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*The state-of-the-art laboratory*

Proposal n°: AD070315C-2

Study n°: AD070315A

**EFFECTS OF CLEAROGEN ACNE LOTION ON  
TESTOSTERONE METABOLISM IN RECONSTRUCTED  
HUMAN EPIDERMIS**

**STUDY REPORT AD070315A-2**

**Study client:** **ADVANCED SKIN AND HAIR Inc.**  
Dr. Alan SHARGANI  
12121 Wilshire Blvd  
Suite 1012  
LOS ANGELES CA 90025  
USA  
E-mail: [alan@clearogen.com](mailto:alan@clearogen.com)

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The investigators and the author of this report hereby certify the validity of the data presented and attest their full agreement with the conclusions presented at the end of the report.

Certified by:

Name: Franck JUCHAUX

Position: Study director

Date: October 18<sup>th</sup>, 2007

Signature

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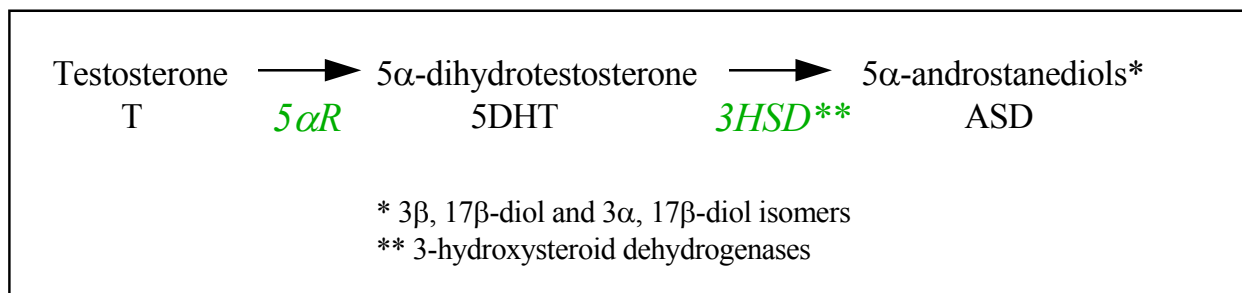
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## 1 - INTRODUCTION

ADVANCED SKIN AND HAIR, INC. has developed the compound **Clearogen Acne Lotion** for treatment of acne. Acne is a multiparametric skin disorder where sebum hypersecretion induced by circulating testosterone is involved. Testosterone is not the active form of this hormone. It is activated by  $5\alpha$  reductase which produces dihydrotestosterone, the efficient steroid. Inhibition of  $5\alpha$  reductase activity is known to reduce acne in human skin.

BIOalternatives performed this study in order to assess the effects of the test compounds on the metabolism of testosterone in reconstructed human epidermis. This model has been shown to be useful for the evaluation of inhibitors of this metabolism (*Bernard et al. 2000; Int. J. Cosm. Sci.,22, 397-407*).

The steroid  $5\alpha$ -reductase isoenzymes ( $5\alpha$ R) transform testosterone (T) into  $17\beta$ -hydroxy- $5\alpha$ -androstan-3-one ( $5\alpha$ -dihydrotestosterone, DHT). This reaction is crucial in the action of androgens.



## 2 - MATERIALS AND METHODS

### 2.1 Biological model

#### Reconstructed Human Epidermis (RHE)

- Tissues: 18 RHE (0.50 cm<sup>2</sup>, 10 days), batch n° 01015-31
- Culture: at 37°C and 5 % CO<sub>2</sub>
- Culture medium: differentiation medium

## 2.2 Test compounds and references

Test compound	Stock-solution	Dilution	Application
<b>Clearogen Acne Lotion</b> batch 0K085B (AD070315/1)	Cream supplied by the study promoter and stored at room temperature.	-	<b>topical at 3 µl/RHE</b>

Reference	Stock-solution	Dilution	Application
<b>Finasteride</b> batch 231664	10 <sup>-2</sup> M in ethanol	In water	<b>topical at 10<sup>-5</sup> M/RHE</b>
<b>Avodart®</b> batch 053721A	10 <sup>-3</sup> M in ethanol	In water	<b>topical at 10<sup>-6</sup> and 10<sup>-5</sup> M/RHE</b>

## 2.3 Testosterone

*Testosterone*: [4-<sup>14</sup>C] testosterone (Amersham B76, 54 mCi/mole, 2.35 nmole/epidermis). [4-<sup>14</sup>C] testosterone stock-solution was dissolved in ethanol and diluted in sterile water (1% ethanol final).

## 2.4 Treatment

The RHE were topically treated (or not, control) with the test compound or the references. Three RHE were used for each experimental condition.

After **24h of treatment**, the RHE were topically re-treated and incubated for **5 hours**. After incubation, the test compound and references were removed from the top of the RHE and 100 µl of the labelled testosterone solution were loaded on the *stratum corneum* of each RHE (127 nCi/epidermis).

After a 24-hour incubation period, the media underneath the RHE were collected for sterols analysis.

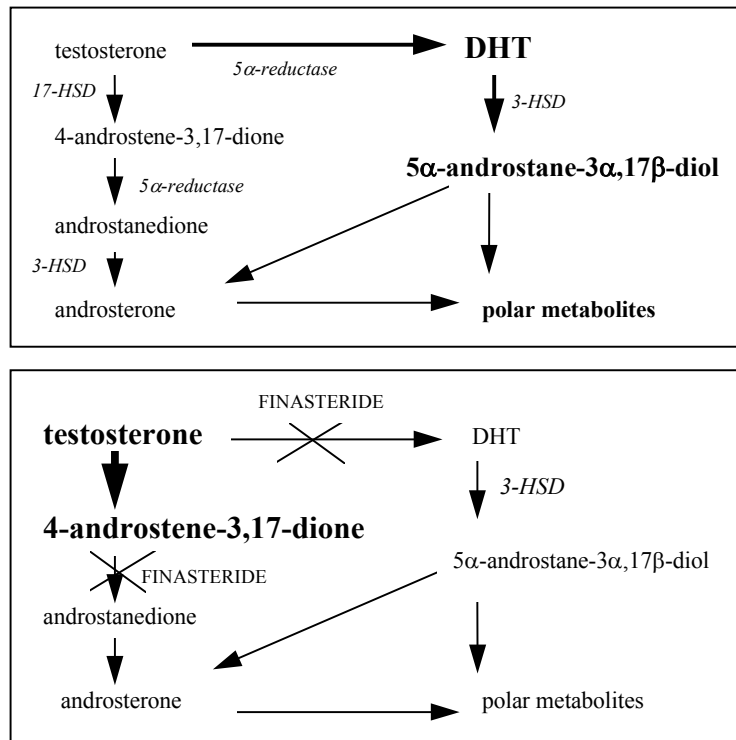
## 2.5 Extractions and analysis

*Transepidermal diffusion assessment*: the amount of testosterone that passed through the epidermal tissues was measured by liquid scintillation counting (LKB 1211 Rackbeta counter) of a fraction of culture medium.

*Metabolism analysis:* the steroid molecules from culture media were extracted by 2 volumes of chloroform/methanol (98:2) and dried. The various molecular species (testosterone metabolites) were separated by thin layer chromatography (TLC) on silica plates (RE/Silice, Whatman) in a solvent system containing dichloromethane, ethylacetate and methanol (85:15:3). The plates were autoradiographed and testosterone metabolites were quantified using a phosphorImager and specific software (Packard instrument).

### 3 - RESULTS AND CONCLUSION

#### Testosterone metabolism



Schematic simplified pathway for testosterone metabolism. Effects of finasteride (from Bernard F-X *et al.*, *Int. J. Cosmetic Science*, **22** 397-407 (2000))

#### Tables 1 and 2

##### **Untreated control:**

After 24h of culture, the rate of testosterone metabolism was very high.

Dihydrotestosterone (DHT) was clearly identified in the steroid profile. DHT was the major metabolite in the control epidermis. After 24h, about 74% of the deposited testosterone was converted into DHT. Other important metabolites were androstane-diols (e) and 4-androstene-3,17-dione (b).

##### **Effects of finasteride:**

**Finasteride** at  $10^{-5}$  M strongly inhibited the transformation of testosterone into DHT (67% inhibition compared to the control). Furthermore, as expected, finasteride decreased the amount of androstane-diols (e) and induced a strong accumulation of 4-androstene-3,17-dione (b) (Figure 1).

##### **Effects of dutasteride:**

**Dutasteride** at  $10^{-6}$  M and  $10^{-5}$  M strongly inhibited dose dependently the transformation of testosterone into DHT (respectively 80% and 86% of inhibition of the DHT production compared to the control). Furthermore, as expected, dutasteride decreased the amount of

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androstane-diols (e) and induced a strong accumulation of 4-androstene-3,17-dione (b) (Figure 1).

***Effects of Clearogen Acne Lotion:***

**Clearogen Acne Lotion** (5 mg/cm<sup>2</sup>) reduced the transformation of testosterone into DHT (49% of inhibition of the DHT production compared to the control) and of DHT into androstane-diols (e). Surprisingly the accumulation of 4-androstene-3,17-dione (b) was not visible.

**To conclude, Clearogen Acne Lotion clearly decreased the production of DHT and therefore could be used to treat acne.**

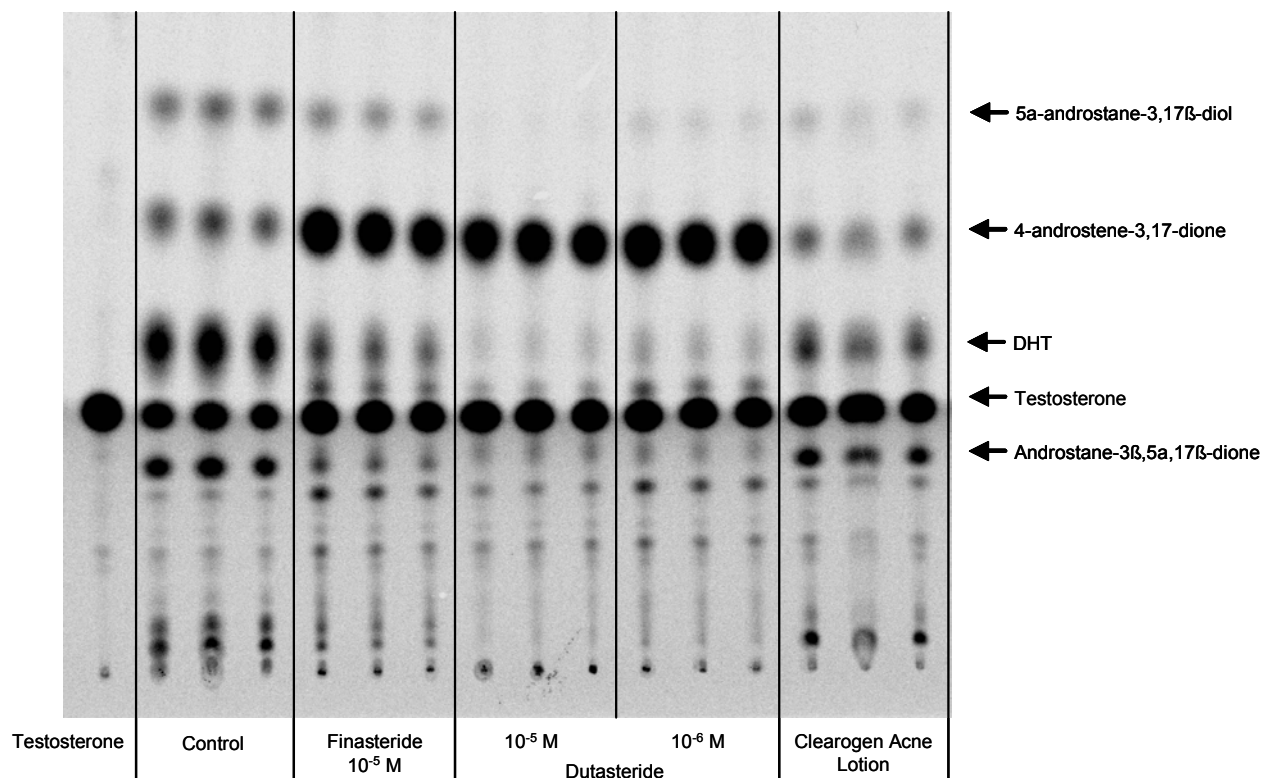


## 4 - TABLES AND FIGURES

**Table 1:** Diffusion of [<sup>14</sup>C]-testosterone (and metabolites) through RHE.

### Trans-epidermal diffusion (24h)

Treatment	Conc.	cpm	sd	n	% control	nmol steroid
Total testosterone	-	<b>326060</b>	-	1	-	2,35
Untreated control	-	<b>125307</b>	14977	3	<b>100</b>	0,9
Finasteride	10 <sup>-5</sup> M	<b>147747</b>	6335	3	<b>118</b>	1,1
Dutasteride	10 <sup>-5</sup> M	<b>150160</b>	10809	3	<b>120</b>	1,1
	10 <sup>-6</sup> M	<b>159213</b>	20653	3	<b>127</b>	1,1
Clearogen Acne Lotion	5 mg/cm <sup>2</sup>	<b>140640</b>	14980	3	<b>112</b>	1,0

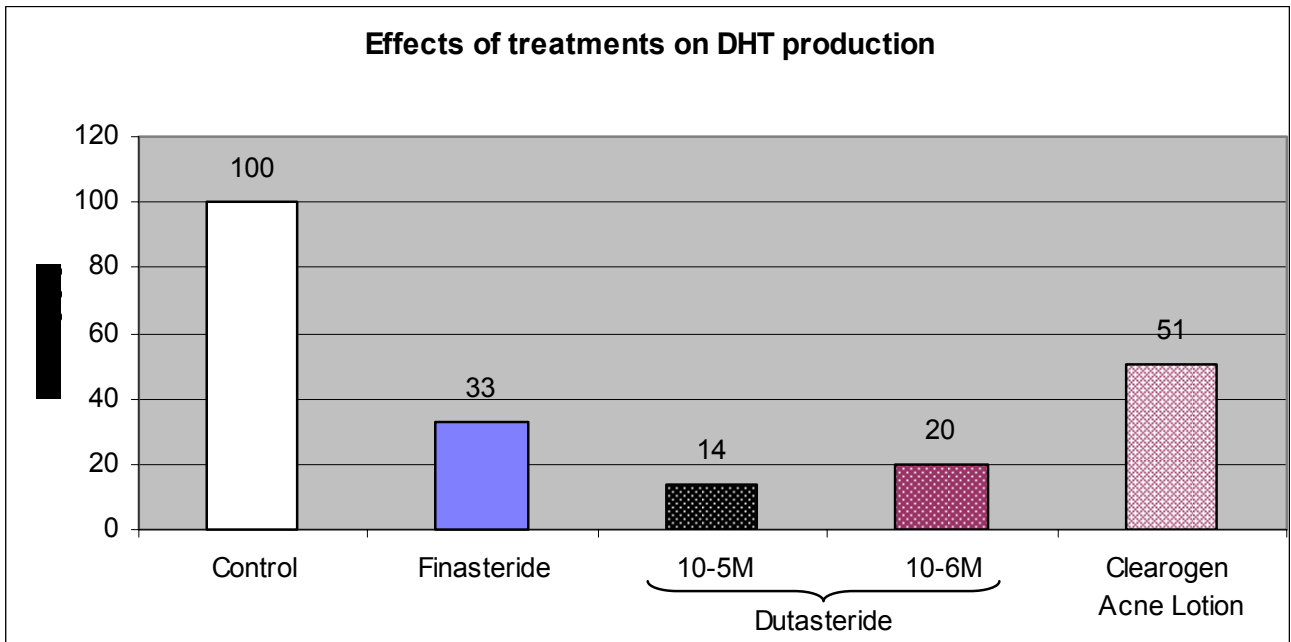


**Figure 1:** Thin layer chromatography and autoradiography of [<sup>14</sup>C]-testosterone and metabolites after transepidermal diffusion (24h).

**Table 2:** Effects of **Clearogen Acne Lotion** and the reference compounds on the production of testosterone metabolites. Instant Imager analysis of TLC in figure 2 (direct radioactivity measurement).

Treatment	Conc.	Lane n°	Total		Testosterone			DHT			DHT/testo
			AU	moyenne	AU	% total	moyenne (%)	AU	% total	moyenne (%)	ratio
Control	-	2	24474	<b>26091</b>	5821	23,8	<b>24,6</b>	4273	17,5	<b>17,5</b>	<b>0,71</b>
		3	29037		7177	24,7		5072	17,5		
		4	24763		6293	25,4		4375	17,7		
Finasteride	10 <sup>-5</sup> M	5	35114	<b>30507</b>	13282	37,8	<b>38,4</b>	3314	9,4	<b>9,1</b>	<b>0,24</b>
		6	30182		11691	38,7		2744	9,1		
		7	26227		10105	38,5		2325	8,9		
Dutasteride	10 <sup>-5</sup> M	8	24240	<b>25230</b>	11298	46,6	<b>46,6</b>	1222	5,0	<b>5,0</b>	<b>0,11</b>
		9	25088		11695	46,6		1282	5,1		
		10	26361		12264	46,5		1298	4,9		
	10 <sup>-6</sup> M	11	29444	<b>28162</b>	12800	43,5	<b>42,7</b>	1650	5,6	<b>5,8</b>	<b>0,13</b>
		12	28026		11772	42,0		1649	5,9		
		13	27017		11520	42,6		1567	5,8		
Clearogen Acne Lotion	5 mg/cm <sup>2</sup>	14	27795	<b>25257</b>	11660	42,0	<b>40,4</b>	2977	10,7	<b>11,4</b>	<b>0,28</b>
		15	24841		9985	40,2		2982	12,0		
		16	23135		9001	38,9		2661	11,5		

AU: Arbitrary Unit for radioactivity quantification



**Figure 2:** Effects of **Clearogen Acne Lotion** and the reference compounds on DHT metabolism.