5α-REDUCTASE DEFICIENCY REVISITED

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On November 8, 1973 I gave these grand rounds on hereditary male pseudohermaphroditism. In that talk I summarized the current level of understanding of androgen action and sexual differentiation and described the genetic and endocrine evidence that we had put together for separation of the hereditary defects in androgen action into four distinct entities -- complete testicular feminization, incomplete testicular feminization, the Reifenstein syndrome, and pseudovaginal perineoscrotal hypospadias. After the rounds Zaven Chakmakjian told me that his brother Souren Chakmakjian had seen two sisters with some unusual form of male pseudohermaphroditism and that he thought the patients would be of interest to us. The girls were subsequently hospitalized by James Marks in the Children's Medical Center and proved to be of interest indeed. As the result of endocrine, phenotypic, and enzymatic characterization of these patients it was established that the recessive disorder previously described under the eponymic term pseudovaginal perineoscrotal hypospadias is due to a defect in the conversion of testosterone to its active metabolite dihydrotestosterone by the 5α-reductase enzyme. In the intervening years we (and others) have spent a great deal of effort in investigating the pathogenesis of this disorder. 5α-Reductase deficiency is of interest not because of its frequency (it is rare) but because of its importance for understanding the mechanism of androgen action, the normal process of sexual differentiation, and the factors that influence human sexual behavior. I propose this morning to review briefly the thinking that went into the recognition of the etiology of this disorder, to summarize our own studies and those of our collaborators on 19 affected individuals from 14 different families with 5α-reductase deficiency, to describe the modes of therapy now available, and to provide a progress report on our current state of thinking about the mechanisms by which dihydrotestosterone formation is important in androgen action and in influencing sexual behavior.

ANDROGEN ACTION

Testosterone, the major androgen secreted by the testis and the major androgen in plasma, is itself a potent hormone and also serves as the precursor (or prohormone) for two other active hormones -- estradiol and dihydrotestosterone (Fig. 1). Dihydrotestosterone is the intracellular mediator of certain androgen actions and also circulates in blood; the circulating hormone is derived primarily by

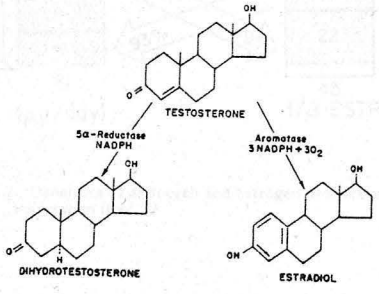


Fig. 1. Testosterone serves as a prohormone for the formation of two other active hormones, dihydrotestosterone and estradiol (Ref. 1).

conversion from testosterone in peripheral tissues and to a lesser extent by direct secretion by the testes into the circulation. The plasma level of dihydrotestosterone is on average about one-tenth that of testosterone (1).

1. Wilson JD: Metabolism of testicular androgens, in Handbook of Physiology Section 7: Endocrinology, Vol V, Male Reproductive System, RO Greep, EB Astwood (eds). Washington: American Physiological Society, 1975, p 491.

The exact function of estrogen in normal men remains to be established; in some species the hormone plays a role in normal male sexual drive. However, excess estrogen -- either relative or absolute -- causes profound feminization in men, particularly the induction of gynecomastia (2). As a consequence, estrogen plays a major role in determining the final phenotype in several disorders of androgen action. For this reason it is necessary to understand the dynamics of estrogen and androgen production and metabolism in the normal man (Fig. 2). As measured by isotope-dilution techniques the production rates of estradiol and estrone, respectively, average about 45 and 60 μg/day in normal men, and plasma production rates of testosterone and androstenedione, respectively, average about 6000 and 3000 μg/day (3). As a consequence, the ratio of the production rate of testosterone to that of estradiol in normal men is about 100 to 1. All the estrone and about 85 percent of the estradiol is derived from peripheral conversion from androstenedione and testosterone. Thus, in normal men an average of only 6 to 10 μg estradiol is secreted directly into the circulation by the testes (3, 4). However, when large amounts of human chorionic gonadotropin (hCG) are administered to normal men (or when plasma luteinizing hormone activity is elevated in pathological states) direct secretion of estrogen by the stimulated testes increases in proportion to the increase in the secretion of testosterone (5). In summary,

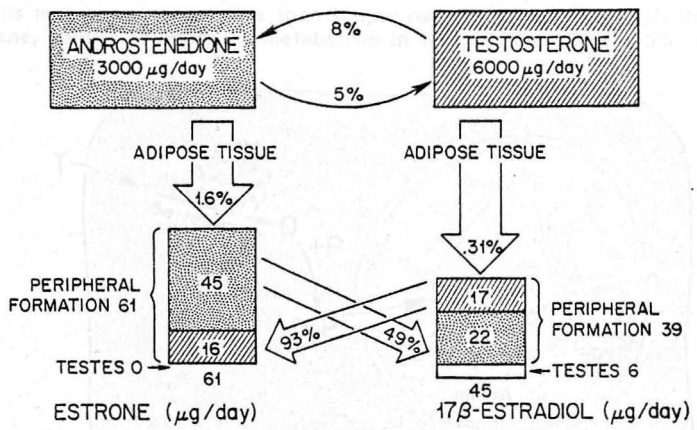


Fig. 2. Dynamics of androgen and estrogen production in 4 normal young men (Ref. 3)

under normal conditions most estrogen in men is formed by peripheral aromatization of circulating androgens, but when gonadotropin concentrations are elevated the testis may secrete significant amounts of estrogen directly into the circulation. Feminization of men can result when the normal hundred-fold excess of androgens to estrogens is disturbed either by an increase in estrogen production or by a decrease in testosterone formation (or action) under circumstances in which estrogen production remains appreciable (2).

2. Wilson JD, Aiman J, MacDonald PC: The pathogenesis of gynecomastia. Adv Int Med 25:1, 1980.

3. MacDonald PC, Madden JD, Brenner PF, Wilson JD, Siiteri PK: Origin of estrogen in normal men and in women with testicular feminization. J Clin Endocrinol Metab 49:905, 1979.

4. Siiteri PK, MacDonald PC: Role of extraglandular estrogen in human endocrinology, in Handbook of Physiology, Section 7: Endocrinology, Vol 2, RO Greep, EB Astwood (eds). Washington: American Physiological Society, 1973, p 615.

5. Weinstein RL, Kelch RP, Jenner MR, Kaplan SL, Grumbach MM: Secretion of unconjugated androgensand estrogens by the normal and abnormal human testis before and after human chorionic gonadotropin. J Clin Invest 53:1, 1974.

The current concepts of the mechanisms by which androgens exert their physiological actions in a target tissue such as the prostate are summarized in Fig. 3. Testosterone, the principal androgen secreted by the testis and the major androgen in plasma of men, circulates bound to two proteins, testosterone-binding globulin (TeBG, also termed sex-hormone binding globulin or SHBG) and albumin. The protein-bound steroid is in dynamic equilibrium with unbound or free hormone, the latter comprising 1 to 3 percent of the total. Although the mechanism of entry into cells may be more complex than simple passive diffusion through the plasma membrane, studies of androgen metabolism in the prostate suggest that the entry

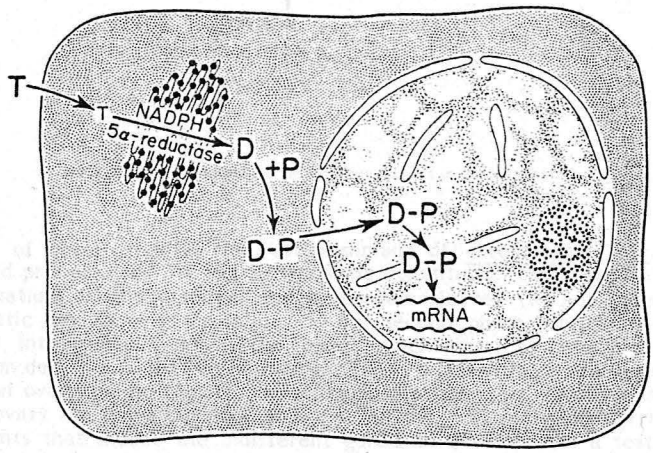


Fig. 3. Schema of androgen action in prostate.

process is not energy dependent and that free steroid, rather than a steroid-protein complex, enters the prostate cell by a passive mechanism. The fact that the concentration of testosterone in most androgen target tissues is lower than that of plasma is in keeping with this interpretation (6).

Inside the cell testosterone can be reduced to dihydrotestosterone by the 5α-reductase enzyme. This serves to keep the intracellular concentration of testosterone low and thus promotes the diffusion of testosterone into the cell down an activity gradient. Dihydrotestosterone is bound to a high affinity receptor protein (P) in the cell cytoplasm. The androgen receptor complex (DP) enter the nucleus, become attached to the chromatin of the cell, and in some way promote the transcription of messenger RNA. There is no uniform agreement on the size or properties of the androgen receptor or on the nature of the chemical transformations (if any) that the hormone-receptor complexes must undergo before becoming active, but it appears that the major native receptor in cytosol is large (8S or greater) and that the form recoverable from the nuclear chromatin is smaller in size (4S or less). The mechanism by which the androgen-receptor complexes effect the transcription of stored genetic information also is not established with certainty. After this schema of androgen action was formulated in the 1960's we turned our attention to androgen action in the male embryo, where the hormone promotes development of the male anatomy.

6. Siiteri PK, Wilson JD: Dihydrotestosterone in prostatic hypertrophy. I. The formation and content of dihydrotestosterone in the hyperprophic prostate of man. J Clin Invest 49:1737, 1970.

NORMAL SEXUAL DEVELOPMENT IN THE MALE

As formulated by Jost, normal sexual development in the mammal consists of three sequential, ordered, and interrelated processes (7).

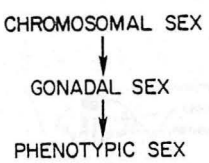


Fig. 4. Jost Paradigm for Sexual Development in the Mammal.

The first of these involves the establishment of chromosomal sex. This is determined primarily by the sex chromosome constitution established at the time of fertilization; in the mammal the heterogametic sex (XY) is male, and the homogametic sex (XX) is female. In the second phase, chromosomal sex is translated into gonadal sex. The exact mechanisms by which the genetic information determines that an indifferent gonad differentiates into a testis in the male or an ovary in the female and secretes the hormones characteristic of the testis or ovary are not understood entirely, but the Y chromosome carries genetic determinants that induce the indifferent gonad to develop into a testis. These determinants are either identical to or closely linked to a differentiative antigen termed H-Y antigen (8). The final process, the translation of gonadal sex into

phenotypic sex, is the direct consequence of the type of gonad formed and the resulting endocrine secretions of the fetal testis. In the formation of phenotypic sex, indifferent internal and external genital anlagen are converted to a male or female form, and the sexual, behavioral, and functional characteristics are ultimately determined.

The events involved in the development of phenotypic sex are summarized in Fig. 5. The internal genitalia arise from the wolffian and mullerian ducts, both of which are present in early embryos of both sexes. The wolffian ducts are the

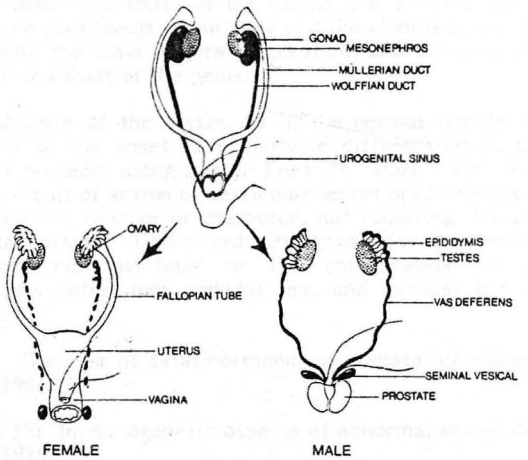


Fig. 5A. Differentiation of the male and female urogenital tracts

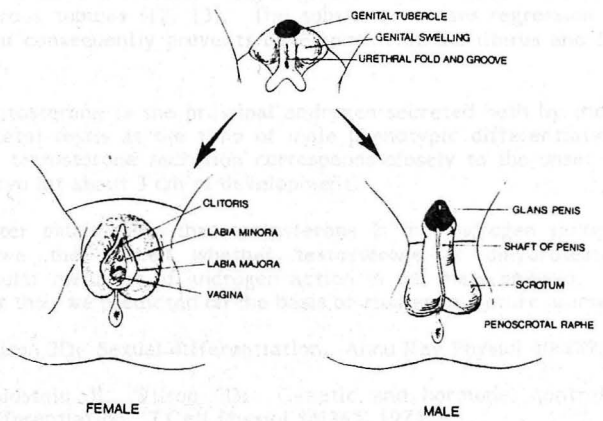


Fig. 5B. Differentiation of the external genitalia in the two sexes.

excretory ducts of the mesonephric kidney and are physically connected with the indifferent gonad. The mullerian duct forms secondarily from the wolffian duct and is not contiguous with the gonad. In the male the wolffian ducts gives rise to the epididymides, vasa deferentia, and seminal vesicles. In the female the mullerian ducts give rise to the fallopian tubes, uterus, and upper vagina. Thus, the internal genital tracts in males and females develop from different anlagen. In contrast, the external genitalia and urethra of both sexes develop from common anlagen, the urogenital sinus and the genital tubercle, folds, and swellings. In the male the urogenital sinus gives rise to the prostate and prostatic urethra and in the female to the lower two-thirds of the vagina and urethra. The genital tubercle is the anlage of the glans penis in the male and the clitoris in the female. The genital swellings become the labia majora or scrotum, and the genital folds develop into labia minora or the shaft of the penis.

In the absence of the testes, as in the normal female or in male embryos castrated prior to the onset of phenotypic differentiation, the development of phenotypic sex proceeds along female lines (7). Thus, masculinization of the fetus is the positive result of action by testicular secretions, whereas development of the female phenotype is passive in character, not requiring the action of a hormone from the fetal ovary. Under ordinary conditions development of the sexual phenotype conforms faithfully to the chromosomal or genetic sex, i.e., chromosomal sex determines gonadal sex, and gonadal sex in turn determines phenotypic sex.

7. Jost A: The role of fetal hormones in prenatal development. Harvey Lect 55:201, 1961.

8. Wachtel SS: Immunogenetic aspects of abnormal sexual differentiation. Cell 16:691, 1979.

Three hormones control the development of the male phenotype (9-11) (Table I). Two of the hormones -- mullerian inhibiting substance and testosterone -- are secretory products of the fetal testes. Mullerian inhibiting substance is an incompletely characterized product of the embryonic testis, probably a macromolecule with a molecular weight greater than 15,000 that is secreted by the seminiferous tubules (12, 13). The substance causes regression of the mullerian ducts and consequently prevents development of the uterus and fallopian tubes in the male.

Testosterone is the principal androgen secreted both by the adult testis and by the fetal testis at the time of male phenotypic differentiation (14, 15). The onset of testosterone secretion corresponds closely to the onset of virilization of the embryo (at about 3 cm of development).

After establishing that testosterone is the androgen secreted by the fetal testis, we then asked whether testosterone or dihydrotestosterone is the intracellular mediator of androgen action in the male embryo. The results were different than we predicted on the basis of studies in mature animals.

9. Wilson JD: Sexual differentiation. Annu Rev Physiol 40:279, 1978.

10. Goldstein JL, Wilson JD: Genetic and hormonal control of male sexual differentiation. J Cell Physiol 85:365, 1975.

11. George FW, Wilson JD: Sexual differentiation, in Fetal Physiology and Medicine, RW Beard, PW Nathanielsz (eds), in press.

12. Picard J-Y, Tran D, Josso N: Biosynthesis of labelled anti-mullerian hormone by fetal testes: evidence for the glycoprotein nature of the hormone and for its disulfide-bonded structure. Mol Cell Endocrinol 12:17, 1978.

13. Donahue PK, Ito Y, Price JM, Herndon WH III: Mullerian inhibiting substance activity in bovine fetal, newborn, and prepubertal testes. Biol Reprod 16:238, 1977.

14. Wilson JD, Siiteri PK: Developmental pattern of testosterone synthesis in the fetal gonad of the rabbit. Endocrinology 92:1182, 1973.

15. Siiteri PK, Wilson JD: Testosterone formation and metabolism during male sexual differentiation in the human embryo. J Clin Endocrinol Metab 38:113, 1974.

As the result of studies of androgen metabolism in embryos of several species including man it was deduced that testosterone promotes virilization of the urogenital tract in two different ways. Testosterone acts directly to stimulate the wolffian ducts and induce development of the epididymides, vasa deferentia, and seminal vesicles. In fact, as illustrated in Fig. 6, differentiation of the wolffian ducts into seminal vesicle and epididymis is completed in the human male embryo

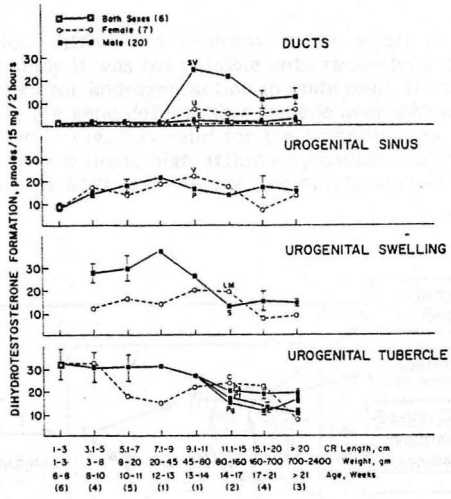


Fig. 6. Dihydrotestosterone formation in the urogenital tissues of the human embryo (Ref. 10).

(≅9 cm of development) before the capacity to form dihydrotestosterone is acquired by these tissues (15). In contrast, in the remaining tissues of the male urogenital tract testosterone acts as a prohormone for the third hormone of fetal virilization, dihydrotestosterone. Dihydrotestosterone, which does not appear to be synthesized in appreciable quantities by the fetal testes at the time of male

phenotypic development, is formed by enzymatic reduction of testosterone within the urogenital sinus and lower urogenital tract before male differentiation of these tissues takes place (15). Dihydrotestosterone acts in the urogenital sinus to induce formation of the male urethra and prostate and in the urogenital tubercle, swelling, and folds to cause the midline fusion, elongation, and enlargement that eventuate in the male external genitalia.

Thus, three hormones control male phenotypic development (Table I).

Table I. Hormonal Control of Male Phenotypic Sex Differentiation

		Phase of Phenotypic Differentiation		
Gonadal Hormone	Intracellular Hormone	Mullerian Duct Regression	Wolffian Duct Differentiation	Virilization of Urogenital Sinus and External Genitalia
Mullerian Inhibiting Substance	?	+		
Testosterone	Testosterone		+	
	Dihydrotestosterone			+

Due to technical difficulties inherent in the small amounts of embryonic tissues available for study it was not possible until recently to characterize directly the receptor machinery for androgen action in embryonic tissues. However, as the result of studies of single gene defects that impede androgen action it was deduced that the schema shown in Fig. 7 is valid for the embryonic as well as for postnatal androgen action, namely a single high affinity cytosolic receptor is responsible for mediating the actions of both testosterone and dihydrotestosterone (16). It is also

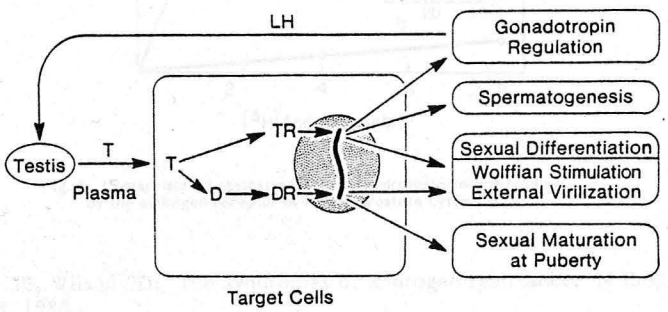


Fig. 7. Current concepts of the roles of testosterone and dihydrotestosterone in androgen physiology.

established that the androgen receptor mechanism is fundamentally the same in male and female embryos; namely, when female embryos are exposed to androgens at the appropriate time in embryonic development both the wolffian duct and external genitalia virilize in the characteristic male fashion (9). Thus, the differences in male and female phenotypic development are due solely to the action of hormones produced by the fetal gonads at the critical period of embryonic development and not to differences in the receptor machinery for the hormones. The validity of this model for androgen action in the embryo has been confirmed only recently. The androgen receptor of human prostate binds dihydrotestosterone approximately 10-fold as well as testosterone (17), and we now believe that the formation of dihydrotestosterone probably magnifies androgen action but is not an absolute requirement.

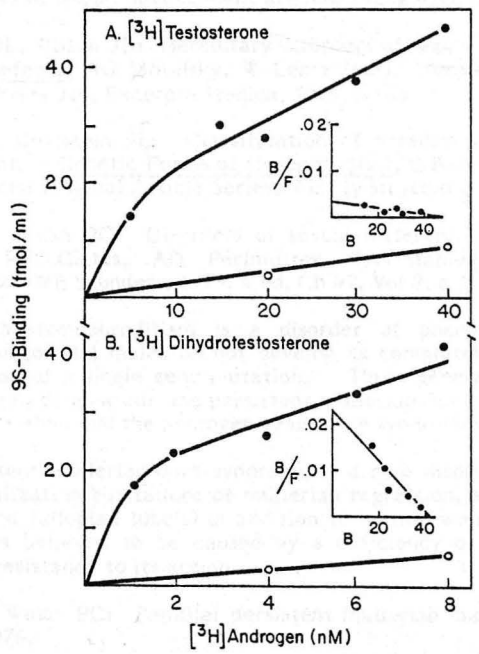


Fig. 8. Comaprison of testosterone and dihydrotestosterone binding by the androgen receptor of human prostate cytosol (17).

16. Griffin JE, Wilson JD: The syndromes of androgen resistance. N Engl J Med 302:198, 1980.

17. Wilbert DM, Griffin JE, Wilson JD: Characterization of the cytosol androgen receptor of the human prostate. Submitted for publication.

DISORDERS OF SEXUAL DEVELOPMENT

A disturbance during embryogenesis at any step of sexual differentiation may give rise to a disorder of sexual development. On theoretical grounds, these disorders can be classified meaningfully in terms of the initial developmental stage influenced by the mutant gene, e.g., errors in chromosomal sex, errors in gonadal sex, or errors in phenotypic sex. Such abnormalities may arise by several mechanisms, such as environmental insult (as ingestion of a virilizing drug during pregnancy), a nonfamilial aberration in the sex chromosomes (as in 45,X gonadal dysgenesis), a developmental birth defect of multifactorial etiology (as in most cases of hypospadias), or a hereditary disorder resulting from a single gene mutation (as in the testicular feminization syndrome). A minimum of 19 simply inherited disorders of sexual development are now recognized (18-20).

18. Goldstein JL, Wilson JD: Hereditary disorders of sexual development in man, in Birth Defects, AG Motulsky, W Lentz (eds). Amsterdam: International Congress Series 310, Excerpta Medica, 1974, p 165.

19. Wilson JD, Goldstein JL: Classification of hereditary disorders of sexual development, in Genetic Forms of Hypogonadism, D Bergsma (ed). New York: Birth Defects: Original Article Series, Vol 11, Stratton Corp, 1975, p 1.

20. Wilson JD, Walsh PC: Disorders of sexual differentiation, in Urology, JH Harrison, RF Gittes, AD Perlmutter, TA Stamey, PC Walsh (eds). Philadelphia: WB Saunders, 1979, 4 ed, Ch 42, Vol 2, p 1484.

Male pseudohermaphroditism is a disorder of phenotypic sex in which chromosomal and gonadal males do not develop as completely normal men, most commonly because of a single gene mutation. Three general categories of such disorders have been delineated: the persistent mullerian-duct syndrome, defects of testosterone formation, and the androgen-resistance syndromes.

The persistent mullerian-duct syndrome is a rare disorder characterized by normal male virilization but failure of mullerian regression, so that affected men have a uterus and fallopian tube(s) in addition to normal wolffian structures (21). This syndrome is believed to be caused by a deficiency of mullerian inhibiting substance or by resistance to its action.

21. Sloan WR, Walsh PC: Familial persistent mullerian duct syndrome. J Urol 115:459, 1976.

Disorders of testosterone formation result either from poorly understood developmental abnormalities in the testis or from a deficiency in any of the five enzymes necessary for testosterone synthesis from cholesterol: 20,22-desmolase, 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase, 17,20-desmolase, or 17β-hydroxysteroid dehydrogenase. The latter disorders result in a spectrum of defects in virilization of affected males and are discussed in detail in Ref. 20.

The third type of disorder, androgen resistance, accounts for approximately three-fourths of cases of male pseudohermaphroditism. Testosterone synthesis and mullerian-duct regression are normal, but because of a defect in some aspect of androgen action, affected persons are resistant to the hormone during embryogenesis as well as in the postnatal state. On the basis of the model shown in Fig. 7 it was predicted on theoretical grounds that androgen resistance could result from

at least three different types of lesions in the pathway of androgen action and that these lesions would have different phenotypic consequences -- namely, defects in the 5α-reductase enzyme, defects in the androgen receptor, or defects in the intranuclear events in androgen action.

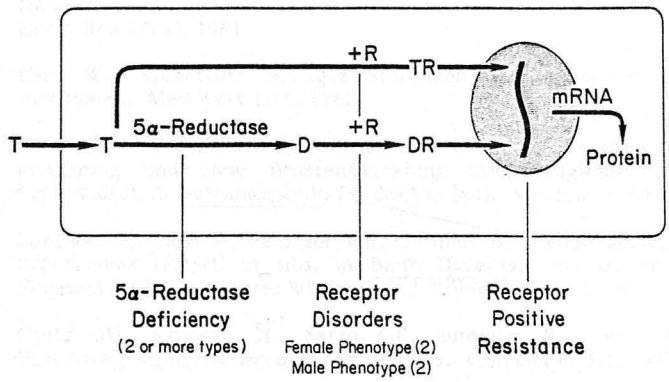


Fig. 9. Types of androgen resistance

The latter two categories of androgen resistance were covered in detail in a recent grand rounds by James Griffin. The focus of this discussion is upon the first category -- 5α-reductase deficiency. After the model of androgen action was formulated and the role of testosterone in virilizing the wolffian duct are recognized, Joe Goldstein pointed out to me that a genetically distinct disorder had been described under the eponymic term pseudovaginal perineoscrotal hypospadias in which the phenotype is what would be predicted for 5α-reductase deficiency (18, 19).

5α-REDUCTASE DEFICIENCY

Clinical Features

Pseudovaginal perineoscrotal hypospadias was defined on clinical and genetic grounds in 1961 by Nowakowski and Lenz (22-24). Subsequent patients were described by Simpson et al (25) and by Opitz and coworkers (26). Affected persons are 46,XY males who have an autosomal recessive disorder characterized by an external female phenotype at birth, bilateral testes and normally virilized wolffian structures that terminate or empty in the vagina. In the original description, normal male testosterone production was assumed to be present, but the disorder was described at a time when plasma testosterone was difficult to measure. As a consequence, in these early studies testosterone production was documented to be normal only in some affected individuals, and one of the families originally described with the syndrome was subsequently shown to have a hereditary defect in

testosterone synthesis (27). There can, however, be little doubt in retrospect that most of the patients described under this nosology do have 5α-reductase deficiency. Since we were on the lookout for such patients, the girls referred by Dr. Chakmakjian were of particular interest since they proved to be the first patients documented to have 5α-reductase deficiency.

22. Nowakowski H, Lenz W: Genetic aspects in male hypogonadism. Recent Prog Horm Res 17:53, 1961.

23. Lenz W: Genetisch bedingte Storungen der weiblichen Fortpflanzungsfunktionen. Med Welt 1:16, 1962.

24. Lenz W: Pseudohermaphroditismus masculinus externus mit Sckundarbehaarung und ohne Brustentwicklung (pseudovaginale, perineoskrotalehypospadie), in Humangenetik, PE Becker (ed). Stuttgart: Verlag, 1964, p 385.

25. Simpson JL, New M, Peterson RE, German J: Pseudovaginal perineoscrotal hypospadias (PPSH) in sibs, in Birth Defects, Original Article Series, D Bergsma (ed). Baltimore: Williams and Wilkins, Vol 7, p 140.

26. Opitz JM, Simpson JL, Sarto GE, Summitt RL, New M, German J: Pseudovaginal perineoscrotal hypospadias. Clin Genet 3:1, 1972.

27. Givens JR, Wiser WL, Summitt RL, Kerber IJ, Andersen RN, Pittaway DE, Fish SA: Familial male pseudohermaphroditism without gynecomastia due to deficient testicular 17-ketosteroid reductase activity. N Engl J Med 291:938, 1974.

Case Reports

Case 1. The index patient, born on August 13, 1959, is a phenotypic black girl and was thought to be normal until the age of 13, when the family noted failure of breast development and of menses, with a deepened voice. About six months later a growth spurt was noted. She had a masculine habitus, low voice, no breast tissue, bilateral inguinal masses, a large phallus (5 by 1.5 cm), and a 5-cm, blind-ending vagina (Fig. 11). Intravenous pyelography, barium-enema study and voiding cystourethrography gave normal results. Cystoscopy demonstrated a normal female urethra. The karyotype was 46,XY. Plasma levels of LH, FSH, testosteorne, dihydrotestosterone, androstenedione, estradiol and estrone were all within the range of normal men. Measurements of testosterone and androstenedione blood production rates and interconversion and of estradiol and estrone production rates and interconversion were performed in the Clinical Research Center.

An exploratory laparotomy was then performed. The testes were dissected from pouches at the external inguinal rings. The vasa deferentia were dissected, and 1 ml of 50 percent diatrizoate sodium was injected into the lumen of each. X-ray study of the abdomen revealed bilateral ampullae of the vasa deferentia, normal seminal vesicles and normal ejaculatory ducts that emptied into the vagina. No uterus or other female structures were found in the pelvis. The testes (4.0 by 2.5 by 1.5 cm) were removed. On microscopical examination, the basic architecture of the testes was normal. The seminiferous tubules contained many well developed Sertoli cells. Many spermatogonia and primitive spermatocytes and some secondary spermatocytes and spermatids were seen, but no spermatozoa were

found. The Leydig cells were normal. The entire histologic picture was consistent with a pubertal testis with early spermatogenesis but without mature sperm. The epididymis was of a normal male character, with columnar development of the tubules. A resection of the clitoral shaft was performed. The patient was placed on cyclical estrogen therapy.

Case 2. This girl, a younger sibling of Case 1, was noted at the time of birth in 1962 to have an enlarged clitoris. At 2 1/2 months of age she was evaluated and found to have palpable gonads in the labial folds, an enlarged clitoris, no evidence of a vaginal orifice and a negative chromatin pattern. An intravenous pyelogram was normal. At two years of age exploratory laparotomy and circumcision were performed at the Ben Taub General Hospital in Houston. No female genitalia were found in the pelvis, and both testes were removed. She was subsequently reared as a female and at no time did any voice change, pubertal growth spurt, breast enlargement, masculine habitus or pubic hair develop. At the age of 11 she was admitted to the Children's Medical Center, Dallas, for evaluation. The karyotype was 46,XY. Voiding cystography revealed a normal bladder and normal female urethra. No vaginal opening could be identified. Cystoscopy demonstrated a female urethra with a shallow, saccular outpouching of the floor of the urethra near the meatus. Because of the enlarged clitoris, a resection of the clitoral shaft was performed.

The family history is compatible with (but not pathognomonic of) an autosomal recessive defect.

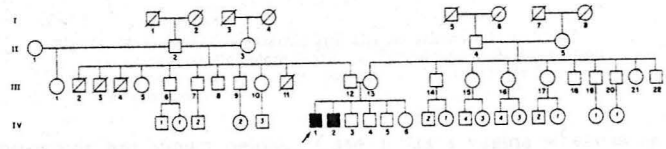


Fig. 10. Family tree of the first Dallas family with 5α-reductase deficiency.

The external genitalia of Case 1 are shown in panel C in the figure below. The clitoromegaly is striking, but separate urethral and vaginal orifices can be seen.

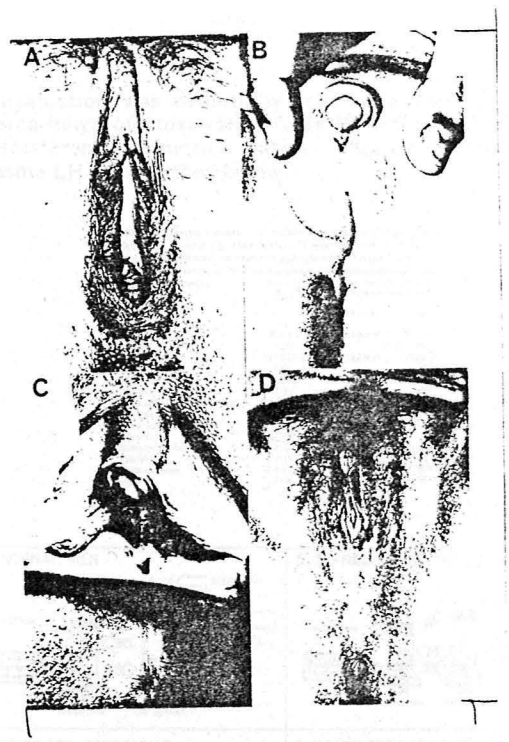


Fig. 11. Representative external genitalia in subjects with androgen resistance. A. Complete Testicular Feminization. B. Reifenstein Syndrome. C. 5α-Reductase Deficiency. D. Incomplete Testicular Feminization.

The urethrograms are shown below. Case 1 has a vagina whereas in Case 2 no vagina is present -- only a dimple in the posterior urethra.

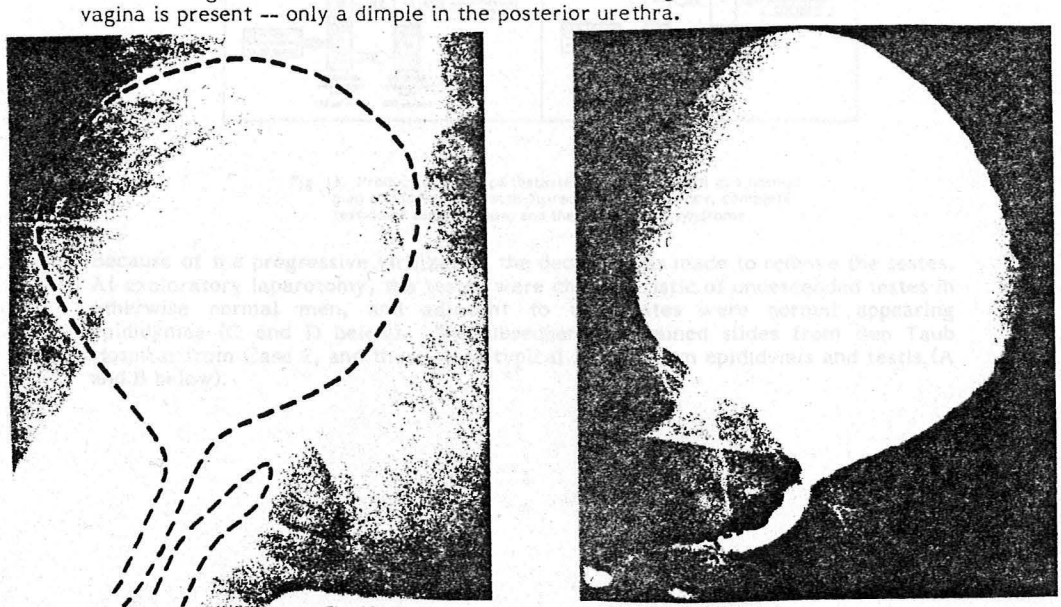


Fig. 12. Urethrograms of Case 1 (left) and Case 2 (right)

The endocrine evaluation was essentially that of a normal man with low (but measurable) plasma dihydrotestosterone (Table II) and normal plasma testosterone and normal testosterone production rates. (Subsequent patients have slight elevations of plasma LH as described below.)

Table II Plasma Levels of Gonadotropins, Androgens and Estrogens in Normal Men and in Patients with Familial Incomplete Male Pseudohermaphroditism, Types 1 and 2.

Measurement	Normal Men	Familial Incomplete Male Pseudohermaphroditism	
		Type 2 (case 1)	Type 1
Plasma LH (ng/ml)	50±3.3 (35)[1]	46.5±5.4[a]	111±9.2 (9)
Plasma FSH (ng/ml)	223±12.5 (33)	254±9.2[a]	150±32 (9)
Plasma testosterone (ng/ml)	6.1±0.5 (10)	6.9	9.2±0.54 (9)
Plasma dihydrotestosterone (ng/ml)	0.53±0.06 (9)	0.45±0.05[a]	—
Plasma androstenedione (ng/ml)	1.35±0.18 (10)	1.06	—
Plasma estradiol (pg/ml)	26.1±2.3 (10)	22	34[a] 66
Plasma estrone (pg/ml)	33.4±2.7 (10)	29	60[a] 49

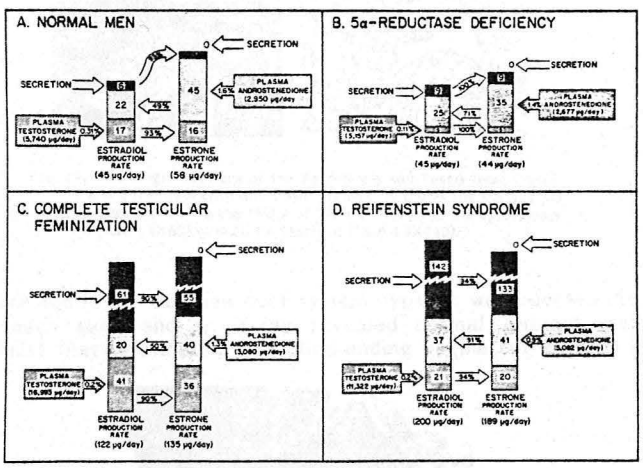


Fig. 13. Production rates of testosterone and estradiol in 4 normal men and individuals with 5α-reductase deficiency, complete testicular feminization, and the Reifenstein syndrome.

Because of the progressive virilization the decision was made to remove the testes. At exploratory laparotomy, the testes were characteristic of undescended testes in otherwise normal men, and adjacent to the testes were normal appearing epididymes (C and D below). We subsequently obtained slides from Ben Taub Hospital from Case 2, and these were typical for newborn epididymis and testis (A and B below).

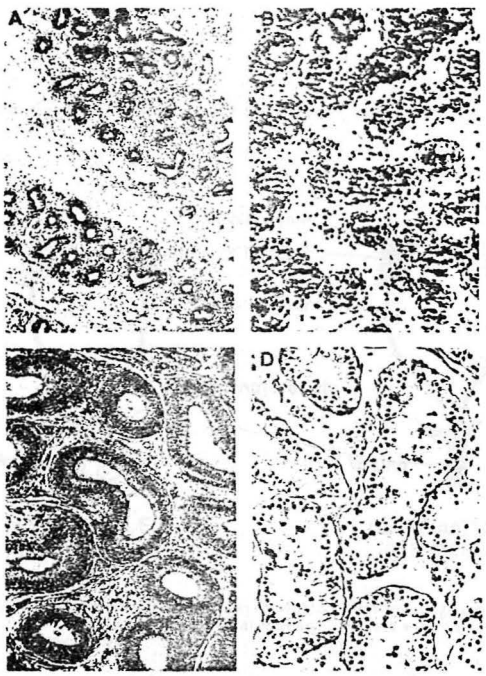


Fig. 14. Histologic Sections of the Epididymis and Testis from Cases 1 and 2 (Hematoxylin and Eosin Stains). A shows the epididymis of Case 2 (X60), B the testis of Case 2 (X140), C the epididymis of Case 1 (X60), and D the testis of Case 1 (X140).

To characterize the lower wolffian duct system hypaque was injected into the vas deferens on each side, and an X-ray revealed normal seminal vesicles and ejaculatory ducts that terminated in a blind-ending vagina rather than in a male urethra.

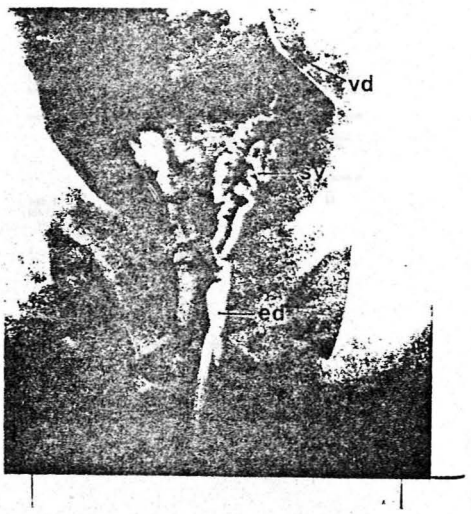


Fig. 15. X-Ray film of the abdomen of Case 1 after injection of contrast dye into the vas deferens at the time of abdominal exploration.

These findings are shown schematically below:

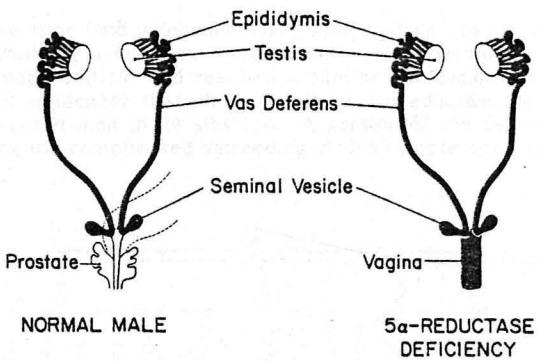


Fig. 16. Schematized comparison of wolffian duct and urogenital sinus structures between a normal man and a subject with 5α-reductase deficiency.

Thus, these individuals had 46,XY male chromosome composition, testes, male testosterone production rates, and male wolffian duct systems, but from the urogenital sinus on out they were predominately female in character. As we predicted dihydrotestosterone formation was less than 5 percent of normal in biopsy taken from foreskin, epididymis, and labia majora of Case 1.

Table III Formation of Dihydrotestosterone by Skin Slices* from Normal Subjects and from Patients with Different Types of Hereditary Male Pseudohermaphroditism.

Datum	Controls	Complete Testicular Feminization	Familial Incomplete Male Pseudohermaphroditism	
			Type 1	Type 2 (Case 1)
Age, yr	6-85	2-56	9	13
Dihydrotestosterone, pmoles/100 mg/hr:				
Scrotum	526±60† (14)		422	
Foreskin	211±26 (7)		87	8
Penis ‡	83±17 (5)			0
Clitoris	210±72 (5)			
Epididymis	142±32 (5)			3
Labia majora	183±25 (9)	101±15 (6)		0
Nongenital body skin	49±6 (20)	72±22 (5)		

*Skin slices (40-100 mg) were incubated with 1,2-^{3}H-testosterone (0.5 μM), glucose (10 mM) & Krebs — Ringer phosphate buffer, pH 7.4 in a total volume of 2.5 ml. After incubation (or 1 hr (genital tissue) or 2 hr (abdominal skin) the steroids were extracted & analyzed as described.[14] Figures in parentheses refer to no. of subjects studied in each group. Data for some of the control patients & some of the subjects with testicular feminization have been presented elsewhere.[14,31]

†Mean±SEM.

‡Corpora cavernosa.

28. Walsh PC, Madden JD, Harrod MJ, Goldstein JL, MacDonald PC, Wilson JD: Familial incomplete male pseudohermaphroditism, Type 2. Decreased dihydrotestosterone formation in pseudovaginal perineoscrotal hypospadias. N Engl J Med 291:944, 1974.

At the same time (and independently) Julianne Imperato-McGinley and Ralph Peterson were studying a more extensive family in the Dominican Republic with male pseudohermaphroditism and reached a similar conclusion (on the basis of a different type of evidence) that they also have 5α-reductase deficiency. They identified 38 affected men in 24 sibships. A portion of the family tree is shown below, illustrating the complicated inbreeding in this remote agricultural village.

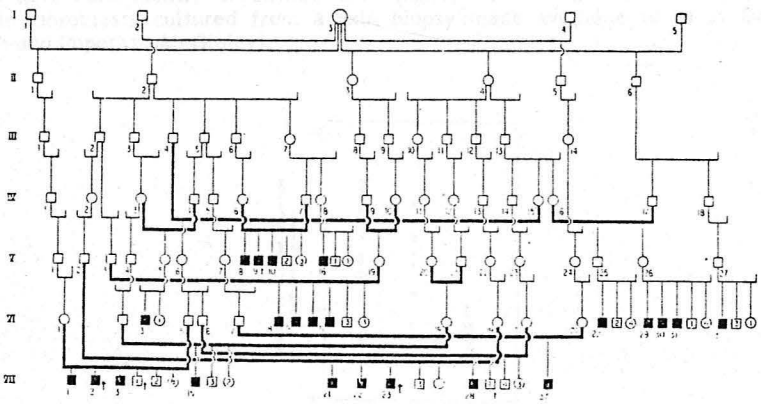


Fig. 17. Pedigrees showing transmission of male pseudohermaphroditism through seven generations.

Their patients, like ours, had low but measurable dihydrotestosterone in plasma, and they established that the appropriate way of making the diagnosis is by measuring the ratio of testosterone to dihydrotestosterone in random plasma samples (after age of patients) or following hCG stimulation in prepubertal individuals.

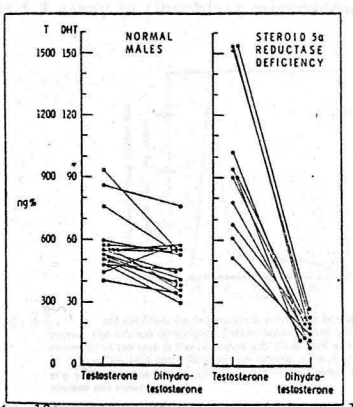
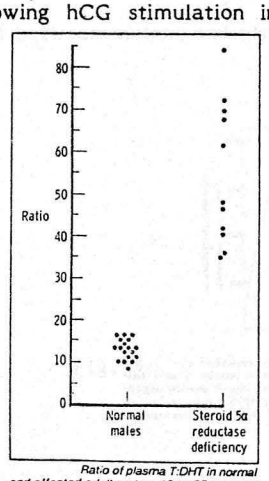
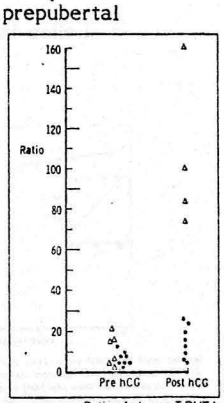


Fig. 18 Plasma concentrations of T and DHT in normal and affected adult males, 18 to 55 years of age.

Fig. 19.



Ratio of plasma T:DHT in normal and affected adult males, 18 to 55 years of



Ratio of plasma T:DHT in prepubertal male controls (●) and prepubertal affected males (△) before and after hCG stimulation.

Fig. 20

29. Imperato-McGinley J, Guerrero L, Gautier T, Peterson RE: Steroid 5α-reductase deficiency in man: an inherited form of male pseudohermaphroditism. Science 186:1213, 1974.

30. Peterson RE, Imperato-McGinley J, Gautier T, Sturla E: Male pseudohermaphroditism due to steroid 5α-reductase deficiency. Am J Med 62:170, 1977.

A second means of diagnosis is by direct assay of the enzyme in fibroblast monolayers cultured from the same areas of skin that normally express the enzyme in vivo, namely the foreskin, labia majora, or scrotum. We documented initially that the enzyme activity was low in foreskin fibroblasts cultured from our original patients (and subsequently confirmed the diagnosis in the Dominican Republic family in fibroblasts cultured from a skin biopsy made available to us by Drs. Peterson and Imperato-McGinley).

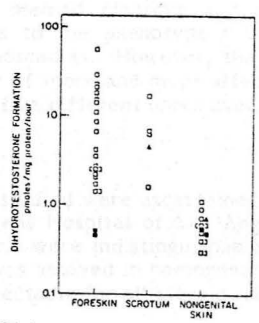


Fig.21. Dihydrotestosterone formation by normal and mutant fibroblasts as a function of the anatomical site of the skin biopsy. Cells were grown in microwells containing standard growth media as described under "Materials and Methods." On day 7 the medium was replaced with 0.4 ml of fresh medium containing 0.05 μM [1,2-^{3}H]testosterone. After 2 hours the dihydrotestosterone content of the medium and the protein content of the cell monolayer were determined. □, normal fibroblasts; ■, fibroblasts from 2 patients with familial incompiete male pseudohermaphroditism, type 2; ▲, fibroblasts from a subject with familial incomplete male pseudohermaphroditism, type 1. Each point represents an average of two or more separate measurements, each of which was performed in triplicate.

The assay condition that discriminates best between mutant and normal cells is a pH 5.5 assay in fibroblast microsomes.

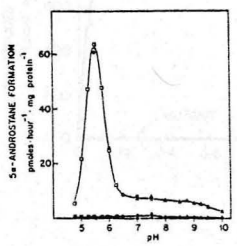


Fig.22. pH optimum for 5α-reductase activity in homogenates fr normal and mutant fibroblasts. Frozen homogenates were assayed described in the text at the indicated pH. □, ■, 0.08 M Na citrate; ●, 0.08 M Tris-HCl and 0.08 M sodium citrate; △, ▲, 0.08 M Tris-H and 0.08 M glycine-NaOH. ○, □, △, normal cell strain 80; ●, ■, mutant cell strain 139.

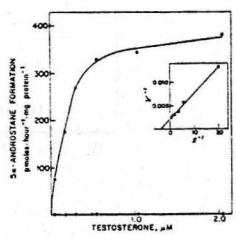


Fig.23. 5α-Reductase activity in particulate fractions from normal and type 2 mutant fibroblasts at varying concentrations of [1,2-^{3}H]testosterone. Frozen particulate fractions were assayed as described in the text using [1,2-^{3}H]testosterone that varied from 0.05 to 2 μM. ○, foreskin cell strain 80; ●, type 2 mutant cell strain 139.

This assay has become the standard one utilized in our subsequent studies. All patients to date have some residual enzyme activity detectable, perhaps explaining why all patients studied so far have measurable dihydrotestosterone levels in plasma.

31. Wilson JD: Dihydrotestosterone formation in cultured human fibroblasts: comparison of cells from normal subjects and patients with familial incomplete male pseudohermaphroditism, type 2. J Biol Chem 250:3498, 1975.

32. Moore RJ, Griffin JE, Wilson JD: Diminished 5α-reductase activity in extracts of fibroblasts cultured from patients with familial incomplete male pseudohermaphroditism, type 2. J Biol Chem 250:7168, 1975.

33. Moore RJ, Wilson JD: Steroid 5α-reductase in cultured human fibroblasts: biochemical and genetic evidence for two distinct enzyme activities. J Biol Chem 251:5895, 1976.

At this point, the issue seemed clearcut and simple, namely profound deficiency of the enzyme leads to the phenotype that previously described as pseudovaginal perineoscrotal hypospadias. However, the subject has become more complex as a result of the study of more and more affected individuals in greater depth. I would like to expand on five different unresolved aspects of the disorder.

1. Genetic Heterogeneity

The next patients that we studied were ascertained by Dr. Maurice D. Kogut and his colleagues at the Childrens Hospital of Los Angeles. Phenotypically and endocrinologically, these two girls were indistinguishable from the original Dallas family, and when 5α-reductase was assayed in homogenates of epididymis removed at surgery the activity was undetectable for all steroid substrates examined.

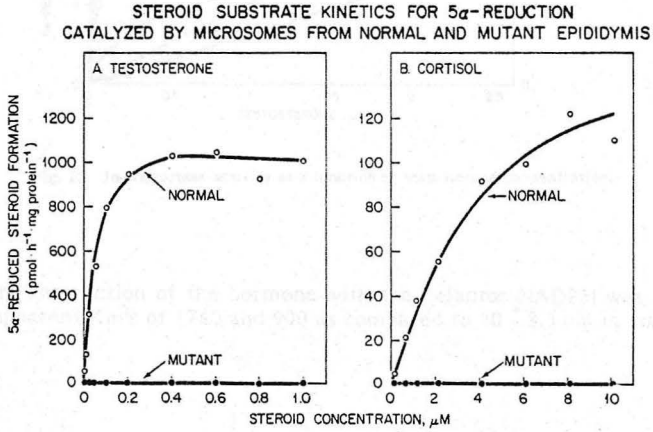


Fig. 24. Steroid substrate kinetics for the 5α-reduction of testosterone and cortisol by microsomal fractions of human epididymis.

Thus, insofar as we could tell, the defect in these siblings was identical to that in the Dallas family.

34. Fisher LK, Kogut MD, Moore RJ, Goebelsmann U, Weitzman JJ, Isaacs H Jr, Griffin JE, Wilson JD: Clinical, endocrinological, and enzymatic characterization of two patients with 5α-reductase deficiency: Evidence that a single enzyme is responsible for the 5α-reduction of cortisol and testosterone. J Clin Endocrinol Metab 47:653, 1978.

However, when Mark Leshin examined the fibroblasts cultured from the skin of the Los Angeles sisters, we found that the enzyme activity and the enzyme kinetics with respect to testosterone were normal -- completely different results than in the biopsied tissues.

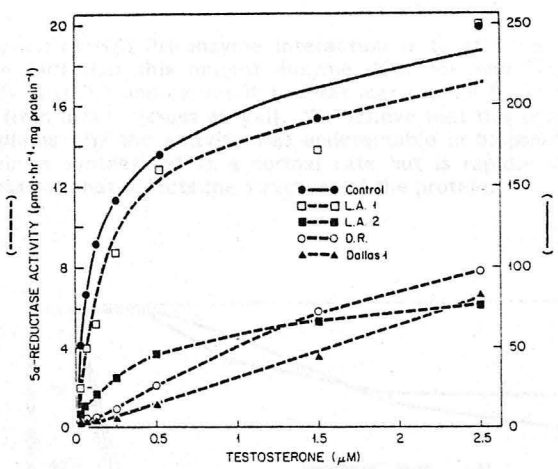


Fig. 25. 5α-Reductase activity as a function of testosterone concentration.

However, the interaction of the hormone with the cofactor NADPH was decidedly abnormal (apparent Km's of 1760 and 900 as compared to 40 ± 8.3 μM in controls).

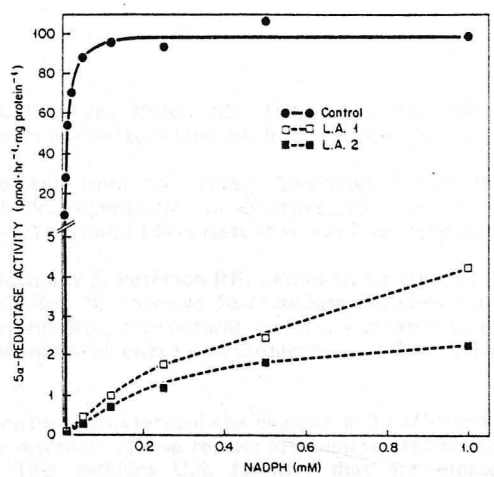


Fig. 26. 5α-reductase activity as a function of NADPH concentration.

A known function of NADPH-enzyme interaction is to stabilize the normal 5α-reductase; the fact that this mutant enzyme does not bind NADPH makes the enzyme grossly unstable and causes it to disappear rapidly from intact fibroblasts and probably from intact tissues as well. We believe that this property of enzyme instability explains why the activity was undetectable in biopsied tissue, e.g. the enzyme protein is synthesized at a normal rate but is rapidly denatured as the result of a mutation that affects the structure of the protein.

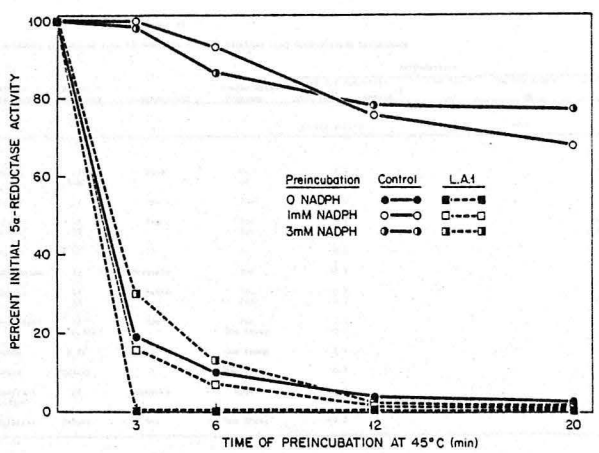


Fig. 27. Effect of NADPH on denaturation of 5α-reductase at 45°C.

35. Leshin M, Griffin JE, Wilson JD: Hereditary male pseudohermaphroditism associated with an unstable form of 5α-reductase. J Clin Invest 62:685, 1978.

A subsequent patient from an Italian American family that we studied in collaboration with Dr. Imperato-McGinley proved to have a third type of mutation, namely an enzyme in cultured fibroblasts that was both deficient and unstable.

36. Imperato-McGinley J, Peterson RE, Leshin M, Griffin JE, Cooper G, Draghi S, Berenyi M, Wilson JD: Steroid 5α-reductase deficiency in a 65 year old male pseudohermaphrodite: the natural history, ultrastructure of the testes and evidence for inherited enzyme heterogeneity. J Clin Endocrinol Metab 50:15, 1980.

We subsequently characterized the enzyme in 19 affected individuals from 14 families with the disorder. These results are summarized schematically in Fig. 28 and Table IV. This includes U.S. families that are ethnically black, Latin, American-Indian, Italian-American and of uncertain backgrounds as well as families from the Dominican Republic, Cyprus, Malta, Pakistan, Jordan, and Egypt. In eleven families the activity of the enzyme is very low. In the twelfth family (Los Angeles) it synthesized at a normal rate but is unstable, and in the remaining 2 families it is both unstable and deficient (the apparent Km's for both testosterone and NADPH being abnormal). One of these families (London 1) has been reported elsewhere (37).

37. Savage MO, Preece MA, Jeffcoate SL, Ransley PG, Rumsby G, Mansfield MD, Williams DI: Familial male pseudohermaphroditism due to deficiency of 5α-reductase. Clin Endocrinol 12:397, 1980.

TABLE IV

5α-Reductase Activity in Genital Skin Fibroblasts from 14 Families with 5α-Reductase Deficiency

Designation	Name	Ethnic Background	Age at Diagnosis	Consanguinity	Gender Role Problems	5α-Reductase: pH 5.8 Activity ($pmol \cdot hr^{-1} \cdot mg\ protein^{-1}$)	5α-Reductase: Km for T (μM)	5α-Reductase: Km for NADPH (μM)	5α-Reductase: Cycloheximide Stability %	Androgen Receptor (fmol/mg protei
Family # Controls (41)						30.6(1.4-215)	0.08±0.01	40±8	>95%	15-50
Class I										
1. Dallas 1	1. S. Jackson	Black	13	None	No	<0.2			>95%	22
	2. J. Jackson		N.B.		No	<0.2				20
2. Dallas 2	3. Bayonne	Black	17	Yes	Yes	0.2				30
3. Dominican Republic	4. Cazazio	Black?	12	Yes	Yes	0.2			>95%	—
	5. Cuevas	Black?	12		Yes	<0.2			>95%	29.6
4. Irvine	6. Urribe	Latin	17	?	No	0.2				41
5. New York 2	7. Hind	Jordanian	15	Probable	Yes	<0.2				81
6. London 1	8. Piritpisi	Cyprus	14	Probable	Yes	<0.2			>95%	
	9. Piritpisi		15		Yes	<0.2			>95%	
7. London 2	10. M. Akhton	Pakistani	15	Yes	Yes	0.2				19.1
	11. S. Akhton		N.B.(6)		Too Young	<0.2				26.9
8. Chicago 1	12. Kearns	White	N.B.	?	Too Young	0.4			>95%	21.4
9. Chicago 2	13. Campbell	White	Infant	?	Too Young	<0.2				
10. Phoenix	14. Robertson	American Indian	15	Probable	None	<0.2				26.5
11. Cairo	15. F	Egyptian	Infant	Yes	Too Young	<0.2				27.7
Class II										
12. Los Angeles	16. C. Cooper	Black	12	None	Uncertain	4.5	0.16	1760	<5%	49.8
	17. S. Cooper		11		Uncertain					
Class III										
13. New York 1	18. Uva	Sicily	17	Probable	Yes	0.6	2.20	425	75%	41
14. London 3	19. Muskat	Malta	16	Probable	Yes	0.6	1.1	780	68%	49.8

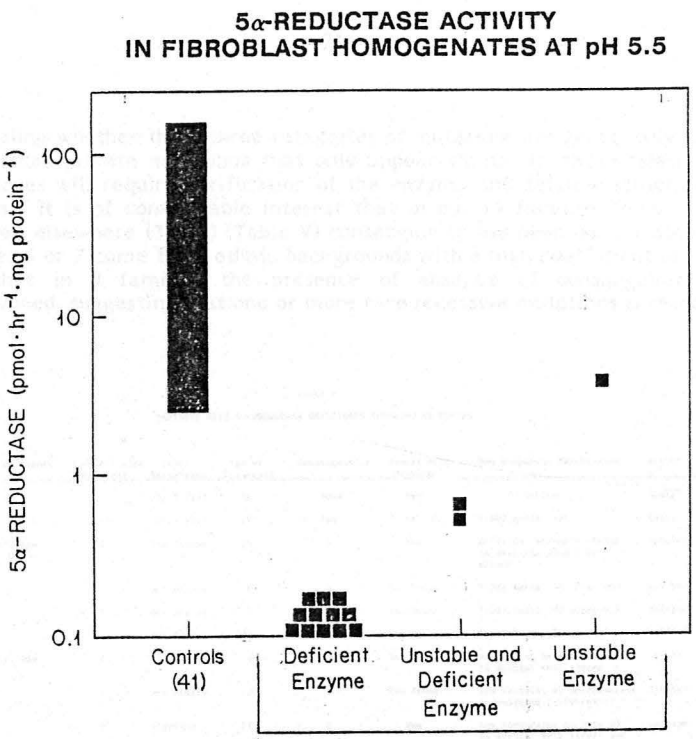


Fig. 28. 5α-Reductase activity in fibroblasts cultured from individuals from 14 families with 5α-reductase deficiency.

A poetic version of our interpretation is shown below. Namely, a variety of mutations that affect the 5α-reductase enzyme lead to a similar phenotype. Those that affect the binding of testosterone to the enzyme are most common, and those that affect solely the binding of NADPH to the enzyme are least common. The third type of mutation affects both binding sites.

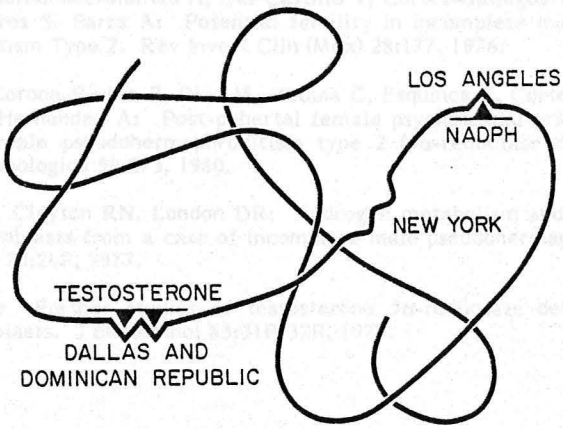


Fig. 29

Unraveling whether these three categories of mutations are genetically identical or are instead private mutations that only appear similar by these relatively simple techniques will require purification of the enzyme and detailed structure-function studies. It is of considerable interest that in our 14 families and in 11 families reported elsewhere (38-48) (Table V) consanguinity has been documented in 6, that another 6 or 7 come from ethnic backgrounds with a high coefficient of inbreeding, and that in 9 families the presence of absence of consanguinity was not ascertained, suggesting that one or more rare recessive mutations is responsible.

TABLE V

PATIENTS WITH 5α-REDUCTASE DEFICIENCY REPORTED BY OTHERS

Family #	Origin of Report	# of Affected Patients	Ethnic Background	Age at Diagnosis	Consanguinity	Gender Role Problems	How Diagnosis Established	Author	Ref.	Unusual Features
1.	Mexico	1	Not Stated	34	None	Yes	Unclear	Cantu	38	Low sperm count
2.	Mexico	1	Not Stated	18	Yes	No	T:DHT Ratio >35	Cantu	39	
3.	Great Britain	1	Not Stated	14	?	Yes	Deficient enzyme activity in cultured skin fibroblasts	Hodgins	40-41	
4.	U.S.A.	1	Not Stated	6	?	Too Young	T:DHT Ratio 35 after hCG	Saenger	42	
5.	U.S.A.	1	Not Stated	7	?	Too Young	T:DHT Ratio 53 after hCG	Saenger	42	
6.	France	1	Not Stated	25	?	Not Stated	T:DHT Ratio 38	Jaffiol	43	
7.	Great Britain	1	Pakistani	2½	None	Too Young	Absence of 5α-reductase in scrotal skin slices	Greene	44	
8.	Canada	1	Not Stated	2½	?	Too Young	Low activity of 5α-reductase in cultured fibroblasts	Pinsky	45	
9.	France	1	Algerian	25	?	Yes	Low conversion of T to DHT in scrotal skin biopsy and T:DHT ratio of 42	Kuttenn	46	
10.	France	1	Not Stated	20	?	No	T to DHT ratio of 26 and low conversion of T to DHT in skin homogenates	Mauvais-Jarvis	47	Absent spermatogenesis despite descended testes
11.	Israel	2	Not Stated	13	Yes	Probably Not	Deficient 5α-reductase activity in cultured genital skin fibroblasts	Oken	48	Absent spermatogenesis despite descended testes

38. Cantu JM, Hernandez-Montes H, Del Castillo V, Cortes-Gallegos V, Sandoval R, Armendares S, Parra A: Potential fertility in incomplete male pseudohermaphroditism Type 2. Rev Invest Clin (Mex) 28:177, 1976.

39. Cantu JM, Corona-Rivera E, Diaz M, Medina C, Esquinca E, Cortes-Gallegos V, Vaca G, Hernandez A: Post-pubertal female psychosexual orientation in incomplete male pseudohermaphroditism type 2 (5α-reductase deficiency). Acta Endocrinologica 94:273, 1980.

40. Hodgins MB, Clayton RN, London DR: Androgen metabolism and binding in skin and fibroblasts from a case of incomplete male pseudohermaphroditism. J Endocrinol 75:24P, 1977.

41. Hodgins MB: Further studies of testosterone 5α-reductase deficiency in human fibroblasts. J Endocrinol 83:31P-32P, 1979.

42. Saenger P, Goldman AS, Levine LS, Korth-Schutz S, Muecke EC, Katsumata M, Doberne Y, New MI: Prepubertal diagnosis of steroid 5α-reductase deficiency. J Clin Endocrinol Metab 46:627, 1978.

43. Jaffiol C, Robin M, Corratge P, Mirouze J: Un cas de pseudohermaphrodisme masculin par defaut de conversion de testosterone en dihydrotestosterone. Ann Endoc (Paris) 39:4, 1978.

44. Greene SA, Symes E, Brook CGD: 5-α-reductase deficiency causing male pseudohermaphroditism. Arch Dis Child 53:751, 1978.

45. Pinsky L, Kaufman M, Straisfeld C, Zilahi B, Hall C St-G: 5α-reductase activity of genital and nongenital skin fibroblasts from patients with 5α-reductase deficiency, androgen insensitivity, or unknown forms of male pseudohermaphroditism. Am J Med Genet 1:407, 1978.

46. Kuttenn F, Mowszowicz I, Wright F, Baudot N, Jaffiol C, Robin M, Mauvais-Jarvis P: Male pseudohermaphroditism: a comparative study of one patient with 5α-reductase deficiency and three patients with the complete form of testicular feminization. J Clin Endocrinol Metab 49:861, 1979.

47. Mauvais-Jarvis P, Kuttenn F, Mowszovicz I, Wright F: Different aspects of 5α-reductase deficiency in male pseudohermaphroditism and hypothyroidism. Clin Endocrinol 14:459, 1981.

48. Okon E, Livni N, Rosler A, Yorkoni S, Segal S, Kohn G, Schenker JG: Male pseudohermaphroditism due to 5α-reductase deficiency. Arch Pathol Lab Med 104:363, 1980.

2. Role of Dihydrotestosterone in Human Gender Behavior

One of the most remarkable features of 5α-reductase deficiency was the observation that 16 of 18 affected individuals in the Dominican Republic who were identified as female at birth and raised as females underwent a change in gender role to that of male as they partially virilized at the time of expected puberty (49, 50).

49. Imperato-McGinley J, Peterson RE, Gautier T, Sturla E: Male pseudohermaphroditism secondary to 5α-reductase deficiency -- a model for the role of androgens in both the development of the male phenotype and the evolution of a male gender identity. J Steroid Biochem 11:637, 1979.

50. Imperato-McGinley J, Peterson RE, Gautier T, Sturla E: Androgens and the evolution of male-gender identity among male pseudohermaphrodites with 5α-reductase deficiency. N Engl J Med 300:1233, 1979.

A similar change has occurred in individuals from six families studied by us (ethnic blacks, Pakistanis, Jordanian, Italian, and Maltese) (Table IV) and in three families studied by others (Mexico, Great Britain, and Algeria) (38, 40, 41, 46) (Table V). In 7 of the 25 families the affected individuals are too young to know whether such changes will occur, and in 5 families affected adults appear to have normal female orientation: (Dallas 1, Irvine, Phoenix, Mexico 2, and France 10). In the remainder of the families sufficient information is not available to be certain whether gender role problems occur. The conclusion seems inescapable that a change in psychosexual orientation is a common occurrence in this disorder. This

phenomenon raises fundamental questions about the factors that regulate human sexual behavior as well as about the appropriate management of the condition. (For the literature on this subject see Ref. 51.)

a. Effect of Hormones on the Sexual Behavior of Animals

The influence of gonadal hormones on behavior in animals is now well established. Many aspects of the problem are beyond the scope of the present discussion, but several issues deserve emphasis:

1) Sexually dimorphic behavior patterns of diverse types are regulated by gonadal steroids, ranging from the songs and mating rituals of birds to copulatory patterns in mammals. For example, male and female rodents differ in the predominant type of sexual postures they assume during coitus as well as the sexual partner they pursue. These behaviors can be changed by appropriate hormonal manipulation. If testosterone is given to rats soon after birth the female is made anovulatory. Likewise, if male rats are castrated during the neonatal period, their sexual response as adults to estrogen and progesterone is like that of females.

2) Although androgens and estrogens are formed in both males and females (with both hormones possibly playing roles in the sexual physiology of both sexes) the general statement can be made that androgens (and androgen metabolites) dictate male behavior patterns and that estrogens (and to a certain extent progesterone) dictate female behavior patterns.

3) These hormones act in the central nervous system via the same molecular mechanisms that operate in peripheral tissues. Steroid hormone receptors in brain have been isolated and characterized and shown to have specific distributions within certain areas of that tissue. Pathological states in which either the hormones or the machinery of hormone action are aberrant may thus cause effects on the central nervous system as well as anatomical and functional defects in peripheral tissues. Steroid hormones may also have effects in the central nervous system in addition to those mediated by the known receptor mechanisms (e.g., effects on cell permeability).

4) In the rodent the neonatal surge of testosterone secretion appears to play a vital role in virilizing hypothalamic function -- namely in determining a tonic pattern of gonadotropin release as compared to the cyclic patterns in females. (This action of testosterone in the central nervous system may be mediated by testosterone metabolites such as estradiol-17β.) A neonatal surge in testosterone secretion also occurs in the human male infant and could conceivably play a similar role.

5) Two general types of effects of gonadal steroids on behavior can be delineated. These phenomena are termed organizational and concurrent. Organizational effects are those that require the presence of the steroid at a specific time of development, that appear to result in a permanent effect on function or behavior, and that persist (to a greater or lesser degree) even after the steroid is no longer present. Such organizational effects are thought to take place during the neonatal surge of testosterone production and may be accompanied by permanent changes in anatomical development and organization of the brain. Concurrent effects require the continued presence of the steroid for the full manifestation of the effects -- for example the mounting response of the female

rodent when in estrus. Although the delineation of these phenomena is of conceptual advantage, there is considerable overlap between them in the sense that "organizational" effects may be silent in the absence of the proper hormonal milieu. Furthermore, some concurrent phenomena such as the typical male behavior involved in intromission and ejaculatory thrusting may persist to a variable degree in the castrated animal.

6) Virtually every behavioral effect of steroid hormones is due to complicated interaction between peripheral and central actions of the hormones. Consider for example, the paradigm of sexual behavior in the mammal that has been best explored, the mounting reflex of the female rat. When the female in estrus is mounted by a male, she extends the hind legs, elevates the rump, and dorsiflexes the vertebral column. This action involves not only sensory input from the rump but a well defined neural arc that includes motor and sensory components and specific estrogen-dependent nuclei in the central nervous system. The threshold of hormone action, however, may differ in the various components of this neurogenic arc. Thus, while the central nervous system plays a vital role in the hormone-mediated control of sexual behavior in animals, individual components of behavior may be influenced to different degrees by central versus peripheral actions of the hormones. Even under defined laboratory conditions, it is difficult to devise experimental conditions that allow one to quantitate the relative contribution of each to a given action.

7) Animal species differ in the extent to which hormones exert a permanent organizational effect on behavior or on gonadotropin production. Specifically, permanent effects of gonadal hormones in primates appear to be less clearcut than in rodents; for example, when estrogens are given in appropriate amounts to male rhesus monkeys of any age a positive release of luteinizing hormone (similar to that of the normal ovulatory surge in females) can be induced.

There also does not appear to be a permanent imprinting by steroid hormones on the pattern of gonadotropin secretion in the human. The normal tonic pattern of gonadotropin secretion in adult men can be altered to the cyclic pattern characteristic of the female by administration to men of ovarian steroids in such a way as to mimic the pattern of secretion of the normal ovary. This suggests that gonadal hormones have no "organizational" effect on central nervous system function in the human since the male pattern can be reversed and suggests that the effects of hormones on central nervous system functions are different in the human than in lower animals.

8) Even when hormones are involved in mediating specific aspects of behavior, stereotyping can also play a role. For example, development of the normal male song pattern in some bird species requires both the action of androgen and exposure of the developing male to a mature male of the same species. In this case the hormone acts both directly and by influencing learning patterns.

In summary, the role of gonadal steroids in sexual behavior involves development and function of the central nervous system, the development of the genital tract in the two sexes, reflex, sensory, and motor aspects of neurosensory arcs, and the integration of the various neural subsystems that constitute the behavioral process.

b. Do Androgens Play a Role in Sexual Identification in the Human?

In contrast to sexual drive -- which is not usually considered to be sexually dimorphic -- aspects of sexual behavior relating to identification are fundamentally different in men and women. For the present purposes the two important concepts are gender identity and gender role. Gender identity is defined as the unified and persistent experience of oneself as male, female or ambivalent. Gender role is composed of the actions, activities, and behavior that indicate to others the degree to which one is male, female or ambivalent. The factors that constitute gender role are obviously influenced by a variety of cultural and social variables, since actions and activities of the two sexes vary in different societies. Consequently, the patterns by which one identifies oneself as male or female vary. Two other aspects of dimorphic behavior are beyond the scope of the present discussion: sexual orientation (whether sexual object choice is heterosexual, homosexual, or bisexual) and parenting (the desire and capacity for the care of children). Knowledge of endocrine influences on the latter processes is sparse.

Vagueness in the definition of gender identity and gender role reflects the fact that it is difficult to quantify these parameters in any meaningful way and more difficult to devise means of investigating their provenance. Appropriately controlled experiments that would allow rigorous elucidation of the determinants of sexual identification and behavior cannot be performed in human beings.

As a consequence, a major emphasis in the study of human sexual behavior has been the analysis of gender role and behavior in subjects with histories of endocrine abnormalities, particularly studies of patients with abnormalities of sexual development. All such studies are subject to problems that make questionable any interpretation:

First, there is considerable variation in the seriousness of the phenotypic defects that eventuate in the various types of abnormal sexual development. For example, men with the 47,XXY Klinefelter syndrome or with the XX male syndrome differentiate as men (albeit infertile because of azoospermia) and express endocrine abnormality only later in life. Likewise, subjects with 45,X gonadal dysgenesis or the syndrome of pure gonadal dysgenesis develop a female phenotype, and most patients with true hermaphroditism have unambiguous male or female phenotypes. Thus, most patients with abnormalities of sexual development end up nevertheless with an unequivocal male or female phenotype. This is the consequence either of the fact that the formation of testicular hormones was sufficient to induce a male phenotype or that the failure of production and/or action of testicular hormones is complete enough to result in formation of a female phenotype. If hormones are involved in the formation of gender identity, in most patients the hormonal tendency would correspond with the anatomical development and hence with the sex assignment at birth.

Second, disorders that appear phenotypically similar can result from very different mechanisms. For example, some patients with errors in chromosomal sex, such as mixed gonadal dysgenesis, have phenotypes similar to those of patients with abnormalities of phenotypic sex such as 5α-reductase deficiency. Since different disorders differ in their pathophysiology, it is essential that the diagnosis be established before any valid interpretation can be drawn as to behavioral consequences of a given pathology.

Third, ambiguity of genital development (and hence confusion as to appropriate gender assignment at birth or in subsequent life) occurs in only a few disorders: 1) the testes produce insufficient hormones to virilize the male embryo -- either because of developmental abnormality of the testes (as in mixed gonadal dysgenesis) or because of a hereditary defect in one of the enzymes required for testosterone biosynthesis; 2) sufficient testosterone is synthesized by the testes, but due to an inherited abnormality that affects the molecular machinery of androgen action (usually the receptor protein or 5α-reductase enzyme) the hormone cannot act to virilize the embryo normally; or 3) overproduction of androgen occurs in the female embryo as in congenital adrenal hyperplasia due to deficiency of the 21-hydroxylase enzyme. If hormones are involved directly or indirectly in development of gender identity one would predict that gender identity would be most imperfect in patients with ambiguous genitalia. However, even if generally true, gender identity would not be expected to be influenced in every patient because all defects that cause ambiguous genitalia vary in severity among affected individuals and consequently result in different degrees of abnormal genitalia. For example, the external phenotypes of chromosomal males with abnormalities of the androgen receptor or of chromosomal females with 21-hydroxylase deficiency can span an entire spectrum from male to ambiguous to female. One would not expect abnormalities of gender identity in those individuals with minor or no defects in genital development.

Fourth, even when the degree of ambiguity of the external genitalia is similar, disorders can have different times of onset and different long-term endocrine consequences. For example, disorders of androgen synthesis and/or action influence embryonic development beginning at the end of the first trimester whereas adrenal function -- and hence adrenal virilization in 21-hydroxylase deficiency -- does not begin until later in embryogenesis. Furthermore, adult males with 17β-hydroxysteroid dehydrogenase deficiency, mixed gonadal dysgenesis, or 5α-reductase deficiency may have a normal endocrine profile for a postpubertal man despite the profound defect in androgen action during embryogenesis, whereas the testicular lesions in the Klinefelter syndrome and in the XX male become progressively severe so that plasma testosterone values, although initially normal, decline with age. Behavioral consequences might or might not occur in these disorders, depending on when in development gonadal steroids normally exert an effect on gender identity.

Thus, abnormalities of sexual development differ in their influence on the sexual phenotypes, their effects on hormone patterns at various times of life, their times of first manifestation during development, and their ultimate metabolic effects. Any interpretation as to possible behavioral consequences of a specific disorder has to take these various factors into account. That is, since various abnormalities have different effects on the anatomic and functional phenotypes, different behavioral consequences would be predicted in various disorders even if hormones are involved in the genesis of human sexual behavior. For these reasons, it is necessary to be especially cautious in interpreting any negative result, specifically one that fails to support an effect of hormones on gender development.

c. Behavioral Studies in Patients with Abnormalities of Sexual Development

While different forms of abnormal sexual development have been lumped together in many behavioral studies, detailed studies have been performed in six groups of patients with specific diagnoses: 1) Females exposed to excess androgens as a result of the syndrome of congenital adrenal hyperplasia develop a variable

degree of virilization of the external genitalia. Gender identity is usually female despite the presence of virilization and despite the fact that discernable effects can be delineated in certain aspects of gender role behavior, generally a tomboyishness and characteristic male energy expenditure. 2) Children who were exposed to exogenous estrogen or progesterone during gestation usually have appropriate male or female phenotypes. These agents at best have minor effects on sexually dimorphic behavior and no discernable effect on gender identity. 3) Males with complete androgen resistance and the syndrome of testicular feminization develop a female phenotype. Such patients have testes and male testosterone levels but cannot respond to androgen and consequently differentiate as phenotypic women. Gender identity and gender role in such patients are unequivocally female in accord with the anatomy and sex assignment, and such patients rank high in all femininity quotients. 4) Men with partial androgen resistance usually differentiate as phenotypic males but with severe hypospadias; their gender role and behavior appear to be concordant with the male phenotype and the male sex of rearing. 5) True hermaphrodites may have male, female, or ambiguous phenotypes. In patients with true hermaphroditism, gender identity and role usually correspond with the sex of rearing. 6) Women with gonadal dysgenesis have female phenotypes and appear to have generally normal female gender role behavior and gender identity. Since such subjects are believed to be profoundly estrogen deficient throughout life it has been inferred that postnatal estrogen plays at best a minor role in the evolution of female gender identity as well as of female libido.

The usual conclusion drawn from these various studies has been that gender role and identity usually differentiate in conformity with the sex of assignment and rearing. In other words, gender role and identity correspond to the predominant anatomical development and hence to the predominant prenatal hormonal milieu. This conformity can withstand various perturbations that include: 1) contradictory hormonal patterns in which a girl virilizes or a boy feminizes at puberty, 2) tomboyish energy expenditure in girls, and 3) imperfect development of the secondary sexual characteristics at puberty. It should be emphasized that the conclusion that gender identity corresponds to sex assignment has been made by workers in different countries using a variety of different patient groups. Despite inherent weaknesses in design in all such studies and despite the fact none of the disorders constitutes a perfect experiment the unanimity of opinion in this regard is impressive.

The problem is that the evidence is open to diametrically opposite interpretations. One view -- most eloquently formulated by John Money and his coworkers -- is that sex assignment at birth influences parental attitudes and the manner by which infants are treated and that these social forces are paramount in determining human gender identity and role, so powerful as to be irresistable after a few years of age. According to this formulation, any effect of hormones in modulating this phenomenon in the human is secondary, indeed probably minor. This interpretation has been dominant for the past 20 years. A second interpretation is possible. Gonadal steroids could be primary determinants of gender development, but since they also determine anatomical development and hence sex assignment and the sex of rearing, gender identity and anatomical sex would almost invariably be the same. It would thus be expected that phenotypic sex (sex assignment) and gender role and identity usually correspond. In such a view, it is impossible to determine the extent to which psychological and social versus biological determinants are more powerful because the psychological and social forces generally correspond with the anatomical and biological ones.

d. Apparent Reversal of Gender Identity

From the first there has been a minority view that biological determinants as well as psychological factors influence human sexual identification. Further, occasional instances have been reported over the years in which individual patients with abnormal sexual development have undergone a change in gender role and sex assignment at some time after gender identity is usually considered to be fixed irreversibly. The majority of these instances were described before the means of making specific diagnoses of the etiology of abnormal sexual development were widely available. It is not possible, in retrospect, to determine the diagnoses in most such reports. Nevertheless, in analyzing these reports two facts seem clear: 1) the majority of patients in whom gender identity does not differentiate in conformity with gender assignment have ambiguous genitalia at birth -- that is, they commonly fall into the category of genetic males in whom male development is incomplete, resulting in an incorrect sex assignment of female at birth, and 2) the decision to change gender role at puberty is most commonly to shift from female to male. In other words, the shift is usually from a misassigned gender in biological/hormonal terms to the correct one; only rarely does a shift occur from the correct biological sex. The reverse sequence, a shift from male to female gender role, has been reported but is rare.

TABLE VI

Occurrence of Ambiguous Genitalia and Changes in Gender Role in Abnormalities of Sexual Development

Type of Disorder	Disorder	Ambiguity of External Genitalia	Reported Change in Gender Role
Chromosomal Sex	Klinefelter Syndrome XY Male		
	Gonadal Dysgenesis Mixed Gonadal Dysgenesis	Common	Occasional Female to Male
	True Hermaphroditism	Occasional	Unusual
Gonadal Sex	Pure Gonadal Dysgenesis Absent Testis Syndrome	Occasional	
Phenotypic Sex	Female Pseudohermaphroditism Congenital Adrenal Hyperplasia	Common in Females	Unusual
	Nonadrenal Female Pseudo-hermaphroditism	Common in Females	Unusual
	Developmental Disorders of the Mullerian Duct		
	Male Pseudohermaphroditism Abnormalities of Androgen Synthesis	Common	Occasional Female to Male
	Abnormalities of Androgen Action		
	5α-Reductase Deficiency	Usual	Female to Male
	Defects of the Androgen Receptor Testicular Feminization Reifenstein Syndrome Male Infertility	Unusual	Rare
	Persistent Mullerian Duct Syndrome		
	Sporadic Hypospadias	Occasional	Rare

These instances of change in gender role/assignment have been noted in many clinics, were clearly recognized by the anthopocentric school including Money, and have been considered by most students to be the secondary consequence of ambiguity in assignment as perceived and practiced by the parents and/or the subjects themselves. However, in the recent past change in gender role and assignment at puberty has been documented not only in 5α-reductase deficiency but also in subjects with a hereditary defect in testosterone synthesis due to deficiency of 17β-hydroxysteroid dehydrogenase (52-53) (Table VI). (Our own unreported clinical experience plus a review of the literature suggests to us that this phenomenon also occurs occasionally in male pseudohermaphrodites with ambiguous genitalia due to mixed gonadal dysgenesis.) It has been implied (but not established) that these individuals have undergone a change in gender identity as well as a change in gender role and assignment, i.e. that a true reversal of gender identity had taken place.

51. Wilson JD: Gonadal hormones and sexual behavior, in Clinical Neuroendocrinology, L Martini, GM Besser (eds). New York, Academic Press, August 1982, in press.

52. Akesode FA, Meyer WJ III, Migeon CJ: Male pseudohermaphroditism with gynecomastia due to testicular 17-ketosteroid reductase deficiency. Clin Endocrinol 7:443, 1977.

53. Imperato-McGinley J, Peterson RE, Stoller R, Goodwin WE: Male pseudohermaphroditism secondary to 17β-hydroxysteroid dehydrogenase deficiency: gender role change with puberty. J Clin Endocrinol Metab 49:391, 1979.

These reports have reactivated (or reinvigorated) the discussion as to the role of biological versus psychosocial factors in determining gender identity. Indeed, the suggestion has been made that androgen action on the brain in utero, during the neonatal period, and at puberty has an impact on determination of a male gender identity and, under certain circumstances, that it can override female sex assignment and female sex of rearing (52). Interesting as these observations may be, they are open to as many problems of interpretation as are all other clinical studies of gender identity and role:

1) No prospective studies have been performed so that it is impossible to ascertain whether the gender identity prior to puberty was in fact unambiguously female. Apparent gender reversal is a prominent feature only of those forms of abnormal sexual development in which genital ambiguity is prominent (Table VI). Many individuals with ambiguous genitalia are aware of their abnormalities from an early age and may consequently be unclear as to their exact gender identity prior to puberty.

2) Most patients in whom gender identity has apparently changed from female to male at puberty have been raised in families or in cultures in which the sexes have fairly rigid stereotypes as to sexual role and in which the traditional female roles centering around home and family life are difficult, if not impossible, for women with phallic enlargements and shallow vaginas. Consequently, in such an environment cultural forces serve to reinforce any biological forces involved in a change from a female to a male role. It is even more difficult to perform prospective studies of sexual behavior in these communities.

3) Even if it were established that the change in gender role in such patients were due to an actual change in gender identity and that the change in identity and

role were determined solely, or significantly, by endocrine or biological forces rather than psychosocial factors, it would still be difficult to ascertain whether the changes are due to effects on the central nervous system or the peripheral target tissues. The development of a functional penis might influence behavioral patterns independent of the central nervous system. It remains plausible to argue that any effects of gonadal steroids on human behavior could be mediated largely, if not exclusively, by their effects on peripheral tissues. These could include such diverse actions as tomboyish behavior which might result from androgen actions on muscle growth as well as adoption of a male gender role due to virilization of the genitalia.

4) If it were true, however, that androgens can override female gender identity -- by whatever mechanism -- it is interesting that those disorders in which reversal apparently occurs (5α-reductase deficiency, defects in testosterone synthesis, and possibly mixed gonadal dysgenesis) are those in which the neonatal surge in testosterone synthesis is probably normal, suggesting the possibility that the neonatal phase of male sexual life might be of importance in influencing development of male gender identity. This would imply that the neonatal surge of testosterone production should be prevented in infants with ambiguous genitalia in whom the sex of assignment is female.

There can be no doubt that confusion about gender role and/or identity is one of the most baffling and frustrating problems of mankind; from every standpoint -- endocrine, psychological, sociological, and legal; there can also be little doubt that endocrine etiologies can account for only a small proportion of gender identity problems. It is possible, however, that male pseudohermaphroditism (e.g. sex misassignment at birth) could be a frequent cause for those individuals who desire to change gender role from male to female. The difficulty involved in making such a transition is illustrated by the recent publication in English translation of the memoirs of Herculine Barbin (54). Herculine Barbin was born in 1838 in Saint-Jean-d'Angely, France and from the age of 7 was raised in a series of convents. In 1860 she told her confessor that she had changed from a woman to a man. After consulting with the Bishop the problem was turned over to the Court to decide. After an extensive workup the presiding judge decreed that her civil status be changed from female to male -- the first instance it is believed for a legal change in gender in the western world. She lived for eight years (rather unsuccessfully) as a man and in 1868 committed suicide, leaving an autobiography that describes in a moving way her frustration and the difficulty of functioning with gender that is neither male or female. A remarkably detailed autopsy was performed. The phallus was bound by a tight chordee; the urethra was female in character, but the vagina was blind-ending. The right testis was in the labia, and the left testis was intraabdominal; normal epididymes, vasa deferentia, and seminal vesicles were present. No spermatogenesis was present in either testis, and no sperm were present in the seminal vesicles. The ejaculatory canals were in the normal position of Gartner's ducts. These findings are compatible with (but not pathognomic of) a diagnosis of 5α-reductase deficiency. The story is a moving and tragic one to read -- a true cry from the heart of one who does not fit a conventional role in life and who does not understand why; it elevates to the level of literature the inherent tragedy and difficulty of adjusting to a society in which only two genders are acceptable. This makes even more critical the appropriate management of such individuals.

54. Foucault M: Herculine Barbin. Being the Recently Discovered Memoirs of a Nineteenth-Century French Hermaphrodite. New York, Pantheon Books, 1980.

In summary, it is impossible on the basis of the evidence at hand to be certain of the extent to which biological factors interact with psychosocial forces to determine sexual behavior of humans, and it is equally impossible to know whether any biological factors that may be involved act directly on the central nervous system. It seems safe to assume that the truth lies somewhere between the extremes of the anthropocentric and zoocentric schools, namely that both biological and psychosocial factors play a role in determining the sexual behavior of humans. Interesting though the quantitative issues may be, definitive studies to resolve this dilemma are difficult, if not impossible, to design because of inherent limitations in the methodologies available for definitive analysis of human behavior. We do know that certain male pseudohermaphrodites who virilize at puberty undergo a change in role. The causes for this change -- and indeed whether the change constitutes a change in gender identity or only the resolution of an ambiguous gender identity -- are not known. The fact that such a change can occur, however, has major implications for the management of these disorders (see below).

3. Why Do Subjects with 5α-Reductase Virilize More Completely at Puberty than During Embryogenesis?

It is a striking and consistent feature that subjects with 5α-reductase deficiency virilize significantly at the time of expected puberty -- in particular growth of the phallus and enlargement of the pectoral muscles are marked (acne does not occur, and the growth of facial and body hair is less than that of unaffected siblings). If testosterone can cause phallic growth postnatally, why does it not cause formation of a male urethra in utero? This puzzling problem is closely related to the question of why dihydrotestosterone formation is necessary for formation of the urogenital sinus and external genitalia but not for virilization of the wolffian ducts, indeed why dihydrotestosterone formation plays a role in androgenization. Provided the interpretation is correct that both hormones bind to the same receptor but that dihydrotestosterone binds more avidly, then one would conclude that 5α-reductase magnifies but is not absolutely required for androgen action; it would follow on a priori grounds that diminution in dihydrotestosterone formation should impair partially all androgen actions. This is clearly not the case during embryogenesis, since 5α-reductase deficiency selectively impairs the action of the hormone on differentiation of the wolffian duct.

Phrased in another way, any model system that purports to explain the role of dihydrotestosterone formation in androgen action must also provide explanations as to 1) why dihydrotestosterone formation is not necessary for virilization of the wolffian ducts and 2) why dihydrotestosterone formation is more important in the embryonic than the postnatal urogenital tubercle. Despite more than a decade of effort we do not know the explanation for any of these phenomena. The following facts seem fairly well established:

1) Both genetic and biochemical evidence indicate that testosterone and dihydrotestosterone bind to the same androgen receptor protein but with differing affinities. The fact that a single gene mutation in the mouse impairs the action of both hormones equally implies that a single protein is involved, but it is possible

that independent receptors for the two hormones have a common subunit (which is absent in the Tfm mouse) and unique subunits so that the actual intact receptor molecules are different; it is also possible that different hormones could have different allosteric effects on the same receptor protein so that complexes of the receptor with the two different hormones (TR and DR) could fit on different acceptor sites within the chromosomes. The fact that the off-time (dissociation rate) of testosterone from the receptor is faster than that for dihydrotestosterone is in keeping with the possibility of a different effect of the two hormones on the tertiary structure of the receptor.

55. Verhoeven G, Wilson JD: Cytosol androgen binding in submandibular gland and kidney of the normal mouse and the mouse with testicular feminization. Endocrinology 99:79, 1976.

Whatever role dihydrotestosterone formation plays in wolffian duct differentiation, it cannot be an exclusive one at the molecular level, namely testosterone is sufficient for virilization of the wolffian duct but is not necessary since the administration of dihydrotestosterone to the pregnant rat (56) or to the pregnant mouse (57) causes virilization of the wolffian ducts in female embryos.

56. Schultz FM, Wilson JD: Virilization of the wolffian duct in the rat fetus by various androgens. Endocrinology 94:979, 1974.

57. Goldstein JL, Wilson JD: Studies on the pathogenesis of the pseudohermaphroditism in the mouse with testicular feminization. J Clin Invest 51:1647, 1972.

2) All attempts to date to explain the role of dihydrotestosterone in androgen physiology in metabolic terms have been unsuccessful. The greater potency of dihydrotestosterone is not due to the fact that, unlike testosterone, it is not converted to estrogens (58), and dihydrotestosterone formation does not appear to be involved in facilitation of transport of androgens from plasma to the cell (59). Malcolm Hodgins has proposed that 5α-reduction is important in utero not primarily to form dihydrotestosterone but rather to inactivate progesterone, the natural inhibitor of testosterone action, but again the evidence does not really support such a concept (60).

58. Milewich L, George FW, Wilson JD: Estrogen formation by the ovary of the rabbit embryo. Endocrinology 100:187, 1977.

59. Lasnitzki I, Franklin HR, Wilson JD: The mechanism of androgen uptake and concentration by rat ventral prostate in organ culture. J Endocrinol 60:81, 1974.

60. Hodgins MB: Binding of progesterone metabolites to androgen receptors of 5α-reductase deficient human fibroblasts. Acta Endocrinol 97(Suppl 243):Abstract 161, 1981.

3) The greater virilization at time of puberty than in utero could be due to any of several possible causes: a) the plasma testosterone at the time of puberty is on average about 50% higher than during the phase of embryogenesis in which male sexual differentiation takes place, and it is conceivable that testosterone can accomplish by mass action over many years time a partial virilization of all androgen target tissues; b) as described, all patients with 5α-reductase deficiency

characterized to date have measurable dihydrotestosterone in the plasma; (the site of origin has not been determined, perhaps from the liver), and it is possible that this small amount of circulating dihydrotestosterone acts like a true hormone to induce a partial virilization again, over a long period of time; c) the third possibility is that the difference between the embryo and puberty is solely an issue of time. As Jost documented many years ago, the events in male differentiation in utero are carefully timed in such a way that the formation of the male urethra has to be completed within a short period of time (once the "window of time" for fusion is past, it can never be accomplished, no matter how much potent androgen is given). Thus, there may be no real difference between the events in utero and at puberty; d) the degree of virilization at puberty is variable, some patients (e.g. patients 3, 6, and 14 in our series) virilizing only to a minor degree whereas other virilize more extensively. This type of variability may be related to the genetic heterogeneity in the disorder.

To summarize, we cannot explain why dihydrotestosterone formation appears to be necessary for virilization of one portion of the male urogenital tract but not for the other. If the identical androgen receptor system is operative for the two hormones in the embryo as in the adult then one must conclude that at the level of the gene some androgen actions can be accomplished by testosterone whereas others require the action of dihydrotestosterone. If this is the case, then the differences in virilization between the embryonic and postembryonic states must be due to some trivial cause.

The way we are currently investigating this problem is as follows: The Merck Company has developed an experimental drug (DMAA) that is a potent competitive inhibitor of 5α-reductase activity. The drug has an apparent Ki of approximately 3 nM and is effective in vivo and in vitro. When administered to inactive male dogs it causes a striking decrease in prostate weight and prostate dihydrotestosterone concentration but no change in prostate or plasma testosterone levels. (Fig. 30)

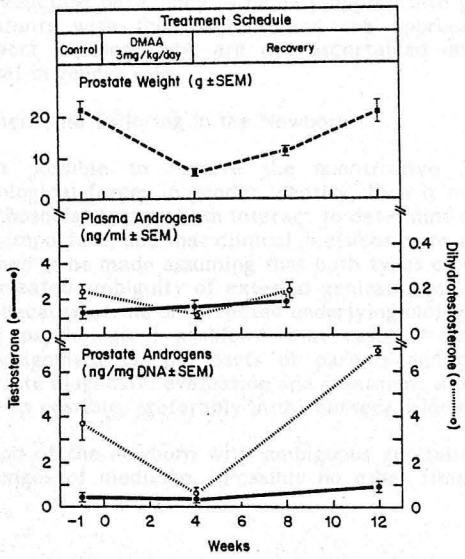


Fig. 30. Effect of DMAA on prostatic hyperplasia in the dog.

61. Brooks JR, Baptista EM, Berman C, Ham EA, Hichens M, Johnston DBR, Primka RL, Rasmusson GH, Reynolds GF, Schmitt SM, Arth GE: Response of rat ventral prostate to a new and novel 5α-reductase inhibitor. Endocrinology 109:830, 1981.

62. Laing T, Heiss CE: Inhibition of 5α-reductase, receptor binding, and nuclear uptake of androgens in the prostate by a 4-methyl-4-aza-steroid. J Biol Chem 256:7998, 1981.

63. Leshin M, Wilson JD: Inhibition of steroid 5α-reductase from human skin fibroblasts by 17β-N,N-diethylcarbmoyl-4-methyl-4-aza-5α-androstan-3-one. J Steroid Biochem (in press).

64. Wenderoth UK, Wilson JD: Plasma testosterone is the precursor for the prostatic dihydrotestosterone responsible for development of prostatic hyperplasia in the dog. Submitted for publication.

When this agent is administered to pregnant animals it produces a phenocopy of 5α-reductase deficiency in male embryos -- namely male embryos with partial impairment of external virilization but with normal wolffian ducts. Fred George has developed microtechniques for measuring the androgen receptor directly in embryonic tissues for the first time and for measuring the actual concentrations of the various hormones in the tissues themselves. By performing both assays in normal and DMAA treated embryos, we should be able to ascertain what actually happens to the hormone levels and to the receptors within the tissues and to determine clearly whether the role of dihydrotestosterone formation is at the level of hormone action or at some prior stage that influences hormone levels or metabolism.

4. Management of 5α-Reductase Deficiency

Two separate issues exist in regard to the appropriate management of patients with 5α-reductase deficiency -- namely appropriate gender assignment in newborn or in infants with the disorder and the appropriate hormonal and psychological support in those who are not ascertained until after they have undergone a reversal in gender role.

a. Gender Assignment and Tailoring in the Newborn

If it is not possible to resolve the quantitative issue regarding the contribution of biological forces in gender identity, then it must be assumed that biological and psychosocial factors both interact to determine sexual behavior, that the role of each is important, and that clinical decisions as to sex assignment in the newborn nursery need to be made assuming that both types of factors are involved. It follows that untreated ambiguity of external genitalia can lead to confusion of gender role either because of the uncorrected underlying biological abnormality per se or because of psychological problems that result from uncertainty as to appropriate sex assignment on the parts of parents and patients themselves. Therefore, appropriate diagnostic evaluation and treatment should be instituted and completed as early as possible, preferably in the nursery prior to discharge.

The evaluation of the newborn with ambiguous genitalia is one of the most complicated challenges of medicine. Possibly no other field required a broader

application of theoretical and practical knowledge, and few decisions have greater social impact on the future of the patient. The issue is not important in those forms of abnormal sexual development in which the phenotypic sex is clearcut such as Klinefelter syndrome or gonadal dysgenesis. This limits the major problem to the four common causes of ambiguous genitalia -- true hermaphroditism, mixed gonadal dysgenesis, female pseudohermaphroditism, and male pseudohermaphroditism (including 5α-reductase deficiency).

While the infant with ambiguous genitalia is in the newborn nursery the family should be informed that the development of the external genitalia is incomplete and that additional studies will be required before correct sex assignment can be made. The investigation of such patients in the newborn nursery includes a properly performed history, pedigree analysis, and physical examination; evaluation of the genetic sex using nuclear chromatin studies, fluorescent staining for a Y chromosome, and analysis of karyotype; biochemical and endocrine evaluation; roentgenographic and endoscopic assessment of the urogenital sinus and internal duct structure, and in some instances laparotomy and gonadal biopsy; and assessment of androgen action in cultured fibroblasts. The diagnosis of 5α-reductase deficiency is established in 46,XY infants either by measurement of plasma testosterone to dihydrotestosterone ratios after hCG or by direct assay of the enzyme in cultured skin fibroblasts. Once these studies are complete, a decision as to gender assignment can usually be made.

In all forms of male pseudohermaphroditism (and in 5α-reductase deficiency as well) the major criterion for sex assignment is the degree of masculinization of the external genitalia. If the patient has an inadequate phallus, the anatomy should probably be corrected to as nearly a female pattern as possible, and the individual should be raised as a female. In the subject with an adequate phallus the decision may be complicated.

As summarized by Money and Ehrhardt (66), the decision as to gender assignment should be made as early as possible but only after involvement of the parents in the decision. We prefer to complete diagnostic workups and perform plastic reconstruction when feasible before the infants are discharged. Orchidectomy should be performed to prevent the neonatal surge of androgen when the decision is made to raise the child as a female. It is our belief that parents usually react more favorably the more normal the infant appears. Obviously, such therapeutic interventions make it necessary to plan lifetime medical followups of patients, with institution of estrogen treatment at the time of expected puberty in those assigned a female role and institution of appropriate vaginal enlargement regimens (when necessary) when patients are ready to commence an active sexual life. After a child has reached the period normally associated with well-differentiated psychosexual identity (1 1/2-2 years) reassignment of gender is unwise and should be undertaken only after careful psychiatric, social, and endocrine evaluation.

This formulation which we follow in assessing male pseudohermaphroditism in the newborn nursery is based upon the unproven assumption (summarized above) that if androgens have a role to play in development of gender identity/role then the effect must be solely related to the increased androgen levels at puberty, e.g., that, no imprinting of the CNS has occurred during embryogenesis. A potentially valuable study in this regard is now being conducted in London; 5α-reductase deficiency was diagnosed in a 15 year old Pakistani immigrant who underwent a

reversal in gender (Subject 10 in Table IV). He was allowed to complete his switch from a female to a male role. An identical diagnosis was made in two younger sisters, but the parents had been embarrassed by the boy's action and decided to have the younger sisters gonadectomized to prevent such an occurrence (one is Subject 11 in Table IV). If either of these girls subsequently undergoes a switch in gender role despite the early castration, it will raise the possibility that some permanent CNS imprinting during embryogenesis or early postnatal life is involved in development of gender. It is conceivable that all patients with 5α-reductase deficiency should be raised as males.

65. Wilson JD, Walsh PC: Disorders of sexual differentiation. In Campbell's Textbook of Urology, JH Harrison, RF Gittes, AD Perlmutter, TA Stamey, PC Walsh (eds), Vol 2, Ch 42: WB Saunders, Philadelphia, 1979, p 1484.

66. Money J, Ehrhardt AA: Man and Woman, Boy and Girl: Johns Hopkins University Press, Baltimore, 1972.

b. Hormonal Management in the Adult

There are many unresolved problems as to the management of those individuals who are raised as males or switch to a male role after the time of expected puberty. To the extent that it is practical the hypospadias should be corrected. In some instances (such as the patient shown below) the correction is fairly successful.

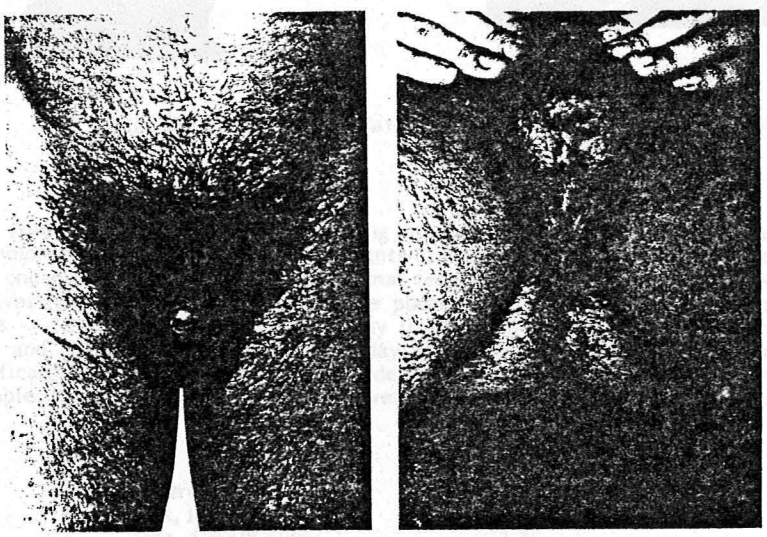


Fig. 31. Photograph of Patient 8, Table IV, following hypospadias repair. Courtesy of M. Besser.

The plasma testosterone and LH in such individuals are those of normal men, and as stated above virilization, albeit incomplete, does occur. However, growth of facial and body hair is usually deficient, there is no temporal hair regression, ejaculate volume is small, and the external genitalia are in the low normal range. Such subjects frequently complain of an incomplete virilization (photographs of the face and chest of one such patient are shown below).

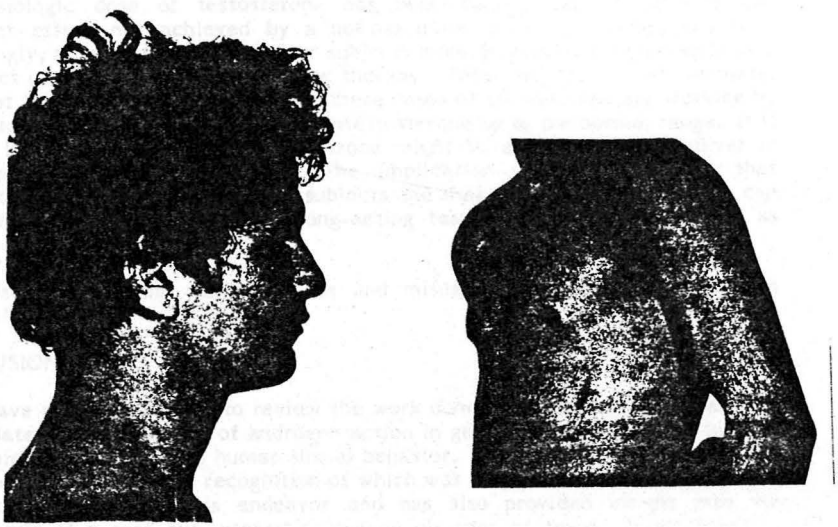


Fig. 32. Photograph of Patient 8, Table IV. Courtesy of M. Besser.

Four such patients are now being studied by the Endocrine Unit at St. Bartholomew's Hospital in London (Patients 8, 9, 10, and 19 in Table IV). Since all were incompletely virilized despite normal testosterone levels Dr. Besser decided to investigate the effect of raising the plasma testosterone to supraphysiologic levels -- first 1 mg testosterone/Kg/day (roughly 10 times the normal secretion rate) and then 5 mg testosterone/Kg/day (50 times the normal secretion rate). Significant positive nitrogen balance developed only on the higher dose; for example, the patient shown in Fig. 31 developed markedly positive N_2 balance:

	N_2 Balance, g/day
Control	-2.33
Test, 1 mg/Kg/day	+1.52
Test, 5 mg/Kg/day	+3.34

The controls for this study were two men with male pseudohermaphroditism due to defects of the androgen receptor who did not develop positive balance on either dose. On the basis of the effects on nitrogen balance, the four patients with 5α-

reductase deficiency have been given an amount of long-acting testosterone for two years that keeps the plasma testosterone in the range of 25-35 ng/ml (three times normal). All four have developed chest and facial hair, increased ejaculate volume, and some phallic growth, and they are uniformly happy with the results of the therapy. To my knowledge, this is the first instance in medicine in which a supraphysiologic dose of testosterone has been documented to produce any significant effect not achieved by a normal male level of the hormone (67). Interestingly, at least three of the four subjects have developed gynecomastia as a side effect of the high dose testosterone therapy. These studies are not complete. We do not know, for example, whether these doses of testosterone are working by mass action or by bringing plasma dihydrotestosterone up to the normal range. It is also not known whether dihydrotestosterone might be a more potent virilizer in these subjects than testosterone. The implication is clear, however, that virilization may be promoted in such subjects and that their function as males can be promoted by heroic doses of a long-acting testosterone preparation such as testosterone cypionate.

67. Wilson JD, Griffin JE: The use and misuse of androgens. Metabolism 29:1278, 1980.

CONCLUSION

I have attempted today to review the work done by us and by others designed to elucidate the mechanisms of androgen action in general and specifically in male phenotypic development and human sexual behavior. Study of the syndrome of 5α-reductase deficiency -- the recognition of which was a byproduct of these rounds -- has been invaluable in this endeavor and has also provided insight into the pathogenesis of a rare but interesting human disorder of sexual development as well.