Testosterone: A Major Determinant of Extragenital Sexual Dimorphism

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Summary. Sexual dimorphism in selected extragenital tissues is described with emphasis on the molecular basis of the differences. Testosterone rather than 5α-dihydrotestosterone appears to be the major intracellular androgen in organs other than skin and reproductive tract, but other steroid metabolites and their receptors are required to produce the diverse tissue differences observed in males and females. There is also evidence that multiple hormones from several endocrine glands are required to act in concert with androgens to produce and maintain their effects. Although many of the consequences of sexual dimorphism, such as body size and strength, have been evident for centuries, other differences between males and females such as disease incidence, response to drugs and toxins, and the metabolism and assimilation of dietary constituents have only recently been discovered.

Sexual dimorphism is obvious in most mammalian species because of differential development of the internal and external genitalia as well as extragenital features such as body size, appendages, and specific cellular components. The term "sexual dimorphism" refers to any differences in form regardless of whether it is manifest at the morphologic or molecular level. In spite of the quantitative differences in the gene products of tissues from males and females, there are few important sexual differences in the genome, with the exception of the male-specific Y chromosome. The major function of this chromosome is to determine testicular differentiation; in its absence, ovaries develop. It is, then, the secretory products of the gonads that are responsible for sexual dimorphism (*1*). Although estrogens secreted by the ovary have a major differentiating effect on the reproductive tract, fat distribution, and certain bones such as the pelvis, they have minor effects on other organs. The ovary also produces testosterone, but the amount secreted in most species is too low to have a biologic effect. In contrast, testosterone, produced in large quantities by the testes, has a major role in the growth and differentiation of many tissues in addition to the organs of reproduction. As a consequence of its widespread action, testosterone is the major hormone responsible for the sexual dimorphism of nonreproductive tissues.

The realization that testosterone has some action on virtually every tissue prompted attempts to classify the effects of this hormone. Historically, the responses associated with masculinization of the reproductive tract were defined as "androgenic," whereas those characterized by growth of nonreproductive tissues, such as bone, muscle, kidney, and liver, were referred to as "anabolic" actions (*2*). Much of the interest in distinguishing between the androgenic and anabolic effects of testosterone came from the clinical need for development of steroids with anabolic, but not androgenic, activity. However, it was soon observed that a single androgen may not have a uniform effect on two closely related parts of the male reproductive tract, indicating that the effects of these hormones are organ-specific rather than steroid-specific (*3*, *4*).

Even though the ultimate effects of testosterone on cellular constituents are tissue-specific, the initial steps of androgen action are common to many organs. These steps include (i) the binding of testosterone and its metabolites to specific cytoplasmic receptors that are transferred to the nucleus of the cell; (ii) an interaction of the steroid receptor complex with acceptor sites on chromatin that is associated with increased RNA polymerase and chromatin template activities; and (iii) an increase in messenger RNA (mRNA) and protein synthesis and, in some tissues, DNA synthesis (*4*, *5*). Which mRNA's and proteins are stimulated and which cells undergo division in response to testosterone depend on the individual tissues. For example, in the mouse, androgens stimulate increases in β-glucuronidase in the kidney (*6*) and epidermal growth factor in the submaxillary gland (*7*), but do not stimulate increases in these substances in the other tissues that produce them. These and other observations suggest that the structure of chromatin is modified during differentiation so that the androgen receptor complex will activate selective tissue responses. We have therefore discarded the term "anabolic," particularly as it refers to the selective action of testosterone on extragenital tissues, and have focused instead on how the actions of testosterone on diverse tissues can be understood in molecular terms.

In this article we describe how testosterone produces sexual dimorphism of nongenital tissues other than brain. We discuss first the early steps of testosterone action that are common to many cells, and then the effects of androgen on selected tissues.

Early Steps of Androgen Action

The metabolism of testosterone. This hormone, the major androgen circulating in the blood of man and most mammals, enters cells by diffusion. Within the cells it may act directly or be metabolized. Depending on the tissue, the testosterone may be converted to steroids that are more androgenic, estrogenic, or not active on the reproductive tract. Since some of the organ-specific effects of testosterone are brought about by its metabolites, it follows that the effects of testosterone are the sum of its direct actions and those of all its metabolites. These metabolites and their receptors are shown in Fig. 1.

In one of the more important pathways of androgen metabolism, the biopotency of testosterone is amplified by 5α-reductase (*8*, *9*). This enzyme reduces testosterone to 5α-dihydrotestosterone (5α-DHT), a steroid that is 2.5 times more potent than testosterone in several bioassay systems (*10*). The amount of 5α-DHT in a given tissue is determined by its rate of formation and reversible conversion to 5α-androstanediols:

Testosterone → 5α-DHT ⇆ 5α-androstanediols

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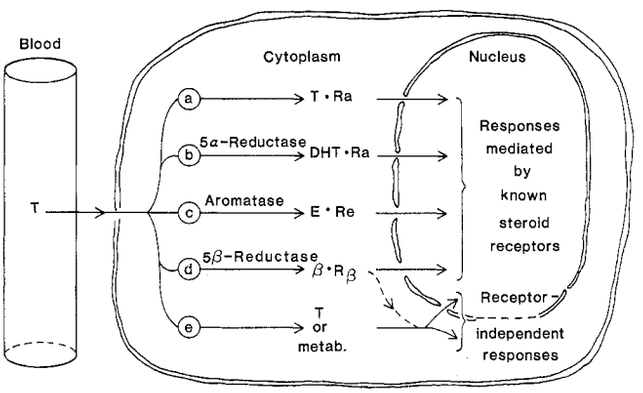

Fig. 1. Testosterone (*T*) metabolism and receptor binding. The known pathways of testosterone metabolism that lead to biological activities in various tissues are summarized as though they occurred in a single cell. Testosterone leaves the blood and enters the cell by diffusion. In the cytoplasm, testosterone can react in the following ways: (a) Without being metabolized, in which case testosterone binds directly to the androgen receptor (*Ra*), and this steroid receptor complex (*T·Ra*) is then transferred to nuclear acceptor sites in chromatin for initiation of the steroid-specific responses; (b) testosterone is metabolized by 5α-reductase to 5α-dihydrotestosterone (*DHT*) which then binds to Ra and the steroid receptor complex (*DHT·Ra*) is bound in the nucleus; (c) testosterone is aromatized to estradiol (*E*) which binds to estrogen receptor (*Re*) which is transferred and bound in nuclei as for (a) and (b); (d) testosterone is metabolized to 5β metabolites (β) which bind to the β steroid receptor (*Rβ*) and the steroid receptor complex (*β·Rβ*) presumably acts in the nucleus; and (e) testosterone or one of its metabolites (*metab.*) acts in the nucleus or cytoplasm by mechanisms which are independent of known receptors (receptor-independent responses).

Although the androstanediols are also potent androgens, their biological activities on most tissues depend on their back conversion to 5α-DHT, which is the favored direction of the reaction in vivo (*11*). In the adult male, the production of 5α-reduced androgens from testosterone occurs most notably in the reproductive tract and skin where these steroids stimulate cell division. Since skin is one of the most accessible organs, much of our knowledge about the significance of 5α-reductase in humans has been derived from studies of testosterone metabolism in vitro with the use of tissue slices, homogenates, hair follicles, or cultured fibroblasts prepared from this organ (*8, 12*). The metabolism of testosterone by skin has also been studied in vivo by Mauvais-Jarvis and his colleagues (*13*). These workers used a double-labeling technique, injecting simultaneously ^{3}H-labeled testosterone and ^{14}C-labeled testosterone by percutaneous and intravenous routes, respectively. Taken together these studies show that the activity of 5α-reductase varies with anatomical location and hormonal status. Skin from the genital tubercle of both sexes has high 5α-reductase activity in the fetus before the onset of testicular testosterone secretion. The organs derived from the genital tubercle in the male require 5α-DHT for differentiation. By contrast 5α-reductase activity is low in the skin and other responsive tissues of androgen-naïve animals and increases only after exposure to testosterone (*14*). In the adult this androgen-stimulated increase in testosterone metabolism results not only in a larger excretion of 5α-androgen metabolites (*15*), but also in a more rapid rate of testosterone clearance in men than in women (*16*). Thus an increased rate of 5α-DHT formation is a dimorphic feature characteristic of the male.

As shown in Fig. 1, both testosterone and 5α-DHT bind to and exert their actions by way of the androgen receptor. Since both steroids use the same receptor it is not certain how the unique effects of 5α-DHT are mediated. For example, decreased 5α-DHT concentrations in patients with 5α-reductase deficiency are associated with reduced beard growth and lack of acne, even though the concentrations of testosterone in the blood of these patients are normal (*17*). By contrast, masculine muscular development in these patients apparently does not have an obligatory requirement for 5α-DHT (*17*). One possible explanation for the effects of 5α-DHT not being mimicked by testosterone is that the 5α-DHT receptor complex has a higher affinity for acceptor sites in chromatin. Alternatively, the androgen receptor may be modified in a minor way in some organs so that it favors 5α-DHT over testosterone. This latter possibility has been discussed by Baker *et al.* (*18*) (Table 1).

Estradiol is another metabolite of testosterone that exerts its effect either in the tissue where it is produced or at distant sites (Fig. 1). In both instances, the hormonal effects are mediated via the estrogen receptor. In men, the importance of estradiol for the regulation of some tissues was only recently established. For example, the major actions of androgen on the brain (*19*) and selected muscles (*20*) of some species are mediated by estrogens produced from testosterone. Those actions of testosterone are blocked by antiestrogens and aromatase inhibitors (Table 1). By contrast, in feminized men with increased production of estradiol (*21*), the action of this steroid is opposite that of testosterone on the

Table 1. Receptors for and responses to testosterone and its metabolites. The actions of testosterone may be mediated directly or by any one of several metabolites.

Intracellular steroid hormones	Cellular receptor or mediator	Response to intracellular steroid in Tfm	Blocked by anti-androgens	Blocked by anti-estrogens or aromatase inhibitor	Examples in mature animals
Testosterone	Androgen receptor	Reduced or absent	Yes	No	Kidneys, submaxillary gland
Testosterone metabolites					
5α-DHT	Androgen receptor*	Reduced or absent	Yes	No	Male reproductive tract, skin
Estradiol	Estrogen receptor	Normal	No	Yes	Brain, levator ani muscle
5β-DHT and other 5β steroids	5β steroid receptor	Normal	Yes	Unknown	Bone marrow, liver
Unknown	Cyclic AMP		No	Unknown	Selected enzymes in prostate
Unknown	Unknown	Normal	No	Unknown	Total liver protein

*The androgen receptor in prostate may have a lower affinity for testosterone than that of kidney [see (*21*)].

breast (*22*) and liver (*13*). In other tissue, such as prostate, estrogens are synergistic with androgens (*19, 23*).

Testosterone has additional actions on bone marrow and liver that are not mediated by either androgen or estrogen receptors. Some of these effects are mimicked by 5β-androgen metabolites (*24*) that have no growth-promoting activity on the male reproductive tract, and thus by definition are not androgens. These steroids mediate their effects by way of the β-steroid receptor which has a greater affinity for the β metabolites of testosterone and progesterone than their 5α epimers (*25*). Animals that have a genetically determined deficiency of the androgen receptors [and therefore exhibit testicular feminization (Tfm)] respond normally to β steroids (*26*) but do not respond, or show reduced responses, to testosterone and its 5α metabolites (*9*) (Table 1).

Several actions of testosterone either have not or cannot be explained by known metabolites or receptors (Table 1, columns 5 and 6). Among these are the actions of testosterone on the pentose phosphate cycle of the prostate that are mediated by adenosine 3′,5′-monophosphate (cyclic AMP) (*27*). These cyclic AMP-mediated responses are not inhibited by antiandrogens, supporting the concept that these actions do not require the androgen receptor (*28*). Furthermore, cyclic AMP cannot mimic the actions of testosterone that depend on this receptor (*29*). Another androgen action that is not explained by known mechanisms is the responses of some liver proteins to testosterone and 5α-DHT in animals with Tfm (*30*). Thus it is evident that a cell can recognize and respond to testosterone by a variety of independent mechanisms, some of which have yet to be defined with regard to metabolite or receptor.

Testicular feminization. Much of our understanding about the molecular basis of sexual dimorphism stems from studies of animals and humans with Tfm. This is an X-linked recessive gene defect that is transmitted by heterozygous females to half of their male offspring. Affected individuals are male pseudohermaphrodites characterized by an XY karyotype female phenotype, short vagina, inguinal or abdominal testes, and absence of the remainder of the reproductive tract (*31, 32*). The external genitalia in persons with Tfm do not become masculinized after treatment with methyl testosterone because of tissue resistance (*33*). In addition, virtually all androgen-responsive organs in these individuals are insensitive to testosterone (*34*). That the genetically determined androgen resistance was found to affect many tissues implied that this defect involves an early step in androgen action. A clear understanding of the Tfm mutation in molecular terms, however, awaited the identification of this disorder in rats (*35, 36*) and mice (*37*). The basis of androgen resistance was first studied in Tfm rats. When it was demonstrated that testosterone conversion to 5α-DHT in resistant tissue was normal (*38*) but that 5α-DHT binding in nuclei was reduced (*39, 40*) (Fig. 2a), a defect of the androgen receptor was proposed as the basis for androgen resistance in this species. Comparable studies were then performed in Tfm mice, and in this species also a receptor defect was proposed as the basis for androgen resistance (*41, 42*) (Fig. 2b). A reduced amount of cytosol androgen receptor was subsequently demonstrated by sucrose gradient analysis in both rats and mice with Tfm (*43*) (Fig. 2, c and d). It is now accepted that the androgen resistance in animals (*44-47*) and many humans (*48, 49*) with Tfm is due to reduced or undetectable amounts of androgen receptor.

The relation of the receptor defect to androgen responsiveness and the Tfm gene is illustrated by comparison of normal and Tfm mice with mice that are heterozygous carriers of the Tfm gene (*46*). Normal male and female mice have normal X chromosomes and a full complement of active androgen receptors and, in turn, are fully responsive to androgens. The androgen-insensitive male pseudohermaphrodite carrying the Tfm gene on its X chromosome in all cells has little or no functional androgen receptor. In contrast, the carrier female mouse has two populations of cells as a consequence of random X inactivation. In some cells, the active X chromosome is normal and in others Tfm is expressed. This correlates with androgen receptor activity and androgen responsiveness which are intermediate between those of Tfm mice and normal mice (*50, 51*). Analogous results are available from a study of cultured human fibroblasts from women who transmit the Tfm gene (*52*). These experiments correlating androgen receptor concentration and androgen responsiveness with Tfm genotype suggest that Tfm is either the structural gene for the receptor or a regulatory gene that controls its activity (*9*). The function of the Tfm gene is one of the more interesting unresolved problems of steroid hormone action.

The major difference between Tfm rats and mice is their degree of androgen resistance. Many of the cellular components of the preputial gland (*53*), kidney (*54*), and pituitary (*55*) in Tfm rats exhibit

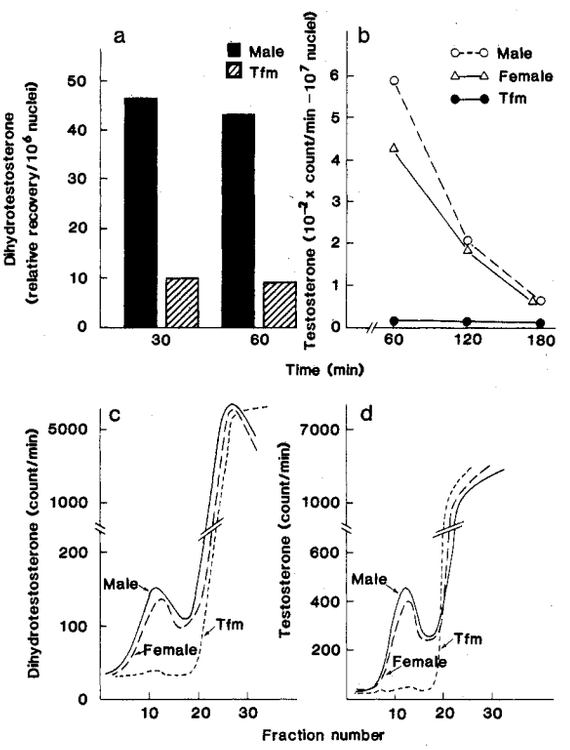

Fig. 2. Androgen receptor deficiency in animals with testicular feminization (*Tfm*). (a) The uptake of 5α-dihydrotestosterone in preputial gland nuclei of normal male and Tfm rats injected intravenously with [^{3}H]testosterone. The preputial gland is a skin derivative with 5α-reductase which is equally active in normal males and animals with Tfm. The reduced uptake of dihydrotestosterone in the nuclei of Tfm rats is due to reduced amounts of androgen receptor. [From Bullock and Bardin (*39*)] (b) Similarly, the uptake of [^{3}H]testosterone in kidney nuclei of Tfm mice is dramatically reduced over that of normal males and females. The mouse kidney has very little 5α-reductase activity so no dihydrotestosterone is detected. [From Bullock *et al.* (*41*)] (c) Binding of [^{3}H]dihydrotestosterone by preputial gland cytosols from male, female, and Tfm rats, and (d) [^{3}H]testosterone by kidney cytosols from male, female, and Tfm mice. Cytosols were incubated with ^{3}H-labeled steroids and ananlyzed on sucrose gradients. The androgen receptors in normal animals sedimented at 7*S* to 8*S* in fractions 10 to 14. No androgen receptor was detected in Tfm animals in this study. [From Bullock and Bardin (*43*)]

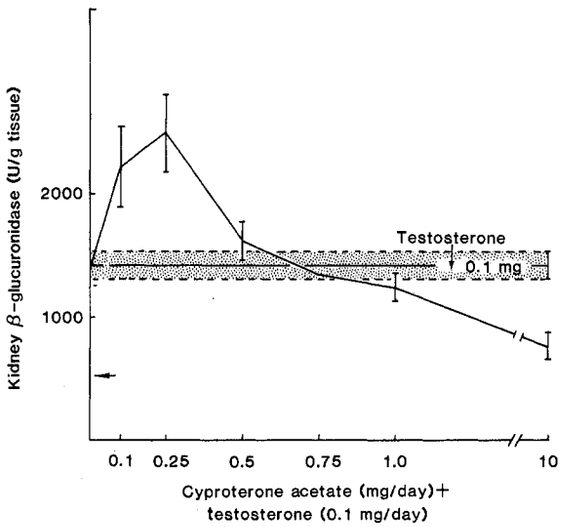

Fig. 3. The syn- and anti-androgenic actions of cyproterone acetate on kidney β-glucuronidase activity of female mice. Testosterone treatment (0.1 mg per day for 6 days) increased β-glucuronidase twofold in this study (horizontal shaded bar shows the mean ± standard error). When this treatment was combined with various doses of cyproterone acetate (0.1 to 10 mg/day) the response was first potentiated and then inhibited (solid line). Cyproterone acetate alone produced no effect on β-glucuronidase. The arrow indicates the enzyme activity in untreated animals. [From Mowszowicz *et al.* (*69*)]

a dose-dependent response to very large doses of testosterone. By contrast, Tfm mice are relatively more resistant in that most gene products are not affected by androgens while a few show a minimal or fractional response (*9*, *56*). These mice and rats may thus be phenotypically similar to humans with the complete and incomplete forms of Tfm, respectively. Rats with Tfm have approximately 10 percent of the receptor found in normal rats. Mice with Tfm also have a small amount of residual androgen receptor (*45*). Although many of the physical properties of this receptor are normal, it does have a different pattern of elution on DNA cellulose (*57*). A detailed comparative study of residual receptors in Tfm rats and mice awaits the isolation of highly active stable preparations of these proteins. It is therefore not possible to relate specific defects to the different degrees of androgen resistance in these species.

The variable phenotype in humans with androgen insensitivity is believed to reflect different degrees of tissue responsiveness which, as noted above, is associated with heterogeneity of androgen receptors. These individuals include those with no measurable receptor, reduced amounts of normal receptor, reduced amounts of heat-sensitive receptor, and normal amounts of "normal" receptor (*58*). These last patients, termed "receptor-positive," do not respond to the full complement of androgen receptor even though it is transferred to the nucleus. Patients with each of these receptor variants (except receptor-negative) may have more than one phenotype, which underscores our lack of understanding of how receptor concentration and structure relate to androgen response.

In spite of the lack of information about the nature of the receptor abnormality in animals with Tfm, the defect can be useful in elucidating how testosterone influences sexual dimorphism in extragenital tissues. Actions of testosterone and 5α-DHT that are attentuated or absent in Tfm are believed to be mediated by androgen receptor. Those that are normal in Tfm are believed to be mediated by estrogen receptor, β-steroid receptor, or some other undefined mechanism. Table 1 summarizes the actions of testosterone and its metabolites as they are mediated by various steroid receptors.

Androgen Responses of the Kidney

Sexual dimorphism of the kidney exists in many species, but such dimorphism has been studied most extensively in the mouse. Morphological studies of mouse kidney indicate that the cells of Bowman's capsule and proximal convoluted tubule are larger in males than females (*59*). Endogenous or exogenous androgens produce these differences and the effects of these androgens are not mimicked by estrogens (*2*). In male mice that have been castrated the size of the kidney regresses to the size of that in the female; and the kidney in female mice is as responsive to androgens as that of the male. Taken together, these observations suggest that many androgenic responses in this organ are not fixed at birth as is the case for brain and liver. Analysis of mouse kidney before and after androgen treatment indicates that renal enlargement is secondary to cellular hypertrophy rather than hyperplasia (*2*, *60*). In this regard, the kidney differs from the male reproductive tract where androgens produce an increase in cell number as well as size. Other experiments on kidney have provided insights into how hormones mimic and modify androgen action, how androgens influence transcriptional events, and how androgens control specific genes.

Hormones that mimic and modify androgen action on kidney. The effects of androgens on many responsive tissues, including kidney, may be blunted in hypophysectomized animals, depending on the end point examined (*61*). Replacement with one hormone, or with a combination of hormones, including growth hormone, prolactin, glucocorticoids, and thyroxin, only partially restores the full effect of testosterone. These observations suggest that an additional factor either produced by the pituitary or under pituitary control may be important for the modulation of androgen action on the kidney. There have been relatively few studies on the mechanism by which pituitary hormones synergize with testosterone; however, prolactin has been shown to facilitate the uptake of testosterone in some tissues (*62*). That these responses are not unique to kidney is suggested by the observations that pituitary, adrenal, and thyroid hormones are required to maintain a full androgen response in other tissues such as liver (*63*) and submaxillary gland (*64*). In addition, testosterone-responsive cells cultured in serum-free medium require multiple hormones and growth factors for maintenance of androgen sensitivity (*65*).

Progestins are another class of hormones that modify the actions of androgens, and some of them also mimic the effects of testosterone. Progestins that are either progesterone or nor-testosterone derivatives have minimal and marked androgenic effects, respectively, when assayed on the male reproductive tract (*66*). By contrast, both types of steroids masculinize the external genitalia (*67*), preputial gland (*9*), submaxillary gland (*68*), and kidney (*69*). Insights into how progestational agents can act like androgens came from the realization that the responses are not observed in mice and rats with Tfm. Subsequent studies with ^{3}H-labeled medroxyprogesterone acetate ([^{3}H]MPA) demonstrated that this androgenic progestin binds to cytoplasmic and nuclear androgen receptors (*70*). The absence of [^{3}H]MPA binding in animals with defective androgen receptors correlates with the absence in these animals of a steroid effect on kidney (*69*) and other tissues (*9*, *68*). These observations led to the study of progestins that modify androgen action.

The progestins that potentiate the action of testosterone are known as synandrogenic steroids (*69*). The synandrogenic effects of progestins on β-glucu-

ronidase activity in mouse kidney have been studied most extensively; however, such effects are by no means limited to this protein (*71*). Many synandrogenic steroids have other biological activities. For example, the potent progestin cyproterone acetate has both synandrogenic and antiandrogenic actions (Fig. 3). Although cyproterone acetate has no effect on kidney β-glucuronidase activity per se, low doses of this and certain other progestins potentiate while larger doses inhibit the action of testosterone (*69, 72*). Since steroids that mimic or modify androgen action bind to the androgen receptor, a steric-allosteric model of this intracellular binding protein was proposed to explain androgen-progestin interaction (*70*). Considerable evidence was marshaled to support the model as it related to the independent androgenic, synandrogenic, and antiandrogenic effects of progestins (*70, 73*), but the model did not explain how potentiation could be followed by inhibition (Fig. 3). Therefore, an effort was made to understand the metabolism and binding of 6α-methyl progesterone (6MP), which has both synandrogenic and antiandrogenic activities (*72, 74*). When [^{3}H]6MP was injected intravenously into animals, both [^{3}H]6MP and its 20α-hydroxy metabolite were found in kidney cell nuclei but not in seminal vesicle nuclei. The renal uptake of both steroids was increased by concomitant administration of testosterone, progestins, or glucocorticoids. This increased uptake of 6MP correlates with its ability to potentiate androgen action on kidney. Other studies with Tfm animals suggest that at least one binding site in addition to the androgen receptor is involved in potentiated uptake of 6MP.

The binding of progestins to the androgen receptor is believed to be partly responsible for the varied effects of these steroids. The ability of progestins to modulate the activity of specific proteins such as β-glucuronidase is, at least in part, a reflection of their ability to increase or decrease the rate of enzyme synthesis (*4*). Progestins can thus be used as probes in concert with other genetic and biochemical approaches to better understand the selective and specific actions of testosterone.

Most of the agents that inhibit androgen action are steroids or closely related compounds. Recent studies indicate that long-acting agonists of gonadotropin-releasing hormone (GnRH) also have antisteroidal activity. In addition to having stimulatory action on the pituitary, GnRH and its agonists block steroid synthesis in testis and ovary (*75*). Several GnRH agonists, including the specific peptides [D-Trp^6,Pro^9-NEt]-GnRH and [(imBzl)D-His^6,Pro^9-NEt]-GnRH, block the direct effect of testosterone on a variety of androgen-responsive tissues (*76*) and, surprisingly, inhibit the effect of estradiol on the vagina and uterus (*76*). Prior to these observations, all steroidal and nonsteroidal compounds with antiandrogenic or antiestrogenic activity were believed to exert their effects by interacting with soluble intracellular androgen or estrogen receptors (*9, 77*). The classic view was that antiandrogens and antiestrogens compete with testosterone and estradiol, respectively, for their intracellular binding sites. The actions of most peptides, however, including those of GnRH and its agonists on the pituitary and gonads, are initiated via binding to membrane receptors. Although many peptides and protein hormones are internalized by endocytosis, it is not clear that this process relates to their hormonal activity (*78*). It remains to be established whether the antisteroidal activity of GnRH agonists reported here is mediated by membrane or intracellular receptors. Nevertheless, it is unlikely that the agent that normally interacts with this binding site is the same as that which acts on the pituitary, because the amount of GnRH in the circulation is too low to produce effects in the reproductive tract. That peptides structurally related to GnRH are present in the male reproductive tract where they presumably modulate the local effects of sex steroids has recently been shown by Sharpe and Fraser (*79*). Studies on the action of these peptides should provide useful insights into how sexual dimorphism is modified in extragenital tissue.

Effects of androgens on transcription. Numerous experiments have indicated that steroid hormones regulate RNA synthesis in reproductive tissues. Increases in RNA have been associated with changes in the transcriptional process as reflected by enhanced chromatin template activity, increase in the number of initiation sites on chromatin, and activation of DNA-directed RNA polymerases (*80*). Studies with mouse kidney indicate that androgens stimulate these transcriptional processes in a similar way. A single injection of testosterone increases the activities of RNA polymerases I and II in kidney nuclei at the same time that these nuclei take up the androgen receptor complex. These enzyme activities are at a maximum after 1 to 2 hours and return toward base line by 4 hours after injection. Both activities increase again between 4 and 24 hours without subsequent hormone treatment. In addition, testosterone increases the template activity of renal chromatin as measured with highly purified renal RNA polymerase II (*81*). The changes in chromatin template capacity are associated with a testosterone-induced increase in the number of initiation sites on kidney chromatin for homologous RNA polymerases I and II (*82*). Finally, testosterone increases the amount of RNA polymerases II and III isolated by diethylaminoethyl-Sephadex chromatography (*82*). These observations indicate that some of the earliest effects of androgens on extragenital tissues are similar to those provided by sex steroids on the reproductive tract.

Although the progestin MPA stimulates early transcriptional events as does testosterone, its actions are different from testosterone in several respects (*82*). First, MPA does not stimulate a secondary increase in RNA polymerase II activity in isolated nuclei. Second, MPA treatment increases extractable RNA polymerase I, whereas no detectable change in this enzyme is observed with testosterone. The fact that neither testosterone nor MPA stimulates these transcriptional processes in Tfm mice indicates that an active androgen receptor is required for both steroids. One of the interesting problems posed by these observations is how two steroids that exert their action by way of the same receptor stimulate different patterns of transcriptional events. The magnitude of the measured changes in polymerase or chromatin activities does not correlate with subsequent protein synthesis. For example, the early responses in RNA polymerase and template activity (between 1 and 4 hours) are similar for testosterone and MPA, whereas kidney proteins such as alcohol dehydrogenase (ADH) and β-glucuronidase are always increased more by testosterone (*69, 72*). Furthermore, it has been observed that cyproterone acetate stimulates transcription (*83*) but has no effect on β-glucuronidase or ADH activity (*69, 72*).

Effects of androgens on specific kidney proteins. Testosterone increases the activities of many specific enzymes in kidney (*4, 6*). However, electrophoretic profiles of soluble renal proteins in extracts made before and after androgen administration revealed no significant differences. By contrast, electrophoretograms demonstrated an increase in proteins T1 and T2 in the mitochondrial-lysosomal fraction and T3 in the microsomal fraction in kidney extracts from testosterone-treated mice (*84*). Even though these proteins are highly in-

duced, they still represent only 1 to 3 percent of total protein, and further experiments will be required to establish them as useful markers of androgen action.

An alternative approach to identifying proteins synthesized in response to androgen treatment is to isolate kidney mRNA's from testosterone-treated mice, translate them in vitro, and analyze the translation products. Using this method, Toole *et al.* (*85*) identified a kidney androgen-dependent protein (KAP) that is highly induced by androgens. The activity of KAP mRNA represents 4 to 5 percent of the total mRNA activity in the androgen-treated kidney. Complementary DNA hybridization studies showed that KAP mRNA is the product of a single-copy gene that is expressed only in the kidney (*85*). Although its function remains obscure, KAP can be used as a marker for androgen effects in differential gene expression.

The most studied and perhaps most important androgen-induced protein in the kidney is β-glucuronidase. This enzyme is stable, easily assayed, and increases markedly during testosterone treatment. An extensive characterization of the genetic locus of β-glucuronidase has also been undertaken, and both structural and regulatory mutants have been identified. The regulatory mutants control the extent to which the β-glucuronidase gene responds to androgen stimulation. In spite of the fact that β-glucuronidase is less than 1 percent kidney protein, study of the enzyme has greatly increased our understanding of hormone action. For this reason we describe β-glucuronidase expression in the kidney in some detail.

β-Glucuronidase is a tetrameric protein (molecular weight, 280,000) found in the lysosomes of most cells. In the kidney and liver, it is also associated with microsomal membranes (*86*). In kidney but not in liver, enzyme activity increases 20- to 50-fold and enzyme synthesis increases 200-fold during testosterone treatment. The disparity between the two figures is accounted for by a large increase in the secretion of the active enzyme into the urine (*6*). A genetic analysis of β-glucuronidase activity in several strains of mice led to the identification of a complex locus designated Gus (*87*). The structural gene, *Gus-s*, is the source of both lysosomal and microsomal glucuronidase. The gene is near the distal end of chromosome 5, and the mutants at this locus code for a thermolabile enzyme (*Gus-s*^{h}) and two electrophoretic variants of β-glucuronidase (*Gus-s*^{a} and *Gus-s*^{b}) (*88-90*).

Mouse strains generally fall into two groups according to their responses to androgens. In some, the β-glucuronidase increases rapidly to a high plateau and, in others, the response is slower and lower. These different responses are defined by the regulatory locus, Gus-r, within the Gus complex. The A/J and C57BL/6J mice are typical of high-inducing and low-inducing strains, respectively (*89*). Strain A/J is homozygous for allele *Gus-r*^{a} and its glucuronidase can be induced to a level several times higher than C57BL/6J which is homozygous for the *Gus-r*^{b} allele as shown in Fig. 4. The regulatory locus acts in a *cis* manner on the structural gene (*89*) and, on the basis of breeding studies, is near or within the *Gus-s* structural gene (*91*). By analogy with bacteria one would expect to find transcription regulatory sequences adjacent to the structural gene that they regulate (*92*). Although several instances of nucleotide sequences similar to bacterial regulators being adjacent to eukaryotic genes have been reported, the only functionally characterized eukaryotic regulatory sequence is the one controlling transcription initiation of 5*S* RNA genes in *Xenopus* oocytes. This regulatory sequence is actually within the 5*S* RNA gene (*93*). Further studies of *Gus-r* and *Gus-s* should, therefore, provide insight into the structure and function of a hormonally controlled regulatory locus.

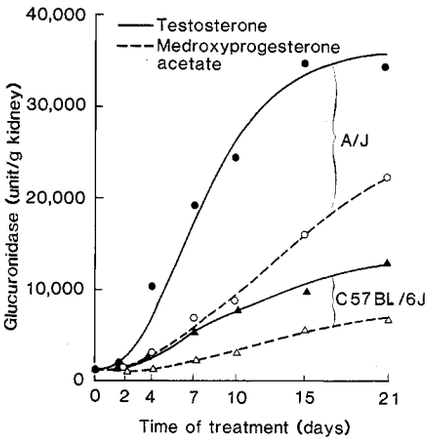

Fig. 4. The effect of testosterone and medroxyprogesterone acetate on β-glucuronidase activity in kidneys of female mice. A/J mice (●,○) are homozygous for the *Gus-r*^{a} allele, and C57BL/6J animals (▲,△) are homozygous for the *Gus-r*^{b} allele. [From Bardin *et al.* (*4*)]

Previous studies showed that *Gus-r* determines the rate of enzyme synthesis from *Gus-s* during androgen treatment (*89*). Recent experiments indicate that this is associated with an increase in β-glucuronidase mRNA activity (*94*). By analogy with other steroid responsive systems, it seems likely that *Gus-r* regulates glucuronidase synthesis, in part by affecting the synthesis of its mRNA. However, the extent to which the appearance of new enzyme molecules in response to testosterone treatment reflects transcriptional as opposed to posttranscriptional control remains to be established.

It is not known how genes are activated by steroid receptors. Acceptor sites in chromatin for androgen receptors are difficult to identify and have not been defined chemically. It is possible, however, that *Gus-r* represents a site of interaction between the hormone receptor complex and the chromosome. That *Gus-r* is a *cis*-acting regulator, that it does not affect the activity of inducible enzymes other than β-glucuronidase, and that it does not influence *Gus-s* in the absence of androgen are all compatible with this hypothesis. Furthermore, if this hypothesis is correct, the activity of androgenic progestins should be modulated by *Gus-r*. That this is, indeed, the case is shown in Fig. 4. Like testosterone, MPA stimulates *Gus-r*^{a} strains more than *Gus-r*^{b} The rate of synthesis and maximum concentrations of glucuronidase are lower in animals treated with MPA than in androgen-treated animals commensurate with the lower potency of this progestin (*4*). In view of these considerations, if *Gus-r* is a recognition site for androgen receptor, there must be separate sites in chromatin for other androgen-responsive genes.

Androgen Responses of the Liver

Sexual differences in liver have been reported in several species including rat, mouse, and man. These differences occur in (i) enzymes involved with drug and steroid metabolism; (ii) neonatal imprinting, that is, effects in the adult determined by early exposure to hormones; and (iii) secretory proteins such as the major urinary proteins (MUP's) in the mouse, and the similar α_{2u}-globulin in the rat. Although the androgenic control of the liver, as well as kidney, is modulated by pituitary, thyroid, and other steroid hormones, we discuss here only the effects of testosterone and its metabolites.

Drug and steroid metabolizing enzymes. The activities of enzymes participating in drug and steroid metabolism vary according to sex and species. The steroid hydroxylases in rat liver are androgen-dependent (*95*). For example, the concentrations of the 2β-, 6β-, and 18-hydroxylases are decreased in castrated animals but can be restored by testosterone treatment (*96*). In contrast, 5α-

reductase activity is higher in females than males and is stimulated by estradiol rather than testosterone (*97*). Thus sexual dimorphism in the liver is, in part, determined by estrogens. In addition to their effects on steroid metabolism, testosterone and estradiol regulate the activities of many microsomal enzymes that metabolize drugs. These dimorphic effects are highly dependent on the species and end point examined.

Agents that alter the activities of hepatic drug metabolizing enzymes are usually divided into three classes depending on the onset and duration of action, the microsomal components affected, and the specific reactions involved (*98*). One class of agents, which includes phenobarbital, enhances almost all the enzyme reactions associated with cytochrome P-450-dependent systems. Another class, which includes the polycyclic hydrocarbons such as 3-methylcholanthrene, stimulate only a few specific enzyme reactions that are dependent on a different hemoprotein, cytochrome P_1-450. The third class includes the steroids; these agents act specifically on certain enzymes although steroids of diverse structure may stimulate similar reactions in liver.

It is widely held that an increase in microsomal enzyme activity after testosterone administration does not represent a true androgenic response comparable to that seen in other tissues. However, other lines of evidence support the hypothesis that in liver some of the androgen-related effects are mediated by mechanisms similar to those in prostate: first, androgen receptors are present in liver (*99-101*), even though they are difficult to measure; second, testosterone does not increase drug metabolism in rats and mice with Tfm (*30*, *36*, *102*); and third, the antiandrogen flutamide blocks the androgen-induced increase of microsomal drug metabolism in normal rats and mice (*103*). The significance of the lack of testosterone effect on some enzymes in Tfm animals is shown by the normal hepatic responses of these animals to estrogens, progestins, phenobarbital, or 3-methylcholanthrene. That androgens stimulate an increase in liver weight and hepatic microsomal protein content in Tfm mice indicates that these responses are independent of the androgen receptor (*30*). Thus it is evident that some of the hepatic effects of testosterone are mediated by the androgen receptor as defined by the Tfm gene and some are mediated by other undefined mechanisms (Table 1).

Hepatic neonatal imprinting. Some steroid metabolizing enzymes in rat liver are androgen-dependent but do not decrease in castrated males (*104*). In such instances, the continued presence of testosterone is not required to maintain sexual dimorphism. Studies of animals castrated at birth indicate that only a brief exposure to androgen early in life is required to maintain the increased enzyme activity in the adult (*105*). This phenomenon has been called imprinting because a single dose of hormone at birth affects later enzyme development. This response is believed to be analogous to the hormonally programmed development in the central nervous system (*19*). In this instance a single exposure to testosterone in utero or at birth produces certain irreversible effects on the brain that may be identified with morphological, behavioral, or hormonal end points. Imprinting in the liver has been studied extensively, and the imprintable enzymes can be subdivided according to the amount of androgen required to produce the effect (*106*). As in other tissue-specific responses to androgens, imprinting is controlled by a multihormonal mechanism, for example, hormones produced in the adrenals, thyroid, and particularly the pituitary affect the imprinting of metabolic enzymes in the liver (*106*). That imprinting also influences drug metabolism is shown by the fact that the androgen response of ethylmorphine *N*-demethylase is reduced in animals deprived of androgen during the neonatal period (*107*).

Hepatic secretory proteins. The synthesis of hepatic proteins other than those involved in drug or steroid metabolism is also regulated by androgens. The best characterized examples are α_{2u}-globulin in the rat and the major MUP's of the mouse. Studies of Roy and colleagues (*63*, *108*) indicate that α_{2u}-globulin is synthesized and secreted by liver into blood. It is then rapidly cleared by the kidney and is, therefore, a major protein in the urine of male rats. The protein is absent in immature and female rats and appears in males at puberty (*109*). Testosterone treatment of ovariectomized females induces α_{2u}-globulin synthesis to approximately 80 percent of that of normal males after 8 days. Castration or estrogen treatment of mature males greatly reduces the concentration of α_{2u}-globulin (*110*).

The effects of both androgen and estrogen on α_{2u}-globulin are mediated through the control of its α_{2u}-globulin mRNA (*63*, *111*). Translation studies in vitro show that the mRNA activity parallels the induction or repression of α_{2u}-globulin during treatment with androgen or estrogen, respectively. In addition to being increased by testosterone, α_{2u}-globulin mRNA activity is increased by thyroid hormones, glucocorticoids, and growth hormone, and all four hormones are required for maximum stimulation of hypophysectomized animals (*112*).

The development of androgen sensitivity in the rat is an age-dependent phenomenon. Prepubertal males, like females, do not synthesize α_{2u}-globulin. However, androgens can induce production of α_{2u}-globulin in female animals but not in males until they are approximately 40 days of age. It is interesting that the senescent rat (> 750 days old) loses the ability to synthesize α_{2u}-globulin which, much as in the prepubertal male, cannot be induced even by high doses of testosterone (*100*). The appearance of α_{2u}-globulin synthesis in the immature male and its disappearance with age is correlated with the presence of a 3.5*S* androgen binding protein in cytosol. It has been suggested that the development of and structural changes in this 3.5*S* receptor are responsible for age-dependent androgen insensitivity (*63*). Regardless of the mechanism responsible for α_{2u}-globulin synthesis, the loss of this mechanism in old rats is of great importance. Since the hormonal responsiveness of many tissues diminishes with advancing age, studies of α_{2u}-globulin synthesis in liver can provide insights into how these changes occur.

Adult mice excrete in the urine a group of proteins with physical properties similar to those of α_{2u}-globulin. Females as well as males excrete MUP's, but males excrete the largest quantitites. However, excretion of MUP's can be increased in female mice by androgen treatment. The MUP's are a complex of at least three proteins of similar or identical molecular weights but differing charges (*113*). The protein sequence of each MUP is different, supporting the idea that each is from a distinct gene. Strain variants in the MUP complex are thought to be related to structural alleles at a genetic locus designated MUP_a. Recently, two components of the MUP phenotype have been described and used to analyze the genetic basis of androgen induction of MUP. The basal levels of MUP vary from strain to strain, and this phenotypic trait is not necessarily hormone-dependent. Androgen administration increases urinary excretion of MUP's, and it is the relative amounts of individual MUP's that are regulated by MUP_a during hormone treatment (*113*). These studies indicate that the MUP_a locus acts as a regulator and not as a site for structural allelism, as previously thought (*114*).

When mouse liver mRNA is translated in vitro, four related proteins that bind

MUP antibody are synthesized. When translated in the presence of a membrane fraction from dog pancreas, these four proteins are reduced in size so that three co-migrate with MUP's 1, 2, and 3, while the fourth represents a molecular weight variant (*115*). These studies suggest that MUP's are synthesized as precursor molecules that are processed upon secretion. They are thus similar to most other secretory proteins that contain an amino terminal signal peptide to facilitate transport into the endoplasmic reticulum (*116*).

The synthesis of MUP's is controlled by androgen regulation of RNA activity, as is the synthesis of other hormonally regulated proteins. Hybridization of MUP complementary DNA with liver RNA and analysis of products translated in vitro show that MUP mRNA is severalfold higher in males than females. In castrated males MUP mRNA is reduced to a level slightly higher than that of females. Whether these effects on mRNA activity occur at the level of transcription or translation, or both, remains to be established. Studies of the control of MUP synthesis are particularly interesting because the MUP complex contains multiple genes. Hybridization studies indicate that there may be as many as 25 MUP genes per haploid mouse genome (*117*). These studies suggest that the relative amounts of MUP's 1, 2, and 3 are related to the number of genes present for each individual MUP. Since MUP's and α_{2u}-globulin share common sequences they probably evolved from a common progenitor sequence. During evolution a single copy of this gene has been maintained in the rat whereas amplification has occurred in the mouse (*117*, *118*). It is not known whether different regulatory mechanisms are necessary for the control of single as opposed to multiple gene systems by androgens.

Androgen Responses of the Erythron

Hemoglobin synthesis in the erythron can be independently controlled by testosterone and erythropoietin (*119*). The secretion of erythropoietin by the kidney is increased by androgens and is believed to be mediated by way of the androgen receptor. In contrast, the direct steroid effects on bone marrow are mediated by 5β-androgen and 5β-progestin metabolites. Steroids with the 5β structure are more potent than their respective 5α epimers in stimulating hemoglobin synthesis in the erythroblasts of the chick blastoderm (*120*), and the effects of 5β steroids are mediated by a specific 5β steroid receptor in this embryonic blood-forming organ (*121*). Both 5β steroids and testosterone stimulate erythropoiesis in mice as measured by iron incorporation into the erythrocytes of polycythemic mice (*122*). Antibodies against erythropoietin abolish the effects of testosterone but not of the 5β steroids. These observations indicated that the 5β steroids have a direct effect on erythropoiesis that is independent of erythropoietin (*123*). A similar observation has been made in squirrel monkeys indicating that 5β steroids are active in a wide variety of species ranging from birds to primates (*124*). In many of these experiments 5β metabolites of both androgens and progestins were capable of stimulating hemoglobin synthesis (*25*).

The studies of Bullock and Besa (*26*) provide conclusive evidence that stimulation of erythropoiesis is not mediated by way of the classic androgen receptor that determines the action of testosterone on the reproductive tract. Twenty times more 5α-DHT is required to cause maximum iron incorporation into hemoglobin than 5β-DHT. The effect of both steroids is blocked by the antiandrogen flutamide, suggesting that this inhibitor can influence both the androgen and the 5β steroid receptors. It is significant that in Tfm mice the responses to 5α- as well as 5β-DHT are the same as in normal animals. Studies of the ability of these steroids to stimulate the pluripotential stem cell to divide provide similar conclusions: 5β-DHT is more effective than 5α-DHT, both effects are blocked by flutamide, and Tfm mice are as sensitive as normal animals.

Thus, observations in many species indicate that the pluripotential stem cell of bone marrow has evolved a unique receptor system that allows it to respond to 5β-androgen and 5β-progestin metabolites that have no measurable effect on the reproductive tract. That this system is not unique to bone marrow was demonstrated when it was found that 5β steroid metabolites stimulate porphyrin and δ-aminolevulinic acid synthetase formation in chick embryo liver cells (*24*), an observation that has subsequently been extended by other investigators (*125*).

Androgen Responses of Muscle

The major extragenital site of action of testosterone is sketetal muscle. Although the magnitude of androgen effect on this tissue is small relative to that on other extragenital sites such as kidney, part of the difference in body weight between men and women can be attributed to muscle mass. The ability of androgens to stimulate muscle growth correlates with their ability to increase the retention of dietary nitrogen. This latter response is of historic interest since it is used to measure the biological action of this class of steroids in humans (*2*). The magnitude of the response produced by testosterone varies widely between muscles and among species (*2*). In addition, cardiac muscle mass and enzyme activities are increased with androgen treatment in parallel with overall skeletal muscle growth (*126*). However, the muscle showing the greatest response to testosterone is the levator ani (dorsal bulbocavernosus) in the pelvic area. Treatment with a wide variety of steroids suggests that the effects of testosterone on the levator ani are greater than would be expected from assays with other muscles (*2*); such assays also indicate that estrogenic metabolites of testosterone have a marked effect on the levator ani but not on other muscles.

Testosterone is not metabolized extensively in skeletal muscle except in the levator ani, which has a limited capacity to convert testosterone to 5α-DHT (*127*) and estrogens (*20*). The levator ani responds to estradiol synthesized from testosterone with an increase in gluco-6-phosphate dehydrogenase (*20*). This effect is not mimicked by the non-aromatizable androgens such as fluoxymesterone or 5α-DHT and is blocked both by aromatase inhibitors and antiestrogens. However, the effects of testosterone on thymidine incorporation into cultured myogenic cells (*128*), RNA synthesis in skeletal muscle (*129*), and general muscle growth cannot be mimicked by estrogens (*2*). It is the inability of muscle to synthesize 5α-DHT that suggests that most of these effects are probably mediated by testosterone per se.

The molecular basis for the myotrophic actions of androgens on skeletal and cardiac muscle has been difficult to understand. Early studies with conventional techniques failed to provide evidence for androgen receptors in these tissues, but subsequent experiments showed that nuclei and cytosols prepared from perineal and skeletal muscles from rodents possess androgen receptors (*127*, *130*). That testosterone rather than 5α-DHT is the dominant receptor-bound steroid in these tissues is a result of the 5α-DHT that is formed being rapidly metabolized to androstanediols (*127*). A similar pattern of androgen metabolism and receptor binding occurs in a variety of other extragenital tissues including kidney, submaxillary gland, pituitary, brain, and some tumors (*131*). Radio-

autographic studies show that androgen receptors are present in atrial and ventrical myocardial cells, suggesting that androgens may affect myocardial cells directly to increase cardiac weight and enzyme activities (*132*).

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Sexual Differentiation of the Central Nervous System

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Summary. Sexual differentiation of reproductive and behavior patterns is largely effected by hormones produced by the gonads. In many higher vertebrates, an integral part of this process is the induction of permanent and essentially irreversible sex differences in central nervous function, in response to gonadal hormones secreted early in development.

In many species marked sex differences in the control of endocrine function and behavior by the central nervous system (CNS) are an integral part of the reproductive process, including the recognition of a suitable sexual partner, in mating, and in the subsequent production and rearing of young. Sex differences in central nervous function represent the outcome of interactions between several different factors, among which the hormones secreted by the gonads are of paramount importance.

Current concepts of CNS sexual differentiation have their origins in a series of experiments, performed almost 50 years ago by Pfeiffer (*1*). His experiments with the laboratory rat showed that the expression of masculine patterns of pituitary gonadotropin secretion in adulthood depended on factors released from the testes during early postnatal life. Thus, the development of masculine patterns of gonadotropin secretion could be induced in genetic females by transplantation of a testis into the neck shortly after birth whereas castration of genetic males at birth resulted in the development of characteristically feminine patterns of gonadotropin release (*1*). With the later demonstration that the functions of pituitary are regulated by the hypothalamus, it became clear that the testes must influence the development of centers located within the brain (*2*).

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Many other sexually differentiated neuroendocrine functions and behaviors are also dependent on early gonadal secretions. A general hypothesis has been formulated for the mechanism of CNS sexual differentiation which has much in common with the model for differentiation of the peripheral reproductive tract (*3*). The intrinsic pattern of CNS development is assumed to be organized along lines that are appropriate for the homogametic sex. In the heterogametic sex, differentiation away from this pattern occurs as a result of hormones produced by the gonads. Thus, in mammals the intrinsic pattern is female, with differentiation toward masculine patterns of gonadotropin secretion and behavior occurring in the male as a result of exposure to testicular hormones during development (*4*). In birds the homogametic sex is male, and differentiation of the female CNS phenotype occurs as a result of exposure to ovarian hormones (*5*).

This simple mechanism is not the sole determining factor in sexual differentiation of the CNS. In many cases, however, there is good evidence that early hormonal experience makes at least some contribution.

Role of Early Gonadal Hormone Secretions

Although sex differences in CNS function exist in a great many vertebrate phyla, it is only in birds and mammals that these differences can be attributed to early gonadal hormone secretions. In fishes and amphibia, there are species in which early exposure to gonadal steroids induces complete sex reversal (*6*); but there is insufficient evidence to ascertain whether these effects involve a permanent developmental change in the CNS, or if they reflect primarily hormone-induced differentiation of peripheral structures.

The effects of early gonadal hormone secretions on mammalian and avian CNS function are extensive and diverse (Table 1). In addition to reproductively oriented functions, such as sex behavior and the control of gonadotropin secretion, sex differences in a large number of other behavioral and neuroendocrine end points to some extent depend on early gonadal hormone secretions.

The diversity in the developmental effects of gonadal hormones raises the question of whether one or many different hormone-sensitive mechanisms are involved in CNS sexual differentiation. Although we cannot answer this question definitively, there is increasing evidence that separation of different developmental responses to gonadal hormones can occur. Species differ in the extent to which CNS functions are influenced by early gonadal hormone exposure. In rodents, early exposure to androgens from the developing testes results in permanent suppression of the capacity to support cyclic feminine pat-

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