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DIFFERENTIAL RATES OF CONVERSION OF TESTOSTERONE TO DIHYDROTESTOSTERONE IN ACNE AND IN NORMAL HUMAN SKIN—A POSSIBLE PATHOGENIC FACTOR IN ACNE*

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ABSTRACT

Dihydrotestosterone has been shown to be a major metabolite of testosterone. Human skin has been shown to activly convert testosterone to dihydrotestosterone. Since the levels of circulating testosterone in normals and in individuals with acne are the same, "end organ sensitivity" has been postulated. To evaluate the possibility that this may be a function of the rate of conversion of testosterone to dihydrotestosterone, a total of 62 biopsies were studied from 32 subjects without and with acne from affected and unaffected areas. Labeled testosterone metabolites were identified by TLC, GLC, and GFP. Acne bearing skin produced from 2 to 20 times more dihydrotestosterone than did normal skin. Normal facial skin produced more dihydrotestosterone than normal back skin. It thus appears that the previously postulated end organ sensitivity, thought to account in part for the occurrence and distribution of acne, may indeed exist and may be mediated by differential rates of conversion of testosterone to dihydrotestosterone in the skin.

Current data strongly support the view that 5α-androstan-17β-ol-3-one (dihydrotestosterone) appears to be the active form of testosterone at the cellular level in the accessory organs of reproduction and in model sebaceous structures such as the preputial gland of the rat and the preen gland of the duck (1–3). Bruchovsky and Wilson have shown that dihydrotestosterone rather than testosterone is the predominant species bound to the chromatin of prostatic nuclei within 15 minutes after testosterone-1,2 H^3 administration (1). These investigators have also shown that in the preputial gland of the rat, 27% of injected testosterone is converted to dihydrotestosterone within 5 minutes, a rate equal to that of the prostatic cells. In the preen gland of the duck (2), following the administration of testosterone-1,2 H^3, there is selective localization of the isotope in the nuclei consisting of testosterone (25–50%) and a single testosterone metabolite, dihydrotestosterone (50–75%). The studies referred to above provide strong evidence that dihydrotestosterone, rather than testosterone, may be the form of the androgen which stimulates sebaceous gland development.

Gomez and Hsia, using human skin, found that 15 to 80% of the testosterone was metabolized and that the major metabolite was dihydrotestosterone (4). Wilson and Walker (5) studied the rate of formation of dihydrotestosterone *in vitro* in 112 skin specimens. All skin sites tested could metabolize testosterone to dihydrotestosterone; however, there were distinct regional differences. Areas such as the palms and soles which contain no sebaceous glands were also able to convert testosterone to dihydrotestosterone.

It has long been known that plasma levels of testosterone and the daily secretion of testosterone are not necessarily higher in males with acne than in normal males despite significantly higher sebum production in those males with acne (6, 7). This has caused investigators to suggest that "end organ sensitivity" may determine the development of acne and the degree of sebaceous gland activity. In order to evaluate the possibility that "end organ sensitivity" may be a function of the rate of conversion of testosterone to dihydrotestosterone in affected skin vs normal skin, a total of 62 biopsies were studied from 32 subjects without acne and with acne from affected and unaffected areas.

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MATERALS AND METHODS

The skin samples used in this study were obtained from normal male and female subjects and males and females with acne. Samples were selected from sites where acne was present and non-acne sites in the subjects with acne. Samples from face and back were also obtained from the normal subjects. Two or three mm punch biopsies were taken. Saline anesthesia was used in all cases.

The skin samples were immediately placed in chilled Krebs-Ringer phosphate buffer and taken to the laboratory in an ice bucket. The underlying fat was removed and 10 mg of minced tissue was routinely used. The incubation mixture, a modification of that used by Wilson (5) consisted of testosterone-1,2 H^3 (300 $\mu\mu$ moles/ml 1×10^6 cpm) penicillin and streptomycin (2500 U) glucose (1.1×10^{-2} mole/liter) and Krebs-Ringer phosphate buffer, pH 7.4 in a total volume of 2.0 ml. The tubes were gassed with 95% O_2, 5% CO_2, capped and incubated at 37° C in a Dubnoff metabolic incubator with shaking for three hours. The testosterone-1,2 H^3 (New England Nuclear) contained 1 mc/5.64 μg and was brought in aqueous solution directly before using by the method of Wilson (5).

The reaction was stopped by the addition of $CHCl_3$ and MeOH and the lipids extracted by the method of Bligh and Dyer (8). The chloroform extract was taken to dryness under N_2 and the residue was taken up in 2 ml of $CHCl_3$ and stored under N_2 at −20° C. Zero time controls were run with tissue and controls containing the incubation mixture minus tissue were run for three hours.

Thin layer chromatography (TLC), glass fiber paper chromatography (GFP) and gas liquid chromatography (GLC) were used to identify and quantify the testosterone metabolites.

Aliquots of the lipid fraction containing radioactive steroids (50,000 cpm of ^3H) were chromatographed on TLC, GFP and GLC along with a mixture containing 20 μg each of testosterone, dihydrotestosterone, androsterone, androstandiol, epiandrosterone, androstenedione and androstandione. The method of Gomez and Hsia (4) and Wilson and Walker (5) was used for TLC.

Glass plates were coated with silica gel G (Brinkman Instruments, Inc., Westbury, N.Y.) and activated at 110° C for one hour. The plates were developed by ascending chromatography in 98.25% chloroform: 1.75% methanol. The plates were dried and spots visualized with iodine. The spots were then analyzed for radioactivity by the zonal scan profile scraping technique. The plates were applied to a machine which automatically scrapes 2 mm sections of silica gel along an ascending column into Packard counting vials. The method and the advantages of using it are discussed in detail by Snyder (9). The counting vials were filled with one ml of methanol and 10 ml of 0.4% diphenyloxazole in toluene and assayed for radioactivity in a liquid scintillation spectrometer. The counting efficiency for ^3H was 65%.

Glass fiber paper chromatography was also used to identify and quantify steroids. Silica gel coated glass fiber paper (ITLC-SG) purchased from Gelman Instrument Company was dipped into a 1% solution of Zn $(NO_3)_2 \cdot 6H_2O$ was allowed to dry in an oven at 110° C for three hours. The paper was then spotted with 5 μg each of the carrier steroids and an aliquot of the tissue extract containing 10,000 cpm of H^3. The paper was developed in benzene, ethyl acetate (90:1.25 v/v). The bands were visualized with iodine and the pieces of the fiber glass paper were cut out and placed in counting vials containing scintillation fluid (10 ml) and assayed for radioactivity in a liquid scintillation spectrometer.

Gas liquid chromatography of steroids was performed on a Varian Aerograph 2100 model gas chromatograph equipped with a hydrogen flame detector. The column (6 feet; ¼ inch diameter) was packed with 3% QF-1 on Gas Chrom Q (100–120 mesh) (Applied Science Laboratories, Inc.).

The carrier gas was N_2 and the flow rate was 35 ml/min. Column temperature was 230° C. The radioactive samples were enriched with carrier steroid mixtures. The effluent was collected in cooled coiled glass capillary tubes containing glass wool. The steroids were then eluted with hexane. The hexane was removed under N_2 and the samples assayed for radioactivity using the liquid scintillation mixture described above.

RESULTS

The results of chromatographic identification and quantitation of testosterone-1,2 H^3 metabolites are shown in Figures 1A and 1B. Lipid extracts containing the testosterone metabolites from the incubations of acne-bearing and normal tissues were identified and quantified by thin layer chromatography. Aliquots of the same sample were chromatographed on TLC and GLC and compared as shown in Figures 1A and 1B. The methods were in close agreement, indicating that ⅛ of the radioactivity injected was recovered in the DHT peak, and $\frac{1}{10}$ in the androstenedione peak when the same sample was analyzed on both TLC and GLC. Since dihydrotestosterone is not separated from androsterone by the thin layer system, the amount of radioactivity in the androsterone peak was measured in most instances by gas chromatography and subtracted from that found in the DHT peak. The amount of radioactivity in the androsterone peak was found to be negligible.

The results of the thin layer and gas chromatography were in close agreement. Our results are similar to and confirm those of Wilson and Walker (5). The identity of the two principal

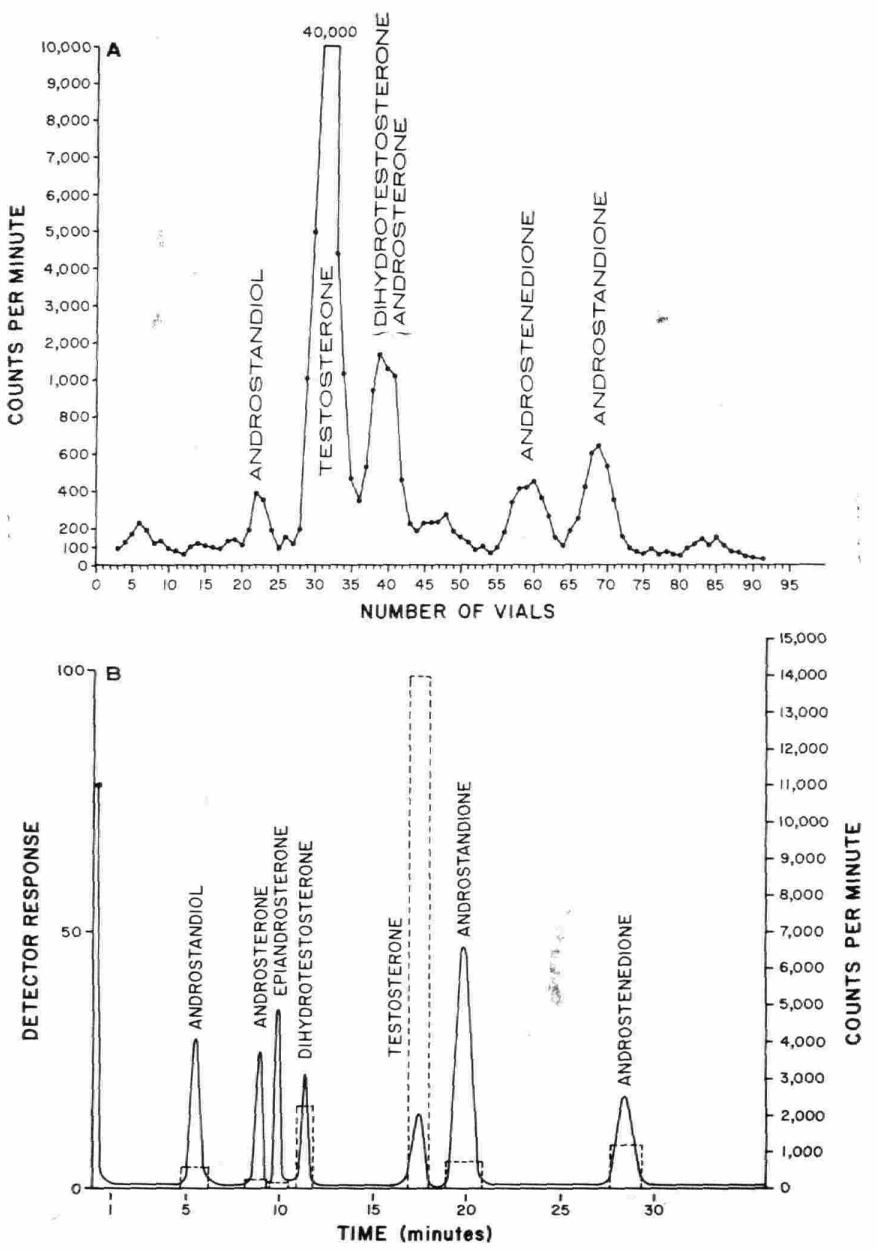

FIG. 1A. Thin layer chromatographic identification of testosterone-1,2 H^3 metabolites.
FIG. 1B. Gas liquid chromatographic identification of testosterone-1,2 H^3 metabolites. The solid line represents the metabolite peaks. The dotted line represents the cpm's contained in the peak.

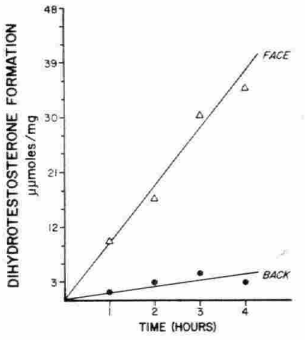

FIG. 2. DHT formation as a function of time

testosterone metabolites in skin has been previously confirmed by recrystallization to constant specific activity (5) and it has been concluded that thin layer chromatographic separation of dihydrotestosterone provides adequate means for routine assay. The time course of the appearance of dihydrotestosterone during the incubation of acne skin slices and normal skin slices with testosterone-1,2 H^3 is illustrated in Figure 2. The reaction appeared to be linear for three hours and all incubations were carried out for this time period.

The routine assay procedure was established from the results demonstrated in Figures 3 and 4. The relationship between the amount of testosterone added and the formation of dihydrotestosterone is shown in Figure 3. The concentration was varied from 150 $\mu\mu$ moles to 900 $\mu\mu$ moles/ml of incubation medium. The rate of dihydrotestosterone formation leveled off at 300 $\mu\mu$ moles and this was the concentration routinely used in subsequent experiments.

The relationship between the weight of the tissue slices and the rate of conversion of testosterone to dihydrotestosterone is shown in Figure 4. The rate of formation of dihydrotestosterone was linear between 5 and 45 mg of tissue. In all instances the tissue weights varied between 5–20 mg. All values were expressed as per one mg wet weight of tissue. The results of 62 measurements on 32 subjects using the standard procedure described is presented in Figures 5 and 6. In the skin samples obtained from the backs of normal female subjects (Fig. 5), the average rate of dihydrotestosterone formation was 0.85 ± 0.23 (SE) $\mu\mu$ moles DHT/mg tissue/3 hrs. The skin biopsies from the chin and forehead of normal females averaged 3.03 ± 0.94 (SE) $\mu\mu$ moles

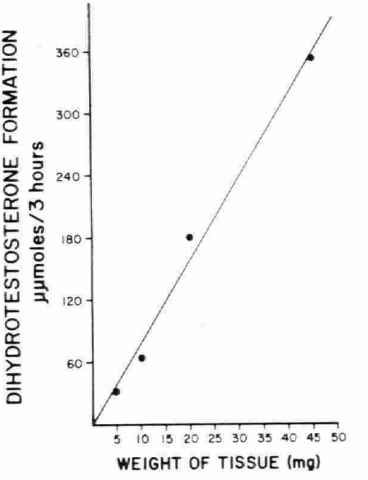

FIG. 3. DHT formation as a function of testosterone 1,2 H^3 concentration.

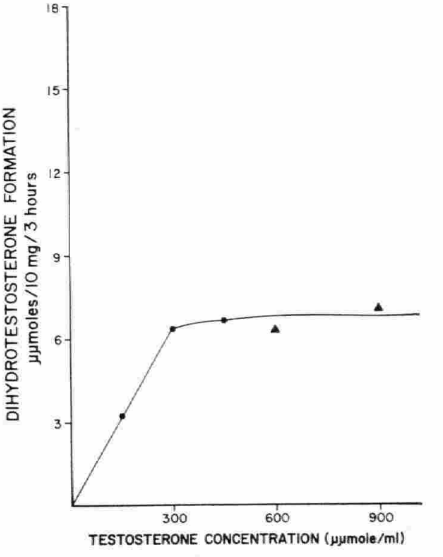

FIG. 4. DHT formation as a function of tissue weight.

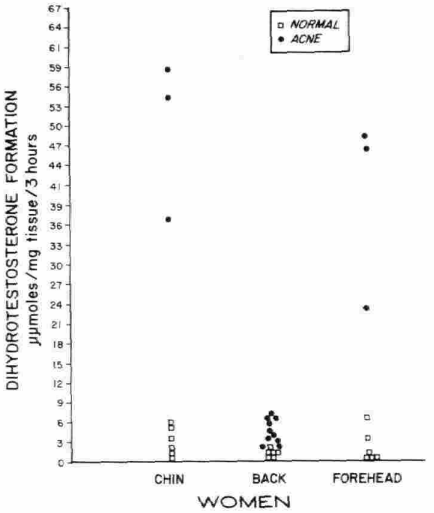

FIG. 5. DHT formation in normal female skin and acne bearing female skin.

DHT/mg tissue/3 hrs and 1.96 ± 1.01 (SE) $\mu\mu$ moles DHT/mg tissue/3 hrs respectively.

The skin samples from the backs of female patients displaying facial acne and minimal acne on the back, converted 4.30 ± 1.95 (SE) $\mu\mu$ moles DHT/mg tissue/3 hrs. However, the rate of formation of dihydrotestosterone in the facial skin of women who had moderate acne on the face was approximately 10–20 fold higher than the normals. The values were 49.56 ± 6.60 (SE) $\mu\mu$ moles DHT/mg tissue/3 hrs on the chin and 39.06 ± 8.05 (SE) $\mu\mu$ moles DHT/mg tissue/3 hrs on the foreheads of acne bearing females.

Similar, although not so spectacular results were found in males. In Figure 6, samples obtained from the backs of normal male subjects averaged 2.48 ± 1.21 (SE) $\mu\mu$ moles DHT/mg tissue/3 hrs. Skin of normal males averaged 15.00 ± 3.00 (SE) and 19.50 ± 1.5 (SE) $\mu\mu$ moles DHT/mg tissue/3 hrs respectively for chin and forehead. Acne bearing backs of males averaged 16.50 ± 4.86 (SE) $\mu\mu$ moles DHT/mg tissue/3 hrs. The skin biopsies from the chin and forehead of acne bearing males averaged 25.16 ± 9.42 and 32.30 ± 3.02 (SE) $\mu\mu$ moles DHT/mg tissue/3 hr respectively. The abdominal skin of males who had acne on backs but none on abdomen averaged 0.83 ± 0.33 (SE) $\mu\mu$ moles DHT/mg tissue/3 hrs which was similar to the backs of normal female subjects.

The differences in conversion of T to DHT in biopsies from all acne bearing sites in the women studied vs conversion in corresponding non-acne bearing sites was statistically significant—$p < .01$—using the Student T test for non-paired samples (see Table). In the males studied the corresponding results were: forehead, $>.01$, $<.02$; back, $>.02$, $<.05$; chin, $>.05$. All males studied were receiving either systemic tetracycline or corticosteroid therapy or both. The influence, if any, of these agents on conversion remains to be determined by future investigations.

DISCUSSION

The results of our study are in agreement with those of Gomez and Hsia (4) in that dihydrotestosterone is one of the major metabolites of testosterone in skin. Furthermore, our results confirm those of Wilson and Walker (5) that the rate of dihydrotestosterone formation differs in skin from different areas of the body. These authors, however, did not report studies on skin of face or back, nor that of acne bearing skin *vs.* normal skin.

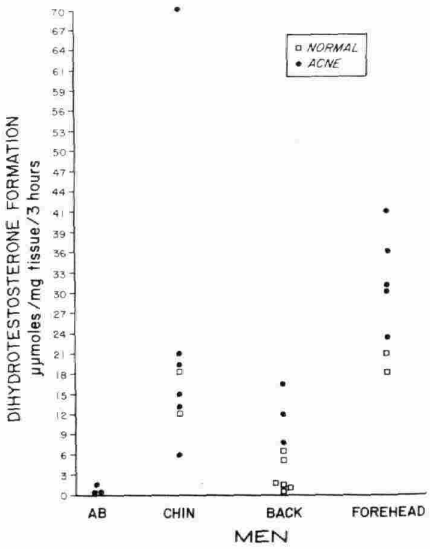

FIG. 6. DHT formation in normal male skin and acne bearing male skin.

TABLE

Conversion of testosterone to dihydrotestosterone by human skin μμ moles of DHT/mg of tissue/3 hours

	Site	Normal	Acne-bearing	Significance of difference—p. values Student T test
Female	Chin	3.03 ± 0.94 (SE)	49.56 ± 6.60 (SE)	$<.01$
	Forehead	1.96 ± 1.01 (SE)	39.06 ± 8.05 (SE)	$<.01$
	Back	0.85 ± 0.23 (SE)	4.30 ± 1.95 (SE)	$<.01$
Male	Chin	15.00 ± 3.00 (SE)	25.16 ± 9.42 (SE)	$>.05$
	Forehead	19.50 ± 1.5 (SE)	32.30 ± 3.02 (SE)	$>.01$ $<.02$
	Back	2.48 ± 1.21 (SE)	16.50 ± 4.86 (SE)	$>.02$ $<.05$

Our data show that, in general, acne bearing skin converted testosterone to dihydrotestosterone 2–20 times greater than did normal skin from a corresponding area. Acne bearing facial skin was more active than acne bearing back skin, and normal facial skin showed more conversion than normal back skin. In general, normal male skin showed higher rates of T to DHT conversion than did female skin from corresponding sites.

Several factors make the comparison between acne bearing female skin and correspondingly located acne bearing male skin in this study difficult to interpret. The first is the relatively small number of individuals in each of the subgroups in this preliminary study. The second is the relatively greater severity of the acne present in the male patients as opposed to the female patients in the study, which presents not only its inherent variable, but also resulted in all the male patients in this initial investigation being studied at a time when they were on concommitant tetracycline and often systemic steroid therapy. What effect these parameters may have on conversion rates of T to DHT is unknown at this time. We are in the process now of accumulating a group of patients with severe pustulo-cystic acne who have not yet been treated, and these patients will be studied both before and during treatment.

It might be anticipated that female patients with acne would have higher rates of conversion of T to DHT than males with corresponding degrees of acne if the sebaceous gland response is related to the amount of dihydrotestosterone present at the cellular level. Higher conversion rates would be necessary to produce similar amounts of DHT in females who start with a much lower T level to convert from than in males in whom much lower conversion rates would result in similar final cellular levels of DHT because of the much larger amounts of T available. While inspection of our data suggests this interpretation, the many variables present do not allow meaningful statistical evaluation and these and many other details remain to be worked out.

An important relationship yet to be studied is that between T to DHT conversion and sebum production. While this may be the central mechanism mediating the effect of DHT production rates on the development of acne it must also be remembered that other androgen effects may play a contributing or even major role. These include such factors as the possible influence of DHT on the thickness, quality and "stickiness" of the keratin produced by the sebaceous and follicle duct epithelia, DHT effect on sebum composition, DHT effect on the permeability of pilosebaceous duct epithelium, etc.

What is clear from this preliminary study is that in the patients studied there is a statistically significant difference between T to DHT conversion rates in biopsies of skin from acne-bearing sites as opposed to biopsies from corresponding non-acne-bearing sites. Further studies are under way to attempt to define more precisely the mechanisms through which this relationship is mediated and the possible modifying roles of therapeutic agents.

The theory of end organ sensitivity was postulated to help explain the lack of correlation between levels of circulating androgens and the occurrence and severity of acne, which has been shown to be an androgen dependent disease. We now suggest that this previously postulated "end organ sensitivity" does in fact exist and may be mediated through higher rates of conversion of testosterone to dihydrotestosterone in acne-bearing as opposed to non-acne-bearing skin.

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