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Research Article

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The Conversion of Testosterone to

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(Dihydrotestosterone) by Skin Slices of Man

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A B S T R A C T The conversion of testosterone-1,2-^{3}H to dihydrotestosterone by slices of human skin obtained from various anatomical sites in 112 normal subjects and three individuals with the syndrome of testicular feminization has been measured under standardized conditions. Very low rates of dihydrotestosterone formation were observed in sites obtained from the mons or from miscellaneous areas of the trunk and limbs of the control subjects. The mean rates of conversion were very high, however, in slices of skin obtained from several perineal sites (labia majora, scrotum, prepuce, and clitoris). Furthermore, as measured here, the rate of dihydrotestosterone formation by prepuce rises during the 3 months after birth and then falls progressively thereafter, reaching a level in the adult that is almost as low as that observed in the slices of nonperineal skin from all ages. In the patients with testicular feminization dihydrotestosterone formation by slices of skin obtained from the mons was within the normal range, whereas the rates observed in labia majora were lower than the average values obtained in the normal subjects.

INTRODUCTION

Considerable evidence has recently accrued to indicate that testosterone and androstenedione are converted in some target tissues to 5α-androstan-17β-ol-3-one (dihydrotestosterone),[1] a metabolite which in certain bioassay systems appears to be a more potent androgen than

[1] The following trivial names are used: testosterone (Δ⁴-androsten-17β-ol-3-one); androstenedione (Δ⁴-androsten-3,-17-dione); dihydrotestosterone (5α-androstan-17β-ol-3-one); androsterone (5α-androstan-3α-ol-17-one); androstandiol (5α-androstan-3α,17β-diol); epiandrostenone (5α-androstan-3β-ol-17-one).

testosterone itself (1, 2). This metabolite, which was originally observed in rat prostate by Pearlman and Pearlman (3), has been found in a variety of tissues (4–9), including the prostate (7, 8) and skin of man (9). And, although an enzyme that performs this reduction is located in the microsomal fraction of liver (10), it has recently been reported from this laboratory that after the intravenous administration of testosterone-1,2-^{3}H, appreciable amounts of dihydrotestosterone were recovered only in certain organs of accessory reproduction in the male rat, and that the only detectable testosterone metabolite recovered within the prostatic nuclei, a presumed site of action of this hormone, was dihydrotestosterone (11). These findings have recently been confirmed by Anderson and Liao (12). Furthermore, we have also demonstrated that prostatic nuclei contain an enzyme that performs this conversion (11), and that dihydrotestosterone rather than testosterone is the predominant species bound to the nuclear chromatin within 15 min after testosterone administration (13). Taken together, these findings suggest that dihydrotestosterone may be an active form of the hormone in some target tissues.

The present study was undertaken to determine whether regional variations also exist among various types of human skin in their ability to convert testosterone to dihydrotestosterone. With this possibility in mind the optimal conditions for assaying this reduction in skin slices have been established, and the conversion of testosterone to dihydrotestosterone has been measured in skin obtained from various anatomical sites in 112 normal individuals of both sexes and varying ages and in three patients with the syndrome of testicular feminization.

METHODS

Incubation procedure. The skin samples used in this study were obtained from a variety of sources: prepuce removed

at circumcision, legs from patients undergoing amputation, perineum from patients undergoing vulvectomy, skin resections at the time of mastectomy, skin revisions of several types, clitoris from a clitorectomy in an 8 yr old girl with the adrenogenital syndrome, and penis from a 36 yr old man who underwent resection because of a carcinoma of the glans penis. In a number of instances small pieces of skin were taken from the edges of surgical incisions in patients undergoing different procedures. Three patients with the syndrome of testicular feminization agreed to biopsy of the labia majora and of the mons at the time of abdominal exploration for the purpose of removal of testis. The endocrinological data for these three unrelated individuals, ages 17, 44, and 56, who all had XY karyotypes, affected siblings with the same disorder, and typical clinical findings for the diagnosis of testicular feminization, will be reported separately. No attempt has been made in this study to evaluate the effects of the type of anesthesia, the various skin sterilization procedures, the underlying clinical state of the control patients, or race on testosterone metabolism. Only skin that appeared grossly normal was utilized for study.

The skin samples were immediately placed in chilled saline and taken to the laboratory in an ice bucket. Under a magnifying glass any underlying fat was cut away, and slices of skin, approximately 0.5 mm in thickness and containing both dermis and epidermis, were prepared by hand. The slices were blotted, weighed, and added to tubes containing the incubation mixture. In two studies of prepuce the mucinous connective tissue which connects the two skin layers was dissected and sliced, and dermis and epidermis were separated by the method of Van Scott (14); full-thickness slices, epidermal scrapings, and slices of dermis and connective tissue were incubated separately.

The standard incubation mixture consisted of testosterone-1,2-^{3}H (9.5 $\times$ 10^{-7} mole/liter containing 5 $\times$ 10^{-7} cpm), potassium penicillin (2500 U), glucose (1.1 $\times$ 10^{-2} mole/liter), and Krebs-Ringer phosphate buffer, pH 7.4, in a total volume of 2.5 ml. The tubes were gassed with 95% O$_2$: 5% CO$_2$, capped, and incubated at 37°C with shaking for varying periods of time. In most instances less than 20 min elapsed between the time of surgical excision and the beginning of the incubation. The testosterone-1,2-^{3}H (New England Nuclear Corp., Boston, Mass., 5 mc/0.034 mg) was brought into aqueous solution each day by adding one drop of a 1:50 dilution of Tween 40 (Atlas Chemical Industries, Inc., New York) to the benzene:ethanol solution in which the hormone was stored, evaporating to dryness, and reconstituting the residue in buffer. At least 91% of the radioactivity in this preparation had the same R_f as testosterone when tested either by thin-layer or by gas-liquid chromatography; the remainder of the radioactivity was spread almost uniformly through the chromatograms. In several batches 3–5% of the radioactivity exhibited the same chromatographic mobility as androstenedione.

In the initial experiments the reaction was stopped at the end of the incubation period by the addition of 0.6 ml of 10 N KOH. The contents were allowed to digest at room temperature for 2 hr; 35 ml of chloroform:methanol (2:1) and 4 ml of 1 N HCl were then added, and the lipids were extracted and backwashed to neutrality as described by Folch, Lees, and Sloane Stanley (15). The alkalinization step in this procedure had no effect on the total recovery of radioactivity or on the percentage of radioactivity in the dihydrotestosterone area. Consequently, the alkalinization procedure was omitted in the remaining experiments, and the reaction mixture was extracted directly with chloroform: methanol. The chloroform:methanol extract was taken to

dryness on a Rinco Evaporator (Rinco Instruments Co., Inc., Greenville, Ill.), and the residue was transferred with chloroform to vials. Zero time controls containing tissue were run with each batch of incubations.

Chromatographic procedures. Aliquots of the radioactivity (containing 10,000–20,000 cpm of ^{3}H) were chromatographed, along with a mixture containing 20 μg each of testosterone, dihydrotestosterone, androsterone, androstandiol, epiandrosterone, androstenedione, and androstandione by a thin-layer procedure and in some experiments by gas-liquid chromatography. For thin-layer chromatography, glass plates 20 $\times$ 20 cm were coated with a suspension of silica gel H (Brinkmann Instruments Inc., Westbury, N. Y.) in distilled water (30 g/75 ml) and activated in an oven at 110°C for 1 hr. After the tissue extracts and standard steroid mixtures were spotted, the plates were developed by ascending chromatography in the 98.25% chloroform:1.75% methanol solution of Gomez and Hsia (9). After chromatography, the plates were dried and sprayed with anisaldehyde, and the color was allowed to develop at 100°C for 20 min. We then divided the plate into 14–15 horizontal bands, taking into account the position of the standards. These bands were scraped individually and transferred to counting vials containing 10 ml. of 0.4% diphenyloxazole in toluene and 1 ml of methanol and assayed for radioactivity in a liquid scintillation spectrometer.

Gas-liquid chromatography of steroids was performed on a Research Specialties Co. apparatus (Richmond, Calif.) equipped with an ionization detector. The column (6 feet $\times$ ¼ inch) was packed with 3% QF$_1$ on Gas Chrom Q (100–120 mesh) (Applied Science Laboratories, Inc., State College, Pa.). The carrier gas was argon, and flow rate was 100 ml/min. Column temperature was 220°C. In each instance carrier steroid mixtures were added to the radioactive samples. For radioactivity determination the effluent was collected directly into the scintillation mixture. The recovery of radioactivity was 85–93% for the thin-layer plates and the gas-liquid method.

For estimation of the amount of end product formed in these experiments, the per cent of the total recovered radioactivity that was present in the metabolite in question was multiplied by the amount of testosterone originally added to the flask.

Recrystallization procedure. The identity of dihydrotestosterone-^{3}H and androstandiol-^{3}H as conversion products was further confirmed in one experiment by adding appropriate carriers after they were isolated by thin-layer chromatography and recrystallizing these materials repeatedly as previously described (11).

RESULTS

The chromatographic procedures that were used for the identification of the end products of the tissue incubations are illustrated in Fig. 1. In this experiment chloroform:methanol extracts obtained after the incubation of slices of prepuce with testosterone-1,2-^{3}H at 37°C for 1 hr were chromatographed both by thin-layer chromatography and by gas-liquid chromatography. In each instance radioactivity was recovered in areas corresponding to androstandione, androstenedione, dihydrotestosterone, testosterone, and androstandiol. Although dihydrotestosterone is not separated from androsterone by the thin-layer system, the purity of this peak was checked in 18 instances by gas-liquid chromatography, and an

TABLE I

Confirmation by Crystallization of the Identity of Dihydro-testosterone-³H and Androstandiol-³H Isolated by Thin-Layer Chromatography from Slices of Skin Incubated With Testosterone-1,2-³H

| Crystal-lization	Solvent	Dihydro-testosterone-³H specific activity	Andro-standiol-³H specific activity
		cpm/mg	cpm/mg
1	Methanol:water	18,400	6500
2	Acetone:water	18,360	5980
3	Benzene:heptane	18,380	6260
4	Ethyl acetate: cyclohexane	17,830	5780
5	Ethyl ether: hexane	17,500	5430

Material tentatively identified by thin-layer chromatography and gas-liquid chromatography as either androstandiol-³H or dihydrotestosterone-³H was added to 100 mg of the appropriate carrier steriod for recrystallization.

average of 84% of the radioactivity (76–95%) was recovered in the dihydrotestosterone area. The finding of a rather small amount of radioactivity with a mobility

similar to androsterone, which must arise from andros-tandiol or androstandione, is different from the observations of Gomez and Hsia (9); the conditions and the duration of the incubation in that study were so different that the two studies are not strictly comparable. The identity of the two principal testosterone metabolites was further confirmed by the recrystallization experiment shown in Table I. In five successive recrystallizations of materials isolated from thin-layer chromatography and added to appropriate carrier steroids, the specific activity of dihydrotestosterone-³H changed only about 5% and that of androstandiol only 10%, findings that are in keeping with the chromatographic identification of these steroids. In addition, as noted in Fig. 1, the percentage of recovered radioactivity in the two chromatographic procedures was similar (15.7 and 15.4% for dihydrotestosterone and 5.2 and 7.0% for androstandiol). It was concluded, therefore, that the thin-layer chromatographic separation of dihydrotestosterone provides an adequate means for the routine assay of this compound under these experimental conditions, and this procedure was used in the subsequent studies.

The time course of the appearance of the various metabolites during the incubation of slices of prepuce with testosterone-1,2-³H is illustrated in Fig. 2. The testoster-

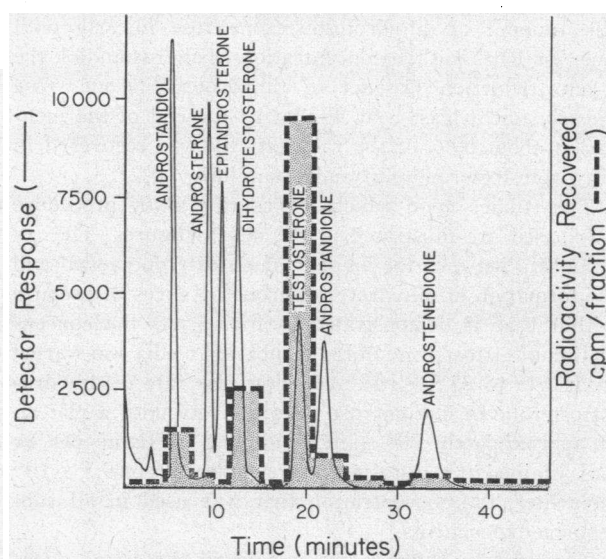## THIN-LAYER CHROMATOGRAM
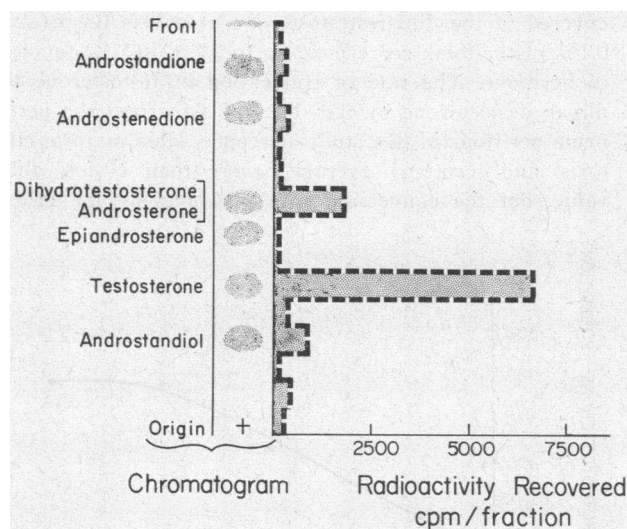
GAS-LIQUID CHROMATOGRAM

FIGURE 1 Comparison by gas-liquid and thin-layer chromatography of the metabolites of testosterone-1,2-³H after incubation with slices of prepuce. Slices of prepuce obtained from a newborn infant (100 mg) were incubated with testosterone-1,2-³H (9.5 × 10⁻⁷ mole/liter), glucose (1.1 × 10⁻³ mole/liter), penicillin (2500 U), and Krebs-Ringer phosphate buffer, pH 7.4, in a total volume of 2.5 ml. After incubation for 1 hr at 37°C, the mixture was saponified, and the steroids were extracted into chloroform: methanol and backwashed as described in the text. Aliquots of the chloroform: methanol extracts and appropriate mixtures of steroid standards were chromatographed either by thin-layer chromatography or by gas-liquid chromatography, and the thin-layer scrapings and gas eluates were then assayed for radioactivity.
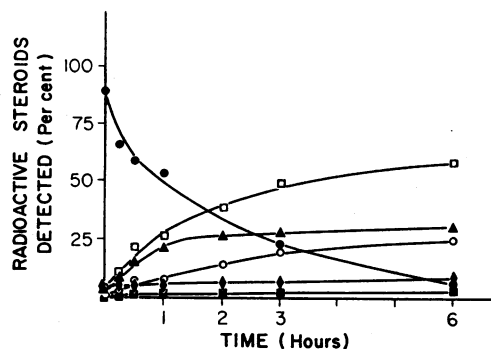
FIGURE 2 Time course of the formation of various metabolites of testosterone-1,2-³H after incubation with slices of prepuce. Slices of prepuce obtained from three newborn infants were pooled. Aliquots of the slices (100 mg) were incubated as described in Fig. 1 for varying periods of time, after which the steroids were separated and analyzed for radioactivity as described in the text. (●——●, testosterone; ▲——▲, dihydrotestosterone; O——O, androstandiol; ◆——◆, androstenedione; ■——■, androstandione; □——□, dihydrotestosterone plus androstandiol).

one-³H preparation contained a small amount of radioactivity with a mobility similar to androstenedione (5%), which rose only slightly during the 6 hr incubation, as did the trace quantities of radioactivity with the mobility of androstandione. The testosterone concentration fell progressively with time so that only about 10% of the radioactivity was recovered unchanged after 6 hr. The amount of dihydrotestosterone rose linearly with time for 1 hr, and the concentration of androstandiol, the 3-keto reduction product of dihydrotestosterone, rose linearly for at least 3 hr so that by the end of the incubation about 60% of the radioactivity was recovered in dihydrotestosterone plus androstandiol.

The studies upon which the routine assay procedure was based are illustrated in the next 3 figures. The relationship between the amount of testosterone added and the formation of dihydrotestosterone by slices of prepuce within 1 hr is demonstrated in Fig. 3. As the concentration of testosterone in the incubation media was varied from 2.45 to 23.5 × 10⁻⁷ mole/liter, the rate of dihydrotestosterone formation rose progressively until a plateau of approximately 700 μμmole/100 mg of tissue per hr was attained at a testosterone concentration of 9.5 × 10⁻⁷ mole/liter, the concentration that was used in all subsequent experiments.

The relation between the weight of the tissue slices and the rate of conversion of testosterone to dihydrotestosterone is illustrated in Fig. 4. In this experiment the rate of formation of dihydrotestosterone was linear between 25 and 150 mg of prepuce slices and plateaued thereafter. In most instances the actual tissue weights in the subsequent studies varied from 93 to 109 mg; in seven cases the tissue weights were smaller (49–72 mg),
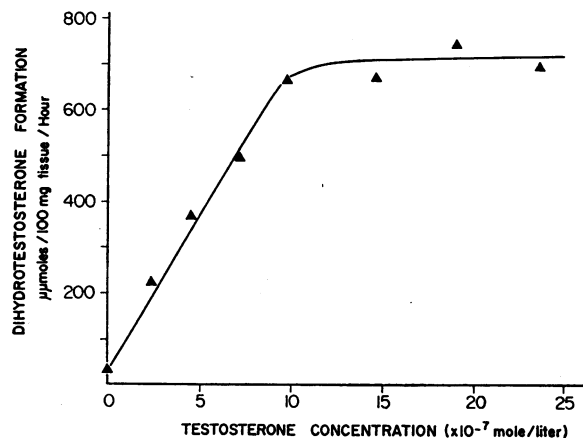

FIGURE 3 Relation between dihydrotestosterone formation and the concentration of testosterone-1,2-³H in the incubation mixture. Slices of prepuce from two infants (30 and 37 days of age) were pooled.

but since all these weights fell on the linear portion of the curve, all the results were normalized to 100 mg of tissue weight.

The time course of dihydrotestosterone-³H formation by various types of slices is shown in Fig. 5. The rate of dihydrotestosterone formation in the slices of prepuce was much more rapid than was observed in the mons preparation; in the latter case, however, the conversion was linear for 6 hr, whereas in the former the reaction rate was linear only for the first few hours. In the 23 zero time tissue controls the amount of radioactivity recovered in the dihydrotestosterone area was 0.9 ±(SE) 0.1% of the total, corresponding to 22 ±(SE) 2 μμmoles of hormone. The rate of conversion of testosterone to dihydrotestosterone by the slices of skin from the perineum per hour in this study (prepuce, labia majora, clitoris, and scrotum) averaged more than 10-fold this value, but the conversion rate per hour by the other
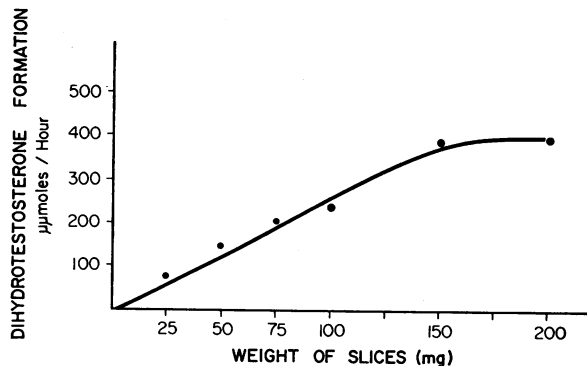

FIGURE 4 Relation between the amount of tissue and the formation of dihydrotestosterone-³H by slices of prepuce. Varying amounts of prepuce obtained from a 6 yr old boy were incubated and analyzed as described in Fig. 1.
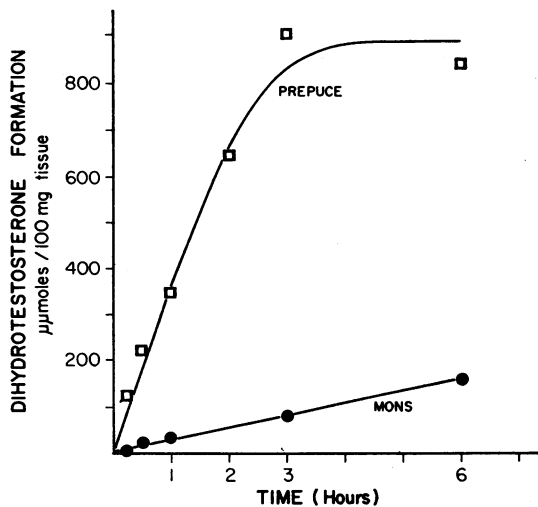
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FIGURE 5 Time course of dihydrotestosterone-[3] formation by slices of prepuce and mons. Tissue slices (100 mg) obtained from different sites in different patients were incubated for varying periods of time as described in Fig. 1. The radioactive steroids were then separated and analyzed for radioactivity as described in the text.

types of skin (obtained from mons and miscellaneous sites) was on an average only two to three times as great as this zero time control. Therefore, when these latter tissues were assayed, the incubations were carried out for 3 hr so that the actual measured value ranged from 7 to 14% in the case of the mons and from 3 to 9% in the case of the miscellaneous samples. In the studies of the prepuce, labia majora, clitoris, and scrotum the incubations were stopped at the end of 1 hr. In all cases, however, the results have been expressed as micromicromoles of dihydrotestosterone formed per 100 mg of tissue per hour.

The results of 112 measurements utilizing this standard incubation procedure are illustrated in Fig. 6. In the 10 skin samples obtained from the mons the rate of dihydrotestosterone formation observed was $72 \pm$ (SE) 6 $\mu\mu$moles/hr. In the specimens of skin labeled miscellaneous sites (thigh, breast, back, inguinal area, sole of the foot, leg, upper abdominal wall, and chest wall), a similar value was obtained ($46 \pm$ (SE) 5 $\mu\mu$moles/100 mg tissue per hr). The values observed in the remaining tissues were strikingly different. The prepuce, which exhibited a wide range of values (from 51 to 884
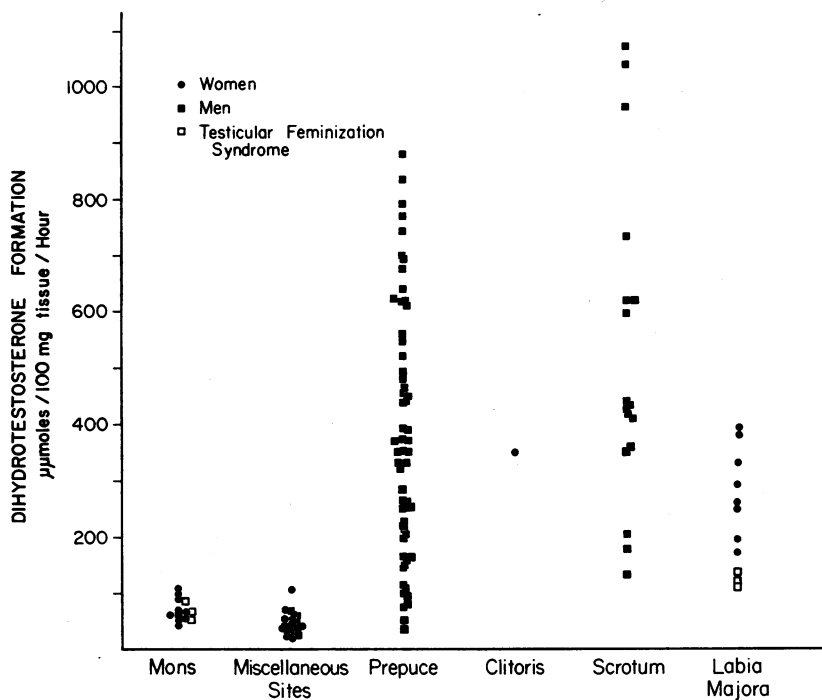

FIGURE 6 Comparison of the rate of formation of dihydrotestosterone-^{3}H by slices of skin obtained from various anatomical sites in normal men and women and in patients with the syndrome of testicular feminization. The incubation conditions are described in Fig. 1. The incubations were carried out either for 3 hr (mons and miscellaneous sites) or for 1 hr (prepuce, scrotum, clitoris, and labia majora), and the radioactive end products were then separated and analyzed as described in the text.

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$\mu\mu$moles/100 mg per hr), had a mean rate of formation of 382 ±(SE) 29 $\mu\mu$moles/100 mg per hr. The single specimen of clitoris fell within this range (350 $\mu\mu$moles). The 17 scrotal samples also showed a wide variation (131–1074 with a mean of 534 ±(SE) 69 $\mu\mu$moles/100 mg per hr). The labia majora, although synthesizing dihydrotestosterone at somewhat lower rates on an average than the scrotum, exhibited values which fell within its range (283 ±(SE) 28 $\mu\mu$moles/100 mg per hr). Thus, on an average the rates of conversion of testosterone to dihydrotestosterone were considerably higher in the 4 perineal skins from both sexes than was observed in the samples from mons and from the miscellaneous sites.

The results obtained from the individuals with the syndrome of testicular feminization are also illustrated in Fig. 6. The rates of formation of dihydrotestosterone in the three samples of skin obtained from the mons, 90, 42, and 52 $\mu\mu$moles/100 mg of tissue per hr, were clearly within the normal range. However, the rates observed in the skin from the labia majora (134, 101, and 111 $\mu\mu$moles/100 mg of tissue per hr) were somewhat lower than the average values for labia majora of normal women and considerably lower than the mean rates observed in the samples of scrotum.

Nevertheless, the range of variation in the prepuce, scrotum, and mons was so great that individual samples fell within or near the normal range, and consequently the data in Fig. 6 was analyzed in relation to the age of the patient. In the case of mons (ages 19–65), miscellaneous sites (ages 1–62), scrotum (ages 9–85), and labia majora (ages 21–56) there was a uniform scatter of the data with age. With prepuce, however, a very interesting relationship was observed between the rate of dihydrotestosterone formation and the age of the patient (Fig. 7). In the newborn (birth up to 5 days), the rate of conversion averaged 413 ±(SE) 26 $\mu\mu$moles/100 mg per hr. In the samples obtained from infants between the ages of 10 days and 3 months the mean value rose
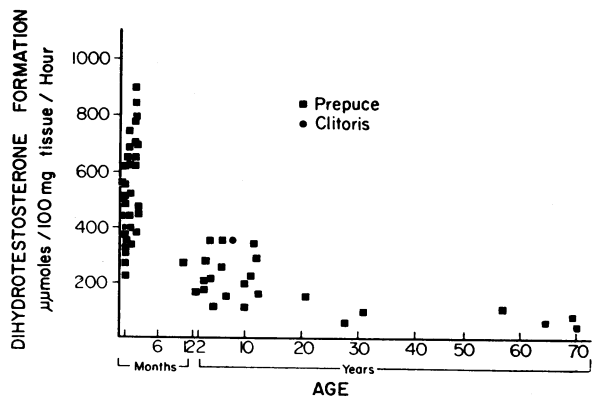

FIGURE 7 Relation between patient age and dihydrotestosterone formation by slices of prepuce and clitoris.

TABLE II
Conversion of Testosterone-1,2-³H to Dihydrotestosterone-³H by Various Layers of Prepuce

Skin preparation	Dihydrotestosterone formation	
	Newborn	12 yr
	$\mu\mu$moles/100 mg tissue per hr	
Full thickness	619	199
"Epidermis"	154	116
"Dermis"	175	117
Underlying connective tissue	847	190

Prepuce from two newborn infants was combined in one experiment, and that from a 12 yr old boy was used in the second. After full-thickness slices were made from each, the underlying mucinous connective tissue was dissected away, the remaining tissue was stretched and pinned on a corkboard, and the epidermis was scraped as described by Van Scott (14). The scrapings were combined and weighed, and the dermis and connective tissue were then sliced. The incubation conditions are described in Fig. 1.

slightly to 636 ±(SE) 36 $\mu\mu$moles/100 mg per hr. In the next age group (3–12 yr) this value fell to a mean of 219 ±(SE) 19 $\mu\mu$moles/100 mg per hr, and in the specimens from older patients the conversion rate was only 90 ±(SE) 13 $\mu\mu$moles/100 mg per hr, a value that was not significantly different than was observed for mons. A variety of uncontrolled variables might have influenced these results. Before 3 months of age the circumcisions were performed without anesthesia, whereas above this age the operations were performed under general anesthesia. The sampling also suffers from the small number of tissue samples available in the older age group, and there is a gap between 3 months and 2 yr of age. In addition, the prepuce from the older age groups contained much thicker connective tissue undercoats which were difficult to dissect away.

In order to characterize the nature of this apparent age relation, in two experiments the rates of dihydrotestosterone formation in full thickness slices of prepuce were compared with that of mechanically separated dermis, epidermis, and connective tissue (Table II). Dihydrotestosterone formation was considerably more active in the full-thickness preparation from the newborn than from the older sample. There was little difference, however, in the activity observed in the preparation of epidermis and dermis. Indeed, the most striking differences observed were in the connective tissue layer in which a 4-fold variation was found (847 in the newborn vs. 190 $\mu\mu$moles/100 mg per hr in the sample from the 12 yr old boy). Whether such differences are due to variations in the water content, to a change in the cellular composition of the tissue, or to an actual decrease in the en-

zyme responsible cannot be determined from this study. With all these limitations, however, it is clear that striking differences do exist between the rates of this conversion in infants and in older individuals as measured in this assay.

In one instance the conversion of testosterone to dihydrotestosterone was studied within the various areas of a penis removed because of a squamous cell carcinoma of the glans. The rates of dihydrotestosterone formation varied from 61 $\mu\mu$moles/100 mg per hr in the corpus spongiosum to 293 $\mu\mu$moles/100 mg per hr in the glans penis (see Table III). This patient had previously been circumcised, but the value observed for skin (91 $\mu\mu$moles) fell within the range which would have been predicted from the data shown in Fig. 6 for foreskin for a man of this age. And, although this is the only sample available for this type of analysis, it is clear that dihydrotestosterone formation in the penis occurs in areas other than the skin itself, notably the fibrous tunica albuginea.

Because the skin is a very heterogenous tissue, it cannot be ascertained from the study of full-thickness slices whether the ability to form dihydrotestosterone is a property of the skin itself or whether instead this reduction takes place in skin organelles which are under the control of androgenic hormones such as sebaceous glands or hair follicles. In an attempt to clarify this issue the rate of dihydrotestosterone formation was compared in skin samples obtained from the side of the leg and the sole of the foot from two amputated legs (Table IV). Since the sole of the foot contains neither hair follicles nor sebaceous glands such a comparison provides a means of differentiating between reactions which take place in these organelles and that occurring in the skin itself.

TABLE III

Dihydrotestosterone Formation in Various Areas of the Penis

Site	Dihydro-testosterone formation
	$\mu\mu$moles/100 mg tissue per hr
Corpus spongiosum	61
Corpus cavernosum	131
Tunica albuginea	175
Skin	91
Glans penis	293
Squamous cell carcinoma of the glans penis	84

The penis of a 36 yr old man who underwent a partial penectomy because of a squamous cell carcinoma of the glans penis was dissected and sliced, and 100-mg aliquot portions of the slices were incubated and analyzed as described in Fig. 1.

TABLE IV

Comparison of the Rate of Conversion of Testosterone-1,2-³H to Dihydrotestosterone-³H by Skin Slices Obtained from the Sole of the Foot and the Side of the Leg in the Same Individual

Patient	Diagnosis	Age Sex	Type of skin	Testosterone-1,2-³H conversion to dihydro-testosterone
				$\mu\mu$moles/100 mg skin per hr
E. L.	Malignant melanoma	55 F	Sole	31
			Leg	26
W. D.	Osteosarcoma	13 F	Sole	24
			Leg	24

Skin biopsies obtained from legs immediately after amputation were sliced by hand. The incubation conditions are described in Fig. 1. After incubation for 3 hr at 37°C, the mixture was saponified, and the steroids were separated and assayed as described in the text.

Although, as expected, the rates of conversion of testosterone to dihydrotestosterone in these samples were low, there was no difference between the two sites in either case, a finding suggesting that in the nonperineal skins at least this conversion is not limited to the sebaceous glands and hair follicles.

DISCUSSION

The results of the present study confirm the report by Gomez and Hsia that testosterone is converted to dihydrotestosterone by human skin (9). Furthermore, the rate of this conversion was considerably higher in four types of skin obtained from the perineum (labia majora, prepuce, scrotum, and clitoris) than in skin obtained from other anatomical sites. In view of the fact that dihydrotestosterone is a potent androgen in man (16) as well as in the rat (1, 2), and in view of the fact that dihydrotestosterone rather than testosterone itself is a predominant form of the hormone bound to the nuclear chromatin of rat prostate, a presumed site of action of the hormone (13), the possibility must be considered that the ability to form this metabolite may be related in some way to the capacity of some tissues to develop an androgenic response to testosterone.

This, of course, does not imply that the 5α reduction is necessarily an obligatory step in every effect of testosterone. It is clear that the skin from the mons did not differ significantly from skin obtained from other, nonperineal areas in the rather slow rate of dihydrotestosterone formation, despite the fact that hair growth in this area and sebaceous gland growth in all areas of skin are known to be under the control of testosterone.

This slow rate of conversion might be due to difficulty in penetration of the hormone to the organelles in question in this type of in vitro preparation, or to the fact that only a fraction of the hormone in skin actually is metabolized in these organelles. It is also possible that testosterone itself or some other metabolite is the active form of the hormone in some tissues. The latter possibility is in keeping with the previous inability in this laboratory to demonstrate dihydrotestosterone in the levator ani muscle of the rat, a tissue that is known to grow under the stimulus of testosterone (11).

It is interesting that the types of skin that demonstrated the most active rates of dihydrotestosterone formation in this study both in men and women arise from common anlage in the urogenital ridge (17), and that each of these tissues grows in response to androgenic stimulae. In view of the fact that the blood testosterone level is much lower in women than in men and, indeed, is low in babies of both sexes, it is likely that the ability of a tissue to perform this conversion is not the result of testosterone action at least in the postnatal period. This is also true in rats in which castration, by reducing the ultimate size of the male organs of accessory reproduction, causes a decrease in total enzyme amount but not in the enzyme concentration in these tissues.[2]

There is now a convincing body of evidence, both as the result of studies showing testosterone blood levels and secretory rates similar to those of normal men (18) and demonstrating a lack of responsiveness to exogenous testosterone after castration (19), that some peripheral defect in androgen action must occur in the syndrome of testicular feminization (17). Whether such a defect could be due to a deficient conversion of testosterone to dihydrotestosterone is unclear, for while the values observed in the labia majora in the present study were lower than average, these results might be secondary to some other defect in testosterone action. Since these measurements were performed at a concentration of testosterone which is probably above the physiological range and represent only a single measurement after the differentiation of the genitalia has been completed, it is clear that no cause and effect relationship can be drawn at present from these observations.

The apparent decrease with age in the enzyme concentration in prepuce also deserves comment. While it is impossible at present to be certain that this apparent fall is not the result of alterations in the cellular populations of the underlying connective tissue or of some other undefined factor, it is intriguing to speculate that the fall may be related to the temporal limitation in the ability of the penis to grow under androgenic challenge. The growth of the penis is, of course, controlled by the concentration of circulating testosterone, but this ability

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[2] Wilson, J. D. Unpublished observations.

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to grow is a limited one. If testosterone is administered to men castrated at an early age, the penis increases in size and then ceases to grow, and when administered to adult, normal men testosterone causes no further growth of the penis (20). It is conceivable that this phenomenon, which is in some ways analogous to the closure of the epiphyses, might be the result of a decrease in the ability of the tissue in question to convert the hormone to a locally active derivative. Inadequate age distribution and the small number of the other perineal skin samples preclude such an analysis in the other skin types with high reductase activity.

Finally, neither the cell type(s) nor the cell organelle(s) responsible for this reaction can be determined from the study of skin slices. The results observed in the studies of the sole of the foot do suggest, however, that the ability to perform this conversion may be a property of the skin itself.

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REFERENCES

1. Dorfman, R. I., and R. A. Shipley. 1956. Androgens. John Wiley & Sons, Inc., New York.
2. Saunders, F. J. 1963. Some aspects of relation of structure of steroids to their prostate-stimulating effects. *In* Biology of the Prostate and Related Tissues. National Cancer Institute Monograph 12. E. P. Vollmer, editor. 139.
3. Pearlman, W. H., and M. R. J. Pearlman. 1961. The metabolism *in vivo* of Δ⁴-androstene-3,17-dione-7-H³; its localization in the ventral prostate and other tissues of the rat. *J. Biol. Chem.* **236:** 1321.
4. Murota, S., and B. Tamaoki. 1967. Metabolism of progesterone and testosterone by chick embryo cartilage *in vitro. Biochim. Biophys. Acta.* **137:** 347.
5. Shimazaki, J., H. Kurihara, I. Yoshikazu, and K. Shida. 1965. Testosterone metabolism in prostate; formation of androstan-17β-ol-3-one and androst-4-ene-3,17-dione, and inhibitory effect of natural and synthetic estrogens. *Gunma J. Med. Sci.* **14:** 313.
6. Shimazaki, J., H. Kurihara, Y. Ito, and K. Shida. 1965. Metabolism of testosterone in prostate. *Gunma J. Med. Sci.* **14:** 326.
7. Farnsworth, W. E., and J. R. Brown. 1963. Testosterone metabolism in the prostate. *In* Biology of the Prostate and Related Tissues. National Cancer Institute Monograph 12. E. P. Vollmer, editor. 323.
8. Farnsworth, W. E., and J. R. Brown. 1963. Metabolism of testosterone by the human prostate. *J. Amer. Med. Ass.* **183:** 436.

9. Gomez, E. C., and S. L. Hsia. 1968. *In vitro* metabolism of testosterone-4-^{14}C and Δ^4-androstene-3,17-dione-4-^{14}C in human skin. *Biochemistry.* **7**: 24.

10. McGuire, J. S., Jr., and G. M. Tomkins. 1959. The effects of thyroxin administration on the enzymic reduction of Δ^4-3-ketosteroids. *J. Biol. Chem.* **231**: 791.

11. Bruchovsky, N., and J. D. Wilson. 1968. The conversion of testosterone to 5α-androstan-17β-ol-3-one by rat prostate *in vivo* and *in vitro*. *J. Biol. Chem.* **243**: 2012.

12. Anderson, K. M., and S. Liao. 1968. Selective retention of dihydrotestosterone by prostatic nuclei. *Nature (London).* **219**: 277.

13. Bruchovsky, N., and J. D. Wilson. 1968. The intranuclear binding of testosterone and 5α-androstan-17β-ol-3-one by rat prostate. *J. Biol. Chem.* **243**: 5953.

14. Van Scott, E. J. 1952. Mechanical separation of the epidermis from the corium. *J. Invest. Dermatol.* **18**: 377.

15. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226**: 497.

16. Dingman, J. F., and W. H. Jenkins. 1962. Dihydrotestosterone-hypocholesterolemic androgenic hormone. *Metabolism.* **11**: 273.

17. Federman, D. D. 1967. Abnormal Sexual Development. W. B. Saunders Company, Philadelphia.

18. Southren, A. L., H. Ross, D. C. Sharma, G. Gordon, A. B. Weingold, and R. I. Dorfman. 1965. Plasma concentration and biosynthesis of testosterone in the syndrome of feminizing testes. *J. Clin. Endocrinol. Metab.* **25**: 518.

19. Wilkins, L. 1965. The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence. Charles C Thomas, Springfield. 3rd edition. 321.

20. Turner, H. H. 1950. The Clinical Use of Testosterone. W. O. Thompson, editor. Charles C Thomas, Springfield.