

Metabolism of Testosterone and Related Steroids in Metastatic Interstitial Cell Carcinoma of the Testis *

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Interstitial cell carcinoma of the testis is a singularly rare steroid-producing cancer. Of the seven reported cases (1-7), urinary 17-ketosteroid (17-KS) excretion was high in the four cases in which it was measured. Abelson, Bulaschenko, Trommer, and Valdes-Dapena (7) fractionated the urinary 17-ketosteroids and corticoids in one recently reported case. There is, however, no comprehensive study of either the production of androgens or related steroids by this tumor. We have had the opportunity to study a patient with metastatic interstitial cell carcinoma, and we have examined the androgens and several related steroids. From these data, we have concluded that interstitial cell carcinoma resembles virilizing adrenal carcinoma in its pattern of biosynthesis of C-19 steroids and 21-deoxypregnane analogues. The high testosterone ¹

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¹ The chemical names for the steroids used in this paper are as follows: 17 β -hydroxy-androst-4-en-3-one (testosterone); 17 α -hydroxy-androst-4-en-3-one (epitesterone); 3 α -hydroxy-5 β -androst-17-one (etiocholanolone); 3 α -hydroxy-5 α -androst-17-one (androsterone); 3 α ,11 β -dihydroxy-5 β -androst-3-one (11-hydroxyetiocholanolone); 3 α -hydroxy-5 β -androst-3,11-dione (11-ketiocholanolone); 3 α ,11 β -dihydroxy-5 α -androst-17-one (11-hydroxy-androsterone); 3 β -hydroxy-androst-5-en-3-one (dehydroepiandrosterone, DHA); androst-4-en-3,17-dione (androstenedione); 3 β ,16 α -dihydroxy-5-androst-17-one (16-hydroxy DHA); androst-5-en-3 β ,17 β -diol (androstenediol); pregn-5-en-3 β ,20 α -diol (pregnenediol); pregn-5-en-3 β ,17 α ,20 α -triol (pregnenetriol); 5 β -pregnan-3 α ,20 α -diol (pregnanediol); 5 β -pregnan-3 α ,17 α ,20 α -triol (pregnanetriol); 3 α ,17 α -dihydroxy-5 β -pregnan-20-one (17-hydroxypregnanolone); 3 α ,17 α ,20 α -trihydroxy-5 β -pregnan-11-one (11-ketopregnanetriol); 5 α -androst-16-en-3 α -ol (androstenol); 11 β ,17 α ,21-trihydroxy-pregn-4-en-

production rate has been shown to be a consequence of metabolism of dehydroepiandrosterone sulfate.

Methods

Routine methods were used to analyze the following: urinary 17-KS (8), urinary 17-hydroxycorticoids (9), plasma Silber-Porter chromogens (10), and plasma testosterone (11).

Gas-liquid chromatography. We carried out gas-liquid chromatography (GLC) in a Glowell Chromolab gas chromatograph utilizing a ⁹⁰Sr ionization detector operating at 1,050 v. The coiled glass columns, 6 feet by $\frac{3}{8}$ inch i.d., were packed with 2% SE-30 on Chromosorb W. Trimethylsilyl derivatives were prepared as noted previously (12).

Fractionation of urinary steroids. Urinary steroid sulfates and glucuronides were separated by thin layer chromatography in system TN (13) after an ether-ethanol extraction (14). Glucuronides and sulfates were hydrolyzed separately either with β -glucuronidase (Ketodase) or by solvolysis (15). In the subsequent discussion, steroid sulfates are defined as those steroids having the mobility of steroid monosulfates in TN and hydrolyzed by solvolysis. Glucuronides are defined as those steroids having the mobility of steroid glucuronides in TN and released by β -glucuronidase. Further steps in purification were chromatography on the silica-alumina column (16) and group fractionation with Girard reagent T and digitonin. For isolation of individual metabolites, the resulting ketonic and nonketonic alpha and beta fractions of differing polarity were then chromatographed in the appropriate systems listed below.

Paper chromatography systems (after Bush) were as follows: A1—ligroin: methanol: H₂O (100:90:10); B $\frac{1}{2}$ —ligroin: toluene: methanol: H₂O (75:25:70:30); B1—ligroin: toluene: methanol: H₂O (50:50:70:30); B4

3,20-dione (cortisol); 3 α ,11 β ,17 α ,21-tetrahydroxy-5 β -pregnan-20-one (tetrahydrocortisol); 3 α ,17 α ,21-trihydroxy-5 β -pregnan-11,20-dione (tetrahydrocortisone); 3 α ,11 β ,17 α ,21-tetrahydroxy-5 α -pregnan-20-one (allotetrahydrocortisol); 3 α ,17 α ,21-trihydroxy-5 β -pregnan-20-one (tetrahydro substance S); 3-hydroxyestratrien-1,3,5 (10)-17-one (estrone); 1,3,5 (10)-estratriene-3,17 β -diol (estradiol); 1,3,5 (10)-estratriene-3,16 α ,17 β -triol (estriol).

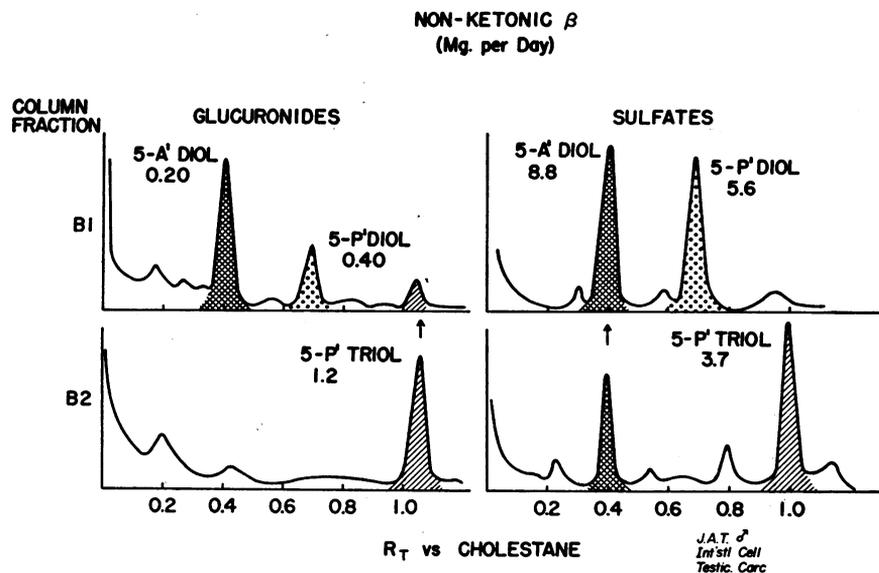


FIG. 1. GAS-LIQUID CHROMATOGRAMS OF NONKETONIC- 3β -HYDROXYSTERIODS. Glucuronides and sulfates were separated before hydrolysis, and each column fraction was treated with Girard's reagent T and digitonin. 5-A'diol = 5-androstenediol; 5-P'diol = 5-pregnenediol; and 5-P'triol = 5-pregnenetriol.

—toluene: methanol: H₂O (100:50:50); and C—toluene: ethyl acetate: methanol: H₂O (90:10:50:50).

Thin layer silica gel systems were as follows: T4—benzene: ethyl acetate (40:60); T6—benzene: ethyl acetate (60:40); TE—benzene: ethanol (90:10); and TN—ethyl acetate: ethanol: ammonia (50:50:10).

3 α -Hydroxyketonic steroids. Etiocholanolone and androsterone were quantified as the trimethylsilyl ethers by GLC of the appropriate fractions after chromatography in A1 and T6. The 11-oxo-17-ketosteroids were measured as the free steroids by GLC after chromatography in B1 and T4.

3 α -Hydroxy nonketonic steroids. Androstenol was measured by GLC of the least polar nonketonic alpha fraction (17). Pregnenediol and pregnetriol were isolated from appropriate nonketonic alpha fraction by chromatography in T4 and estimated by GLC as the free steroids. We could not identify 11-ketopregnenetriol by GLC after chromatography in T4.

Δ^5 -3 β -Hydroxy nonketonic steroids. Androstenediol, pregnenediol, and pregnetriol were measured by GLC directly after column chromatography and Girard and digitonin fractionation. Figure 1 is an example of the chromatograms obtained from two such fractions. The clearly defined peaks, well separated from contaminants, permitted accurate measurements.

Cortisol metabolites. Cortisol and its metabolites, tetrahydrocortisol, tetrahydrocortisone, and allotetrahydrocortisol, were measured together as phenylhydrazine chromogens after silica-alumina column fractionation and thin layer chromatography in TE. Tetrahydro substance S was sought in another column fraction after paper chromatography in B4.

Estrogens. The three classical urinary estrogens were isolated from the phenolic fraction as suggested by Engel (18). Tritiated estrone, estradiol, and estriol² were added to the phenolic extract to correct for losses occurring after β -glucuronidase hydrolysis. We separated estriol from estrone and estradiol by partitioning between petroleum ether-benzene and water. Estrone and estradiol were chromatographed on thin layer silica gel plates in TE, and estriol was chromatographed in 100% ethyl acetate. All samples were acetylated and chromatographed on thin layer silica gel. Chloroform was used to develop the chromatograms. After saponification, paper chromatography was performed as follows: estrone, B \ddagger ; estradiol, B1; and estriol, C. Subsequent thin layer chromatography of estrone and estradiol in T6 and estriol in T4 was performed. Gas-liquid chromatography of these fractions resulted in single clean peaks for estrone and estriol. Estradiol could not be identified.

16-Hydroxy DHA. Since this steroid has been found only rarely in urine, its identity was assured by the correspondence of the isolated material with authentic standard³ in paper, thin layer, and gas-liquid chromatography as the free steroid and the acetate. The color changes produced by the Allen reagent and the sulfuric acid spectra were identical for the presumptive and authentic 16-hydroxy DHA.

² We wish to thank Dr. Mortimer Levitz, New York University Medical School, for a gift of estriol-³H.

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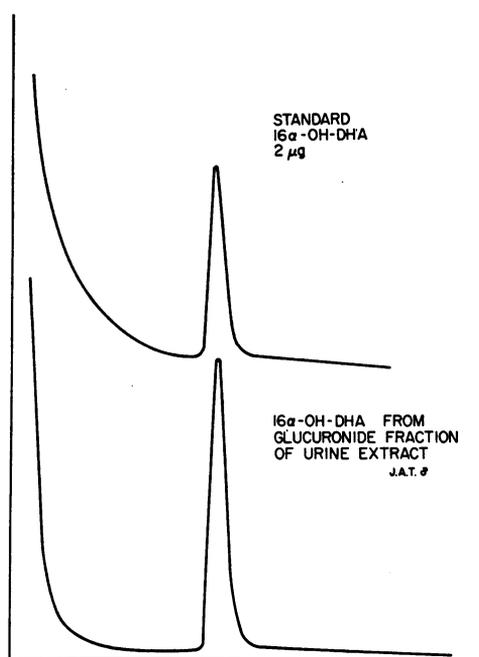


FIG. 2. GAS-LIQUID CHROMATOGRAM OF 16 α -HYDROXY DHA. Comparison of authentic standard and steroid isolated from urine.

Radioactivity counting. Radioactivity measurements were made on a Packard liquid scintillation spectrometer, model 314EX, with discriminator and gain settings to give an efficiency of 45% for ^{14}C and 19% for ^3H . Sufficient counts were accumulated to give a standard error of no more than 2%.

Preparation of tracers. Testosterone-7 α - ^3H ,⁴ dehydroepiandrosterone-4- ^{14}C ,⁵ and dehydroepiandrosterone sulfate-7 α - ^3H ⁵ were chromatographed before use. DHA sulfate was extracted with ether shortly before infusion into the patient. Epitestosterone- ^3H was prepared by the Wilzbach technique and purified by column, paper, and thin layer chromatography of the free steroid and its acetate (19).

Production rates. To measure testosterone production rate, we injected 10.0 μc of testosterone- ^3H in saline intravenously and determined the specific activity of testosterone in urinary testosterone glucuronide (16). Epitestosterone production rate was estimated similarly after injection of 5 μc of epitestosterone- ^3H and isolation of epitestosterone from a 3-day urine collection. Blood testosterone production rate was measured by the metabolic clearance technique (20); a Bowman infusion pump was used for the constant infusion. It was found subsequently that 26% of the testosterone infused via the venoclysis set was absorbed by the tubing, and appropriate corrections were made. Blood samples were

obtained at 50, 60, and 70 minutes of the infusion. The plasma was separated, and 400 dpm of testosterone- ^{14}C and 50 μg of testosterone were added. Testosterone extracted from the plasma was purified through five chromatography systems. The specific activities and the $^3\text{H}/^{14}\text{C}$ ratios agreed within 5% during the last three chromatographies, in which the testosterone was measured successively as the acetate, the free alcohol, and androstenedione.

We determined the production rates of DHA and DHA sulfate with 20.45 μc of DHA sulfate- ^3H and 2.77 μc of DHA- ^{14}C injected intravenously over a 5-minute period. Urine was collected for 3 days, processed as described above, and the specific activity of the DHA sulfate and DHA glucuronide was measured.

Specific activities of DHA, 16-hydroxy DHA, and androstenediol. The appropriate fractions containing DHA, 16-hydroxy DHA, and androstenediol were chromatographed on 45-cm thin layer silica gel plates in T4, and they were then chromatographed in systems A1, B1, and B1, respectively. The DHA was chromatographed on T6, and the 16-hydroxy DHA and androstenediol were chromatographed in T4. Specific activities of the DHA and 16-hydroxy DHA were determined at this point, the mass being measured with the Allen reagent and by GLC. Agreement between the two estimates was good. Figure 2 demonstrates the purity of the 16-hydroxy DHA; comparable tracings were obtained for androstenediol and DHA. The mass of androstenediol was measured by GLC only. The coefficient of variation of estimates of mass by GLC averages 7% in our hands. This is the largest error associated with the determination of the specific activities calculated in this paper.

Results

17-Ketosteroids and corticoids. The 17-KS excretion varied between 200 and 400 mg daily during the control period and was not suppressed by either dexamethasone, 8 mg daily, or fluoxymesterone, 40 mg daily (Figure 3). The administration of human chorionic gonadotropin (HCG) in doses of 4,000 international units (IU) daily for 5 days was associated with an increase in 17-KS excretion to an average level of 690 mg per day. Since chemotherapy could not be delayed further, a subsequent control period was not obtained; hence, the decreased excretion of 17-KS after cessation of HCG could conceivably be attributed to the chemotherapy. However, the lack of response of the tumor to the chemotherapeutic regimens that were tried makes this an unlikely possibility.

Urinary 17-hydroxycorticoid excretion averaged 22 mg per day. Since the excretion of pregnane-triols, which are also measured by the method em-

⁴ Nuclear-Chicago Corp., Des Plaines, Ill.

⁵ New England Nuclear Corp., Boston, Mass.

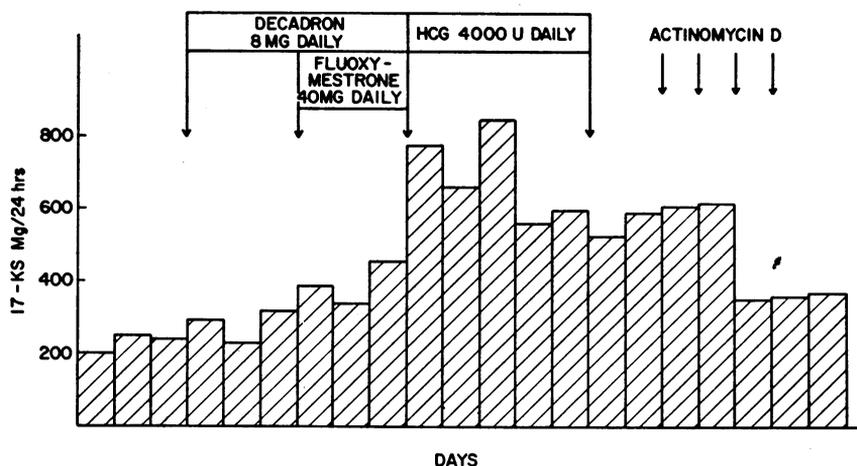


FIG. 3. EFFECT OF EXOGENOUS HORMONES AND ACTINOMYCIN D ON 17-KETOSTEROID EXCRETION. HCG = human chorionic gonadotropin.

ployed, was increased to 6 mg per day, urinary corticoid excretion was actually within the normal range. Plasma Silber-Porter chromogen levels were normal.

Testosterone. Total excretion of testosterone ranged between 500 and 1,500 μg per day, or some 10 to 30 times normal. These values are not corrected for losses, and the recovery was probably between 40 and 50%. When the sulfates and glucuronides were separated before hydrolysis, the excretion of testosterone isolated from the sulfate fraction was 690 μg per day.

Plasma testosterone concentration was 1.96 μg per 100 ml. The metabolic clearance rate of testosterone was 2,700 L per day, about three times the normal clearance rate (21), indicating extensive extrahepatic clearance of testosterone. As the tumor at autopsy was six times as large as the liver, it was the likely site of the extrahepatic metabolism. Other studies in this patient likewise suggest active participation of the tumor in metabolism of plasma steroids.

The testosterone production rate calculated by the urinary isotope dilution method from the specific activity of urinary testosterone glucuronide was 53 mg per day, or ten times normal. When the blood testosterone production rate was estimated as the product of the metabolic clearance rate and the plasma level (20), the value was 48 mg per day. The reasonable agreement between these two production rates indicates that the urinary testosterone glucuronide originated almost

entirely from the blood testosterone compartment. If a significant fraction of the urinary testosterone glucuronide had been derived from a testosterone compartment other than the plasma testosterone compartment, the production rate estimated by urinary isotope dilution would have been larger than that measured by the metabolic clearance technique (22, 23).

Origin of plasma testosterone. It could not be established from these data whether the plasma testosterone resulted from "secretion" by the tumor or peripheral production from precursors. The high urinary excretion of DHA sulfate suggested that this conjugate could serve as a precursor of the plasma testosterone. To examine this, we isolated testosterone glucuronide from urine and measured its specific activity after the intravenous infusion of DHA sulfate- ^3H and DHA- ^{14}C (Table I). It is apparent that the specific activity of testosterone glucuronide was the same as

TABLE I
Specific activity of urinary testosterone glucuronide and urinary DHA sulfate after administration of DHA sulfate- ^3H and DHA- ^{14}C *

	Testosterone glucuronide	DHA sulfate	% testosterone glucuronide derived from DHA sulfates
	<i>dpm/μg</i>		
^3H	1.2	1.1	100
^{14}C	0.65	0.72	90

* DHA = dehydroepiandrosterone.

TABLE II

Specific activities of testosterone sulfate, DHA glucuronide, and DHA sulfate after infusion of DHA sulfate-³H and DHA-¹⁴C

	Specific activity			Production rate	
	DHA glucuronide	DHA sulfate	Testosterone sulfate	DHA	DHA sulfate
	<i>dpm/μg</i>			<i>mg/24 hr</i>	
³ H	10.3	1	18.9		2,270
¹⁴ C	7.5	0.72	1.04	286	

that of DHA sulfate with respect to tritium and was 90% of the specific activity of DHA with respect to ¹⁴C. This means that plasma DHA sulfate must have been the unique precursor of essentially all of the urinary testosterone glucuronide and hence, of the plasma testosterone, for if there had been a precursor of testosterone not derived from DHA sulfate, the urinary testosterone glucuronide would have been diluted by this other source, and its specific activity would have been lower than that of DHA sulfate. The good agreement between the specific activities of ³H and ¹⁴C is additional evidence that the plasma DHA sulfate was the precursor of the urinary testosterone glucuronide and thus the source of the plasma testosterone.

This conclusion is supported by considering the conversion of DHA sulfate to urinary testosterone glucuronide after the infusion of DHA sulfate-³H. By reverse isotope dilution, it was found that 0.03% of the DHA sulfate-³H was converted to urinary testosterone glucuronide. Since about 1% of plasma testosterone is metabolized to urinary testosterone glucuronide (24), and since plasma DHA sulfate was the precursor of plasma testosterone in this subject, the production of plasma testosterone could be estimated at 68 mg per day from the production rate of DHA sulfate (Table II) ($2,270 \times 0.0003 \times 100$). The difference of 30% from the experimental values may be due to the assumed rate of metabolism of plasma testosterone to urinary testosterone glucuronide.

Origin of urinary testosterone sulfate. After administration of testosterone-³H, the specific activities of urinary testosterone sulfate and glucuronide were measured. Testosterone sulfate had a specific activity of 31.2 dpm per μg , whereas testosterone glucuronide had a specific activity of

146 dpm per μg . Had both steroids been derived exclusively from the same precursors, their specific activities would have been equal. Thus, 80% of the urinary testosterone sulfate must have been diluted by unlabeled testosterone sulfate.

In view of the significance of the sulfoconjugates in steroid biosynthesis (25) and the demonstration that an adrenocortical carcinoma could synthesize testosterone sulfate (26), it may be reasonably assumed that the testosterone sulfate was synthesized by the tumor. The data of Table II support this assumption. After the infusion of DHA sulfate-³H, the specific activity of ³H of the testosterone sulfate was 18 times as great as that of DHA sulfate. If urinary DHA sulfate is derived entirely from plasma DHA sulfate, the testosterone sulfate must have been synthesized from infused DHA sulfate-³H that was selectively metabolized by the tumor before equilibration with plasma DHA sulfate had occurred. Siiteri and MacDonald (27) used a similar explanation to account for similar apparently anomalous findings after infusion of labeled DHA sulfate. It is not easy to invoke this explanation for the finding that the ¹⁴C specific activity of testosterone sulfate was twice the ¹⁴C specific activity of DHA sulfate, since free plasma DHA is rapidly metabolized.

Production rates of DHA and DHA sulfate. An attempt was made to use the two compartment model of Gurpide, MacDonald, Vande Wiele, and Lieberman (28) to calculate secretion rates, production rates, and rates of conversion of DHA and DHA sulfate. From the data of Table II, we calculated that the production rate of DHA sulfate was 2,270 mg per day and that of DHA was 286 mg per day. The calculation of secretion and conversion rates was not possible due to the excess of tritium in the DHA glucuronide.

The reason for this finding has been alluded to. The analysis of Gurpide and co-workers (28) was predicated on a two compartment model, although it seems probable that in this patient the tumor acted as a third compartment. If, as suggested earlier, the tumor was removing DHA sulfate from plasma and secreting DHA, then plasma DHA and urinary DHA glucuronide could contain an excess of tritium. There would then be no way to estimate secretion and conversion rates.

If injected DHA sulfate-³H was metabolized by the tumor before equilibration with plasma DHA

sulfate occurred, then one of the criteria of the urinary isotope dilution method was not fulfilled, namely, that the tracer be metabolized identically with the unlabeled steroid. Thus, even calculation of production rates would be theoretically unsound. The divergence between the true and calculated blood production rates would depend on such incalculable factors as how much tracer was metabolized by the tumor and the fate of this tracer. Nevertheless, the internal consistency of the testosterone production rate calculated from the dilution of testosterone-³H and that estimated from the conversion of DHA sulfate to urinary testosterone glucuronide suggests that DHA sulfate production rates cannot have been greatly overestimated.

Epitestosterone. The production rate of epitestosterone estimated from the specific activity of urinary epitestosterone glucuronide after the injection of epitestosterone-³H was 495 μ g per day, or two to three times normal. We have shown previously (29) that epitestosterone is not a peripheral metabolite of DHA or testosterone, and these data suggest that increased synthesis of these steroids is not accompanied by a proportionate increase in epitestosterone synthesis.

Excretion of Δ^5 steroids. The excretion of several steroids retaining the Δ^5 - 3β -hydroxy function is presented in Table III. As expected, the larger

TABLE III
Urinary steroid excretion

	Glucur- onide	Sulfate	Total	Normal (sulfate plus glucuronide)
	<i>mg/24 hr*</i>			
Androstenol	1.2	†	1.2	1-2
DHA	3.5	45.0	48.5	0.2-2.0
16-Hydroxy DHA	5.0	6.8	11.8	0.1-0.2
Androstenediol	0.2	8.8	9.0	0.1-0.2
Pregnenediol	0.4	5.6	6.0	0.1-0.4
Pregnenetriol	1.2	3.7	4.9	0.2-0.4
Pregnanediol	2.1	†	2.1	0.4
Pregnanetriol	6.0	†	6.1	0.5
17-Hydroxypregnanolone	2.8	†	2.8	0.2
Androsterone	65	7.3	72.3	1-3
Etiocholanolone	119	1.8	120.8	2-4
11-Ketoetiocholanolone	1.4	0	1.4	0.5
11-Hydroxyetiocholanolone	3.1	0	3.1	0.4
11-Hydroxyandrosterone	4.5	2.2	6.7	0.5
Estrone			0.075	†
Estradiol			<0.013	†
Estriol			0.029	†

* These values are not corrected for losses during isolation.

† Not searched for.

‡ Normal values have not been measured in this laboratory.

TABLE IV
Specific activities of sulfates of DHA, androstenediol, and 16-hydroxy DHA

	DHA	Andros- tenediol	16-Hy- droxy DHA
	<i>dpm/mg</i>		
³ H	1,070	1,060	1,080
¹⁴ C	720	700	730

fraction was excreted as the sulfate, although in the case of 16-hydroxy DHA, the glucuronide almost equaled the sulfate. Dehydroepiandrosterone was excreted in amounts similar to those noted in adrenal carcinoma. The urinary levels of pregnenediol, pregnetriol, and androstenediol were many times the normal values. Similar findings have been described in adrenocortical carcinoma (30). A high excretion of 16-hydroxy DHA has been described previously in a patient with adrenocortical carcinoma (31).

The origin of the androstenediol sulfate and the 16-hydroxy DHA sulfate was examined by comparing their specific activities to that of DHA sulfate after the infusion of DHA sulfate-³H and DHA-¹⁴C (Table IV). Their specific activities were the same within the limits of error of measurement, which made it likely that the plasma DHA sulfate was the sole precursor of the urinary androstenediol sulfate and 16-hydroxy DHA sulfate. Roberts and associates (32) reported similar findings in a subject with adrenocortical carcinoma.

Excretion of 3α -hydroxysteroids. Urinary androsterone and etiocholanolone levels were greatly elevated, although they totaled only 9% of the DHA sulfate production. These are uncorrected figures, however, and the true excretion may be twice the estimated values. The metabolites of 17-hydroxyprogesterone, pregnetriol and 17-hydroxypregnanolone, were excreted in increased amounts (Table III). Pregnanediol excretion was increased proportionately less than the metabolites of other biosynthetic intermediates, suggesting that either progesterone was efficiently utilized by the tumor, or that biosynthesis occurred predominantly via the pathway from 17-hydroxypregnenolone to 17-hydroxyprogesterone, thus bypassing progesterone.

Excretion of 11-oxo-steroids. Of considerable interest were the increased urinary titers of the

three 11-oxo-17-ketosteroids. The excretion of 6.7 mg per 24 hours of 11-hydroxyandrosterone represented a thirteenfold increase above normal. Since synthesis of this steroid presumably required an 11 β -hydroxylating system in the tumor, the general question of 11 β -hydroxylation was examined. As pregnanetriol excretion was elevated and we could infer 17-hydroxyprogesterone synthesis, we attempted to measure 11-ketopregnanetriol in the urine. However, none was found in this subject's urine, indicating that 11 β -hydroxylation of 17-hydroxyprogesterone did not occur to any measurable extent.

To ascertain whether the urinary 17-hydroxycorticoids were metabolites of cortisol produced by the tumor or by the adrenal cortex, we measured tetrahydrocortisol, tetrahydrocortisone, allotetrahydrocortisol, and cortisol together. During a control period, the 24-hour excretion was 7.8 mg; this was decreased to 3.8 mg by the daily administration of 8 mg of dexamethasone. Thus, the titers of cortisol metabolites paralleled adrenocortical function, whereas the excretion of 11-hydroxyandrosterone measured simultaneously was unchanged by dexamethasone. Hence, it seems that the tumor demonstrated some substrate specificity in 11-hydroxylation, a conclusion that has some precedence in a similar study by Savard and associates (33) of a benign interstitial cell tumor.

Tetrahydro substance S was not detected in the urine of this patient. In view of the probable excess of 17-hydroxyprogesterone produced by the tumor, it must be concluded that the tumor did not possess a 21-hydroxylating system and differs in this regard from many adrenocortical tumors.

Estrogens. The excretion of estrone, estradiol, and estriol was measured in four consecutive 24-hour urines. The average excretion of estrone was 75 μ g per 24 hours; estriol, 29 μ g per 24 hours; and estradiol, less than 13 μ g per 24 hours. Other patients with interstitial cell carcinoma have had an increased excretion of estrogens determined either by bioassay (4, 7) or chemically (5).

The failure to find estradiol may have been due to the relatively small samples used and the large losses before the measurement of estrogen. It cannot be assumed from these high levels that the tumor produced estrogens, since the data are

equally compatible with estrogen production peripherally from any of the C₁₉O₂ precursors.

Discussion

The rarity of interstitial cell carcinoma has precluded adequate study of its steroid synthetic activity. The 17-KS excretion ranged from 20 to 1,000 mg daily in four reported cases (1, 4, 5, 7) and in our case, and it was thus within the range usually found with adrenocortical carcinoma (34). Although the steroid biosynthetic activity of the interstitial cell tumor was not suppressed by exogenous androgen as is the normal interstitial cell (35), the response to HCG demonstrated that the tumor was not completely autonomous. Occasional adrenocortical carcinomas have similarly shown ACTH responsiveness, but there has been a uniform inability to suppress their steroid output with exogenous corticoids (34).

This patient and the patient described by Abelson and co-workers (7) had a high plasma concentration of testosterone, the characteristic secretory product of the Leydig cell. Since this patient's remaining testis was atrophic, it seems unlikely that it could have accounted for a significant fraction of the plasma testosterone.

In spite of a high metabolic clearance rate of testosterone, the plasma level was maintained at 1.96 μ g per 100 ml, due to a high testosterone blood production rate. An unexpected conclusion was that almost all of this testosterone had its origin from plasma DHA sulfate, and the close agreement between the specific activities of tritium and carbon in urinary DHA sulfate and in urinary testosterone glucuronide left no alternative. This conclusion was strengthened by the estimate of testosterone production obtained from the independent measurement of DHA sulfate production and its conversion to testosterone glucuronide. This emphasizes the hazards of assuming synthesis of a steroid by a tumor when such a steroid can be synthesized peripherally from other steroid precursors.

The agreement between the two methods of estimating testosterone production rates is of considerable interest in view of prior studies demonstrating that, in general, only a small moiety of plasma testosterone is due to hepatic testosterone synthesis from other precursors (23, 35, 36).

This phenomenon most probably reflects rapid conjugation of the newly synthesized testosterone. This has not been invariable, however, and in one patient it was shown that all of the testosterone synthesized peripherally from androstenedione appeared in the plasma as free testosterone (23). Thus, variability in rates of conversion to glucuronide of testosterone synthesized in the liver could have a decisive role in determining plasma testosterone levels. Had the production rates not been measured by both techniques, it would have been concluded that plasma DHA sulfate could not have been the source of plasma testosterone.

The measurement of urinary metabolites of known intermediates of biosynthetic sequences permits an informed guess about *in vivo* synthetic activity. Thus, the excretion of large amounts of pregnenediol, pregnetriol, androstenediol, and DHA reflected the production of proportionately increased amounts of their respective precursors, pregnenolone, 17-hydroxypregnenolone, and DHA. A subsequent inference is that tumor 3β -hydroxysteroid dehydrogenase activity was limited, since these steroids were not converted to the corresponding Δ^4 -3-ketosteroids. This pattern, too, is characteristic of adrenocortical carcinoma (37).

The excretion of individual 11-oxo-17-ketosteroids was measured by Abelson and associates (7), and the excretion of each was increased. The findings in our case are similar, but the largest increase was in the excretion of 11-hydroxyandrosterone. This steroid is the principal metabolite of 11β -hydroxyandrostenedione (38). Since suppression of the adrenal cortex did not decrease the excretion of 11-hydroxyandrosterone, we have concluded that 11β -hydroxyandrostenedione was synthesized by the tumor.

In three cases of benign interstitial cell tumor (33, 39, 40) 11-hydroxyandrosterone was also excreted in increased amounts. Savard and associates (33) suggested that the 11β -hydroxylating system showed substrate specificity, since they were unable to demonstrate 11β -hydroxylation of progesterone. Our findings support this suggestion, since we were unable to find 11-ketopregnanetriol in spite of an abundant supply of the substrates for 11β -hydroxylation, 17-hydroxypregesterone, and 17-hydroxypregnenolone. It should be noted, however, that Smith, Breuer, and

Schriefers (40) demonstrated that a homogenate of a benign interstitial cell tumor could 11β -hydroxylate both androstenedione and deoxycorticosterone.

Although we have suggested that the 3β -hydroxysteroid dehydrogenase activity of the tumor was limited, it should be pointed out that the evidence for any 3β -hydroxysteroid dehydrogenase activity of the tumor is limited. It is known that the liver also possesses this enzyme system so that progesterone or 17-hydroxypregesterone could have been synthesized there from their respective Δ^5 - 3β -hydroxy precursors. The finding that tends to refute this possibility is the synthesis of 11β -hydroxyandrostenedione, since if androstenedione were synthesized only in the liver, 11β -hydroxylation would not be expected.

The resemblance of this tumor to adrenocortical carcinoma in its functional aspect is striking. The high production rate of DHA sulfate and the excretion of greatly increased amounts of DHA and other Δ^5 steroids are in the usual pattern of functional adrenal carcinoma (37). Many adrenal carcinomas likewise demonstrate a decreased activity of 3β -hydroxysteroid dehydrogenase and, in some, this enzyme appears to be absent. The occurrence of 11β -hydroxylation of androstenedione and the simultaneous lack of 11β -hydroxylation of C-21 pregnane derivatives have also been described in adrenocortical carcinoma (37). The interstitial cell carcinoma synthesized small amounts, if any, of testosterone, the important secretory product of the interstitial cell; similarly, some adrenal cancers that synthesize several corticoids fail to synthesize the characteristic adrenal cortical secretory product, cortisol.

A singular point of difference between the two cancers is the development of an 11β -hydroxylating system in the interstitial cell carcinoma. As far as we are aware, 11β -hydroxylation has not been shown to occur in any normal mammalian testis. Thus, this interstitial cell cancer is an exception to the generalization that the development of neoplasia is not accompanied by the appearance of new enzymes. Rather, the activity of normal enzyme systems may be greatly reduced or disappear, as so often happens to 11β -hydroxylation in adrenocortical carcinoma.

These comparisons rest on the unequivocal identification of the tumor in this case as an interstitial

cell carcinoma. This question will be considered in detail by Horner and Evans (41), but it should be stated that there was no disagreement among experienced pathologists regarding the origin of the cancer. One could, however, make a convincing argument that biochemical and morphologic criteria have equal validity.

Summary

Steroid studies in a patient with metastatic interstitial cell carcinoma have been described. The patient excreted large amounts of 17-ketosteroids, comprising both the 11-deoxy- and the 11-oxy-steroids. The tumor responded to human chorionic gonadotropin but was not suppressed by exogenous androgen.

The plasma testosterone level was four times normal, and it was demonstrated that plasma dehydroepiandrosterone sulfate was the source of the plasma testosterone. The dehydroepiandrosterone sulfate production rate was 2,270 mg per day. Evidence was presented that the tumor secreted testosterone sulfate as well.

The excretion of metabolites of the biosynthetic precursors of testosterone demonstrated that there was a low activity of tumor 3β -hydroxysteroid dehydrogenase. The steroid synthetic activity of this cancer resembled closely that of adrenocortical cancer.

Appendix

Case report

J.A.T. (NIH, 05-84-62) was a 67-year-old white man who had a left orchietomy for a testicular mass in 1963. In June 1964 an abdominal mass was noted; this was biopsied 3 months later, and he was referred from the Savannah U. S. Public Health Service Hospital to the Endocrinology Branch, National Cancer Institute. On admission, the only significant physical finding was a 15- \times 15-cm lower abdominal mass.

The routine laboratory studies including hematology, liver function tests, protein-bound iodine, and total gonadotropin excretion were within normal limits. The biopsy specimen of the tumor and the material obtained at autopsies were reported as interstitial cell carcinoma by pathologists at the National Institutes of Health and the Armed Forces Institute of Pathology. The clinical and pathological aspects of this case will be reported by Horner and Evans (41).

References

1. Masson, P. Deux cancers Leydigiens de l'homme. *Rev. canad. Biol.* 1943, 2, 168.
2. Gharpure, V. V. A case of malignant interstitial-cell tumour of the testis in man. *J. Path. Bact.* 1950, 62, 113.
3. Ohlsen, A. S., and G. Rønn. Leydig-cell carcinoma. With report of a case. *Acta chir. scand.* 1957, 112, 411.
4. Largiader, F. Maligner Tumor der Leydigischen Zwischenzellen des Hodens. *Frankfurt. Z. Path.* 1960, 70, 630.
5. Ward, J. A., S. Krantz, J. Mendeloff, and E. Haltiwanger. Interstitial-cell tumor of the testis: report of two cases. *J. clin. Endocr.* 1960, 20, 1622.
6. Short, M., and J. I. Coe. Malignant interstitial cell tumor of the testis: a case report. *J. Urol. (Baltimore)* 1963, 89, 851.
7. Abelson, D., H. Bulaschenko, P. R. Trommer, and A. Valdes-Dapena. Malignant interstitial-cell tumor of the testis treated with o,p'-DDD. *Metabolism* 1966, 15, 242.
8. Peterson, R. E., and C. E. Pierce. Determination of urinary neutral 17-ketosteroids in Lipids and the Steroid Hormones in Clinical Medicine, F. W. Sunderman and F. W. Sunderman, Jr., Eds. Philadelphia, Lippincott, 1960.
9. Wilson, H., and M. B. Lipsett. Use of periodate oxidation in the clinical analysis of urine corticoids. *Analyt. Biochem.* 1963, 5, 217.
10. Peterson, R. E., A. Karrer, and S. L. Guerra. Evaluation of Silber-Porter procedure for determination of plasma hydrocortisone. *Analyt. Chem.* 1957, 29, 144.
11. Kirschner, M. A., M. B. Lipsett, and D. R. Collins. Plasma ketosteroids and testosterone in man: a study of the pituitary-testicular axis. *J. clin. Invest.* 1965, 44, 657.
12. Kirschner, M. A., and M. B. Lipsett. Gas-liquid chromatography in the quantitative analysis of urinary 11-deoxy-17-ketosteroids. *J. clin. Endocr.* 1963, 23, 255.
13. Sarfaty, G. A., and M. B. Lipsett. Separation of free and conjugated 11-deoxy-17-ketosteroids by thin-layer chromatography. *Analyt. Biochem.* 1966, 15, 184.
14. Edwards, R. W. H., A. E. Kellie, and A. P. Wade. The extraction and conjugation of urinary steroid conjugates. *Mem. Soc. Endocr.* 1953, 2, 53.
15. Burstein, S., and S. Lieberman. Hydrolysis of ketosteroid hydrogen sulfates by solvolysis procedures. *J. biol. Chem.* 1958, 233, 331.
16. Korenman, S. G., H. Wilson, and M. B. Lipsett. Testosterone production rates in normal adults. *J. clin. Invest.* 1963, 42, 1753.
17. Wilson, H., M. B. Lipsett, and S. G. Korenman. Evidence that 16-androsten-3 α -ol is not a peripheral metabolite of testosterone in man. *J. clin. Endocr.* 1963, 23, 491.

18. Engel, L. L. The chemical estimation of steroid hormone metabolites. *Recent Progr. Hormone Res.* 1950, **5**, 335.
19. Wilson, H., and M. B. Lipsett. Metabolism of epitestosterone in man. *J. clin. Endocr.* 1966, **26**, 902.
20. Tait, J. F. Review: the use of isotopic steroids for the measurement of production rates *in vivo*. *J. clin. Endocr.* 1963, **23**, 1285.
21. Horton, R., J. Shinsako, and P. H. Forsham. Testosterone production and metabolic clearance rates with volumes of distribution in normal adult men and women. *Acta endocr. (Kbh.)* 1965, **48**, 446.
22. Tait, J. F., and R. Horton. Some theoretical considerations on the significance of the discrepancy in urinary and blood production rate estimates of steroid hormones particularly in those of testosterone in young women. *Steroids* 1964, **4**, 365.
23. Korenman, S. G., and M. B. Lipsett. Is testosterone glucuronoside uniquely derived from plasma testosterone? *J. clin. Invest.* 1964, **43**, 2125.
24. Camacho, A. M., and C. J. Migeon. Studies on the origin of testosterone in the urine of normal adult subjects and patients with various endocrine disorders. *J. clin. Invest.* 1964, **43**, 1083.
25. Calvin, H. I., R. L. Vande Wiele, and S. Lieberman. Evidence that steroid sulfates serve as biosynthetic intermediates: *in vivo* conversion of pregnenolone-sulfate-S³⁵ to dehydroisoandrosterone sulfate-S³⁵. *Biochemistry* 1963, **2**, 648.
26. Dixon, W. R., J. G. Phillips, and N. Kase. *In vitro* production of testosterone sulfate from progesterone-4-¹⁴C in an adrenal tumor. *Steroids* 1965, **6**, 81.
27. Siiteri, P. K., and P. C. MacDonald. The utilization of circulating dehydroisoandrosterone sulfate for estrogen synthesis during human pregnancy. *Steroids* 1963, **2**, 713.
28. Gursipide, E., P. C. MacDonald, R. L. Vande Wiele, and S. Lieberman. Measurement of the rates of secretion and of peripheral metabolism of two interconvertible compounds: dehydroisoandrosterone-dehydroisoandrosterone sulfate. *J. clin. Endocr.* 1963, **23**, 346.
29. Korenman, S. G., H. Wilson, and M. B. Lipsett. Isolation of 17 α -hydroxyandrost-4-en-3-one (epitestosterone) from human urine. *J. biol. Chem.* 1964, **239**, 1004.
30. Wilson, H., M. B. Lipsett, and D. W. Ryan. Urinary excretion of Δ^5 -pregnenetriol and other 3 β -hydroxy- Δ^5 steroids by subjects with and without endocrine disease. *J. clin. Endocr.* 1961 **21**, 1304.
31. Okada, M., D. K. Fukushima, and T. F. Gallagher. Isolation and characterization of 3 β -hydroxy- Δ^5 -steroids in adrenal carcinoma. *J. biol. Chem.* 1959, **234**, 1688.
32. Roberts, K. D., L. Bandi, H. I. Calvin, W. D. Drucker, and S. Lieberman. Evidence that steroid sulfates serve as biosynthetic intermediates. IV. Conversion of cholesterol sulfate *in vivo* to urinary C₁₉ and C₂₁ steroidal sulfates. *Biochemistry* 1964, **3**, 1983.
33. Savard, K., R. I. Dorfman, B. Baggett, L. L. Fielding, L. L. Engel, H. T. McPherson, L. M. Lister, D. S. Johnson, E. C. Hamblen, and F. L. Engel. Clinical, morphological, and biochemical studies of a virilizing tumor in the testis. *J. clin. Invest.* 1960, **39**, 534.
34. Lipsett, M. B., R. Hertz, and G. T. Ross. Clinical and pathophysiologic aspects of adrenocortical carcinoma. *Amer. J. Med.* 1963, **35**, 374.
35. Horton, R., and J. F. Tait. Androstenedione production and interconversion rates measured in peripheral blood and studies on the possible site of its conversion to testosterone. *J. clin. Invest.* 1966, **45**, 301.
36. Korenman, S. G., and M. B. Lipsett. Direct peripheral conversion of dehydroepiandrosterone to testosterone glucuronoside. *Steroids* 1965, **5**, 509.
37. Lipsett, M. B., and H. Wilson. Adrenocortical cancer: steroid biosynthesis and metabolism evaluated by urinary metabolites. *J. clin. Endocr.* 1962, **22**, 906.
38. Bradlow, H. L., and T. F. Gallagher. Metabolism of 11 β -hydroxy- Δ^4 -androstene-3,17-dione in man. *J. biol. Chem.* 1957, **229**, 505.
39. Camin, A. J., R. I. Dorfman, J. H. McDonald, and I. M. Rosenthal. Interstitial cell tumor of the testis in a seven-year-old child. *Amer. J. Dis. Child.* 1960, **100**, 389.
40. Smith, E. R., H. Breuer, and H. Schriefers. A study of the steroid metabolism of an interstitial-cell tumor of the testis. *Biochem. J.* 1964, **93**, 583.
41. Horner, C. E., and G. Evans. Personal communication.