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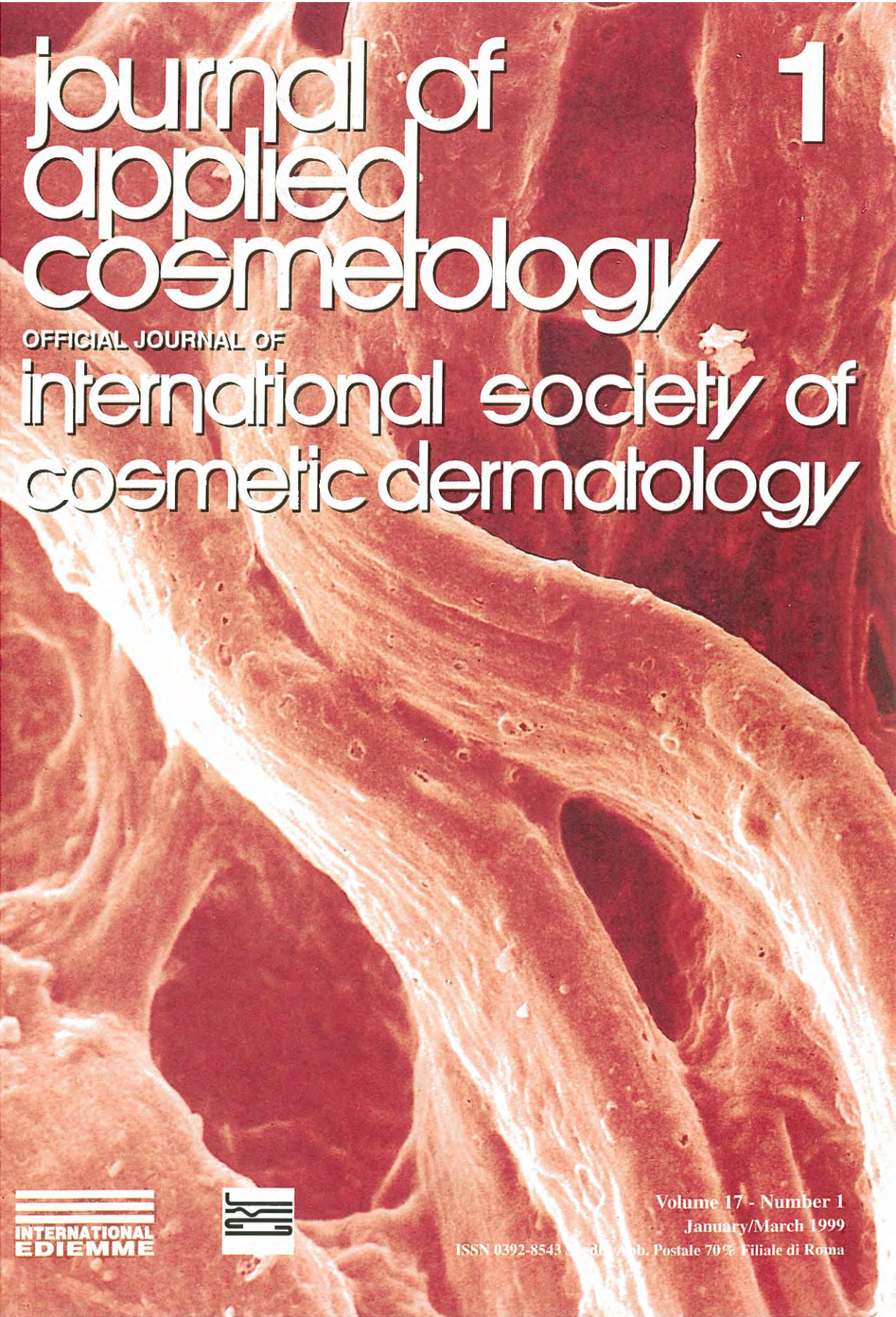

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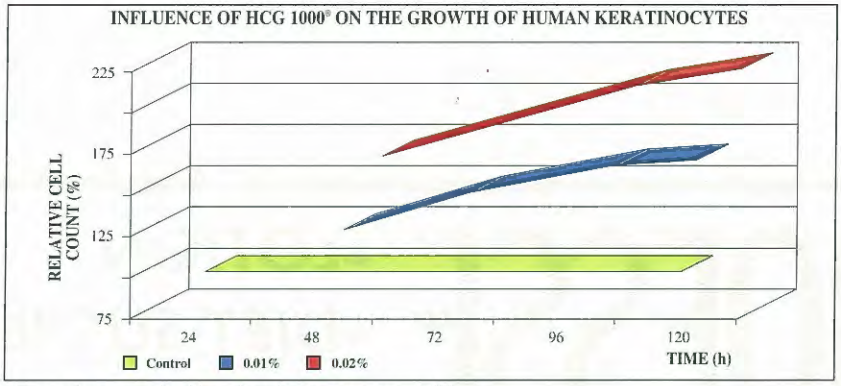

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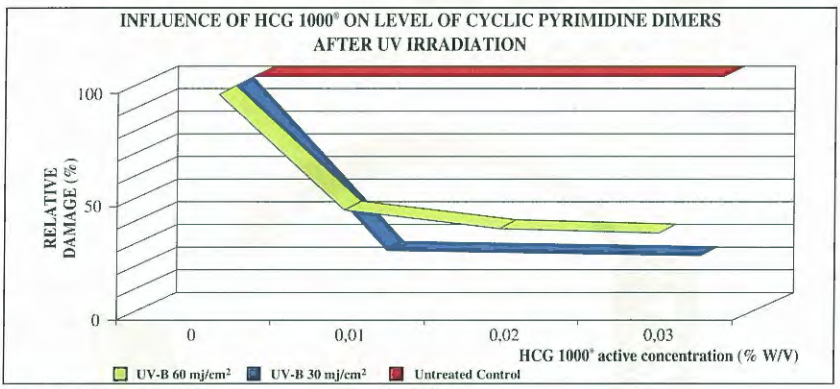

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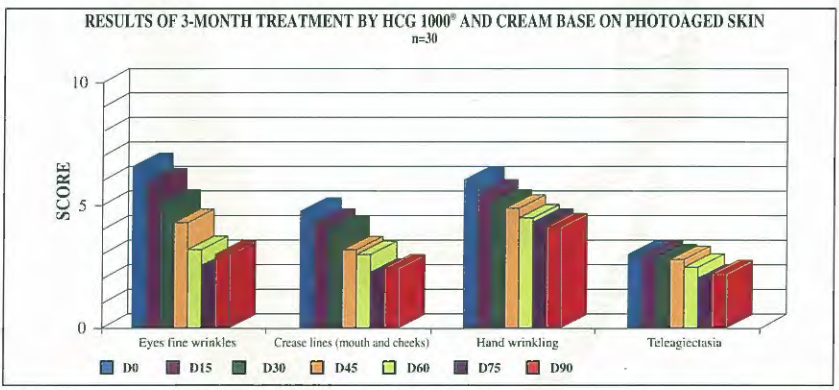
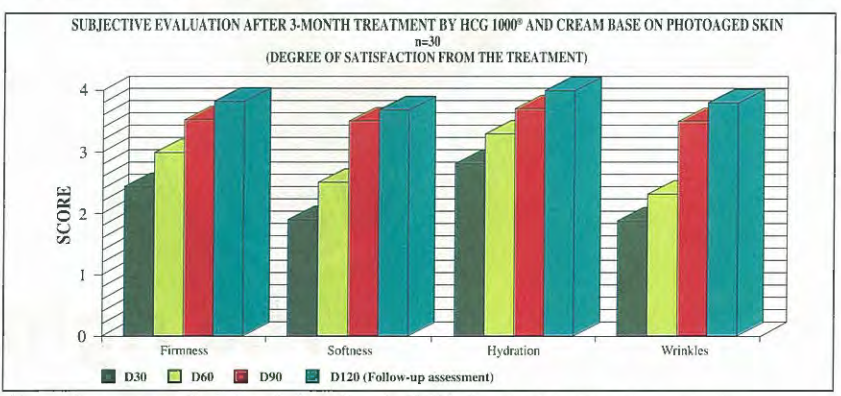


All p values are significant as to baseline (p<0.05) *(0 = No Signs of Photoageing • 10 = Many Signs of Photoageing)*



All p values are significant as to baseline (p<0.05) *(0 = Unsatisfaction • 4 = Satisfaction)*

REFERENCE: A. Di Pietro, G. Fabrizi, U. Giaroli, L. Tiberi, C. Bruno and P. Morganti (1998) Role of hyaluronic acid and vitamin C in photoageing • J.Appl. Cosmetol. 16, 125-133

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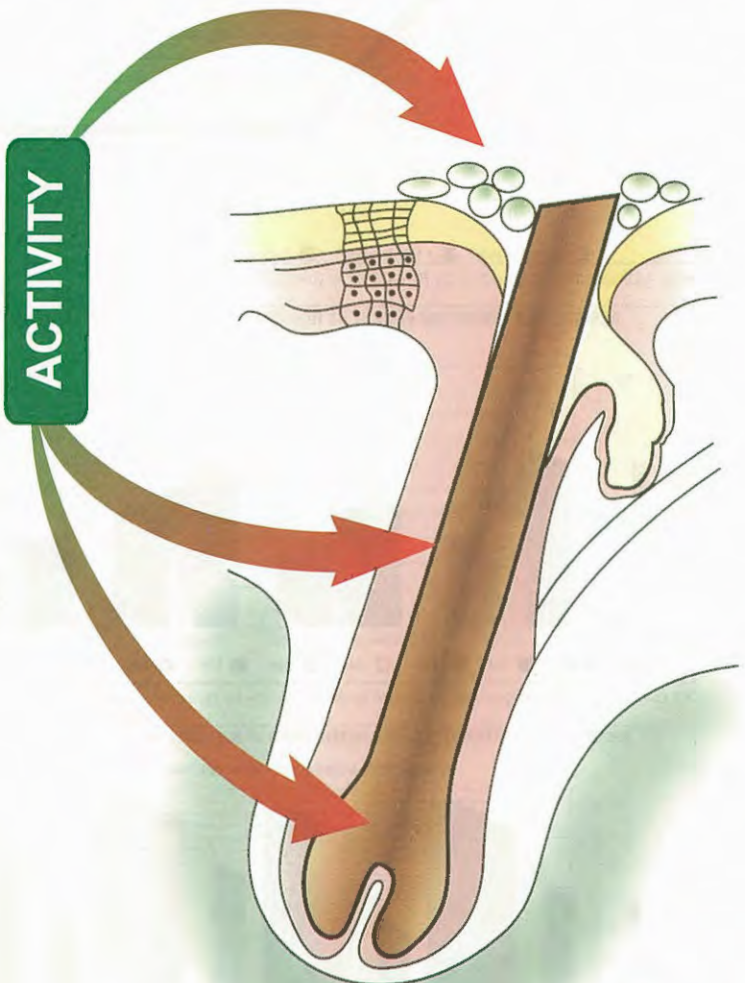

The right reply to an old problem

Clinical studies established efficacy with mild to moderate hair loss in the frontal - parietal scalp[1]

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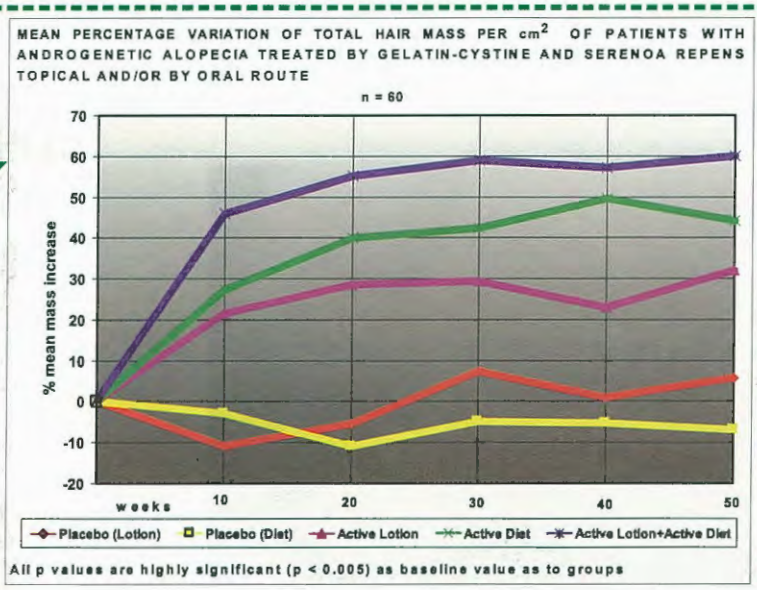
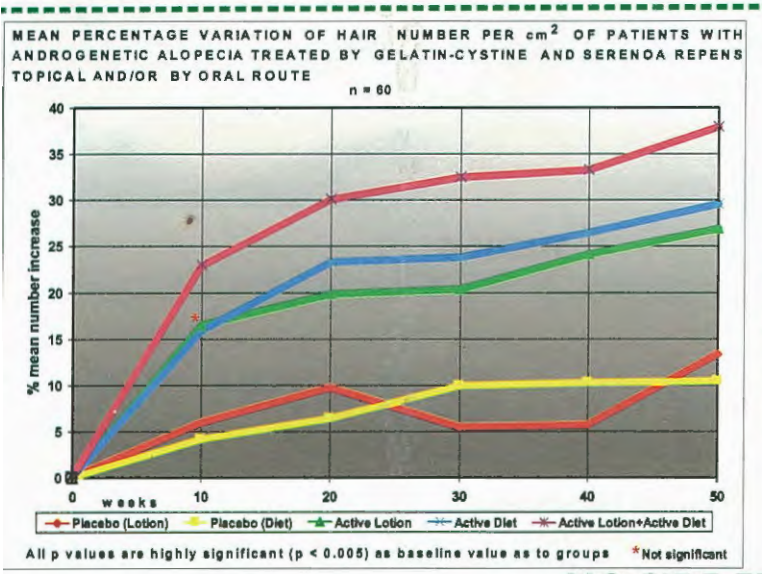


60%

increase in hair mass



38%

increase in hair number

NO SIDE EFFECT WAS RECORDED

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) Morganti P., Fabrizi G., James B., Bruno C. *J. Appl. Cosmetol.* **16**,57,1998

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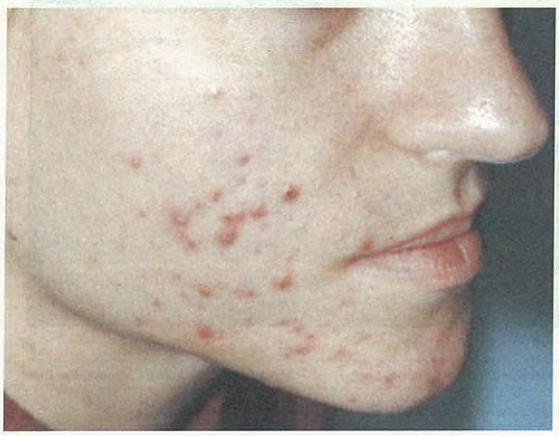

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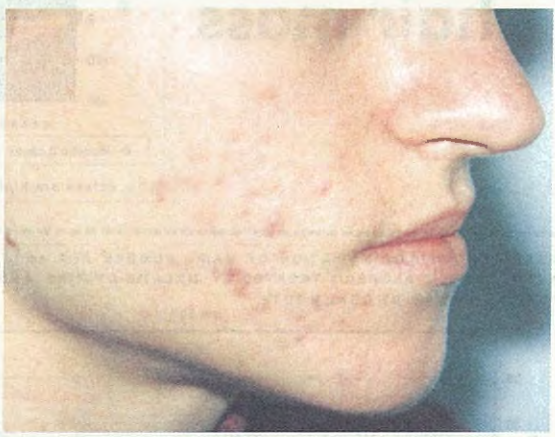

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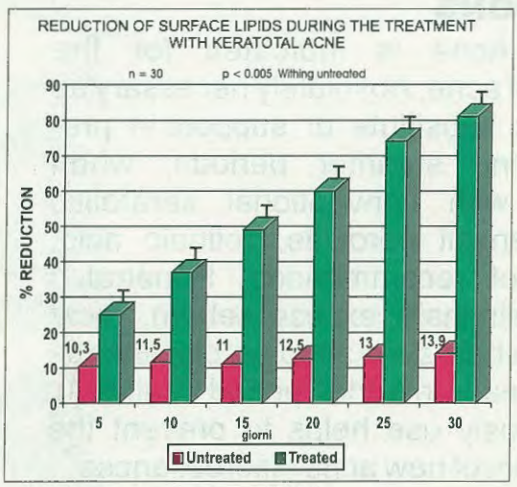
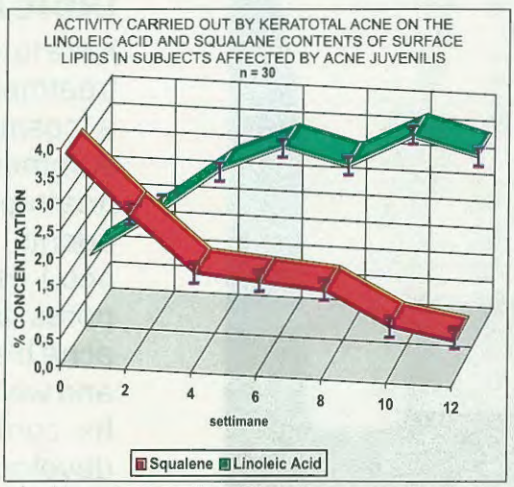
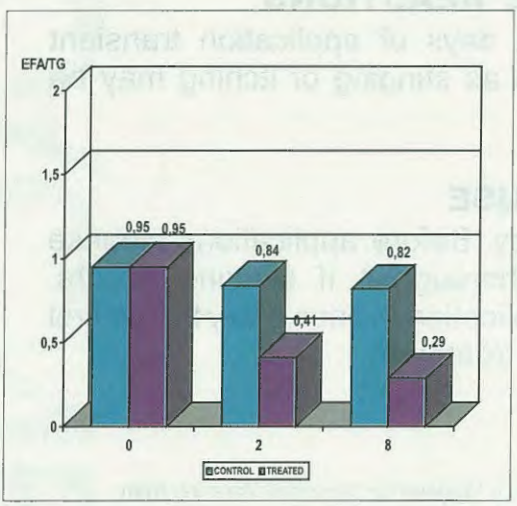


⇒ *Reduces excess lipids*



⇒ *Decreases the Squalene content of acne affected skin*



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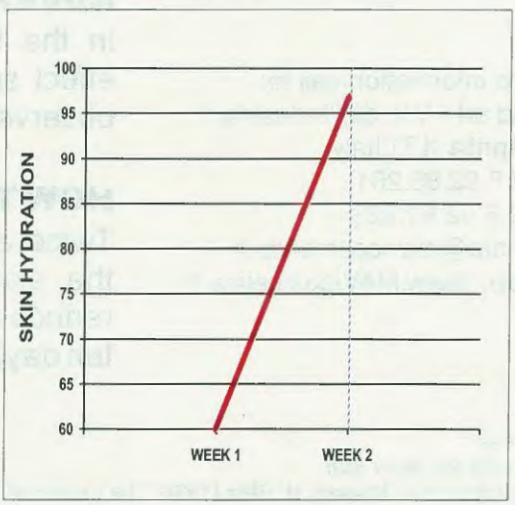

⇒ *Increases skin hydration by 97%*

Please see a brief summary of prescribing information on next page →

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DESCRIPTION

Keratotal Acne is a special fat-free lamellar phosphatidylcholine emulsion developed for the treatment of acne. It is delivered in a special phospholipidic-vehicle linoleic acid rich which contains glicolic acid and salicilic acid partially neutralized by a special patented blend of aminoacids

INDICATIONS

Keratotal Acne is indicated for the treatment of acne. Absolutely necessary as a cosmetic substitute or support in pre-summer and summer periods, when treatment with conventional keratolitic agents (benzoil peroxide, retinoic acid, ecc.) is not recommended. Penetrates pores to eliminate excess sebum, most acne blemishes, acne pimples, blackheads and whiteheads in a short period treatment. Its continously use helps to prevent the development of new acne efflorescences

ADVERSE REACTIONS

In the first days of application transient effect such as stinging or itching may be observed

HOW TO USE

Twice a day. Before applications cleanse the skin thoroughly; if stinging occurs, reduce application to once a day for the first ten days of treatment

REFERENCES:
1,2 - Data on file Mavi Sud
3 - **M. Ghiczy, H.P. Nissen, H. Biltz (1996)** The treatment of Acne Vulgaris by phosphatidilcholine from Soybeans, with a high content of linoleic acid. *J. Appl. Cosmetol.* **14,** 137-145

journal of applied cosmetology

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Quarterly Review of Cosmetic Dermatology

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2) Strehler BL (1977) Time, cells and aging 2nd edn. Academic Press, New York
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Contents

J. Appl. Cosmetol. 17, 1-9 (January - March 1999)

SAFETY EVALUATION OF PHYTOSPHINGOSINE AND CERAMIDES OF PHARMACEUTICAL GRADE

P. Morganti* and G. Fabrizi**

* President/Director, R. & D. - Mavi Sud S.r.l., Viale dell'industria, 1 - 04011 Aprilia (LT) - Italy
Dept. of Internal Medicine, Aesthetic Medicine Training School, University of Rome "Tor Vergata" - Italy
Secretary Generol, International Society of Cosmetic Dermotology (ISCD)

** Professor of Paediatric Dermotology, Dermotol. Dpt., Catholic University "Sacro Cuore", Rome - Italy

Received: *February, 10 1999*
Presented at The International Conference on "In Vitro Cytotoxicity Mechanism" January 25-27,1999 Istituto Superiore di Sanità, Rome, Italy.

Key words: *Phytosphingosine, Ceramides, Betacarotene, Vitamin C, Skin barrier, Photoageing, Skin hydration, Toxicological safety.*

Synopsis

Ceramides are the most prominent lipids found in the stratum corneum and play a fundamental role in mantaining in healthy condition the skin barrier. Their decreasing levels, securely impairing this barrier, are accompanied by skin dryness, scaling, roughness that lead to pathological conditions.
For these reasons ceramides are considered important raw materials of pharmaceutical and cosmetic use. The aim of this study was to evaluate the toxicological and dermatological safety of phytosphingosine and ceramide-identical 1, 2, 3A, 3B and 6 produced by fermentation methodology.
Raw material' toxicological safety was performed using Umes test, acute oral and dermal toxicity (rat), eye skin irritancy and skin sensitization (rabbit) and human skin membrane irritancy.
Safety and efficacy of the cosmetic preparation, containing ceramide 6 and phytosphingosine with Vitamin C or Betacarotene, was controlled in a double blind randomized study on 30 volunteers (female subjects, 37 to 53 years old) and evaluated during a three months period using both clinical scores and the 3C System instrumental method. According to EEC Criteria (DIR 93/21, 27/4/93) the controlled ceramides have no obligatory labelling requirements for oral and dermal toxicity, eye irritation as well as for skin sensitization. The cosmetic preparations studied did not lead to any unwanted skin reactions and a progressive and significant ($p<0.005$) increase of skin hydration (+87%), skin suppleness and firmness (from 23 to 42%) and fine wrinkle improvement (from 18 to 29%), with a TEWL decrease of 40% has been observed during the treatment period.

Riassunto

Le cerammidi rappresentano la parte predominante dei lipidi presenti a livello dello Strato Corneo. Una ridnzione del loro livello sicuramente influenza l'integrità della barriera cutanea che può apparire secca, fessnrata ed irritata, fino a raggiungere condizioni patologiche. Per queste ragioni le cerammidi sono considerate importanti materie prime d'uso sia farmaceutico che cosmetico.

Lo scopo di questo studio è stato di valutare la sicurezza tossicologica e farmacologica della fitosfingosina e delle cerammidi-identiche 1, 2, 3A, 3B e 6, prodotte per fermentazione batterica.
La sicurezza tossicologica di queste materie prime è stata valutata in-vitro mediante l' UMES Test, ed in-vivo, valutando la tossicità orale acuta, la tossicità dermica, quella oculare su animali, ed eseguendo le prove di irritabilità e di sensibilizzazione allergica sull'uomo.
La sicurezza e l'efficacia di un prodotto cosmetico finito, contenente cerammide-6 e fitosfingosina associate con Vitamina C o Betacarotene, è stata controllata a doppio cieco, su 30 volontarie donne di età compresa tra 37 e 53 anni, durante un periodo di 3 mesi, utilizzando sia la metodica del punteggio che il 3C System.
In accordo con i criteri europei (DR 93/21, 27/4/93) le cerammidi utilizzate non richiedono un'etichettatura particolare. Le creme cosmetiche studiate si sono rivelate sicure nell'uso ed hanno mostrato di incrementare rispettivamente del 87% ($p<0.005$) l'idratazione cutanea, dal 23 al 42% l'elasticità e di ridurre dal 18 al 29% le rughe sottili, riducento la TEWL del 40% circa.

INTRODUCTION

Ceramides, a special class of sphingolipids, are the most prominent lipids found in the stratum corneum and play a fundamental role in maintaining in healthy condition the skin barrier. Epidermal sphingolipids represent 7,3% of total lipid in the basal layers, increasing to about 15% in the stratum granulosum, 30% in lower stratum corneum, and reaching 40% in the other stratum corneum. (1,2).
Their decreasing levels, moreover, impair the skin barrier function involving skin dryness, scaling, roughness and various skin disorders (3-6).
Decreased levels of ceramides have been also correlated with increasing age and seasonal variation (7-9). For these reasons ceramides are considered important raw material of pharmaceutical and cosmetic use.

AIMS

The aim of this study was to evaluate the toxicological safety and the dermatological efficacy of different kind of ceramides (1, 3, 3A, 3B, 6) and phytosphingosine obtained biotechnologically by fermentation methodologies and, therefore, having the same molecular configuration as human ceramides.

MATERIAL AND METHODS

Materials

Phytoceramide 1, Ceramide 3, 3A and 3B, 6 and Phytosphingosine were of pharmaceutical grade (Cosmoferm Gist-Brocades group NL).

TOXICITY STUDIES AND SAFETY EVALUATION

All the studies were perfomed according to international accepted guidelines (OECD/EU) and are in compliance with the principles of Good Laboratory Practice (FDA/OECD). (10-12)
The following safety studies were carried out:

Ames Test

The compounds were tested in the Salmonella typhimurium reverse mutation assay with two or four histidine-requiring strains of Salmonella typhimuriu strains. Phytoceramide 1, was also tested in Escherichia coli reverse mutation assay with tryptophan-requiring strain of Escherichia coli WP2uvrA.
Two indipendent experiments were performed.
Negative and positive controls were run simultaneously with tests.

Acute oral toxicity

The compounds were administrated by oral gavage to three (or five) Wistar rats of each sex at 2000 mg/kg body weight (or 5000 mg/kg body weight, Ceramide 3).
Animals were subjected to daily observations and weekly determination of body weight. Macroscopic examination was performed after terminal sacrifice (day 8 or 15).
The result of this test is expressed in a LD50 value (lethal dose for 50% of test animals).
The obtained results are reported in Table I.

Primary skin irritation

Three rabbits were exposed to 0.5 grams of Ceramide, applied to the intact skin of the shaved area on one flank for 4 hours using a semi-occlusive dressing. Observations were made 1, 24, 48 and 72 hours after exposure.
The obtained results are reported in table 1.

Eye irritation

Single samples of the test substances were instilled into the eye of each of the three rabbits. Observation were made 1, 24, 48 and 72 hours after instillation.
The obtained results are reported in table 1.

Skin sensitisation

The potential of the compounds to cause delayed contact hypersensitivity in guinea-pigs was assessed

Table I

TOXICITY TESTS RESULTS

	Mutagenicity Ames Test	Acute oral toxicity	Primary skin irritation	Eye irritation	Skin sensitisation
Phito Ceramide	Non mutagenic SALMONELLA TYPHIMURIUM ESCHERICHIA COLI In presence and absence of S9 mix	No mortality Wistar Rat > 2000 mg/kg NO OBLIGATORY LABELLING REQUIREMENT	Non irritating Rat > 2000 mg/kg NO OBLIGATORY LABELLING REQUIREMENT	Light irritation Rabbits 28 mg NO OBLIGATORY LABELLING REQUIREMENT	No sensitisation Guinea-pig conc. 50% mg NO OBLIGATORY LABELLING REQUIREMENT
Ceramide-3	Non mutagenic SALMONELLA TYPHIMURIUM In presence and absence of S9 mix	No mortality Female Rats > 5000 mg/kg NO OBLIGATORY LABELLING REQUIREMENT	Non irritating Rabbits > 5000 mg/kg NO OBLIGATORY LABELLING REQUIREMENT	Light irritation Rabbits 27 mg NO OBLIGATORY LABELLING REQUIREMENT	No sensitisation Guinea-pig conc. 5/25% mg NO OBLIGATORY LABELLING REQUIREMENT
Ceramide-3A	Non mutagenic SALMONELLA TYPHIMURIUM In presence and absence of S9 mix	No mortality Rats > 2000 mg/kg NO OBLIGATORY LABELLING REQUIREMENT	Midly irritation Albino Rabbits > 5000 mg/kg NO OBLIGATORY LABELLING REQUIREMENT	Light irritation Rabbits 47 mg NO OBLIGATORY LABELLING REQUIREMENT	
Ceramide-3B	Non mutagenic SALMONELLA TYPHIMURIUM In presence and absence of S9 mix	No mortality Rats > 2000 mg/kg NO OBLIGATORY LABELLING REQUIREMENT	Non irritating Albino Rabbits > 5000 mg/kg NO OBLIGATORY LABELLING REQUIREMENT	Light irritation Rabbits 47 mg NO OBLIGATORY LABELLING REQUIREMENT	
Ceramide-6	Non mutagenic SALMONELLA TYPHIMURIUM In presence and absence of S9 mix	No mortality Rats > 2000 mg/kg NO OBLIGATORY LABELLING REQUIREMENT	Non irritating Albino Rabbits > 5000 mg/kg NO OBLIGATORY LABELLING REQUIREMENT	Light irritation Rabbits 51 mg NO OBLIGATORY LABELLING REQUIREMENT	
Phyto Sphingosine	Non mutagenic SALMONELLA TYPHIMURIUM In presence and absence of S9 mix	No mortality Rats > 2000 mg/kg	Non irritating Albino Rabbits > 5000 mg/kg NO OBLIGATORY LABELLING REQUIREMENT FOR PREPARATION CONTAINING 5% (v/v)	Severely irritation Rabbits 41 mg CARE SHOULD BE TAKEN WHEN HANDLING POWDER	

Table I.

by the Magnusson-Kligman Maximization Test. Test substance concentrations selected for the main study were based on the results of a preliminary study. The obtained results are reported in table 1.

HUMAN PATCH/TEST

The potential of the test compounds to cause irritation on the healthy human skin was assessed by the Human patch test. A total of 44 subjects (males and females) were subjected to the test substances in 5% vaseline solution. 0.1 g of the test sample was placed in a finn chamber and 24-hour occlusive application on the flexor side of the subject's upper arm was concluded. After 24 hours, the test sample was removed and the skin condition was examined, both 1 hour and 24 hours after removal.
Skin reactions as erythema and oedema were scored.

COSMETIC PREPARATION

A cosmetic formulation was formulated using liposomes composed of an high proportion of lipophilic phospholipids.
The liposomal fraction of the emulsion tested contained: ceramide-6 and Phytosphingosine, cholesterol, linoleic acid and ascorbic acid (active 1)[1] - cream A) or the same components plus betacarotene instead of vitamin C (active 2)[2] - cream B).

STUDY DESIGN

Thirty healthy volunteers (female subjects, age range 37 to 53) partecipated in the study. Each women had at least a moderate degree of photodamage as defined by an overall score of 5 with separate scores for each side of the face on a visual analogue scale 0 (none) to 10 (severe).
The number of wrinkling improvement were also counted. No topical retinoid use or other topical medications or cosmetic products were allowed for 1 month prior to study initiation.
The subjects were observed weekly for three months (september-november 1988) always by the same investigator. Each volunteer was supplied with two tube A (active 1) or B (active 2) together with a cleansing cream.
All were instructed to apply the test creams on their face twice a day (morning and evening) for three months and they were not allowed to use any other skin care product during the study period. Each subject was used as her own control; the test cream (A and B) being applied, always on a randomized basis, on the same right or left area of the face.

CLINICAL EVALUATION

Clinical evaluation were performed on the day 1 (baseline), and 2,4,6,8,10 and 12 weeks(end of treatment). Control of photoaging was controlled evaluating, by a visual analogue scale, the degree of fine wrinkles in the crow's feet area (lateral-periorbital area) and overall photoaging in the whole face right or left of the subjects. Finally the means for each criterion was calculated.
The obtained results are reported on figure 4.

1. Marketed under the name Idroskin C®/ Kera C®
2. Marketed under the name Kera A®

BIOPHYSICAL MEASUREMENTS

Skin surface lipids

The skin surface lipids were controlled by means of the computerized 3C System (13) (Dermotech, Roma, Italy). On the 1st day (baseline) and at 2,4,6,8,10 and 12 week. Determination is based on photometric measurement of light trasmission through a surface imprint obtained applying to the designed skin area a frosted plastic foil.
It allows adherence of skin lipids in a 1 cm2 area. The obtained readings (4 means values) automatically converted into μ g/ cm2 are reported in figure 1.

Skin hydration

Skin hydration of the horny layer was assessed by measuring electrical capacitance of the skin surface by the 3C System (13).
When the probe is applied to the skin (recording time 0.5 s.), the capacitance is displayed digitally in arbitrary 3C units. The results, expressed as means values of the measurements performed on 4 different right or left sites (cheek, forehead, chin and nose), are reported in figure 2.

Trans Epidermal Water Loss (TEWL)

Water evaporating from the skin (TEWL) was measured quantitatively by the 3C System methodology (13), after a 30-minute acclimatization period in a room at 22± 2°C and 50% humidity. Measurements were performed on the 1st day (baseline value) and after 2,4,6,8,10 and 12 week (end of treatment).
The TEWL probe consists of a cylindrical open chamber measuring system diameter 14mm. height 10mm. and skin area 0.95 cm2.
Two sensor units, containing thin capacitative film transducer are placed at 3 and 7 mm. distance from

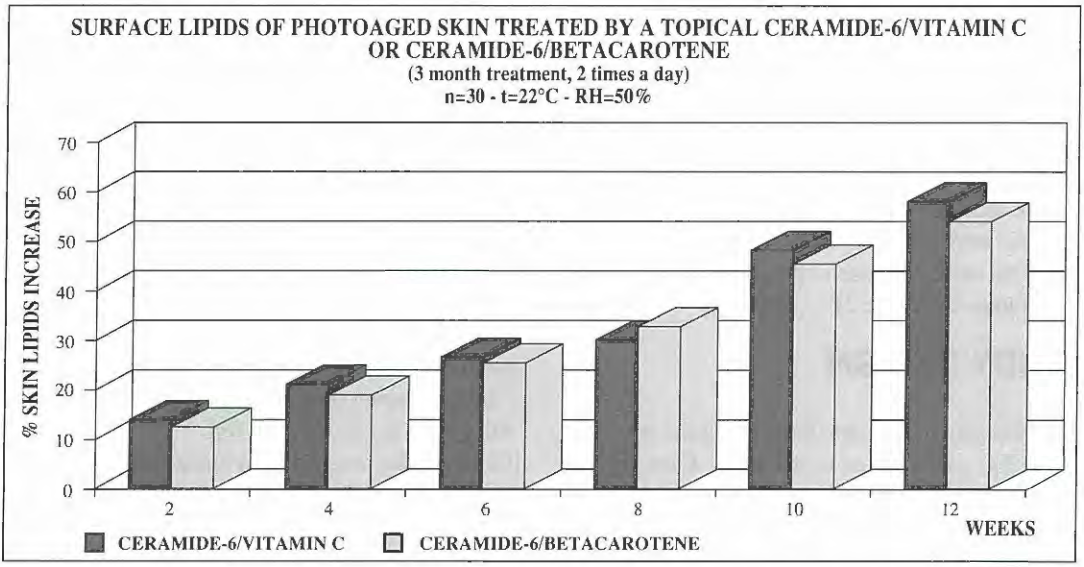

*Fig. 1. All **p** values are highly significant (p<0.005) as baseline values. **VITAMIN C/CERAMIDE-6** versus **CERAMIDE-6/BETA-CAROTENE** not-significant.*

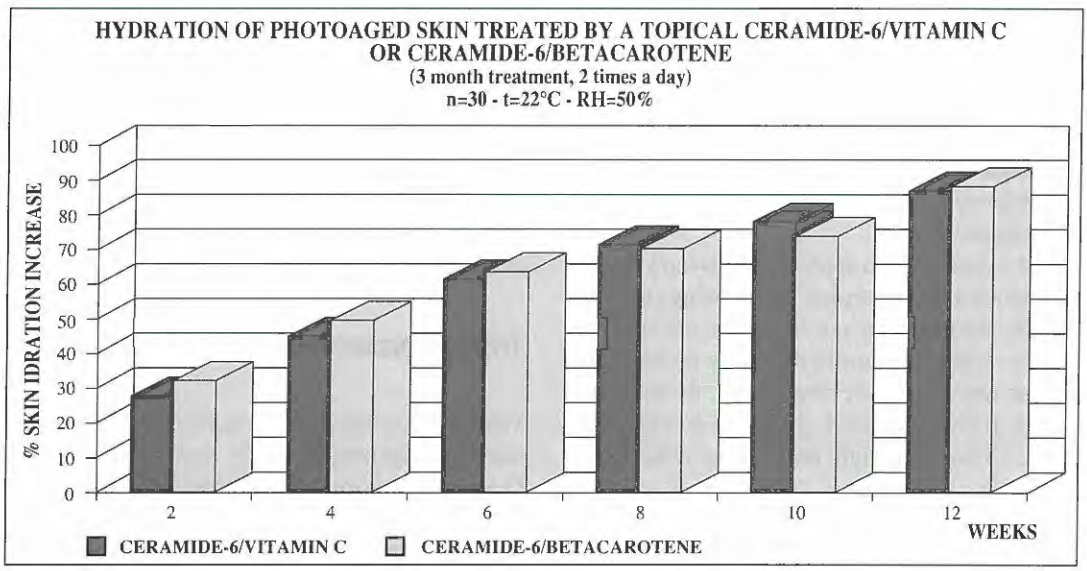

*Fig. 2. All **p** values are highly significant (p<0.005) as baseline values. **VITAMIN C/CERAMIDE-6** versus **CERAMIDE-6/BETA-CAROTENE** not-significant.*

the skin surface. TEWL is calculated automatically in g/ m2 h.

The obtained results are reported in figure 3.

Skin firmness

Skin firmness was evaluated measuring the skin elasticity by the use of a torsional equipment (14).(Der-

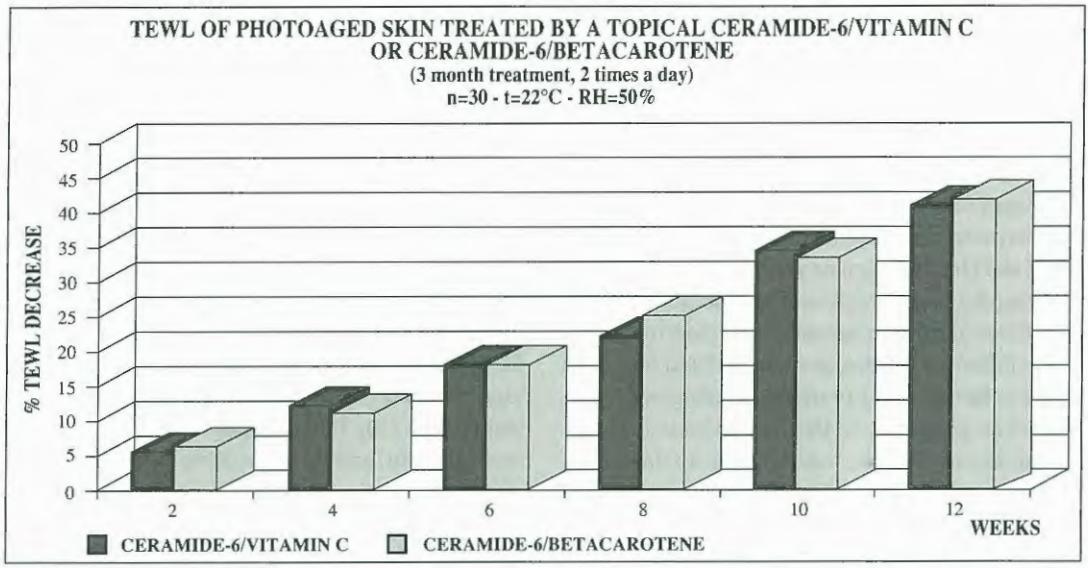

*Fig. 3. All **p** values are highly significant (p<0.005) as baseline values. **VITAMIN C/CERAMIDE-6** versus **CERAMIDE-6/BETACAROTENE** not-significant.*

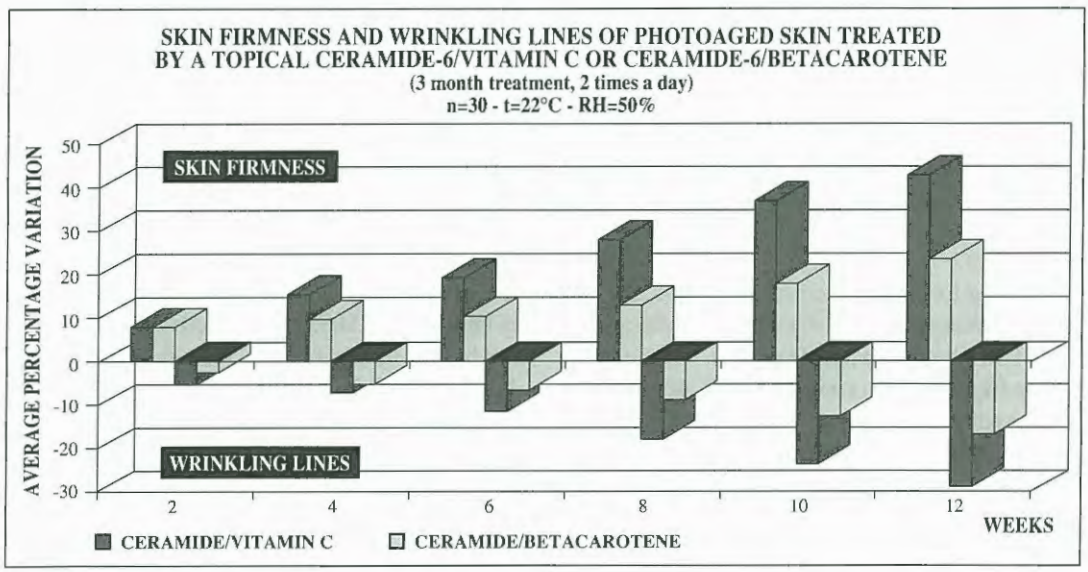

*Fig. 4. All **p** values are highly significant (p<0.005) as baseline values and as to groups. **VITAMIN C/CERAMIDE-6** versus **CERAMIDE-6/BETACAROTENE** is also significant (p<0.005).*

mal Torque Meter, Diastrum, Ltd, Andover U.K.) Torsional equipment acts through a disk glued to the skin, which is rotated by a motor powered by a controlled voltage, thereby loading the peripheral skin with a torque, the value of which can be evaluated.

The relative obtained signals are digitalized and a micro processor both computes the main parameters

and controls automatically the measurement phases. The obtained results are reported in figure 4.

RESULTS AND COMMENTS

None of the compounds tested was mutagenic in the Ames test.
Furthermore, the oral LD50 value of all ceramides tested and phytosphingosine was considered to exceed 2000 mg/kg body weight and the compounds were found not to be skin sensitisers or skin irritants.
The studied ceramides, in unformulated form, are found to be minimally or mildly irritating to the eye. However, according to the EEC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC), they do not have to be classified and have no obligatory labelling requirements for eye irritation.
Phytosphingosine (the pure, unformulated product), in the contrary, was considered to be a severely irritating to the rabbit eye.
Based on European legislation, however, preparations containing less that 5% (volume/volume) of Phytosphingosine need not to be classified and has no obligatory labelling requirement for eye irritancy (possible hazardous properties of other ingredients in the final preparation are not taken into account). See table 1.
According to the obtained results it appears also that ceramide-6 seems to be able to emiliorate the TEWL of photoaged people (fig. 3), when used in a right formulation in combine with l-ascorbic acid and or with betacarotene.
Moreover after the treatment with the emulsion A (Vitamin C-Cream) and B (Betacarotene-Cream), it has been obtained a significant ($p<0.005$) increase of about 30% of skin hydration still in the second week of treatment (fig.2). This observed amelioration increases of about 40% at the week 12. Same results have been obtained with the surface skin lipids which increase from 15 to about 60% ($p<0.005$) at week 12 (fig.1).
No differences have been noted between ceramide-6/vitamin C and ceramide-6/betacarotene treatment in TEWL, skin hydration or surface lipids.
However, both the clinical evaluation of the number of the present face wrinkles, and the elasticity measurements, set in evidence how the addition of vitamin C notably increases these parameters unlike what happens with the betacarotene (fig.4).
Probably because the ascorbic acid, penetrating quickly through the skin like recently shown (15), immediatly protects the cutaneous cells from the environmental pollutants and from the UV strenghtening its abilities in recovering.
Moreover, according to recent studies, it seems that vitamin C plays a key role in the formation of SC barrier lipids (16) The tolerability of both formulations was excellent, and no undesireble side effects were observed.
Therefore, the use of ceramides in the future formulations will be surely increased when the role of the skin lipids will be better understood together with the role of antioxidant vitamins.
Clarified these roles, it will be certainly easier to formulate proper vehicles able to rebalance an altered cutaneous barrier, and/or to allow an easier and selective cutaneous absorption of the active principles of cosmetic use.

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A RANDOMISED PLACEBO CONTROLLED DOUBLE-BLIND PARALLEL GROUP STUDY IN THE TREATMENT OF AGING SYMPTOMS OF THE SKIN.

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Key words: *Marine proteins, aging skin, placebo, skin elasticity, skin thickness.*

Synopsis

The present placebo controlled double blind study was carried out in 59 non smoking females with aging symptoms of the skin. Skingain©, a new preparation, containing marine proteins, vitamins and minerals, was given twice daily to 29 persons, and an identical placebo capsule was given twice daily to 30 persons in a randomised way. The study had a duration of four months. The two treatment groups were comparable at the outstart of the study with respect to the main parameters such as age and grade of aging symptoms of the skin.
Objective measurements, skin thickness and elasticity, as well as subjective observations in form of clinical parameters were used for evaluation of the effect of the treatments. In addition the participants made their own self evaluation of the effect. Photos were taken of all participants initially and after 2 and 4 months treatment.
The results show a significant improvement in skin quality both with regard to objective parameters as well as in subjective parameters after treatment with Skingain©. No significant treatment effects were detected after treatment with placebo. The participants self evaluation showed also a highly statistical difference in favour of the active treatment. The majority of the participants taking Skingain© would like to continue with the treatment after the study was concluded.
The tolerability of the treatments were excellent. Two patients in the active treatment group stopped the treatment due to rash like symptoms during the first two months of the study.

Riassunto

Il presente studio a doppio cieco e controllato con placebo è stato condotto su 59 donne non fumatrici che presentavano sintomi di invecchiamento della pelle. È stata somministrata una nuova preparazione denominata Skingain© e contenente proteine marine, vitamine e minerali. Skingain© è stato somministrato due volte al giorno a 29 persone, e contemporaneamente un'identica capsula placebo è stata data due volte al giorno a 30 persone in modo randomizzato. Lo studio ha avuto una durata di quattro mesi. All'inizio dello studio i due gruppi erano paragonabili per quanto riguarda i parametri principali quali età e livello dei sintomi di invecchiamento della pelle.

Misurazioni obiettive dello spessore e elasticità della pelle e osservazioni soggettive sotto forma di parametri clinici sono state usate per valutare gli effetti del trattamento. Inoltre i partecipanti facevano delle autovalutazione degli effetti. Sono state scattate delle foto di tutti i partecipanti all'inizio e dopo 2 e 4 mesi di trattamento. I risultati hanno mostrato un miglioramento significativo nella qualità della pelle sia con riferimento al paramentri oggettivi che a quelli soggettivi dopo il trattamento con Skingain®. Nessun effetto significativo è stato invece riscontrato dopo il trattamento con placebo. Anche l'autovalutazione dei partecipanti ha indicato una differenza altamente significativa a favore del trattamento attivo. La maggioranza dei partecipanti che ha assunto Skingain® vorrebbe continuare il trattamento anche dopo la fine dello studio.
La tollerabilità del trattamento si è dimostrata eccellente. Due pazienti nel gruppo di trattamento attivo hanno dovuto interrompre il trattamento a causa di prurito durante i primi due mesi dello studio.

INTRODUCTION

Several treatment opportunities are available for the antiaging treatment of the skin. Prescription drugs, cosmoceutic and cosmetic agents as well as food supplements are claimed to have antiaging effects on the skin. However, for most of these agents, at least for the last two groups, acceptable scientific efficacy and safety documentation is lacking. Launching highly priced preparations without effect are unethical to the consumers, and have therefore also been heavily criticised and even stopped by consumer authorities in some countries. One exception, however, is retinoic acid, which has been convincingly documented to have effect on aging skin symptoms in a number of well performed clinical studies (1-4). Retinoic acid is in most countries a prescription drug and thus not possible to advertise directly to the consumers or sell without a prescription from a physician.

The investigational preparation is a food supplement mainly based on marine proteins from a deep water fish living along the Norwegian coastline[1]. In addition the product is containing minerals and vitamins. In open studies the preparation has been shown to have a favourable effect on the aging skin. No controlled studies have so far been carried out. The tolerability of the preparation has been reported to be excellent, while it is well known that treatment with retinoic acid can give side-effect in some patients (4-10%) in form of soreness and redness of the skin (2). We decided, based on the above mentioned to carry out a controlled study to investigate the efficacy and tolerability of the preparation in females with aging symptoms of the skin.

MATERIAL AND METHODS

The study was carried out as a randomised placebo controlled double blind study in 63 non smoking females aged 35-70 years. Thirty four of the participants received the active preparation, while 29 were randomised to placebo. Blinding of the treatments was achieved by using identical appearing active and placebo capsules. The total treatment period was 4 months and the participants came to follow-up controls after 2 and 4 months.

The participants were asked to take two capsules per day (one in the morning and one in the evening) together with food and to swallow them with water. Each capsule contains 250 mg of the marine protein. All patients received verbal and written information about the aim of the study before they gave their informed consent to participate.

The aging symptoms of the skin were evaluated clinically by use of a five point scale. 0 = Absent, 1 = Very modest, 2 = Modest, 3 = Moderate, 4 = Pronounced.

The following symptoms were scored: fine wrinkles, coarse wrinkles, tactile roughness and teleangiectasia. On the two follow-up visits, after 2 and 4 months, the effect of the treatment was evaluated by using VAS (Visual Analogue Scales) of 10 cm with defined endpoints "no change" and "pronounced change" for the four symptoms listed above. An overall rating of the extrinsic aging was also performed using a 3 point scale; 0 = Modest, 1 = Moderate, and 2 = Pronounced. In addition, a global evaluation of the effect of the treatment was made also by using VAS with the same defined endpoints.

In addition, the patient themselves performed self evaluation by using VAS with defined endpoints "no change" and "very pronounced change" when returning to the follow-up visits.

Objective measurements of the skin thickness and skin elasticity were performed initially and at each of the follow-up visits using two measuring sites, right, and left sides of the face (lateral angel of the eyes). Each measurement was done in duplicate and the mean value was registered. In the following the mean values of the measurements on the right and left of the face are listed. Measurements were carried out with Dermascan A and Dermaflex (Cortex; Århus, Denmark).

In order to avoid interobserver variability all objective skin measurements were carried out by the same person.

Close-up pictures of the face of the participants were taken initially and at each of the follow-up visits under standardised conditions.

1. Marketed under the name Skingain®

Statistical methods

Mean was used for estimation of continuous and near - continuous variables and Student procedure was used for construction of confidence interval of mean. The one-sample t-test was used for analysing change over time within groups. Analysis of covariance and two-sample tests were used to compare between groups with regard to continuous variables. Categorical variables were reported using contingency tables. Fisher's exact test was applied when testing 2 x 2 tables. A Network Algorithm for Performing Fisher's Exact Test in r x c contingency tables was used when testing tables greater than 2 x 2.
A significance level of 5% was used in tests, and two-tailed tests were applied.

RESULTS

Subject population

A total of 59 subjects concluded the study according to the protocol, 29 in the active and 30 in the placebo group. Five persons, three on active preparation and two on placebo withdrew or dropped out of study at the first follow up visit. Two persons in the active group got skin rash, while one could not tolerated the taste of the capsules and stopped the treatment. The 2 persons on placebo did not show up at the control visit. These persons are not included in the statistical evaluation.
In table 1 the demographic as well as the global rating of the aging symptoms initially in the two treatment groups are shown.
As can be seen from the table the two treatment groups are comparable initially with respect to age and the severity of the aging symptoms. No statistical differences either in age or distribution of aging symptoms are detected between the groups.
The results of the global clinical evaluation are shown in table 2. Following a four months treatment period no significant differences in the global evaluation could be detected in the persons receiving placebo, while a significant effect could be seen in the group receiving the active preparation. It worthwhile to stress that this effect was more pronounced after four months than after two months as can bee seen from the table.

Objective skin measurements

In table 3 the measurements of the skin thickness and elasticity are presented respectively. When comparing the two treatment groups with regard to change in skin thickness, a significant increase between the initial value and the value seen after 4 months was found in the group treated with the active preparation, but not in the group treated with placebo. The difference in the increase in skin thickness between the groups is significant. The increase in skin elasticity in the active group was significant when comparing the initial value and the value after four months treatment. The change in skin elasticity in the same period was not significant in the placebo group. The dif-

Table I

Group	Age (yrs)	The grade of the aging symptoms (No of patients)		
		Modest	Moderate	Pronounced
Active	49,6 (9,2)	10	15	4
Placebo	47,0 (9,5)	11	14	5

Table I. Demographic data for the participants in the two treatment groups (SD in parentheses).

Table II		
Group	**After 2 months (cm)**	**After 4 months (cm)**
Active	5,0 (1,9)	7,8 (3,2)
Placebo	0,6 (0,7)	0,9 (0,8)

Table II. *Global assessment of the skin aging symptoms as function of the treatment duration using VAS (SD in parentheses).*

Table III				
	Active		**Placebo**	
	Skin thickness (mm)	**Elasticity %**	**Skin thickness (mm)**	**Elasticity %**
Initially	0,85 (0,12)	47 (8,1)	0,90 (0,15)	48 (7,6)
8 Weeks	0,97 (0,15)	50 (8,5)	0,89 (0,13)	47 (8,2)
16 Weeks	1,20 (0,17)	62 (8,4)	0,87 (0,12)	44 (8,5)

Table III. *Mean skin thickness and elasticity index of 59 females treated with active capsules or placebo for a period of 4 months (SD in parentheses).*

ference in the increase in skin elasticity was significant between the two groups.

Participant's assessment of effect

The self-evaluation of the effect of the treatment by the participants revealed a significant difference in favour of the active group after a treatment period of four months, as shown in table 4. Of the 29 persons in the active group, 20 persons felt that they saw an improvement in their skin quality on self inspection, while one of the 30 placebo treated persons felt that she had a small effect.

The 20 persons in the active group that had a positive effect of the treatment would like to continue the treatment while none of the placebo treated persons would like to continue.

Tolerability

The overall tolerability was good. Two participants in active group stopped the treatment due to rash and one because she did not like the taste and smell of the capsules. None of the persons on placebo reported any side-effects.

Table IV		
Group	**After 2 months (cm)**	**After 4 months (cm)**
Active	4,5 (1,7)	7,4 (2,7)
Placebo	0,2 (0,7)	0,3 (0,8)
p-value	< 0,001	< 0,0001

Table IV. *The results of the participants self evaluation of the effect of the active treatment and placebo as function of treatment duration on VAS (SD in parentheses).*

DISCUSSION

The results from this study indicate that the active capsules have a significant positive effect on the skin quality in middle-aged females with skin aging symptoms as compared to a placebo treated group. The effect of the treatment seems to develop over time being less pronounced after two months than after four months. By comparing the objective measurements of skin thickness and skin elasticity with the clinical observations and the participants own evaluations show that the efficacy of the treatment as revealed by the changes in these parameters is highly correlated. No pronounced effect of the active treatment can be expected before the treatment has been applied for two months or more, even if it was significant also after a treatment period of two months Even after 4 months a number of the participants in the active group (approx. 30%) could not detect any change in skin quality by self evaluation. However, by looking at this subgroup we found a smaller, but still a significant positive change in the skin thickness and elasticity, as compared to the group reporting a positive self evaluation. By comparing the age and the grade of aging symptoms we could not detect any significant differences between this group of none responders and the 20 persons responding to the treatment. We have no reasons to believe, after asking them about compliance to the intake of the preparation, that the none responders had not taken the preparation as recommended. It might be that they have to use the preparation for a longer time than the responders to obtain a similar effect. Although the mode of action of Skingain® is unclear, the results of the present study indicate that the preparation seems to have a favourable effect on degenerated elastic and collagen tissue of the dermis. Skingain® can therefore, based on its efficacy and excellent tolerability, be an attractive alternative in the treatment of aging symptoms of the skin.

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DERMATOLOGICAL APPLICATIONS OF OLIVE OIL

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Synopsis

In this paper the bibliography of dermopharmaceutical preparations with olive oil is reviewed. In recent years it is evident that it is mainly the unsaponifiable fraction that is used and that the ozonation of this oil has increased considerably, together with that of sunflower oil.

Riassunto

Nel seguente articolo viene fatta una revisione bibliografica di preparati dermofarmaceutici con olio di oliva. Negli ultimi anni si sono scoperte le proprietà della frazione non saponificabile dell'olio di oliva e i suoi usi. È stata aumentata inoltre la ozonificazione di questo olio con quella dell'olio di girasole.

INTRODUCTION

Within the realm of cosmetic preparations, unguents comprise an important domain given their marked protective and emollient nature for the skin. An ideal unguent is compatible with the skin, stable, permanent soft, inert, and non-irritating. Oleaginous unguents contain fixed oil of vegetable origin in their composition.

The cosmetic use of vegetable oils can be complicated and in many cases there is a possibility of their acting as "attractives" that supposedly have surprising functions in cutaneous corrections (1).

The cosmetic interest of these vegetable oils resides primarly in obtaining the unsaponifiable fraction, since in topical applications this fraction activates the cutaneous metabolism in the zone of application, producing an emollient, hydrating, dermoprotective, and photoprotective effect (2).

Table 1 gives the unsaponifiable content for various oils employed in cosmetics. In general, those most used are the least expensive ones and those with a high unsaponifiable propotion of the fat being extracted.

Olive Oil, apart from the interest in it due to its aforementioned high unsaponifiable fraction, can be found in many dermatological preparation, as well as in the form of ozonated olive oil, obtained by combining it with ozone.

Table I

UNSAPONIFIABLE CONTENT

Karité Lard	3,5 - 12
Avocado Oil	3,0 - 10
Soya Oil	0,5 - 1,5
Sesame Oil	1,0 - 1,5
Corn Oil	1,5 - 3,0
Rice Oil	1,0 - 3,0
Olive Oil	0,6 - 1,2
Peanut Oil	0,2 - 0,9
Castor Oil	0,3 - 0,8
Coconut Oil	0,1 - 0,3

OLIVE OIL: PHARMACEUTICAL AND COSMETIC APPLICATIONS

Olive oil considered officinal in most pharmacopoeias (3) is a slightly yellow liquid with a characteristic odour that easily goes rancid. It comprises distinct acids, such as oleic (65-80%), palmitic (7-20%), linoleic (4%), estearic (2-4%), miristic (1%) and occasionally lauric and arachidonic acids (4, 5).

Olive oil is employed in the fabrication of many pharmaceutical preparations (6, 7) and cosmetics (8, 12) due to its unsurpassable quality in soap, creams, skin oils, liniments, and sunscreens preparations (13). It is also used in the preparation of Salicylic Oil and Camphorated Oil (14).

OZONE AND OLIVE OIL

Ozone is a contaminating agent that can generate unsafe oxidizing compounds. It has been known as a powerful oxidant since its discovery by Christian Friedrich in 1840, capable of producing adverse effects in humans when inhaled in appreciable amounts. In small doses it has been shown to reduce the ventilatory function, increasing the permeability and reaction of the respiratory tree. It has also been observed to increase the endogenous mediators in inflamed cells.

It has been demonstrated that the damage caused by ozone when inhaled is directly related to the arachidonic acid of the cellular membranes in the lung, producing an increase in the leukotriene levels. Other researchers correlate exposure to ozone with a rise in the infiltration of neutrophils, inflammatory mediators, and cytokines.

Ozone passed through olive oil is converted into ozonid, the polymer of a long chain. Ozonated olive oil is thus obtained, with a final semisolid consistency, like vaseline, but preserving the odour of ozone.

The process consists of sending a continuous flow of gas through the oil and takes several days (15). Ozonated olive oil is stable when kept under refrigeration, maintaining nearly all its potency in spite of having no stabilizers or preservatives. It can also be obtained as "Oil Sticks", or small cylinders, for implants. The amount of oil need is cut off and the implant is ready for use (16).

BIOLOGICAL ACTION AND THERAPEUTIC PROPERTIES OF OZONATED OLIVE OIL

Ozonated vegetable oils contain a mixture of chemical compounds with considerable germicidal effect. The results of topical applications in dermatological diseases due to viruses, fungi, bacteria, etc. have been studies for years.

Ozonated Olive Oil has been utilized for skin treatments since the end of the 19th century, being particularly popular in the 1950s, which saw the appearance of many articles on the subject of its medical applications.

Currently, it is used in the treatment of both contact skin inflammations (dermatitis) and of atopical ones such as seborrhoea, etc. It has also been shown to be of use in skin infections caused by *Staphylococcus*, as well as in cellulitis, impetigo, folliculitis, furuncules, and carbuncies, infections of the sweat glands and of the nail bed, and in numerous fungal infections such as "athlete's foot", primarly due to *Epidermophyton*, *Candidiasis* and *Pityrosporum*. Its main use is undoubtedly for infections produced by herpes simplex.

Also worth noting is its employment in the treatment of acne and other skin-related problems such as psoriasis or inflammations caused by intoxication of a drug. It is also used to prevent secondary postoperative infections. Finally, Cuban researchers (Ozone Research Center, La Havana) have used it in capsules for treating gastroduodenal ulcers, gastritis, etc. (16).

OZONE AND SUNFLOWER OIL

"OLEOZON", wich is ozonated sunflower oil, deserves special mention.

Cuban investigators (op. cit. 16) have mainly used it, as it is more economical and easily obtainable. OLEOZON has a different consistency since olive oil has a spiral structure that allows the ozone to place itself in the free spaces in the spiral.

OLEOZON has been employed to treat alveolitis (17) , dyschromia (18), gynaecological problems, subprosthesic estomatitis (19), giardiasis (20, 21), pioderma (22), haemorroids, gingivostomatitis and dermatological problems (23).

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SKIN DISEASES DUE TO INCREASED ANDROGEN SENSITIVITY

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Synopsis

In an inherited genetic hypersensitivity, normal levels of androgens may cause cosmetic disturbances or even dermatological diseases: acne and alopecia. The cosmetic dermatologist is called for help.
Androgenetic stimulation leads to increased levels of sebum, to an imbalance of lipids on the skin surface, to follicular hyperkeratosis and, finally, to the formation of comedones. In those patients with hypersensitive sebocytes, only cosmetic disturbances are present at the beginning but a rapid progression towards an embarassing skin disease with severe symptoms in the face is possible. An aggravation of Acne comedonica into Acne papulopustulosa and Acne conglobata has to be considered in each case. Therapy starts with adequate skin cleansing and comedonolytic skin care, e. g. with topical adpalene, and may continue in severe cases with oral isotretinoin. In females, antiandrogens may be given to reduce the androgenic stimulation of sebocytes.
Other patients reveal high levels of androgen receptors and testosterone-activating steroid-5α-reductase in the frontal areas and in the vertex of the scalp (balding areas). As a consequence, androgenetic alopecia develops. Although hair loss is generally regarded as a cosmetic disturbance, the patients afflicted from this disease suffer from their reduced self-esteem and their impaired psychological and social well-being. This matters for women and men, especially for young men. Androgenetic alopecia is a disease that needs help, from the doctor, from the pharmacist, and, on the first hand, from the cosmetic dermatologist. But only suppressive measures are known today. Topical minoxidil or topical 17α-estradiol will stop hair loss in the vast majority of cases. Life-long treatment may be necessary, however. Only recently, oral finasteride has been shown to be highly effective in suppressing hair loss in androgenetic alopecia. However, the undesirable actions of this drug warrant a most critical and cautious application.

Riassunto

Nella ipersensibilità genetica ereditata, i normali livelli di androgeni possono provocare lievi alterazioni cutanee o addirittura malattie dermatologiche di acne e perdita di capelli. Si ricorre, allora, all'aiuto del dermatologo-cosmetologo.
La stimolazione androgenica porta ad un aumento dei livelli di sebo, ad uno squilibrio dei lipidi della

superficie cutanea, alle ipercheratosi follicolari ed infine, alla formazione di comedoni. Nei pazienti con sebociti ipersensibili, all'inizio si verificano soltanto lievi alterazioni cutanee, che possono rapidamente progredire verso una vera e propria patologia della pelle, con evidenti sintomi soprattutto sul viso. In ogni caso, ci si deve aspettare un'aggravarsi dall' acne comedonica all'acne papulo/pustulosa o all'acne conglobata.

La terapia comincia con un'adeguata pulizia della pelle e con un trattamento comedolitico, quale ad esempio l'adapalene topico, e può continuare, nei casi gravi, con isotretinolo orale. Nelle donne, possono essere somministrati antiandrogeni per ridurre la stimolazione androgenica dei sebociti .

Altri pazienti mostrano di avere alti livelli di recettori androgeni della 5α-reduttase che attiva il testosterone nei follicoli delle aree frontali e sulla sommità della testa (aree calve). Come conseguenza si sviluppa l'alopecia androgenetica.

Sebbene la perdita dei capelli generalmente sia ritenuta un distnrbo di carattere cosmetico, i pazienti che ne sono colpiti soffrono di una ridotta autostima che minaccia il loro benessere psicologico e sociale. Ciò interessa l'uomo, la donna, ma specialmente i ragazzi.

L'alopecia androgenetica è un disturbo che richiede l'aiuto del medico, del farmacista e, in primo luogo del dermatologo-cosmetologo. Ma tutt'oggi sono conosciute soltanto metodologie soppressive. Minoxidil topico o il 17α-estradiolo topico sono in grado di fermare la caduta dei capelli nella maggioranza dei casi, senza però eliminare il problema. E' necessario utilizzarli per tutta la vita. Solo di recente, il finasteride orale ha dimostrato di essere molto efficace nel sopprimere la caduta dei capelli provocata dall'alopecia androgenetica.

Comunque, gli effetti indesiderati di questo rimedio farmacologico richiedono una applicazione consapevole e cauta.

INTRODUCTION

Due to genetic factors, two types of skin cells may reveal an abnormally high sensitivity to androgens. Even normal levels of testosterone may provoke specific disturbances: the stimulation of sebocytes leads to acne, the stimulation of dermal papilla hair root cells causes androgenetic alopecia.
In figure 1, the pathways of androgenetic stimulation of the two types of skin cells is depicted. Testosterone from testes, ovaries and adrenal glands must be converted into dihydrotestosterone in order to act on the androgen response element of nuclear DNA. This conversion is effected by a specific enzyme, the steroid- 5α-reductase. Two isoenzymes exist (22): type I in skin and sebocytes, type II in hair root cells and prostatic glands. Inhibition of steroid-5α- reductase as well as the presence of antiandrogens protect sebocytes and hair root cells from androgenic stimulation. Both modalities are used for therapeutic purposes.

ACNE

Acne is one of the most frequent skin diseases, especially in the age group between 12 and 24 years (incidence rate 80%). The most important pathogenetic factor in acne is the androgen-induced increase in sebum production which leads to an imbalance of lipids on the skin surface (Figure 2). Furthermore, an abnormally high adherence of epithelial cells lining the follicles provokes follicular hyperkeratosis. Microcomedones and comedones are formed. In this anaerobic medium numerous Propionibacteria grow the products of which pass the follicular walls. Chemotactic factors, enzymes and antigenic proteins evoke dermal inflammation: acne papules develop. - " Body builder acne " following the ingestion of anabolic hormones is an excellent proof for the importance of androgens in the pathogenesis of acne. Stress aggravates acne by inducing the production of adrenal hormones.
Therapy of acne in its four stages is directed against four pathogenetic factors : androgen- induced sebum production, follicular hyperkeratosis, bacterial growth, and inflammation (9, 10, 18).
In females, the oral administration of antiandrogens may reduce the high rate of sebum secretion due to androgenic stimulation. Cyproterone acetate or chloromadinone acetate were used preferably under the form of a contraceptive pill. It must be mentioned that cyproterone acetate inhibits steroid-5α-reductase in the sebaceous follicles (10). Spironolactone in daily doses between 100 and 200 mg is less effective in acne of females than the two other antiandrogens mentioned above. For the protection of a male fetus, females under antiandrogenic treatment must use an effective contraceptive. - So far, no positive results were obtained with topical applications of cyproterone acetate. Maybe the use of a liposomal lotion will improve the efficacy.
Systemic administration of estrogens is ineffective in acne. Retinoids are the most potent and most widely used anti-acne drugs. They reduce sebum production to about 10% of its original value, sebaceous glands decrease dramatically in size, proliferation and differentiation of epithelial cells is normalized (action on α-and γ-retinoic acid receptors) (1, 22).
Furthermore, it has been shown that retinoids inhibit steroid-5α-reductase (20), another antiandrogen and antiacne effect of this class of drugs.
In acne therapy, retinoids were used systemically and topically. 13-cis-retinoic acid is administered orally in doses of 5 mg/d/kg bodyweight over at least six months. In topical acne therapy adapalene has displaced all -trans-retinoic acid (tretinoin, vitamin A-acid). Adapalene exhibits far better tolerability, higher stability and somewhat more favorable biochemical- pharmacological actions than tretinoin. Adapalene is used as 0.1% gel (1, 22).
Steroid-5α-reductase is an important enzyme for the elicitation of androgenic effects. Inhibition of this enzyme is provoked by cyproterone acetate and by retinoids, both columns of antiacne treatment. Oral finasteride and 17α-estradiol (cf. later) have not been tried in acne.

ANDROGENETIC ALOPECIA

Androgenetic alopecia may occur in women and

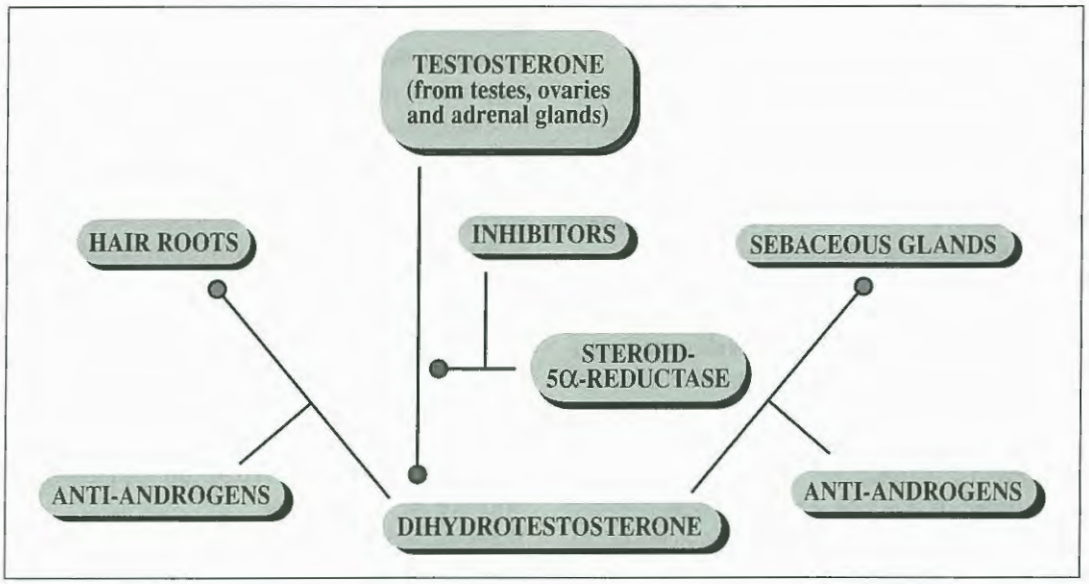

Fig. 1. Androgenic Actions on Skin Cells

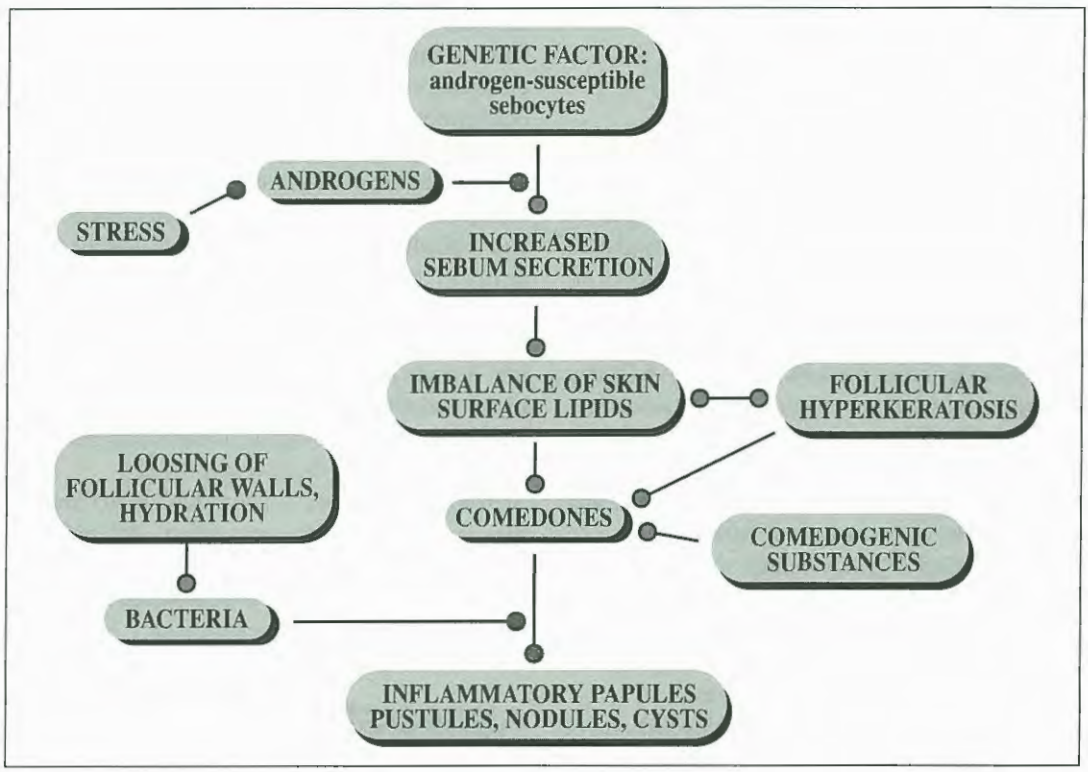

Fig. 2. Pathogenesis of Acne

men as a result of the effects of androgens on the genetically predetermined hair follicles. In Caucasians, the prevalence of this disease (" disease" according to the classification of the World Health Organization) approaches 100%. Although androgenetic alopecia is regarded as a normal variant of aging of most individuals, it causes psychological distress, especially in women and in young men. Hair root cells belong to the mitotically most active cells of the organism. The daily production of 20 to 30 m of hair is a great achievement. But on the other hand, hair root cells are classed with the most sensitive cells. The sensitivity against androgens leads to a gradual replacement of the large, pigmented hairs of the scalp by fine, insignificant hairs.

In recent years, this specific sensitivity of the balding hair dermal papilla cells has been the target of biochemical investigations. It was shown that these cells contain significantly higher androgen receptor levels than cells from non-balding areas (5, 22). Details are shown in figures 3 and 4. Furthermore, androgen-sensitive areas of the scalp possess more steroid-5α-reductase (type I and II) than the occipital regions where balding due to androgenetic alopecia never occurs (22). Details are shown in figure 5. It should be stressed that these biochemical differences between balding and non-balding areas are present in men and women.

Steroid-5α-reductase seems to play an important role in the pathogenesis of androgenetic alopecia:

- in men and women, balding areas of the scalp exhibit increased levels of steroid-5α-reductase
- in genetic deficiency of steroid-5α-reductase, no androgenetic alopecia develops. However, an analysis of the pertinent genes failed to show any association (2)
- a strong association was observed between male baldness and benign prostatic hyperplasia (12)
- inhibitors of steroid-5α-reductase may stop the continuous hair loss in men and women.

Finally, a last enzyme has to be mentioned which shows different activities in balding and non-balding areas, namely aromatase. This enzyme converts testosterone into estrogens. In men and in women with androgenetic alopecia, balding areas reveal significantly lower activities of aromatase (22). Details are shown in figure 6. – No therapeutic consequences resulted from this observation, so far.

The genes encoding the two steroid-5α-reductase isoenzymes are not associated with the appearance of androgenetic alopecia (2). A polygenic etiology should be considered, involving other enzymes and steroid receptors. Steroid-5α-reductase plays an important role as outlined above but the high activity of this enzyme can not be regarded as the only underlying cause of androgenetic alopecia.

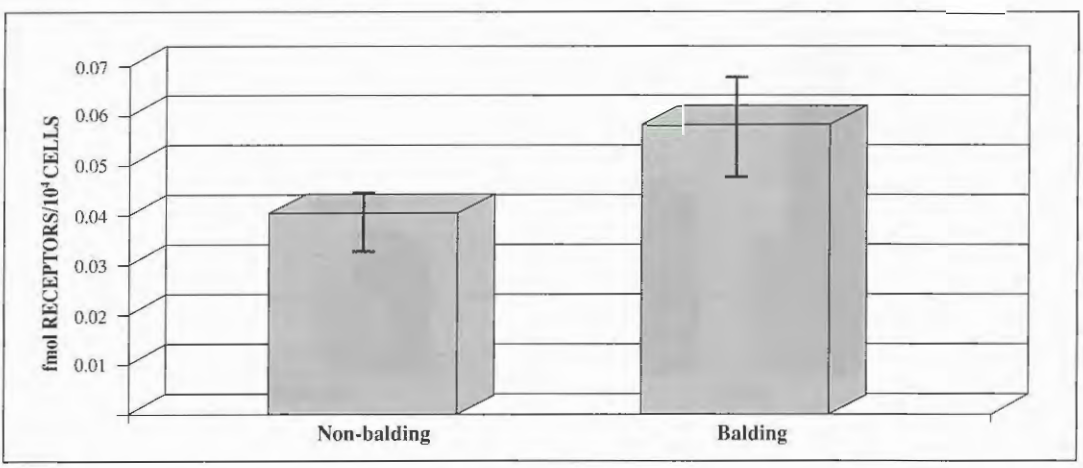

Fig. 3. Androgen Receptors in Cultured Dermal Papilla Cells (5).

THE HAIR CYCLE

Human hair grows in aperiodic cycles. Normal hair cycles last about seven years. The longest period of about six years is the anagen phase, the phase of active hair growth. After a short catagen phase, one year of telogen phase follows. This phase ends with hair loss. Then the follicle moves deeper into the dermis again, and another anagen phase starts (17, 19). In androgenetic alopecia, the hair cycles are becoming shorter and shorter, due to a shorter anagen phase, and visible hair loss occurs. The hair follicles continuously move towards the skin surface and undergo progressive miniaturization. Terminal hair is replaced by vellus hair and balding spots develop due to telogen hairs not replaced in time (4). In many instances, a complete atrophy of the hair follicles is the endpoint.

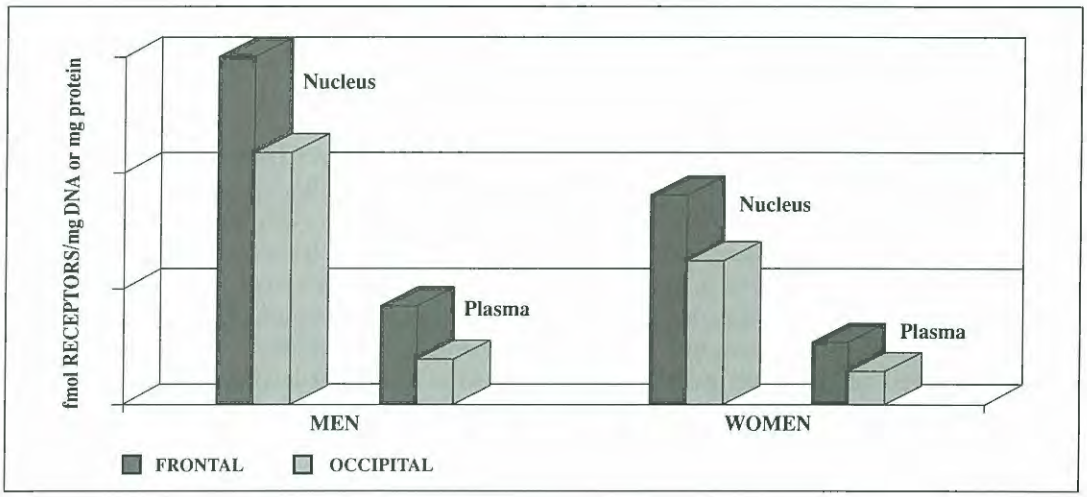

Fig. 4. Androgen receptors in Hair Follicles of Women and Men with Androgenetic Alopecia (22).

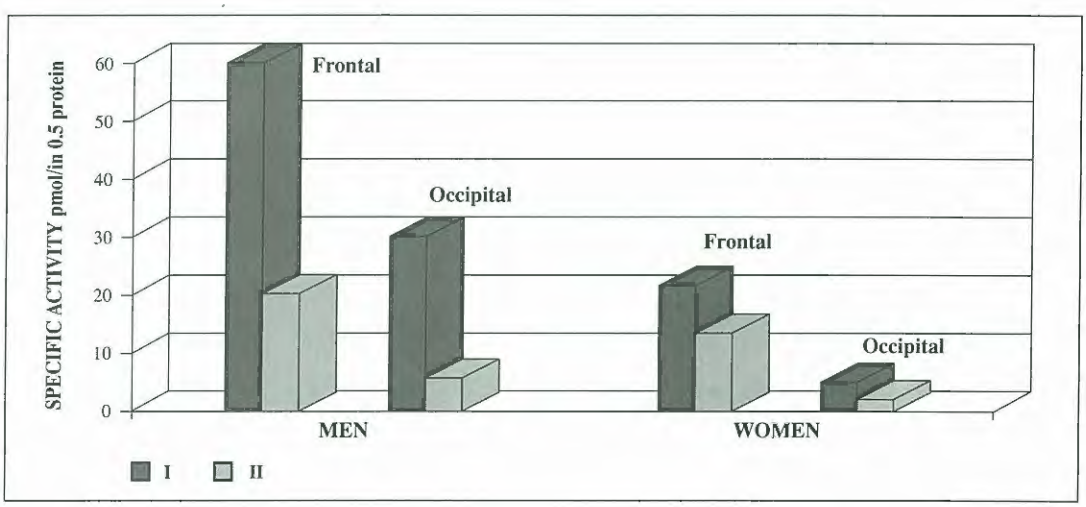

Fig. 5. Steroid-5α-reductase Type I and II in Hair Follicles of Men and Women with Androgenetic Alopecia (22).

ANDROGENETIC ALOPECIA IN MEN

In men, androgenetic alopecia causes the typical "male pattern baldness". At the end of the second decade of life, the hairline recedes in the frontal and frontoparietal region; at the same time, vertex thinning starts. Hairloss progresses continuously, with short phases of arrest. At the age of 40 to 50 years, complete baldness may be present. However, the parietal and occipital regions remain unaffected of the balding process. As quoted above, these regions reveal lower levels of androgen receptors and steroid-5α-reductase, and higher levels of aromatase. This is the reason why hair transplants from the occipital region into balding areas never reveal hair loss but continuously grow hair.
In castrates or in individuals with steroid-5α-reductase deficiency androgenetic alopecia never occurs. The different stages of androgenetic alopecia in men were classified according to HAMILTON (figure 7).

ANDROGENETIC ALOPECIA IN WOMEN

Women with androgenetic alopecia generally have diffuse hair loss or hair thinning of the temporal and parietal areas with retention of the frontal hair line. In postmenopausal women, however, typical "male pattern baldness" may occur. The different stages of hair loss in women were classified according to LUDWIG (figure 7).
In women with higher levels of androgens, either idiopathic or symptomatic (tumors of the ovaries or of the adrenal glands; hormone therapy), hirsutism may develop: terminal hair grows on the face, on

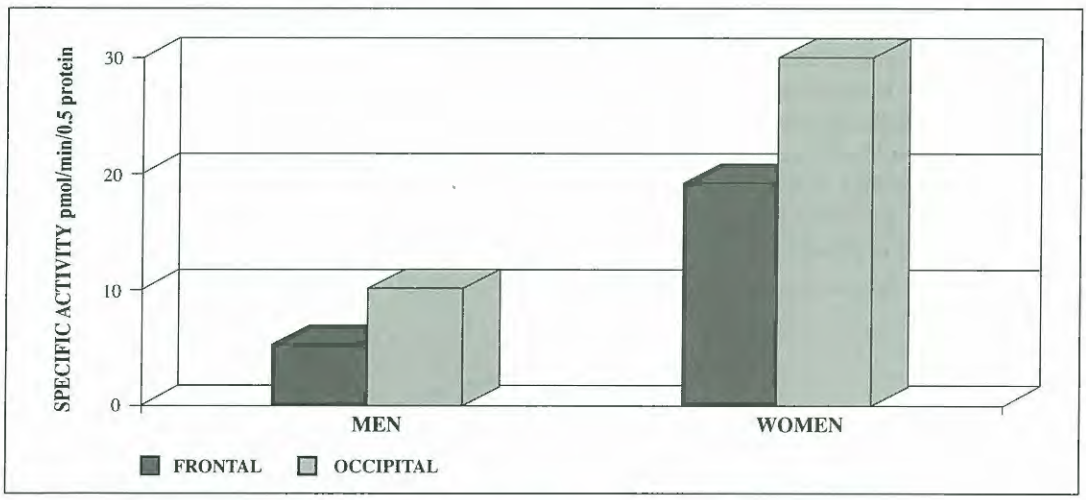

Fig. 6. Aromatase Activity in Hair Follicles of Men and Women with Androgenetic Alopecia (22).

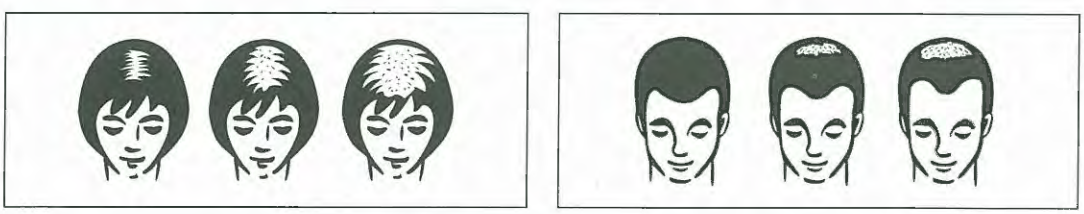

Fig. 7. Pattern of Androgenetic Alopecia in Women and Men, Stages by LUDWIG, resp. HAMILTON.

the body, and on the genital region as in males. In some instances, hirsutism may be accompanied by androgenetic alopecia. – In virilism, in addition to the symptoms of hirsutism, other signs of the male sex appear. In women with virilism, male pattern baldness may develop. In hirsutism and virilism, the causes for the hyperandrogenemia must be searched for.

(Hypertrichosis is characterized by an increased hair growth in women but the developing terminal hairs reveal the typical female distribution pattern. Hypertrichosis is generally due to genetic factors. No specific therapy is known, depilation in its different forms is the only advice that can be given to affected subjects (19). No hormonal deviations are present in hypertrichosis.)

TREATMENT OF ANDROGENETIC ALOPECIA

There are means for a successful treatment of androgenetic alopecia (stop of hair loss, increasing the mass of hair). No healing can be expected. Some hair loss is inevitable in aging, for both men and women. About balding, special anxiety develops in young men, and they seek medical advice. Until recently, doctors did not have much to offer (21). The situation has improved but it is important to state that only symptomatic treatment regimens are known. If the treatment is ended, massive balding comes back.

Topical applications of various products with irritants, konakion, rhodanides and others did not exert any significant influence on androgenetic alopecia in progression. Oral applications of vitamins, iron, zinc, selenium, gelatine, amino acids such as cystine, yeast extracts, biotin and others more may support hair regrowth in some instances. Significant effects in androgenetic alopecia were not recorded.

An effective treatment of androgenetic alopecia can be performed by topical applications of minoxidil, of estrogens, and of 17α-estradiol or by systemic administration of antiandrogens or finasteride.

MINOXIDIL

Minoxidil has been introduced into medical therapy as a powerful antihypertensive drug upon oral administration. Quite unexpectantly, patients taking this drug experienced massive hypertrichosis. This observation initiated clincal trials with 2 to 5% minoxidil for topical applications in various forms of hair loss. In androgenetic alopecia, such treatment proved to be effective (16). Details are shown in figure 8. It is still not known via which pharmacological mechanism minoxidil stops hair loss. As already stated,

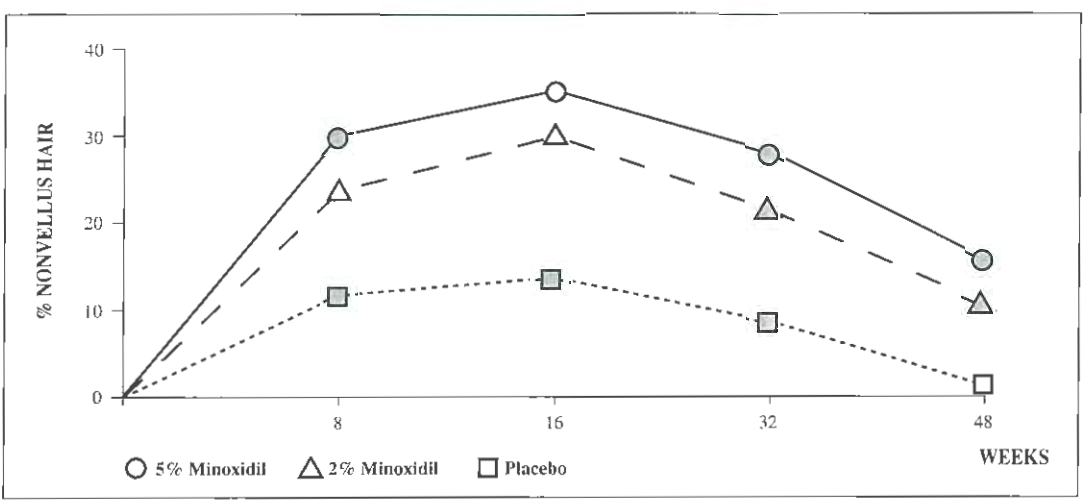

Fig. 8. Efficacy of topical Minoxidil in Androgenetic Alopecia (321 Men) Mean Change from Baseline (16).

minoxidil applications have to be continued as long as hair loss should be suppressed.
In some rare instances, hypotensive effects have been evoked with topical minoxidil.

TOPICAL ESTROGENS

Topical applications of estrogens (e. g. 1% β-estradiol) arrested hair loss in androgenetic alopecia. However, the hormonal effects caused by systemically absorbed estrogen prohibit its use (12, 14, 15). In women, disturbances of the menstrual cycle developed, men experienced gynecomastia.

ANTIANDROGENS

Oral antiandrogens have sucessfully been used in androgenetic alopecia of women. Cyproterone acetate

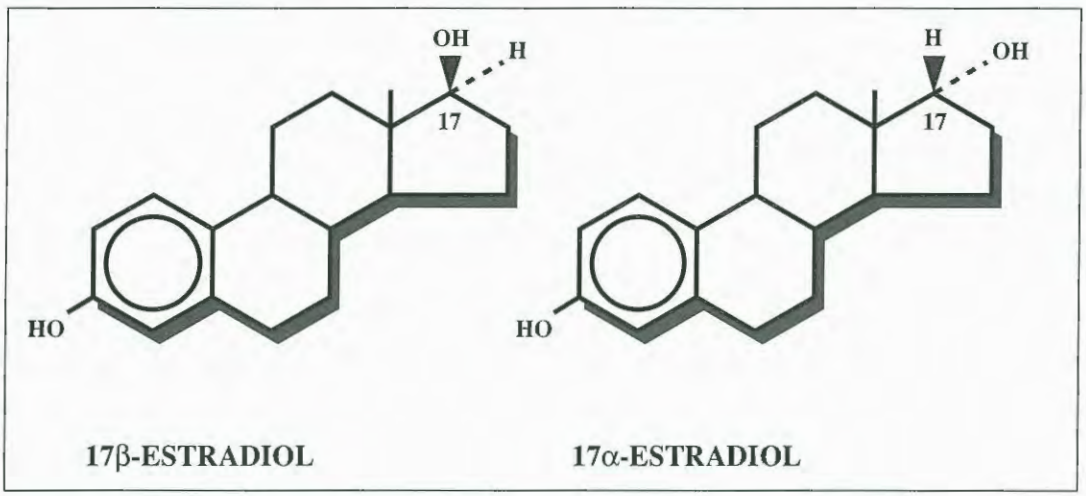

Fig. 9. The structural Formulas of 17β-Estradiol and its hormonally inactive Enantiomer17α-Estradiol.

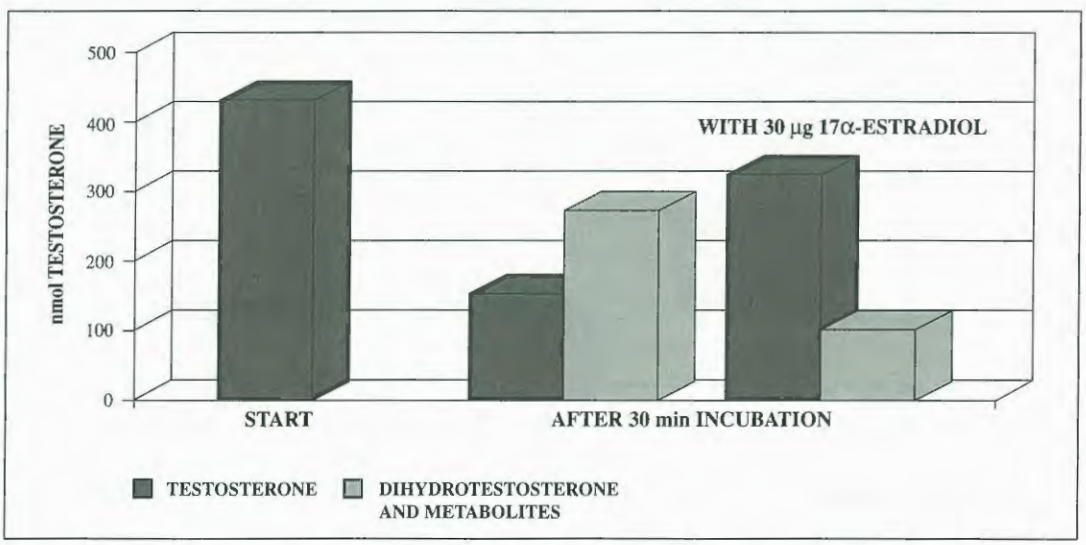

Fig. 10. The Metabolization of 14C-Testosterone in Liver Slices of Female Rats. The Influence of 17α-Estradiol.

was given either as monotherapy (10-50 mg/d) or, better, under the form of a contraceptive pill (3). Cyproterone acetate changes the configuration of the androgen receptor. Binding to the androgen-respose-element of DNA is no longer possible. Prolonged treatment with antiandrogens should be considered with caution.

For topical applications on the scalp, cyproterone acetate is not yet available.

Topical applications of 3.3% spirinolactone had no significant therapeutic action in androgenetic alopecia.

17α-ESTRADIOL

17α-Estradiol is the hormonally inactive enantiomer of the classical estrogen 17β-estradiol (figure 9). For the elicitation of estrogenic effects, the steric position of the substitutes on C-17 of the sterol skeleton is of great importance. A hydroxyl group in β-position on C-17 is necessary for estrogenic action. The same sterol with a hydroxyl group in α-position on C-17 lacks hormonal/estrogenic activity. Therefore, 17α-estradiol is only a steroid of the estra-type but not an estrogenic hormone.

In numerous biochemical and animal experiments, any relevant estrogenic activities of 17α-estradiol could be ruled out. The binding affinity of 17α-estradiol for the specific cytosolic estrogen receptor measured o.8 to 9% of the affinity of 17β-estradiol. But in most experiments, no estrogenic activity of 17α-estradiol could be recorded, the compound was even used as negative control. A few tests revealed a very weak activity of 17α-estradiol with a maximum of o.4% of 17β-estradiol. These data permit the conclusion that although 17α-estradiol binds to the specific receptor at a very weak degree, the resulting complex is not capable to react with the specific site on nuclear DNA.

As hormonal actions of 17α-estradiol could be ruled out, the question arose of how this steroid exerts it therapeutic action in androgenic alopecia. In biochemical investigations, an inhibition of steroid-5α-reductase was recorded (24). For details see figure 10. This action could be confirmed in cell cultures, measuring growth of human hair matrix cells in the presence of testosterone, dihydrotestosterone, 17β- and 17α-estradiol (7). The incorporation of 17α-estradiol in this test system neutralized the growth decreasing action of testosterone but not that of dihydrotestosterone (details in figure 11).

After local application, 17α-estradiol penetrates into the epidermis and dermis (23). The incidence of undesirable topical effects is extremely low (0.0007%);

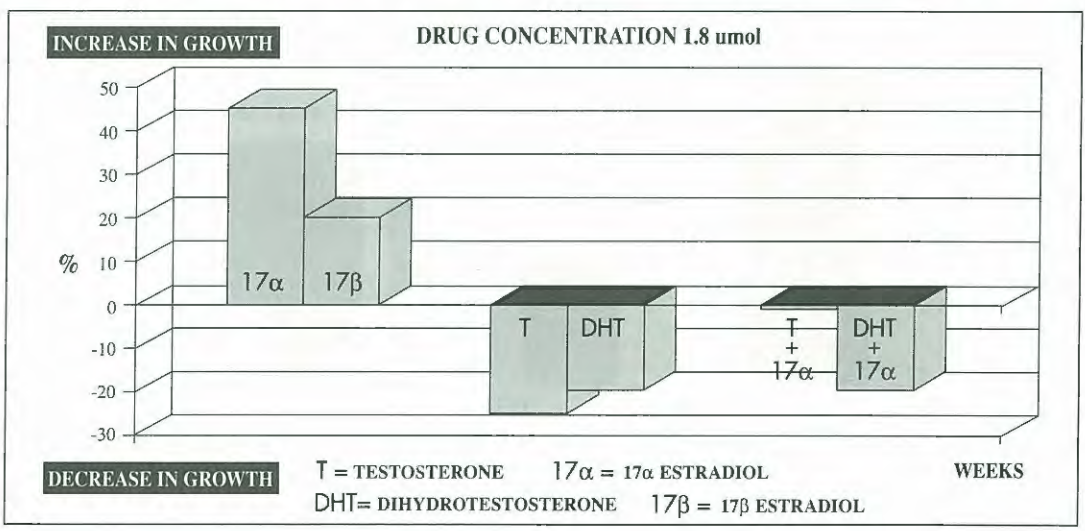

Fig. 11. Proliferation of Human Hair Matrix Cells in vitro (7).

the slight burning sensation is caused by the solvent propanol/water in which 17α-estradiol is applied in a concentration of 0.025%. In contrast to 17β-estradiol (cf. above), 17α-estradiol never caused any systemic estrogenic effects not even on long term use over years. This observation confirms the fact that 17α-estradiol is not a hormone.

Controlled clinical trials with topical 17α-estradiol have been performed in men and women over periods of at least one year. The uniform outcome of the

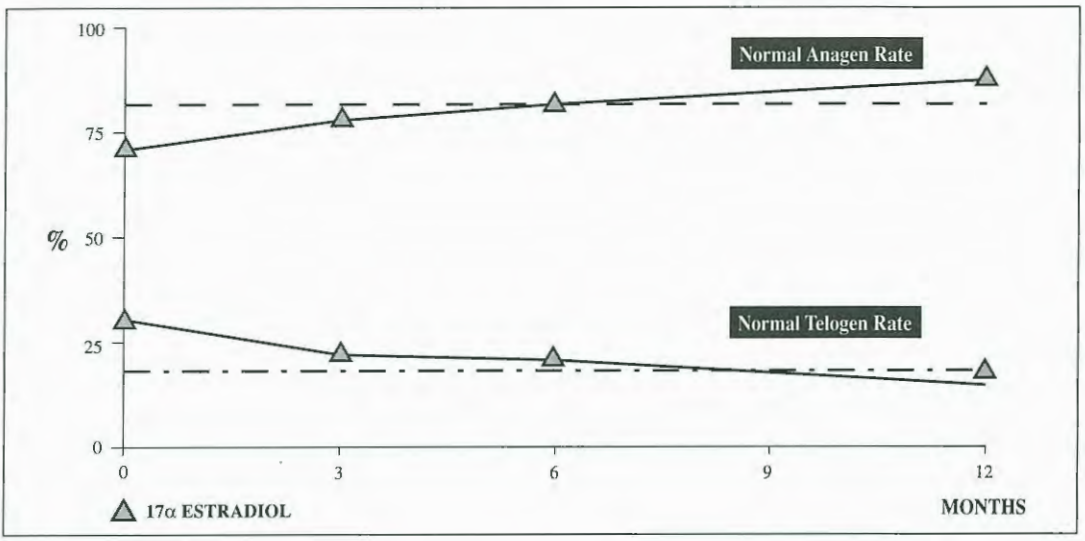

Fig. 12. Changes in Hair Morphology under a Treatment with topical 17α-Estradiol. 96 Patients, 1 Year (8).

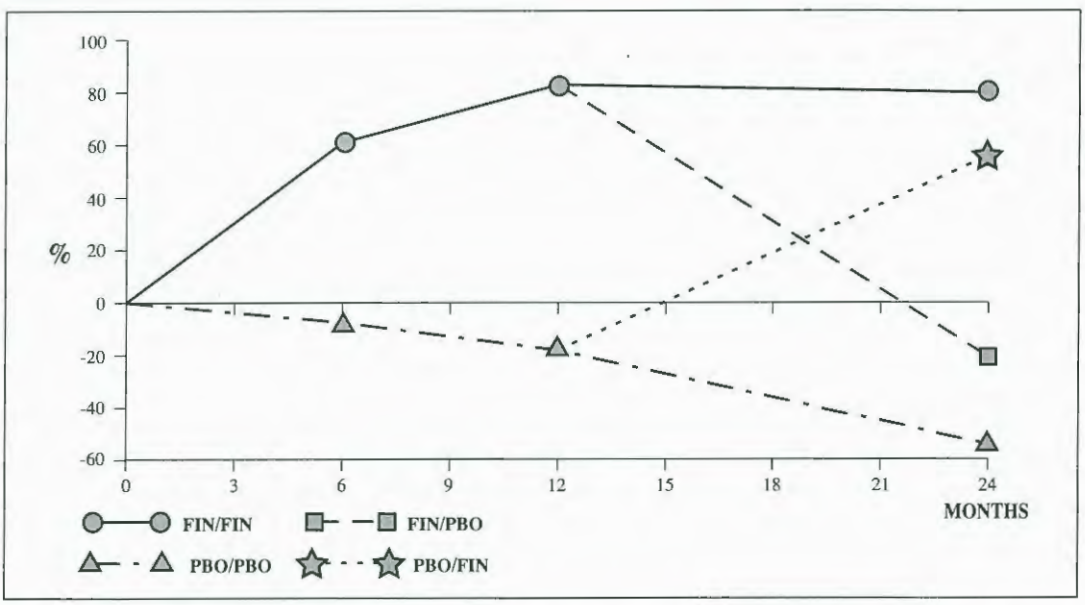

Fig. 13. Hair Count Mean Change from Baseline Finasteride vs. Placebo. Double-blind, 2 years, 1553 Men, Crossover after 12 months (6).

studies was a decrease in telogen hairs (e. g. in one study by 63% in comparison to a placebo rate of 37%), and anagen hairs increased by 20% (8, 11, 13). For details see figure 12.

FINASTERIDE

Finasteride is a potent inhibitor of steroid-5α-reductase, mainly of type II which is found in prostatic glands and in hair root cells. For years finasteride has been used already in daily doses of 5 mg for the treatment of benign prostatic hyperplasia.
Only recently, finasteride at daily doses of 1 mg was tested in androgenetic alopecia of men as steroid-5α-reductase has been shown to be of high pathogenetic importance in this type of hair loss. The clinical outcome of a large international trial was surprising: in controlled studies over two years, finasteride retained hair count in 83% of cases (placebo rate: 28%); a regrowth of natural visible hair was found in 66% of the patients (placebo rate: 7%). It can be concluded that undoubtedly finasteride is most effective in androgenetic alopecia of men. For details of the study outcome see figure 13 (6).
However, finasteride exerts major undersirable effects:

- finasteride is teratogenic
- finasteride may cause abnormalities of the extemal genitalia of a male fetus if the mother comes into contact with the drug. Therefore, women who are or who may be pregnant should not ingest finasteride nor should they handle crushed or broken tablets. The amount of finasteride in sperm of a sexual partner was reported to be too low to damage the male fetus after vaginal absorption.
- Finasteride even at low doses may decrease libido in men and may cause erectile dysfunction and ejaculatory disorders (incidence 1-2%). In some rare instances, gynecomastia was evoked.

Taking all those undesirable effects together finasteride should be recommended for the treatment of androgenetic alopecia only with uttermost caution. One should never forget that finasteride as all the other in androgenetic alopecia effective measures acts only symptomatically and must, therefore, be used as long as hair loss should not come back. It goes without saying that the patient has to be informed about all the possible undesiderable effects.

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ATLAS OF CONTACT DERMATITIS

by Robert L. Rietsched MD, An Goosseas PhD, Luis Conde-Salazar MD, Niels K. Veien MD.
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It represents a real editorial jewel to be always in the library as a supplement of other textbooks.
What caracterizes this atlas is the concise way in which the fundamental aspects of contact dermatitis are reported.
The description of the different typologies of contact dermatitis is essential and defined with concise and easily understandable words, even for non specialists.
In fact, it can be also clearly understood by cosmetic chemists, completely devoid of allergological bases.
The strenght point of this textbook is represented by the plain pictures both of the contact dermatitis different typologies affected areas and the relative histological preparations.
The legenda of all pictures are concise and clear too.

In the first chapter the contact dermatitis is defined describing the diagnostic criteria, the epidemiology, the biology and the immunology matched at well- described pictures of histopathology.
At the end, all the risk factors for the develcpement of irritant contact dermatitis or allergic contact dermatitis are reported. Moreover, it talks about how to prevent this kind of pathologies.

In the second chapter all the technics and the systems adopted to carry out the patch test are fairly showed.
More and more important than the text itself, are undoubtly the pictures showing "themselves" the technics and the methodologies used to identify the patch test positivity.
The reader is led step by step to recognize the different reactions through the coulored pictures, of great impact because well carried out.
Even a non technician could try to use the same methodology paying attention at the execution as described in the text !

In chapters three and four the reader is led to establish the correct diagnosis of contact dermatitis confined to specific anatomical regions, in order to start the correct treatment plan.
The evidence of the pictures results to be always the keystone for an appropriate final diagnosis of the pathology.

In fact the different pathologies of contact dermatitis affecting the different body regions are well documented: from the dermatitis of the hands, provoked by topically applied substances, to a chronic dermatitis of the foot, caused by chromate contained in the leather or due to an azodye.

The fifth chapter is entirely dedicated to the systemic contact dermatitis, such as the symmetrical recurrent vesicular palmar and/or interdigital dermatitis or a more widespread dermatitis.
In this chapter also many and well detailed are the pictures.

In the sixth chapter are reported the specific categories of contact allergens such as different kind of medications, medical devices, metals, plants and woods, textiles, clothing, shoes and, of course, cosmetics.

The seventh chapter is entirely dedicated to the occupational contact dermatitis.
Allergic reactions due, for example, to chromate in the cement used in the construction industry or to disinfectant used in the unit nurse, or to acrylate used in the dental profession, etc., are reported.

This interesting textbook ends with the eight chapter reporting Contact Dermatitis present with unusual morphology or due to an unusual set of circumstances.

As previously said the reading of this textbook is very fluent and instructive.
Dermatologists and allergologists are adviced to buy it because it represents a fundamental book for their own library but it is also useful for people directly involved in cosmetics dermatology: cosmetic chemists and all the technicians belonging to both chemical raw materials and finished product industries.

The textbook could be also of great support for the students of medicine, who want to deepen the wide branch of contact dermatitis, as well as for the pharmacists and aestheticians in their daily activities.

P. MORGANTI
Editor in Chief

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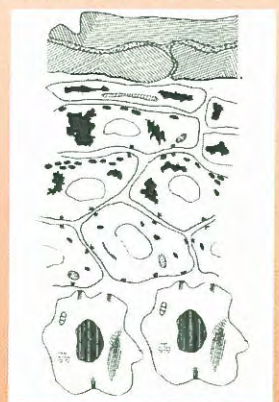

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