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Characterization of the Testicular Abnormality in 5α-Reductase Deficiency*

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ABSTRACT. The testes of five phenotypic women (from four families) with 5α-reductase deficiency were studied. In one of the patients, the enzyme deficiency was similar in the testis and epididymis and in fibroblasts cultured from the labia majora. In testes from four of the patients, the concentrations of the 5α-reduced steroids dihydrotestosterone and 3α-androstanediol were less than 10% of those in normal subjects. We conclude that the testis is involved in 5α-reductase deficiency. Impaired spermatogenesis was evident in testicular biopsies from all five subjects, and in two, sperm production, as estimated in testicular homogenates, was less than 10% of normal. The extent to which spermatogenic arrest is due to 5α-reductase deficiency or testicular maldescent is not clear. (*J Clin Endocrinol Metab* **63:** 1091, 1986)

THE 5α-reductase deficiency is a rare autosomal recessive mutation that impairs the 5α-oxidoreduction of steroid hormones, including the conversion of testosterone to dihydrotestosterone (1). In affected females, the mutation is phenotypically silent, implying that 5α-reduced steroid hormones play no obligatory role in the endocrinology and/or reproduction of women (2, 3). In males, however, dihydrotestosterone mediates virilization during embryogenesis of the external genitalia and urethra and a major portion of virilization in postnatal life (4). As a consequence, males with 5α-reductase deficiency have a characteristic form of male pseudohermaphroditism that was originally termed pseudovaginal perineoscrotal hypospadias (1, 5). Despite a 46,XY karyotype, testes that secrete normal male levels of testosterone, and a normal male excretory duct system (epididymides, vasa deferentia, seminal vesicles, and ejaculatory ducts), their ejaculatory ducts terminate in a vagina, and the external genitalia are either ambiguous or female in character. Approximately 60 patients with this disorder have been characterized, including 22 patients from 18 families studied in Dallas (1, 2, 6–14). Two enzymatic variants of the disorder are recognized. In the usual form, 5α-reductase activity is so low that the residual protein cannot be easily characterized; in the other, binding of steroid and/or NADPH to the mutant enzyme is impaired (15). The phenotypic manifestations of these 2 types of defect appear to be identical. In all patients studied to date, however, 5α-reductase activity, although low, was not absent, and some dihydrotestosterone formation could be demonstrated.

Several aspects of 5α-reductase deficiency are unresolved. One has to do with the role of dihydrotestosterone in spermatogenesis. Androgen action is essential for the formation of sperm, and since 5α-reductase activity in normal testis is low (16), it has been generally assumed that testosterone is the active androgen that performs this function. The availability of 46,XY individuals with 5α-reductase deficiency should make it possible to examine the relation between dihydrotestosterone formation and spermatogenesis, but study of sperm production is hampered by three features of the disorder. First, the common anatomical abnormalities of the ejaculatory

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duct system preclude quantitative collection of the ejaculate, making it difficult to quantify sperm production directly. Second, histological techniques for quantifying spermatogenesis can rarely be applied. The testes are frequently left intact, since at the time of expected puberty, some affected individuals change gender behavior pattern from female to male (17); when this occurs, the partial virilization that eventuates is allowed to proceed unimpaired. Third, the degree of testicular descent varies. In some patients, the testes descend into the labia majora, whereas in others, there is a variable failure of the terminal phase of descent. When testicular descent is incomplete, secondary effects of maldescent on spermatogenesis must be separated from any direct effects of 5α-reductase deficiency itself.

As a result of these problems, quantitative studies of sperm production in mature patients with 5α-reductase deficiency and descended testes are limited to histological studies of spermatogenesis in four subjects (2, 10, 12, 18), an assessment of sperm numbers in a partial ejaculate from another patient (11), and measurements of sperm production in two brothers in whom it was possible to collect ejaculates after surgical repair of the urethra (14). In five of these patients, spermatogenesis was profoundly impaired.

We report here studies of the testes of five 46,XY phenotypic women with 5α-reductase deficiency who elected to lead lives as women and undergo castration. In two patients, one or both testes were in the labia majora, and in three, they were in the inguinal canal. We quantified sperm production in two patients using techniques of tissue analysis. In addition, we analyzed 5α-reductase activity in the testis from one patient and the content of the 5α-reduced androgens dihydrotestosterone and 3α-androstanediol in the testes of four patients. The findings document that 5α-reductase deficiency affects the testes as well as extragonadal tissues, confirm previous reports that impaired spermatogenesis is common, and suggest a role for dihydrotestosterone formation in normal spermatogenesis.

Case Reports

Patient 1, a 16-yr-old girl, was referred to the University of Illinois Hospital because of primary amenorrhea, virilization, and failure of breast development. She was born in Mexico. The external genitalia at birth had been thought to be those of a normal female. During the preceding 3 yr, a masculine pattern of muscular development had been noted, and she became aware of clitoral enlargement. She has two younger normal sisters and two normal brothers. The family history was uninformative.

On physical examination she was 160 cm in height and weighed 58 kg. A moderate degree of facial acne was present, but there was no beard. Muscular development of the arms and chest resembled that of an adolescent male. There was no female breast development. Pubic hair was Tanner stage IV, with male type distribution, and some axillary hair was present. The clitoris measured 4 cm in length. There were separate urethral and vaginal orifices. Gonads were palpable in the inguinal canals. The vagina was short and had a narrow orifice. No cervix was present.

Karyotype was 46,XY. Serum testosterone was 5.8 ng/ml, and dihydrotestosterone was 0.2 ng/ml. FSH was 11 mIU/ml (normal, 5–20 mIU/ml), and LH was 7.8 mIU/ml (normal, 5–20 mIU/ml). Serum 17-hydroxyprogesterone, PRL, GH, cortisol, T_4, electrolytes, and glucose were normal. Ultrasound of the pelvis showed a vagina. No uterus or Fallopian tubes were identified by ultrasonography or computerized tomography of the pelvis. On the basis of the clinical and laboratory findings, a tentative diagnosis of 5α-reductase deficiency was made.

Psychological evaluation indicated normal female gender identity and gender role. A psychiatric consultant concurred in these findings and found no evidence of depression. There were no contraindications to corrective surgery of the genitalia and hormone therapy for female maturation, as requested by the patient and her parents.

Cystourethroscopy revealed a 3-cm female urethra with poor trigonal formation and absence of an interureteric ridge. The bladder wall mucosa and bladder neck were normal. The urethral orifice was located on the anterior wall of the vagina. At surgery a gubernaculum-like structure was found to connect each testis to the ipsilateral labial structure. The epididymis, vas deferens, and spermatic vessels appeared normal. The anatomy of the Wolffian duct-derived structures was examined in detail. After injection of indigo carmine dye into each vas, dye entered the vagina through small orifices in its lateral walls. Contrast material was then injected into the vasa, and a radiograph was taken. Each vas deferens and seminal vesicle was opacified, and the seminal vesicles were found to terminate in the region of the vagina. Bilateral orchiectomy and clitoroplasty were performed. Each testis measured 1.8 × 1.5 × 1.0 cm. On histological examination, the basement membrane of the spermatogenic tubules was normal, and the tubules were lined by Sertoli cells. No spermatogenesis was seen in one testis, and only spermatogonia and primary spermatocytes were seen in the other testis. Both testes contained foci of Leydig cell hyperplasia. The resected shaft of the phallus measured 3 × 2 × 1 cm; histological sections showed a segment of erectile tissue surrounded by fibrous tissue. At surgery, biopsy of the foreskin was obtained for propagation of fibroblasts. Portions of the testes and epididymides were used for measurement of 5α-reductase and androgen content and for electron microscopy.

After surgery, the patient was given conjugated estrogens, first 0.625 mg daily and later 1.25 mg daily. The patient returned to school with full activity, including participation in sports. Examination 4 months later revealed marked improvement in facial acne. Breast development had reached Tanner stage II.

Patient 2, an American Indian, was referred at age 16 yr to the St. Joseph's Medical Center (Phoenix, AZ) because of clitoromegaly. She had been raised as a female, with no recognized ambiguity of the genitalia. At the age of 13 yr, her voice

began to deepen, and clitoromegaly was noted. At age 14 yr, the clitoris measured 1 × 3 cm. She had no breast enlargement, pubic hair and urethral orifice were female in character, and a blind-ending vaginal pouch was present. Testes were palpable in the right labioscrotal fold (3 × 5 cm) and the left inguinal canal (2 × 3 cm). The karyotype was 46,XY, and the plasma testosterone level was 10 ng/ml. Eight siblings have normal genitalia, and one sibling with ambiguous genitalia died in infancy. Because of apparent apprehension on her part, further workup was delayed. At age 16 yr, she was 178 cm in height, thin and well muscled, and had fine hairs on the upper lip and sideburn areas but none on the chin or cheeks. No breast tissue was palpable. The pubic hair was Tanner stage I, and the clitoris measured 2 × 6 cm. One testis was present in the right labial fold, and the other was in the left inguinal canal. There was a single perineal opening, which was 3 cm deep. No uterus could be felt on bimanual examination. Repeat karyotype was 46,XY. Her serum hormone levels were as follows: LH, 7 mIU/ml; FSH, 4.5 mIU/ml; testosterone, 11.4 ng/ml; and dihydrotestosterone, 0.33 ng/ml. A diagnosis of 5α-reductase deficiency was made. Because of the desire of the patient and her parents that she continue life as a woman, the decision was made to remove the testes and perform a clitoral resection. At the time of surgery, contrast medium was injected into the vasa deferentia, and the presence of seminal vesicles and ejaculatory ducts that terminated in the vagina was documented by x-ray. Spermatogenesis was ongoing in both testes, as evidenced by the presence of spermatogonia, spermatocytes, and spermatids. Samples of testes were processed for electron microscopy, and a biopsy of labia majora was obtained for propagation of skin fibroblasts. After surgery, the patient was treated with ethinyl estradiol.

Patient 3 was thought to be a normal female until the age of 16 yr, when she sought medical advice because of failure of breast development and primary amenorrhea. The parents are unrelated. She has two siblings, a sister who has regular menses and an affected sister who is described below. Physical examination revealed no signs of feminization; no breast tissue was palpable. Her voice was deep, but no acne or temporal recession of the hairline was present. Testes were palpable in the inguinal canals. Clitoromegaly and a blind-ending vagina were present. Laboratory analyses performed at the Universities of Bonn and Innsbruck revealed the following: serum testosterone, 9 ng/ml (normal, 3.5–7); dihydrotestosterone, 0.04 ng/mg (normal, 0.35–0.9); LH, 11.6 mIU/ml (normal, 3–10); and FSH, 8.7 mIU/ml (normal, 2–10). Cystourethrography showed a female urethra. The karyotype was 46,XY. Exploratory laparoscopy of the pelvis revealed bilateral testes and epididymides and the absence of a uterus and Fallopian tubes. Injection of contrast medium into the vasa deferentia showed seminal vesicles and ejaculatory ducts that terminated in the vagina. Since the psychosexual orientation of the patient was female, orchiectomy and clitoral resection were performed, at which time a biopsy of labia majora was obtained for the propagation of skin fibroblasts. On histological examination of the testes, spermatogonia were identified, but no spermatocytes or spermatids were present. One testis was frozen and shipped to Dallas for analysis. This histology of the epididymis was normal. Since surgery, the patient has been treated with estrogens and progestogens and has made a successful adjustment as a woman.

Patient 4, the youngest sibling of patient 3, was thought to be a normal girl. She was first evaluated at the age of 14 yr because of failure of breast development and amenorrhea. Physical examination revealed a boyish habitus, low voice, no breast tissue, bilateral inguinal testes, a large phallus, a normal female urethral orifice, and a blind-ending vagina. Laboratory analyses at the Universities of Bonn and Innsbruck revealed a serum testosterone level of 3.6 ng/ml (normal, 3.5–7), dihydrotestosterone level of 0.1 ng/ml (normal, 0.35–0.9), LH level of 9.1 mIU/ml (normal, 3–10), and FSH level of 20 mIU/ml (normal, 2–10). A cystourethrogram showed a female urethra. Since the psychosexual orientation was female, an orchiectomy was performed. On this occasion, an x-ray contrast study revealed bilateral seminal vesicles and ejaculatory ducts that emptied into the vagina. Histological examination of the testes revealed spermatogonia but no spermatocytes or spermatids; the structure of the epididymis appeared normal. One testis was frozen and shipped to Dallas for analysis. Since the time of surgery, the patient has been treated with estradiol. A clitoroplasty is planned.

Patient 5 was a 35-yr-old Vietnamese woman seen at the Harbor/UCLA Medical Center because of primary amenorrhea. A younger sibling was known to have male pseudohermaphroditism. This patient was noted to have no palpable breast tissue, normal axillary and pubic hair, no facial hair, a 4-cm clitoris, a deepened posterior fourchette and small vaginal orifice, a blind-ending vagina that measured 1 × 3.5 cm, and testes in both labia majora. The karyotype was 46,XY. Serum testosterone was 6.7 ng/ml, dihydrotestosterone was 0.2 ng/ml, LH was 30 mIU/ml, and FSH was 32 mIU/ml. A biopsy of the labia majora was obtained for propagation of skin fibroblasts. Because of female gender identity, the decision was made to remove the testes and perform a clitoral resection. Histological examination of the testes (each of which measured 4 × 2 × 2 cm) revealed that spermatogenesis was defective, in that some tubules contained Sertoli cells only, some tubules were hyalinized, and only occasional tubules contained all types of germ cells from spermatogonia to mature spermatids. The histology of the epididymis was that of a normal male. The lower Wolffian duct system was not characterized. At the time of surgery, one testis was frozen and shipped to Dallas for analysis.

Materials and Methods

Assay of 5α-reductase and androgen receptor in cultured skin fibroblasts

The techniques for the propagation of fibroblasts and assay of 5α-reductase and androgen receptor were described previously (15, 19).

Assay of 5α-reductase in testis and epididymis

Testes from patient 1 obtained at surgery and a normal testis obtained at postmortem examination were dissected free of epididymis, immediately frozen in dry ice, and stored in liquid nitrogen. To prepare microsomes, aliquots of testis and epididymis were minced and homogenized in 3 vol 10 mM Tris-Cl

buffer, pH 7.4, containing 0.25 M sucrose and 1 mM EDTA, first in a Polytron (Brinkmann Instruments, Westbury NY) and then in a Dounce homogenizer (Kontes Co., Vineland, NJ). The homogenates then were centrifuged at 200,000 × *g* for 30 min, and the pellets (termed microsomes) were washed three times in 10 mM Tris buffer, pH 7.4, resuspended in the same buffer containing 20% glycerol, and either assayed immediately or frozen at −70 C for subsequent assay.

Assays for 5α-reductase activity contained 0.25 μM testosterone, 0.4 μM (10,000 dpm) [4-^{14}C]dihydrotestosterone, 1 mM NADPH, 100 μg BSA, microsomes from testis (0.7–1.2 mg protein) or epididymis (0.01–0.02 mg protein), and 0.1 M Tris-citrate buffer, pH 5.5, in a total volume of 0.1 ml. Initial incubation, extraction, and analysis of dihydrotestosterone formation were as described previously (15). In incubations containing testicular microsomes, more than 90% of the [^{14}C] dihydrotestosterone was metabolized to 5α-androstane-3α,17β-diol (3α-androstanediol), and a different method was used for assessing 5α-reductase activity. The area containing [^{14}C]3α-androstanediol was scraped, and the steroid(s) was eluted into ethyl acetate. After evaporation, 3α-androstanediol was acetylated by incubation with acetic anhydride and pyridine, and the diacetate was purified by sequential thin layer chromatography in dichloromethane-ethyl ether (98:2, vol/vol) and dichloromethane-ethyl acetate (99:1, vol/vol). No change in the ratios of ^{3}H to ^{14}C 3α-androstanediol diacetate occurred between the first and second thin layer chromatographic procedures or in a subsequent recrystallization step (data not shown). 5α-Reductase activity was calculated using the ^{3}H to ^{14}C ratio of the 3α-androstanediol diacetate after the second chromatographic procedure.

RIA of testicular androgens

Testes obtained at autopsy within 12 h of death or within 20 min of removal at surgery were stored frozen. To assay androgen content, 0.25- to 1-g portions were minced and transferred to extraction tubes; after the addition of recovery tracers (~5000 dpm each of [1,2,4,5,6,7-^{3}H]dihydrotestosterone and [1,2,4,5,6,7-^{3}H]3α-androstanediol and 2500 dpm [1,2,6,7-^{3}H]-testosterone), the samples were digested and extracted, as described previously (20). Separation of testosterone, dihydrotestosterone, and 3α-androstanediol was achieved by chromatography columns containing 1 g of a Celite-ethylene glycol mixture (1 g-0.75 ml) in 5-ml pipets. After washing the columns with 5 ml isooctane, the samples were loaded on the columns in 1 ml 2% ethyl acetate in isooctane and sequentially eluted with 5 ml isooctane (discarded), 5 ml 5% benzene in isooctane (collected for dihydrotestosterone), 10 ml 20% benzene in isooctane (discard 3 ml, collect 4 ml for testosterone, discard 3 ml), and 5 ml 7.5% ethyl acetate in isooctane (collected for 3α-androstanediol). To minimize cross-contamination of the samples, the dihydrotestosterone and 3α-androstanediol fractions were pooled separately after the first chromatography, and each was rechromatographed on Celite columns. In this system, the potential contamination of dihydrotestosterone by testosterone was approximately 0.2%, and the contamination of 3α-androstanediol by testosterone was about 0.9% (results not shown).

After chromatography, the various androgen-containing fractions were dried and reconstituted in 1.0 ml phosphate-buffered saline containing 0.1% gelatin. Aliquots were assayed for radioactivity to estimate recoveries (which ranged from 50–80% for testosterone and from 10–50% for dihydrotestosterone and 3α-androstanediol). Testosterone and 3α-androstanediol were measured by RIA using a commercial antiserum (Radioassay Systems Laboratories, Inc., Carson, CA), which cross-reacts with dihydrotestosterone (19%), 3α-androstanediol (3%), and 1% or less with other steroids. Dihydrotestosterone was measured with antiserum from radioassay Systems Laboratories that cross-reacts with testosterone (23%), 5α-androstane-3,17-dione (17%), androstenedione (2%), and 1% or less with other steroids.

Histometric analysis of Leydig cells and seminiferous tubules

The techniques used in the histometric analysis of Leydig cells (21) and seminiferous tubules (22) have been described. In brief, 5–10 pieces (~3 mm^3) of testicular tissue were fixed in glutaraldehyde and then in osmium, dehydrated in alcohol, embedded in Epon 812, sectioned at 0.5 μm, stained with toluidine blue, and observed under bright field microscopy. The percentage (volume density) of testicular tissue occupied by various components of Leydig cells and seminiferous tubules, the number of Leydig cells per testis (22), the volume of an individual Leydig cell nucleus and the volume of a single Leydig cell were estimated. For comparison with normal testes, 95% confidence limits were calculated for a group of 15 previously studied men (33 ± 9 yr of age) (21, 22).

Estimation of daily sperm production

Daily sperm production, an estimate of the total number of sperm produced per testis/day, was determined from testicular homogenates prepared as described previously (23). To express spermatogenesis on a daily production basis, the number of spermatids was divided by the lifespan (duration of stages of the cycle of seminiferous epithelium in which the spermatids occur) of the spermatids.

Electron microscopy

Ultrathin Epon sections were stained with uranyl acetate and lead citrate before being examined by electron microscopy, as described previously (24).

Results

The patients reported here have typical phenotypic and endocrine features of 5α-reductase deficiency, and the studies of 5α-reductase activity and androgen binding in fibroblasts cultured from the labia majora of four of the patients are in keeping with this diagnosis (Table 1). Androgen receptor levels were normal in the fibroblasts. In three of the women (patients 1, 2, and 5), 5α-reductase activity was low and was not studied further; these findings are characteristic of the most common form of 5α-reductase deficiency (1). In patient 3, the activity of the enzyme at pH 5.5 was normal, but the enzyme had

TABLE 1. 5α-Reductase activity and androgen receptor in genital skin fibroblasts from four women with 5α-reductase deficiency

Subjects	5α-Reductase					Androgen receptor B_{max} (fmol/mg protein)
	Activity, pH 5.5 (pmol/h·mg protein)	% Thermolability at 45 C	Apparent K_m		pH optimum	
			Testosterone (μM)	NADPH (μM)		
Normal	>1.0	<20	0.08	40	5.5	>15
Patient 1	0.3	—	—	—		47.5
Patient 2	<0.2	—	—	—		26.5
Patient 3	1.6	76	0.46	45	6.5	45.4
Patient 5	<0.2	—	—	—		55.4

Methods for the propagation of fibroblasts and for the assay of 5α-reductase and androgen receptor were described previously (15, 19). —, Not done. B_{max}, Maximum binding.

abnormal properties, as evidenced by enhanced thermolability at 45 C, a high apparent K_m for testosterone, and an abnormal pH optimum; the defect in this patient falls into the category of qualitatively abnormal 5α-reductase (1, 10, 15). A skin biopsy was not obtained from patient 4.

In one experiment, aliquots of testis and epididymis from patient 1 and from a normal testis and epididymis obtained at autopsy were stored in liquid nitrogen and subsequently assayed for 5α-reductase (Table 2). As reported for another patient (9), enzyme activity was similarly low in the epididymis and cultured fibroblasts from the same patient. In addition, the enzyme was undetectable in the testis from patient 1. We conclude that the enzyme deficiency previously characterized in biopsies of genital skin and epididymis (7, 9) and in fibroblasts cultured from genital skin (25) is also expressed in the testis in this patient.

This conclusion is strengthened by studies of the androgen content of testis from six normal men, one woman with testicular feminization, and four of the patients with 5α-reductase deficiency (Fig. 1). In these studies, the average hormone levels in normal testes were: testosterone, 70 ng/mg protein; dihydrotestosterone, 0.5 ng/mg protein; and 3α-androstanediol, 2.5 ng/mg protein. The total concentration of 5α-reduced metabolites in the testis (dihydrotestosterone plus 3α-androstanediol) was about 4% that of testosterone itself, a percentage of 5α-reduced metabolites similar to that in the testis of the patient with testicular feminization. In the patients with

TABLE 2. 5α-Reductase activity in testicular and epididymal microsomes

Subject	5α-Reductase (pmol/h·mg protein)	
	Epididymis	Testis
Normal	486	3.5
Patient 1	<0.2	<0.2

The preparation of microsomes and the assay of 5α-reductase are described in the text.

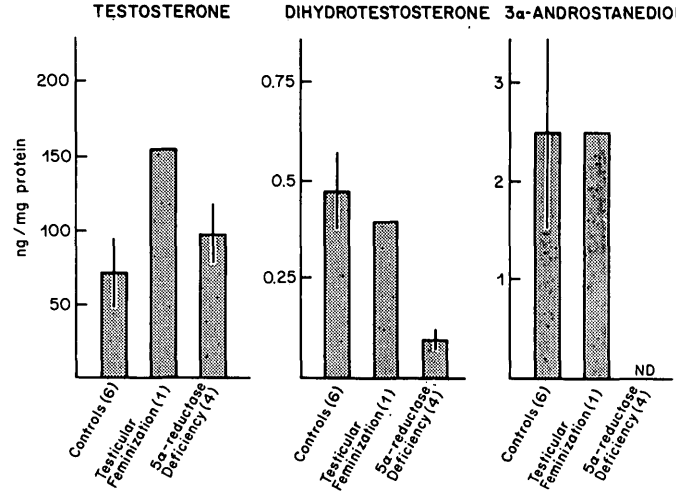

FIG. 1. Mean (±SEM) testosterone, dihydrotestosterone, and 3α-androstanediol levels in the testes of six normal men, one patient with testicular feminization, and four patients with 5α-reductase deficiency. The six normal testes included three samples removed at autopsy from men who died accidental deaths and three testes removed at surgery from men with prostatic carcinoma. The patient with testicular feminization was a patient of Dr. LaDonna Immken, University of Texas Health Science Center (Houston, TX), and the sample was frozen and shipped to us in dry ice. The patients with 5α-reductase deficiency were patients 1, 3, 4, and 5. The procedures for the collection and storage of samples and extraction and analysis of steroids are described in the text. ND, Not detectable.

5α-reductase deficiency, the mean testosterone content was similar to that of the normal testes, but the levels of the two 5α-reduced androgens were low. The finding of low testicular levels of 5α-reduced androgens is in keeping with the low 5α-reductase activity in patient 1.

The histological characteristics of the testes of patients 1 and 2 are shown in Figs. 2 and 3. Spermatogenesis was quantified in both (Table 3). By light and electron microscopy, the Leydig cells and interstitial compartment were unremarkable. The seminiferous epithelium comprised Sertoli cells, spermatogonia, and primary spermatocytes. In patient 1, no mature spermatids were visible (Fig. 2), whereas in patient 2, some immature and

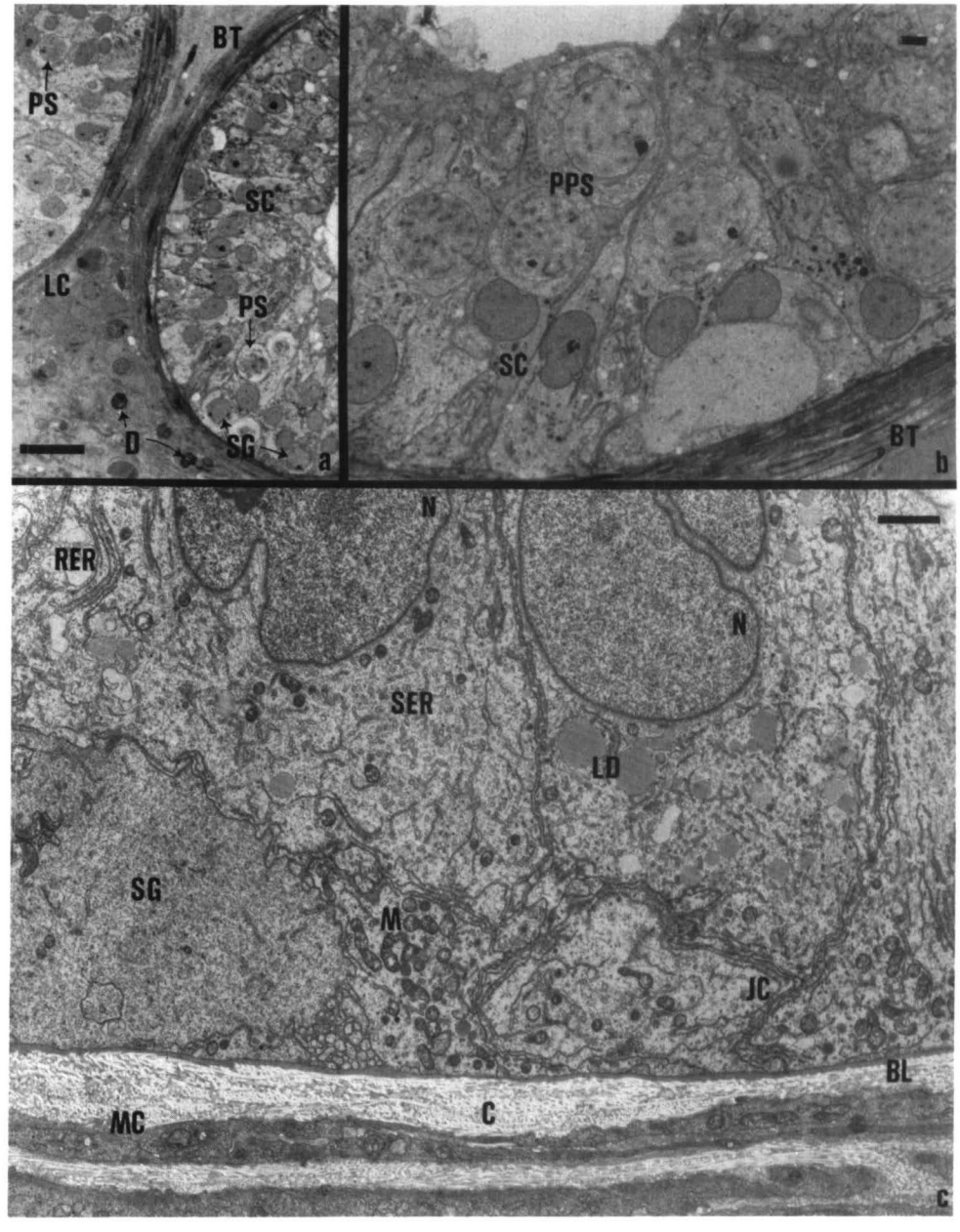

FIG. 2. Photomicrographs (a and b) and electron micrograph (c) of the interstitium and seminiferous tubules in the right testis from patient 1 with 5α-reductase deficiency. a) The interstitium surrounding tubules contains Leydig cells which appear normal (LC). However, occasionally, degenerative Leydig cells with dark to pyknotic nuclei (D) are present. The boundary tissue (BT) around the tubules appears of normal thickness, with two to four layers of myoid cells. The seminiferous epithelium is composed of Sertoli cells (SC), spermatogonia (SG), and primary spermatocytes (PS). b) A higher magnification photomicrograph reveals the presence of apparently normal, well developed pachytene primary spermatocytes (PPS) and Sertoli cells (SC) in the seminiferous epithelium. The lumen is present, and the boundary tissue (BT) appears normal. c) This electron micrograph portrays the upper portion of the boundary tissue and the lower portion of the seminiferous epithelium. The boundary tissue is characterized by myoid cells (MC), collagen fibers (C), and a basal lamina (BL). Within the seminiferous epithelium is the cytoplasm of a spermatogonium (SG) and the basal region of several Sertoli cells. Junctional complexes (JC) between adjacent Sertoli cells have the typical apposition of plasma membranes paralleled by profiles of endoplasmic reticulum. Typical profiles of rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), mitochondria (M), and lipid droplets (LD) are present. The indented nuclear envelope, euchromatic nucleoplasm, and large nucleolus are characteristic of Sertoli cell nuclei (N). *Bar* lengths are 10, 5, and 1 μm for a, b, and c, respectively.

mature spermatids were present (Fig. 3). Quantitative morphometric studies were performed on these testes (Table 3). The testis size was normal in patient 2 and small in patient 1. The Leydig cell histology was normal, and the number of Leydig cells per g tissue and per testis was high (possibly due to the young age of the patients compared to the normal men). The seminiferous epithelium was relatively normal, whereas the lumen and diameter of the seminiferous tubules were small. Daily sperm production in patient 1 was undetectable and in patient 2 was about 10% of normal.

Discussion

The testes from five 46,XY individuals with 5α-reductase deficiency were used for histological, enzymatic, and androgen assays. In contrast to many untreated patients with this disorder, who undergo apparent reversal of gender role behavior from female to male at the time of expected puberty (13, 17), these five women were studied because of primary amenorrhea. These patients elected to undergo a treatment regimen designed to reinforce the female phenotype. Postpubertal function as women has been described previously in this disorder (1, 7). It is not known at present why some individuals undergo reversal of gender role behavior and some do not, although it is our impression that the group who elect to maintain female gender behavior have more profound defects in dihydrotestosterone formation and, hence, virilize to a lesser degree at the time of expected puberty than do

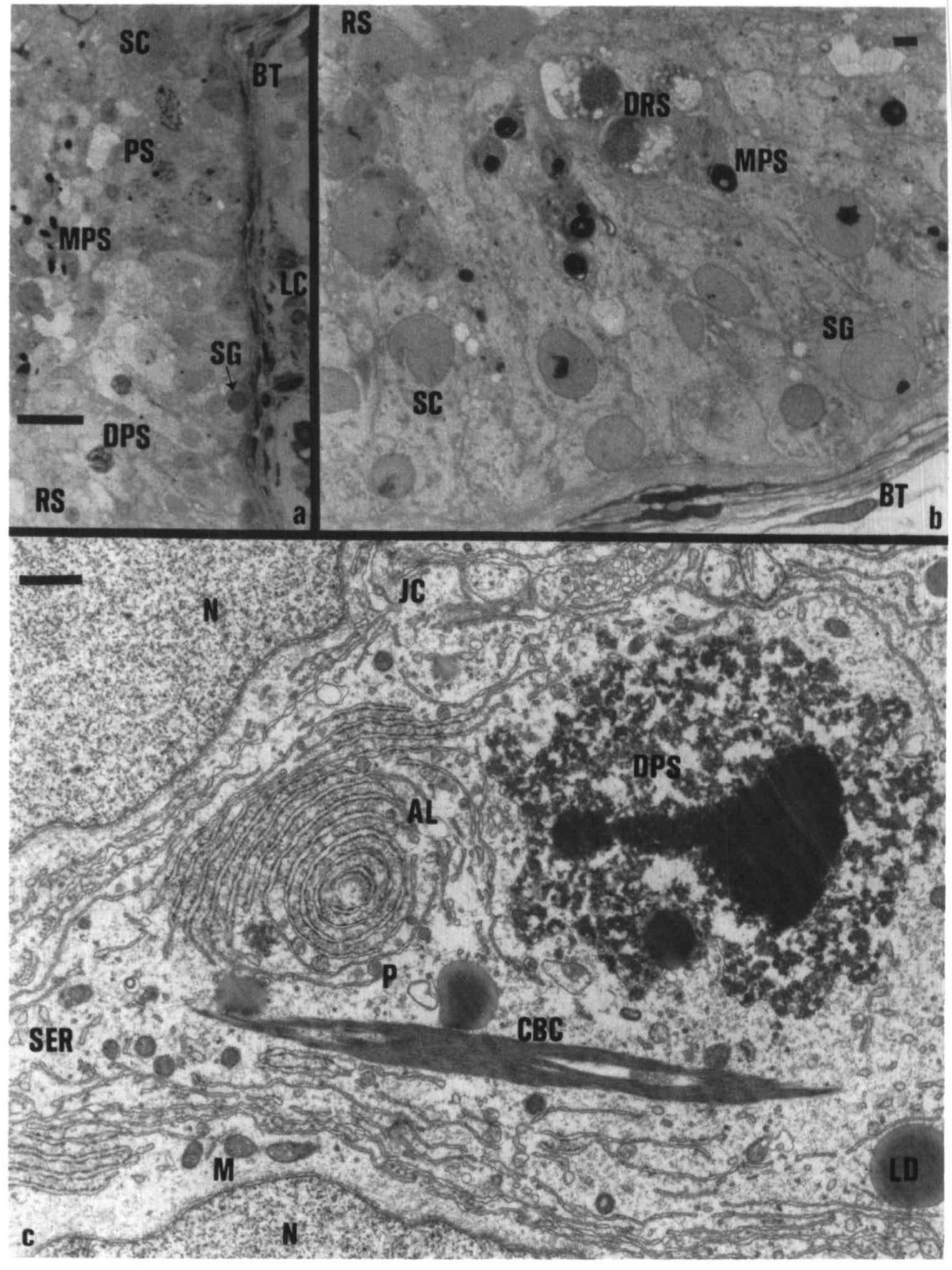

FIG. 3. Photomicrographs (a and b) and electron micrograph (c) of the interstitium and seminiferous tubules in the testis from patient 2 with 5α-reductase deficiency. a and b) Leydig cells (LC) in the interstitium appear normal. The boundary tissue (BT) constituting the outer portion of the tubule is of normal thickness. In addition to Sertoli cells (SC), the seminiferous epithelium includes spermatogonia (SG) and primary spermatocytes (PS), as well as younger spermatids with round nuclei (RS) and mature maturation phase spermatids (MPS). Degeneration of germ cells appears at the level of primary spermatocytes (DPS) and spermatids with round nuclei (DRS). c) Sertoli cells are characterized by the euchromatic nuclei (N) with irregular nuclear surfaces. Mitochondria (M) are long and thin. Specific organelles, including several profiles of smooth endoplasmic reticulum (SER), a Charcot-Boettcher's crystalloid (CBC), lipid droplets (LD), and concentrically arranged cisternae of rough endoplasmic reticulum (AL) are present. Large vesicles containing fine granular materials and resembling peroxisomes (P) were found between cisternae of the rough endoplasmic reticulum. Part of a junctional complex between adjacent Sertoli cells (JC) appears normal. The phagocytic function of Sertoli cells is indicated by the presence of the nuclear remains of a degenerating primary spermatocyte (DPS). *Bar* lengths are 10, 5, and 1 μm for a, b, and c, respectively.

patients who change gender role from female to male (13). If this is the case, the subset of patients reported here may not be representative of the syndrome as a whole. Nevertheless, the present findings document that the defect in dihydrotestosterone formation in these patients involves the testis as well as extragonadal tissues and are in keeping with some previous reports (10, 12, 13) that impairment of spermatogenesis is common in the disorder.

The measurement of 5α-reductase in testicular tissue is technically difficult. The large pool of testosterone within the testis interferes both with indirect assessment of 5α-reductase, such as measurement of dihydrotestosterone content, as well as with direct assays of the enzyme itself. Indeed, in the initial studies of 5α-reductase from this laboratory, it was not possible to document the presence of the enzyme in testes (16). However, Oshima *et al.* (26) and Payne *et al.* (27) subsequently found the enzyme in the rat testis. Furthermore, Ito and Horton (28) reported that small amounts of dihydrotestosterone are secreted by the human testis. In the rabbit, a 5α-reduced androgen, 3α-androstanediol, is secreted by the testis at a high rate, second only to testosterone (29). In the present study, we documented 5α-reductase activ-

TABLE 3. Leydig cells and seminiferous tubule morphometrics and daily sperm production in 2 patients with 5α-reductase deficiency and 15 normal adult men

Item	Normal men[a]			Patients with 5α-reductase deficiency	
	Mean ± SEM	95% confidence limit		1	2
		Low	High		
Average testicular wt (g)	24.5 ± 1.3	21.7	27.3	15.5	22.1
Leydig cells					
Nuclei					
Vol density	0.0040 ± 0.0004	0.0031	0.0049	0.0110	0.0058
Diameter (μm)	8.73 ± 0.06	8.60	8.86	9.20	8.56
Vol of single nucleus (fl)	355.8 ± 7.1	340.6	371.0	412.10	333.3
Cell					
Vol density	0.0461 ± 0.0009	0.0442	0.0480	0.0935	0.0692
Vol of single cell (pl)	4.26 ± 0.24	3.74	4.77	4.14	4.32
No. ($\times 10^{-6}$)					
Per g parenchyma	10.36 ± 1.03	8.15	12.57	21.49	15.25
Per testis	215.9 ± 22.6	167.4	264.4	271.20	273.6
Interstitial vol density	0.402 ± 0.018	0.363	0.441	0.501	0.424
Seminiferous tubules					
Volume density					
Boundary tissue	0.0913 ± 0.0049	0.0807	0.1018	0.1689	0.1492
Seminiferous epithelium	0.423 ± 0.0152	0.390	0.456	0.325	0.418
Lumen	0.0881 ± 0.0153	0.0553	0.1209	0.0052	0.0092
Components combined	0.598 ± 0.018	0.559	0.637	0.499	0.576
Diameter (μm)	227.8 ± 4.7	217.7	237.9	191.3	206.7
Daily sperm production ($\times 10^{-6}$)					
Per g parenchyma	7.03 ± 0.44	6.09	7.97	0	0.81
Per testis	160.9 ± 16.1	126.4	195.4	0	14.5

[a] n = 16, based on the data of Neaves *et al.* (21) and Johnson *et al.* (22).

ity in washed microsomes from normal human testis. Two 5α-reduced androgens, dihydrotestosterone and 3α-androstanediol, were present in the normal testis at levels about 4% that of testosterone. The measurements in the present study were in extracts of whole tissue, and it is possible that 5α-reduced androgens in some compartments of the testis, such as the spermatogenic tubules, may constitute an important physiological pool. Indeed, these findings raise the possibility that 5α-reduced steroids play a role in spermatogenesis.

The nature of androgen control of spermatogenesis is unclear. The developing spermatocytes themselves do not require the direct action of androgen (30), and it is assumed that the hormonal effect is exerted at the level of the Sertoli cell. In the rat, the androgen receptor in the spermatogenic tubule (31) is similar in its binding specificity to that in the prostate, in that dihydrotestosterone is the preferred binding ligand. If the action of androgen on spermatogenesis, like that of most other androgen effects, is mediated by dihydrotestosterone, then the only known direct physiological actions of testosterone are to mediate virilization of the Wolffian ducts during embryogenesis and regulate the production of LH in males (1).

The histological studies in these patients confirm previous reports that spermatogenesis is markedly impaired in some patients with this disorder. The two patients who had quantitative estimates of sperm production (patients 1 and 2) and three patients previously reported (10, 12, 13) all had major defects in spermatogenesis. Two of the five produced no sperm, and in the other three sperm production was less than 10% of normal. Furthermore, by light microscopy the remaining three patients (patients 3–5) and most, but not all, of those previously described (2, 9, 18) also had defective spermatogenesis. It is possible that profound impairment of spermatogenesis is a general property only in the subset of individuals with the most severe impairment of dihydrotestosterone formation. Unfortunately, it is not possible to be certain whether the defect in spermatogenesis is the consequence of dihydrotestosterone deficiency or is, instead, a secondary phenomenon. All five of these patients had partially descended testes, the testis being located in the inguinal canal or in the labia majora. Testicular maldescent is known to be associated with impairment of spermatogenesis, although the degree of impairment in high scrotal testes is not necessarily complete (32), and it is unclear whether descent into the labia majora is equivalent to descent into a scrotum. Thus, we cannot exclude the possibility that thermal

injury associated with a high scrotal or labial testis, rather than 5α-reductase deficiency *per se*, might contribute to or cause the impaired spermatogenesis in patients with 5α-reductase deficiency. To determine the precise role of dihydrotestosterone in spermatogenesis it will be necessary to study the effects of potent 5α-reductase inhibitors on testicular 5α-reductase and spermatogenesis (20). Whatever the mechanism, the possibility of fertility in men with this disorder appears unlikely.

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