ANDROGEN RESISTANCE SYNDROMES

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"in spite of plaits and a girl's name the structure

in the inguinal hernial sac may even be a testicle"

(De Quervain, 1923)

Patient 1

S.J., a 15 year-old black phenotypic female was thought to be normal until the age of 13 when the family noted failure of breast development and of menses, and the presence of 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 phallus that measured 5xl.5 cm, and a blind-ending vagina. Cystoscopy demonstrated a normal female urethra. The karyotype was 46,XY. Plasma levels of LH, FSH, testosterone, androstenedione, estradiol, and estrone were all within the range of normal men. No mullerian derivatives were present on exploratory surgery but well-developed wolffian structures were present so that the vas deferens could be injected with contrast material and its course into ejaculatory ducts emptying in the vagina followed. Testis histology was similar to otherwise normal undescended testes. She had a similarly affected younger sister.

Patient 2

S.V., a 26 year-old Latin-American phenotypic female was not seen by a physician during infancy but was considered by her family to have normal growth and development as a child. Breast development began at age 14 and was accompanied by axillary and pubic hair growth. She was referred for evaluation of primary amenorrhea. Family history was unremarkable. Height was 167 cm and weight 80 kg. There was no facial hair. Axillary and pubic hair and breast development were considered to be those of a normal woman. Masses presumed to be gonads were palpable in both inguinal regions. The genitalia were remarkable for clitoromegaly, hypoplastic labia minora, and posterior labial fusion. The vagina was blind-ending and no uterus was palpable. Plasma LH and FSH were each about twice normal, and plasma testosterone was 1600 ng/dl (normal male 300-1000 ng/dl). Karyotype was 46,XY. No mullerian duct structures were found at laparotomy, but bilateral testes and underdeveloped but unequivocal wolffian derivatives were detected.

Patient 3

J.W., a 15 year-old white male, was noted to have perineoscrotal hypospadias at birth. He had two maternal uncles and two half-brothers (same mother) with third degree hypospadias and cryptorchidism. At age 11 he had pre-pubertal prophylactic breast excision in anticipation of problems with gynecomastia. In spite of this he was concerned about recent breast enlargement. Exam included 8 ml testes in a bifid scrotum a urethral opening 2 cm below the base the glans (after 4 hypospadias repair procedures), and bilateral glandular breast tissue. Karyotype was 46,XY. Plasma hormone values included an LH three times the upper normal limit, a normal FSH, testosterone 1570 ng/dl (normal male 300-1000) and a slightly elevated estradiol.

Patient 4

J.B., a 38 year-old black male, was seen because of infertility and no sperm in two semen samples collected two weeks apart. He had no children in two marriages. His first wife had subsequently borne children by another husband. Libido and sexual potency were normal. There was no family history of gynecomastia or genital abnormality. He was a muscular man with a beard. No physical abnormalities were found, and gynecomastia was not present. Exam of the genitalia was normal with no hypospadias or bifid scrotum. Testicular volume was 20-25 ml. Fructose was present in the ejaculate. Karyotype was 46,XY. Plasma hormone values included an LH three times normal, a normal FSH, and testosterone of 1380 ng/dl.

I. INTRODUCTION

In 1957 Lawson Wilkins reported that administration of methyltestosterone in large doses to a patient with testicular feminization following castration failed to result in growth of sexual hair, clitoral enlargement, or change in the voice (1). This provided the first evidence for resistance to exogenous androgens in this best known form of hereditary male pseudohermaphroditism. Since then we have come to recognize that androgen resistance syndromes extend beyond the usual definitions of male pseudohermaphroditism (46,XY males with testes who fail to develop as totally normal phenotypic men), ranging from the almost normal-appearing phenotypic female with testicular feminization to the otherwise normal but infertile man (see Patient Summaries).

There are at least four developments in the last 20 years that have aided in the elucidation of androgen resistance syndromes:
1) The recognition that incomplete male pseudohermaphroditism in many families is the result of a single gene mutation (2).
2) The description of the principal processes involved in normal male embryonic sexual development (3).
3) The study of androgen-estrogen dynamics in normal individuals and the ability to assess the quantitative aspects of these two classes of hormones in patients with disorders of sexual development (4).
4) The unraveling of molecular processes by which androgens act within cells (5) and the recent adaptation of these methods to cultured fibroblasts from affected individuals.

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II. PERIPHERAL METABOLISM OF ANDROGENS

Testosterone, secreted by the testes in response to luteinizing hormone (LH) is the principal circulating androgen in men. An understanding of its action requires a consideration of its peripheral metabolism. Testosterone serves as the prohormone

for the formation of two types of active metabolites which mediate many of the processes involved in androgen action (Fig. 1). Testosterone can undergo irreversible reduction to dihydrotestosterone which serves as the intracellular mediator of many testosterone actions (6). On the other hand circulating testosterone can be converted to estradiol in the peripheral tissues of both sexes (4). This conversion of androgen to estrogen is termed aromatization and takes place in many peripheral tissues of which adipose tissue is probably the most important. The estradiol formed in some instances may act in concert with androgens to influence physiological processes but may also exert independent actions or have effects opposite to those of androgens (7). Thus the physiological consequences of circulating testosterone are the result of the combined effects of testosterone itself plus those of the active androgen and estrogen metabolites.

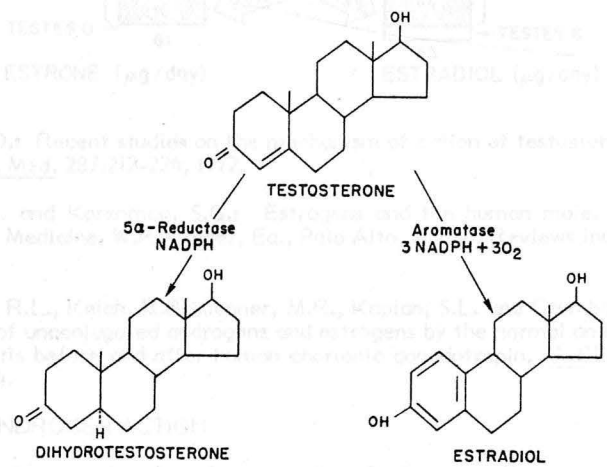

Figure 1

In considering the quantitative aspects of androgen-estrogen dynamics, the role of the primary adrenal androgen, androstenedione, and its conversion to the weak estrogen estrone must also be included. Androstenedione and testosterone as well as estrone and estradiol may be reversibly interconverted by the ubiquitous 17 β-hydroxysteroid dehydrogenase enzyme. In normal young men (Fig. 2) of an average 45 μg of estradiol produced daily, 17 μg (or about 35-40%) are derived from aromatization of circulating testosterone, 22 μg (about 50%) are derived from the weak estrogen estrone, and only 6 μg (about 10-15%) are secreted directly into the plasma by the testes (4). However, when plasma LH is elevated for any reason, direct secretion of estradiol into the plasma increases (8).

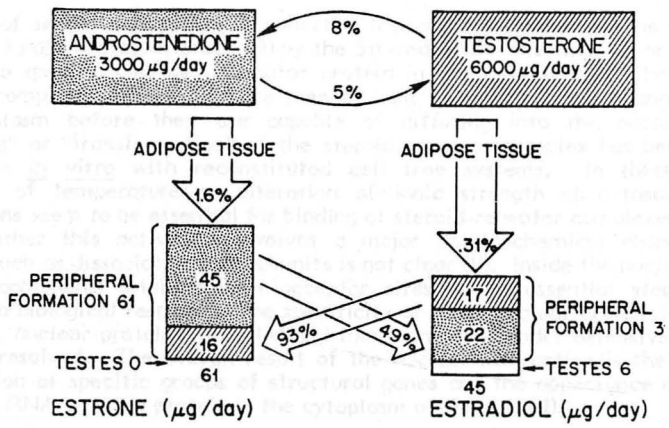

Figure 2

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III. NORMAL ANDROGEN ACTION

The current concepts of androgen action in target cells are summarized schematically in Fig. 3. Testosterone is secreted by the Leydig cells and is present in high concentrations (approximately 100 times plasma levels) in the testis. Although specific androgen binding proteins have been identified in the testis and epididymis of lower animals (apparently serving a transport function for testosterone through the Wolffian duct-derived structures), such tubular binding proteins have not been identified in higher primates and man (9). In the plasma, testosterone is principally bound to two proteins, testosterone-binding globulin and albumin. The protein bound steroid is in dynamic equilibrium with unbound or free hormone (10). It is felt that only the free fraction is able to enter cells and produce effects attributed to androgens. Although the entry of androgens (and other steroids) into target cells may be more complex than simple passive diffusion, the existence of specific transport mechanisms operating at physiological concentrations has yet to be demonstrated convincingly (11).

As indicated in Fig. 3 the major functions of androgen in men include the regulation of gonadotropin secretion, the initiation and maintenance of spermatogenesis, the formation of the male phenotype during sexual differentiation, and the

induction of sexual maturation at puberty. Inside the cell testosterone (T) can be converted to dihydrotestosterone (D) by the 5α-reductase enzyme. T or D is then bound to a specific androgen receptor protein in the cytosol (R). The hormone-receptor complexes (TR or DR) are then thought to undergo some change while in the cytoplasm before they are capable of diffusing into the nucleus. This "activation" or "transformation" of the steroid-receptor complex has been studied extensively in vitro with reconstituted cell free systems. In these systems elevations of temperature or alteration of ionic strength of cytosol receptor preparations seem to be essential for binding of steroid-receptor complexes to nuclei (11). Whether this activation involves a major physicochemical change in the receptor such as dissociation into subunits is not clear (11). Inside the nucleus steroid receptor complexes interact with acceptor sites as an essential step prior to effecting a biological response. The specificity of these nuclear acceptor sites (i.e. chromatin, nuclear proteins, or DNA) and their number is under extensive study but not fully resolved. The overall result of the nuclear interaction is the increased transcription of specific groups of structural genes and the appearance of specific messenger RNA and new protein in the cytoplasm of the cell (11).

Available evidence suggests that the testosterone-receptor complex regulates gonadotropin secretion, spermatogenesis, and the wolffian stimulation during sexual differentiation, whereas the dihydrotestosterone-receptor complex is responsible for external virilization during embryogenesis and the major portion of androgen action during sexual maturation and adult sexual life. The reason that testosterone seems to be the mediator of some androgen effects and dihydrotestosterone the mediator of others is not entirely clear. It may involve some subtle difference in receptor affinity that we are unable to detect, the transient presence of a specific testosterone binding protein in embryogenesis, or a phenomenon of the metabolism of testosterone and the local concentration in the genital tract. The mechanisms by which estrogens act to augment or block androgen action have not been totally defined. However, in the case of the prostatic growth in the aging male, small increases in estradiol may increase the level of androgen receptors in the prostate and thus enhance androgen action (12).

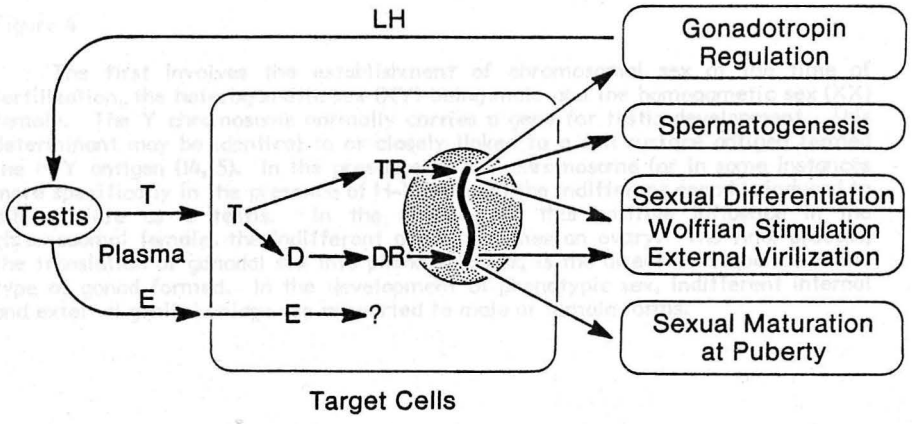

Figure 3

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IV. NORMAL MALE SEXUAL DEVELOPMENT

The embryos of both sexes develop in an identical fashion until approximately 40 days of gestation, and only thereafter does development diverge to result in the formation of the male and female phenotypes. As formulated by Jost (3,13), normal sexual development consists of three sequential processes (Fig. 4).

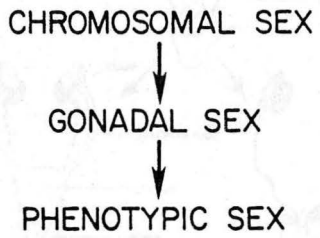

Figure 4

The first involves the establishment of chromosomal sex at the time of fertilization, the heterogametic sex (XY) being male and the homogametic sex (XX) female. The Y chromosome normally carries a gene for testis development. This determinant may be identical to or closely linked to a cell surface antigen termed the H-Y antigen (14,15). In the presence of a Y chromosome (or in some instances more specifically in the presence of H-Y antigen) the indifferent gonad is induced to differentiate as a testis. In the absence of this positive influence in the chromosomal female, the indifferent gonad becomes an ovary. The final process, the translation of gonadal sex into phenotypic sex, is the direct consequence of the type of gonad formed. In the development of phenotypic sex, indifferent internal and external genital anlage are converted to male or female forms.

The internal genitalia in the two sexes are derived from separate anlage, the mullerian and wolffian ducts that exist side by side in early embryos of both sexes (Fig. 5A) (16). The wolffian ducts are the excretory ducts of the mesonephric kidney, and the mullerian ducts are formed from the wolffian ducts. In the male the wolffian ducts give rise to the epididymis, vas deferens, and seminal vesicle, and the mullerian ducts disappear. In the female the fallopian tubes, uterus, and upper vagina are derived from the mullerian ducts, and the wolffian ducts regress. In contrast, the external genitalia and the urethra in the two sexes develop from common anlage (Fig. 5B). The urogenital sinus gives rise in the male to the prostate and the prostatic urethra and in the female to the urethra and a portion of the vagina. The genital tubercle is the origin of the glans penis in the male and the clitoris in the female. The urogenital swelling becomes the scrotum or the labia majora, and the genital folds develop into the labia minora or the shaft of the penis.

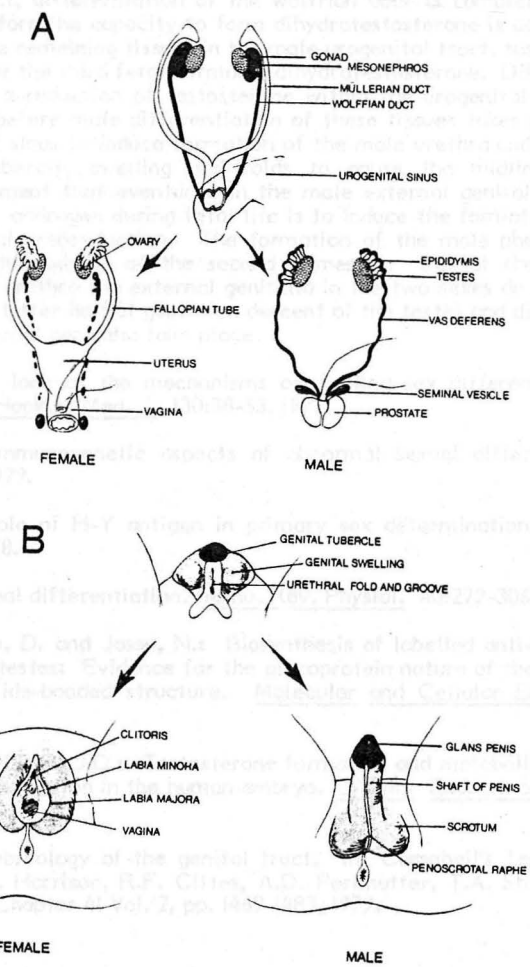

Figure 5

In the absence of the testis, as in the normal female or in the male embryo castrated prior to the onset of phenotypic differentiation, the development proceeds along female lines (13). Thus, masculinization of the fetus is the positive result of action of a hormone from the fetal gonad whereas female development does not require the presence of a gonad. Under ordinary circumstances development of the sexual phenotype conforms to chromosomal sex. That is chromosomal sex determines gonadal sex, and gonadal sex in turn determines phenotypic sex.

Control over the formation of the male phenotype is vested in the action of three hormones. Two of the three -- mullerian regression factor and testosterone are secretory products of the fetal testis. Mullerian regression factor is an incompletely characterized peptide hormone which acts to suppress the mullerian ducts thereby preventing development of the uterus and fallopian tubes in the male (17). Testosterone promotes virilization of the urogenital tract in two ways. It acts directly to stimulate the wolffian ducts to form the epididymis, vas deferens, and seminal vesicle. In fact, differentiation of the wolffian duct is completed in the human male embryo before the capacity to form dihydrotestosterone is acquired by these tissues (18). In the remaining tissues in the male urogenital tract, testosterone acts as a prohormone for the third fetal hormone, dihydrotestosterone. Dihydrotestosterone is formed by 5 α-reduction of testosterone within the urogenital sinus and lower urogenital tract before male differentiation of these tissues takes place (18). It acts in the urogenital sinus to induce formation of the male urethra and prostate, and in the genital tubercle, swelling and folds to cause the midline fusion, elongation, and enlargement that eventuate in the male external genitalia. Thus, the primary function of androgen during fetal life is to induce the formation of the accessory organs of male reproduction. The formation of the male phenotype is largely completed by the middle of the second trimester. But at the time of completion of the male urethra the external genitalia in the two sexes do not differ in size (19). During the latter half of gestation descent of the testes and differential growth of the male external genitalia take place.

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V. CLASSIFICATION OF DISORDERS OF SEXUAL DEVELOPMENT IN MAN AND THE ROLE OF ANDROGEN RESISTANCE

For the purposes of analysis and understanding of specific defects in sexual development as they occur in man, a classification based on the Jost model of the ordered sequential process of normal sexual development can be used. Thus disorders can be considered to be abnormalities of chromosomal, gonadal, or phenotypic sexual differentiation (Table I). This is obviously not meant to be all inclusive because it omits the disorders resulting from environmental insults as in the ingestion of drugs during pregnancy and poorly understood multifactorial developmental birth defects such as most cases of hypospadias.

TABLE I DISORDERS OF SEXUAL DEVELOPMENT IN MAN

- Disorders of Chromosomal Sex
  - Klinefelter Syndrome
  - XX Male Syndrome
  - Turner Syndrome
  - Mixed Gonadal Dysgenesis
  - True Hermaphroditism
- Disorders of Gonadal Sex
  - Pure Gonadal Dysgenesis
  - Absent Testis Syndrome
- Disorders of Phenotypic Sex
  - Female Pseudohermaphroditism
    - Congenital Adrenal Hyperplasia
    - Non-adrenal Female Pseudohermaphroditism
  - Male Pseudohermaphroditism
    - Testosterone Synthesis Defects (5)
    - Androgen Resistance Syndromes
    - Persistent Mullerian Duct Syndrome
  - Isolated Defects in Mullerian and Wolffian Development

Thus androgen resistance syndromes are forms of male pseudohermaphroditism and disorders of phenotypic sex. The karyotype of affected patients is 46, XY (normal chromosomal sex), bilateral testes are present (normal gonadal sex), but the phenotype that develops is not that of a normal male. Since the Persistent Mullerian Duct syndrome is a disorder characterized by normal virilization but failure of mullerian regression, the primary differential diagnosis in consideration of androgen resistance syndromes is one of the testosterone synthesis defects. These disorders also result in a spectrum of defective virilization secondary to a deficiency of one of the five enzymes necessary for testosterone synthesis: 20,22-desmolase, 3β-hydroxysteroid dehydrogenase, 17-hydroxylase, 17,20-desmolase, or 17β-hydroxysteroid dehydrogenase (20,21). The proportion of male pseudohermaphrodites who have a testosterone synthesis defect to those who have androgen resistance has not been precisely determined by studies at the molecular level. However, a recent endocrine assessment of fifty patients with male pseudohermaphroditism evaluated in childhood and adolescence could find no evidence for a testosterone synthesis defect in 88% of the patients (22). This study did not include any patients with complete testicular feminization, the most severe form of androgen resistance and, until recently, considered to be the most common. Thus androgen resistance may be more

common than previously recognized. The most common testosterone synthesis defect is probably 17 β-hydroxysteroid dehydrogenase deficiency. Postpubertal individuals with this disorder can be detected by measurement of plasma androstenedione, the immediate precursor to the enzyme block. The concentration is usually elevated to ten times the normal level. However, because of the elevations of the precursor and partial enzyme deficiency, the plasma testosterone level may be in the normal range (20). In prepubertal individuals an hCG stimulation test may be used to determine whether the ability of the testis to synthesize testosterone is normal (23).

The molecular defects that result in androgen resistance can be viewed as occurring at three steps in the pathway of normal androgen action (Fig. 6). There are abnormalities in the 5 α-reductase enzyme, receptor disorders, and disorders in which the 5 α-reductase and the androgen receptor are apparently normal. We have termed this last category receptor postive resistance and indicate that the defect may reside at a step distal to the receptor such as generation of messenger RNA. We currently recognize two or more different mutations in the 5 α-reductase enzyme: diminished enzyme activity and unstable enzyme. Receptor disorders can be associated either with a predominantly female or predominantly male phenotype. Each of these categories encompasses at least two mutations. Although individuals with a female phenotype (testicular feminization) are the best known form of receptor disorders, patients with a male phenotype may actually be more common (see below).

MOLECULAR BIOLOGY OF ANDROGEN RESISTANCE

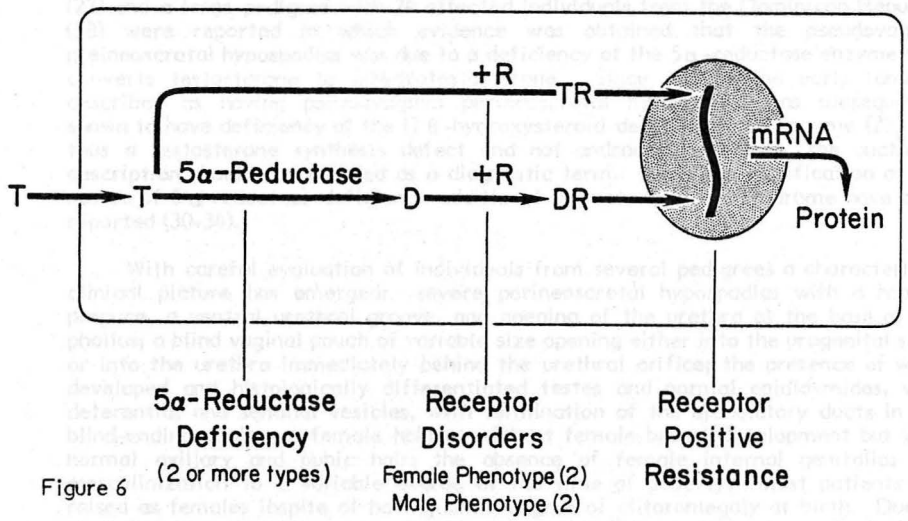

Figure 6

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VI. 5α-REDUCTASE DEFICIENCY

In 1961 Nowakowski and Lenz provided the first clinical and genetic definition of a specific type of hereditary male pseudohermaphroditism which they termed "pseudovaginal perineoscrotal hypospadias" (24). Five additional families with nine affected individuals were reported subsequently (25,26). As described initially, the characteristics of the syndrome include autosomal recessive inheritance, defective virilization of the external genitalia, normal virilization of the internal genitalia (wolffian duct-derived structures), 46,XY karyotype, bilateral testes, a female phenotype at birth with virilization at puberty, absence of gynecomastia, normal axillary and pubic hair, and apparently normal testosterone production. At approximately the same time in 1974 a family with two affected individuals in Dallas (27) and a large pedigree with 24 affected individuals from the Dominican Republic (28) were reported in which evidence was obtained that the pseudovaginal preineoscrotal hypospadias was due to a deficiency of the 5α-reductase enzyme that converts testosterone to dihydrotestosterone. Since one of the early families described as having pseudovaginal perineoscrotal hypospadias was subsequently shown to have deficiency of the 17β-hydroxysteroid dehydrogenase enzyme (29) and thus a testosterone synthesis defect and not androgen resistance, the anatomic description should not be used as a diagnostic term. Since the clarification of the nature of 5 α-reductase deficiency additional patients with the syndrome have been reported (30-34).

With careful evaluation of individuals from several pedigrees a characteristic clinical picture has emerged: severe perineoscrotal hypospadias with a hooded prepuce, a ventral urethral groove, and opening of the urethra at the base of the phallus; a blind vaginal pouch of variable size opening either into the urogenital sinus or into the urethra immediately behind the urethral orifice; the presence of well-developed and histologically differentiated testes and normal epididymides, vasa deferentia, and seminal vesicles, with termination of the ejaculatory ducts in the blind-ending vagina; a female habitus without female breast development but with normal axillary and pubic hair; the absence of female internal genitalia; and masculinization to a variable degree at the time of puberty. Most patients are raised as females inspite of having some degree of clitoromegaly at birth. Due to partial virilization at the time of puberty, castration is usually performed relatively early inhibiting an understanding of the natural history of the disorder. Insight has been gained by observations of the large kindred from the Dominican Republic

(28,35,36) and a recently described untreated older man with the disorder in the United States (34). At puberty, there is virilization with the development of a male muscular pattern, growth of the phallus and scrotum, and deepening of the voice, but no development of gynecomastia. Simultaneously there appears to be a change in gender role from female to male in the setting of an absence of medical intervention. The individuals have erections and are able to ejaculate. Body hair is decreased and the beard growth is scanty. Testicular histology and sperm densities may be normal. Prostate development is rudimentary or absent. Thus, an affected newborn presents with failure of the external genitalia (urogenital folds, urogenital tubercle, and urogenital sinus) to virilize completely but is an otherwise normal, testis-bearing male. In contrast, at puberty, considerable masculinization may take place.

The disorder is due to the homozygous state of an autosomal recessive gene that only causes abnormal sexual development in 46,XY individuals. Homozygous 46,XX individuals have been identified but are apparently totally normal including normal fertility. No clear-cut clinical evidence of heterozygous manifestations of the gene has been reported. Gonadotropin levels are either normal or only slightly elevated, and testosterone and estrogen production are those of normal men, providing an explanation for the failure to undergo female breast development at puberty. Plasma levels of dihydrotestosterone are usually only slightly lower than those of normal men. The fact that the defective virilization during embryogenesis is limited to the urogenital sinus and the anlage of the external genitalia has provided insight into the fundamental abnormality responsible for the disease. As indicated above, testosterone, the androgen secreted by the fetal testes, appears to be the intracellular mediator for differentiation of the wolffian ducts, whereas dihydrotestosterone is responsible for virilization of the urogenital sinus and external genitalia. Consequently, a failure of dihydrotestosterone formation should result in the precise phenotype observed in these patients, namely normal male wolffian duct derivatives but defective virilization of the structures originating from the urogenital sinus, tubercle, and swelling. In confirmation of this interpretation two different methods were utilized to demonstrate deficiency of the 5 α-reductase in the first two descriptions of the syndrome. 5 α-reductase activity was measured in tissue slices of genital skin and epididymis from one of the Dallas patients (27). In contrast the same conclusion was reached in the Dominican Republic patients by measurements of ratios of 5 β- to 5 α-reduced androgen metabolites in the urine (28). These patients and subsequent patients identified as having the disorder have had elevated ratios of etiocholanolone (5 β) to androsterone (5 α). In adult individuals the plasma dihydrotestosterone is somewhat lower than normal. However, in itself this is not diagnostic. The ratio of plasma testosterone to dihydrotestosterone in the Dominican patients was clearly higher than normal men. This same ratio was useful in distinguishing prepubertal affected individuals from normals if assessed after hCG administration (35).

The observation that 5 α-reductase activity was diminished in cultured genital skin fibroblasts from affected patients (37,38) provided the opportunity for studying the molecular characteristics of the mutation in individuals from different pedigrees. The enzyme in normal genital skin fibroblasts has a pH optimum of 5.5; measurement of this activity has proved to be the most sensitive assay for detecting enzyme deficiency. [Since nongenital skin and nongenital skin fibroblasts lack this activity at pH 5.5 and have lower 5 α-reductase activity in general, it is necessary to study the enzyme in cells cultured from explants of genital skin (foreskin,

scrotum, or labia majora) (37-40).] We have had the opportunity to study the residual enzyme activity in cultured cells from eight patients from six different families with 5α-reductase deficiency (Fig. 7) (Table II). The activity in the majority of families has been severely deficient, about one tenth the lower limit of control subjects. However, in patients from the Los Angeles pedigree (32), the activity in cultured cells was in the lower normal range in spite of severe deficiency of enzyme activity in fresh tissues (41). The enzyme in cells from these patients was found to have a normal pH optimum and Km for the substrate testosterone in contrast to cells from the first families described with the disorder (Dallas and Dominican Republic) (Table II). However, because of reduced affinity for the cofactor for the reaction (NADPH), the enzyme activity was found to be unstable and to have a rapid turnover (41). Recently cells from a patient from New York have been found to have intermediate levels of enzyme activity and altered affinity for both testosterone and NADPH with intermediate instability (34) (Fig. 7, Table II).

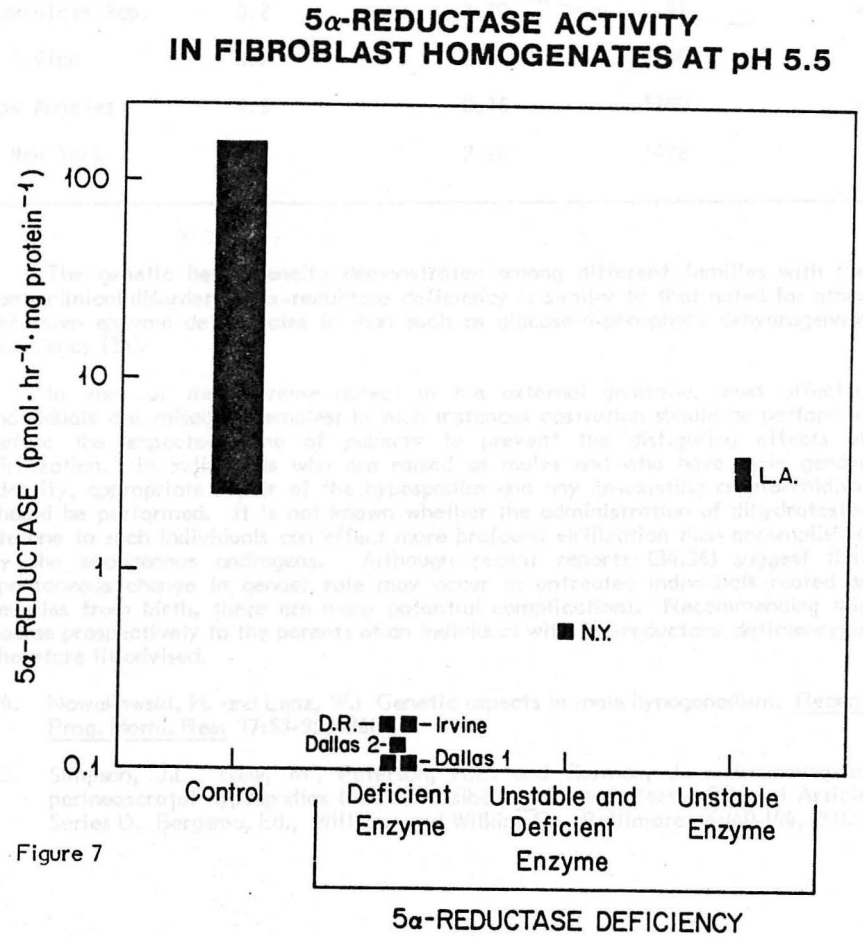

Figure 7

TABLE II. CHARACTERISTICS OF RESIDUAL 5α-REDUCTASE ACTIVITY IN SIX FAMILIES WITH 5α-REDUCTASE DEFICIENCY

Family	Activity pH 5.5 pmol/mg protein/h	Km for testosterone, μM	Km for NADPH, μM	Stability after cyclohexim
Controls (12)	33(2-150)	0.08 (±.01)	40 (±8)	> 95%
Dallas 1	0.2	1.80	250	> 95%
Dallas 2	0.2	1.30		
Dominican Rep.	0.2	3.40	97	> 95%
Irvine	0.2	2.90	140	
Los Angeles	4.5	0.16	1760	< 5%
New York	0.6	2.20	425	75%

The genetic heterogeneity demonstrated among different families with the same clinical disorder of 5α-reductase deficiency is similar to that noted for other recessive enzyme deficiencies in man such as glucose-6-phosphate dehydrogenase deficiency (34).

In view of the extreme defect in the external genitalia, most affected individuals are raised as females; in such instances castration should be performed before the expected time of puberty to prevent the disfiguring effects of virilization. In individuals who are raised as males and who have male gender identity, appropriate repair of the hypospadias and any co-existing cryptorchidism should be performed. It is not known whether the administration of dihydrotestosterone to such individuals can effect more profound virilization than accomplished by the endogenous androgens. Although recent reports (34,36) suggest that spontaneous change in gender role may occur in untreated individuals reared as females from birth, there are many potential complications. Recommending this course prospectively to the parents of an individual with 5α-reductase deficiency is therefore ill-advised.

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VII. RECEPTOR DISORDERS

A. Female Phenotype - Testicular Feminization

The syndrome of testicular feminization has been recognized as a distinct entity for a long time with clinical descriptions of this medical "curiosity" in the early 1800's (for review of early reports see Ref. 42). Major insight into the pathophysiology of the disorder came initially from careful pedigree analysis in 1937 in which Petterson and Bonnier deduced that affected individuals "must" be genetic males, that the defect could be due either to an X-linked recessive defect or a sex-limited autosomal dominant mutation, and that the syndrome might result from failure of male induction in an embryo in which the fundamental trend is toward the female phenotype (43). The term "testicular feminization" was first applied by Morris in 1953 (44). The disorder is generally considered to be the most common form of familial male pseudohermaphroditism with estimates of frequency varying from 1 in 2,000 (42) to one in 64,000 male newborns (45). Some authors claim that one in five intersex patients (excluding gonadal dysgenesis) have this diagnosis (46). Examined another way, testicular feminization is thought to rank third after gonadal dysgenesis and congenital absence of the vagina as a cause of primary amenorrhea, accounting for approximately 11% of these patients (47).

The major clinical features of the complete form of the disorder are quite uniform (42,48-51). A phenotypic female is seen by the physician because of primary amenorrhea (postpubertal) or inguinal hernia (prepubertal). The development of the breasts at the time of expected puberty and the general habitus and distribution of body fat are female in character. Many patients have a truly feminine appearance. Axillary and pubic hair are usually absent or scanty. Scalp hair is that of a normal woman, and facial hair is absent. The external genitalia are unambiguously female, and the clitoris is normal in size. The vagina is short or blind-ending; it may be absent or rudimentary. All internal genitalia are absent except for gonads. The testes may be located in the abdomen, along the course of the inguinal canal, or in the labia majora. Occasionally remnants of mullerian or wolffian duct origin can be identified in the paratesticular fascia or the fibrous bands extending from the testis. The histology of the testes is similar to cryptorchid testes of other causes but differs in that spermatogenesis is always absent. The karyotype is 46,XY (53) and

there is usually a history of similarly affected family members. As has been demonstrated in the best studied animal model of the disorder, the Tfm mouse (54), the gene for the disorder is X-linked (55). As in other disorders with decreased reproductive fitness, about a third of patients have negative family histories and are presumed to represent new mutations.

The hormone profile of patients with testicular feminization is characteristic of androgen resistance. Plasma testosterone levels and rates of production by the testes are normal or higher than in normal men (56-59). Observations of decreased dihydrotestosterone formation in these patients is probably secondary to the primary defect in androgen action and the resulting decreased mass of androgen target tissues that metabolize testosterone (60). It is likely that the elevated testosterone production rate is secondary to a high mean plasma LH (58,61,62) with more frequent secretory episodes (63), which in turn is due to defective feedback regulation because of resistance to testosterone action at the hypothalamic-pituitary level (62). Elevated LH levels are also probably responsible for the elevated estrogen production by the testes (64) and elevated blood estradiol concentrations in the spermatic vein (65). On average testosterone production is about 50% greater than normal and estradiol production about twice normal, but the estradiol component secreted directly by the testes is about seven to eight times normal (64). Thus, androgen resistance coupled with enhanced estradiol production results in the development of female secondary sex characteristics at the expected time of puberty as well as the formation of a female phenotype during embryogenesis.

About ten percent or less of the patients with testicular feminization have an incomplete or partial form of the disorder. Patients with incomplete testicular feminization resemble patients with testicular feminization except for some degree of ambiguity of the external genitalia and the development of slight virilization as well as feminization at puberty (66-69). The term has been used to describe many cases of incomplete male pseudohermaphroditism, and undoubtedly many of these patients do not have androgen resistance. However, some of the patients described under this term constitute a distinct phenotype, and their separation into a distinct androgen resistance syndrome may be justified (70).

Affected individuals have the habitus and general appearance of women, and, like patients with the complete disorder, most commonly present with primary amenorrhea. The karyotype is 46,XY. Testes are present in the abdomen or in the inguinal canals and are indistinguishable histologically from those in the complete form. The external genitalia are distinctive in that there is partial fusion of the labioscrotal folds and a variable degree of clitoromegaly. The vagina is short and blind-ending. At the time of expected puberty, variable feminization and partial virilization may both take place. At laparotomy all mullerian duct derivatives are absent, but the presence of wolffian duct structures is a distinctive feature that (together with the partial virilization of the external genitalia) clearly separates the phenotype from the complete form of the disorder. Although the wolffian duct structures are male in character, they are underdeveloped in comparison with those of normal men. The family history in most cases is uninformative. However in at least one of the families studied adequately to exclude a testosterone synthesis defect the presence of two affected half-siblings with the same mother and a different father support X-linked inheritance (A. DiGeorge, unpublished observations). No convincing pedigree has been reported in which the complete and incomplete forms of testicular feminization co-exist in the same family. The endocrine findings are similar to those in the complete form of the disorder (70). In one well-studied patient dihydrotestosterone formation was normal in fresh tissue slices (70) and cultured cells (37,38).

The nature of the androgen resistance has been elucidated only recently. Initial reports of the amount of specific high affinity dihydrotestosterone binding in patients with the complete disorder documented a severe deficiency of the androgen receptor (71,72) similar to the defect demonstrated in the Tfm mouse (73-78). These observations in the human disorder were confirmed by other laboratories (79,80) and extended to include partial receptor deficiency in one patient with the incomplete form of the disorder (79). Although near absence of the androgen receptor was thought to be the predominant abnormality in testicular feminization, at least one family with apparently the typical complete form of the disorder was noted to be receptor positive with no demonstrable abnormality of binding or nuclear localization (81) (see Receptor Positive Resistance below). Recently evidence for two types of abnormalities of the androgen receptor in testicular feminization has been obtained (82) (Fig. 8). A number of patients with both the complete and incomplete forms of testicular feminization were noted to have only partial receptor deficiency (about half-normal levels of binding) under the usual monolayer binding assay conditions. Evidence was sought for qualitative abnormalities in the measurable receptor by studies of affinity, specificity, turnover, nuclear localization, and thermolability. The last parameter was the only one found to be abnormal in these testicular feminization patients with dihydrotestosterone binding (Bmax or the amount of receptor) falling more than 80% when the binding reaction was performed at an elevated temperature (42°C) (Fig. 8). In other systems thermolability has proved to be a sensitive marker for structural abnormalities in enzymes and other protein. The thermal inactivation was rapidly reversed on lowering the assay temperature back to 37°C suggesting temporary alteration of the tertiary structure of the binding protein at elevated temperature. The patients with this temperature sensitive receptor included three pairs of siblings. The family studies suggest X-linked inheritance. Thus two different molecular abnormalities of the receptor can lead to the syndrome of testicular feminization: absence of binding or an unstable receptor. Although the unstable receptor has been associated with both the complete and incomplete form of the disorder, thus far only patients with the complete form have been shown to have complete absence of binding. Whether the qualitative abnormality is allelic to the receptor negative mutation is not known.

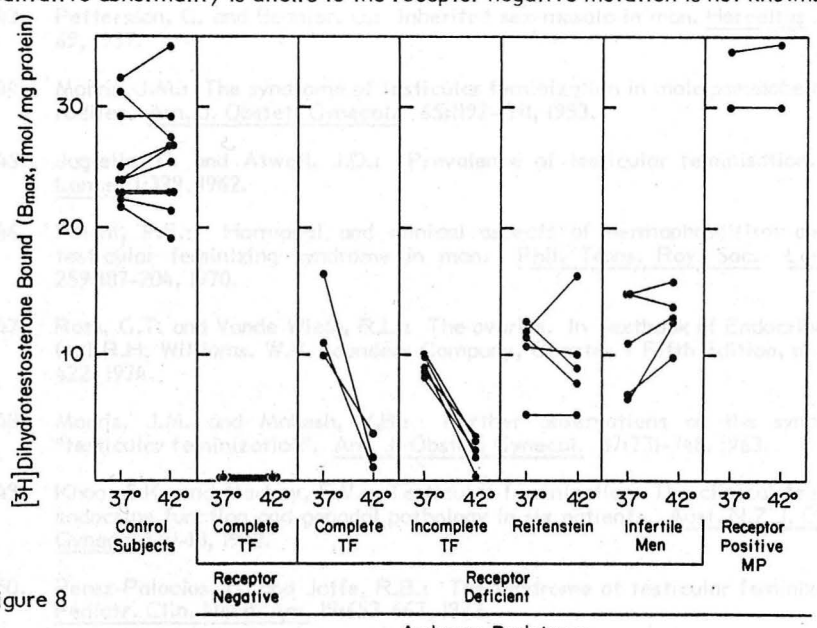

Figure 8

The treatment of patients with testicular feminization is different for the complete and incomplete forms. For those patients who have totally normal female external genitalia (the complete form), castration is indicated in the prepubertal patients if the testes are present in the inguinal region or the labia majora and cause discomfort or if there is associated hernia formation. Since affected individuals undergo a normal pubertal growth spurt and feminize at the expected time of puberty and since testicular tumors rarely develop until after the completion of puberty in patients with intra-abdominal or otherwise asymptomatic testes (83,84), it is common to delay castration until after adolescence. The most serious complication in the untreated patient is the development of tumors in the undescended testes. It is not known whether tumor development is more common than in cryptorchidism of other causes, but many such tumors do behave as true malignancies, and consequently the testes should be removed after secondary sex development is complete (84). Since patients with the incomplete disorder virilize (as well as feminize) at puberty, any prepubertal patient with clitoromegaly or posterior labial fusion should have gonadectomy prior to the expected time of puberty. After castration postpubertally or at the age of usual puberty in individuals castrated prepubertally, replacement estrogens should be given. The blind-ending vagina is usually of sufficient depth to permit intercourse and will usually increase in depth with repeated intercourse. In those patients in whom there is inadequate vaginal depth to allow intercourse, either operative or nonoperative techniques may be used to increase depth as is done for patients with congenital absence of the vagina (85). Studies of psychosexual function suggest that these patients are unmistakably feminine in behavior and outlook and can function as normal adoptive mothers (86).

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B. Male Phenotype

Reifenstein syndrome Several X-linked recessive forms of incomplete male pseudohermaphroditism have been described in which the predominant phenotype is male. This includes the syndromes described by Reifenstein (87,88), Rosewater (89), Gilbert-Dreyfus (90), Lubs (91) and their colleagues. Each of these disorders was originally assumed to be a distinct nosological entity, but three fairly extensive pedigrees have been described in which affected members of the same family exhibit variable phenotypes that incorporate the defects described (92-94). Since all these pedigrees are compatible with X-linkage, it is our interpretation that these syndromes likely constitute variable manifestations of a single mutation and can be termed "Reifenstein syndrome" (94). Additional patients who seem to be examples of this syndrome have subsequently been described (95-99).

The spectrum of defective virilization ranges from a male with gynecomastia and azoospermia through more severe defects such as hypospadias to individuals with pseudovagina formation. The most common presentation is that of a male with perineoscrotal hypospadias, azoospermia, and gynecomastia that develops at or after puberty. Puberty is characterized by the development of axillary and pubic hair but minimal development of chest and facial hair. Temporal recession of the hairline is minimal, and the voice is often pre-pubertal in character. Cryptorchidism is frequent, and the testes are smaller than normal (although usually larger than in the Klinefelter syndrome). Leydig cells appear normal, and the tubules contain both germinal cells and Sertoli cells but usually no maturation of the germinal cells beyond the primary spermatocyte. The exception to this has been a recently reported family with normal sperm densities but decreased ejaculate volume in whom normal spermatogenesis was found on biopsy (99). Since some individuals with Reifenstein syndrome have defects in wolffian duct-derived structures such as absence or severe hypoplasia of the vas deferens, the infertility which is a consistent finding may be due to anatomical changes in the ejaculatory system in addition to defective spermatogenesis. Although affected individuals may exhibit ambiguous genitalia at birth, most patients are raised as men. The psychological development in most appears to be unambiguously male, and phenotypic men have had successful marriages.

Plasma LH and testosterone levels are elevated (94). The increased frequency and magnitude of the LH secretory episodes are similar to those seen in testicular feminization (63). Quantitative studies of androgen and estrogen dynamics have demonstrated increased testosterone and estradiol production similar to testicular feminization. In fact in the few patients studied the total amount of estradiol produced and the quantity secreted by the testes was greater than the mean value of these parameters in patients with complete testicular feminization. In spite of this the degree of feminization at puberty is not so pronounced. This is probably because the androgen resistance is incomplete, and, as a consequence, the imbalance of the two hormones within the cell is not so severe.

Studies designed to examine the molecular basis of the androgen resistance have used cultured fibroblasts from affected patients. Levels of dihydrotestosterone binding from patients with the typical familial disorder have been partially deficient (79,82,100,101) (Fig. 8). Attempts to demonstrate qualitative abnormalities in the residual dihydrotestosterone binding in the form of alterations in affinity, specificity, or turnover of the receptor were unsuccessful (100). Unlike the receptor deficient testicular feminization patients, the receptor in cells from these patients was not thermolabile (82) (Fig. 8). Since the mutation has been shown to involve a protein that is known to be X-linked, we can conclude that the inheritance is X-linked.

The hypospadias and cryptorchidism should be corrected surgically. The gynecomastia, which is believed to result from increased estrogen production acting in concert with androgen resistance, may be disfiguring. The only form of therapy that has been successful for the gynecomastia is surgical removal.

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Infertile Male Syndrome The second major category of receptor disorders with a male phenotype has only recently been recognized (102); and, unlike the other types of androgen resistance, it is not actually a form of male pseudohermaphroditism. However, this form of androgen resistance may eventually be shown to be the most common. In evaluating the variable presentation of the Reifenstein syndrome, some men with an X-linked disorder of gynecomastia and infertility were encountered. It was thus anticipated that similar minimally affected patients might be found. However, few such patients have been identified. Instead patients without a positive family history or gynecomastia have been found to have androgen resistance when ascertained solely on the basis of infertility. These 46,XY men do not have hypospadias or detectable abnormalities of wolffian duct structures but have infertility due to azoospermia or oligospermia and androgen-estrogen dynamics similar to those identified in the patients with the other receptor disorders described above (102). Mean plasma concentrations and production rates of testosterone were approximately twice the average found in normal men. Plasma LH levels were also elevated in two of these first three patients studied in detail. The amount of high affinity dihydrotestosterone binding is diminished to less than half normal and similar to that present in patients with Reifenstein syndrome (82,102) (Fig. 8). Also like the patients with Reifenstein syndrome, the androgen receptor in the cells from these patients does not appear to be qualitatively abnormal by any of the current methods of evaluation. There is no consistent fall in receptor level with elevated temperature.

The major unanswered question about this form of androgen resistance is its frequency in the large population of infertile men. About 15% of all marriages are involuntarily childless. In at least 30% of these marriages the husband can be identified as the responsible factor. Thus, as many as 5% of married men are infertile. Although large series of consecutive patients with male infertility claim a documented etiologic factor in over 80% of the individuals (103), careful evaluation of the categorization makes this seem unlikely. For example varicocoele is claimed to be the causative factor in 39% of cases. But over half of the patients with varicocoele have sperm densities less than 10 million and a response to varicocoelectomy in terms of pregnancy that may not be different than that anticipated in a control group (104,105). Thus it is probably reasonable to conclude that at least one fourth of men with infertility do not have a known explanation for the problem.

As suggested by Walsh (106), one approach to try to assess the percentage of infertile men with androgen resistance might be to look for patients with both increased plasma LH and testosterone levels. Elevated plasma testosterone levels were noted in 2% of men with hypospermatogenesis in one study (107). Since both testosterone and LH concentrations are elevated in androgen resistance, Aiman has evaluated the product of the plasma LH and testosterone in a number of normal and infertile men. The mean of this product was 102 in normal men, 287 in 10 subjects with Reifenstein syndrome or incomplete testicular feminization, and 233 in 7 subjects with azoospermia and decreased androgen receptor levels. This index was more than twice normal (i.e. greater than 200) in 23% of 67 men with azoospermia or severe oligospermia, but in only 6% of 78 men with sperm counts greater than 10 million/ml (108). Thus androgen resistance may be a common cause of male infertility. Unfortunately, at the present time, as in the other receptor disorders, no specific therapy is known for the infertility.

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VIII. RECEPTOR POSITIVE RESISTANCE

When one family with the syndrome of complete testicular feminization was noted to have normal amounts of whole cell dihydrotestosterone binding in cultured fibroblasts and normal nuclear localization (81), a new category of molecular abnormalities leading to androgen resistance was identified. However, the clinical phenotype in this category is the least well defined. All patients are 46,XY chromosomal males with bilateral testes and no evidence for a testosterone synthesis defect. Plasma testosterone levels are those of normal men or higher and plasma LH is elevated. But while the first patients reported with receptor positive resistance appeared to have typical testicular feminization, subsequent patients have had an incomplete form of male pseudohermaphroditism varying from clitoromegaly (109) to a phallus with perineal hypospadias (98). In addition some patients reported as having a phenotype similar to Reifenstein syndrome but a negative family history were also noted to have normal androgen receptor levels (101). 5α-reductase activity has been normal in all of the patients in whom it has been measured (98,101,109).

The site of the molecular abnormality in these patients is still unclear. Although receptor levels are normal, it is possible that the receptor is qualitatively abnormal. In the two patients we evaluated no abnormality of affinity, turnover, or nuclear localization could be found (109). In addition there is no thermolability of the androgen binding (82) (Fig. 8). Either more subtle qualitative abnormalities of the receptor must be present such as inability to bind to the appropriate high affinity sites within the nucleus, or the defect must reside at some site distal to the receptor such as the generation of specific messenger RNA. What is needed to aid in the in vitro assessment of androgen resistance in these patients is some marker of androgen action in the cultured cell. Unfortunately such a marker has not been identified to date.

The treatment of these patients depends on the phenotype of the external genitalia and in most cases involves a sex of rearing of female with castration to prevent virilization at puberty and reconstructive surgery of the external genitalia when indicated.

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In summary the spectrum of patients with these disorders of androgen resistance spans all of sexual differentiation from almost normal men to phenotypic women. It has been possible to classify these apparently overlapping disorders into 5α-reductase deficiency, receptor disorders, and receptor positive resistance. In view of the genetic heterogeneity already demonstrated in this group of disorders, it is certain that additional variants will be described for each of these three steps and for as yet uncharacterized steps in androgen action. The classification of androgen resistance proposed here (Table III) provides a suitable framework upon which the pathogenesis of new variants can be defined.

TABLE III CLASSIFICATION OF ANDROGEN RESISTANCE

- 5 α-Reductase Deficiency
  - Decreased enzyme activity
  - Unstable enzyme
- Receptor Disorders
  - Female Phenotype - Testicular Feminization
    - Receptor Negative TF
    - Unstable Receptor TF
  - Male Phenotype
    - Reifenstein Syndrome
    - Infertile Male Syndrome
- Receptor Positive Resistance