion technique still has the advantages of: (1) establishing equilibrium between all metabolites; (2) giving a precise estimate of the tissue hormonal pool; (3) maintaining the relative concentrations of metabolites within the tissue; and (4) not changing the relative level of accumulation from one tissue to another as occurs following a single injection of tracer (Tait and Horton, 1966; Gurpide, 1971).

Infusion and steady-state kinetics have been primarily used to assess the rate of conversion of one compound to another, estimate metabolic clearance rate, and establish hormone production and secretion rates. Since the use of a constant infusion technique has been relatively ignored in investigations of testosterone metabolism, one of the objectives of the present study was to assess the validity of using

continuous infusion to detect changes in testosterone metabolism in animals exposed to different androgen levels.

Summary

The preceding review has demonstrated that testosterone diffuses through subdermal Silastic capsules at predictable rates and illustrates the advantages and potential usefulness of the constant infusion technique for studying androgen metabolism *in vivo*. In addition, the extent to which testosterone is accumulated and retained by a variety of tissues (brain, pituitary, testis, liver and prostate) has been discussed. Comparison of the uptake and retention of testosterone illustrates that (1) hepatic testosterone metabolism is primarily reductive and oxidative and may be easily modified by the hormonal mileau; (2) the prostate converts testosterone to dihydrotestosterone which is responsible for the transcriptional and translational action of androgens in this tissue; (3) the testis metabolizes testosterone to 5 -reduced products and estrogens which may be involved in intratesticular regulation of spermatogenesis; and (4) the brain and pituitary may metabolize testosterone to affect regulation of gonadotropin secretion.

To gain a better understanding of the relationship between circulating testosterone levels and the regulation of gonadotropin secretion, the following must be determined: (1) testosterone release rate from Silastic capsules and its effect on seminal vesicle weight; (2) the validity of using continuous infusion to assess $[^3H]$ testosterone retention and metabolism and (3) where and in what form testosterone acts in the brain and pituitary gland to control gonadotropin secretion.

CHAPTER III

MATERIALS AND METHODS

Materials

Animals

Male albino rats (300 to 400 g and 2 1/2 to 3 1/2 months old) were used in this study. They were raised in the laboratory from animals originally obtained from the Holtzman Co., Madison, Wisconsin.

Silicone Rubber

Polydimethylsiloxane capsules were prepared from Dow-Corning medical grade Silastic tubing (cat. no. 602-305) (0.6 mm wall thickness, 1.98 mm i.d., 3.18 mm o.d.; 1 mm long = 10 mm^2) and sealed with Dow-Corning Medical Adhesive Silicone type A.

Infusion Materials

Tubing. Clay Adams (New York, N.Y.) Intramedic Polyethylene tubing PE 20 (0.381 mm i.d. and 1.09 mm o.d.) and PE 100 (0.864 mm i.d. and 1.52 mm o.d.) were used in preparing the indwelling catheter and connecting tubing.

Pump. An Infusion-Withdrawal Pump, Model 600-900 (Harvard Apparatus Co., Millis, Mass.) was used to infuse radioisotope at a constant rate.

Restraining Cage. A restraining cage for the rats was constructed from two plastic jars with the bottoms cut out. One was large-mouthed (tail-end) with an opening cut in the cap for the rat's tail. The other plastic jar was small-mouthed (head-end) and uncapped for the animal to breathe. The two jars were of slightly different diameters, allowing the open bottom of one jar to slide inside the other, thus permitting the cage to be adjustable to the length of the animal. The plastic jars were held in place around the animal by tape on the outside.

Nonradioactive Steroids

Testosterone (see Appendix A for abbreviations and systematic names), estrone, estradiol, androstenedione, epitestosterone, and 5β-androstanediol were purchased from Steroloids, Inc., Pawling, N.Y. 5α- and 5β-dihydrotestosterone, androsterone, 5α- and 5β-androstanedione, 3α- and 3β-androstanediol, 5α- and 5β-epiandrosterone were obtained from Sigma Chemical Co., St. Louis, Missouri.

Steroid Derivatives

Acetates. Testosterone acetate, 5α-dihydrotestosterone acetate, estrone acetate, estradiol acetate, androsterone acetate, and 3α-androstanediol acetate were purchased from Steraloids, Inc., Pawling, N.Y. and stored at 5 C at a concentration of 1 mg/ml in benzene:ethanol (9:1).

Methoximes. Methoxime derivatives of androstenedione and 5α-androstanedione were prepared in the laboratory (see Methods).

Derivatization Compounds

Pyridine (Matheson, Coleman and Bell, Baton Rouge, La.), acetic anhydride (Fisher Scientific Company, Fairlawn, N.J.) and methoxyamine hydrochloride (Eastman Kodak Co., Rochester, N.Y.) were stored at room temperature in a desiccator over calcium chloride.

Scintillation Counting Material

Scintillation counting was performed in 12 ml of toluene (Beckman) Instruments, Inc.) based scintillation fluid containing 0.004% p-bis-2-(5 phenyl-orazoyl) benzene (POPOP) and 0.4% 2,5-diphenyloxazole (POP) (New England Nuclear, Boston, Mass.). The mixture was allowed to equilibrate at least 24 hr before use and stored in the dark.

Reagents

The nanograde solvents (Mallinckrodt Chemical Works, St. Louis, Mo.) included acetone, benzene, chloroform, ether, ethyl acetate, hexane and methanol. Methylene chloride was purchased from Burdick and Jackson Laboratories (Muskegon, Mich.) and ethanol was obtained from Commerical Solvents Corp. (Harvey, La.). All solvents were fractionally distilled through a column packed with glass helices (1/4 in i.d., Scientific Glass Apparatus Co., Bloomfield, N.J.) just prior to use. Methanol was fractionally distilled over 2,4 dinitrophenylhydrazine and sulfuric acid (Haltmeyer and Eik-Nes, 1972). The methanol distillate was stored under N_2 at 4 C and fractionally redistilled prior to use. Water used in the procedures was distilled and filtered through two filters (Continental Water Supply, Austin, Texas). The filters included an activated charcoal filter to remove organic material and a combination anionic-cationic

resin filter to remove minerals and ions.

Glassware

Immediately after use, glassware was rinsed, soaked in detergent for 24 hr, and placed in dichromate-sulfuric acid solution overnight. Following ten tap water rinses, the glassware was soaked in detergent, rinsed again with tap water and placed in dilute hydrochloric acid. The 24 hr acid bath was followed with rinses as follows: ten times with tap water, ten times with deionized water and two times with fractionally distilled methanol. The glassware was subsequently air dried at room temperature.

Chromatographic Materials

Adsorbents. Silica gel (Silica TLC-7GF, Mallinckrodt) plates (20 cm x 20 cm) were poured (0.25 mm thick) from a slurry of 24 g silica gel in 64 ml water. Aluminum oxide (Merck 1101, neutral type T) plates (20 cm x 20 cm) were prepared (0.25 mm thick) from a slurry of 60 g aluminum oxide in 84 ml water.

Development. Thin layer plates were developed in rectangular tanks lined with Whatman 3M (W. R. Balston, Ltd., England) filter paper. Solvents were allowed to equilibrate for at least 1 hr.

Visualization Spray. Primulin (Pfaltz and Bauer, Inc., Flushing, N.Y.) at 0.002% in acetone:water (4:1) was used to detect reference steroids.

Radioactive Isotopes

[1,2,6,7-^{3}H] testosterone (85-100 Ci/mM), [7-^{3}H] testosterone (25 Ci/mM), [4-^{14}C] testosterone (57.5 mCi/mM), [1,2-^{3}H] dihydrostestosterone (40 Ci/mM), [4-^{14}C] dihydrostestosterone (50.6 mCi/mM), [2,4,6,7-^{3}H] estradiol-17β (52 mCi/mM), [2,4,6,7-^{3}H] estrone (106 Ci/mM), [4-^{14}C] estrone (58.2 mCi/mM), [1,2-^{3}H]-androsterone (40 Ci/mM), [1,2-^{3}H]5α-androstan-3α,17β-diol (44 Ci/mM), and [4-^{14}C] androstenedione (50 mCi/mM) were purchased from New England Nuclear, Boston, Mass.

Since [^{14}C]5α-androsterone and [^{14}C]5α-androstanedione were not available commercially, these compounds were synthesized from [4-^{14}C] testosterone (see Methods).

Solution for Tissue Homogenization

The solution used for homogenization of tissues was prepared by mixing 109.539 g sucrose (0.32 M), 0.2033 g $MgCl_2 \cdot 6H_2O$ (0.001 M), 0.1109 g $CaCl_2$ (0.001 M) and 0.1361 g KH_2PO_4 (0.001 M) in 1 liter of distilled water. This buffered sucrose solution was adjusted to pH 6.5 and stored at 5 C.

Methods

Animal Housing and Preparation

The rats used in this study were housed in groups of four or five per cage in an air-conditioned (22 ± 2 C) room on a 14:10 hr light:dark schedule and provided with Wayne Lab Blox (Texas Feed and Ranch Supply) and water *ad libitum*. Castration and subcutaneous implantation of testosterone-filled Silastic capsules were performed under light ether

anesthesia.

Polydimethylsiloxane Capsule Preparation and Implantation

Silastic (Dow-Corning) medical grade silicone rubber tubing was used to prepare all capsules. The tubing was rinsed with hot tap water, distilled H_2O, and air dried. The desired length of tubing (30 mm) was cut, one end sealed with approximately 3 mm of Silicone Medical Adhesive type A and allowed to cure at room temperature for 24 hr. Silastic tubing with one end sealed and cured was filled to 20 mm with chromatographically pure crystalline testosterone. The remaining end was sealed with Silicone Medical Adhesive type A and cured as previously described. The sealed ends were trimmed to 2 mm. The capsules were then rinsed in ethanol and dried at 55 C for 24 hr.

After weighing to the nearest 1/100th of a mg, the capsules were placed subcutaneously along the dorsal midline of the rats. Testosterone release rate (μg/day) was determined by subtracting post-implantation weight from pre-implantation weight of the silastic capsule.

Recrystallization of Nonradioactive Steroids

Nonradioactive steroids were recrystallized by placing 100-1000 mg of the desired steroid in a minimal volume of aqueous methanol (70%) (Axelrod, *et al.*, 1965). The tube containing the dissolved steroid was allowed to come to room temperature for 2 hr, followed by 5 hr at 5 C and then 12 hr at -20 C. The methanolic solution was removed and the tube containing the crystals was placed in a vacuum jar containing phosphorus

pentoxide and dried for 72 hr. The crystals were transferred to a screw cap vial and stored at 5 C. Standard solutions of the 15 nonradioactive steroids were made at a concentration of 1 mg/ml in either benzene:ethanol (9:1) for androgens or in ethanol for estrogens and stored at 5 C. These solutions were checked periodically for chromatographic purity using separate solvent systems described below.

Preparation of [4-^{14}C]5α-androstanedione and [4-^{14}C]-androsterone

Carbon labeled 5α-androstanedione and androsterone were synthesized by a procedure adapted from Ota, *et al.* (1972). Briefly, 0.5 g of liver from an adult female rat was homogenized in 3 ml of 0.1 M Krebs-Ringers phosphate (KRP) buffer (pH 7.4). The homogenate was incubated with [4-^{14}C] testosterone and 500 μg NADPH (dissolved in 0.5 ml 0.1 M KRP) for 60 min at 37 C in an atmosphere of 95% O_2:5% CO_2. [^{14}C]5α-androstanedione and [^{14}C] androsterone were extracted, isolated and purified by the procedures described below.

Preparation of Indwelling Catheters and Connecting Cannulas

The indwelling catheter (PE 20, 15 cm long and beveled at one end) was prepared by bending the tube around a 0.5 cm steel rod in boiling water to conform to the shape of the animal. Three cuffs (PE 100, 1 cm long and flared at both ends) were enlarged by forcing on a 19 gauge needle and dipped in boiling water. The cuffs were arranged on the catheter to facilitate anchoring the catheter to the surrounding musculature and to allow approximately 1 cm of the beveled end to be introduced

into the jugular vein. Cuffs were made stationary on the catheter by flaring slightly. The connecting tubing (PE 20, 35 cm long) was encased in a small, hollow steel spring to prevent the animal from chewing through the tubing. A different indwelling catheter and connecting tubing was used for each animal.

Surgical Procedure

Animals were anesthetized with ketamine hydrochloride (Ketalar, Parke Davis and Co., Detroit, Mich., 60 mg/kg IM) followed 5 min later by sodium pentobarbital (Nembutal, Abbot Laboratories, North Chicago, Ill., 21 mg/kg IP) (Youth, et al., 1973). The combined use of ketamine and pentobarbital sodium was found to be safe and rapid, and yet maintained a state of deep surgical anesthesia for at least 1 hr.

Following clipping of hair from the back of the neck and over the left jugular vein, the skin was incised transversely between the shoulder blades and longitudinally over the jugular vein. Approximately 1 cm of the jugular vein was exposed by blunt dissection. The salivary glands and subcutaneous tissue 1 to 2 cm cephalad on the midline were freed to allow later placement of the U-portion of the cannula. A trocar (made by cutting the hub off a 6 in, 13 gauge hypodermic needle) was passed subcutaneously, starting at the midline of the neck, posteriorly, then around the left foreleg to the back skin incision. The cannula was inserted into the trocar and the trocar removed. The cannula was attached to the connector tubing and filled with isotonic saline using a 1 ml syringe. The vein was raised slightly, cut with iris scissors, and the cannula introduced to a distance of 1 cm. The cannula was anchored to the neck muscles by suture around the cuffs and the incision closed. The back

skin incision was closed around the adaptors attaching the cannula to the connector tubing. The animal was placed in the plastic restraining cage and infused with isotonic saline (0.3 ml/hr) until recovery from anesthesia was complete (approximately 2 1/2 hr).

Infusion of Radiolabeled Testosterone

Following purification and purity verification, the isotope to be infused was diluted to a stock concentration of 1 μCi per 10 μl. Each animal was infused at a rate of 3.5 μCi/hr/100 g body weight. The correct volume of stock solution was placed in a small tube, evaporated to dryness, and resuspended in 2.5 ml of a 5% ethanol-saline solution. Two 10 μl aliquots were taken and counted after addition of scintillation fluid. The infusion rate was determined by converting cpm of this known volume to μCi/hr, assuming the pump rate was constant. The remaining infusate was transferred to a 3 ml syringe and attached to the connecting tube. An initial infusion of 0.6 ml (0.3 ml equivalent to approximately 12% of the total dose plus 0.3 ml of dead space in the connecting tube and catheter) was quickly administerd to the animal. All animals were infused at a rate of 0.5 ml/hr. The calibration and consistent rate of the Harvard Pump are shown in Table I.

Tissue Collection and Homogenization

At the end of the infusion the animal was anesthetized with ether, removed from the restraining cage, and the connecting tube removed. The chest cavity was opened and approximately 10 ml of blood was collected in heparin (1 mg) by heart puncture and stored on ice. The animal was perfused with 50 ml cold 0.85% saline via the left ventricle. The right

TABLE I

CALIBRATION OF HARVARD INFUSION PUMP (SERIES 900)[1]

Time Interval (minutes)	Radioactivity (dpm) Delivered/ Time Interval	µl/Time Interval	µl/hr
0- 19	26810	156	468
20- 39	27278	158	474
40- 59	28208	164	492
60- 79	26003	151	453
80- 99	28270	164	492
100-119	29200	170	510
120-139	28160	164	492
140-159	28535	166	498
160-179	29537	172	516
180-199	29417	171	513
200-219	29132	169	507
220-239	30118	175	525
			495±6.15[2]

[1]Calibrated by placing [1,2,6,7-^{3}H]-testosterone (17,177 dpm/100 µl 5% ethanol-saline) in 3 ml B-D Luer-Lok glass syringe and pumping through 50 cm polyethylene tubing (Intramedic Clay-Adams PE 20) into a scintillation vial.

[2]Mean ± standard error of the twelve time intervals.

atrium was cut allowing almost all blood to be drained from the animal. The prostate, seminal vesicles, brain, anterior pituitary, testes, and a portion of liver were removed and placed in a beaker of cold saline. All tissues were blotted slightly and weighed to the nearest 1/100th of a mg. A portion of the ventral prostate was separated from the other lobes and weighed. The testes were weighed, and the left testis was decapsulated and a portion of the parenchyma weighed. The other testis was placed in an oven for 48 hr at 50 C to obtain a dry weight. Dry weight of the testes (right = r and left = ℓ) was determined by the following formula:

$$\text{Dry weight of testes } (r + \ell) = \frac{\text{Dry weight of testis } (r) \times \text{Wet Weight of testes } (r + \ell)}{\text{Wet weight of testis } (r)} .$$

After the expression of fluid, both seminal vesicles were weighed, dried and reweighed. The brain was dissected according to McEwen and Pfaff (1970) to obtain samples of hypothalamus and cerebral cortex. Heparinized blood was centrifuged at 1500 rpm for 15 min at 5 C to obtain plasma.

Tissue samples were placed in ice cold glass homogenizing tubes (80 ml). Buffered sucrose solution was added to give a minimum of either 4 ml of homogenate (including a wash of the homogenizing tube) or a concentration of 50 mg/ml. Tissue homogenates and plasma were added to 80 ml extraction tubes containing each of the nonradioactive standards (50 μg), and ^{14}C radioactive standards (5000 dpm) that were isolated to minimize and correct for losses incurred in the isolation of ^{3}H metabolites of testosterone.

Extraction of Testosterone Metabolites

Plasma and tissue homogenates were extracted three times with 12 ml of dichloromethane, and the combined extracts evaporated under nitrogen at 45 C. The residue was dissolved in 15 ml of hexane and partitioned five times between 10 ml of 70% aqueous methanol. The combined dichloromethane extracts were evaporated under nitrogen and the residue concentrated to the tip of the tube with benzene:ethanol (9:1) (Table II).

Separation of Testosterone Metabolites by Thin-Layer Chromatography (TLC)

The overall scheme of the thin-layer chromatographic procedures used to separate 14 metabolites of testosterone from tissue extracts is shown in Figure 2. The residue remaining after extraction was chromatographed on silica-gel and developed twice in dichloromethane:ether (85:15). Adsorbent from all the zones shown in Figure 2 was scraped and aspirated into a disposable pipette packed with glass wool and eluted with warm benzene:ethanol (9:1). Appropriate standards were spotted on a single lane that was sprayed after chromatography with primulin and viewed under long- and short-wave ultraviolet light (UV). Characterization of the materials visualized under UV light in the five zones from the first TLC plate was as follows.

Zone 1 contained radioactivity possibly associated with 5β-androstanediol (R_F = 0.21). This zone was scraped from the plate, eluted and rechromatographed on aluminum oxide and developed twice in dichloromethane:ether (9:1) (R_F = 0.09).

Zone 2 contained radioactivity possibly associated with testosterone epitestosterone, 3α- and 3β-androstanediol. The respective R_F values

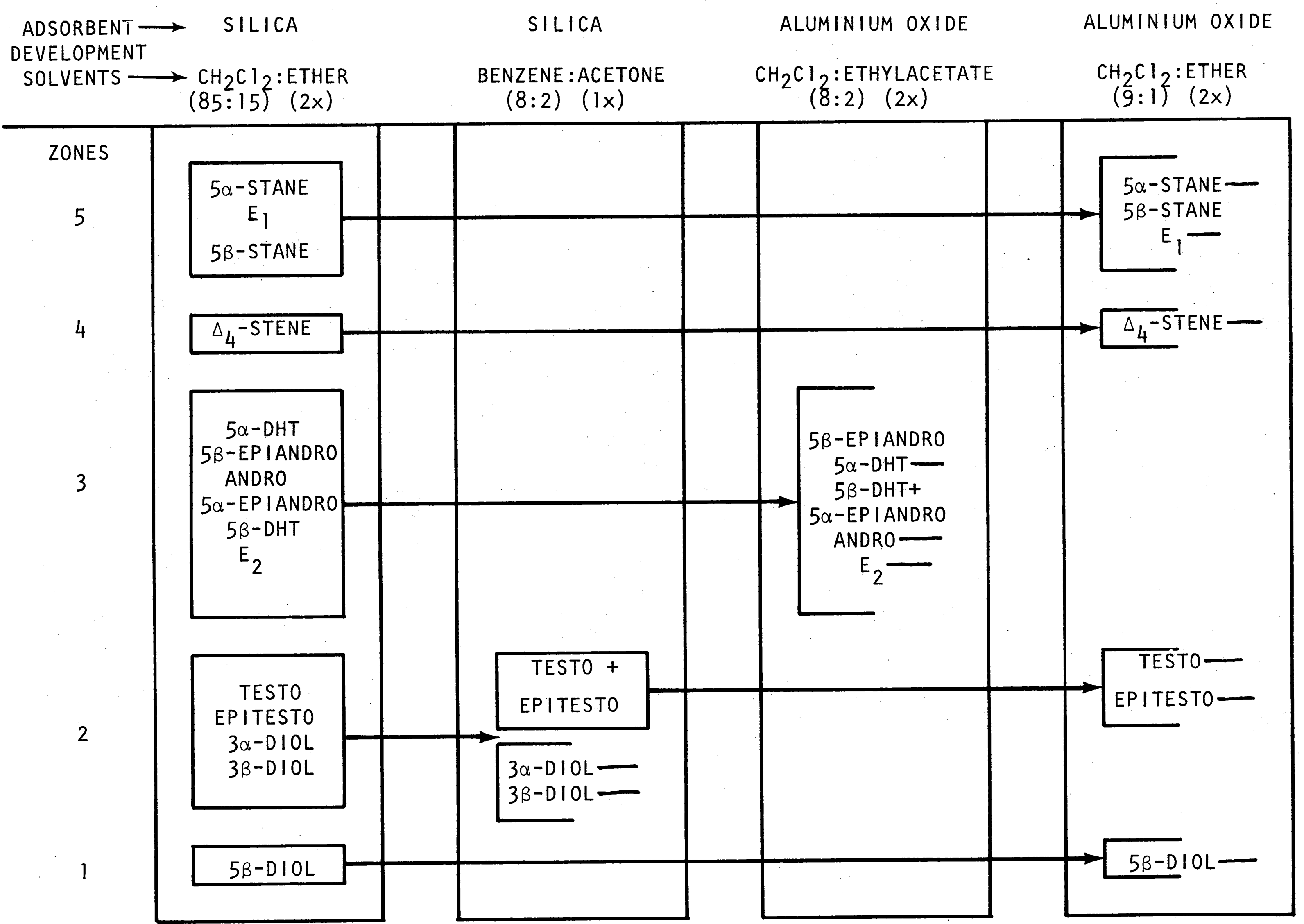
TABLE II

VERIFICATION OF PROCEDURES USED TO EXTRACT AND ISOLATE TESTOSTERONE AND SOME OF ITS METABOLITES[1]

Steroid	dpm Added	Water	Homog-enizing Solution	Prostate Homog-enate	Plasma
[1,2-^{3}H] Androstanediol	27,331	54.6 ±4.9	56.7 ±0.6	62.7 ±5.3	54.0 ±0.4
[1,2-^{3}H] Androsterone	28,998	67.2 ±1.6	60.8 ±4.7	62.7 ±1.8	60.7 ±1.1
[4-^{14}C] Androsterone	4,926	--	--	57.6 ±1.6	55.4 ±2.7
[4-^{14}C] Androstenedione	27,115	80.3 ±5.1	79.0 ±2.2	80.0 ±0.1	76.2 ±3.1
[1,2-^{3}H] Dihydrotestosterone	19,965	75.3 ±0.4	73.4 ±6.4	78.0 ±3.1	70.2 ±0.1
[4-^{14}C] Dihydrotestosterone	3,260	--	--	74.2 ±2.5	66.3 ±4.4
[2,4,6,7-^{3}H] Estradiol	28,722	67.9 ±0.2	51.4 ±4.0	68.9 ±1.2	67.9 ±1.2
[4-^{14}C] Estradiol	3,820	--	--	64.2 ±3.5	54.5 ±1.9
[2,4,6,7-^{3}H] Estrone	28,763	51.4 ±6.5	58.0 ±2.8	64.3 ±0.9	55.4 ±4.4
[4-^{14}C] Estrone	4,919	--	--	57.6 ±2.5	57.1 ±4.6
[1,2,6,7-^{3}H] Testosterone	24,529	75.9 ±4.0	73.9 ±5.3	76.3 ±3.4	75.7 ±1.9
[4-^{14}C] Testosterone	1,963	--	--	71.4 ±2.7	66.7 ±3.8

[1]Values are mean percent recovery ± standard error of five determinations.

Figure 2. Thin Layer Chromatography Systems Used to Separate Testosterone and Some of its Metabolites. (Dash following the steroid abbreviation indicates separation, for systematic name see Appendix.)


ADSORBENT
DEVELOPMENT SOLVENTS
SILICA
CH2Cl2:ETHER (85:15) (2x)
SILICA
BENZENE:ACETONE (8:2) (1x)
ALUMINIUM OXIDE
CH2Cl2:ETHYLACETATE (8:2) (2x)
ALUMINIUM OXIDE
CH2Cl2:ETHER (9:1) (2x)
ZONES
5
4
3
2
1
5α-STANE
E1
5β-STANE
Δ4-STENE
5α-DHT
5β-EPIANDRO
ANDRO
5α-EPIANDRO
5β-DHT
E2
TESTO
EPITESTO
3α-DIOL
3β-DIOL
5β-DIOL
TESTO +
EPITESTO
3α-DIOL
3β-DIOL
5β-EPIANDRO
5α-DHT
5β-DHT+
5α-EPIANDRO
ANDRO
E2
5α-STANE
5β-STANE
E1
Δ4-STENE
TESTO
EPITESTO
5β-DIOL

were 0.43, 0.40, 0.37, and 0.32. The entire zone was scraped, eluted and rechromatographed on silica gel and developed in benzene:acetone (8:2). This system allowed separation of 3β-androstanediol (R_F = 0.30), 3α-androstanediol (R_F = 0.38) and a zone containing both testosterone (R_F = 0.48) and epitestosterone. The later zone was rechromatographed on aluminum oxide and developed twice in dichloromethane:ether (9:1). Testosterone (R_F = 0.37) was adequately separated from epitestosterone (R_F = 0.20).

Zone 3 contained radioactivity possibly associated with estradiol, 5β-dihydrotestosterone, 5α-epiandrosterone, androsterone, 5β-epiandrosterone, and 5α-dihydrotestosterone. The respective R_F values were 0.56, 0.57, 0.59, 0.63, and 0.64. The entire zone was scraped, eluted and rechromatographed on aluminum oxide and developed twice in dichloromethane:ethylacetate (80:20). Five distinct zones were formed in this system. The zones were associated with estradiol (R_F = 0.29), androsterone (R_F = 0.49), both 5β-dihydrotestosterone and 5α-epiandrosterone (both R_F values were 0.56), 5α-dihydrotestosterone (R_F = 0.64 and a zone containing 5β-epiandrosterone (R_F = 0.71).

Zone 4 contained radioactivity possibly associated with androstenedione (R_F = 0.71). This zone was scraped, eluted and rechromatographed on aluminum oxide and developed twice in dichloromethane:ether (9:1) (R_F = 0.63).

Zone 5 contained radioactivity possibly associated with 5β-androstanedione, estrone and 5α-androstanedione. The respective R_F values were 0.84, 0.86, and 0.88. The entire zone was scraped, eluted and rechromatographed on aluminum oxide and developed twice in dichloromethane:ether (9:1). Three zones were evident in this system: one

associated with estrone (R_F = 0.48), another with 5β-androstanedione (R_F = 0.70) and finally one associated with 5α-androstanedione (R_F = 0.79).

Preliminary determinations showed that the majority of the unconjugated radioactivity was associated with 5β-, 3β- and 3α-androstanediol testosterone, estrone, androsterone, 5α-dihydrotestosterone, androstenedione, estrone and 5α-androstanedione. Thus only those steroids indicated in Figure 2 by a dash after the name of the steroid were examined for radioactive purity. Each steroid was eluted from the final plate and concentrated to the tip of the tube. An aliquot of each steroid from the seven tissues of one animal was pooled for proof of purity by derivative formation (Table III). The remainder was assayed for radioactivity by liquid scintillation counting.

Derivative Formation

Steroids containing hydroxyl-groups were acetylated using a mixture of pyridine:acetic anhydride (5:1) (Dominquez, _et al._, 1963). Pooled aliquots of tentatively identified steroids of radioactive (10,000 dpm) plus nonradioactive (50 μg) steroid standard were added to a 15 ml conical tube and concentrated with benzene:ethanol (9:1) under nitrogen at 45 C. Following evaporation to dryness, 0.2 ml of acetylation mixture was added to each tube. Tubes were mixed with a Vortex mixer, capped, and the reaction allowed to proceed in the dark at room temperature for 24 hr. The reaction was stopped by adding five drops of water and the mixture extracted three times with 2 ml of benzene:ethanol (9:1). The combined extracts were concentrated as described above and chromatographed on silica gel in benzene:ethylacetate (9:1) with appropriate

TABLE III

EVIDENCE FOR THE RADIOCHEMICAL PURITY OF TESTOSTERONE AND RELATED STEROID DERIVATIVES[1]

Steroid	Total dpm Chromatographed	dpm Recovered as Steroid Derivative	Percent Derivatized
[7-^{3}H] 5β-Androstanediol	4,501	4,075	96.9
[7-^{3}H] 3α-Androstanediol	15,361	15,045	97.9
[7-^{3}H] 3β-Androstanediol	12,209	11,651	95.4
[7-^{3}H] 5α-Androstanedione	694	684	98.5
[4-^{14}C] 5α-Androstanedione	2,426	2,319	95.6
[4-^{14}C] Androsterone	2,185	2,091	95.7
[7-^{3}H] Androstenedione	1,540	1,465	95.2
[4-^{14}C] Androstenedione	16,927	16,640	98.3
[4-^{14}C] Dihydrotestosterone	15,167	14,469	95.4
[4-^{14}C] Estradiol	16,928	16,115	95.2
[4-^{14}C] Estrone	12,922	12,521	96.9
[4-^{14}C] Testosterone	16,682	16,165	96.9

[1]All derivatives are acetates except androstanedione and androstenedione which are methoxamines.

acetate standards chromatographed in separate lanes. Ten milligram quantities of 5β- and 3β-androstanediol acetate standards, not commercially available, were synthesized as above. The absorbent was scraped and eluted with benzene:ethanol (9:1) into a scintillation vial. The solvent was air dried and 12 ml scintillation fluid added to determine the amount of radioactivity present. For 5 of the 10 isolated steroids a tritium labeled internal standard was used to correct for recovery during acetate formation as shown in Table IV. The rate of recovery during acetate formation for the remaining steroids was not determined.

Androstenedione and 5α-androstanedione were derivatized using methoxylamine hydrochloride (Horning, _et al._, 1968). An unknown sample or radioactive plus nonradioactive standard was placed in a tube and evaporated as above. Following the addition of 0.5 ml of 16% methoxylamine hydrochloride in pyridine, the mixture was heated at 60 C for 3 hr. The reaction was stopped by the addition of 2 ml of water, and the methoxime derivative extracted three times with 5 ml benzene:ethanol (9:1). The combined extracts were evaporated to approximately 5 ml and washed with 1 N hydrochloric acid, with distilled water, with 1% sodium bicarbonate solution, and finally with distilled water. The organic solution was dried and concentrated as above and chromatographed on silica gel in benzene:acetone (4:1) with an androstenedione methoxime or 5α-androstanedione methoxime standard (prepared in our laboratory by the above procedure) in separate lanes. Since the two isomers formed during the above reaction conditions were not separated in this chromatographic system, the methoxime derivatives migrate as a single spot. The silica gel was scraped, eluted, dried and counted as above. The purity was determined by scraping the entire lane and expressing the purity of an

TABLE IV

EVIDENCE FOR THE RADIOCHEMICAL PURITY OF TESTOSTERONE AND RELATED STEROID DERIVATIVES BY USING AN INTERNAL STANDARD

Steroid	Internal Standard	Total dpm Prior to Acetylation	Total dpm Chromatographed Excluding Internal Std.	dpm Recovered as Derivative	% Derivatized	% Recovery	Calculated dpm Steroid Prior to Acetylation
E_2		50,414	41,400	39,827	96.2	--	50,233
	E_1	20,791	--	16,487	--	79.3	
And		30,922	23,024	22,264	96.7	--	30,752
	T	3,511	--	2,532	--	72.4	
T		8,713	6,323	6,140	97.1	--	8,709
	DHT	7,807	--	5,505	--	70.5	
E_1		10,411	8,427	8,199	97.3	--	10,405
	E_2	14,396	--	11,341	--	78.8	
DHT		39,728	30,380	29,651	97.6	--	39,747
	T	3,511	--	2,619	--	74.6	

isolated compound as the percentage of dpm steroid derivative divided by the total dpm in the lane. Tables III and IV show evidence of the radiochemical purity of the acetate and methoxime derivatives.

Quench Correction and Calculations to Arrive at dpm of an Isolated Steroid

The counting efficiency of each sample was determined by using an Automatic External Standard (^{226}Ra) of a Packard Tri-Carb Liquid Scintillation Spectrometer, Model 3220.

A rectangular coordinate system was used to construct quench correction curves from a one-to-one correspondence between sets of points in a plane. Percent of counting efficiency was designated as points on the ordinate (y-axis) and counts per minute in the Automatic External Standard was designated as points on the abscissa (x-axis).

For the present study, quench correction curves for [^{3}H] and for [^{14}C] were constructed using [^{3}H] toluene and [^{14}C] toluene as sources of radioactivity. Nitromethane was used as a quenching agent, and the measurements were made with double label settings on the liquid scintillation spectrometer in a toluene based scintillation fluid.

The following materials were added to 30 scintillation vials: 0.05 ml [^{3}H] toluene to 14 vials, 0.05 ml [^{14}C] toluene to another 14 vials, nitromethane in graduated amounts (0.005, 0.01, 0.02, 0.03, 0.04 and 0.05 ml) to duplicate vials (12 vials each for [^{3}H] toluene and [^{14}C] toluene), and 12 ml toluene scintillation fluid to all vials.

Thus two vials contained only toluene scintillation fluid to provide background measurements, four vials contained no quenching agent to provide radioactivity measurement of the [^{3}H] toluene (2 vials) and [^{14}C]

toluene (2 vials) used as standards and 24 vials contained [^{3}H] toluene (12 vials) or [^{14}C] toluene (12 vials) plus graduated amounts of quenching agent. Each vial was counted three times for ten minutes each.

The density of toluene equals 0.8669 g/ml. The radioactivity of the tritium standard equalled (2.28 x 10^{6} dpm/g times 0.794 decay correction factor) 1.81032 x 10^{6} dpm/g. The 0.05 ml used in each vial represents (1.81032 x 10^{6} dpm/g times 0.043345 g/0.05 ml) 78,468 dpm/0.05 ml of tritiated toluene. The radioactivity of the [^{14}C] standard equalled 4.94 x 10^{5} dpm/g. The 0.05 ml used in each scintillation vial represents (4.94 x 10^{5} dpm/g times 0.043345 g/0.05 ml) 21,412 dpm/0.05 ml of [^{14}C] toluene.

To determine the efficiency of the counting apparatus and the dpm of ^{3}H and ^{14}C of an isolated steroid the following formulas were used:

$$^{3}\text{H efficiency in } ^{3}\text{H channel} = \frac{\text{cpm } ^{3}\text{H in } ^{3}\text{H channel}}{\text{dpm } ^{3}\text{H expected}}$$

$$^{14}\text{C efficiency in } ^{14}\text{C channel} = \frac{\text{cpm } ^{14}\text{C in } ^{14}\text{C channel}}{\text{dpm } ^{14}\text{C expected}}$$

$$^{14}\text{C dpm isolated steroid} = \frac{\text{cpm in } ^{14}\text{C channel}}{^{14}\text{C efficiency in } ^{14}\text{C channel}}$$

$$^{3}\text{H dpm isolated steroid} = \frac{\text{net cpm in } ^{3}\text{H channel} - {}^{14}\text{C dpm x } ^{14}\text{C efficiency in } ^{3}\text{H channel}}{^{3}\text{H efficiency in } ^{3}\text{H channel}}$$

Experimental Design

The present study was designed to compare the uptake, retention and metabolism of [^{3}H] testosterone in normal, castrate and testosterone

treated rats. The rationale for this study is based on the finding that testosterone treated rats (subdermal testosterone-filled Silastic capsule) are azoospermia and have suppressed pituitary LH and FSH secretion even though plasma testosterone remains within the normal range. The observation that testosterone is not elevated in rats experiencing a reduction in circulating LH and FSH titers cannot be explained on the basis of current concepts of how testosterone exerts its negative feedback on the hypothalamo-hypophyseal axis. To investigate this problem, [^{3}H] testosterone was infused under steady state conditions and some of its tritiated metabolites isolated from various tissues.

Experiment 1: Determination of Testosterone Release Rate and the Influence of Different Circulating Androgen Titers on the Testis and Seminal Vesicle Weights

The objectives of this experiment were to: (1) determine the amount of testosterone released from subdermal Silastic capsules (200 mm^2) for comparison with previously determined release rates and (2) to determine the influence testosterone treatment has on testis and seminal vesicle weights. The results were expressed as mg wet and dry testis and seminal vesicle weights ± standard error and as μg testosterone released per day ± standard error. The results were analyzed by a one-way analysis of variance (AOV) (Snedecor and Cochran, 1967). When the AOV revealed significant variation, the differences between treatment means were assessed by Duncan's New Multiple Range Test (Steel and Torrie, 1960).

Experiment 2: Determination of the Time Required to Establish Steady-State Conditions for Plasma Testosterone

This experiment was performed to determine the amount of time required for [^{3}H] testosterone in plasma to be in an isotopic steady state. This was accomplished by infusing [1,2,6,7-^{3}H] or [7-^{3}H] testosterone into four or five male rats from each treatment group (normal, castrate, and testosterone treated for 3 or 4 hr. Approximately 10 ml of blood was obtained from each animal and centrifuged. The plasma was extracted, and testosterone isolated. The rate of testosterone infusion (dpm/100 g body weight/hr) was divided by the plasma testosterone concentration (dpm/ml) to give a radiotestosterone clearance rate (RCR). A mean RCR ± standard error for each treatment group and time period was obtained. Differences between treatment groups and times were determined as in Experiment 1.

Experiment 3: Differential Metabolism of [^{3}H] and [^{14}C] Testosterone Following Constant Infusion for 3 Hours

The purpose of this experiment was to determine whether tritiated forms of testosterone ([1,2,6,7-^{3}H] and [7-^{3}H]) were metabolized differently than the carbon 14 (4-^{14}C) isotope of testosterone. This experiment was performed by infusing radioactive testosterone at two different ratios of [^{3}H] to [^{14}C] into the three treatment groups. The first experiment was carried out by infusing [1,2,6,7-^{3}H] and [4-^{14}C] testosterone at a ratio of 18 to 1 into two animals per group for 3 hr. The second experiment was performed by using a ratio of 8 to 1 of [7-^{3}H] to [4-^{14}C] testosterone infused into four animals per group for 3 hr. Testosterone

was isolated from the pituitary gland hypothalamus, cerebral cortex, prostate, testis, liver and plasma and counted for double label. The results were expressed as the mean tissue:infusion ratio ± standard error. This ratio is defined as the ratio of $^{3}H:^{14}C$ in the tissue divided by the ratio of $^{3}H:^{14}C$ infused. Failure to detect a difference in the metabolism of the two isotopic forms of testosterone should result in a ratio that is approximately equal to 1.00. The results were analyzed as in Experiment 1.

Experiment 4: Distribution of [^{3}H] Testosterone and Some of its Tritiated Metabolites Following Infusion of [1,2,6,7-^{3}H] Testosterone in Normal, Castrate and Testosterone Treated Male Rats

This experiment was performed to determine whether or not castration or testosterone treatment modified the uptake and/or metabolism of testosterone in the pituitary gland, hypothalamus, cerebral cortex, prostate, testis, liver and plasma of 3 animals from each group. Testosterone and 9 of its metabolites were isolated from each tissue following constant infusion of [1,2,6,7-^{3}H] testosterone for 3 hr. The results were expressed in terms of dpm per 100 mg tissue or as the mean relative percentage of the isolated compounds (calculated by dividing dpm/100 mg of a compound by the total radioactivity isolated in that tissue). Treatment differences were analyzed as in Experiment 1.

Experiment 5: Distribution of [^{3}H] testosterone and Some of its Tritiated Metabolites Following Constant Infusion of [7-^{3}H] Testosterone in Normal, Castrate and Testosterone Treated Male Rats

This experiment was performed exactly as Experiment 4 except that four animals per treatment group were infused with [7-^{3}H] testosterone. The results were expressed and analyzed as in Experiment 4.

CHAPTER IV

RESULTS

The purpose of the present study was to determine the relationship of endogenous testosterone levels on the capacity of androgen dependent tissue to concentrate, retain and metabolize [^{3}H] testosterone under equilibrium or steady state conditions. To accomplish this objective preliminary experiments were performed to establish the criteria needed to administer testosterone and to validate the constant infusion procedure used to assess the uptake, retention and metabolism of [^{3}H] testosterone.

Experiment 1: Determination of Testosterone Release Rate and the Influence of Different Circulating Androgen Titers on the Testis and Seminal Vesicle Weights

Testosterone was released from subdermal Silastic capsules at a rate of 120.96 μg/day ± 5.78 (Table V). Rats receiving this 200 mm^2 testosterone-filled Silastic capsule for four weeks experienced a significant ($p < 0.005$) reduction in testicular wet and dry weight, but the wet and dry weight of the seminal vesicles remained ($p > 0.05$) near normal. As expected, castration significantly ($p < 0.005$) decreased the seminal vesicle wet and dry weights.

TABLE V

TESTIS AND SEMINAL VESICLE WET AND DRY WEIGHTS OF NORMAL, CASTRATE AND TESTOSTERONE TREATED MALE RATS AND THE MEAN RELEASE RATE OF THE 200 mm^2 SILASTIC[1] CAPSULES

Treatment	n	Testes Weight (mg)		Seminal Vesicle Weight* (mg)		μg Testosterone Released/Day
		Wet	Dry	Wet	Dry	
Normal	12	3304.92[2] ±81.87	447.64 ±12.13	321.67 ±19.40	70.57 ±4.24	--
Castrate	12	--	--	79.06 ±3.94	16.78 ±0.86	--
Testosterone Treated	20	2344.52 ±64.99	342.75 ±9.18	384.80 ±15.86	84.50 ±4.08	120.96 ±5.78

[1]Polydimethylsiloxane.

[2]Each value is the mean ± standard error of the indicated number of animals.

Experiment 2: Determination of the Time Required to Establish Steady State Conditions for Plasma Testosterone

The results of this experiment showed that the radiotestosterone clearance rate (RCR) remained relatively constant ($p > 0.05$) between 3 or 4 hr after infusing [1,2,6,7-^{3}H] testosterone to either normal, castrate or testosterone treated rats (Table VI). The RCR was slightly lower in testosterone treated rats than in normal or castrate animals, but this difference was not significant ($p > 0.05$).

The RCR of rats infused with [7-^{3}H] testosterone was slightly lower than that obtained with [1,2,6,7-^{3}H] testosterone but was not significantly ($p > 0.05$) different. Thus, the concentration of radiolabeled testosterone in plasma was in a steady state 3 hr after the onset of constant infusion. As a result, 3 hr infusion intervals were used in all subsequent experiments.

Experiment 3: Differential Metabolism of [^{3}H] and [^{14}C] Testosterone Following Constant Infusion for 3 Hours

Radiolabeled testosterone ([7-^{3}H] or [1,2,6,7-^{3}H] and [4-^{14}C]) was infused for 3 hr at two different ratios to establish that the ratio of ^{3}H:^{14}C in the tissues investigated remained relatively constant (Table VII). The tissue:infusion ratio should be equal to approximately 1.00 in order to show that [^{3}H] and [^{14}C] testosterone were not differentially metabolized.

The results of this experiment showed that the tissue:infusion ratio

TABLE VI

RADIOTESTOSTERONE CLEARANCE RATE[1] OF [^{3}H] TESTOSTERONE OF ADULT MALE RATS AT THE END OF 3 OR 4 HOUR INFUSIONS WITH [1,2,6,7-^{3}H] OR [7-^{3}H] TESTOSTERONE

Infused Isotope	Infusion Time (hr)	Number of Animals	Treatment		
			Normal	Castrate	Testosterone Treated
[1,2,6,7-^{3}H]	3	5	665[2] ±38	694 ±40	566 ±39
	4	4	660 ±65	672 ±27	576 ±76
[7-^{3}H]	3	4	587 ±33	607 ±30	568 ±32
	4	4	587 ±24	582 ±27	563 ±37

[1]Radiotestosterone clearance rate is defined as the ratio of the rate of infusion of labeled testosterone (dpm/100 g body weight/hr and the plasma testosterone concentration (dpm/ml).

[2]Each value (ml/hr/100 g body weight) is expressed as the mean ± standard error of the indicated number of animals.

TABLE VII

THE TISSUE:INFUSION RATIO[1] FOLLOWING INFUSION OF [1,2,6,7-^{3}H] OR [7-^{3}H] AND [4-^{14}C] TESTOSTERONE FOR 3 HOURS IN VARIOUS TISSUES OF NORMAL, CASTRATE AND TESTOSTERONE TREATED MALE RATS

Treatment	Tissues						
	Prostate	Plasma	Pituitary	Hypothalamus	Cortex	Testis	Liver
Normal	1.11[2] ±0.10	0.97 ±0.06	0.82 ±0.16	1.07 ±0.04	1.01 ±0.03	1.00 ±0.03	1.23 ±0.33
Castrate	1.14 ±0.13	0.96 ±0.01	1.16 ±0.19	1.08 ±0.17	0.85 ±0.06	--	1.77 ±0.33
Testosterone Treated	0.90 ±0.13	0.98 ±0.05	1.04 ±0.07	0.98 ±0.02	1.11 ±0.20	0.80 ±0.12	1.08 ±0.19

[1]The tissue:infusion ratio is defined as the isolated testosterone ratio (^{3}H dpm/^{14}C dpm) and the infused testosterone ratio (^{3}H dpm/^{14}C dpm) in the various tissues.

[2]Each value is the mean tissue:infusion ratio ± standard error of six determinations (two using [1,2,6,7-^{3}H] testosterone and four using [7-^{3}H] testosterone.

was approximately equal to 1.00 in all of the tissues investigated. In addition, no significant differences ($p > 0.05$) in the tissue:infusion ratio were apparent for any of the tissues exposed to different endogenous testosterone levels. Furthermore, there was no measurable difference in this ratio when [1,2,6,7-^{3}H] or [7-^{3}H] testosterone was infused with [^{14}C] testosterone. Since the results failed to reveal any differences in the metabolism of the two isotopic forms of testosterone, it was assumed that both forms of testosterone were metabolized in the same manner as endogenous testosterone.

Experiment 4: Distribution of [^{3}H] Testosterone and Some of its Tritiated Metabolites Following Infusion of [1,2,6,7-^{3}H] Testosterone for 3 Hours in Normal, Castrate and Testosterone Treated Male Rats

Pituitary Gland

Estraction of [^{3}H] testosterone and other tritiated metabolites established that 35-40% of the total radioactivity isolated in the pituitary gland of all animals was present as testosterone. In all the animals dihydrotestosterone accounted for 20-30% and the two estrogens 10-20% of the isolated radioactivity (Figure 3). The concentration of [^{3}H] testosterone (dpm/100 mg) retained in the pituitary gland of testosterone treated rats was significantly ($p < 0.05$) higher than that found in the pituitary gland of either normal or castrate animals (Figure 4 and Table VIII). The pituitary gland of castrate animals also was capable of retaining 10% of the isolated radioactivity as androstenedione.

Figure 3. Relative Percentage of Isolated Compounds in Cerebral Cortex, Hypothalamus and Pituitary of Normal [dotted], Castrate [hatched], and Testosterone Treated [cross-hatched] Male Rats Following 3 Hr Infusion of [1,2,6,7-^{3}H] Testosterone

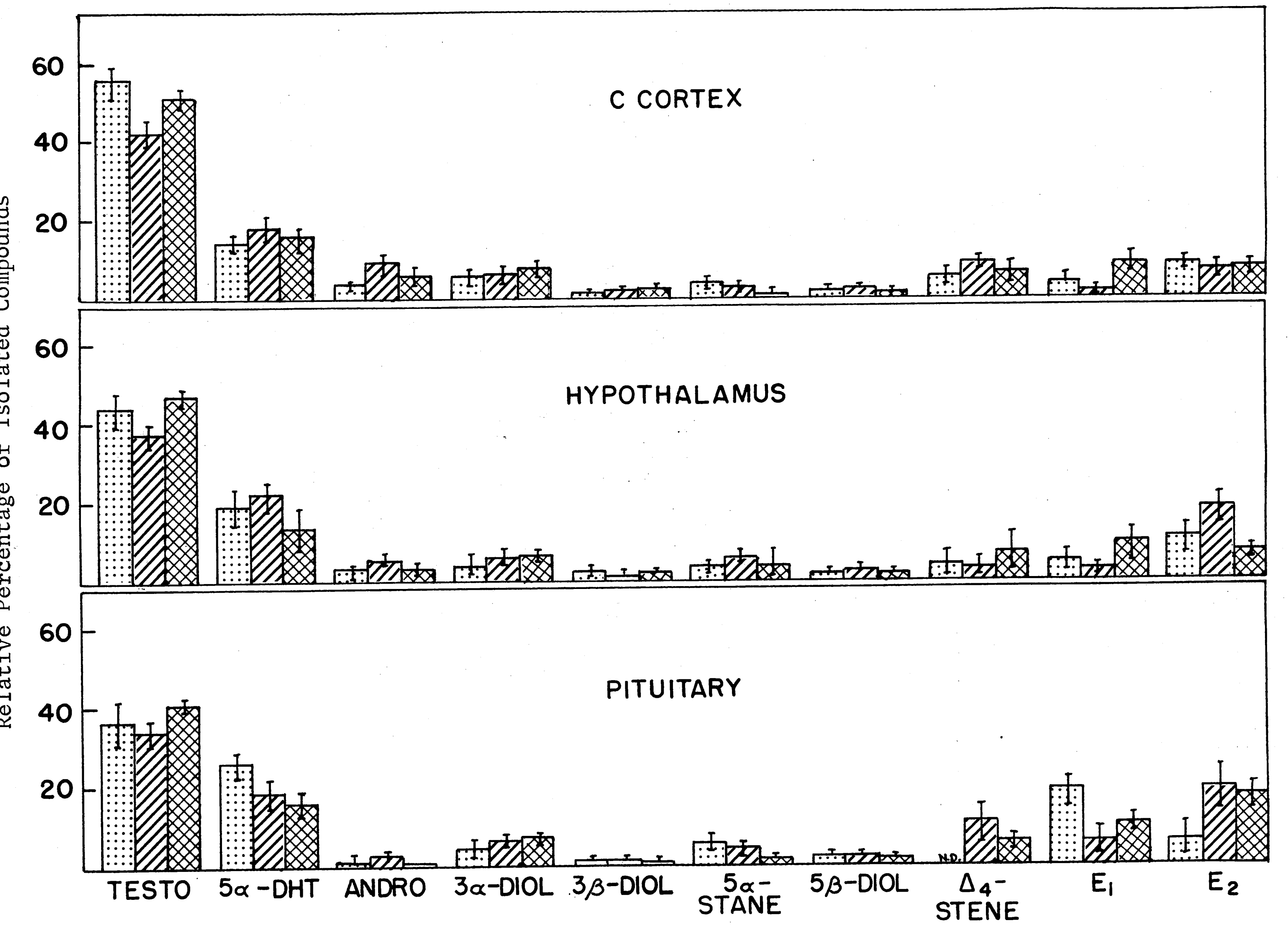

Relative Percentage of Isolated Compounds
C CORTEX
HYPOTHALAMUS
PITUITARY
60
40
20
N.D.
TESTO
5α -DHT
ANDRO
3α-DIOL
3β-DIOL
5α-
STANE
5β-DIOL
Δ4-
STENE
E1
E2

Figure 4. Radioactive Concentration of Testosterone (dpm x 10^3/100 mg Tissue) in Various Tissues of Normal, Castrate and Testosterone Treated Male Rats Following 3 Hr Infusion of [1,2,6,7-^{3}H] Testosterone

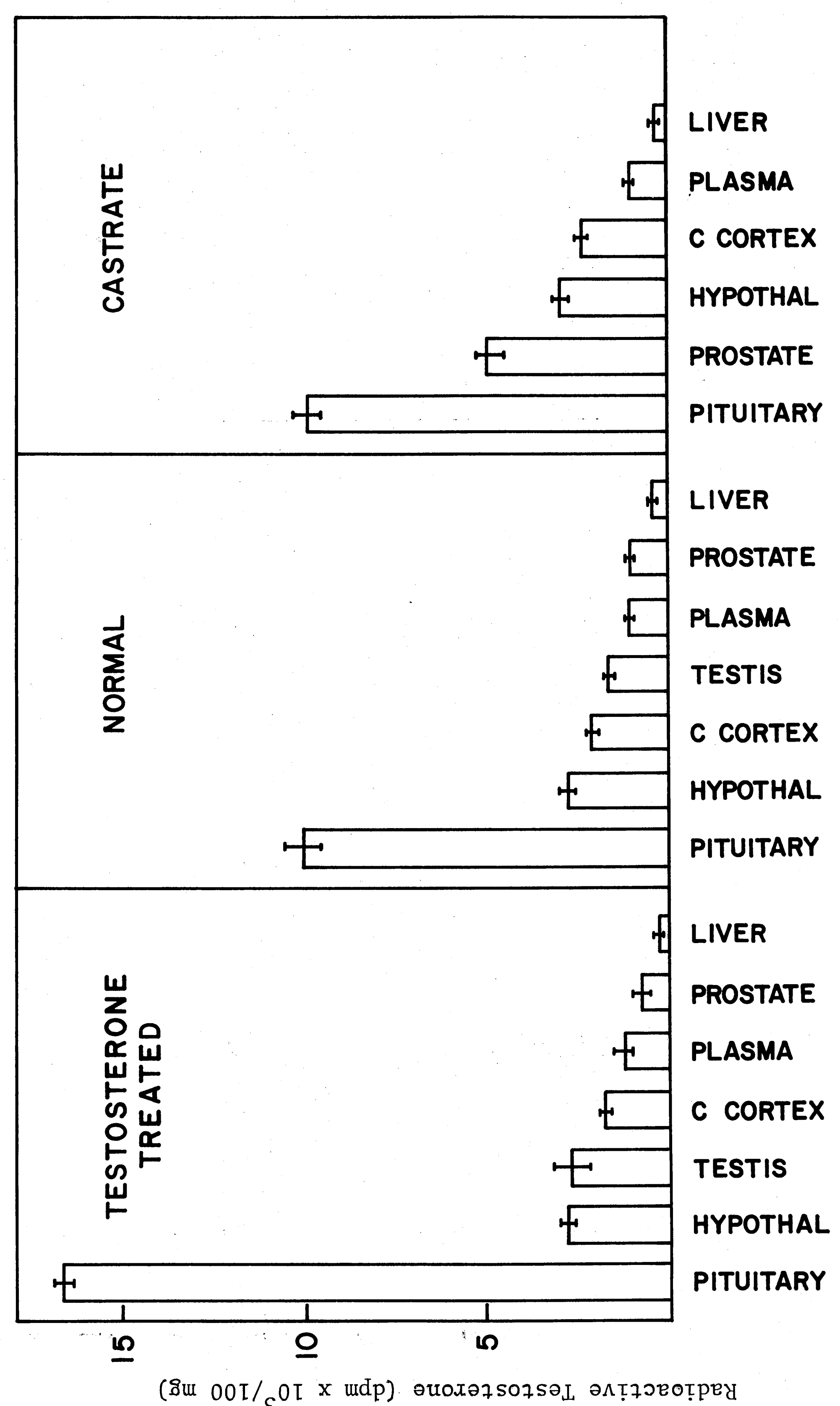

CASTRATE
LIVER
PLASMA
C CORTEX
HYPOTHAL
PROSTATE
PITUITARY
NORMAL
LIVER
PROSTATE
PLASMA
TESTIS
C CORTEX
HYPOTHAL
PITUITARY
TESTOSTERONE TREATED
LIVER
PROSTATE
PLASMA
C CORTEX
TESTIS
HYPOTHAL
PITUITARY
15
10
5
Radioactive Testosterone (dpm x 10^3/100 mg)

TABLE VIII

DISTRIBUTION OF TESTOSTERONE AND SOME OF ITS TRITIATED METABOLITES FOLLOWING INFUSION OF [1,2,6,7-^{3}H] TESTOSTERONE FOR 3 HOURS INTO NORMAL (N), CASTRATE (C) AND TESTOSTERONE TREATED (TT) MALE RATS

Tissue	Treatment	Isolated Steroids									
		Testo	5α-DHT	Andro	3α-diol	3β-diol	5α-stane	5β-diol	Δ_4-stene	E_1	E_2
Pituitary	N	10071[1] ±497	7711 ±2087	131 ±98	1135 ±380	215 ±14	1694 ±1047	598 ±115	--	5441 ±910	2287 ±1816
	C	9873 ±470	5320 ±853	709 ±513	1602 ±242	366 ±98	1205 ±157	619 ±103	3169 ±1675	1581 ±1491	5695 ±1341
	TT	16632 ±450	6771 ±1591	803 ±448	2547 ±100	256 ±40	4965 ±2735	754 ±80	3270 ±2211	5334 ±3483	6681 ±1087
Hypothalamus	N	2734 ±148	1221 ±320	204 ±52	228 ±90	144 ±87	231 ±22	137 ±28	280 ±147	407 ±204	695 ±266
	C	2908 ±207	1701 ±135	447 ±35	524 ±83	135 ±32	501 ±235	194 ±27	311 ±176	265 ±131	1249 ±446
	TT	2884 ±147	846 ±236	176 ±31	411 ±47	96 ±4	241 ±136	125 ±5	291 ±190	675 ±144	384 ±152
C. Cortex	N	2126 ±111	569 ±106	128 ±16	194 ±66	40 ±14	157 ±0	81 ±16	219 ±82	132 ±13	342 ±58
	C	2319 ±71	1038 ±345	544 ±166	329 ±74	78 ±5	220 ±94	140 ±14	539 ±34	156 ±101	402 ±83
	TT	1685 ±35	541 ±28	157 ±38	265 ±36	59 ±3	35 ±15	55 ±13	237 ±51	420 ±71	279 ±12

TABLE VIII (Continued)

Tissue	Treatment	Testo	5α-DHT	Andro	3α-diol	Isolated Steroid 3β-diol	5α-stane	5β-diol	Δ_4-stene	E_1	E_2
Prostate	N	975	8942	550	405	25	389	58	184	101	111
		±123	±1513	±181	±232	±15	±105	±8	±150	±65	±78
	C	4956	28900	1250	980	603	1108	411	1386	1247	3607
		±554	±5730	±291	±8	±227	±559	±45	±598	±878	±535
	TT	710	4695	254	587	165	140	53	170	191	139
		±216	±107	±30	±50	±97	±52	±6	±29	±59	±70
Testis	N	1615	139	80	234	62	45	66	193	182	124
		±48	±53	±10	±74	±23	±25	±12	±100	±45	±121
	TT	2834	264	43	400	102	5	55	240	249	141
		±442	±35	±8	±102	±22	±5	±55	±97	±111	±68
Liver	N	318	460	1417	416	1179	48	1315	248	141	119
		±20	±143	±721	±72	±431	±23	±381	±77	±23	±37
	C	232	1198	25229	8228	40355	164	1610	240	82	186
		±9	±337	±13095	±3228	±2374	±74	±110	±33	±80	±79
	TT	201	253	1228	645	1783	49	1059	29	132	163
		±35	±77	±467	±45	±86	±35	±296	±29	±68	±16
Plasma	N	996	26	20	106	35	13	45	29	26	18
		±88	±14	±4	±7	±15	±3	±13	±15	±1	±7
	C	1007	67	93	136	53	8	124	95	25	15
		±71	±1	±14	±0	±3	±1	±19	±16	±14	±7
	TT	1201	62	35	203	91	4	74	60	12	27
		±212	±13	±6	±8	±19	±4	±31	±12	±9	±5

[1]Each value is the mean dpm/100 mg ± standard error of 3 animals.

Hypothalamus

The data demonstrated that the hypothalamus of all the animals retained 15-20% of the isolated radioactivity as [^{3}H] dihydrotestosterone and 15-20% in the form of the two estrogens (Figure 3). However, the majority (35-45%) of radioactivity present in the hypothalamus of all animals was retained as [^{3}H] testosterone (Figure 3). Castration slightly increased ($p > 0.05$) the retention of tritiated 5α-reduced metabolites and tritiated estradiol above that found in the hypothalamus of normal or testosterone treated rats (Table VIII). The total amount of the isolated radioactivity (dpm/100 mg) retained in the hypothalamus of all animals was much lower than that found in the pituitary (Table VIII).

Cerebral Cortex

In all animals this tissue retained 40-50% of the isolated radioactivity as [^{3}H] testosterone, but also retained 25-35% as 5α-reduced androgen metabolites and 10-15% as the two estrogens (Figure 3). Castration caused a significant increase in the retention of testosterone ($p < 0.05$), androsterone ($p < 0.05$), 5β-androstane-3α, 17β-diol ($p < 0.025$) and androstenedione ($p < 0.025$) above that found in the cortex of normal or testosterone treated animals (Table VIII). The amount (dpm/100 mg and relative distribution (%) of isolated radioactivity was comparable to that found in the hypothalamus (Table VIII and Figure 3).

Prostate

The majority (60-75%) of the radioactivity in the prostate of all animals was in the form of [^{3}H] dihydrostestosterone and 10% was present

as $[^3H]$ testosterone (Figure 5). The concentration (dpm/100 mg) of $[^3H]$ testosterone, $[^3H]$ 5β-androstane-3α, 17β-diol and $[^3H]$ estradiol were all significantly higher ($p < 0.05$) in castrate animals than both the normal and implanted animals. Castration also greatly increased the radioactivity retained as $[^3H]$ dihydrostestosterone ($p < 0.025$) and $[^3H]$ androsterone ($p < 0.05$) above that found in prostatic tissue from testosterone treated but not normal animals (Table VIII).

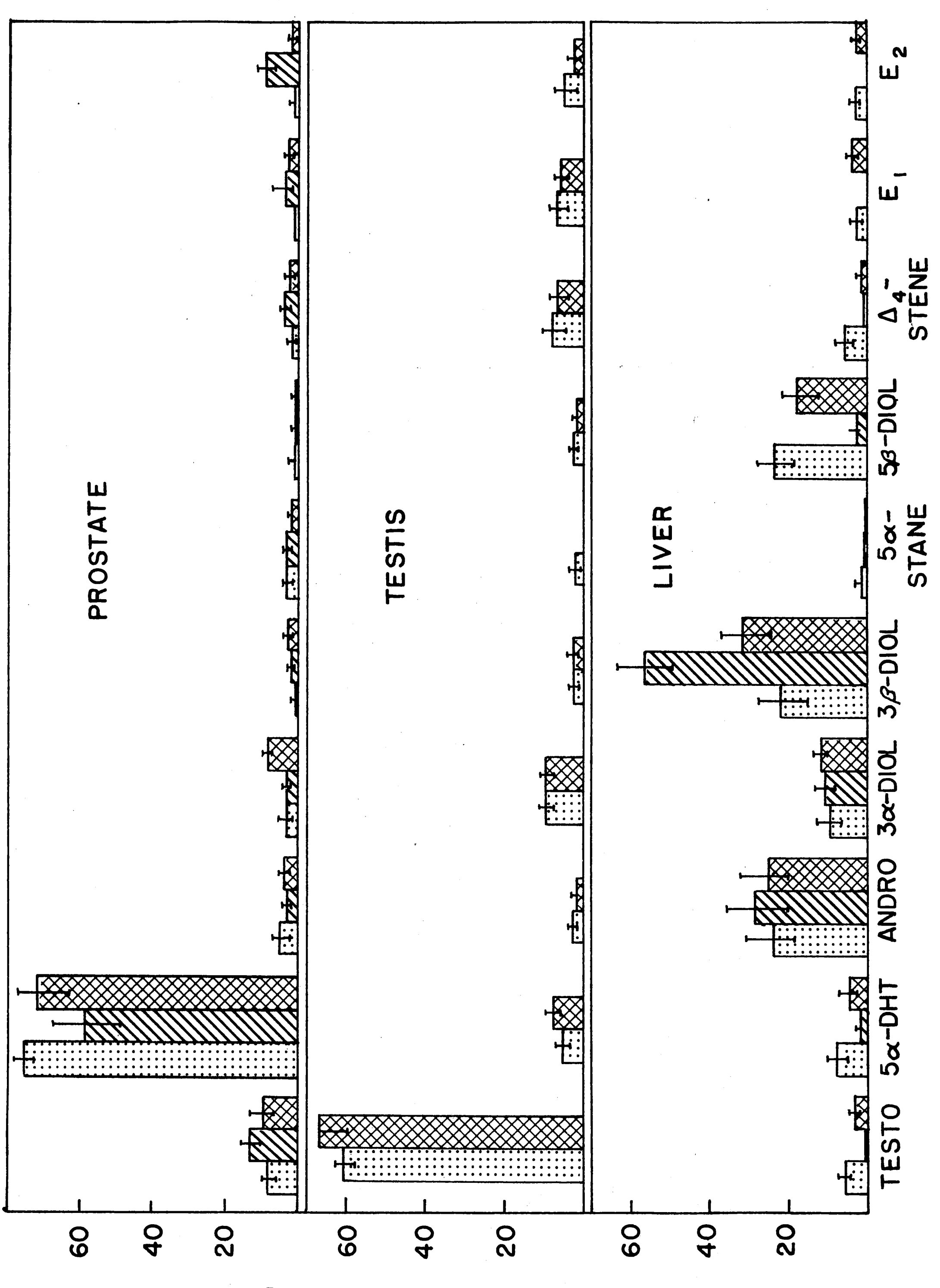
Testis

The testis of both normal and testosterone treated animals showed the capacity to retain infused testosterone as $[^3H]$ dihydrostestosterone (5-7%) and 5α-androstane-3α, 17β-diol (9%), but 60-65% of the radioactivity isolated was retained as $[^3H]$ testosterone (Figure 5). Moreover, radioactive testosterone (dpm/100 mg) in the testis of testosterone treated animals was almost twice ($p < 0.05$) that found in normal animals (Table VIII). Testosterone treatment did not affect the retention of radioactivity as estrogens (Table VIII).

Liver

Hepatic tissue from normal, castrate and testosterone treated rats freely metabolized infused testosterone. The majority of radioactivity isolated from hepatic tissue of all animals (Figure 5) was in the form of $[^3H]$ androsterone (25%), $[^3H]$ 5α-androstane-3β, 17β-diol (30-50%) and $[^3H]$ 5β-androstane-3α, 17β-diol (15-20%). Castration significantly increased ($p < 0.005$) the amount of radioactivity (dpm/100 mg) isolated in the form of $[^3H]$ androsterone, $[^3H]$ 5α-androstane-3α, 17β-diol and $[^3H]$ 5α-androstane-3α, 17β-diol (Table VIII). Testosterone treatment did not

Figure 5. Relative Percentage of Isolated Compounds in Prostate, Testis and Liver of Normal [dotted pattern], Castrate [hatched pattern], and Testosterone Treated [cross-hatched pattern] Male Rats Following 3 Hr Infusion of [1,2,6,7-^{3}H] Testosterone



Relative Percentage of Isolated Compounds
PROSTATE
TESTIS
LIVER
60
40
20
TESTO
5α-DHT
ANDRO
3α-DIOL
3β-DIOL
5α-STANE
5β-DIOL
Δ4-STENE
E1
E2

significantly affect ($p > 0.05$) the hepatic metabolism of infused testosterone (Table VIII and Figure 5).

Plasma

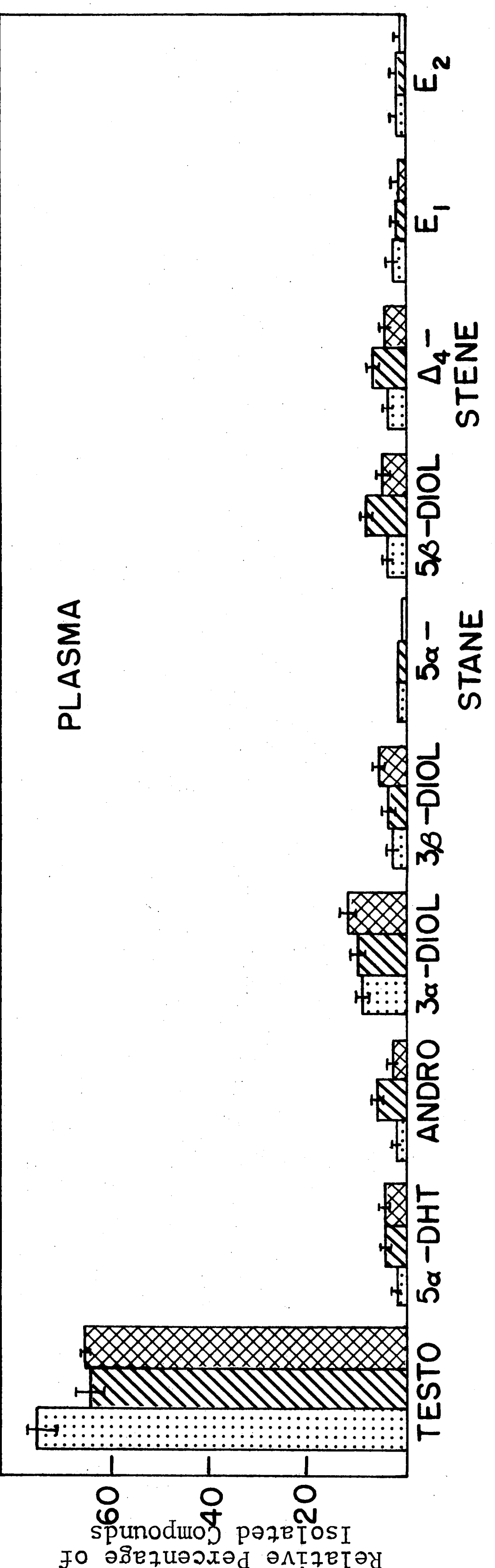
The majority of radioactivity found in plasma of all animals (Figure 6) was isolated as [^{3}H] testosterone (65-75%). Any other single radioactive metabolite present in plasma from all animals accounted for less than 5% of the total isolated, except for [^{3}H] 5α-androstane-3α, 17β-diol (8-11%) and [^{3}H] 5β-androstane-3α-17β-diol (5-7%).

Experiment 5: Distribution of [^{3}H] Testosterone and Some of its Tritiated Metabolites Following Infusion of [7-^{3}H] Testosterone in Normal, Castrate and Testosterone Treated Male Rats

Pituitary Gland

After constant infusion of [7-^{3}H] testosterone, pituitary tissue demonstrated the capacity to retain radioactivity as [^{3}H] dihydrotestosterone (5-15%) and the two estrogens (10-20%). The majority of infused [7-^{3}H] testosterone retained as [^{3}H] testosterone (40-60%) was higher than [^{3}H] testosterone (35-40%) retained by the pituitary gland after infusion of [1,2,6,7] testosterone (Figures 3 and 7). As in Experiment 4, [^{3}H] testosterone (dpm/100 mg) retained in the pituitary gland of testosterone treated animals following [7-^{3}H] testosterone infusion was significantly higher ($p < 0.05$) than that found in the pituitary of either castrate or normal animals (Figure 8 and Table IX).

Figure 6. Relative Percentage of Isolated Compounds in Plasma of Normal [dotted pattern], Castrate [hatched pattern] and Testosterone Treated [cross-hatched pattern] Male Rats Following 3 Hr Infusion of [1,2,6,7-^{3}H] Testosterone



PLASMA
Relative Percentage of Isolated Compounds
20
40
60
TESTO
5α-DHT
ANDRO
3α-DIOL
3β-DIOL
5α-STANE
5β-DIOL
Δ4-STENE
E1
E2

Figure 7. Relative Percentage of Isolated Compounds in Cerebral Cortex, Hypothalamus and Pituitary of Normal [dotted box], Castrate [hatched box] and Testosterone Treated [cross-hatched box] Male Rats Following 3 Hr Infusion of [7-^{3}H] Testosterone

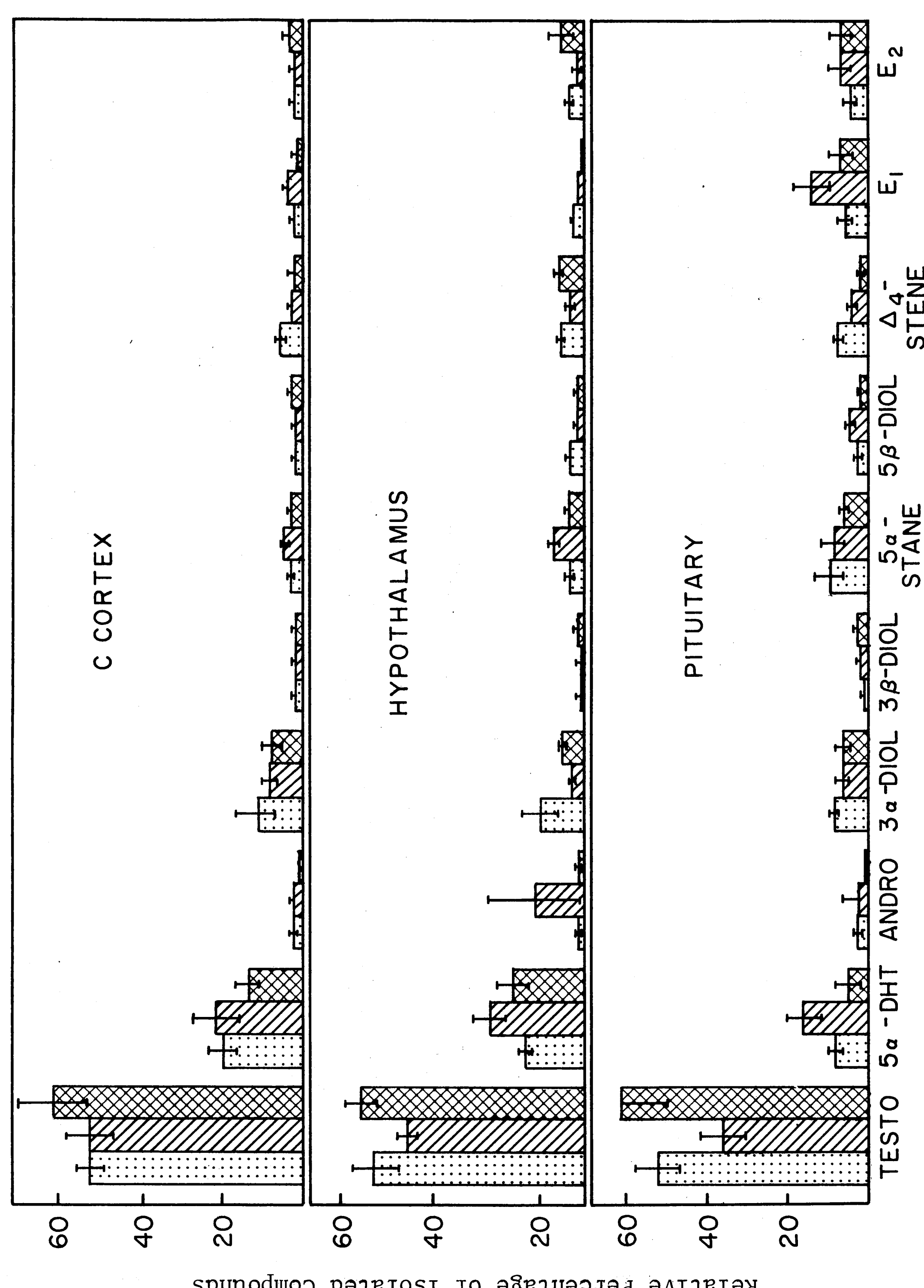

C CORTEX
HYPOTHALAMUS
PITUITARY
60
40
20
TESTO
5α -DHT
ANDRO
3α -DIOL
3β-DIOL
5α- STANE
5β-DIOL
Δ4- STENE
E1
E2
Relative Percentage of Isolated Compounds

Figure 8. Radioactive Concentration of Testosterone (dpm x 10^3/100 mg Tissue) in Various Tssiues of Normal, Castrate and Testosterone Treated Male Rats Following 3 Hr Infusion of [7-^{3}H] Testosterone

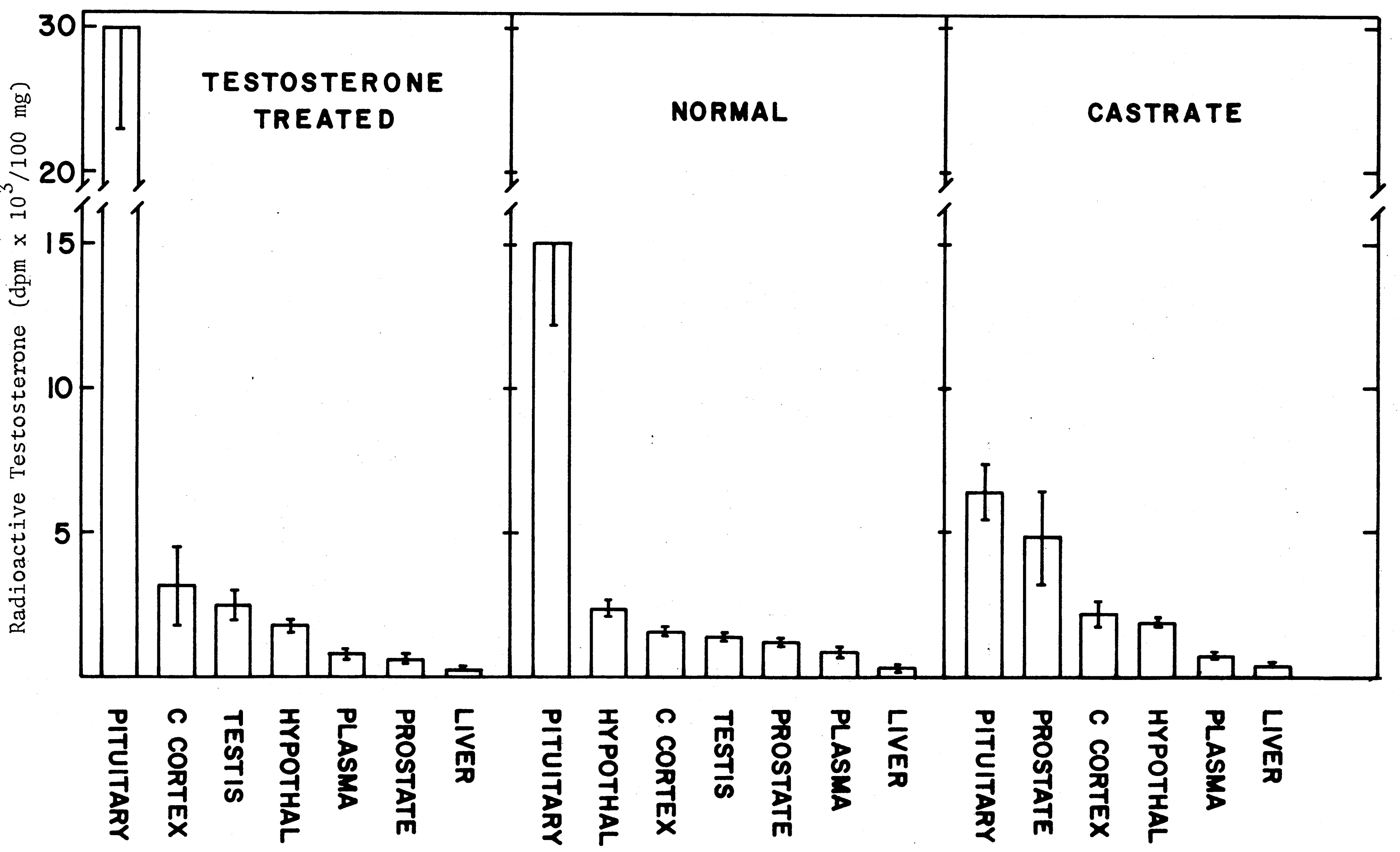

Radioactive Testosterone (dpm x 10^3/100 mg)
30
20
15
10
5
TESTOSTERONE TREATED
NORMAL
CASTRATE
PITUITARY
C CORTEX
TESTIS
HYPOTHAL
PLASMA
PROSTATE
LIVER
PITUITARY
HYPOTHAL
C CORTEX
TESTIS
PROSTATE
PLASMA
LIVER
PITUITARY
PROSTATE
C CORTEX
HYPOTHAL
PLASMA
LIVER

TABLE IX

DISTRIBUTION OF TESTOSTERONE AND SOME OF ITS TRITIATED METABOLITES FOLLOWING INFUSION OF [7-^{3}H] TESTOSTERONE FOR 3 HOUR INTO NORMAL (N), CASTRATE (C) AND TESTOSTERONE TREATED (TT) MALE RATS

Tissue	Treatment	Isolated Steroids									
		Testo	5α-DHT	Andro	3α-diol	3β-diol	5α-stane	5β-diol	Δ_4-stene	E_1	E_2
Pituitary	N	15042[1]	2197	733	2635	270	2325	722	2028	1440	1627
		±2824	±759	±313	±386	±36	±1162	±118	±400	±695	±323
	C	6396	3112	409	979	264	1073	684	1509	2421	1129
		±1040	±1060	±409	±306	±76	±382	±212	±490	±937	±491
	TT	30100	1621	--	2070	756	2398	710	2041	2176	2231
		±7153	±950		±509	±320	±464	±60	±87	±819	±809
Hypothalamus	N	2313	702	67	516	41	166	160	274	264	188
		±140	±55	±38	±186	±10	±60	±80	±10	±34	±29
	C	1868	880	419	136	44	300	89	219	68	72
		±39	±135	±320	±6	±3	±45	±31	±71	±42	±39
	TT	1789	631	46	194	50	106	59	198	--	170
		±259	±226	±17	±36	±8	±41	±11	±26		±102
C. Cortex	N	1634	601	338	319	57	79	58	172	82	63
		±148	±181	±181	±97	±11	±15	±13	±17	±15	±16
	C	2182	599	569	323	62	210	71	116	175	85
		±482	±339	±283	±116	±13	±90	±13	±22	±89	±27
	TT	3215	558	20	281	72	172	80	175	80	108
		±1292	±150	±13	±27	±14	±51	±19	±41	±61	±36

TABLE IX (Continued)

Tissue	Treatment	Isolated Steroids									
		Testo	5α-DHT	Andro	3α-diol	3β-diol	5α-stane	5β-diol	Δ_4-stene	E_1	E_2
Prostate	N	1222	8093	342	1117	104	437	154	128	123	294
		±23	±466	±78	±162	±11	±105	±48	±19	±43	±268
	C	4817	11757	1499	775	71	257	192	600	491	337
		±1693	±1136	±638	±173	±5	±112	±13	±131	±220	±223
	TT	628	5121	255	494	98	352	60	259	32	43
		±172	±1279	±39	±40	±17	±62	±6	±70	±6	±15
Testis	N	1324	85	17	308	92	92	83	93	27	40
		±83	±11	±17	±62	±13	±54	±18	±7	±22	±17
	TT	2438	58	92	324	99	22	67	74	90	52
		±571	±15	±51	±22	±10	±6	±24	±16	±9	±20
Liver	N	244	188	106	203	267	72	759	81	42	41
		±57	±68	±58	±38	±62	±22	±110	±18	±28	±8
	C	345	693	1557	1447	10176	45	913	75	67	80
		±199	±376	±532	±426	±4291	±24	±169	±36	±16	±10
	TT	144	69	94	480	922	47	492	46	45	58
		±87	±59	±24	±131	±239	±22	±94	±18	±17	±25
Plasma	N	853	26	25	109	39	4	154	83	6	5
		±40	±6	±9	±30	±5	±3	±31	±22	±2	±2
	C	731	13	32	75	34	6	55	40	3	5
		±32	±5	±12	±4	±2	±3	±15	±12	±1	±1
	TT	816	18	60	164	74	5	65	67	9	7
		±39	±4	±40	±24	±12	±3	±4	±5	±5	±4

[1]Each value is the mean dpm/100 mg ± standard error of 4 animals.

Hypothalamus

After infusion of [7-^{3}H] testosterone hypothalamic tissue demonstrated the capacity to retain radioactivity in the form of both dihydrotestosterone (15-25%) and the two estrogens (2-6%) (Figure 7). The radioactivity isolated as the two estrogens (2-6%) in the hypothalamus of all animals after infusion of [7-^{3}H] testosterone was much less than in the hypothalamus (10-20%) after [1,2,6,7-^{3}H] testosterone infusion (Figures 3 and 7). The majority (45-55%) of the radioactivity retained in the hypothalamus of all animals was in the form of [^{3}H] testosterone. The total quantity (dpm/100 mg) of isolated radioactivity retained in the hypothalamus of all animals was much lower than the total amount retained by the pituitary (Table IX).

Cerebral Cortex

This supposedly androgen insensitive tissue in all animals demonstrated (Figure 7) the capacity to retain infused [7-^{3}H] testosterone in the form of [^{3}H] 5α-reduced products (30%) and the two estrogens (10%). The majority (50-60%) of radioactivity isolated in the cortex of all animals was retained as testosterone which agrees with the results of Experiment 4. A comparison of the uptake and metabolism of [7-^{3}H] testosterone (Table IX) and [1,2,6,7-^{3}H] testosterone (Table VIII) shows that after either radiolabeled testosterone was infused the cerebral cortex from all animals demonstrated an increased retention due to castration but only after [1,2,6,7-^{3}H] testosterone was this increase significant ($p < 0.05$) for a number of compounds isolated. The total amount of radioactivity (dpm/100 mg) isolated in the cortex of all animals was much less than that found in the pituitary gland but slightly higher than

that present in the hypothalamus following [7-^{3}H] testosterone infusion (Table IX).

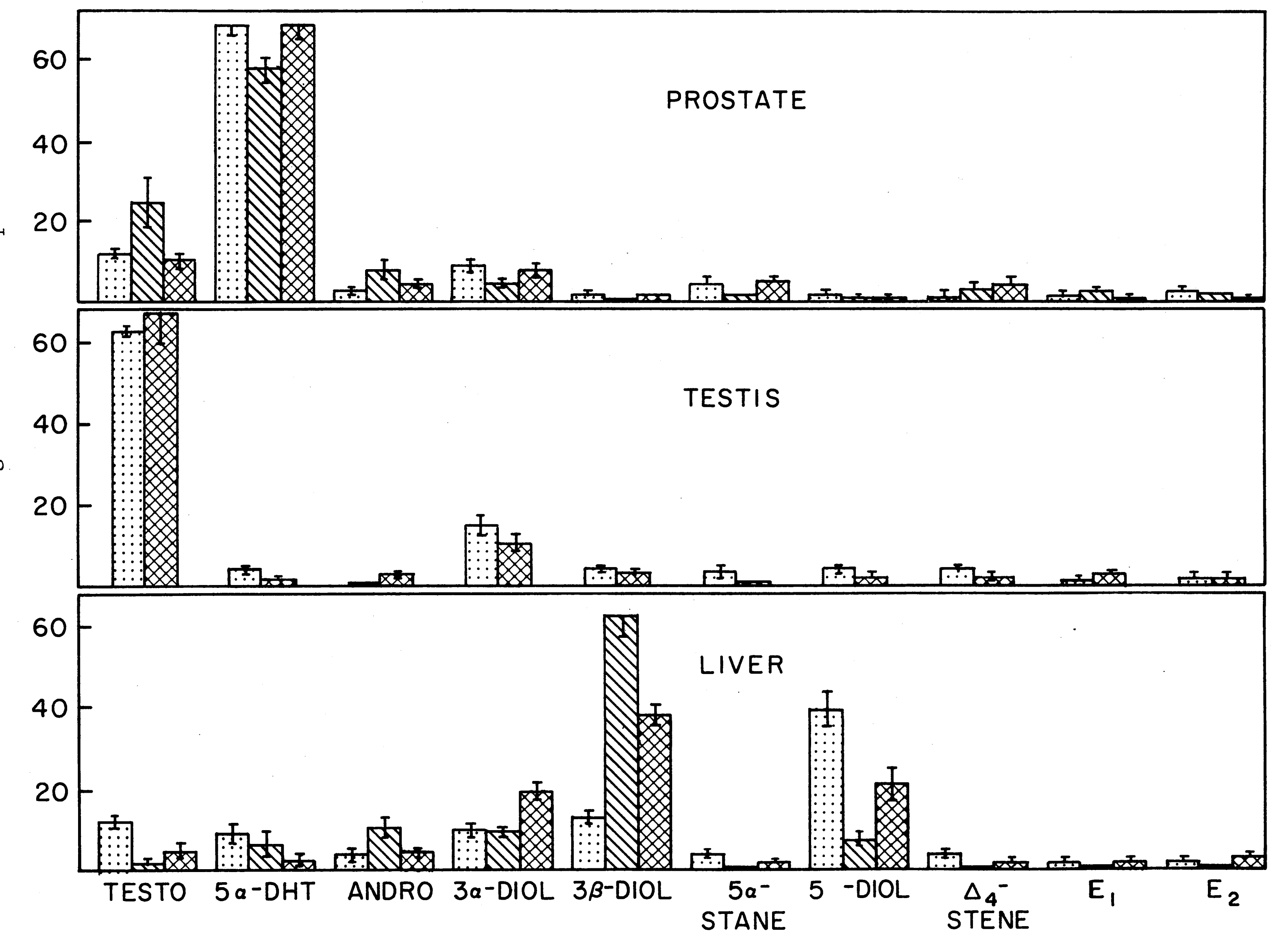
Prostate

In agreement with the results of Experiment 4, the majority (57-68%) of the radioactivity isolated in prostatic tissue from all animals was in the form of [^{3}H] dihydrotestosterone and 10-20% was present as [^{3}H] testosterone (Figure 9). The quantity (dpm/100 mg) of radioactivity isolated from the prostate as [^{3}H] testosterone was significantly higher ($p < 0.01$) in castrate animals than both the normal and testosterone treated animals. Castration also increased the retention of [^{3}H] dihydrotestosterone ($p < 0.01$), [^{3}H] androstenedione ($p < 0.01$) and [^{3}H] estrone ($p < 0.05$) in the prostate above testosterone treated but not normal animals (Table IX). Testosterone treatment did not significantly alter the retention or metabolism in the prostate following [7-^{3}H] testosterone infusion.

Testis

The testis from both normal and testosterone treated rats demonstrated the capacity to retain very little [^{3}H] dihydrotestosterone (24%) but a greater amount was retained as 5α-androstane-3α, 17β-diol (10-15%). However, the majority (60-70%) of the radioactivity isolated in this tissue was [^{3}H] testosterone (Figure 9). The concentration of [^{3}H] testosterone (dpm/100 mg) isolated from the testis of testosterone treated animals was almost twice that found in the testis of normal animals (Table IX).

Figure 9. Relative Percentage of Isolated Compounds in Prostate, Testis and Liver of Normal [dotted pattern], Castrate [hatched pattern] and Testosterone Treated [cross-hatched pattern] Male Rats Following 3 Hr Infusion of [7-^{3}H] Testosterone


Relative Percentage of Isolated Compounds
PROSTATE
TESTIS
LIVER
60
40
20
60
40
20
60
40
20
TESTO
5α-DHT
ANDRO
3α-DIOL
3β-DIOL
5α-STANE
5 -DIOL
Δ4-STENE
E1
E2

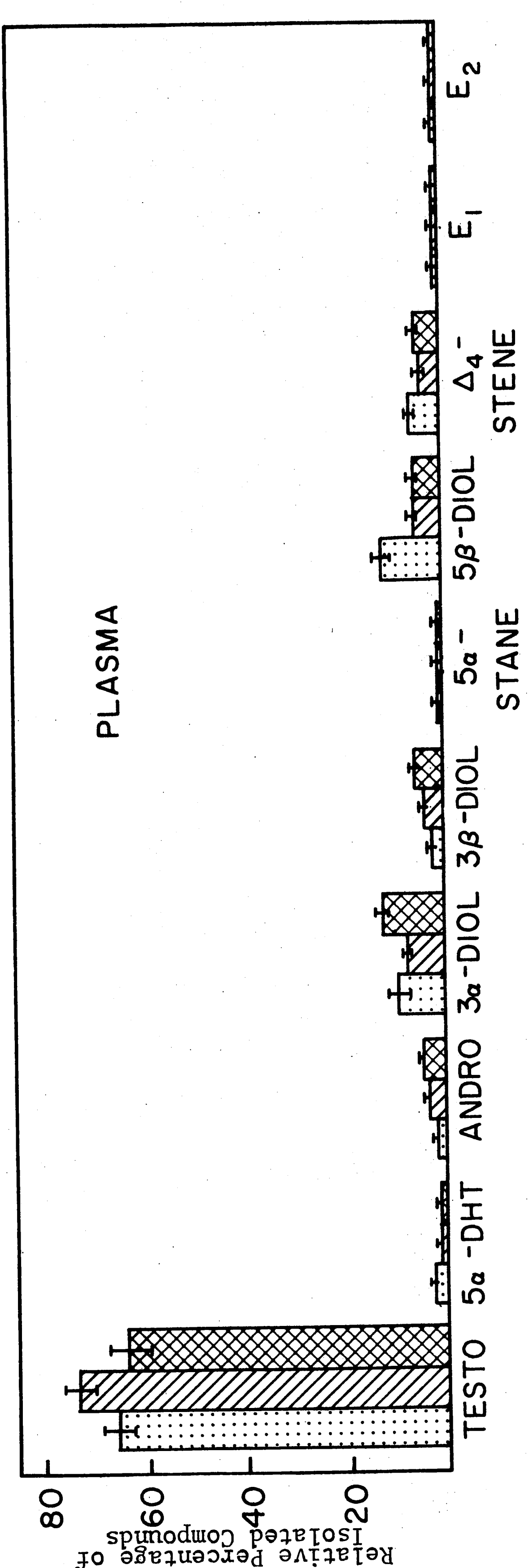
Liver

The majority of radioactivity isolated from the liver of all animals (Figure 9) was in the form of [^{3}H] 5α-androstane-3β, 17β-diol (20-60%), [^{3}H] 5β-androstane-3α, 17β-diol (10-40%), [^{3}H] 5α-androstane-3α, 17β-diol (10-20%) and [^{3}H] androsterone (5-10%). Castration significantly ($p < 0.025$) increased the amount of radioactivity (dpm/100 mg) isolated from liver in the form of [^{3}H] 5α-androstane-3α, 17β-diol and [^{3}H] 5α-androstane-3β, 17β-diol above that of hepatic tissue from normal and testosterone treated animals (Table IX). Testosterone treatment did not significantly ($p > 0.05$) affect the hepatic metabolism of infused testosterone. The total amount of radioactivity retained in the liver after [7-^{3}H] testosterone infusion was lower than after [1,2,6,7-^{3}H] testosterone infusion (Tables VIII and IX).

Plasma

The majority of radioactivity found in plasma of all animals (Figure 10) was isolated as [^{3}H] testosterone (64-73%). Any other single radioactive metabolite investigated in plasma of all animals accounted for less than 5% of the total isolated, except for [^{3}H] 5α-androstane-3α, 17β-diol (7-12%) and [^{3}H] 5β-androstane-3α, 17β-diol (5-12%).

Figure 10. Relative Percentage of Isolated Compounds in Plasma of Normal [dotted pattern], Castrate [hatched pattern] and Testosterone Treated [cross-hatched pattern] Male Rats Following 3 Hr Infusion of [7-^{3}H] Testosterone



PLASMA
Relative Percentage of Isolated Compounds
80
60
40
20
TESTO
5α -DHT
ANDRO
3α-DIOL
3β-DIOL
5α-STANE
5β-DIOL
Δ4-STENE
E1
E2

CHAPTER V

DISCUSSION

The regulatory effects of circulating testosterone on pituitary gonadotropin secretion has been studied extensively in recent years and resulted in the description of a "negative feedback loop" (Davidson, 1969). This feedback theory postulates that if circulating testosterone levels increase, plasma gonadotropin levels will decrease; but if plasma testosterone levels remain within the normal range, circulating gonadotropin levels will also be normal. However, using testosterone-filled Silastic capsules of appropriate wall thickness and surface area, different laboratories have shown that normal plasma testosterone levels can be maintained but plasma concentrations of LH and FSH in rats (Berndtson, *et al.*, 1974), rabbits (Desjardins, *et al.*, 1974) and men (Frick, 1973) fall far below normal. The failure to detect an elevation in plasma testosterone concentration coincides with the finding that the weight of the seminal vesicles and ventral prostate gland in testosterone treated animals are similar to those found in untreated animals despite a significant reduction in testis size.

The results obtained from this study (Experiment 1) confirm the observations of Berndtson, *et al.* (1974) that testosterone-filled Silastic capsules caused testicular atrophy without affecting seminal vesicle weight in adult rats. Testosterone release rate found in the present study (121 μg/day) agrees closely with the value reported by Berndtson,

et al. (1974) and was near that value of 138 μg/day needed to cause infertility in male rats (Reddy and Prasal, 1973). As expected, castrate animals with low levels of circulating testosterone (Verjans, et al., 1975) showed extreme atrophy of the seminal vesicles. The physiological significance of these findings is that plasma testosterone levels may not reflect the regulatory role this steroid proportedly plays in modulating pituitary gonadotropin secretion.

The present investigation was designed to study two possible explanations for the failure of the testosterone treated rats to exhibit the normal testosterone-gonadotropin relationship. One possibility is that testosterone from the Silastic capsules is metabolized to another hormonal steroid (Berndston, et al., 1974) which inhibits gonadotropin secretion or secondly, testosterone-filled Silastic capsules causes an increased uptake of androgens in the pituitary gland or brain. Prior to examining these possibilities, experiments were conducted to validate the continuous infusion procedure used to assess the uptake, retention and metabolism of [^{3}H] testosterone.

As the specific activity of an infused material increases in plasma, the amount of radioactivity removed from the circulation also increases since the total rate of removal of the compound is assumed to be constant. After a predetermined length of infusion, the rate of exit of the labeled compound will approximately equal its rate of entry into the circulation, and an isotopic steady state is established. The distribution of tritiated testosterone and its labeled metabolites during an isotopic steady state can be expected to approximately mimic the distribution of endogenous androgens (Gurpide, 1971).

A radiotestosterone clearance rate (RCR) was used in the present

study (Experiment 2) to determine that plasma radiolabeled testosterone was in a steady state three hours after infusing either [1,2,6,7-^{3}H] or [7-^{3}H] testosterone. It should be noted that a correction for any difference in body weight has been included in the RCR. The RCR is related to the metabolic clearance rate (MCR) for testosterone but the calculations for RCR were performed on the assumption that testosterone is distributed in only one compartment. However, testosterone may be distributed in two or more compartments or pools with the "inner" pool in rapid equilibrium with plasma and an "outer" pool in much slower equilibrium (Tait and Horton, 1966). This outer pool may involve conjugation to gluronides or sulfates. In order to arrive at a value for the MCR of testosterone it would be necessary to obtain accurate measurements of both the inner and outer pools. This was impossible in the present experiment. Thus, the RCR is an approximation of the MCR for testosterone. The plasma concentration of radioactive testosterone was essentially the same for all groups indicating that all animals were in a steady state for radioactive testosterone. In addition, the RCR was not significantly different for any of the groups indicating that the different levels of exposure to testosterone experienced by these animals did not exceed their capacity to clear unconjugated radioactive testosterone.

Assuming an 8% blood volume and an average RCR of 600 ml/hr/100 g body weight, a half-life of 30 seconds for unconjugated radioactive testosterone in plasma can be calculated. A similar half-life (50 seconds) can be calculated for estrone (MCR = 550 ml/hr) in the female rat from previously published data (DeHertogh, _et al._, 1970). Thus, this high clearance rate and short half-life may account for the much lower plasma level of endogenous testosterone (1-10 ng/ml) than endogenous levels of

other steroids.

Protein binding of testosterone in the plasma of man decreases MCR (Verneulen and Verdonch, 1969) and thus increases the half-life of this steroid. The finding that the RCR of testosterone treated animals was slightly but not significantly lower than castrate or normal animals suggests that testosterone filled Silastic capsules may increase the capacity of plasma proteins to bind testosterone. However, the plasma of rats does not contain a testosterone binding globulin as does the plasma of man (Corvol and Borden, 1973). This fact could explain why the rat exhibits a much higher clearance rate and shorter half-life of testosterone.

The validity of any experiment using labeled steroids to investigate steroid metabolism depends on the assumption that the labeled and endogenous material are metabolized in a similar manner. This assumption was verified in the present study by infusing two different ratios of [^{3}H] testosterone:[^{14}C] testosterone. A tissue:infusion ratio (TIR) of tritium to carbon-14 would ideally be equal to 1.00 if both isotopic forms of testosterone are metabolized in a similar manner. The results of Experiment 3 demonstrate that the TIR of all tissues studied was approximately equal to 1.00 regardless of the treatment imposed on the animal. The variability in the TIR could be due to: (1) the inherent variability between animals used for an <u>in vivo</u> experiment; (2) the inability to monitor recovery via an internal standard; and (3) the necessity to infuse a small amount of radioactivity in an attempt to maintain the mass of testosterone within the normal range, thereby decreasing the sensitivity of the counting procedure.

The hypothalamus, testis and cerebral cortex from all animals had

the lowest variability and a TIR closest to 1.00. This finding suggests that there is a limited testosterone metabolism in these tissues. The higher variability of the TIR noted in the prostate, pituitary gland and liver of all animals suggests that these tissues have a higher capacity to metabolize infused testosterone. As seen below, the capacity of the liver to metabolize infused testosterone is so high and [^{3}H] testosterone isolated from liver so low that the determination of TIR approaches the limit of sensitivity of the counting procedures. Measurement of the total radioactivity rather than only testosterone concentration in the tissues would have been more advantageous because the use of total radioactivity would be indicative of the true TIR and the higher radioactivity would increase the sensitivity of the counting procedures. However, the accepted procedure (DeHertough, _et al._, 1970) used to extract the total radioactivity from tissues and plasma (acetone extraction) did not repeatedly remove at least 80% of both organic and aqueous soluble tritium-labeled standards added to the tissues. Therefore, the use of the radioactive testosterone isolated in the tissues had to be used in determining TIR. Since the TIR was close to 1.00, it was assumed that tritium-labeled testosterone and endogenous testosterone were metabolized in a similar manner.

The results of Experiments 2 and 3 established that [^{3}H] testosterone was in an isotopic steady state and was metabolized in a similar manner to endogenous testosterone. Therefore, it was decided to study the relationship of endogenous testosterone levels on the capacity of androgen dependent tissue to retain and metabolize tritiated testosterone under these steady state conditions. [1,2,6,7-^{3}H] testosterone was chosen for use in Experiment 4 because it had the highest specific activity

available. [7-^{3}H] testosterone (Experiment 5) was chosen because aromatization of androgens involves the removal of hydrogens at the 1β and 2β positions (Kelly, 1974), and thus aromatization of [1,2,6,7-^{3}H] testosterone would result in a 50% reduction of the amount of estrogens detected. However, [7-^{3}H] testosterone has the disadvantage of only having one-fourth the specific activity of [1,2,6,7-^{3}H] testosterone.

The results of Experiments 4 and 5 will be discussed together, and the similarities and differences between using either isotopic form of testosterone noted. An obvious difference between Experiments 4 and 5 is the lower uptake of radioactivity after infusion of [7-^{3}H] testosterone. This can be explained by the much lower specific activity of this radioisotope of testosterone.

The radioactivity present in organic extracts of blood plasma of all animals was primarily attributed to [^{3}H] testosterone. The [^{3}H] testosterone was slightly, but not significantly, increased in the testosterone treated animal. The increase in plasma [^{3}H] testosterone coincided with a slight decrease in the RCR of rats receiving testosterone implants (Experiment 2). The increase in plasma [^{3}H] testosterone concentration and the decrease in RCR suggests that testosterone filled Silastic capsules may increase the capacity of plasma proteins to bind testosterone.

Small amounts of both 3α-androstanediol and 5β-androstanediol were detected in the organic extracts of plasma from all animals. While the source of these steroids can be attributed to leakage or secretion by some tissue it is also possible that these steroids were derived from 5α- and 5β-reduction or 3α-hydroxysteroid dehydrogenation of testosterone by cellular elements in the blood. The latter possibility appears likely because of the presence in whole blood and erythrocyte suspensions of a

17β-hydroxysteroid dehydrogenase (van der Molen and Groen, 1968).

No other metabolite of testosterone isolated in the organic extracts of blood plasma was in high enough concentration to indicate that the [^{3}H] testosterone was metabolized in blood and subsequently taken up by the tissues.

The radioactive metabolites present in tissues after infusion of [^{3}H] testosterone were probably not due to blood remaining in the tissues since each animal was perfused with saline to avoid such contamination. Any radiolabeled metabolite remaining in the circulation system after perfusion with saline was probably higher in the liver than in any other tissue because the extensive vascular sinuses in the liver. However, the concentration of [^{3}H] testosterone isolated from liver was extremely low, and the majority of radioactivity present in the liver of all treatments was retained as testosterone metabolites. Castration increased the amount of radioactivity retained by hepatic tissue in the form of 5α-androstane-3β, 17β-diol, 5α- and 5β-androstane-3α, 17β-diol and androsterone. Thus castration caused a feminization of the liver by apparently increasing the 5α-reductase (Shrieffers, 1967). In addition, the results of this investigation agree with others showing that the liver of castrated animals had a greatly increased 3β-hydroxysteroid dehydrogenase activity (Ota, _et al._, 1972). The apparent increased activity of these two enzyme systems in response to castration shows the sensitivity of hepatic tissue to decreased circulating testosterone levels. On the other hand, testosterone treatment did not affect the metabolism of testosterone when compared to the liver of normal animals. This emphasizes that plasma testosterone levels are normal in the testosterone treated animals, and a continuous release of testosterone from the

Silastic capsule does not influence the steroid metabolizing enzymes in the liver. However, the liver of testosterone treated animals could still be involved in homeostasis by an increased conjugation and elimination of testosterone. This possibility was not tested in the present investigation but appears to represent an important physiological mechanism by which the body can keep the circulating titer of testosterone within a normal range. The finding that testosterone promotes an increase in conjugation of testosterone to sulfate (Burnstein, 1968) supports this possiblity.

The prostate also responds to castration by showing an increased uptake of radioactivity. This retention was mainly in the form of DHT and androsterone, demonstrating the presence of 5α-reductase. The data of both Experiments 4 and 5 revealed that the prostate metabolizes testosterone to DHT (Liao and Fang, 1969) and DHT may be necessary in the prostate for androgen activity (Massa and Martini, 1974).

The metabolism of testosterone to DHT in the prostate of castrate animals also indicates that infusion is a reliable method for studying steroid metabolism because the results of the present study agree with the findings _in vitro_ or following a single injection _in vivo_ which demonstrated the potential necessity of metabolism to DHT (Gloyna and Wilson, 1969; Bruchovsky and Wilson, 1968).

The prostate of castrate animals also metabolized part of the infused testosterone to estradiol and estrone. A function for this aromatization cannot be deduced from the present investigation, although the estrogens may function in the prostate as in the testis (deJong, _et al._, 1974) to regulate the metabolism of testosterone within the prostate. This could be expected since numerous steroids have been found to inhibit

5α-reductase activity in the prostate (Massa and Martini, 1971).

The radioactivity present in the testis after infusion of either [7-^{3}H] or [1,2,6,7-^{3}H] testosterone was relatively low and the testis from both normal and testosterone treated animals showed a tendency to retain radioactive testosterone in an unmetabolized form (60%). Testis of testosterone treated rats appeared to accumulate and retain more [^{3}H] testosterone than those of normal rats. Differences in the amount of [^{3}H] testosterone retained may be due in part to the loss of germ cells in the testis of testosterone treated animals or it may be a function of the constant exposure to testosterone experienced by animals receiving a testosterone-filled Silastic capsule. Testis from testosterone treated and normal animals contained small amounts of radiolabeled dihydrotestosterone, 3α-androstanediol and the two estrogens. The presence of these metabolites coincides with _in vitro_ studied (Rivarola and Podesta, 1972; Sowell, _et al._, 1974; deJong, _et al._, 1974). The 5α-reduced metabolites may be acting in the testis to control spermatogenesis while the estrogens may function as regulators of intratesticular testosterone synthesis (deJong, _et al._, 1974).

The majority (40-50%) of the radioactivity in the cerebral cortex was retained as [^{3}H] testosterone, but a small amount of [^{3}H] dihydrotestosterone and [^{3}H] 3α-androstanediol was present, indicating the presence of 5α-reductase and 3α-hydroxysteroid dehydrogenase in cerebral cortex tissue (Rommerts and van der Molen, 1971). Castration increased the uptake of almost all of the compounds isolated indicating that the cerebral cortex may be dependent upon androgens. Although the uptake of radioactivity in the cerebral cortex was relatively low, the demonstration in the present study that the cortex was capable of uptake and a small

amount of metabolism of infused testosterone indicates that androgens may in fact be acting on the cerebral cortex to regulate behavior (Sar and Stumpf, 1973). Thus, the cortex should not be considered a control tissue when investigating uptake and metabolism of testosterone in neural tissues because the above results indicate that further studies should be conducted on the cerebral cortex to determine the significance of the binding of radioactivity to macromolecules in this tissue (Noess and Attramadal, 1974). A better control may be muscular tissue such as the diaphragm (Sar and Stumpf, 1973).

Although uptake of radioactivity in the hypothalamus was primarily in the form of testosterone (40-50%), hypothalamic tissue from all groups metabolized both [7-^{3}H] and [1,2,6,7-^{3}H] testosterone to [^{3}H] dihydrotestosterone and [^{3}H] estradiol. The exact extent to which these metabolites regulate secretion of gonadotropins (Jaffe, 1969; Naftolin, et al., 1972) cannot be determined from the present study. However, since castration did increase the formation of dihydrotestosterone but neither treatment affected estradiol formation, it seems possible that if testosterone does act on the hypothalamus to regulate gonadotropin secretion, it does so via dihydrotestosterone or in combination with testosterone (Jaffe, 1969). Although testosterone-filled Silastic capsules did not affect the amount of [^{3}H] testosterone converted to estrogens, the role of estradiol and estrone in decreasing gonadotropin secretion cannot be ruled out since estradiol has been found to be 60 times more potent than testosterone in inhibiting gonadotropin secretion (Verjans, _et al._, 1974).

After the infusion of both [7-^{3}H] and [1,2,6,7-^{3}H] testosterone, a majority (40%) of the radioactivity found in the pituitary gland of the three groups investigated was [^{3}H] testosterone. A small quantity of

radioactivity was retained as [^{3}H] dihydrotestosterone, [^{3}H] estrone and [^{3}H] estradiol, indicating the presence of 5α-reductase and aromatizing enzymes. However, the 5α-reductase was not increased by castration since this treatment did not increase the radioactivity isolated as [^{3}H] dihydrotestosterone. Thus, the failure to detect a castration response following infusion of [^{3}H] testosterone was contrary to the _in vitro_ findings (Massa, _et al_., 1972). The radioactivity isolated as estrone and estradiol in the pituitary gland was higher after infusion of [1,2,6,7-^{3}H] than after [7-^{3}H] testosterone. This can be explained by the much higher specific activity of [1,2,6,7-^{3}H] testosterone even though the tritiated molecules at positions 1 and 2 are possibly lost during aromatization.

Infusion of [^{3}H] testosterone did, however, demonstrate that the pituitary gland of testosterone treated animals retained almost twice the amount of radioactive testosterone (dpm/100 mg) as did the pituitary of normal animals. Since circulating LH levels are low in testosterone treated animals (Berndtson, _et al_., 1974), testosterone released from Silastic capsules most likely acts on the pituitary gland in the form of testosterone to inhibit gonadotropin secretion. Thus, the results of Experiments 4 and 5 point to a direct action of testosterone on the pituitary gland to regulate the secretion of gonadotropins.

This investigation has presented quantitative _in vivo_ information to show that the pituitary gland from animals receiving testosterone-filled Silastic capsules is unique. Testosterone can enhance its own uptake in the pituitary gland. The continuous release of testosterone from Silastic capsules rather than a quick pulse after a single injection may induce an increase in a "receptor" for testosterone in the pituitary

gland. This seems to be a very real possibility because a receptor protein for testosterone in the pituitary gland of rats has been demonstrated (Jouan, *et al.*, 1973). Thus, the capacity of pituitary tissue to bind testosterone to a receptor may be more important for regulation of gonadotropin secretion than circulating testosterone concentrations.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Numerous investigators, using different species, have shown subdermal steroid filled Silastic capsules offer promise as potential male contraceptives (Berndtson, *et al*., 1974; Desjardins, *et al*., 1974; Frick, 1973). Using capsules of appropriate sizes, these investigators have been able to maintain normal plasma testosterone levels and accessory sex gland weights, yet, significantly decrease plasma gonadotropin levels and testis weights. The present study was designed, in part, to investigate two closely related hypotheses of how testosterone filled Silastic capsules act to decrease the gonadotropin secretion without any apparent reduction in plasma testosterone levels. One hypothesis was that the additional testosterone released from Silastic capsules is metabolized to a different hormonal steroid which decreases gonadotropin secretion and the other hypothesis was that this extra testosterone increased the uptake of one of the metabolites of testosterone in the brain or pituitary gland of the testosterone treated animal thereby decreasing gonadotropin secretion. To test these hypotheses, it was decided to localize testosterone and some of its possible tritiated metabolites in various tissues of normal, castrate and testosterone treated male rats following continuous infusion of radiolabeled testosterone.

The results of Experiment 1 show that testosterone treated animals have normal accessory sex gland weights but testicular atrophy caused by

a Silastic capsule that released 120 μg testosterone per day.

Experiments 2 and 3 validate the use of constant infusions to study the uptake and metabolism of radioactive testosterone. Experiment 2 showed that radiolabeled testosterone was in a steady state after 3 hr infusion and Experiment 3 indicated that radiolabeled and endogenous testosterone were metabolized in a similar manner.

Experiments 4 and 5 showed a two-fold increased in the uptake of radioactivity as [^{3}H] testosterone in the pituitary of the testosterone treated animal. The results of Experiments 4 and 5 also demonstrated that the hypothalamus and cerebral cortex was capable of uptake and metabolism of [^{3}H] testosterone. The data for the prostate, as expected, showed an increased uptake of radioactivity in the form of [^{3}H] dihydrotestosterone after castration and the testis demonstrated the capacity to retain a small amount of [^{3}H] estradiol and [^{3}H] 5α-androstane-3α, 17β-diol. Testosterone was highly metabolized in the liver and castration caused an increase in the uptake of radioactivity by hepatic tissue. The radioactivity in blood plasma was primarily in the form of [^{3}H] testosterone. The failure to demonstrate the presence of testosterone metabolites found in the various tissues were not due to contamination of plasma remaining in the tissues after cold saline perfusion.

In conclusion, the present investigation has demonstrated the validity of using the more advantageous constant infusion technique to study testosterone metabolism. In addition, this investigation has shown that testosterone from Silastic capsules enhances the uptake of testosterone in the pituitary gland to cause a decrease in gonadotropin secretion. Whether this increased uptake is due to an implant-induced increase in a "receptor" for testosterone remains to be determined.

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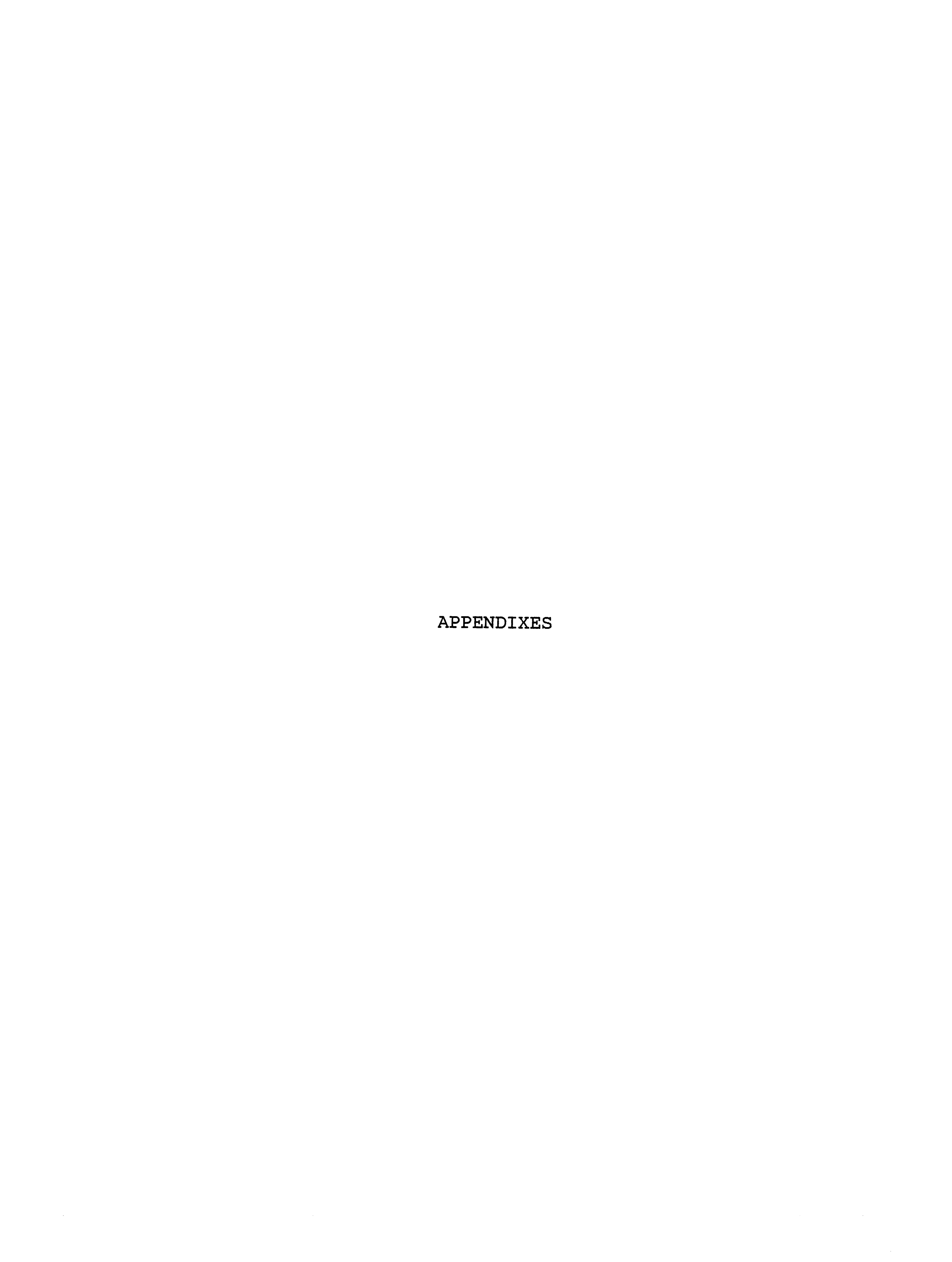

APPENDIXES

Trivial Names, Abbreviations and Systematic Names of Steroids and Steroid Derivatives

Trivial Names (Abbreviation)	Systematic Name
3α-androstanediol (3α-diol)	5α-androstane-3α, 17β-diol
3β-androstanediol (3β-diol)	5α-androstane-3β, 17β-diol
5β-androstanediol (5β-diol)	5β-androstane-3α, 17β-diol
3α-androstanediol acetate (3α-diol-A)	5α-androstane-3α, 17β-diol diacetate
5α-androstanedione (5α-stane)	5α-androstane-3, 17-dione
5β-androstanedione (5β-stane)	5β-androstane-3, 17-dione
Androstenedione (Δ_4-stene)	4-androstene-3, 17 dione
Androsterone (andro)	3α-hydroxy-5α-androstan-17-one
Androsterone acetate (andro-A)	3α-hydroxy-5α-androstan-17-one acetate
5α-dihydrotestosterone (5α DHT)	17β-hydroxy-5α-androstan-3-one
5β-dihydrotestosterone (5β DHT)	17β-hydroxy-5β-androstan-3-one
5α-Dihydrotestosterone acetate (DHT-A)	17β-hydroxy-5α-androstan-3-one acetate
5α-epiandrosterone (5α-epiandro)	3β-hydroxy-5α-androstan-17-one
5β-epiandrosterone (5β-epiandro)	3β-hydroxy-5β-androstan-17-one
Epitestosterone (epitesto)	17α-hydroxyandrost-4-en-3-one
Estradiol (E_2)	1,3,5(10)-estratriene-3, 17β-diol
Estradiol acetate (E_2-A)	1,3,5(10)-estratriene-3, 17β-diol acetate
Estrone (E_1)	3-hydroxy-1,3,5(10)-estratrien-17-one

Estrone acetate (E_1-A)	3-hydroxy-1,3,5(10)-estratrien-17-one acetate
Testosterone (testo)	17β-hydroxyandrost-4-en-3-one
Testosterone acetate (testo-A)	17β-hydroxyandrost-4-en-3-one acetate

VITA

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