Persistent Testicular Δ⁴-Isomerase-3β-Hydroxysteroid Dehydrogenase (Δ⁴-3β-HSD) Deficiency in the Δ⁴-3β-HSD Form of Congenital Adrenal Hyperplasia

GEORGE SCHNEIDER, MYRON GENEL, ALFRED M. BONGIOVANNI, ALLEN S. GOLDMAN, and ROBERT L. ROSENFIELD

From the Departments of Pediatrics and Medicine, Yale University School of Medicine, New Haven, Connecticut 06510, the Children's Hospital of Philadelphia and the Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, and the Department of Pediatrics, University of Chicago, Pritzker School of Medicine, Chicago, Illinois 60637

Abstract A partial testicular defect in testosterone secretion has been documented in a pubertal male with a congenital adrenal hyperplasia due to hereditary deficiency of the Δ⁴-isomerase-3β-hydroxysteroid dehydrogenase enzyme complex (Δ⁴-3β-HSD). Diagnosis of the enzymatic defect is based on the clinical picture of ambiguous genitalia and salt-losing crisis in infancy, together with high urinary Δ⁴-pregnenetriol and plasma dehydroepiandrosterone when the patient was taken off replacement corticoid treatment. No hormonal response to ACTH or salt deprivation was demonstrable. In addition, in vivo studies revealed a partial enzymatic defect in the testis. Although plasma testosterone was low-normal (250 ng/100 ml), plasma Δ⁴-androstenediol was markedly elevated and rose to a greater extent than testosterone after human chorionic gonadotropin administration. In vitro testicular incubation studies suggested a testicular Δ⁴-3β-HSD enzyme defect with less Δ⁴ products formed from Δ⁴ precursors than in a control testis. Histochemical studies of the testis were also consistent with this defect. Testicular biopsy revealed spermato-}

genic arrest, generally diminished Leydig cells, but with focal areas of Leydig cell hyperplasia as well as benign Leydig cell nodules within the spermatogenic cord. In vivo studies of steroid metabolism suggested intact peripheral or hepatic Δ⁴-3β-HSD activity. These studies imply that Δ⁴-3β-HSD activity differs in the gonad, adrenal, and peripheral organs. These findings are compatible with the concept that the enzyme complex consists of subunits and/or that enzymes in these organs are under different genetic control.

INTRODUCTION

The first description of the Δ⁴-isomerase-3β-hydroxysteroid dehydrogenase (Δ⁴-3β-HSD) deficiency form of congenital adrenal hyperplasia was made by Bon-giovanni in 1962 (1). The first direct demonstration of the enzymatic defect in both the adrenals and testes of a 6-wk-old male with the disease was reported in 1964 (2). Since then, a number of other cases have been described, including two achieving spontaneous puberty (3, 4), both males with hypospadias, gynecomastia, and appropriate secondary sexual development. In one, however, a normal testosterone response to human chorionic gonadotropin (HCG) stimulation at

1 Abbreviations used in this paper: Δ⁴-3β-HSD, Δ⁴-isomerase-3β-hydroxysteroid dehydrogenase enzyme complex; DHA, dehydroepiandrosterone; DHAS, dehydroepiandrosterone sulfate; DOCA, desoxycorticosterone acetate; FSH, follicle-stimulating hormone; HCG, human chorionic gonadotropin; IRP, International Reference Preparation, LH, luteinizing hormone.
age 13 suggested postnatal normalization of testosterone secretion and reserve (3).

We have investigated another pubertal boy with clinical evidence of Δ4-3β-HSD deficiency. Studies of his pituitary-Leydig cell axis in vivo and of testicular biosynthesis in vitro indicate that, although this enzyme defect seems complete in the adrenal, it is partial in the testes. In contrast, peripheral enzyme activity appears to be intact.

METHODS

Plasma cortisol, 17α-hydroxyprogesterone (DHA), dehydroepiandrosterone sulfate (DHAS), Δ5-androstenediol, and testosterone were measured by competitive protein binding (5-9). Plasma estradiol, estrone, lutetinizing hormone (LH), and androstenediol, were measured by gas chromatography with propylene glycol as stationary phase and carbon tetrachloride-cyclohexane (90:10) as mobile phase. Label peaks were also identified by NMR. Puberty was defined by gas chromatography with 3% OV-210 and by recrystallization to constant specific activity, as previously described (21).

Histochemistry. Activity of Δ5-3β-HSD was determined as previously described (22) in the testis of this patient and in a control testis obtained at gonadectomy from a 12-yr-old patient with incomplete testicular feminization.

Case summary. M.D., a 15-yr-old white male, was previously described as a case of the salt-losing 21-hydroxylase form of congenital adrenal hyperplasia (23). A third-degree hypoplasia with a bifid scrotum was noted. At birth he required admission to the Yale-New Haven Hospital for therapy of severe dehydration associated with projectile vomiting. Serum sodium was 114 meq/liter, potassium 9.6 meq/liter, and chloride 85 meq/liter. Buccal smear was normal. Chromatin negative, and intravenous pyelogram and bone age were normal. 17-Ketosteroid excretion was 19.7 mg/24 hr. Fractionation of the 17-ketosteroids revealed DHA to be 3.3 mg/24 hr, androstosterone 6.0 mg/24 h, etiocholanolone 2.4 mg/24 h, 11-oxyl-17-ketosteroids 4.0 mg/24 h, and pregnenolone 4.0 mg/24 h, all elevated. Neither pregnenediol nor 11-ketopregnenetriol, usually increased in the 21-hydroxylase form of congenital adrenal hyperplasia (24), were detected. Family history was negative for consanguinity, congenital adrenal hyperplasia, ambiguous genitalia, salt-losing states, and sudden death in infancy. The only sibling was a normal female. The diagnosis of salt-losing congenital adrenal hyperplasia with male pseudohermaphroditism was made, and he was referred to saline, desoxycorticosterone acetate (DOCA), and cortisone therapy clinically and with decreased 17-ketosteroid and pregnenolone excretion.

He was maintained on varying doses of hydrocortisone, depending on 17-ketosteroid excretion and growth, with DOCA pellets for 4 yr, after which 9α-fluorohydrocortisone was substituted. 10 urological procedures were performed in his first decade to correct the hypoplasias. Puberty began at age 10 yr, and gynecomastia at 11. Over the next 18 mo, the gynecomastia, public hair, and acne increased, and axillary hair developed. Bilateral mastectomy was performed with a tissue diagnosis of ductal hyperplasia.

During the next 10 mo pubic and axillary hair increased, and his testes increased in size from 3 × 3 cm to 6 × 4 cm, as New England Nuclear, Boston, Mass.


682 Schneider, Genel, Bongiovanni, Goldman, and Rosenfeld
becoming nodular and hard in consistency. Three small 0.5-1.0-cm nodules became palpable in the left spermatic cord. The left testis and its blood supply were not involved, and there was minimal change in the left testis size, with 0.5-0.25-0.25 mg/24 h. Plasma testosterone was 400 ng/100 ml (normal adult, 240-1,000 ng/ml). When hydrocortisone replacement was increased from 20 to 35 mg/24 h, the right testis shrank to 5 X 2.5 cm, but the left did not change.

Physical examination at age 14 yr and 7 mo revealed a well-developed pubertal boy with acne. Height was 158 cm (height age = 13 yr). Bilateral subareolar mastectomy scars were hyperpigmented with slight keloid formation. There was a moderate amount of pubic and axillary hair but little beard development. The penis was 6 cm in length with a first-degree hypospadias, the result of multiple surgical procedures. The right testis was 5 X 2.5 cm and was normal in consistency, whereas the left testis was 6 X 3 cm and was firm and nodular. The left spermatic cord contained three to four hard 0.5-cm nodules. The scrotum was rugated. The prostate was not palpable on rectal examination.

Clinical studies. The patient was admitted to the Yale Children’s Clinical Research Center after he and his parents were informed of the nature, purpose, and possible risks of the studies, and voluntary consent to participate was obtained. 1 wk before admission, replacement therapy was changed from 35 mg of hydrocortisone in divided doses to dexamethasone, 1 mg/day (0.5-0.25-0.25 mg). Biopsy of the left testis was performed, and one of the nodules in the left spermatic cord was removed under general anesthesia with parenteral dexamethasone and DOCA coverage. After uneventful recovery, the dexamethasone dose was increased to 8 mg/day (2 mg every 6 h) to assure complete suppression of ACTH secretion. 2 days later response to administration of HCG was studied according to the protocol depicted in Table I. Response of the renin-aldosterone system to acute salt deprivation was then studied after a return to 1 mg/day replacement dexamethasone dose for 48 h. Mineralocorticoid therapy with 9a-fluorohydrocortisone was discontinued, and dietary sodium was restricted to 1 g/day. 24-h urines were collected on the 2nd and 3rd days of this regimen for aldosterone and electrolyte determinations. Blood was obtained for plasma renin activity, both supine and after 4 h of exercise on the 3rd day.

At a later date, adrenal function was evaluated according to the protocol in Table II. The patient was taken off 9a-fluorohydrocortisone and hydrocortisone and given 50 mg i.m. of long-acting desoxycorticosterone pivalate. After 21 days off oral therapy, urine was collected for pregnanetriol, 11-ketopregnanol, 17-hydroxyprogrenolone, 11-pregnenetriol, 17-ketosteroids, and 17-hydroxycorticoids. Blood was also obtained for various steroid determinations. In addition, blood for LH and FSH was obtained every 10 min for 3 h during the morning. The patient was then given ACTH gel, 25 U every 12 h for 3 days with concurrent blood and urine collections. After this he was again treated with 8 mg/day of dexamethasone for 9 days.

At a later date, while continued on his usual maintenance therapy of 35 mg of hydrocortisone and 0.05 mg of 9a-fluorohydrocortisone, the patient received oral 17a-hydroxyprogrenolone, 1 mg/kg body weight, in a dilute alcohol solution. 24-h urine collections were obtained for 2 days before, during, and 2 days after the day of administration of the steroid for determination of 11-pregnenetriol and pregnanetriol. Blood for LH and FSH determinations was again obtained every 10 min for 3 h during the morning during maintenance therapy.

RESULTS

Testicular pathology. The predominant histologic picture was of an immature testis inconsistent with the patient’s state of advanced pubertal development. Seminiferous tubules showed spermatogonial arrest with predominantly Sertoli cell lining. For the most part, Leydig cells appeared prominent though relatively diminished in number, although occasional areas of focal Leydig cell hyperplasia were noted. The nodule removed from the left spermatic cord demonstrated be-

**TABLE I**

Response to HCG Stimulation under Dexamethasone Suppression*

<table>
<thead>
<tr>
<th>Study days</th>
<th>Normal values</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone (2 mg every 6 h)</td>
<td>x x x x x x x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9a-Fluorohydrocortisone (0.1 mg/day)</td>
<td>x x x x x x x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCG (2,000 U i.m. every 12 hr)</td>
<td>x x x x x x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol, µg/100 ml</td>
<td>5-20</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androstenedione, ng/100 ml</td>
<td>70-160</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>DHA ng/100 ml</td>
<td>220-800</td>
<td>227</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>293</td>
<td></td>
</tr>
<tr>
<td>DHAS, µg/100 ml</td>
<td>115-265</td>
<td>92</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>157</td>
<td></td>
</tr>
<tr>
<td>Δ4-Androstenediol, ng/100 ml</td>
<td>80-170</td>
<td>285</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>712</td>
<td></td>
</tr>
<tr>
<td>Testosterone, ng/100 ml</td>
<td>240-1,000</td>
<td>250</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>475</td>
<td></td>
</tr>
<tr>
<td>Estrone, pg/ml</td>
<td>&lt;50</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Estradiol, pg/ml</td>
<td>&lt;50</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>LH, mIU/ml</td>
<td>10-20</td>
<td>10</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>FSH, mIU/ml</td>
<td>10-20</td>
<td>13</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

* Study commenced 7 days after substitution of 1 mg/day dexamethasone (0.5-0.25-0.25 mg every 8 h) for routine suppressive therapy with 35 mg/day hydrocortisone (15 mg-10 mg-10 mg).
† All plasma was obtained at 8 a.m. before instituting changes indicated in the study protocol.
nign Leydig cell hyperplasia, although crystalloids of Reinke were not observed.

Response to HCG stimulation (Table I). After 2 days of high-dose dexamethasone suppression, plasma Δ4-androstenediol was elevated, whereas plasma testosterone was low normal and plasma androstenedione, DHA, and DHAS were low. After 4 days of 4,000 U of i.m. HCG, plasma Δ4-androstenediol more than doubled to 712 ng/100 ml, whereas plasma testosterone rose less than twofold to 475 ng/100 ml. Plasma estradiol was low but was stimulated by HCG. Estrone remained low both before and after HCG.

Mineralocorticoids. While on ordinary replacement therapy, aldosterone excretion was only 1.7 μg/day (normal = 5–20 μg/day). During the period of sodium restriction, while off 9α-fluorohydrocortisone therapy, urinary aldosterone failed to rise (1.3 and 0.7 μg/day), despite a significant rise in plasma renin activity from 2.3 ng of angiotensin I generated/ml/h to 4.8 supine and 9.4 after exercise. Urinary Na during this period rose to 250 meq/liter from previous values of 130 and 158 meq/liter. Serum Na did not change significantly, but serum K rose from 4.3 to 5.3 meq/liter, and the blood-urea nitrogen rose from 14 to 23 mg/100 ml. This aspect of the study was terminated after 72 h because of clinical signs of mild dehydration.

Adrenal studies (Table II). When the patient was taken off hydrocortisone for 3 wk, his urinary Δ5-pregnenetriol excretion was 7.42 mg/day (normal 0.1–0.4 mg/day for adults) and urinary pregnanetriol was 8.42 mg/day (normal 0.8–3.1 mg/day for adults). Total 17-ketosteroids were 13.5 mg/day, and 17-hydroxycorticoids were 0.1 mg/day. Neither 11-ketopregnenetriol nor 17-hydroxyprogrenalone, two compounds usually seen in 21-hydroxylase deficiency, were found. On the 2nd day of ACTH gel, the patient's urinary volume fell from 1,926 cm3 to 850 cm3, and Δ5-pregnenetriol excretion was 4.73 mg/day while pregnanetriol excretion was 5.17 mg/day. On the 3rd day of ACTH gel, the urinary volume fell further to 620 cm3, while Δ5-pregnenetriol excretion was 7.24 mg and pregnanetriol excretion was 7.97 mg. The 17-ketosteroids also fell to 9.2 and 3.2 mg on the 2nd and 3rd days of ACTH, respectively. The 17-hydroxycorticoids were always 0.1 mg. The lack of a rise in urinary pregnanetriol and Δ5-pregnenetriol during ACTH probably reflects maximal adrenal stimulation by endogenous ACTH, while the drop in 17-ketosteroid excretion during this same time is most likely related to the drop in urine volume (25, 26), probably attributable to vasopressin contamination of ACTH preparations.

At that time when the patient was off hydrocortisone, plasma DHA was almost twice normal (1416 ng/ml), whereas DHAS was normal and androstenedione was low normal. Both Δ4-androstenediol and testosterone were significantly higher than at the beginning of the initial study, but the ratio of the two compounds remained approximately the same. In addition, plasma estrone and estradiol, although within normal limits, were significantly higher than when the patient was on glucocorticoid therapy. 3 days of ACTH stimulation led to a minimal rise in DHA, DHAS, and an-

### Table II

<table>
<thead>
<tr>
<th>Study days</th>
<th>Normal values</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone (2 mg every 6 h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACTH (25 U i.m. every 12 h)</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Plasma†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol, μg/100 ml</td>
<td>5–20</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androstenedione, ng/100 ml</td>
<td>70–160</td>
<td>93</td>
<td>119</td>
<td>119</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHA, ng/100 ml</td>
<td>220–800</td>
<td>1,416</td>
<td>1,065</td>
<td>1,736</td>
<td>118</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHAS, μg/100 ml</td>
<td>115–265</td>
<td>227</td>
<td>172</td>
<td>265</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ5-Androstenediol, ng/100 ml</td>
<td>80–170</td>
<td>436</td>
<td>449</td>
<td>397</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone, ng/100 ml</td>
<td>240–1,000</td>
<td>471</td>
<td>450</td>
<td>365</td>
<td>184</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrone, pg/ml</td>
<td>&lt;50</td>
<td>28</td>
<td>29</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol, pg/ml</td>
<td>&lt;50</td>
<td>36</td>
<td>33</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH, mIU/ml</td>
<td>10–20</td>
<td>10.3</td>
<td>9.7</td>
<td>25.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH, mIU/ml</td>
<td>10–20</td>
<td>10.7</td>
<td>9.3</td>
<td>20.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-hydroxycorticoids, mg/day</td>
<td>3–10</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>17-ketosteroids, mg/day</td>
<td>5–15</td>
<td>13.5</td>
<td>9.2</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>Pregnanetriol, mg/day</td>
<td>0.8–3.1</td>
<td>8.4</td>
<td>5.2</td>
<td>8.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ5-Pregnenetriol, mg/day</td>
<td>0.1–0.4</td>
<td>7.4</td>
<td>4.7</td>
<td>7.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Study commenced 21 days after cessation of hydrocortisone and 9α-fluorohydrocortisone therapy and administration of 50 mg desoxycorticosterone pivalate i.m.
†All plasma was obtained at 8 a.m. before instituting changes indicated in the study protocol. Urine collections began at 8 a.m. on the day designated, concluding at 8 a.m. the following day.

Schneider, Genel, Bongiovanni, Goldman, and Rosenfield
drostenedione, a slight fall in testosterone and Δ₄-androstenediol, and no change in estrone or estradiol, again probably reflecting maximal endogenous ACTH stimulation. Plasma cortisol was very low both before and after ACTH. After 9 days of high-dose dexamethasone, all androgens and estrogens fell significantly.

Gonadotropin secretion. Sequential plasma LH and FSH were always within normal limits during the two study periods on and off therapy, and there was no difference in either the LH or FSH levels during these two periods. Mean LH±SE off corticoid therapy was 13.35±2.85 mIU/ml, while on therapy it was 13.67±3.03 mIU/ml. Mean FSH±SE off therapy was 12.37±2.55 mIU/ml and 14.41±0.85 mIU/ml on therapy.

In addition, random plasma LH and FSH determinations obtained at various times during the study were always within normal limits, except for one occasion after 9 days of high-dose dexamethasone suppression (Table II). At this time, both plasma LH and FSH were slightly elevated, corresponding to a simultaneous decrease in the levels of plasma androgens and estrogens. Urinary gonadotropins, measured by bioassay, were slightly elevated (>48, >96 mouse uterine U/day on three of four 24-h collections), although simultaneous plasma LH and FSH levels were normal.

Metabolism of 17α-hydroxyprogrenenolone (Fig. 1). As indicated, while continued on routine hydrocortisone replacement of 35 mg/day, base-line Δ₄-pregnenetriol was undetectable and pregnanetriol was within normal limits, thus indicating adequate pituitary-adrenal suppression. After the administration of an oral load of the Δ₄ steroid, 17α-hydroxyprogrenenolone, the patient excreted the major amount as pregnanetriol and only a minor amount as Δ₄-pregnenetriol.

In vitro studies (Figs. 2 and 3). With pregnenolone and DHA as substrates, the patient's testis was able to make a small amount of testosterone, but this was only one-third that of the control. Although conversion to Δ₄-androstenediol was not quantitated in the study depicted, a compound with the mobility of Δ₄-androstenediol on both paper and thin-layer chromatography consistently had four times more ¹⁴C counts in the patient's incubation, as compared to the control. With the micro method, the patient's testis formed similar amounts of testosterone (0.2% of substrate) and androstenedione (4.2% of substrate) as in the macro method. In addition, Δ₄-androstenediol was the major metabolite formed from DHA by the patient's testis, representing 6.4% of the substrate with its identity confirmed by radio-gas-liquid chromatography and recrystallization to confirm specific activity.

Histochernistry. Histochemical studies of the patient's testis demonstrated undetectable Leydig cell Δ₄-3β-HSD activity with DHA as the substrate. In contrast, intense staining for this enzyme was readily demonstrated in the Leydig cells from the control testis. Identical results were obtained in both testes with pregnenolone as the substrate.

Discussion

Hereditary Δ₄-3β-HSD deficiency in males is characterized by adrenal insufficiency, pseudohemaphroditism, and marked salt-wasting, generally leading to death in early infancy (1, 27). However, long-term survival has been reported in a number of cases thought to represent partial deficiency states (3, 28-32). Recently, Finnish investigators have published studies obtained during the natural puberty (4) of their earlier reported case (31). As in the first-described pubertal male (3), their patient's adolescence was characterized by gynecomastia but otherwise showed normal secondary sexual development.

Our patient presented in the newborn period with a typical picture of complete adrenal insufficiency. Although he was originally reported as a case of 21-hydroxylase deficiency with salt-losing and hypoplasia (23), the present studies clearly indicate an adrenal deficiency of the Δ₄-3β-HSD complex. After 3 wk off routine adrenal suppressive therapy, urinary Δ₄-pregnenetriol, plasma DHA, and Δ₄-androstenediol were grossly elevated, and corticoid excretion was low. Moreover, the urinary Δ₄-pregnenetriol:pregnenetriol ratio was nearly 1(0.88), whereas this ratio ranges from 0 to 0.28 in patients with the more common 21-hydroxylase deficiency form of congenital adrenal hyperplasia (17). Absence of 11-ketopregnenetriol and 17-hydroxyprogrenenolone is another strong point against the diagnosis of a 21-hydroxylase deficiency. ACTH administration did not lead to the normal, appreciable
Figure 2. Testicular conversion of pregnenolone to various \( \Delta^5 \) and \( \Delta^4 \) products in vitro, expressed as percent recovery of original substrate. 60 mg of minced testis were incubated with 4.0 \( \mu \)g, 0.67 \( \mu \)Ci of \([^{14}C]\)pregnenolone in 1.0 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing 120 mg of glucose/100 ml for 1 h. D, dehydroepiandrosterone; A, androstenedione; T, testosterone.

Figure 3. In vitro testicular recovery of androstenedione (A) and testosterone (T) after incubation of minced testis with 0.98 \( \mu \)g, 0.2 \( \mu \)Ci of \([^{14}C]\)dehydroepiandrosterone (D). Conditions identical to Fig. 2.

increase in \( \Delta \) or \( \beta \) steroids (33), consistent with already maximum endogenous ACTH stimulation typical of congenital adrenal hyperplasia. As in the neonatal period, glucocorticoids reversed the biochemical abnormality. Furthermore, aldosterone excretion was low and remained low when the mineralocorticoid therapy was discontinued, and dietary salt restriction led to negative sodium balance, a rise in serum potassium, and an appropriate increase in plasma renin, also consistent with adrenal \( \Delta^5 - \beta \)-HSD deficiency.

Since testicular \( \Delta^5 - \beta \)-HSD activity is necessary for production of testosterone, the main secretory product of the human testes, male pseudohermaphroditism in the syndrome has been ascribed to a coexisting deficiency of this enzyme complex (2). This concept has not been proven by studies of the \( \Delta^5 \) pathway from progesterone via 17-hydroxyprogesterone and androstenedione to testosterone. However, the \( \Delta^4 \) pathway from 17-hydroxypregnenolone and DHA via \( \Delta^4 \)-androstenediol to testosterone now seems of major importance in man (34). Several steroids have been found to be secreted directly by the testes. In males approximately 40% of 17a-hydroxypregnenolone secretion is of testicular origin (35), and DHA, though mainly secreted by the adrenal, has also been found in the spermatic vein effluent of man (36). \( \Delta^5 \)-androstenediol.
diol is the second most abundant steroid in spermatic
vein plasma; the ratio of testosterone to DHA is 37:1,
compared to 4:1 for testosterone to Δ\(^{4}\)-androstenediol
(36). This latter ratio corresponds to that found in
the peripheral plasma by Rosenfeld and Otto (8) and
confirmed by recent studies from two separate labora-
tories (37, 38). After HCG stimulation of similar dos-
age and duration as performed in our patient, this ratio
was either maintained (37) or somewhat increased
(38). Furthermore, the ratio was maintained in a vari-
ty of testicular disorders before and after HCG stimu-
lulation (38). Our own studies demonstrate a similar
secretory pattern after HCG administration to children
but also suggest even less Δ\(^{4}\) androgen response with a
resultant testosterone:Δ\(^{4}\)-androstenediol ratio of 10:1
(39).

The in vivo data in our patient provide evidence that
the testicular Δ\(^{4}\)-3β-HSD enzyme complex is also
deficient. Although plasma testosterone is low normal, Δ\(^{4}\)-
androstenediol is elevated, and the plasma testosterone:
Δ\(^{4}\)-androstenediol ratio was 0.88 compared to a normal
ratio of at least 4:1. After HCG, Δ\(^{4}\)-androstenediol
rises more than testosterone, so that the resultant test-
osterone:Δ\(^{4}\)-androstenediol ratio is further reduced to
0.67. The increases in these steroids after HCG may
be artificially low, since the base-line values were ob-
tained after only 2 days of high-dose dexamethasone
suppression. In view of the lower values later observed
after 9 days of dexamethasone administration, the HCG
stimulation very likely was begun at a time of incom-
plete adrenal suppression. Subsequent HCG testing
after prolonged dexamethasone administration has sup-
ported this conclusion (39). These data are consistent
with a partial block in testosterone synthesis and indi-
cate that Δ\(^{4}\)-androstenediol is a major secretory product
of the testes in this patient. Since only 1–2% of Δ\(^{4}\)-
androstenediol is normally converted peripherally to
testosterone (40), the majority of this patient’s test-
osterone is most likely secreted by the testes, con-
sistent with a partial rather than complete testicular
Δ\(^{4}\)-3β-HSD block.

Deficiency of the testicular enzyme complex is further
suggested by the absence of Δ\(^{4}\)-3β-HSD activity histo-
chemically and by the in vitro studies. However, our
data must be interpreted cautiously because of the lack
of extensive control testes. With either pregnenolone or
DHA as a substrate, the control testis consistently
formed 3–10 more Δ\(^{4}\) products than that of our patient.
These differences may even be minimized, since the
control testis was from a patient treated with estrogens,
shown by both in vivo and in vitro studies to suppress
Δ\(^{4}\)-3β-HSD enzyme activity (41–43). In addition, the
micro method demonstrated that Δ\(^{4}\)-androstenediol is
the major product in the patient’s testicular incuba-
tions. The ratio of Δ\(^{4}\)-androstenediol:testosterone pro-
duced by incubation of our patient’s testis with DHA
was 32:1, whereas almost equal amounts of these two
steroids are reported to be produced from DHA after
incubation of normal human testes (34). Thus, in vivo
and in vitro data in our patient are consistent with
data obtained from rats in which Δ\(^{4}\)-3β-HSD activity
has been experimentally inhibited by injecting the
pregnant mother with a Cα substrate analog, cyanoke-
tone (2α-cyano-4,4,17a-trimethyl-5-androstene-17β-ol-3-
one) on either the 16th or the 19th day of gestation
(44). In this experimental situation, Δ\(^{4}\)-androstenediol
is the major product formed in vitro by the cyanoketone-
inhibited testes (45) and is the major androgen in
the circulation of the newborn males (46).

For the most part, plasma estrogens were normal
and estradiol levels were increased by exogenous go-
nadotropins. These studies do not indicate, however, if
estrogens are secreted directly by either the testes or
the adrenals or are derived from peripheral metabolism
of androgens. The intact nature of the pituitary-gonadal
axis was further confirmed by the finding of normal
levels of gonadotropins, except for the rise associated
with the decline of plasma androgens and estrogens
after 9 days of high-dose dexamethasone suppression.
These findings indicate that this patient’s gonadotropins
were responsive to negative feedback by circulating
sex steroids.

Including our patient, the oldest pubertal boys thus
far described have developed gynecomastia (3, 4). This
breast development could represent that seen in other-
wise normal male adolescents, in whom steroid studies
have not demonstrated a characteristic pattern (47).
Alternatively, the gynecomastia may reflect fetal tes-
tosterone insufficiency, with failure of inhibition of the
female breast anlage (3). Animal experiments where
either androgen antagonist (cyproterone acetate) or a
3β-HSD inhibitor was administered to fetal male rats
support the latter concept, since these rats also de-
veloped breast hypertrophy (48, 49), which can be
prevented by simultaneous testosterone treatment (49).
Alternatively, Δ\(^{4}\)-androstenediol itself, in view of its
feminizing effect on vaginal mucosa (50), might pro-
mote breast development.

The testicular biopsy revealed spermatogenic arrest
with primarily areas of diminished Leydig cells but
with occasional focal Leydig cell hyperplasia. Although
this may be considered appropriate for the patient’s
stage of puberty, the studies of Goldman (51) suggest
it may be abnormal. In these latter studies of the cyano-
ketone-inhibited rat, persistent Δ\(^{4}\)-3β-HSD deficiency is
produced in the progeny when the mother is injected
with this Cα substrate analog on the 16th-19th day of
gestation. If the mothers are treated on the 16th day,
testes of the male offspring fail to undergo enlargement at puberty, and the testes have a complete arrest of tubular development, with hypoplastic Leydig cells. When the mother is treated on the 19th day, the offspring's testes have tubular atrophy and hyperplastic Leydig cells. In both situations, enzyme activity in the testis is reduced, and hypospadias is present. The hypospadias can be partially prevented with in utero testosterone treatment, implying that testosterone deficiency is responsible for this defect (44). The histological features and absent histochemical $\Delta^4$-$3\beta$-HSD activity seen in the testis of our patient represent changes similar to those found by Goldman in cyano-ketone-treated male offspring rats and suggest that normal in utero testosterone production is necessary for normal testicular maturation and spermatogenesis.

The nodules noted in the testis and spermat cord in this patient proved to represent Leydig cell hyperplasia. There is controversy in the literature as to whether these tumors in congenital adrenal hyperplasia are of adrenal or of testicular origin (52-56). However, prolonged ACTH stimulation appears to lead to the development of these tumors (57), and whatever the cell of origin, they have the capacity to make cortisol or other steroid precursors (55-57). Although testicular nodule tissue from our patient was not incubated, these nodules appear stimulated by ACTH, as evidenced by a decrease in size, at least in the right testis, with increased glucocorticoid therapy.

The pattern of simultaneous adrenal and testicular enzyme deficiency in this pubertal patient is similar to that of a neonate (2) but may differ from that of the pubertal boy studied by Parks, Bermudez, Anast, Bongiovanni, and New (3). Their patient had a normal testosterone level that rose ninefold after HCG, a brisk response suggesting that testosterone synthesis was intact at puberty. However, as discussed earlier, $\Delta^4$-androstenediol is the major $\Delta^4$-$C_{21}$ androgen secreted by the testes, and was not measured in this pubertal male. Recently, opposite conclusions were reached from studies in another pubertal male (4), in whom a subnormal testosterone response to HCG and elevated spermatic and peripheral venous concentrations of $\Delta^4$-pregnenolone were demonstrated. The elevation of $\Delta^4$-androstenediol observed in our patient, as well as its disproportionate rise relative to testosterone after HCG stimulation, provides substantive evidence for a functional impairment in testosterone secretion. If the enzymatic defect in these three reported pubertal males is similar, testicular function clearly is less compromised than that of the adrenal glands. It is conceivable that the enzyme complex may have a lower affinity for $C_{19}$ than $C_{21}$ steroids to account for the seeming differences in enzyme activity in the adrenal versus the testis. Our in vitro data are against this explanation. The possible explanation that the adrenal and testicular enzymes are under different genetic control seems unlikely, since adrenal insufficiency and pseudohermaphroditism indicate deficiency of adrenal and fetal testicular $\Delta^4$-$3\beta$-HSD. Alternately, the enzyme complex may consist of isoenzymes, one subunit of which is mutant and comprises all of the adrenal activity, whereas testicular activity represents portions of normal as well as mutant subunits, as previously postulated (47).

In this patient, as in some others, urinary pregnan-triol, androsterone, and etiocholanolone are elevated (30-32). The increase in these metabolites has been attributed to peripheral conversion of $\Delta^4$ compounds to $\Delta^4$ compounds by hepatic $\Delta^4$-$3\beta$-HSD activity (3, 27). Bongiovanni, Eberlein, and Moshang (17) administered 17$\alpha$-hydroxy-pregnenolone orally to two normal subjects and found the excretion of more pregan-triol than $\Delta^4$-pregnenetriol, suggesting an hepatic or other extra-adrenal site of conversion. Our patient handled oral 17$\alpha$-hydroxy-pregnenolone in a qualitatively similar manner as the two normal volunteers studied by Bongiovanni et al. These data would suggest that hepatic or other peripheral $\Delta^4$-$3\beta$-HSD activity is intact in this patient and responsible for the increased excretion of $\Delta^4$ steroid metabolites, although it could be argued that the methods used to assess peripheral $\Delta^4$-$3\beta$-HSD activity might not be sufficiently sensitive to detect small differences in enzymatic activity. However, our data are consistent with conclusions deduced from gas-liquid chromatography and mass spectometry analysis of bile secretions in one other patient with a $\Delta^4$-$3\beta$-HSD defect (38). In addition, treatment with cyano-ketone does not affect hepatic $\Delta^4$-$3\beta$-HSD activity despite 90% inhibition of both the testicular and adrenal enzyme complexes (59). These findings, together with our patient's ability to metabolize orally administered 17$\alpha$-hydroxy-pregnenolone, suggests that the liver or other peripheral enzyme systems are either different or under different genetic control from that of the gonad and adrenal.

ACKNOWLEDGMENTS

The authors are grateful for the assistance and cooperation of the nursing and general staff of the Yale Children's Clinical Research Center. We are indebted to Dr. Martin Schiff, Jr., for performing the testicular biopsy, to Dr. Michael Kashgarian for assistance in interpretation of the histologic specimens, to Dr. Judith L. Vaitukaitis for performing the LH and FSH determinations, to Dr. Leon Speroff for determining plasma estrogens, and to Ms. Barbara Bahrs and Ms. Donna Arre for their excellent secretarial assistance in preparation of the manuscript. Dr. Patrick J. Mulrow pro-
vided encouragement and laboratory facilities throughout the course of this study.

Clinical studies were performed at the Yale Children's Clinical Research Center, supported by grant RR-125 from the General Clinical Research Centers Program, Division of Research Resources, National Institutes of Health. This research was supported by Training Grant AM-05015 and Research Grants HD-4683, HD-00371, and HD-06308 from the National Institutes of Health, United States Public Health Service, and by grants from the Rockefeller Foundation and the National Foundation—March of Dimes.

REFERENCES


