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17-BETA-HYDROXY-STEROID-DEHYDROGENASES IN HAIR FOLLICLES OF NORMAL AND BALD SCALP: A HISTOCHEMICAL STUDY*

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ABSTRACT

Testosterone, dihydrotestosterone, and estradiol were used as substrates to evaluate 17-beta-hydroxy-steroid-dehydrogenases in the hair follicles of normal human scalp and scalps of patients affected by initial or advanced male pattern alopecia (MPA).

Trace to strong reactions were found in follicles and moderate reactions were found in the dermal and hypodermal structures immediately surrounding the follicles.

With testosterone and estradiol, the outer sheath of the late anagen follicles in initial MPA showed greater reactivity than did similar follicles of normal subjects.

With dihydrotestosterone, reactivity in the outer sheath of normal late anagen follicles was strong and became progressively less strong in similar follicles from initial and advanced MPA subjects.

The study of the metabolism of steroid sex hormones within the skin, which was previously neglected, has recently become the object of a number of fruitful studies which have been reviewed by Hsia [1]. Among other findings, these studies established that the skin metabolizes many steroids including testosterone [2–4]. They further showed that the rate of oxidation of certain steroids, e.g., estradiol into estrone, varies from one skin region to another [5]. Beta-hydroxy-steroid-dehydrogenases were detected histochemically in the sebaceous glands and in human and animal hypodermis [6–12].

The study of the metabolism of steroid sex hormones in relation to hair growth is very recent. Takashima et al found that testosterone is metabolized by the outer root sheath and the bulb of hair follicles of hairy and bald stump-tailed macaques [13]. In these animals the rate of metabolism of testosterone is higher in the frontal than in the occipital areas and is greater in young macaques than in adult animals. The ratio of metabolized to nonmetabolized testosterone, and the amount of 5-alpha-dihydrotestosterone, is higher in the frontal than in the occipital areas even though the ratio in young and adult macaques remains the same [14].

Rampini et al recently demonstrated that during three consecutive, experimentally provoked hair cycles in rats, estradiol was oxidized to estrone [15] and testosterone was oxidized and/or reduced to various 17-ketosteroids and 5-alpha-metabolites. The rate of these reactions varied with the growth and regression of hair follicles [16].

In this paper the sites of hydroxy-steroid-dehydrogenase activity for testosterone, dihydrotestosterone, and estradiol in the hair follicles of the human scalp have been histochemically determined using testosterone, dihydrotestosterone, and estradiol as substrates. An attempt has been made to evaluate quantitative differences among the enzymatic activities in normal as compared to bald subjects.

MATERIALS AND METHODS

Several round samples of vertex skin measuring 12 mm in diameter were excised by means of a hand punch (after local anesthesia with 1% xylocaine) from the scalps of 23 male individuals aged 16 to 53 years. Nine of these subjects had a normal trichogram and no clinical signs of male pattern alopecia (MPA), seven had more than 15 percent telogen hairs and clinical signs of initial MPA corresponding to degrees II or III (Hamilton's classification), the remaining seven individuals presented a practically glabrous vertex, i.e. degrees V to VI [17, 18]. The skin specimens were frozen and stored at −30°C. 25-μ sections could be used since the numbers of granules in the outer root sheath and papilla of hair follicles are quite small. The sections were incubated for 2 hours at 37° C with the substrates as suggested by Muir et al [8] to reveal the activity of hydroxy-steroid-dehydrogenase (HSD) histochemically, using as substrates estradiol (1,3,5 [10] estratrien-3, 17β-diol); testosterone (4-androstan-17β-ol-3-one); and 5α dihydrotestosterone (5α-androstan-17β-ol-3-one). Control sections from each specimen were also incubated in a medium from which either the substrate [8] or NAD had been omitted.

After incubation, the sections were counterstained with a saturated solution of picric acid, dehydrated, and mounted in Eukitt balsam. With each substrate the 17-beta-HSD activity appeared in the light microscope as a blue granular precipitate, and three observers independently evaluated the localization, amount, and relationship of the precipitate to the stages of the hair cycle.

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Terminal hairs were classified as follows:

I: Late anagen, (stage VI) according to Chase [19, 20] including those hairs which according to Braun-Falco and Kint are in early catagen [21]. This was done in order to avoid possible mistakes arising from difficulties in distinguishing these two types of hairs morphologically.

II: Late catagen, according to Montagna [22] and Flesch [23].

III: Telogen, according to Chase et al [19, 20].

17-beta-HSD reactivity was evaluated by direct histologic observation on a scale from – to ++++. Trace reactions have been classified as ±.

A more quantitative evaluation was attempted by one observer in 14 late anagen follicles from normal scalps and initial cases of MPA by counting the clearly distinguishable granules in the outer sheath and in the papilla, at a magnification of 800×. In the outer sheath, granules were counted within a microscope field, centered on the region immediately adjacent to the keratogenous zone and at a distance from the upper tip of the papilla which was equal to the height of the papillary structure. In the papilla, the granules were counted in an area covered by 16 squares of a Reichert 8 x M reticulum, the center of which was placed at the intersection of the maximal transverse and the maximal longitudinal diameter of the papilla. All values were analyzed statistically. Data on the reactivity of sebaceous glands have been reported previously [24].

RESULTS

Depending upon the substrate employed, HSD reactivity was always shown by a precipitate consisting of intra- and extracytoplasmic granules of varying shape and size, usually isolated but often clumped together (Figs. 1–4). Dehydrogenase reactivity varied from ± to ++++ in hair

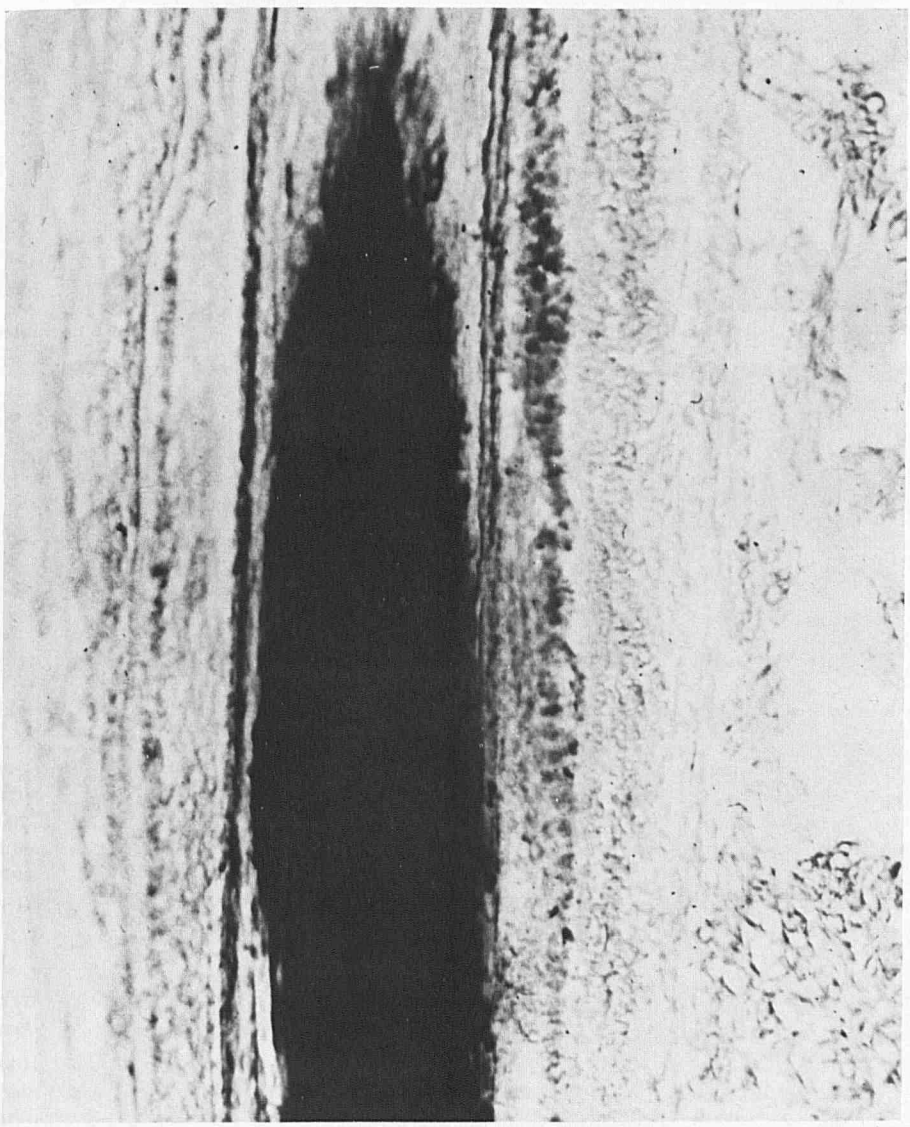

FIG. 1. Testosterone-dehydrogenase: Reactivity in the lower half of the outer sheath in a late anagen hair from a subject with initial MPA.

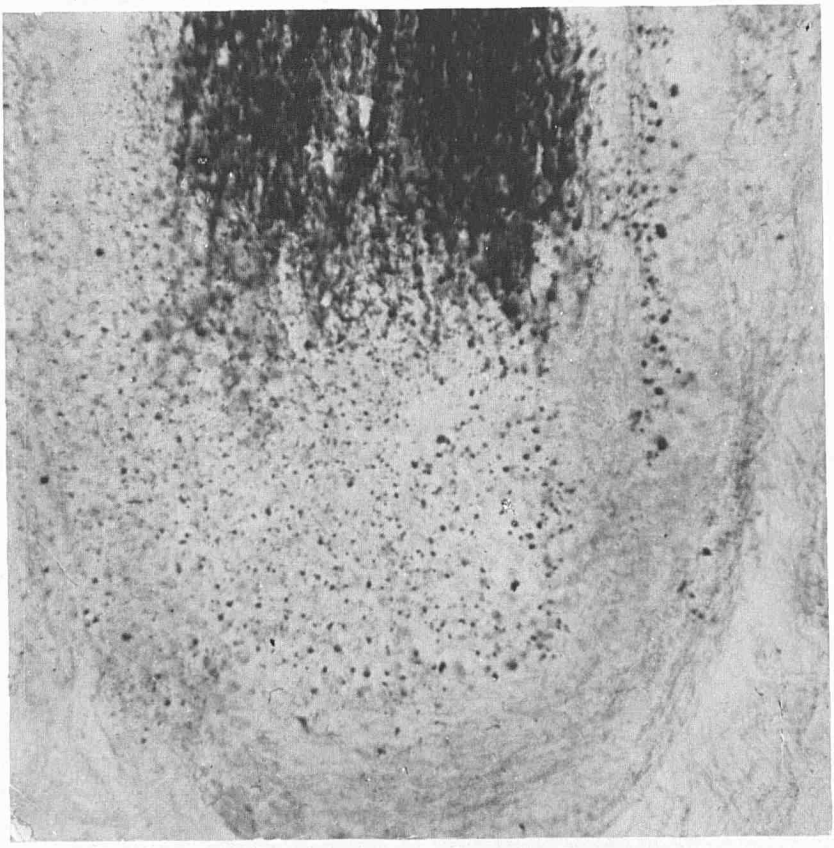

FIG. 2. Testosterone-dehydrogenase: Reactivity in the bulb and papilla of anagen hair from a normal subject.

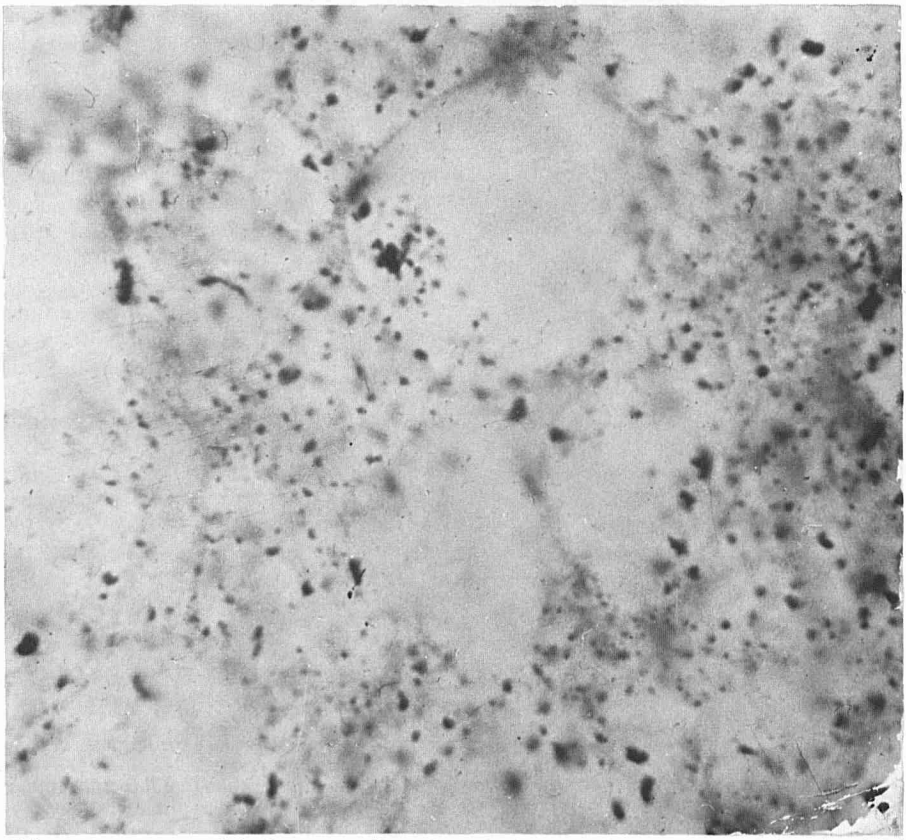

FIG. 3. Dihydrotestosterone-dehydrogenase: Reactivity in the hypodermis of a subject with initial MPA.

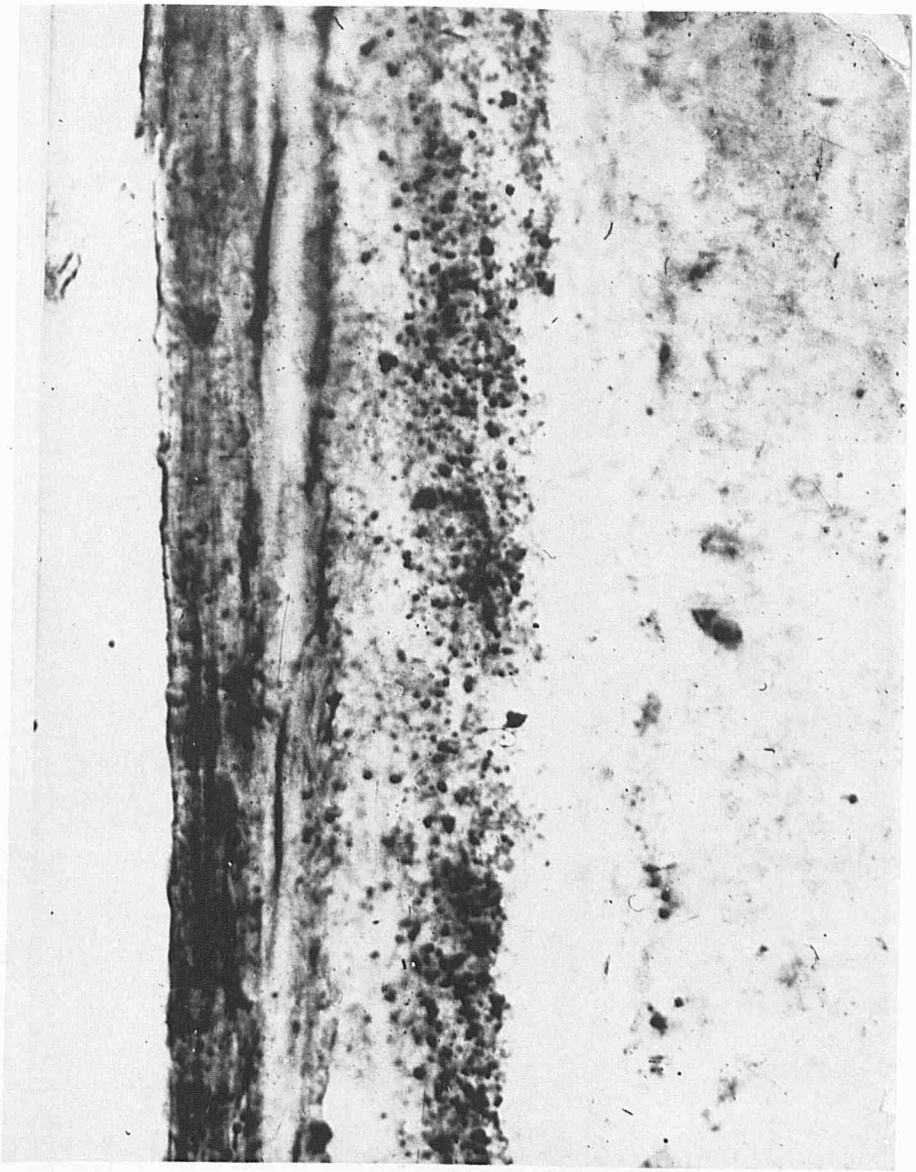

FIG. 4. Estradiol-dehydrogenase: Reactivity in the lower outer sheath of anagen hair from a normal subject.

follicles, was +++ in the dermal and hypodermal structures immediately surrounding the follicles, and traces of activity were occasionally found in the eccrine sweat glands. Except for a few granules scattered in the hypodermis with all substrates, no precipitate was observed in control sections.

Reactivity of 17-Beta-Hydroxy-Steroid-Dehydrogenase with Testosterone as Substrate (Tables I, II)

Normal scalp. In late anagen hairs, +++ reactivity was confined mainly to the peripheral cells of the lower outer sheath surrounding the keratogenous zone (Fig. 1). In most cases during late catagen and throughout telogen, enzymatic reactivity quickly disappeared from the wrinkled outer root sheath between the stages of the epithelial column attached to the ascending club and that of the "nipple." In a few cases of late telogen, however, some granules were found directly surrounding the club.

Initial MPA. In late anagen, the same follicle zones showed a considerably stronger reactivity. However, no reactivity was found in late catagen and telogen, as was the case with normal subjects.

Advanced MPA. Reactivity in all sites was low. The different degrees of reactivity found in normal and initial MPA subjects by simple microscopic observation were statistically confirmed by counting the granules as described above. In cases of initial MPA a higher number of granules were found in the outer sheath in late anagen than in the corresponding follicles from normal persons.

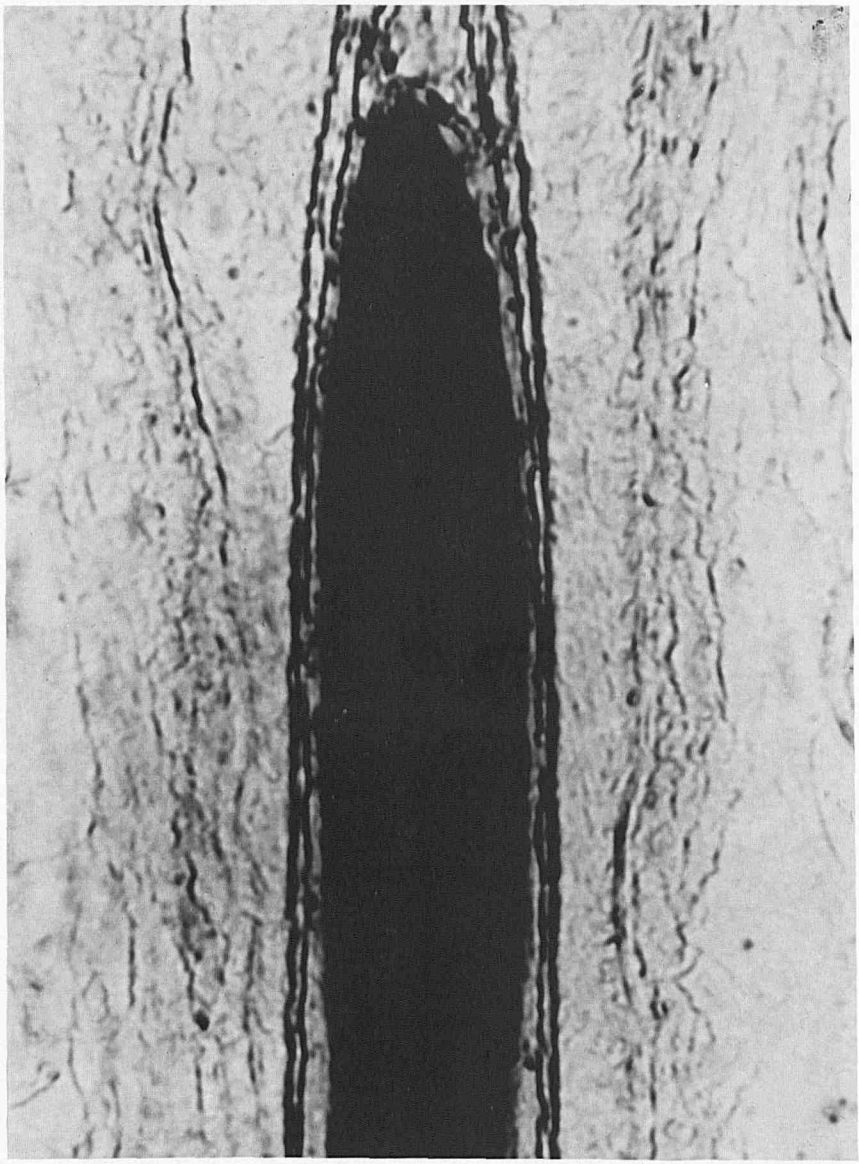

FIG. 5. Testosterone-dehydrogenase: Control section. Lack of reactivity in the lower half of the outer sheath in a late anagen hair from a subject with initial MPA.

On the other hand, though variably positive, no difference in reactivity of late anagen hairs was found when the papillae from normal subjects (Fig. 2) were compared with those of subjects with initial MPA. The reactivity of hypodermal perifollicular fat, though variable, was generally +++ in both normal and alopecic subjects and independent of the cycle phase.

Reactivity of 17 Beta Hydroxy-Steroid-Dehydrogenase Using Dihydrotestosterone as Substrate (Tables I, II)

Normal scalp. In late anagen follicles, the enzymatic reactivity of the lower part of the outer sheath, particularly that surrounding the keratogenous zone was +++ and localized within the cytoplasm of the most peripheral cells. This reactivity tended to disappear rapidly in the subsequent catagen stages. Thus, while some blue granules could be seen in the wrinkled outer sheath surrounding the club at the beginning of catagen, no granules were to be seen in the stages between the hair stem and the "nipple."

Initial MPA. Reactivity was found in anagen follicles in the same sites but was of considerably lower intensity. The reactivity of catagen follicles was quantitatively and qualitatively similar to that of a normal catagen hair.

Advanced MPA. An even lower degree of reactivity was noted in the anagen follicles of these subjects. These observations were confirmed by counting the granules in the outer sheath. The

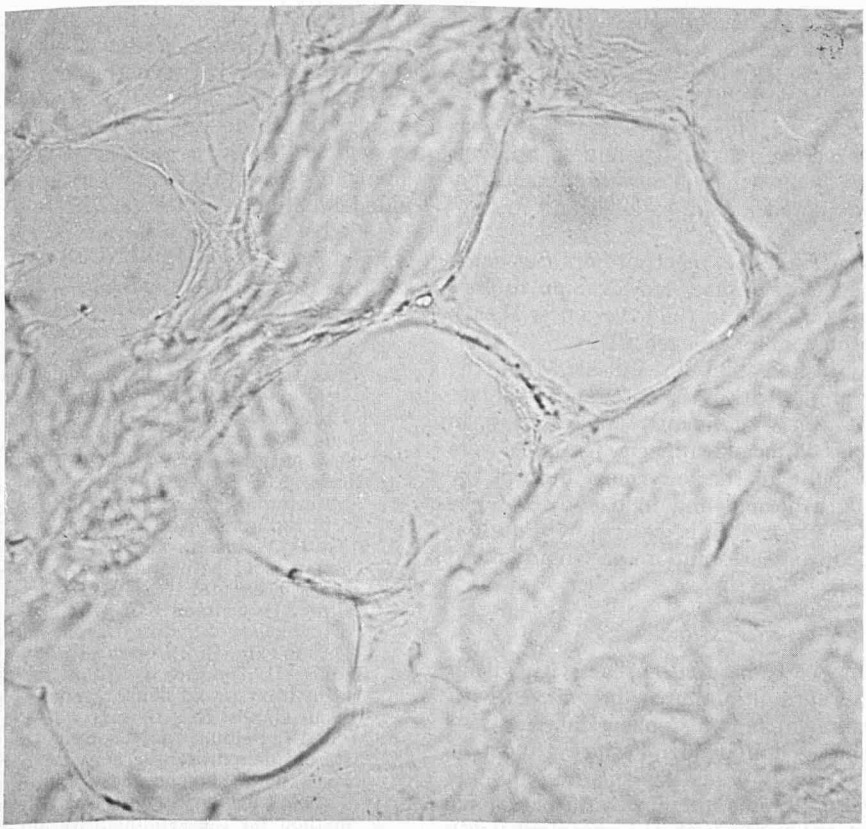

FIG. 6. Testosterone-dehydrogenase: Control section. Substantial lack of reactivity in the hypodermis of subject with initial MPA.

TABLE I

17-beta-HSD activity within the outer sheath

	Stage of cycle	Substrate		
		Testosterone	Dihydrotestosterone	Estradiol
Normal subjects	I	+++	+++	+++
	II	–	–	–
	III	–	–	–
Initial MPA subjects	I	++++	+	++++
	II	–	–	–
	III	–	–	–
Advanced MPA subjects	I	+	±	±
	II	–	–	–
	III	–	–	–

I = late anagen; II = late catagen; III = telogen

For significance of symbols, see *Materials and Methods*.

counts were significantly less in the anagen follicles of normal individuals than in those of subjects with initial alopecia. Reactivity was found only occasionally in the matrix of the bulb and in the papilla of all hair types and was not different in normal subjects as compared with scalp from patients with alopecia. +++ reactivity of the hypodermis was found in both normal and alopecic subjects (Fig. 3).

TABLE II

Statistical evaluation of granule counts (mean values +/– standard deviation)*

	Anagen	
	Normal Subject	Initially Bald Subject
Testosterone	125 ± 8	173 ± 6†
Estradiol	114.5 ± 5.5	170 ± 8†
D.H.T.	121 ± 10	74 ± 7†

* Of lower outer sheaths only. Data on papilla are not in the table because no significant differences were established.

† For each hormone, the values indicated are statistically different from anagen of a normal subject with a P value of less than .01.

Reactivity of 17-Beta-Hydroxy-Steroid-Dehydrogenase with Estradiol as Substrate (Tables I, II)

Normal scalp. +++ reactivity was found in the most peripheral cells of the lower part of the outer sheath surrounding the keratogenous zone mainly in late anagen follicles (Fig. 4). As with testosterone and dihydrotestosterone, reactivity to es-

tradiol was also localized in the wrinkled outer sheath surrounding the hair club and was no longer found in the following stages of catagen and in telogen.

Initial MPA. In late anagen, reactivity was found at the same sites of the follicle but was substantially stronger than in normal subjects. No reactivity was noted in late catagen and in telogen.

Advanced MPA. The reactivity in the same zones in anagen was trace. However an intense reactivity was observed in the lower outer sheath of occasional new hairs (anagen III).

Counts confirmed that in initial MPA the number of granules within the outer sheath of late anagen hairs was significantly higher than in anagen follicles of normal subjects. Granules were occasionally found in the lowermost bulb of the follicles in both normal and bald subjects. The reactivity of the hypodermis was +++ in normal subjects and initial cases and absent in advanced MPA.

DISCUSSION

Our findings of 17-beta-HSD reactivity within the follicles of normal human scalp contrast with those studies in which no histochemical reactivity of this type was found [6]. Failure to detect 17-beta-HSD reactivity within the human hair follicles may be explained in part by the occasional variability of reactivity with the same substrate and by the weak enzymatic reactivity of hairs in comparison with the higher reactivity of sebaceous glands [6–12] on which the attention of previous observers has been focused.

Apart from the proof given by the negative controls (Figs. 5, 6), one must remember that in the follicles of the stump-tailed macaque the 17-beta-HSD activity was mainly localized within the same sites which we found reactive in the human scalp, namely the outer root sheath and the bulb [14]. Furthermore, histochemical reactivity of the hypodermis, especially around growing hairs, confirms our similar findings in rat skin [12]. The differences in enzymatic reactivity found between normal and alopecic skin appear to be reliable. The optical evaluation of histochemical reactivity was confirmed by three observers working independently and the values agree with the data from granule counting.

Regarding the significance of our findings, we are aware that the presence of enzymatic reactivity does not necessarily indicate the presence of the corresponding substrates. The 17-beta-HSD reactivity which is considerably higher in growing than in resting hairs, basically agrees, nevertheless, with Rampini's findings which showed that testosterone and estradiol were metabolized chiefly in the anagen phase of the rat hair cycle [15, 16]. The increased reactivity with testosterone dehydrogenase in scalp affected by initial MPA also agrees with the accumulation of androstenedione found in the bald regions of the macaque [13]. In initial baldness, on the other hand, the discrepancy between the higher reactivity of 17-beta-HSD with testosterone and estradiol coupled with the decline of that acting upon DHT, casts doubt on the hypothesis of the existence of a single 17-beta-HSD in man as suggested by biochemical studies on the rat [25].

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