ENZYMATIC DETERMINATION OF HYDROXYSTEROIDS IN HUMAN SKIN SURFACE LIPIDS*

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(With the technical assistance of ALAN R. SPECTOR, B.S.)

For many years investigations regarding the chemical composition of sebum have been carried out (1, 2, 3). The first claim for the presence of steroid hormone or hormone metabolites in human skin surface lipids was made in 1954 by Dubovii (4), who asserted that 17-ketosteroids were present in small amounts, on the basis that a fraction obtained by Girard separation from the lipids gives a positive Zimmerman reaction. This finding was confirmed in a series of 61 patients whose surface fat was examined by Carrié and Ruhrmann the following year (5). In a report published in October 1960, Dubovii expanded the work and presented values for 17-ketosteroids, 3-hydroxysteroids, 17-hydroxysteroids, and glucocorticoids in human hair fat (6). All of these values were based on chemical determinations using a combination of paper chromatography, column separations and various color reactions for groups of steroidal substances. This introduces the possibility of materials other than those under consideration reacting with the reagents used, which are not steroid-specific (11); hence, the values presented by these authors may be considerably higher than the actual concentrations of such steroids in hair fat.

Recently, steroid-specific dehydrogenase enzymes derived from *Pseudomonas testosteroni* have become available. Extracts of skin surface lipids, therefore, could be and were subjected to analysis with 3α-hydroxysteroid dehydrogenase and 3,17 β-hydroxysteroid dehydrogenase. (Fig. 1). This was done in an attempt to confirm the results of Dubovii and Carrié and Ruhrmann, to establish normal values by this method, and ultimately to check for variability in concentration of these steroids in different dermatoses and diseases as a possible aid to diagnosis and understanding of their pathophysiology, and even, perhaps, in designing therapy.

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MATERIALS AND METHOD

The surface lipids used in these studies were obtained from scalp soaks of young (ages 19–29) adult male volunteers, who used no cosmetic hair preparations. The samples were obtained by the method of Bloom and Nicolaides (7) except that a 3:1 ethyl ether:ethanol mixture was substituted for ethyl ether. The fat yield from this procedure in 27 scalp soaks ranged from 52.28 to 799.24 mg, with a mean of 257.99 mg and a median of 252.85 mg.

The nonsaponifiable fraction of a lipid was obtained by essentially the method reported by Cocks (8), and this fraction was partitioned between aqueous methanol and n-hexane as described by Talalay (9) for urine studies. The residue of the final methanol portion was redissolved in methanol of such volume that 0.1 ml of solution would be equivalent to approximately 30 mg sebum, based on the initial sample weight. If more than this is used, turbidity becomes a complicating factor in accurately reading the final reaction.

The enzyme reaction itself was performed as outlined by Talalay, (9) who kindly provided the enzymes for this study. This is an indirect assay, depending upon the conversion of hydroxysteroids to their corresponding ketosteroids, and the accompanying reduction of DPN to DPNH (Fig. 2). This is a mole per mole conversion, and the amount of steroid present can be calculated from the amount of DPNH formed. A molecular weight of 300 was assumed for the steroids in calculating the weight of steroid present in the samples.

The α-enzyme is added first because it is a highly pure preparation. The β-enzyme is contaminated with small amounts of α-enzyme, but since the α-enzyme reaction is allowed to go to completion before the β-enzyme is added to the system, this is of no importance in the final results.

On 5 of the samples reported, internal controls were performed to test the α-enzyme by adding a known amount of androsterone. Calculated results were 98.6% of actual amounts added. Similar internal controls were carried out on the β-enzyme in 6 of the samples, utilizing dehydroepiandrosterone (DHEA) as the known substance. In these

FIG. 1. Examples of 3α- and 3β-hydroxy compounds.

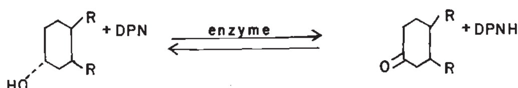

FIG. 2. Reaction with 3α-hydroxysteroid dehydrogenase

cases, calculated results were 86.1% of actual amounts added.

On one of the samples in which internal controls were not used, the test solution was run against the test blank in a Spectronic 505 Recording Spectrophotometer. The absorption curves confirmed the presence of DPN and DPNH, and showed no other peaks, and the amount of DPNH present corresponded almost exactly to that read on the Beckman Spectrophotometer.

To check recovery, 2 sebum samples were enriched with C^{14}-labeled DHEA of known activity, subjected to the entire extraction procedure, and examined for remaining radioactivity in a liquid scintillator. Recovery by this method was 96.2% and 93.2% on the 2 samples, for a mean of 94.7%.

In order to determine whether or not the steroids were altered by the extraction procedure, a known amount of testosterone was added to 2 sebum samples, and once again a complete extraction was carried out. The final solutions and controls were analyzed on the recording spectrophotometer, and the resultant spectra showed only a peak at 244 mμ, the wave length of maximum absorption of testosterone in methanol, with no evidence of other products.

RESULTS AND DISCUSSION

The results of the study are shown in Table I. 3α-hydroxysteroids were present in all samples and were always in greater concentration than the 3,17β-OH steroids, which were present in all but one sample. The ratio of the mean concentrations of the 3α-hydroxy- to the 3β-hydroxysteroids was 3.5:1, but the individual ratios varied from 2.2:1 to 5.3:1, except in the one instance where no β-OH steroid was found.

In reality the ratios may be twice as great as those reported. The β-enzyme can oxidize the hydroxysteroids at the 3 and 17 positions, and the conversion at each of these positions requires one molecule of DPN and produces one molecule of DPNH. The actual concentration of β-OH steroids may lie, therefore, between the figure shown in the table and one-half of that figure.

TABLE I

3α- and 3,17β-hydroxysteroid concentration in skin surface lipid

Patient No.	Age	3α	3,17β	Total	Ratio α:β
		mg. hydroxysteroids/gm. sebum			
1	26	0.22	0.08	0.30	2.7
2	29	0.13	0.00	0.13	
3	19	0.26	0.05	0.30	5.3
4	21	0.19	0.05	0.23	4.0
5	21	0.24	0.10	0.34	2.4
6	22	0.30	0.14	0.44	2.2
7	20	0.22	0.04	0.26	4.9
8	22	0.11	0.02	0.13	4.8
9	22	0.12	0.03	0.14	4.8
10	19	0.35	0.11	0.46	3.2
Means		0.21	0.06	0.28	3.5
Medians		0.22	0.05	0.28	—

Also, a few known steroidal compounds have both 3α-OH and 17β-OH groups, and it is therefore possible that the same steroidal compound might be reported in both columns on the table. In this instance, as in the former, the figures for total steroid concentration would be higher than the actual values.

Since the commonly occurring steroidal compounds are neither 3α, 17β-dihydroxysteroids nor 3,17β-dihydroxysteroids, it is unlikely that the results shown here are abnormally high, if increased at all.

Two of the results shown here were from 24-hour sebum samples, and in one case a random sample and a 24-hour sample were both analyzed. The results are not significantly different in any of these samples indicating that there is probably no significant change in hydroxysteroid concentration of skin surface lipids, regardless of the time they are left on the scalp.

Three of the patients in this series had acne vulgaris, and the results in each of these patients were very close to the mean value for all of the samples. This would tend to indicate that in acne there is no increase in 3α-hydroxy- or 3,17β-

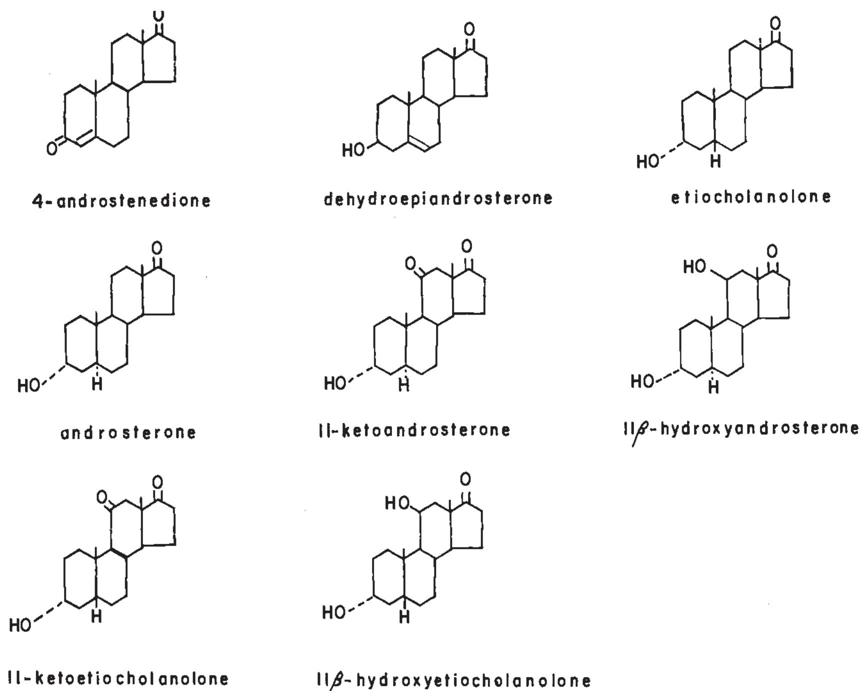

FIG. 3. Commonly occurring 17-ketosteroids

	Mg steroids/Gm sebum
Cook and Lorincz	0.13–0.46 (3α- and $3,17\beta$-hydroxysteroids)
Dubovii	0.3–4.6 (17-ketosteroids)
Carrié and Ruhrmann	7.3–394 (17-ketosteroids)

FIG. 4. *Steroid Concentration in Mg per Gm Sebum*

hydroxysteroids, although the series is too small definitely to warrant such a conclusion, and more work will have to be done on this subject.

Although the reaction upon which this assay is based is reversible, and can be used to detect 3-keto- and 17-ketosteroids, no such analysis was attempted in this study. It is therefore, difficult to compare our results with those of Dubovii and Carrié and Ruhrmann. However, all of the commonly occurring 17-ketosteroids except 4-androstene-dione can be measured by the technic we used, because of the presence of a 3α- or 3β-hydroxyl group on these compounds (10). (See Fig.3.) It may be of benefit, therefore, to compare our results with those of Dubovii and Carrié and Ruhrmann in just this one category of steroidal compounds. (See Fig. 4.)

The results reported in this paper are generally of a considerably lesser magnitude than those reported by the other investigators. And, since by the enzyme method we are measuring a far wider range of compounds than those with a 17-keto grouping, our figures are undoubtedly high for comparison with those results from the other studies. The nonspecific nature of the chemical assays was mentioned previously and may account for the high figures reported by Dubovii and Carrié and Ruhrmann.

SUMMARY

In this paper a new and relatively simple steroid-specific enzyme method for determining 3α-hydroxysteroids and $3,17\beta$-hydroxysteroids in skin surface lipids has been presented. It has been shown that these steroids do occur in human hair fat, with the 3γ-hydroxysteroids being present in the greater concentration. It has been suggested that the concentrations of these steroids in patients with acne vulgaris are not higher than those in patients without acne. The figures reported here tend to indicate that previously reported results for 17-ketosteroids in skin surface fat as determined by chemical means are high.

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DISCUSSION

Dr. Irwin M. Freedberg (Boston, Mass.): Is it known that the steroids found on human skin are substrates for these bacterial enzymes?

Dr. Thomas J. Cook (in closing): Dr. Paul Talalay has tested hundreds of different steroid compounds as substrates for these enzymes. With the exception of those with long chains on the 17-carbon atom, such as cholesterol, all 3α- and $3,17\beta$-hydroxy compounds proved to be satisfactory substrates for their specific enzyme.