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In Vitro Metabolism of Testosterone-4-^{14}C and Δ^4-Androstene-3,17-dione-4-^{14}C in Human Skin*

E. C. Gomez and S. L. Hsia

ABSTRACT: Testosterone-4-^{14}C was incubated with specimens of human skin. Metabolites were separated by paper chromatography and thin layer chromatography. Androstenedione, 5α-dihydrotestosterone, 5α-androstanedione, androsterone, and epiandrosterone were identified as metabolites. The metabolites were identified by reverse isotopic dilution and the preparation of derivatives (formation of acetates or reduction with $NaBH_4$). Incubation of androstenedione-4-^{4}C with human skin produced metabolites with similar chromatographic mobilities, and the formation of testosterone was established by reverse isotopic dilution. No radioactive etiocholanolone or etiocholanedione could be detected among the metabolites.

Human skin has been shown to metabolize testosterone at a rate considerably greater than liver (Wotiz *et al.*, 1956). This finding is of particular interest because the skin is one of the largest organs of the body. Wotiz *et al.* (1956) detected several metabolites by autoradiography after testosterone-4-^{14}C was incubated with human skin *in vitro*, and one of these was identified as androstenedione[1] by mixed chromatography and infrared spectrophotometry. The identification of several additional metabolites by mixed chromatography and reverse isotopic dilution was reported in a preliminary communication from our laboratory (Gomez and Hsia, 1966), but no evidence was found which supported the formation of etiocholanolone, a major urinary metabolite of testosterone. Independently Rongone (1966a,b), using gas chromatography, identified etiocholanolone as one of several metabolites of testosterone in the skin of a patient with Klinefelter's syndrome. In an effort to ascertain if normal skin transforms testosterone to etiocholanolone or any other 5β metabolites, we have studied skin specimens from several subjects of both sexes and varying ages. This paper reports our findings on the metabolism of testosterone-4-^{14}C and the conversion of androstenedione to testosterone in human skin.

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[1] The following abbreviations and trivial names are used: NAD, nicotinamide–adenine dinucleotide; NADH, reduced nicotinamide–adenine dinucleotide; NADP, nicotinamide–adenine dinucleotide phosphate; 5α-androstanedione, 5α-androstane-3,17-dione; androstenedione, Δ^4-androstene-3,17-dione; androsterone, 5α-androstan-3α-ol-17-one; 5α-dihydrotestosterone, 5α-androstan-17β-ol-3-one; epiandrosterone, 5α-androstan-3β-ol-17-one; etiocholanedione, 5β-androstane-3,17-dione; etiocholanolone, 5β-androstan-3α-ol-17-one.

Experimental Section

Chemicals. Testosterone-4-^{14}C and androstenedione-4-^{14}C with specific activities of 45.2 mc/mmole were purchased from New England Nuclear Corp. and purified by chromatography in the ligroin–propylene glycol system described by Savard (1953). Stock solutions

were prepared to contain 10^6 dpm in 0.1 ml of methanol and stored at −10°. Coenzymes were products of Sigma Chemcial Co. The reference steroids were purchased from Southeastern Biochemicals, Mann Research Laboratories, and Sigma Chemical Co. $NaBH_4$ was a product of Metal Hydrides, Inc. Solvents for crystallization were redistilled before use. Reagents used were of analytical grade.

Specimens. Skin specimens were obtained at surgery, circumcision of the newborn, and autopsy. Surgical and autopsy specimens were trimmed of the subcutaneous fat tissue and cut into small pieces. Foreskin specimens contain little subcutaneous fat and were cut without trimming.

Incubation. The tissue (0.2–1.5 g) was suspended in 5 ml of Krebs–Ringer phosphate buffer (pH 7.4) with the following additions: NAD, 3.3 μmoles; NADH, 3.3 μmoles; NADP, 3.3 μmoles; glucose 6-phosphate, 12 μmoles; glucose 6-phosphate dehydrogenase, 2 units; penicillin G, 500 units; streptomycin, 500 μg; and gentamicin sulfate, 200 μg. Immediately prior to incubation 0.1 ml of stock solution of radioactive substrate (1.0×10^6 dpm) was added to each flask. Incubations of 4–6 hr were carried out at 37° in a Dubnoff shaking incubator.

Control of Bacteria Growth. Bacterial growth was adequately controlled by the three antibiotics added to the incubation medium. At the end of each incubation, a drop of the incubate was cultured. No growth of either aerobic or anaerobic bacteria was detected in the experiments reported in this paper. Thus the observed metabolic changes were not due to bacterial activities.

Extraction. The incubation was terminated by the addition of 15 ml of a mixture of methanol–dichloromethane (2:1). The tissue and small amounts of precipitate were removed by filtration and washed with the same solvents. The filtrate and washings were combined, concentrated under vacuum, and then extracted with dichloromethane. The extract was filtered through anhydrous sodium sulfate and then evaporated to dryness under vacuum. The residue routinely contained more than 95% of the ^{14}C used in the incubation.

Chromatography. The residues were subjected to thin layer chromatography and paper chromatography. Four solvent systems were used in paper chromatography.

SYSTEM PG was the ligroin–propylene glycol system described by Savard (1953).

SYSTEM EG was similar to system PG except using petroleum ether (bp 30–60°) and ethylene glycol instead of ligroin and propylene glycol. This system allows faster development. The relative mobilities in this system are different from those in system PG, *e.g.*, androstenedione has a mobility similar to androsterone in system PG but moves with the more polar 5α-dihydrotesterone in system EG.

SYSTEM PE. The ligroin–2-phenoxyethanol system was described by Savard (1963). This system was used for nonpolar compounds, such as steroid acetates.

BUSH SYSTEM A was heptane–methanol–H_2O (5:4:1) (Bush, 1952). The chromatograms were developed at ambient temperature.

After development, the strips were either dried for 30 min under vacuum or allowed to dry in air for 24 hr. The distruibtion of radioactivity was monitored by a Vanguard Autoscanner 880 with automatic data system. The amount of radioactivity represented by each peak was calculated and expressed as a percentage of the total radioactivity in the chromatogram.[2] Reference compounds with an α,β-unsaturated ketone structure were detected by examining the strip under a mineralight UVS 12 lamp. Diketones were detected by staining with 2,4-dinitrophenylhydrazine in HCl (Axelrod, 1953). Other keto steroids were detected by a modified Zimmerman reaction using 2.5 *N* KOH in 90% aqueous methanol and *m*-dinitrobenzene in methanol and developing the characteristic color by heating at 100–120°.

Thin-layer chromatography was performed on 40 × 5 cm glass plates coated with silica gel H (thickness 3/8 mm) prestained with Rhodamine 6G (Avigan *et al.*, 1963). Plates were air dried for 30 min, activated at 80° for 30 min, and stored in a desiccator until used. The solvent system used was chlorform and methanol (99:1). The chloroform was stabilized by 0.75% ethanol immediately after distillation. Plates were allowed to develop until the solvent front had ascended 35 cm.

Isotopic Dilutions. The different radioactive areas of the paper were eluted with methanol. In addition to comparison of chromatographic behaviors, identification of metabolites was established by dilution with carrier steroids and crystallization to constant specific activity. After examination with a magnifying glass, the well-formed crystals were used for the determination of specific activity. Details of the procedure were previously described (Hsia and Hao, 1966). If constant specific activity was obtained, derivatives were prepared and their specific activities were determined. Radioassays were carried out with a Tri-Carb liquid scintillation counter Model 314 EX. The counting efficiency for ^{14}C was 69%.

Preparation of Derivatives. ACETATES. The acetates were prepared by reaction in a mixture of acetic anhydride and pyridine (1:1) at room temperature overnight and then crystallized from suitable solvents.

$NaBH_4$ REDUCTION. Reduction of 5α-androstanedione to 5α-androstane-3β,17β-diol was carried out in

[2] The accuracy of the method was tested in the following experiment. A mixture of androstenedione-4-^{14}C (8.69×10^4 dpm) and testosterone-4-^{14}C (1.42×10^5 dpm) was applied to each of four paper strips, two of which were prepared for development in system PG and two prepared for system EG. After development, one strip from each system was dried in air and the other in a vacuum oven at 40°. The total counts per minute in the chromatograms registered by the scanner was (1) PG, air dried 1.38×10^4; (2) PG, oven dried 1.37×10^4; (3) EG, air dried 1.38×10^4; and (4) EG, oven dried 1.51×10^4. The distribution (per cent) of ^{14}C in these chromatograms were: (1) peak IIa 38.7, peak IV 61.1; (2) peak IIa 37.5, peak IV 61.3; (3) peak IIa 36.1, peak IV 63.8; and (4) peak IIa 39.8, peak IV 60.4. It was found that the scanning speed was critical in such assays. A speed of 0.75 in./min was used in our assays; at higher speeds, the variations in results were considerably higher.

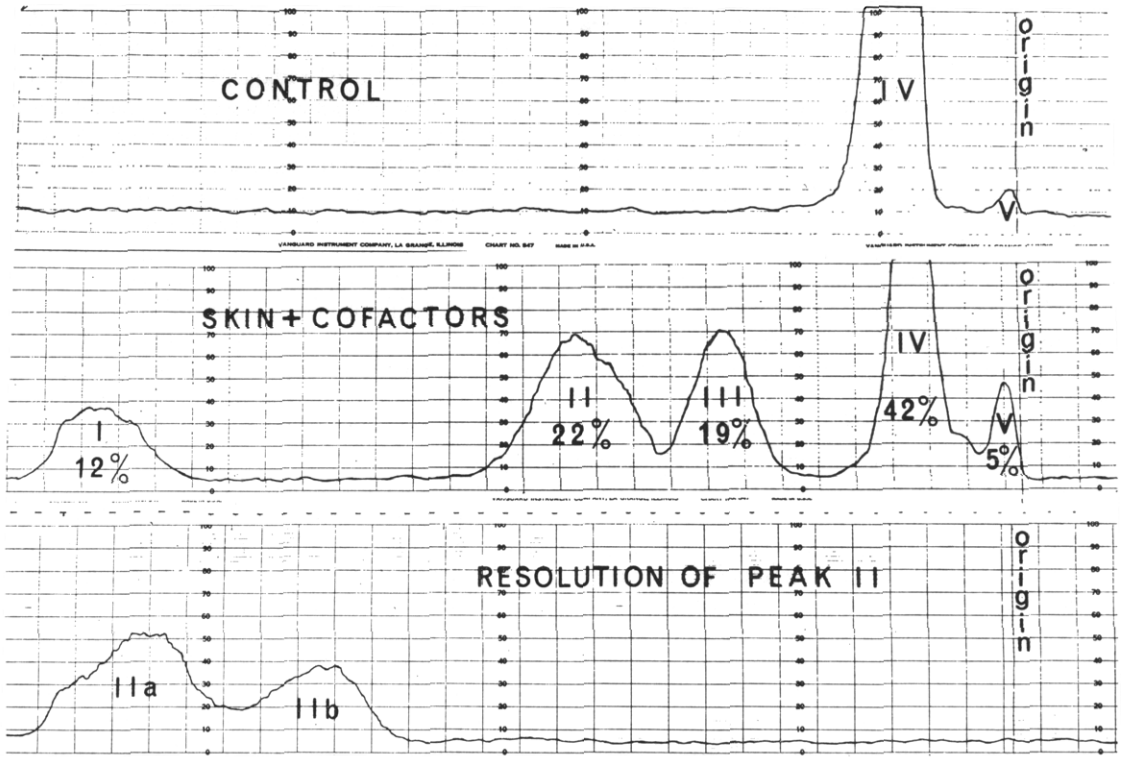

FIGURE 1: Radiochromatograms obtained after incubation of testosterone-4-^{14}C with female neonatal skin. Sliced whole skin (1. 3 g) obtained at autopsy from the abdomen of a 4-day-old infant was incubated with testosterone-4-^{14}C. The top tracing, from the control experiment, shows a large peak (IV) having the mobility of testosterone. Peaks I, II, III, and V of the middle tracing indicate metabolites of testosterone-4-^{14}C. The bottom tracing shows the resolution of peak II into two components (IIa and IIb) after developing for a longer period of time.

methanolic solution with $NaBH_4$ at room temperature as described by Thomas and Dorfman (1961).

Results

Incubation of Testosterone-4-^{14}C. Incubation of testosterone-4-^{14}C with neonatal foreskin and neonatal female abdominal skin produced metabolites with similar chromatographic patterns. A typical chromatogram is shown in Figure 1, obtained after an incubation with female neonatal skin. The top tracing from the control incubation which contained no skin shows a large peak of radioactivity (IV) indicating unchanged testosterone-4-^{14}C. The small peak (V) at the origin could be due to a decomposition product as it was not present prior to incubation. The middle tracing obtained after testosterone was incubated with skin shows three additional peaks having mobilities greater than testosterone. The radioactive materials were eluted separately from the paper and chromatographed again in the same system. The material from peak II was further resolved into two components, IIa and IIb, as shown in the lower tracing.

The chromatographic mobilities of the various metabolites were compared with reference compounds by mixed chromatography (Figure 2). Peak I had a mobility similar to 5α-androstanedione, IIa to androstenedione, IIb to androsterone, III to 5α-dihydrotestosterone, and IV to testosterone.

In a number of studies with both male and female adult skin from the abdominal and inguinal areas, metabolites with similar chromatographic mobilities were produced. The amounts of testosterone-4-^{14}C metabolized by the various specimens ranged between 15 and 80%, the largest amounts being metabolized by neonatal foreskin. In a few experiments the antibiotics were omitted from the incubation mixture, and no noteworthy difference in the results was observed.

Identification of Unmetabolized Testosterone-4-^{14}C (Peak IV). The radioactive material from the area corresponding to peak IV in the middle tracing of Figure 1 was eluted and examined by reverse isotopic dilution with testosterone. The final specific activity of the acetate was 90% of the calculated value (Table I).

Identification of Metabolites of Testosterone-4-^{14}C. The radioactive materials from the areas of peaks I, IIa, IIb, and III were examined by reverse isotopic dilution with appropriate nonradioactive steroids. The specific activities and other details are given in Tables II–VI. These findings, in addition to findings of thin-layer chromatography and chromatography of the free steroids in systems PG, EG, and Bush A, and the acetate of androsterone in system PE gave evidence for the

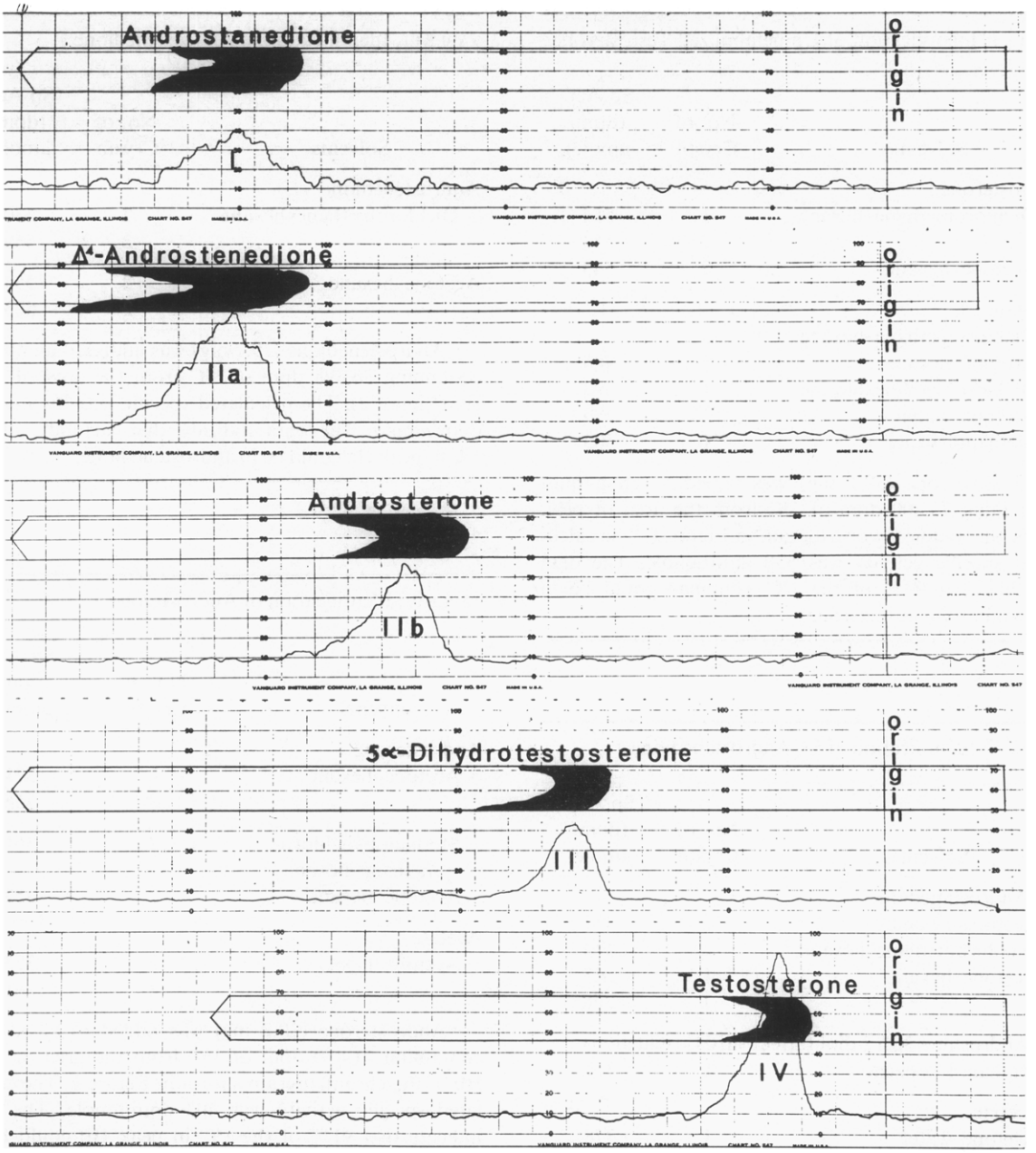

FIGURE 2: Chromatographic identification of metabolites. The radioactive materials indicated by the peaks shown in Figure 1 were eluted from the paper and an aliquot of each was mixed with a suitable reference steroid. The mixture was then chromatographed in system PG. The positions of the reference steroids and radioactive metabolites were determined as described in the text.

identification of 5α-androstanedione (peak I), androstenedione (peak IIa), androsterone (peak IIb), 5α-dihydrotestosterone (peak III), and epiandrosterone (peak III) as metabolites of testosterone in human skin.

Incubation of Androstenedione-4-^{14}C. Androstenedione-4-^{14}C was incubated with male adult skin from the inguinal area and the results are compared with those obtained after testosterone-4-^{14}C was incubated with the same specimen (Figure 3). Metabolites from both substrates had similar mobilities in system PG. Similar results were obtained when androstenedione-4-^{14}C was incubated with specimens of neonatal foreskin or female abdominal skin. The identification of testosterone as a metabolite of androstenedione is shown in Table VII.

Examination for Etiocholanolone and Etiocholanedione. Etiocholanolone, a major urinary metabolite of testosterone, has a chromatographic mobility similar to peak III in the paper chromatography systems used. An aliquot of peak III obtained from incubation of testosterone-4-^{14}C with neonatal foreskin was examined

TABLE I: Identification of Unmetabolized Testosterone.[a]

Solvents	No. of Crystn	Sp Act. (dpm/ μmole)
Testosterone		
Dichloromethane–hexane	1	554
	2	553
Acetone–hexane	2	543
Acetate		
Dichloromethane–hexane	1	
Acetone–hexane	1	486
Dichloromethane–hexane	1	492
Acetone–water	2	499
Methanol–water	1	503

[a] Testosterone (39.1 mg) was added to an aliquot containing 75,400 dpm of ^{14}C from the area of peak IV of the middle chromatogram in Figure 1. The calculated specific activity was 558 dpm/μmole. The data indicate that 90% of the ^{14}C examined could be in unmetabolized testosterone.

TABLE II: Identification of 5α-Androstanedione.[a]

Solvents	No. of Crystn	Sp Act. (dpm/ μmole)
5α-Androstanedione		
Acetone–hexane	2	374
Dichloromethane–hexane	2	370
Acetone–hexane	1	367
	1	368
5α-Androstane-3β,17-diol (after $NaBH_4$ reduction)		
Methanol–benzene	1	372
Diacetate		
Acetone–water	1	378
	1	360

[a] 5β-Androstanedione (31.1 mg) was added to an aliquot containing 40,900 dpm of ^{14}C from the area of peak I of the upper chromatogram in Figure 3. The calculated specific activity was 379 dpm/μmole. The data indicate that virtually all the ^{14}C in peak I could be in 5α-androstanedione.

TABLE III: Identification of Androstenedione.[a]

Solvents	No. of Crystn	Sp Act. (dpm/ μmole)
Androstenedione		
Dichloromethane–hexane	2	530
	2	559
Acetone–hexane	2	554
	2	523

[a] Androstenedione (19.7 mg) was added to an aliquot containing 36,898 dpm of ^{14}C from the area of peak IIa (Figure 2). The calculated specific activity was 535 dpm/μmole. The data indicate that virtually all the ^{14}C in peak IIa could be in androstenedione.

TABLE IV: Identification of Androsterone.[a]

Solvents	No. of Crystn	Sp Act. (dpm/ μmole)
Androsterone		
Acetone–hexane	1	371
	3	356
Methanol–dichloro-methane–hexane	2	361
Acetone–hexane	2	353
Acetate		
Acetone–hexane	2	355
	2	361

[a] Androsterone (30.2 mg) was added to an aliquot containing 41,870 dpm of ^{14}C from the area of peak IIb of the chromatogram shown in Figure 1. The calculated specific activity was 403 dpm/μmole. The data indicate that 91% of the ^{14}C in peak IIb could be in androsterone.

for the presence of etiocholanolone by reverse isotopic dilution (Table VIII). The specific activity dropped continuously to about 1% of the calculated value without arriving at a constant level. These data are consistent with the finding that essentially all the ^{14}C in peak III was in dihydrotestosterone (84%, Table V) and epiandrosterone (17%, Table VI). To determine whether etiocholanolone could be formed in skin of other anatomical sites, this experiment was repeated with radioactive materials indicated by peak III in Figure 3. Immediately following the incubation, 200 μg of carrier etiocholanolone was added to the incubation mixture to prevent decomposition of any radioactive etiocholanolone. After chromatography and elution the material corresponding to peak III was analyzed by dilution with etiocholanolone. Again there was a rapid drop in specific activity; further crystallizations reduced the ^{14}C to background counting levels but without achieving constant specific activity.

Etiocholanolone, 5α-dihydrotestosterone, and epi-

TABLE V: Identification of 5α-Dihydrotesterone.[a]

Solvents	No. of Crystn	Sp Act. (dpm/μmole)
5α-Dihydrotestosterone		
Dichloromethane–hexane	3	263
Acetone–hexane	1	269
	1	258
Acetone–water	2	239
	1	251
Acetate		
Methanol–water	2	243
Acetone–water	1	255
	1	252

[a] 5α-Dihydrotesterone (27.4 mg) was added to an aliquot containing 28,200 dpm from the area of peak III of the chromatogram shown in Figure 1. The calculated specific activity was 299 dpm/μmole. The data indicate that 84% of the ^{14}C in peak III could be in 5α-dihydrotestosterone.

TABLE VI: Identification of Epiandrosterone.[a]

Solvents	No. of Crystn	Sp Act. (dpm/μmole)
Epiandrosterone		
Acetone–hexane	2	85
Dichloromethane–hexane	2	44
Acetone–hexane	2	45
Dichloromethane–hexane	2	42
Acetone–hexane	2	46
Acetate		
Acetone–water	2	41
Acetone–methanol–water	2	37
Methanol–water	1	36
Acetone–water	1	36

[a] Epiandrosterone (27.9 mg) was added to an aliquot containing 21,100 dpm of ^{14}C from the area of peak III of the chromatogram shown in Figure 1. The calculated specific activity was 219 dpm/μmole. The data indicate that 17% of the ^{14}C in peak III could be in epiandrosterone.

androsterone could not be separated from each other in the paper chromatographic systems used; separation was achieved by thin-layer chromatography. The material from peak III from an incubation with foreskin was mixed with carrier 5α-dihydrotestosterone, epiandrosterone, and etiocholanolone. Androstenedione was

TABLE VII: Identification of Testosterone.[a]

Solvents	No. of Crystn	Sp Act. (dpm/μmole)
Testosterone		
Acetone–hexane	2	223
	2	213
	1	213
	1	195

[a] Testosterone (37.1 mg) was added to an aliquot containing 29,900 dpm of ^{14}C from the area of peak IV of the bottom chromatogram in Figure 3. The calculated specific activity was 233 dpm/μmole. The data indicate that 90% of the ^{14}C examined could be in testosterone.

TABLE VIII: Examination for Etiocholanolone.[a]

Solvents	No. of Crystn	Sp Act. (dpm/μmole)
Etiocholanolone		
Acetone–hexane	1	78.9
Dichloromethane–hexane	1	67.3
Acetone–hexane	1	20.0
	1	5.5
	1	3.6

[a] Etiocholanolone (31.2 mg) was added to an aliquot containing 36,500 dpm of ^{14}C from the area of peak III of the upper chromatogram in Figure 3. The calculated specific activity was 339 dpm/μmole.

added as a marker for the movement of the steroids in the chromatogram. The mixture was applied to a 40 × 5 cm thin-layer chromatography plate which was placed diagonally in a 28 × 30 × 7.5 cm tank containing chloroform–methanol (99:1). The cover of the tank was left slightly ajar so that the plate protruded outside the tank through a narrow opening. Thus the solvents were allowed to evaporate as the front approached the opening, permitting continued migration of slow-moving substances. At intervals, the location of androstenedione was observed *in situ* under a mineralight UVS-12 lamp. When it had migrated two-thirds to three-fourths the length of the plate (approximately 5 hr) the locations of all compounds were determined by exposure to iodine vapor, and 5α-dihydrotestosterone, epiandrosterone, and etiocholanolone were found to have separated (Figure 4). The majority of the radioactivity was in the area of 5α-dihydrotestosterone (IIIa), and a small amount in the area of epiandrosterone (IIIb). No radioactivity could be detected in the area of etiocholanolone.

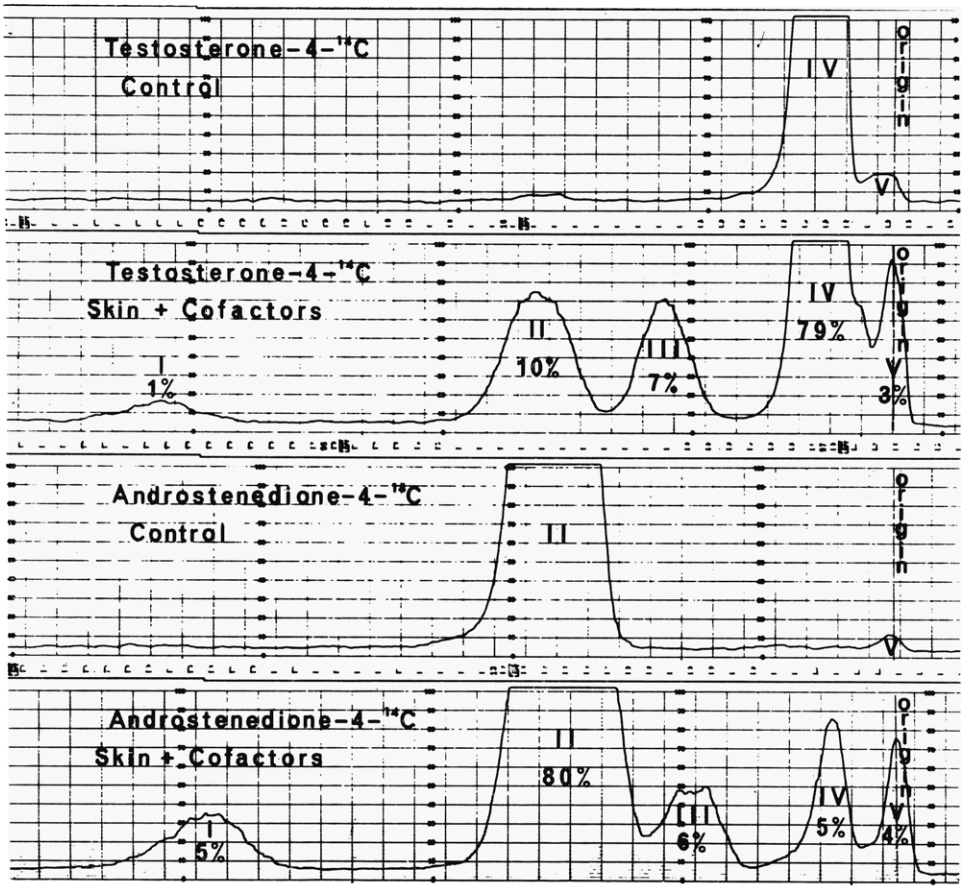

FIGURE 3: Comparison of metabolism of testosterone-4-^{14}C and androstenedione-4-^{14}C in adult male skin. Sliced whole inguinal skin (440 mg) obtained from a 55-year-old white male at herniorrhaphy was incubated with testosterone-4-^{14}C and androstenedione-4-^{14}C. The chromatograms were obtained in system PG. The tracings indicate active metabolism of both substrates, and that the metabolites have similar mobilities.

The area corresponding to epiandrosterone was scraped from the plate and the radioactivity eluted with methanol. This eluate was subjected to chromatography in system PG, and again to thin-layer chromatography. All of the radioactivity present was found in the area of epiandrosterone (lower tracing, Figure 4). An aliquot of the radioactivity in IIIb was then subjected to reverse isotopic dilution with epiandrosterone. The specific activity remained constant at the calculated value after four crystallizations, confirming the data in Table VI.

Etiocholanedione has mobility similar to that of 5α-androstandeione (peak I). An aliquot of radioactive material from peak I (Figure 1) was examined by dilution with carrier etiocholanedione (Table IX). The specific activity declined precipitously after two crystallizations and the ^{14}C remaining with the crystals approached background after five crystallizations. Results of the above experiments do not support either etiocholanolone or etiocholanedione being a metabolite of testosterone in human skin.

TABLE IX: Examination for Etiocholanedione.[a]

Solvents	No. of Crystn	Sp Act. (dpm/μmole)
Etiocholanedione		
Acetone–hexane	2	23.2
Acetone–water	1	9.4
	2	2.6

[a] Etiocholanedione (38.8 mg) was added to an aliquot containing 19,130 dpm of ^{14}C from the area of peak I of the chromatogram shown in Figure 1. The calculated specific activity was 142 dpm/μmole.

Discussion

In the skin, the reduction of the Δ^4 bond of testosterone appears to be in the 5α orientation as we have found no evidence of 5β metabolites in these studies. This is in contrast to the systemic metabolism of testosterone (Slaunwhite and Sandberg, 1957), where the major

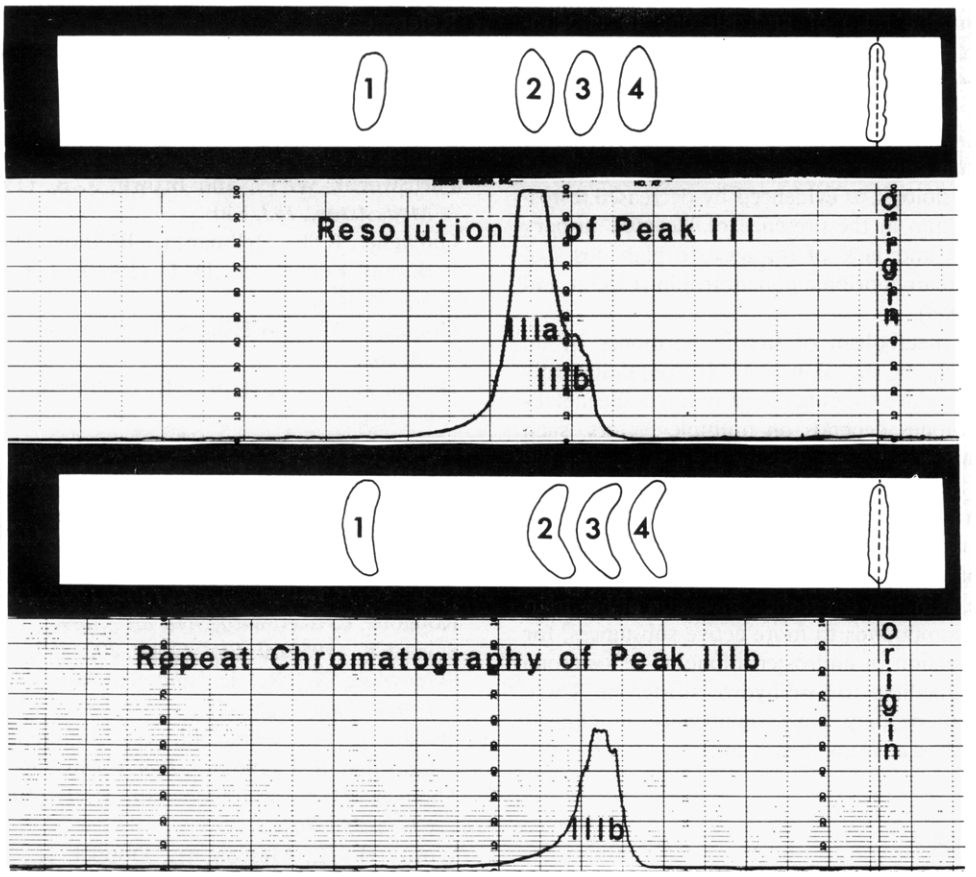

FIGURE 4: Separation of etiocholanolone from components of peak III by thin-layer chromatography. The upper radiochromatogram demonstrates the resolution of peak III into two components (IIIa and IIIb). The lower radiochromatogram demonstrates complete absence of IIIa in IIIb after repeated chromatography (see text). Drawings of the thin-layer chromatographic plates trace the locations of reference steroids as visualized by staining with iodine vapor: 1, androstenedione; 2, 5α-dihydrotestosterone; 3, epiandrosterone; and 4, etiocholanolone. 5α-Dihydrotestosterone moved with IIIa and epiandrosterone with IIIb, but no radioactivity could be detected in the area of etiocholanolone.

urinary metabolites are androsterone and etiocholanolone in approximately equal amounts.

In vitro studies with human liver (Stylianou *et al.*, 1961) have demonstrated active metabolism of testosterone to both 5α and 5β steroids. However, in studies with prostate (Farnsworth and Brown, 1963) no 5β metabolite was found, the pattern of metabolites reported being similar to that in skin. Studies of the cutaneous metabolism of hydrocortisone (Hsia and Hao, 1966) and progesterone (E. C. Gomez, P. Frost, G. D. Weinstein, and S. L. Hsia, unpublished data) have identified several 5α but no 5β metabolites, despite the predominately 5β reduction of these compounds systemically.

At the time of our previous report (Gomez and Hsia, 1966) Rongone (1966a,b) simultaneously reported his study on the metabolism of testosterone by mammary skin of a patient with Klinefelter's syndrome and the identification of etiocholanolone as a metabolite in addition to androsterone, 5α-androstanedione, and androstenedione. His study differed from ours in several respects other than the source of tissue; he used larger amounts of testosterone (50 μg) without radioactive tag and analyzed the products by silica gel column and gas–liquid partition chromatography. It is interesting to note that etiocholanolone was the only 5β steroid found and no 5β intermediate leading to etiocholanolone was identified. It is possible that regional variations and/or endocrine status may account for the differences between his results and ours. A difference in the 5α-reductase activity of skin from different anatomical sites has been demonstrated in the metabolism of hydrocortisone (Hsia and Hao, 1966), the reductase being present in foreskin but not in skin from other sites. No such difference in 5α-reductase has been noted in the present study, but it is possible that a similar heterogeneity may exist with respect to 5β-reductase.

The levels of steroid reductases in tissues are also known to be influenced by endocrine factors. The ratio of 5α- to 5β-reductase activity in experimental animals differs in males and females (Yates *et al.*, 1958; Hagan and Troop, 1960), and is changed by treatment with steroid hormones or thyroid hormones (Gallagher *et al.*, 1960). The latter effect has been confirmed in humans by

differences in the ratio of androsterone to etiocholanolone produced by the systemic metabolism of testosterone-4-^{14}C in euthyroid, hyperthyroid, and hypothyroid subjects (McGuire and Tomkins, 1959). Individuals with Klinefelter's syndrome (testicular dysgenesis) are known to have a considerably altered endocrine physiology, as evidenced by decreased testosterone production in the presence of elevated urinary gonadotropins and lack of response to high doses of exogenous human chorionic gonadotropin (Lipsett and Korenman, 1964).

The active metabolism of steroid hormones by an organ as large as the skin may be physiologically significant, but since the role of any organ in the metabolism of hormones depends on multiple factors, such as blood flow, specific binding by the tissue, etc., the significance is difficult to assess at the present. The presence of an active catabolic pathway is likely to affect the hormonal microenvironment of a tissue. Likewise, since most of the reactions demonstrated are known to be reversible, skin may effect the conversion of hormonally inactive compounds to more active substances, for instance conversion of androstenedione to testosterone or 5α-androstanedione to 5α-dihydrotestosterone. These biotransformations may have significance in clinical conditions such as acne and hirsutism.

Acknowledgments

The authors are grateful to Mrs. Joshephina Lamas for her expert technical assistance, and to Dr. William L. Nyhan for his continued interest in this work.

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