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AMMONIO LATTATO "ATTIVATO" LA RISPOSTA DERMATOLOGICA ALLE IPERCHERATOSI



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Ipercheratosi estrinseca

"ACTIVATED" AMMONIUM LACTATE THE RIGHT REPLY TO HYPERKERATOSIS



KERATOTAL 1

Emulsione - Ammonio Lattato 14%

KERATOTAL 2

Emulsione - Ammonio Lattato 8%

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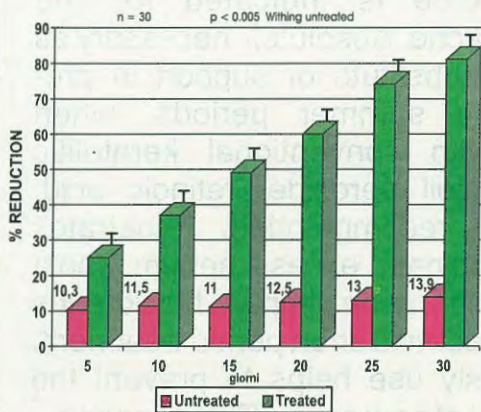
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Dermatologically tested

- Effective for initial and maintenance therapy ^(1,2,3)
- Compatible with all the drugs and cosmetics
- Formulated to treat mild-to-moderate inflammatory acne, indispensable for patients with sensitive skin

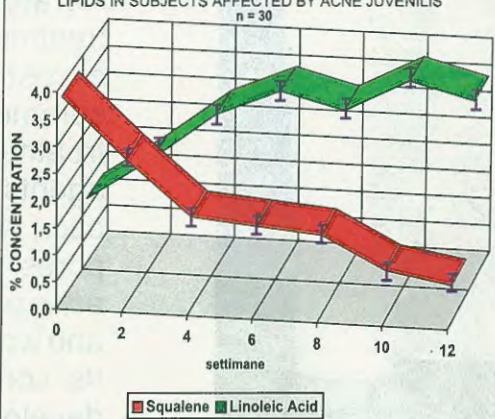
CLINICAL RESULTS ^(1,2,3)

REDUCTION OF SURFACE LIPIDS DURING THE TREATMENT WITH KERATOTALACNE

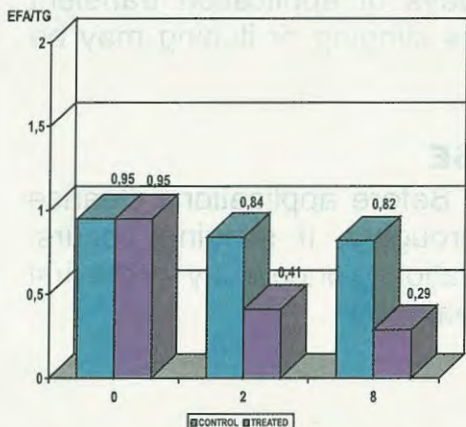


⇒ Decreases the Squalene content of acne affected skin

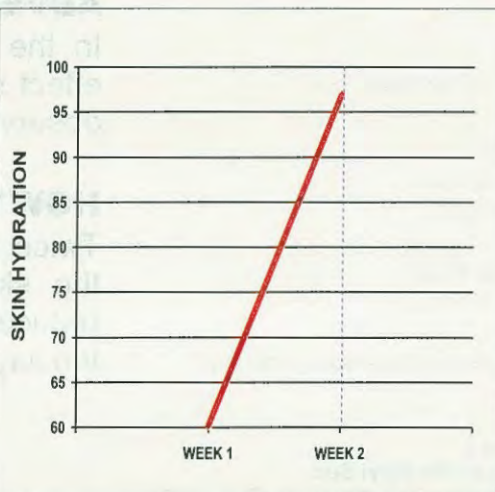
ACTIVITY CARRIED OUT BY KERATOTALACNE ON THE LINOLEIC ACID AND SQUALANE CONTENTS OF SURFACE LIPIDS IN SUBJECTS AFFECTED BY ACNE JUVENILIS



⇒ Reduces excess lipids



⇒ Significantly reduces EFA/TG ratio



⇒ Increases skin hydration by 97%

BRIEF SUMMARY

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TREATMENT WITH
NO-DRUG CONTENT



For more information
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Fax +39.6.92.81.523
E-Mail: mavi @colosseum.it
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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 glycolic acid and salicylic 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 keratolytic agents (benzoyl 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 continuous 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 phosphatidylcholine from Soybeans, with a high content of linoleic acid. *J. Appl. Cosmetol.* **14**, 137-145

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Lip protective with Glycoaminoacids(*)

INDICATIONS

Cosmetic adjuvant in all the forms of cheilitis and lips dryness caused by:

- | | | | |
|----------------------------|-----|----------------|---------------------------------------|
| • Retinoids | ... | Such as | Cheilitis or chapped lips |
| • UV rays | ... | | Actinic cheilitis (acute and chronic) |
| • Wind | ... | | Allergic cheilitis |
| • Weather | ... | | Exfoliative cheilitis |
| • Environmental pollutants | ... | | Angular cheilitis |

HOW TO USE

Use day and night as a regular lipstick

(*) partially neutralized by a special patented blend of aminoacids

Please see a brief summary of prescribing information on next page



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Glycoaminoacids(*)

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MUCOCUTANEOUS INTEGUMENT OF
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patented blend of aminoacids

BRIEF SUMMARY

DESCRIPTION

Keratotal Labbra is a fast-acting, uncoloured treatment to protect the lips from premature ageing and skin cancer due to UV rays. It helps to keeps the lips very moist and well protected from the dryness caused by UV, wind, weather and environment.

INDICATIONS

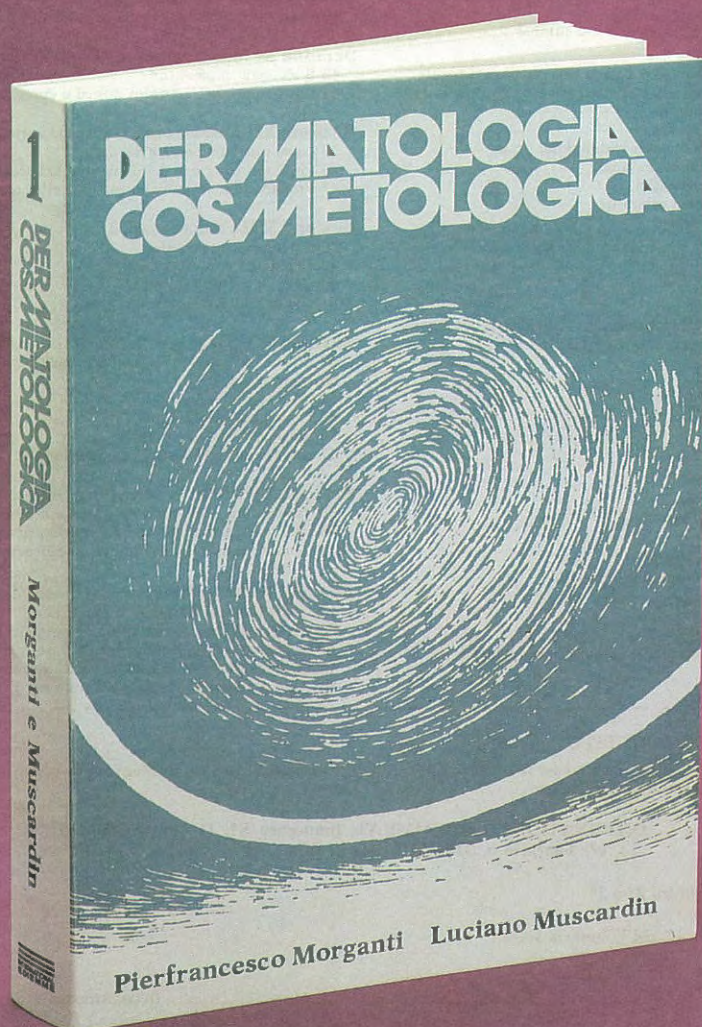
In all forms of dryness caused by the use of retinoids or other drugs, or by environmental pollutants. To avoid the premature lips ageing caused by UV activity.

ADVERSE REACTIONS

No adverse reactions to the use of this product are known.

HOW TO USE

Apply as a regular lipstick. Keratotal Labbra is intended for round-the-clock use.



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DERMATOLOGIA COSMETOLOGICA

A cura di P. Morganti e L. Muscardin
Ed. International Ediemme

Indice 1° Volume

Sezione I Considerazioni Generali

- 1 Cenni storici
- 2 La bellezza della figura umana

Sezione II Fisiologia e Biologia della cute

- 3 Sviluppo della pelle
- 4 La struttura della cute
- 5 Biochimica e Fisiologia dell'epidermide
- 6 Biologia del tessuto connettivo
- 7 Sistema Vascolare ed innervazione della cute

Sezione III La Cute come organo di assorbimento

- 8 Nozioni basilari sulla permeabilità e sull'assorbimento
- 9 Membrane e assorbimento
- 10 Metabolismo della cute e degli annessi cutanei

Sezione IV Chimica e Chimico-Fisica dei preparati topici

- 11 Materie prime e principi attivi di uso cosmetologico
- 12 Emulsioni ed emulsionanti
- 13 Tensioattivi di uso cosmetico
- 14 Gli antiossidanti e i fenomeni ossidativi dei grassi
- 15 Antimicrobici e preservanti cutanei
- 16 La profumazione dei cosmetici
- 17 Chimica e tossicologia dei coloranti
- 18 Prodotti cosmetici in aerosol

Indice 2° Volume

Sezione V Trattamenti dermocosmetici del viso e del corpo

- 19 Detersione, protezione e normalizzazione della pelle
- 20 La cosmesi per l'uomo
- 21 Cosmetici per bambini
- 22 Preparati per il bagno
- 23 Maschere e peeling
- 24 I Depilanti

Sezione VI La cute senile

- 25 Invecchiamento cutaneo
- 26 Il trattamento della cute senile

Sezione VII Cosmetici e Psiche

- 27 Aspetti psicosomatici e somatopsichici in dermatologia cosmetologica

Sezione VIII I danni cutanei

- 28 Patologia cutanea da cosmetici su base immunologica
- 29 Danni da cosmetici

Sezione IX Annessi cutanei e dermocosmesi

- 30 Ghiandole sudoripare e sebacee
- 31 Deodoranti e antisudore
- 32 Struttura e proprietà dei capelli
- 33 Detersione, protezione e normalizzazione dei capelli e del cuoio capelluto
- 34 Cosmetici decorativi ad effetto duraturo
- 35 Le unghie
- 36 Prodotti decorativi ad effetto temporaneo superficiale

Indice 3° Volume

Sezione X Seborrea e dermocosmesi

- 37 Caratteristiche chimico-fisiche e funzioni fisiologiche del sebo
- 38 Produzione e modificazioni del sebo nel sano e nel seborroico
- 39 Influenza dei trattamenti cosmetologici sui lipidi di superficie del viso e del capillizio
- 40 Attività ormonale e ghiandole sebacee
- 41 Il problema terapeutico dell'acne
- 42 Possibilità terapeutiche nella seborrea

Sezione XI Melanogenesi e dermocosmesi

- 43 Il sistema pigmentario
- 44 Filtri solari, pigmentanti diretti e depigmentanti

Sezione XII Mucose orali e dermocosmesi

- 45 La salute della bocca e dei denti
- 46 Profilassi ed igiene dei denti e della bocca
- 47 Preparazioni cosmetiche per la cavità orale

Sezione XIII Prodotti speciali

- 48 Omeopatia e cosmetici
- 49 Soluzioni per lenti a contatto
- 50 Cosmetici ipoallergenici
- 51 Cosmesi su basi naturali

Sezione XIV Trattamenti estetici correttivi

- 52 Interventi correttivi di chirurgia plastica
- 53 Laserterapia
- 54 Crioterapia
- 55 Principi di mesoterapia
- 56 Ionoforesi
- 57 Interventi correttivi di "camouflage"

Sezione XV Controlli dermatossicologici

- 58 Valutazione delle materie prime e dei cosmetici finiti
- 59 Controlli tossicologici delle materie prime e del prodotto finito
- 60 Cosmetognosia. Funzionalità ed efficacia dei prodotti cosmetici

Sezione XVI Problemi normativi e di Marketing

- 61 Nozioni di marketing e di pubblicità
- 62 Grafica pubblicitaria: implicazioni psicologiche
- 63 Normative di legge sui cosmetici nei vari paesi del mondo
- 64 La responsabilità civile dei trattamenti cosmetici
- 65 Giudizio medico-legale del danno estetico

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Trimestrale di Dermatologia Cosmetologica Quarterly Review of Cosmetic Dermatology

Contents

Original Laboratory Studies

- 127** Vacuum skin-abrasion versus glycolic acid peeling
in the treatment of atrophic acne scars
S. Jurassich, A. Lo Schiavo, F. Pinto, M. Nacca
- 133** A video-microscope study of "hair knots"
R. Strumia, C. Roveggio
- 137** The treatment of Acne Vulgaris by Phosphatidylcholine from Soybeans,
with a high content of Linoleic Acid
M. Ghyczy, H-P. Nissen, H. Biltz
- 147** On the heavy metals content in cosmetic formulation:
an atomic absorption spectroscopy investigation
Marcelo E. Conti, Francesco Botrè, Franco Mazzel
- 155** Application of a film method for microbial monitoring
of cosmetic raw materials
L. Piu, C. Juliano, G. Pirisino, P. Minghetti
- 163** Index to Volume 14, 1996
-

XIX Announcements

IN-COSMETICS CONFERENCE 1997
"Modern challenges to the cosmetic formulation"
Düsseldorf (Germany) - May 5-7, 1997

In close cooperation with IN-COSMETICS:
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VACUUM SKIN-ABRASION VERSUS GLYCOLIC ACID PEELING IN THE TREATMENT OF ATROPHIC ACNE SCARS

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Received: May 21st, 1996

Key words: *Glycolic acid peeling and microabrasion.*

Synopsis

Glycolic acid, an α -hydroxyacid, is a natural ingredient derived from sugar-cane. The cosmetic use of AHA dates back to thousands of years ago. Cleopatra was used to taking a bath in the sour milk (lactic acid) and to put the improved red wine (tartaric acid) on her face. This habit made her skin fresh and downy.

The glycolic acid reduces the thickening of the corneum and facilitates the brushing off of the superficial cells, since it weakens the cohesion of corneocytes.

The vacuum demabrator avails of the abrasion effect produced by the microcrystals shot on the epidermis which, delicately and uniformly, peel off the superficial layers.

Riassunto

L'acido glicolico, un α -idrossiacido, è una sostanza naturale estratta dalla canna da zucchero.

L'uso cosmetico degli AHA risale a migliaia di anni fa. Cleopatra era solita fare il bagno nel latte acido (acido lattico) ed applicare sul viso vino rosso (acido tartarico), al fine di rendere la pelle liscia e vellutata.

Gli effetti dell'acido lattico sull'epidermide sono soprattutto cheratoplastici ed esfolianti dal momento che riduce la coesione cheratinocitaria.

Il dermoabrasore esplica il suo effetto abrasivo attraverso un getto di microcristalli che, colpendo l'epidermide delicatamente ed uniformemente, ne rimuovono gli strati superficiali.

Introduction

Scarring occurs to some degree in about 95 per cent of acne subjects^(1,2). The most common types of post-acne scarring may present as thickened scars (hypertrophic or keloid scarring) or as tissue loss (atrophic ice-pick, macular atrophic and follicular macular atrophic scars)⁽¹⁻³⁾.

The atrophic scars have a hollow, whitish and persistent appearance which make the skin appear irregular.

These unsightly lesions have induced dermatolo-

Patients and methods

Twenty patients, 10 males, 10 females, aged from 20 to 30 years, with atrophic acne scars were enrolled in the study. Ten were treated with vacuum microabrasion, 10 with 70% glycolic acid peeling. All patients had type III Fitzpatrick skin. Scar lesions were no greater than 0,5 cm and persisted for over six months.

We used a vacuum-operated skin abrader type (V.M. - Medicale) that projects a sterile microcrystal flow over the skin, which removes uniform surface layers without causing any heat damage

Table 1

MAIN TREATMENTS USED FOR POSTACNE ATROPHIC SCARS

Intralesional therapy	placenta extracts jaluronic acid collagen
Surgery	punches elevation
Physical treatments	cryotherapy dermoabrasion motor-driven - diamond milling cutter - metal brush microabrasion vacuum operating
Chemical peeling	resorcin trichloroacetic acid 70% glycolic acid

gists to seek more than one method (table I) to alleviate the problem⁽⁴⁾. Glycolic acid peeling and vacuum skinmicroabrasion are the most popular techniques employed today to treat acne atrophic scarring. Although based on different mechanisms, both of them stimulate epithelial proliferation and raise the depressed surface to the surrounding skin⁽⁵⁻⁷⁾.

This study compares the two methods by evaluating their beneficial effects as well as their drawbacks.

(microabrasion). We operated with a 0,5-0,7 atm vacuum, and a 0,5 cm throw-away head. The handle was first used along the major axis of the lesion to abrade the bottom. After, it was passed perpendicularly from the axis to level the edges to the bottom. It was then rotated to flush the edges and bottom to the surrounding surface. The handle was sometimes pressed to increase the abrasive effect and to use the sharp rim of the head as a chisel⁽⁵⁾.

When bleeding appeared, we stopped microabra-

sion. Bleeding is necessary because it indicates that the epithelial proliferative layer has been reached, and must not be surpassed to avoid new scarring⁽⁵⁾. Glycolic acid disrupts ionic linking among corneocytes (epidermolysis), stimulates the proliferation of the basal layer and increases penetration of other substances. It also causes the building up of collagen fibres in the papillary dermis, elastic fibres in the reticular dermis and glycosaminoglycine in the dermal matrix⁽⁸⁻¹⁰⁾. Epidermolysis is obtained by applying the acid at a 70% concentration over the skin. It reaches the stratum granulosum after 60 seconds, and the dermo-epidermal junction 5 minutes later⁽⁹⁻¹²⁾. Throughout the application, frosting of the treated surface and later erythema, more or less associated with a dotted hemorrhaging, occur. Redness persists for 48 to 90 hours. Crusts appear after 24-48 hours and treated surface re-epithelialization is completed within 5 to 7 days.

We limited the number of microabrasion and glycolic acid applications to ten and the interval between two treatments ranged from 10 to 15 days. The patients were advised to clean the skin with an abrasive substance for about 5 minutes once a day to detach the crust and prolong the therapeutic effect. They were also instructed to apply antibiotic and non-steroid antinflammatory creams and to avoid photoexposure or to shelter from it by using total block screens. The reduction in the number of lesions was evaluated by a macrophotographic fieldmetre. Two field samples, measuring 4 x 4 cm in diameter, with the greatest number of lesions were considered in each photograph. A simple millimetric probe provided the depth and the diameter of the lesions. Four lesions were sampled. Measurements at the beginning and the end of the therapeutic cycle were expressed in percentages.

Table 2

MICROABRASION VERSUS GLYCOLIC ACID PEELING IN THE TREATMENT OF POSTACNE ATROPHIC SCARS

	<i>Skin-abrader (microabrasion)</i>	<i>70% glycolic acid peeling</i>
action	mechanical	chemical
preoperative treatment	not needed	topical keratolytic for 15 days
square area treated each time	< 10 cm ²	whole face
time for each treatment	10'-15'	3'-5'
interval between treatments	10-15 days	10-15 days
postoperative treatment	abrasive detergent	gentle topical remover
post-treatment clinical features	erythema	erythema
	exudation	slight exudation
	bleeding	frost
	pain	burning
	moderate oedema	possible crusting
	crusting	
clinical improvement (means)	79,16%	75,70%
number	75,24%	70,80%
depth	80,15%	77,60%
diameter	82,10%	78,70%
manageability	good	excellent
cost	considerable	low

Furthermore the presence of erythema and hyperpigmentation together with their intensity were measured by a chromameter at the beginning and at the end of therapy.

Results

For both techniques, the results were based on the decline in number, in depth and in diameter of the lesions and on a whole evaluation of side effects, cure time and operating costs. The results are summarized in table II.

Discussion

The mechanical action of microabrasion causes hemorrhage. This gives us an indication of the depth reached during an application. Glycolic acid, on the other hand, produces epidermolysis which appears like frost. This can only give us a rough estimate of the depth reached.

Microabrasion brought about a 79,16% recovery of the lesions. This was 4% higher than the 75,6% produced by glycolic acid. The better result was from its mechanical action. The head of the microabrasor acted on the lesion surface alone, while the acid had to be spread on the entire skin surface to be treated. Glycolic acid peeling gave us a wider treatment surface, one covering the whole face, within five minutes. Microabrasion, instead, called for a more limited treatment surface (<10 cm²) and took an average of 10-15 minutes to perform. The well-tolerated burning sensation caused by the acid started about 2 minutes after an application and was maximum at 5 minutes. The pain incurred from microabrasion was immediate and increased as the application went on. It was, however, reduced by a topical anaesthetic.

In contrast to traditional dermoabrasion with milling cutters, persistent erythema, oedema and hyperpigmentation were rarer side effects. There was only one case of conjunctivitis as a result of acid application. Since microabrasion works with inert crystal, it could not cause irritant chemical dermatitis.

The microabrasor is a simple, though expensive, instrument. It requires routine maintenance of a pneumatic circuit and the purchase of steril crystals. Glycolic acid is a one time expense which is more or less the same as microcrystals alone.

Regardless of the technique, the resolution of the scar tissue and adverse effects, depend on patient age and phototype, the site and the age at onset. The younger the patient is, the quicker and the better the healing occurs⁽⁵⁾. When scars are old they call for longer treatment schedules, while those of recent onset require fewer applications⁽⁵⁾. Moreover, since IV and V skin phototypes have more frequent hyperpigmentations, we dealt with Fitzpatrick type III subjects.

Conclusions

As previously described⁽⁴⁾ microabrader is a handy tool for the operator. Furthermore, its closed circuit does not damage the environment. The throw away heads and inert sterile crystal make it preferable to peeling with glycolic acid. However, to treat wide surfaces it requires more time and many therapeutic applications. A single treatment lasts 10 minutes, and intervals between applications take 10-15 days.

Peeling treatment with 70% glycolic acid is simple to carry out. Its evaporation however may cause conjunctivitis and it is not available in sterile packs. The entire face can be treated quickly but it also calls for numerous applications.

Both techniques can be carried out in an out patient therapeutic application and although neither can fully heal the scars, they at least give the patient relief. These therapies have no major influence whatever on lifestyle. When erythema and edema occur, no special treatment is normally needed.

Furthermore patients can interrupt or return to treatment at any given time.

We recommend microabrasion for deep and wide atrophic macular scars. In contrast, we prefer glycolic acid peeling for follicular and ice-pick atrophic acne scars, which are generally smaller and shallower.

For the best results we suggest that both techniques be used on alternative way.

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A VIDEO-MICROSCOPE STUDY OF "HAIR KNOTS"

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Received: November 20th, 1995

Key words: "hair knots", video-microscopy, pseudofolliculitis.

Synopsis

The purpose of this work was to investigate, using a video-microscope system, the morphology of burrowing hairs after shaving in the legs of young women. We examined ten young women who regularly removed the hair from their legs by razors or wax. All the patients referred that they had noticed that their hair curved after shaving, re-entering the skin. To the naked eye, the hairs appeared twisted and partially included into the skin. By video-microscope we observed that they formed true "hair knots" partially enclosed in the skin. The knots were rather entangles and their pattern was extremely variable. The methods used to remove unwanted hair - razors or wax - did not influence the morphology of knots. "Hair knots" develop after shaving only in some individuals and each person had the same type of knot in all the shaved areas. In our opinion, this is further evidence of the influence of genetic factors on the hair morphology.

Riassunto

Lo scopo di questo lavoro è stato quello di studiare la morfologia dei cosiddetti *peli incarniti* che compaiono in alcune giovani donne dopo la depilazione delle gambe. Sono stati esaminati, mediante un video-microscopio a sonda ottica, dieci soggetti che depilavano regolarmente le gambe mediante rasoi o cerette e che avevano notato questo fenomeno alcune settimane dopo la depilazione. Mentre a occhio nudo si osservava soltanto la presenza di peli arricciati parzialmente inclusi nella cute, con il video-microscopio si metteva in evidenza che tali peli tendevano a formare veri e propri nodi estremamente bizzarri e complessi la cui morfologia differiva da persona a persona ma era sempre la stessa nelle varie aree cutanee dello stesso individuo. Questo rilievo è, a nostro avviso, un'ulteriore conferma dell' influenza dei fattori genetici sulla morfologia del pelo; d'altro canto, una predisposizione genetica era già stata ipotizzata per la comparsa della pseudofollicolite da depilazione.

The penetration of human hair under the skin has been reported in the literature (1) but it is a rare phenomenon. The name of "burrowing hair" (*pili cuniculati*) was suggested for the condition given its likeness to the burrows of scabies (*cuniculi*) which run tunnel-like, horizontally and superficially. Burrowing hairs are sometimes detectable in the plants of barbers' feet due to the continuous contact with cut hair, but the most common causes are shaving and depilation. In fact, curved shaved hairs reenter the skin at short distance from the follicle especially in black men with curly hair. Sometimes, acting as a foreign body, they incite an inflammatory reaction resulting in papule and pustule development (pseudofolliculitis). In young women, who remove the hair from their legs, burrowing hairs and pseudofolliculitis are frequently seen (2,3,4,5). We observed them morphology by a video-microscope.

Material and methods

The apparatus (Moritex Video Microscope System Scopeman, MS-504, Meisei Bldg., Japan) is composed of a processing unit and a color monitor (14" 1TTL CVS); light from the light source (a 100W mercury vapour lamp) of the processing unit is guided with the optic fiber to the probe end. Objectives are equipped with non contact lens (x25, x50) and with contact lens (x200). A still video recorder and a colour printer may be attached. We examined ten young women who regularly removed the hair from their legs by razors or waxes. All the patients referred that they had noticed that their hair curved after shaving, re-entering the skin.

Results

With the naked eye the hairs appeared twisted and partially included into the skin. By video-microscope we observed that they formed true "hair knots" partially enclosed in the skin. The knots were rather entangles and their pattern was extremely variable (Fig. 1). However, every patient had the same type of knots in all the cutaneous areas examined. Small

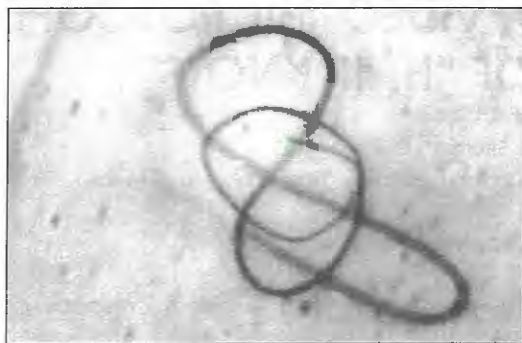


Fig. 1: example of "Hair knot"

haemorrhages were present where the hair penetrated the skin and dilated capillaries were visible nearby. Slight hyperkeratosis was also detectable.

Discussion

There is evidence of genetic predisposition for pseudofolliculitis after shaving. This arises in persons predisposed to it by the anatomy of their hair follicle. For example, tightly curved hairs are a Negro characteristic and so pseudofolliculitis barbae is almost exclusively limited to blacks who shave. Alexander (6) reported a case of pseudofolliculitis localized in the pubic area in a woman who shaved regularly. Indeed, the hair covering this area tends to be very curly or kinky. Pseudofolliculitis has also been described in a set of identical twins, both having the affliction for approximately the same duration and both equally affected.

In the present report, we observed that the methods used to remove unwanted hair - razors or wax - did not influence the morphology of knots.

"Hair knots" develop after shaving only in some individuals and each person has the same type of knot in all the shaved areas. In our opinion, this is further evidence of the influence of genetic factors on the hair morphology.

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THE TREATMENT OF ACNE VULGARIS BY PHOSPHATIDYLCHOLINE FROM SOYBEANS, WITH A HIGH CONTENT OF LINOLEIC ACID

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Key words: *Acne vulgaris, Linoleic Acid, Squalene, Skin Lipids, Phosphatidylcholine*

Synopsis

The efficacy of phosphatidylcholine from soya was evaluated in the treatment of acne vulgaris in 7 studies on 77 subjects.

Results of clinical trials:

- Reduction of the number of comedones and efflorescences;
- Decrease squalene concentration and
- Increase linoleic acid concentration in skin surface lipids;
- The efficacy of phosphatidylcholine is equal if used as liposome or in nonliposomal form.

Riassunto

L'efficacia della fosfatidilcolina di soya è stata valutata nel trattamento dell'acne vulgaris in 7 studi su 77 pazienti.

Risultati dei test clinici:

- Riduzione del numero di comedoni ed eruzioni cutanee;
- Diminuzione della concentrazione di squalene;
- Aumento della concentrazione di acido linoleico nei lipidi di superficie della pelle;
- L'efficacia della fosfatidilcolina è uguale se usata in forma liposomica o non liposomica.

Introduction

Acne vulgaris, with an incidence of nearly 100% in the second decade of life, is the most common skin disorder. The manifestations can differ considerably. According to Gollnick 30% of the affected subjects requires medical consultation and treatment [1]. Nevertheless, the less severe forms, also known as physiological acne, can be a relevant psychological problem for a large proportion of the remaining 70% of the teenage population. OTC drugs and cosmetic products are available to satisfy the demand this creates.

The pathogenesis of acne has not been elucidated in detail. However, four factors are regarded as being of clinical relevance and are, hence, the target of therapeutic intervention. They are differentiated as primary and secondary factors [1].

The primary factors are:

1. Elevated sebum production.
2. Disturbance of follicular keratinization.

The secondary factors are:

1. Follicular inflammation and immune response,
2. Hyperproliferation of *Propionibacterium* acnes.

The ideal treatment for acne is causal therapy for the primary factors. The only drug substance to afford this is isotretinoin, a substance which has to be taken orally and which is associated with severe side effects.

On the other hand, there are several substances whose effects make them suitable for treatment of the secondary factors. Recent studies have suggested the importance of linoleic acid in the process of follicular hyperkeratosis, suggesting a new therapeutic possibility through normalizing the reduced linoleic content in the sebaceous follicle by external application.

Phosphatidylcholine, also misleadingly called lecithin, from soybeans contains chemically bound fatty acids, of which nearly 70% is linoleic acid. Pure phosphatidylcholine and fractions from soylecithine with a high content of phosphatidylcholine, the Phospholipons[®], have been used in drugs and cosmetic products for decades. They are regarded as being safe; this is manifested in their GRAS-status

[2]. They form liposomes with a typical particle size of 0.1 to 0.3 μm when formulated in water. Since the diameter of the sebaceous duct is 5 to 15 μm , liposomes should enter the sebaceous gland readily, transporting the linoleic acid.

Phosphatidylcholine-bound linoleic acid has several advantages compared to the free form. The linoleic acid bound to phosphatidylcholine is more resistant to oxidation, is nearly colourless, has little odor and can formulate itself without further additives. It is beneficial if superfluous chemicals are omitted in the formulation since any chemical substance, used as formulation additive, can have an adverse effect on skin which is already irritated and inflamed as it is in acne.

Triglyceride-bound linoleic acid would add more lipid to the acne-affected skin, which is already suffering from an overproduction of sebum.

Linoleic Acid and Acne

The wax ester content of the sebum and of the epidermal acylceramides containing linoleic acid has been found to be inversely proportional to the activity of the sebaceous glands [3], with sebum fatty acids also being incorporated in epidermal lipids.

An analysis of the lipids in comedones and on the surface of the skin of acne patients and on the surface of the skin of persons not suffering from acne revealed that the acylceramides of the comedones and the skin surface of acne patients contained much less linoleic acid than the acylceramides from the skin surface of control subjects [4,5].

This finding gave rise to the hypothesis that the concentration of linoleic acid in sebum from human sebaceous glands depends both on the amount of linoleic acid that is present in every sebum-producing cell at the start of its differentiation and on the extent to which this amount is reduced by the endogenous lipid synthesis that follows [4,6].

The treatment of female acne patients with a combination of the antiandrogen cyproterone acetate and the estrogen ethinylestradiol led to a reduction of sebum secretion and to a simultaneous increase in the concentration of linoleic acid in all classes of lipids

[3]; a similar effect was also achieved with 13-cis-retinoic acid [7].

All these findings emphasize that sebum production, the development of acne and the concentration of linoleic acid in the sebum and in the epidermal lipids are all closely related, but do not provide any evidence of the pathogenic importance of linoleic acid for the disorder itself.

As early as 1929 Burr and Burr undertook fundamental diet studies in animals, which led to the recognition of the importance of polyunsaturated fatty acids - of linoleic acid in particular [8]. Rats that were fed a fat-free diet for several weeks developed the characteristic symptoms of an essential fatty acid deficiency; these were primarily characterized by an increase in the transepidermal water loss (TEWL) [9] and epidermal hyperproliferation [10].

The topical application of linoleic acid to the skins of rats suffering from essential fatty acid deficiency normalized the transepidermal water loss [11]. This curative effect is not prevented even by large doses of indomethacin, a potent inhibitor of cyclooxygenase, but at least in part by eicosatetraenoic acid, a potent inhibitor of both cyclo- and lipoxygenase [12]. This finding suggests that a lipoxygenase product formed from linoleic acid is of essential importance in addition to linoleic acid, being an essential component of the epidermal lipid barrier in the form of acylceramides.

This substance was later identified as 13-hydroxyoctadecadienoic acid (13-HODE). The application of 0.1% 13-HODE to the skin of guinea-pigs suffering from an epidermal hyperproliferation as a result of the administration of eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids, led to the re-establishment of a histologically normal epidermis and to a normalization of the incorporation of radioactively labelled thymidine [13].

In addition, 13-HODE exhibits anti-inflammatory properties since it inhibits the formation of LTB_4 by human neutrophils [14]. Linoleic acid itself, or a not yet definitely identified reaction product, possibly 13-HODE, inhibits phagocytosis and the formation of reactive oxygen species in the case of neutrophils [15].

Thus, linoleic acid or linoleic acid bound to phosphatidylcholine is able to exhibit the following properties:

1. Linoleic acid is essential for the formation of the epidermal lipid barrier, whose inadequate function is the decisive stimulus for the proliferation, metabolic re-adjustment and keratinization of epidermal cells.
2. Linoleic acid is the precursor of 13-HODE, a product with antiproliferative properties, that also inhibits the production of the inflammation mediator LTB_4 .
3. Linoleic acid itself, or a not yet characterized reaction product, inhibits phagocytosis and the production of aggressive oxygen metabolites by neutrophilic granulocytes and, hence, inhibits the inflammatory process.

These findings suggest the potential of the anti-acne efficacy of linoleic acid bound to phosphatidylcholine from soybeans [16].

Biological Evaluations

The concentration of linoleic acid and squalene was determined in Studies 1 and 2 in order to evaluate the alteration of skin surface lipids as the result of phosphatidylcholine treatment. In Study 3 the anti-acne efficacy was proven, by pooling the results of 7 studies.

PROTOCOL OF STUDY 1

Aim of the study: to evaluate the content of linoleic acid in skin surface lipids

Test product: liposome containing 10% lecithin fraction with 80% phosphatidylcholine (tradename: Natipide® 08010A)

Dosage: 1 mg/cm² of lecithin fraction with 80% phosphatidylcholine; the molecule containing chemically bound linoleic acid (tradename: Phospholipon® 80)

Duration of the treatment: 8 weeks

Number of subjects: 14, 4 female and 10 male, aged 14 to 17 years, affected by acne grade 1 and 2 [18].

Method of evaluation: the skin surface lipids were sampled by direct skin contact with a mixture of n-hexane/2-propanol (3:2, v/v). This was done by

placing 2 ml of the solvent mixture in a test tube and applying this to the skin so that the solvent mixture made contact with the skin for 2 min. The solvent mixture was then evaporated off in a stream of nitrogen, the residue was dissolved in 2 ml methanol/0.5 ml n-hexane and converted to the corresponding methyl esters with the aid of 0.3 ml boron trifluoride methanol complex. Linoleic acid and squalene were determined quantitatively by gas chromatography [17].

PROTOCOL OF STUDY 2

Aim of the study: to evaluate the content of squalene in skin surface lipids

Test product: liposome containing 10% lecithin fraction with 80% phosphatidylcholine (tradename: Natipide® 08010A)

Dosage: 1 mg/cm² of lecithin fraction with 80% phosphatidylcholine; the molecule containing chemically bound linoleic acid (tradename: Phospholipon® 80)

Duration of the treatment: 8 weeks

Number of subjects: 7, 3 female and 4 male, aged 14 to 17 years, affected by acne grade 1 and 2 [18].

Method of evaluation: see Study 1

RESULT OF STUDIES 1 AND 2

The results of Studies 1 and 2 are summarized in Fig. 1.

PROTOCOL OF STUDY 3

Number of studies: 7

Test products: liposome containing 10% lecithin fraction with 80% phosphatidylcholine (tradename: Natipide® 08010A) and the adequate liposome formulations containing 10 and 20% Phospholipon® 90 [16].

Dosage: 1 mg/cm² of lecithin fraction containing 80% phosphatidylcholine and pure phosphatidylcholine (tradename: Phospholipon® 80/90) skin, once per day

Control: no treatment on the contralateral side of the face

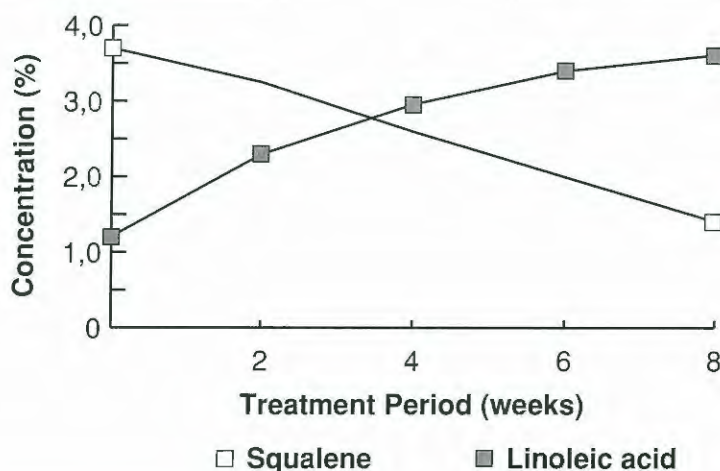
Study design: open, single-center

Duration of the treatment: 20 days to 8 weeks

Number of subjects: between 5 and 15 per study (total number of subjects n=77, age of subjects between 13 and 18 years)

Indication: acne vulgaris

Efficacy variables: number of comedones and efflo-



The values were determined for linoleic acid at 0, 2, 4, 6 and 8 weeks, squalene at 0 and 8 weeks.

Figure 1: Effect of treatment with Natipide 08010A on the linoleic acid and squalene content of the skin surface lipids of acne-affected subjects.

rescences

Evaluation: a projection foil was laid on the test area and a black marker was used to mark the comedones and a red marker to mark the inflamed lesions on the foil on the same 3 x 3 cm area throughout the course of the study. The marks were counted. In all studies an intrasubject control was provided by the corresponding figures for the untreated contralateral side of the face [18].

Exclusion criteria: -acne vulgaris requiring medication, -treatment of the acne with medicaments or cosmetics during the 2 months prior to the study, -consumption of ovulation inhibitors.

Side effects: the subjects merely noticed a somewhat drier and less oily skin and moderate peeling. These effects were enhanced by the use of liposome containing 20% lecithin fraction with 80% phosphatidylcholine (tradename: Natipide® II), which is a gel and can easily be overdosed. However, these observations did not lead to a negative assessment on the part of the subject or to abandonment of the study.

RESULT OF STUDY 3

The results of the studies were compiled and evaluated in a meta-analysis. The average effects of all the studies (Fig. 2, 3) are outlined in the following graphs:

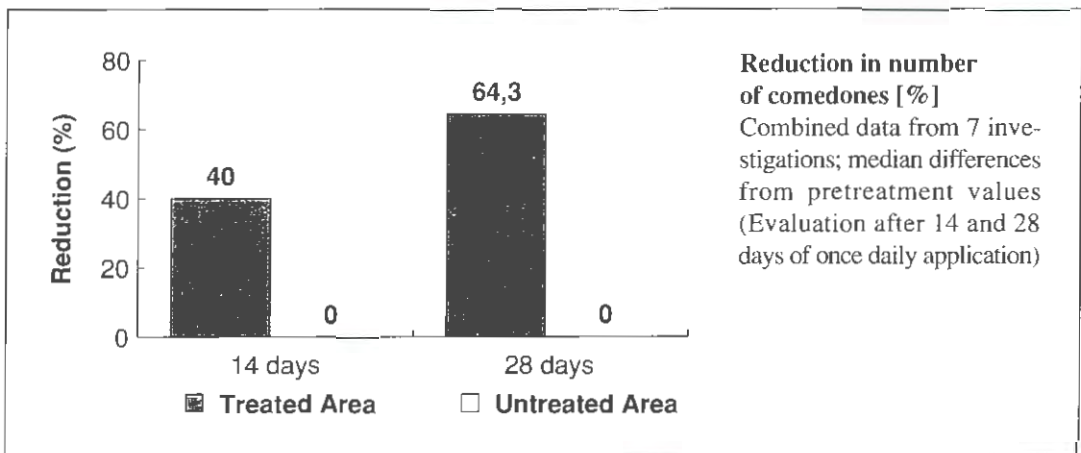


Figure 2: Reduction in number of comedones.

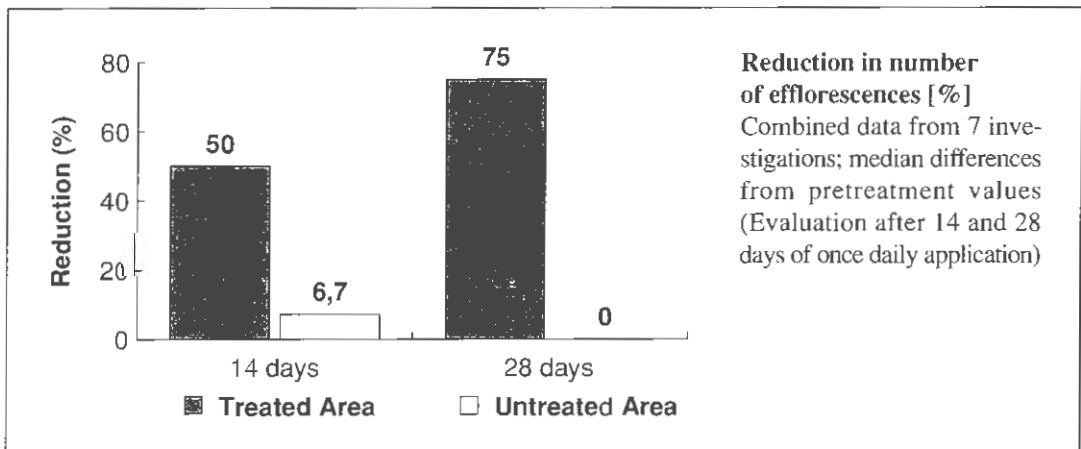


Figure 3: Reduction in number of efflorescences.

The following bar charts (Fig. 4, 5) document the studies separately and give the relative number of subjects where the improvement was more than 60%.

Discussion

Recent investigations suggest that overproduction of sebum leads to a deficiency of linoleic acid and, hence, to disturbance of lipid synthesis and differentia-

tion in the epithelium of the mouth of the sebaceous follicle. This is supported by the analysis of the lipids of the blackheads and of the skin surface of acne patients and of healthy control persons, whereby therapeutic intervention with antiandrogens and 13-cis-retinoic acid lead to the normalization of the linoleic acid content of the lipids in acne patients. Furthermore, linoleic acid is the precursor of 13-hy-

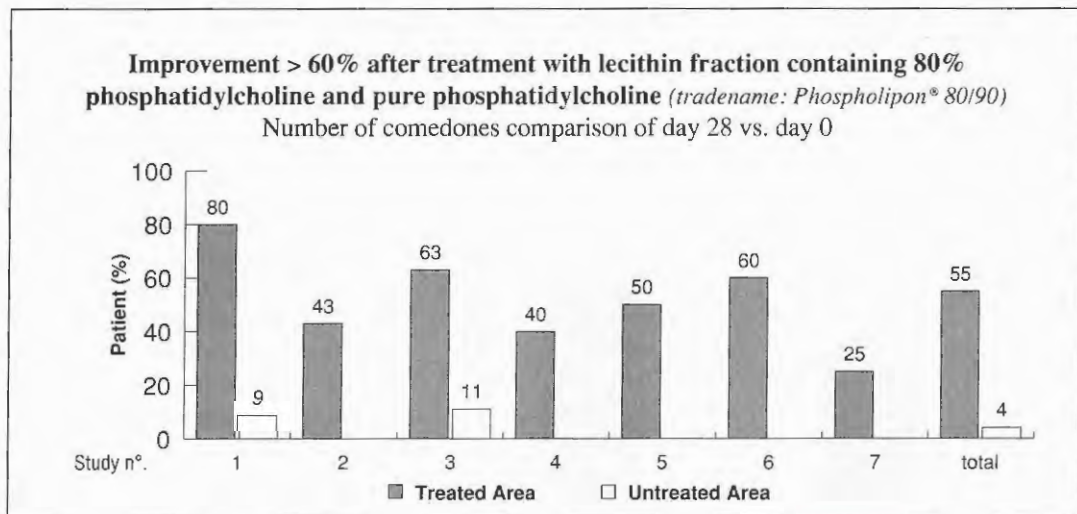


Figure 4: Improvement > 60% with regard to number of comedones.

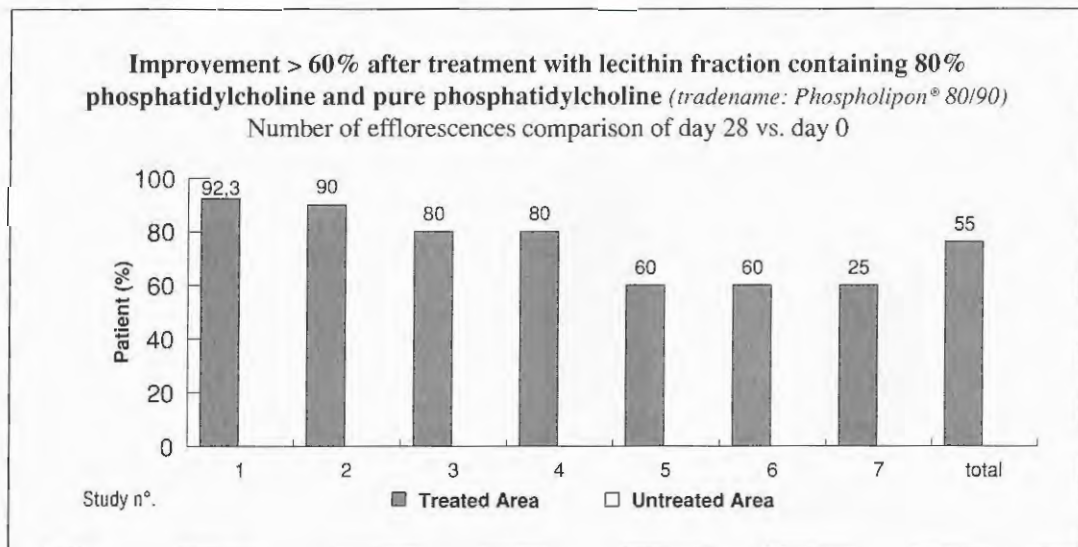


Figure 5: Improvement > 60% with regard to number of efflorescences.

droxyoctadecadienoic acid (13-HODE), a substance with antiproliferative properties, that also inhibits the production of the inflammation mediator LTB₄.

The literature contains no descriptions of the successful treatment of acne by linoleic acid or by derivatives.

Phosphatidylcholine from vegetable sources, especially from soybeans, contains bound fatty acids, of which 70% is linoleic acid. Phosphatidylcholine can be formulated in water to liposomes with a particle size of 0.15 to 0.25 µm without the need for additional substances. These particles may readily pass the sebaceous duct and transport the linoleic acid into the sebaceous gland. The omission of superfluous chemicals in the formulation is beneficial since any chemical substance used as a formulation additive can have adverse effects on skin which is irritated or inflamed as it is in acne.

Seven studies, on 77 subjects, affected by physiological acne, were carried out on the basis of this rationale. Pure phosphatidylcholine and a fraction with 80% phosphatidylcholine, were tested in two different formulations, as liposome with 10% and 20% phospholipids respectively. Formulation containing the same phosphatidylcholine but no water (these are non-liposomal solution) were also investigated.

An average reduction of the comedones by 65% and a reduction of the efflorescences by 75% was recorded for all formulations tested.

In an independent study, the alteration of the skin lipids as the result of treatment by liposome containing 10% lecithin fraction with 80% phosphatidylcholine (tradename: Natipide® 08010A) was studied. A concomitant increase of the linoleic acid content and decrease of squalene content was observed in the course of the treatment. Since squalene is one of the most important indicators for greasy and acne-affected skin, and since the increased sebum production is a primary factor in the pathogenesis of acne, these findings are relevant for the use of phosphatidylcholine; with high content of esterified linoleic acid, thus, suitable for use as a drug substance and as an active ingredient for cosmetics.

These results prove that phosphatidylcholine, purified from soybeans, constitutes a new therapeutic

principle for the treatment of acne [19].

The mode of action suggests the use of soybean-derived phosphatidylcholine

> in physiological acne, which is 70% of the affected persons, as a drug or as an active ingredient in cosmetics,

> or in acne needing medical attention, with the combination of phosphatidylcholine and a drug substance with a complementary mode of action.

The results also suggest its application for normalizing greasy skin, using cosmetics to apply adequate concentrations of phosphatidylcholine in topical formulations.

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ON THE HEAVY METALS CONTENT IN COSMETIC FORMULATIONS: AN ATOMIC ABSORPTION SPECTROSCOPY INVESTIGATION

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Synopsis

The levels of four heavy metals (Pb, Cd, Hg and Cr), have been measured by means of an atomic absorption spectroscopy (AAS) method in 18 different cosmetic formulations, (8 body creams, 3 of them being purchased from herbal shops; 6 hands creams, 2 intime hygiene soaps, 1 face cream, 1 foam bath soap), periodically sampled over a total period of time of 10 months.

The levels of heavy metals have been shown to be in the great majority of samples extremely reduced and in some instances below the detection limits of the applied technique. More precisely, levels of Hg were below the detection limit of the AAS technique in all samples, while mean levels of Pb, Cd and Cr never exceeded 0.184 ppm, 0.035 ppm and 0.270 ppm respectively. However, since the frequency of administration of cosmetic products is very high and it is generally protracted for prolonged periods of time, the toxicologic significance of the present results should be further evaluated, also in the light of a more exhaustive study, carried out on the basis of a more interdisciplinary approach.

Riassunto

Questo articolo riporta i risultati di uno studio spettrofotometrico per assorbimento atomico (AAS) riguardante la determinazione della concentrazione di metalli pesanti in formulazioni cosmetiche. L'indagine, condotta in seguito ad un periodo di campionamento di 10 mesi, ha considerato quattro specifici metalli pesanti (piombo, cadmio, cromo e mercurio) ed è stata condotta su 18 differenti campioni di formulazioni cosmetiche: 8 creme per il corpo, 3 delle quali di erboristeria; 6 creme per le mani, 2 saponi per l'igiene intima, 1 crema per il viso, 1 bagno schiuma. Nella maggioranza dei campioni analizzati la concentrazione di metalli pesanti è risultata estremamente bassa, ed in alcuni casi inferiore al limite di rilevabilità della tecnica AAS impiegata. Più precisamente, i livelli di Hg sono stati sempre al di sotto del limite di rilevabilità in tutti i campioni considerati, mentre i livelli medi di Pb, Cd e Cr non hanno mai superato i valori di 0.184 ppm, 0.035 ppm e 0.270 ppm rispettivamente.

Poiché la frequenza di somministrazione di un prodotto cosmetico è generalmente molto alta, oltre che protratta per lunghi periodi di tempo, il significato tossicologico dei risultati presentati in questo lavoro dovrebbe comunque essere ulteriormente approfondito, preferibilmente alla luce di studi più interdisciplinari.

Introduction

Cosmetic formulations are a class of products whose peculiar features can be considered, from a biochemical and toxicological point of view, as intermediate between foods and drugs.

For indeed, the frequency of administration of cosmetic formulations is generally scheduled on a daily basis, and in some instances several cosmetic products, like, for example, a lipstick or a hand cream, can be applied to the body twice or more times a day. At the same time, the techniques that are generally used to produce a great variety of cosmetic formulations are directly derived from the experience of pharmaceuticals.

It is therefore self evident that any activity devoted to the definition of the possible risks for the consumer due to the presence of toxic substances in a cosmetic formulation is to be strongly supported. In this light, the Italian Law (Legge 713/86) states the procedures to be followed for the commercialization of cosmetic products. More specifically, the supplement II ("Allegato II") of the same law lists a broad group of chemicals whose cannot be employed for the production of any cosmetic formulation.

The limited amount of information, available both from a normative and from a technical point of view, concerning the presence of heavy metals in the cosmetic products and the possible effects of this elements by transcutaneous absorption following topical administration, has suggested to us to plan an experimental study, whose aim was to quantitatively evaluate the levels of some representative heavy metals in various cosmetic formulations.

The assayed samples have been obtained from different suppliers (mainly department stores) located in the East area of Rome, within the inner city limits. Samples of 18 different cosmetic formulations, (8 body creams, 3 of them being purchased from herbal shops; 6 hands creams, 2 intime hygiene soaps, 1 face cream, 1 foam bath soap) were collected, in triplicate, every two months for ten months, so that a total of 270 samples (18x3x5)

were assayed by AAS. The obtained results were compared in order to assess the variability over the period of sampling of the single heavy metals concentrations in each cosmetic formulation.

Results of this preliminary investigation are presented and discussed taking in the due account the indications given by the Italian Law concerning the presence of heavy metals in cosmetic formulations.

In the light of the results obtained in the present work, the need for a more precise definition of heavy metals toxicity, i.e. by considering also the possible presence in the cosmetic formulations of other components, which could, in turn, either reduce or enhance the toxicity of heavy metals, is also stressed.

Materials and methods

Instrumentation

The AAS system is constituted by a Perkin Elmer mod. 1100B atomic absorption spectrometer, equipped with an HGA-700 graphite furnace, a deuterium-arc background correction, and a computer-driven Perkin Elmer Model AS-70 autosampler, (Perkin Elmer Italia SpA, Monza, Italy); the results have been recorded by an Epson EX-850 dot-matrix printer (Epson Italia S.p.A., Milano, Italy).

The detection of mercury, by the cold vapor technique, has been carried out by a Varian Techtron mod. AA-475 atomic absorption spectrometer (Varian Italia, Milano).

Materials

Samples of cosmetic formulations.

The AAS study was carried out on eighteen samples of cosmetic formulations (**#1-18**), indicated as follows:

- 5 body creams (**#1-5**);
- 3 body creams, purchased by herbal shops (**#6-8**);
- 6 hands creams (**#9-14**);
- 2 intime hygiene soaps (**#15-16**);
- 1 face cream (**#17**);
- 1 foam bath (**#18**).

All samples were purchased by different commercial suppliers located in the East area of Rome; more precisely, sampling was repeated five times, once every two months, so that the assayed samples cover a total interval of time of ten months, precisely in the period january-october 1995. It follows that for each one of the 18 cosmetic formulations 15 samples were collected. Each specific sample was always purchased by the same dealer.

The levels of the four representative heavy metals (Pb, Cd, Cr and Hg) have been determined on all the above mentioned samples, according to a procedure recently described (Conti et al., 1996).

The determination of Pb, Cd and Cr was carried out following mineralization of samples, according to the dry mineralization procedure, carried out in order to fully destroy the organic matter. More in details, the mineralization procedure was carried out according to the following stages:

- 1 weight calibration of quartz weighting bottles;
- 2 transfer of samples in the quartz weighting bottles and determination of the humid weight;
- 3 dessication of the sample in oven ($T = 105\text{ }^{\circ}\text{C}$; time of treatment: 12 hours);
- 4 slow thermal treatment ($\Delta T/\Delta t = 50\text{ }^{\circ}\text{C}/\text{hour}$); then
- 5 calcination at $T = 450\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$ in a special oven with the internal walls covered by a layer of quartz;
- 6 collection of white ashes, or, alternatively, repetition of the last passage;
- 7 dissolution of the ashes in 1 mL nitric acid at

$T=40\text{ }^{\circ}\text{C}$;

- 8 transfer of final solutions in 50 mL volumetric flasks and dilution to volume with deionized water.

The determination of Hg was carried out following a wet mineralization procedure, according to the following stages:

- 1 transfer of samples in 25.0 mL volumetric flasks;
- 2 addition of 5.0 mL of concentrated $\text{HNO}_3/\text{H}_2\text{SO}_4$ (1:1);
- 3 boiling to reflux until a clear solution is obtained;
- 4 cooling of the solution;
- 5 concentration of the solution on heating plate;
- 6 transfer of the solution into 50.0 mL volumetric flasks and dilution to volume with bidistilled/deionized water.

Laboratory glassware, reagents and standards of heavy metals.

All the glassware used for the preparation of stock and standard solutions was decontaminated from the possible presence of heavy metals by overnight treatment with metal-free, concentrated HNO_3 (Merck, Darmstadt, Germany).

All reagents were analytical grade. Cadmium, chromium, lead and mercury standards were prepared by dilution with 2% HNO_3 from stock standard solutions of their respective nitric salts ($1000 \pm 2\text{ ppm}$ in 0.5 M HNO_3), supplied by Merck (Darmstadt, Germany); water ultrapure grade by Milli-Q from Millipore (Millipore Corporation U.S.A.) was used for the preparation of all solutions.

Table 1

OPERATIVE CONDITIONS IN THE SPECTROMETRIC ASSAYS

Element	Wavelength (nm)	Slit width (nm)	Matrix modifier	Graphite tubes
Pb	283.3	0.7	0.2 mg/10 μl $\text{NH}_4\text{H}_2\text{PO}_4$	pyrolytic/wall
Cd	228.8	0.7	0.2 mg/10 μl $\text{NH}_4\text{H}_2\text{PO}_4$	pyrolytic/wall
Cr	357.9	0.7	0.05 mg/10 μl $\text{NH}_4\text{H}_2\text{PO}_4$	pyrolytic/wall
Hg	253.7	0.5	-----	-----

Operative conditions in the spectrometric assays.

The quantitative determination of each analyte was carried out by applying the method of the linear regression to the calibration plot following the addition of different aliquots of known standards to the samples under investigation.

The determination of cadmium, chromium and lead has been carried out with the graphite furnace, while the determination of mercury has been carried out according to the cold vapor technique.

The instrumental specification of the method are given in table 1, while the description of the ther-

mal programs followed for the determination of each single metal are reported in tables 2-4. Results of the recovery tests and of the precision tests are given in tables 5 and 6 respectively.

Results

Tables 5-6 show that the analytical procedures followed in the present work allow to obtain reliable results, as indicated by both the values of the recovery assays (Table 5) and of the precision study (Table 6).

All results of the present study are reported in table

Table 2

FURNACE PROGRAM FOR THE DETERMINATION OF Pb
(atomization occurs at step 6; volume of sample injected = 20 μ l)

Step	Temperature ($^{\circ}$ C)	Ramp (s)	Hold (s)	Argon flow (ml/min)
1	80	20	40	300
2	100	20	40	300
3	120	20	60	300
4	300	30	30	300
5	750	30	45	300
6	2000	0	4	0
7	2650	1	5	300
8	20	1	10	300

Table 3

FURNACE PROGRAM FOR THE DETERMINATION OF Cd
(atomization occurs at step 4; volume of sample injected = 10 μ l)

Step	Temperature ($^{\circ}$ C)	Ramp (s)	Hold (s)	Argon flow (ml/min)
1	120	20	10	300
2	350	10	10	300
3	600	10	10	300
4	2100	0	3	0
5	2650	1	5	300
6	20	1	10	300

Table 4

FURNACE PROGRAM FOR THE DETERMINATION OF Cr
(atomization occurs at step 5; volume of sample injected = 15 µl)

Step	Temperature (°C)	Ramp (s)	Hold (s)	Argon flow (ml/min)
1	120	20	10	300
2	300	10	20	300
3	450	10	20	300
4	1650	10	20	300
5	2500	0	5	0
6	2700	1	3	300
7	20	5	5	300

Table 5

RECOVERY TESTS

Element	N. of tests	Concentration added (µg/L)	Recovery (mean ± SD)
Pb	11	10	96.8 ± 4
Cd	10	1	93.7 ± 2
Cr	11	5	96.0 ± 3
Hg	9	2	94.8 ± 5

Table 6

PRECISION TESTS

Element	N. of tests	Concentration (µg/L)	Variation coefficient (%)
Pb	10	4.2	6.1
Cd	10	1.0	3.6
Cr	10	8.2	4.7
Hg	8	1.5	8.4

7. All data are expressed as µg of heavy metal/100.0g of cosmetic formulation. The analytical results are here discussed for each one of the considered analytes.

Lead

Nine out of eighteen samples of cosmetic formu-

lations have shown levels of lead that lie below the detection limit of the instrumental technique. In the other nine samples the values varied from a minimum of 14.2 ppb to a maximum of 184.4 ppb. As it can be seen by the values of the standard deviations, levels of lead show a very narrow variability over the period of time of sampling (10 months),

Table 7

CONCENTRATION OF HEAVY METALS
ON SAMPLES OF COSMETIC FORMULATIONS.
RESULTS (MEAN OF FIVE INDEPENDENT SAMPLE PREPARATIONS $\pm$ SD)
ARE EXPRESSED IN $\mu\text{g}/100\text{ g}$ OF SAMPLE.

Sample	Lead	Cadmium	Chromium	Mercury
1	n. d.	n. d.	2.38 ± 0.82	n. d.
2	1.42 ± 0.40	n. d.	24.70 ± 5.20	n. d.
3	n. d.	n. d.	1.21 ± 0.60	n. d.
4	4.06 ± 1.12	0.08 ± 1.25	0.44 ± 1.35	n. d.
5	1.86 ± 0.76	n. d.	0.84 ± 0.47	n. d.
6	n. d.	0.34 ± 1.80	0.69 ± 2.13	n. d.
7	n. d.	1.17 ± 1.20	4.70 ± 0.95	n. d.
8	n. d.	3.50 ± 0.70	9.50 ± 2.43	n. d.
9	2.47 ± 2.11	0.07 ± 0.65	0.56 ± 0.35	n. d.
10	18.44 ± 0.60	0.09 ± 0.40	0.39 ± 0.25	n. d.
11	n. d.	0.09 ± 1.35	0.38 ± 0.40	n. d.
12	18.43 ± 1.23	n. d.	0.69 ± 0.15	n. d.
13	9.06 ± 0.22	0.19 ± 0.58	1.15 ± 0.22	n. d.
14	1.96 ± 0.35	n. d.	3.74 ± 1.70	n. d.
15	10.95 ± 1.40	1.40 ± 0.46	1.40 ± 2.20	n. d.
16	n. d.	n. d.	12.06 ± 3.50	n. d.
17	n. d.	n. d.	5.60 ± 4.12	n. d.
18	n. d.	0.67 ± 0.24	27.07 ± 6.15	n. d.

n.d.: not detectable ($[\text{Pb}] < 5\text{ }\mu\text{g/L}$; $[\text{Cd}] < 0.1\text{ }\mu\text{g/L}$; $[\text{Cr}] < 4\text{ }\mu\text{g/L}$; $[\text{Hg}] < 1\text{ }\mu\text{g/L}$ in the measuring solution).

thus indicating that the concentration of Pb is virtually independent of seasonal events.

Cadmium

Eight out of eighteen samples of cosmetic formulations have shown levels of cadmium that lie below the detection limit of the instrumental technique. In the other ten samples the values varied from a minimum of 0.7 ppb to a maximum of 35.0 ppb. As it can be seen by the values of the standard deviations, unlike the case of lead, levels of cadmium show a broad variability over the period of time of sampling.

Chromium

Levels of chromium in the eighteen assayed samples varied from a minimum of 3.8 ppb to a maximum of 270.7ppb. Also in this case there is a marked variability of the values along the time.

Mercury

In all the eighteen samples the levels of mercury lied below the sensitivity limits of the instrumental technique.

Discussion

The complete set of experimental results obtained on the 18 different cosmetic formulations and summarized by data reported in table 7 allows to draw the following conclusions:

- the concentration of mercury has been found to lie below the detection limits of the experimental technique in all the 18 assayed samples;
- the presence of chromium was detected in all the 18 assayed samples;
- apart from lead, all the assayed elements showed a marked variability of the concentration values over the time, thus indicating that the differences in the concentration values could be due to environmental factors;
- in four samples (**#1, 3, 16, and 17**, that is 2 out of 5 body creams, 1 out of 2 soaps for the intime hygiene and the only assayed body cream) chromium was the only element to be detected;

The overall picture deriving from the experimental results seems to indicate that no ultimate conclusions can be driven by a simple determination of the concentration of heavy metals in cosmetic formulations, since there is a very broad variability of the results as a function of the sampling period and, at the same time, a non uniform distribution of the single heavy metals in the different samples. The average value are however low enough to ensure the safety of use, provided the cosmetic is applied in the most correct way and, obviously, only on intact skin.

Moreover, the intrinsic toxicity of each heavy metal is a necessary but not a sufficient information in order to quantitatively assess the risk for the consumer, since the bioavailability of a heavy metal can be markedly affected by the presence in the cosmetic formulation of other compounds (e.g. EDTA), which could act as carriers of the metal cation.

Finally, it seems worthwhile to us to highlight that, as of now, no indication is given by the Italian Law concerning the accidental presence of heavy metals in a cosmetic formulation, the only statement being that heavy metals are included in the list of chemicals that cannot be present in any ingredients of a cosmetic product.

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APPLICATION OF A FILM METHOD FOR MICROBIAL MONITORING OF COSMETIC RAW MATERIALS

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Synopsis

The possibility of using a film plate-count method, instead of the too complex and lengthy traditional methods, has been evaluated in cosmetic products. Petrifilm® is a ready-to-use system for detecting any microbial contamination. It consists of a flexible polypropylene film supporting on suitable dehydrated medium, a second support containing guar (a cold-water soluble gel-former) and an indicator on the internal surface.

Petrifilm® proved to have a good sensitivity and to be more convenient than the method recommended by the Italian National Pharmacopoeia. Results showed that it is possible to detect microbial and fungal contamination of raw materials of natural origin, which are frequently used in cosmetic preparations. Positive results were obtained on finished products (oil in water emulsions).

Riassunto

E' stata valutata la possibilità di utilizzare, per la determinazione della carica microbica di materie prime impiegate nei cosmetici e di prodotti finiti, un sistema pronto di conta in piastra (Petrifilm®, 3M). Esso comprende un adatto terreno di coltura su un film di polipropilene, un supporto contenente guar ed indicatore per evidenziare la crescita microbica. Il sistema ha mostrato una buona sensibilità e appare più conveniente rispetto al metodo tradizionale riportato nella Farmacopea Ufficiale Italiana. I risultati del lavoro, seppure preliminari, dimostrano la sua applicabilità nel controllo della contaminazione microbica di materie prime ampiamente usate nell'industria e delle emulsioni O/A.

Introduction

The criteria for microbial purity of cosmetic products are not exactly defined by Italian legislation. The national law 713 of 11.10.1986 states that cosmetic products "must not cause damage to human health when applied under normal conditions of use and should conform to criteria of microbial purity". These criteria have not yet been defined by the Minister for Health, so that recommended microbial limits and official control procedures are not yet available at present. The Minister's decree of 9.7.1987, reporting general instructions concerning manufacturing, handling and storage areas and equipments for manufacturing industries, states that containers and water utilized must be such as to avoid any risk of microbial contamination: a "qualified person" is also responsible for the microbial characteristics of the finished products.

The situation is expected to change rapidly, as the VI amendment (council directive 93/35/EEC) to the cosmetic council directive 76/768/EEC imposes the microbial analyses of raw materials and of the finished products for the protection of the consumer. While waiting for definitive instructions from the Public Administration it is possible to refer to the criteria recommended by the English and American associations, CTFA and CTPA, and to the proposed EC directive relating to microbial purity criteria which however has not yet been promulgated.

In this paper we have chosen as a reference the monograph in the Italian Pharmacopoeia (IX edition): "Microbial contamination of products not required to comply with the test for sterility". This monograph provides the relevant microbial standards and control procedures (1). The Pharmacopoeia test for the detection of microbial contamination consists in the traditional quantitative techniques for the count of bacteria, moulds and/or yeasts using agar media and qualitative tests for the absence of certain specified micro-organisms.

The execution of these tests is complex and lengthy, moreover it requires specialized personnel and suitable equipment both for the preparation of the test materials and for the execution itself. A more con-

venient procedure that would simplify and reduce analysis time is desirable.

Petrifilm[®] is a ready-to-use system for detecting any microbial contamination. It consists of:

1. a suitable dehydrated medium (one of the two types: SM for total aerobic bacteria, YM for yeasts and moulds) supporting on a polypropylene film.
2. a second support containing guar (a cold-water soluble gel-former) and a growth indicator on the internal surface.

As growth indicator two reagents are used: tetrazolium for the total aerobic count in Petrifilm[®] SM or a phosphatase-sensitive indicator for the mould and the yeast count in Petrifilm[®] YM. The medium is rehydrated simply by inoculating the sample.

This system has already been shown to provide a valid alternative to the traditional methods in detecting microbial contamination in a wide variety of foods, e.g. fish (2), milk (3, 4), meat (5), cheese, poultry and frozen vegetables (6).

The Petrifilm[®] system has been accepted and adopted by AOAC to monitor milk and cheese products (7).

These results suggested the extension of these methods to other fields, such as the cosmetic industry.

The aim of the present work was to verify sensitivity and convenience of the Petrifilm[®] plate-count methods compared to a traditional plate-count method in routine analyses of raw materials used in cosmetic products. Preliminary analyses were conducted on finished products (oil in water emulsions).

Materials and methods

Materials

The materials tested are listed in **Table 1**.

Tests were performed on 17 vegetable or animal raw materials employed as functional substances in cosmetic products (4 of them being preservative-free) (nos. 1-17), on 8 plant glycolic extracts (all by their nature containing preservative) (nos. 18-25), and on 7 commercial cosmetic products all of which were o/w emulsions (nos. 26-32).

Table 1**DESCRIPTION OF EXAMINED PRODUCTS****RAW MATERIALS**

n°	Functional substance	Preservative
1	Soya seeds, specific fraction	Parabens; phenoxyethanol 0,8 %
2	Skin hydration factor	Not declared
3	Brain extract crusca	Propyleneglycol
4	Hydrolyzed collagen	None
5	Vegetable polyoses	Parabens and phenoxyethanol 0,4-0,6 %
6	<i>S. cerevisiae</i> cell extract	Phenonip 0.5%
7	Calf thymus peptides	Phenonip 0.3%
8	Bovine ligament elastin	None
9	Bovine brain phospholipids	Phenonip 0.5%
10	<i>S. cerevisiae</i> oligopeptides	Parabens and phenoxyethanol 0,5%
11	Fish glycosaminoglycans	Phenonip 0.4%
12	Blood dialysate	None
13	Bovine brain liposomes	Phenonip 0.5%
14	Tyrosinase-inhibiting peptides	Phenonip 0.45-0.55%
15	Bovine heart extract containing Q. 10	None
16	Bovine brain liposomes	Not declared
17	Fibronectin derivatives	Not declared

GLYCOLIC EXTRACTS

18	<i>Arundo donax</i>	Propyleneglycol
19	<i>Thymus vulgaris</i>	Propyleneglycol
20	<i>Urtica dioica</i>	Propyleneglycol
21	<i>Anthemis nobilis</i>	Propyleneglycol
22	<i>Rosmarinus officinalis</i>	Propyleneglycol
23	<i>Salvia officinalis</i>	Propyleneglycol
24	<i>Malva silvestris</i>	Propyleneglycol
25	<i>Opuntia ficus-indica</i>	Propyleneglycol

EMULSIONS O/W

n°	Main constituent	Preservative
26	Not declared	Not declared
27	Jobba oil-almond oil	Not declared
28	Glycerin	Not declared
29	Macadamia nut oil	Not declared
30	Tocopherol	Not declared
31	Vegetables extracts	Not declared
32	Vegetables extracts	Not declared

Table 2

MICROBIAL COUNTS (mean $\pm$ SE $\times 10^5$ cfu/ml) AFTER SPECIFIC CONTAMINATION OF PRESERVATIVE-FREE RAW MATERIALS (Standard inocula of 10^4 to 10^6 /ml)

CONTAMINANT	PRODUCT (n°)		
	4	8	12
E.COLI			
TSA	1.4 $\pm$ 0.1	13 $\pm$ 0.1	2.5 $\pm$ 0.2*
PETRIFILM® SM	1.3 $\pm$ 0.1	14 $\pm$ 0.2	3.7 $\pm$ 0.3*
S.AUREUS			
TSA	4.8 $\pm$ 0.6	4.7 $\pm$ 0.8	4.6 $\pm$ 0.5
PETRIFILM® SM	6.4 $\pm$ 0.7	6.2 $\pm$ 0.2	4.2 $\pm$ 0.4
C.ALBICANS			
SDA+CAF	0.71 $\pm$ 0.5	1.6 $\pm$ 0.3	8.0 $\pm$ 0.1
PETRIFILM® YM	0.73 $\pm$ 2.4	2.7 $\pm$ 0.4	8.3 $\pm$ 2

*Statistically significant differences for $p = 0.01$

Microbial analyses for the detection of the total aerobic microbial and fungal counts

Initial samples were prepared by diluting 10 ml of each product with 90 ml of sterile peptone dilution water (Peptone Water, Difco). A suitable surfactant, 5% of sorbitan monooleate (Tween 80, Atlas Euro-pol), was added to assist the suspension of poorly water-soluble products. If necessary, the pH of the dispersion was adjusted to about 7.

Samples 1,6,7,10 and 11 contained as preservative various parabens and phenoxyethanol (as shown in Table I). In this case microbial contamination was detected after inactivation of the preservative system by addition of 0.1% TRITON X-100 plus 0.5% TWEEN 80.

The analyses were carried out under conditions designed to avoid any accidental contamination of the samples, in a laminar airflow cabinet.

The conventional "pour plate" test for the total aerobic microbial and fungal count was performed by inoculating decimal serial dilutions of sample in Tryptone Soya Agar (TSA, Oxoid) and in Sabouraud Dextrose Agar (SDA, Oxoid) with added chloramphenicol (100 mg/l). Phosphate-buffered dilution water (Phosphate Buffered Saline, Dul-

becco "A" Oxoid; pH 7.3) was used to prepare the samples. The plates for the total aerobic microbial count were incubated at $37 \pm 1^\circ\text{C}$ for 5 days, and those for the fungal count at $25 \pm 1^\circ\text{C}$ for 5 days. According to instructions from the manufacturer, analyses by Petrifilm® method were performed by pipetting 1ml of the original sample and each of its serial decimal dilutions to a Petrifilm® Aerobic Count Plate (SM) and 1ml to a Petrifilm® Yeast and Mould Count Plate (YM). All plates were incubated at $32 \pm 1^\circ\text{C}$ or at $25 \pm 1^\circ\text{C}$ for 48 hours as appropriate. Analyses for all methods tested were performed in duplicate, three times each. Student's *t* test was applied.

Contamination Test

Because of the very low microbial contamination detected in all materials examined we decided to contaminate some of the raw materials prior to testing by inoculation of bacteria or yeasts.

Inocula of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), a strain of *Candida albicans* clinically isolated, and *Bacillus* spp. isolated from no.18 were used.

Samples 4,8 and 12 of preservative free materials were contaminated with 10^4 to 10^6 micro-organisms/ml. Samples characterized by a self-preservative activity of solvent such as glycolic extracts were contaminated by an inoculum of about 10^3 to 10^4 spores per ml. Samples containing preservative systems (samples 6, 10, 11 and 26-32) were contaminated only after we had added TWEEN 80 and TRITON X-100 as neutralizers.

Results

Total aerobic microbial and fungal counts

All the samples examined except no. 18 showed total aerobic microbial and fungal counts of less than 10 cfu/ml. The total aerobic microbial count of no. 18 Provençal Cane, by both the "pour plate" technique in TSA and the Petrifilm® SM method, was 45 cfu/ml and 60cfu/ml respectively (no statistically significant difference). The contaminating bacterium was *Bacillus* spp.

Application of the contamination test to preservative-free raw materials

Microbial counts on antibacterial free samples 4, 8 and 12 after contamination by inoculation of standard bacterial suspensions (Table 2) were not significantly different in the two methods except for *E. coli* count in sample 12. Results show a higher sensitivity for Petrifilm®.

Application of the contamination test to glycolic extracts of plants

After contamination of all glycolic extracts except no.18 with *Bacillus* spp., we determined microbial counts by the two methods. Results were again not statistically different (Table 3) except for sample 19, where the Petrifilm® method was more sensitive.

Application of the contamination test to raw materials containing preservatives

Raw materials 6, 10 and 11, which contained a preservative system, were inoculated with standard suspensions of *E.coli* and *C.albicans* after neutralization of the preservative system. Again the results

Table 3

MICROBIAL COUNTS
(mean $\pm$ SE $\times 10^4$ cfu/ml)
AFTER CONTAMINATION
OF NATURAL GLYCOLIC EXTRACT
WITH BACILLUS SPP.
(Standard inocula of 10^3 to 10^4 /ml)

Prod. (n°)	TSA	PETRIFILM® SM
19	3.3 $\pm$ 0.2*	5.2 $\pm$ 0.4*
20	0.29 $\pm$ 0.1	0.33 $\pm$ 0.7
21	3.8 $\pm$ 0.2	4.5 $\pm$ 0.2
22	0.32 $\pm$ 0.1	0.37 $\pm$ 0.9
23	0.45 $\pm$ 1	0.51 $\pm$ 1.1
24	3.8 $\pm$ 0.3	4.5 $\pm$ 0.3
25	3.2 $\pm$ 0.5	2.6 $\pm$ 0.4

*statistically significant differences for $p=0.01$

(Table 4) were identical.

Application of the contamination test to o/w emulsions

After contamination of the selected emulsions, where the preservative system was neutralized, if necessary, (nos. 30-32), the microbial counts were not statistically different (Table 5) except for sample 28 inoculated with *P. aeruginosa*. In this case the Petrifilm method was more sensitive.

Conclusions

The results show that the Petrifilm® system and the conventional methods showed no significant statistical differences except in two cases where the Petrifilm® method was the more sensitive.

The new procedure is more convenient than the traditional one in the following ways:

-Petrifilm® does not require preparation of the component parts requiring specialized staff or suitable equipment and requires less time for the procedure itself;

-it is a compact system requiring less space for sto-

Table 4

MICROBIAL COUNTS (mean $\pm$ SE $\times 10^5$ cfu/ml) AFTER SPECIFIC CONTAMINATION OF PRESERVED RAW MATERIALS (Standard inocula of 10^4 to 10^6 /ml.)

CONTAMINANT	PRODUCT (n°)		
	6	10	11
E.COLI			
TSA	4.1 $\pm$ 0.8	3.6 $\pm$ 0.6	2.0 $\pm$ 0.3
PETRIFILM® SM	4.1 $\pm$ 0.7	3.6 $\pm$ 0.7	1.8 $\pm$ 0.2
C.ALBICANS			
SDA+CAF	42 $\pm$ 0.2	50 $\pm$ 0.8	50 $\pm$ 0.8
PETRIFILM® YM	43 $\pm$ 0.7	58 $\pm$ 0.8	52 $\pm$ 0.6

Table 5

MICROBIAL COUNTS (mean $\pm$ SE $\times 10^5$ cfu/ml) AFTER CONTAMINATION OF EMULSIONS WITH STAPHYLOCOCCUS AUREUS AND PSEUDOMONAS AERUGINOSA (Standard inocula of 10^4 to 10^6 /ml)

CONTAMINANT	PRODUCT (n°)						
	26	27	28	29	30	31	32
S.AUREUS							
TSA	6.8 $\pm$ 1.3	14 $\pm$ 0.6	8.1 $\pm$ 1.0	15 $\pm$ 0.5	11	14 $\pm$ 0.8	7,4 $\pm$ 0.2
PETRIFILM® SM	7.5 $\pm$ 1.4	15 $\pm$ 2	7,9 $\pm$ 0.7	15 $\pm$ 1	11	15 $\pm$ 2	7,2 $\pm$ 0.4
P.AERUGINOSA							
TSA	26 $\pm$ 2	11 $\pm$ 3	19 $\pm$ 0.7*	20 $\pm$ 2	11 $\pm$ 2	8.3 $\pm$ 0.7	15 $\pm$ 5
PETRIFILM® SM	27 $\pm$ 1	13 $\pm$ 2	24 $\pm$ 0.3*	27 $\pm$ 2	13 $\pm$ 1	11 $\pm$ 0.5	18 $\pm$ 6

*statistically significant differences for $p=0.01$

rage and during the incubation period.

-Petrifilm® plates are easier to read than agar plates. Sample n.18, for example, showed colonies which became confluent in 24 hours in the traditional agar media, whereas in Petrifilm® plates colonies were always clearly defined and more easily identified. This last is especially advantageous in the investigation of cosmetic powders which often cause difficulties in colony detection because they alter the medium.

No incompatibilities among Petrifilm® and the raw

materials tested were detected.

The Petrifilm® system is more economical if the total test cost is considered, because of the shorter time required to perform the procedure.

Further investigations have to be conducted especially on finished products; in fact, these preliminary data show the applicability of the method only to o/w emulsions.

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Index to Volume 14, 1996

Contents:

Book Review

Hair transplantation

Edited by Walter P. Unger

XIX

Original Laboratory Studies

Alpha Hydroxy Acids in the cosmetic treatment of photo-induced skin ageing

P. Morganti, S.D. Randazzo and C. Bruno

1

Examination of fingernail plates by means of polarized light videomicroscopy

R. Strumia

9

Use of vitamin-A gel for the prophylaxis of post-partum vulvo-vaginitis: a pilot study - 1° note

L. Armino - P. Morganti

15

Enhanced antiinflammatory activity of Diclofenac in jojoba oil submicron emulsion cream

J. S. Schwarz, M. R. Weisspapir, A. Shani and S. Amselem

19

Decreasing the stinging capacity and improving the antiaging activity of AHAs

P. Morganti, S.D. Randazzo, G. Fabrizi, C. Bruno

79

About claims substantiation for topical formulations: an objective approach to skin care product's biological efficacy

L. Rodrigues, I. Jaco, M. Melo, R. Silva, L.M. Pereira, N. Catorze, E. Barata, H. Ribeiro, J. Morais

93

Vacuum skin-abrasion versus glycolic acid peeling in the treatment of atrophic acne scars

S. Jurassich, A. Lo Schiavo, F. Pinto, M. Nacca

127

A video-microscope study of "hair-knots"

R. Strumia, C. Roveggio

133

The treatment of Acne Vulgaris by Phosphatidylcholine from Soybeans, with a high content of Linoleic Acid

M. Ghyczy, H-P. Nissen, H. Biltz

137

On the heavy metals content in cosmetic formulation: an atomic absorption spectroscopy investigation

M. E. Conti, F. Botrè, F. Mazzei

147

Application of a film method for microbi monitoring of cosmetic raw materials

L. Piu, C. Juliano, G. Pirisino, P. Minghetti

155

General Articles

Cosmetic products for the body: an economic study of their distribution and consumption

A. Ghi, R. Jirillo

25

Alpha Hydroxy Acid in cosmetic dermatology

P. Morganti

35

Validation tests and cell cultures in cosmetology: the present and prospects

M.G. Tucci, R. Solmi, L. Simonelli, P. Morganti, G. Ricotti,

M.G. Gandolfi, G. Biagini

43

Role of epidermal ceramides in barrier function

B. Berra, E. Veggetti

51

Transdermal drug delivery by iontophoresis.

I. Fundamentals and theoretical aspects

R. Pignatello, M. Fresta, G. Puglisi

59

Is there any risk of allergic reaction in creno-cosmetology?

G.A. Vena, M. Mastrodonardo, C. Foti

73

Transdermal drug delivery by iontophoresis.

II. Techniques and "*in vitro-in vivo*" models

R. Pignatello, M. Fresta, G. Puglisi

99

The scope of mineral oil in personal care products and its role in cosmetic formulation

D. S. Morrison, J. Schmidt, R. Paulli

111

Techniques of skin correction using bovine collagen: is it possible under analgesia?

G. Sito, L. Sorrentino

119

Author Index

- Amselem S.**, see Schwarz J.S., 19
- Armino L.**, Use of vitamin-A gel for the prophylaxis of post-partum vulvo-vaginitis: a pilot study - 1° note, 15
- Barata E.**, see Rodrigues L., 93
- Berra B.**, Role of epidermal ceramides in barrier function, 51
- Biagini G.**, see Tucci M.G., 43
- Biltz H.**, see Ghyczy M., 137
- Botrè F.**, see Conti M.E., 147
- Bruno C.**, see Morganti P., 1; see Morganti P., 79
- Catorze N.**, see Rodrigues L., 93
- Conti M.E.**, On the heavy metals content in cosmetic formulation: an atomic absorption spectroscopy investigation, 147
- Fabrizi G.**, see Morganti P., 79
- Foti C.**, see Vena G.A., 73
- Fresta M.**, see Pignatello R., 59; see Pignatello R., 99
- Gandolfi M.G.**, see Tucci M.G., 43
- Ghi A.**, Cosmetic products for the body: an economic study of their distribution and consumption, 25
- Ghyczy M.**, The Treatment of Acne Vulgaris by Phosphatidylcholine from Soybeans, with a High Content of Linoleic Acid, 137
- Jaco I.**, see Rodrigues L., 93
- Jirillo R.**, see Ghi A., 25
- Juliano C.**, see Piu L., 155
- Jurassich S.**, Vacuum skin-abrasion versus glycolic acid Peeling in the treatment of atrophic acne scars, 127
- Lo Schiavo A.**, see Jurassich S., 127
- Mastrolonardo M.**, see Vena G.A., 73
- Mazzei F.**, see Conti M.E., 147
- Melo M.**, see Rodrigues L., 93
- Minghetti P.**, see Piu L., 155
- Morais J.**, see Rodrigues L., 93
- Morganti P.**, Alpha hydroxy Acids in the cosmetic treatment of photo-induced skin ageing, 1; see Armino L., 15; Decreasing the stinging capacity and improving the antiaging activity of AHAs, 79; Alpha Hydroxy Acid in cosmetic dermatology, 35; see Tucci M.G., 43
- Morrison D.S.**, The scope of mineral oil in personal care products and its role in cosmetic formulation, 111
- Nacca M.**, see Jurassich S., 127
- Nissen H-P.**, see Ghyczy M., 93
- Paulli R.**, see Morrison D.S., 111
- Pereira L.M.**, see Rodrigues L., 93
- Pignatello R.**, Transdermal drug delivery by iontophoresis. I. Fundamentals and theoretical aspects, 59; Transdermal drug delivery by iontophoresis. II. Techniques and "in vitro-in vivo" models, 99
- Pinto F.**, see Jurassich S., 127
- Pirisino G.**, see Piu L., 155
- Piu L.**, Application of a film method for microbi monitoring of cosmetic raw materials, 155
- Puglisi G.**, see Pignatello R., 59; see Pignatello R., 99
- Randazzo S.D.**, see Morganti P., 1; see Morganti P., 79
- Ribeiro H.**, see Rodrigues L., 93
- Ricotti G.**, see Tucci M.G., 43
- Rodrigues L.**, About claims substantiation for topical formulations: an objective approach to skin care product's biological efficacy, 93
- Roveggi C.**, see Strumia R., 133
- Schmidt J.**, see Morrison D.S., 111
- Schwarz J.S.**, Enhanced antiinflammatory activity of Diclofenac in jojoba oil submicron emulsion cream, 19
- Shani A.**, see Schwarz J.S., 19
- Silva R.**, see Rodrigues L., 93
- Simonelli L.**, see Tucci M.G., 43
- Sito G.**, Techniques of skin correction using bovine collagen: is it possible under analgesia?, 119
- Solmi R.**, see Tucci M.G., 43
- Sorrentino L.**, see Sito G., 119
- Strumia R.**, Examination of fingernail plates by means of polarized light videomicroscopy, 9; A video-microscope study of "hair knots", 19
- Tucci M.G.**, Validation tests and cell cultures in cosmetology: the present and prospects, 43
- Veggetti E.**, see Berra B., 51
- Vena G.A.**, Is there any risk of allergic reaction in creno-cosmetology?, 73
- Weissapir M.R.**, see Schwarz J.S., 19

Subject Index

- 3C System[®]**, 1; 85
13-hydroxyoctadecadienoic acid, 139
 β -glycosidase, 55
A Vitamin, 15; trophic activity of, 17
Absorption, transcutaneous, 148
Acetic acid, 38
Acne, and linoleic acid, 138; and phosphatidylcoline, 139
Acne vulgaris, 137
Age-spots, 1; and AHAs, 80
Aging, 1; 79
AHAs, 1; How to use, 35; 80; chemistry and activity, 36; and pH, 38; alpha hydroxyenoic acids, 36; how synergize the activity of, 38; depigmenting activity of, 82; and arginine, 82; and lysine, 82; and skin hydration, 85; and TEWL, 85; activity on hydroperoxides of, 85
Animal testing, alternative to, 43
Acid Fibroblast Growth Factor (a-FGF), 46
Actinic Keratosis, 1
Anti-cellulite, consumption, 32
Arginine, to reduce the stinging activity of AHAs, 80; to increase the AHAs activity, 80; to reduce the AHAs side effects, 80; to enhance the anti-free radical activity of AHAs, 80
Atomic absorption spectroscopy, 147
Biological activity, and cell culture, 48; of linoleic acid, 138
Biological efficacy, 93
Body odours, 26
Bovine collagen, as skin corrector, 119
Carboxylic Acid, 2
Cell culture, types of, 44; fibroblasts for, 45; in cosmetological research, 45; and phototoxicity, 47; as biological model, 48
Cell turnover, speeding up, 7
Cellulite, treatment, 30
Ceramide, 52; 60
Ceramides, and barrier function, 52
Chemicals, in cosmetic product, 153
Citric acid, 37
Cleansing, skin, 93
Comedogenicity, cosmetic, 116
Comedones, and linoleic acid, 141
Contamination test, 158
Skin turnover, controlling, 38; pH and, 38; synergizing activity of, 38;
Corneocyte, cohesion, 36; 38; disaggregation, 38
Cosmetic, classification, 26; labelling, 32; supply, 29; principal companies, 31; market segment, 30; advertising, 30; consumption, 30; packaging, 32; and heavy metals, 147
Cosmetic Dermatology, 36
Cosmetic products, definition, 26; classed of, 27; consumption in Italy, 28; VIth modification of directive 76/768/EC concern, 32
Cosmetic objectivation, 93
Cosmetic vehicle, a new, 19
Creno-cosmetology, 73
Crenotherapy, 74
Deodorant, consumption, 32
Depigmenting, activity of glycolic acid as, 82
Dexamethasone, 46
Diclofenac, 19
Diethylammonium, 19
EMEM, Earle's modified Eagles medium, 46
E Vitamin, 15
Efficacy, concept of, 94
Epidermal Growth Factor, 47
Episiorrhaphy, 15
Episiotomy, topical treatment of, 17
Essential fatty acid, in the skin, 54
Fibroblasts, growth, 46; and collagen, 46
Film, hydrolipidic, 53; dry rehydratable, 155
Firmness creams, consumption, 30
Free radicals, and AHAs, gelatin-glycine and arginine, 86
Fucoido-cosmesis, 74
Gamma linolenic, acid for skin dryness, 36
Gaucher's disease, 51
Gelatine/Arginine, 35; to increase the AHAs activity, 80
Gelatine-glycine, 1; 6; 35; to reduce the aHAs side effects, 80
Gelatine/Lysine, to increase the AHA's activity, 35
Glucosylceramide, 52
Glycerophospholipids, 54

- Glycolic Acid**, 1; 35; 37; stinging activity of, 81; depigmenting activity of, 82; arginine to reduce the stinging activity of, 83; 119; as peeling, 127
Hair, evaluation of, 12
Hair knots, 133
Heavy metals, in cosmetic formulation, 147
Horny layer, surface lipids of, 53; the hydrolytic enzymes of, 55
Hydration, skin, 7
Hydration activity, of AHAs, 80
Hydrocortisone, 46
Hyperkeratosis, 35
Ichthyosis, 36
Iontophoreic, therapy, 60
Iontophoresis, 59; as transdermal drug delivery, 60; and erythema, 65; side effect of, 65; the limits of, 65; definition and advantages, 63; as a drug delivery device, 100; principal factor influencing, 100; and pH, 102; and current applied, 102; and drug structure, 103; 104
Jojoba oil, 23
Keratinization, process, 52
Keratinocyte, cohesion, 39, culture, 46; culture validation, 48; and keratins, 52
Labelling, cosmetic products, 25
Lactic acid, 37
Langerhans cells, 47
Linoleic acid, 54; 137
Lipids, synthesis, 52; epidermal, 53
Lipid removal capacity, by cleansing cosmetics, 93
Malic acid, 37
Mandelic acid, 37
Microabrasion, 127
Microbial monitoring, of cosmetics raw materials, 155
Microbial contamination, criteria, 156
Microbial purity, of cosmetics products, 156
Mineral oil, as "natural cosmetic compound, 111; purity of, 112; application in cosmetics, 113; and moisturization, 114; and emolliency, 115; to balance spreadability and moisturization, 115
Mineral water, 73
Minolta chromameter CR 200, 83
Muds, natural, 73
Nail, 9; brittle, 13; evaluation of, 12
Natipide® 08010A, 140
NME, 53
Odland bodies, 52
Onyoschizia, 12
PCA, sodium, 2
Peloido-cosmesis, 74
Perfumes, consumption, 29; distribution channels, 28
Petrifilm®, 155
Phosphatidylcholine, and acne, 54; 137; 140
Phosphatidylethanolamine, and acne, 54
Phosphatidylserine, and acne, 54
Phospholipase, and acne, 54
Phospholipids, and acne, 54
Phospholipon® 80, and acne, 140
Phospholipon® 80/90, and acne, 140
Photoaging, 1; 35; 79
Plankton-cosmesis, 74
Pseudofolliculitis, 133
Pufa, 36
Pyrrolidone Carboxylic acid, 1
Sanitary pads, and cosmesis, 15
Sebum, production, 52; composition, 52; and linoleic acid, 138
Simmondsia chinensis, 19
Skin, and AHA's, 1; 35; 79; aging 1; 79; hydration, 1; 35; 79; dryness and AHA's, 1; 35; 79; dryness, 36; peeling, 35; turnover, 35; hydration, 53; and iontophoresis, 60; and elastic property of AHAs, 83; barrier, function, 83; cleansing activity evaluation, 93; bioengineering, 94; cleansing, 94; microabrasion, vacuum, 128; lipids, 137
Sodium PCA, 2
Sphingolipids, 54
Squalene, 137
Status cosmeticus, 38
Steroid sulphatase, 55
Stinging capacity, of AHA's, 81
Stinging test, 81
Sub-Micron Emulsion, 22
Sun Products, consumption, 30

Tartaric acid, 37
Tattooing, and iontophoresis, 60
Tomesa therapy, 74
Toxicity, intrinsic, 153
Transdermal drug delivery, 59
Triacylglycerol hydrolase, 55
Trichloroacetic acid, 38
Twistometer, 83
Videomicroscopy, 9; 133
Vulvar epidermis, dermatites, 16
Vulvo-vaginitis, 15; topical treatment of, 17
Wrinkling, skin, 1
Xerosis, cosmetic treatment of, 39



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IN-COSMETICS Conference 1997

“MODERN CHALLENGES TO THE COSMETIC FORMULATION”

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Colipa (The European Cosmetic, Toiletry and Perfumery Association),

ISCD (International Society of Cosmetic Dermatology)

IASC (International Aloe Science Council)

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The 1997 IN-COSMETICS Conference in Düsseldorf, 5-7 May promises to be the highlight of the cosmetic year.

DAY 1. The Colipa speakers will concentrate on the challenges of cosmetic regulations. They will discuss the work of SCAAT, Colipa's steering committee on Alternatives to Animal Testing. Also the reality of ingredient labelling in the EU will be highlighted. What are the facts about BSE and Cosmetics? If you want to know May 5 in Düsseldorf will show. New sunscreens regulations and the role of Colipa's cosmetic ingredient Committee round out this most interesting day. In a poster session Colipa will also introduce its inventory CD.

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The themes of the ISCD Day are:

The Dossier - Quality & Safety Aspects
Scientific Backgrounds
and
Evaluation of cosmetic formulations

Experimental and dermatological approaches

Scientific topics sponsored by ISCD are the following:

- **Basic requirements**
- **Uv Filters as skin protectors**
- **Hair growth**
- **Allergic reactions to cosmetics**
- **Natural products**
- **Environmental impacts**
- **Activity evaluation of raw materials**
- **Activity evaluation of finished products**
- **Cosmetic approaches in pigmentary disorders**
- **Alternatives to animal experiments.**

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In close cooperation with **IN-COSMETICS**



International Society of
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THE I.S.C.D. DAY **Düsseldorf - 6 May 1997**

*President: Prof. Dr. Rodolfo Paoletti,
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SECOND ANNOUNCEMENT **THE DOSSIER - Quality & Safety Aspects** **Scientific Backgrounds** **Chairs: P. Morganti, C.E. Orfanos**

Lecturers:

Prof. Dr. Mercedes **DE SOLA** (Belgium); Prof. Dr. Hans **SCHAEFER** (France);
Prof. Dr. Nicola **LOPRIENO** (Italy)

EVALUATION OF COSMETIC FORMULATION **- Experimental and Dermatological Approaches -** **Chairs: B. Giannotti, C.E. Jacobson, F. Kerdel Vegas, F. Kemper, H. Schaefer**

Lecturers:

Dr. Jochen **SPENGLER** (Germany); Prof. Dr. Helmut **IPPEN** (Germany);
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Prof. Luis **RODRIGUES** (Portugal); Prof. Coleman **JACOBSON** (USA); Prof. Brodie **JAMES** (USA).

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Organizing secretariat:

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I.S.C.D. Day

President: Prof. Dr. Rodolfo Paoletti

THE DOSSIER - Quality & Safety Aspects Scientific Backgrounds

Chairs: P. Morganti, C.E. Orfanos

- 8,30** Prof. Dr. Rodolfo PAOLETTI, *Italy*
Introductory remarks
- 9,00** Prof. Dr. Mercedes DE SOLA, *EU Brussels*
Scientific demand of the Dossier - VI Amendment
- 9,20** Prof. Dr. Hans SCHAEFER, *France*
Skin barrier
- 9,40** Prof. Dr. Nicola LOPRIENO, *Italy*
Alternatives to Animal Experiments
- 10,00 - 10,30** *Coffee Break*

EVALUATION OF COSMETIC FORMULATION - Experimental and Dermatological Approaches -

Chairs: C.E. Jacobson, F. Kemper

- 10,30** Dr. Jochen SPENGLER, *Germany*
Basic requirements
- 10,50** Prof. Dr. Helmut IPPEN, *Germany*
UV Filters as skin protectors
- 11,10** Prof. Dr. Benvenuto GIANNOTTI, *Italy*
Cosmetic approaches in pigmentary disorders
- 11,30** Prof. Dr. Constantine E. KOUSKOUKIS, *Greece*
Allergic reactions to cosmetics

11,50 - 13,00 *Lunch*

Chairs: B. Giannotti, H. Schaefer, F. Kerdel Vegas,

13,00 Prof. Dr. Constantin E. ORFANOS and Dr. Ulrike BLUME, *Germany*
Hair growth

13,20 Prof. Dr. Pierfrancesco MORGANTI, *Italy*
Natural products

13,40 Prof. Dr. Fritz H. KEMPER, *Germany*
Enviromental impacts

14,00 Prof. Dr. Luis RODRIGUES, *Portugal*
Activity evaluation of raw materials

14,20 Prof. Dr. Coleman JACOBSON, *USA*
Products for skin aging: activity evaluation

14,40 Prof. Dr. Brodie JAMES, *USA*
Hair products: activity evaluation

15,00 - 16,00 General discussion and poster-discussion

End of ISCD Day

CALL FOR POSTERS

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