AMMONIO LATTATO “ATTIVATO”
LA RISPOSTA DERMATOLOGICA
ALLE IPERKERATOSI

Ipercheratosi intrinseca

Ipercheratosi estrinseca

“ACTIVATED” AMMONIUM LACTATE
THE RIGHT REPLY TO HYPERKERATOSIS

KERATOTAL 1
Emulsione - Ammonio Lattato 14%
KERATOTAL 2
Emulsione - Ammonio Lattato 8%
KERATOTAL SHAMPOO
Ammonio Lattato 7%
KERATOTAL BAGNO
Olii lineari e ramificati - Ammonio Lattato 5%

Modo d’uso: localmente 2 volte al di.

COLLAGEN RESEARCH CENTER srl
Via Innocenzo XI, 41 - 00165 ROMA
Distribuito da MAVI sud - Aprilia (LT) Italy
A NEW MAVICEUTICAL®

KERATOTALACNE™

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\(^{(1,2,3)}\)

⇒ Decreases the Squalene content of acne affected skin

⇒ Reduces excess lipids

⇒ Significantly reduces EFA/TG ratio

⇒ Increases skin hydration by 97%

Please see a brief summary of prescribing information on next page →
**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 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 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 continuously 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 
A NEW MAVICEUTICAL®

Lip protective with Glycoaminoacids(*)

INDICATIONS

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

• Retinoids
• UV rays
• Wind
• Weather
• Environmental pollutants

Such as

Cheilitis or chapped lips
Actinic cheilitis (acute and chronic)
Allergic cheilitis
Exfoliative cheilitis
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
KERATOTAL Labbra

Lip protective with Glycoaminoacids (•)

In all the disorders of the mucocutaneous integument of the lips

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 keep 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.

For more information call to:
Mavi Sud Srl
V.le dell'Industria 104011 Aprilia (Lt) Italy
Tel.+39.6.92.86.261
Fax +39.6.92.81.523
E-Mail:mavi@colosseum.it
URL=http://www.colosseum.it/st81/mavi

(•)partially neutralized by a special patented blend of aminoacids
DERMATOLOGIA COSMETOLOGICA
A cura di F. Morganti e L. Mucardini
Ed. Internazionali Ediemme

Indice 1° Volume

Sezione I Considerazioni Generali
1 Consegni 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 inservazione della cute

Sezione III La cute come organo di assorbimento
8 Nozioni basilari sulla porosità 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 Antimicrubi e preservanti cutanei
16 La prefumazione 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 psicocosmetici e somatoestetici in dermatologia cosmetologica

Sezione VIII I danni cutanei
28 Patologia cutanea da cosmetici su base immunologica
29 Danni da cosmetici

INFORMAZIONI PER L'ACQUISTO
Il pagamento di Lire 120.006 (Centoventi mila) per l'acquisto del 1° volume di Dermatologia Cosmetologica può essere effettuato mediante assegnazione di conto corrente o per contanti indirizzandoli a:

INTERNATIONAL EDIEMME Via Innocenzo XI, 41 - 00165 ROMA
c/c bancario n. 3184/51 Banca di Roma Ag. 1, Aprilia (LT)

☐ Prenotato fin dal ora i volumi 2° a 3°
Con la presente richiesta:
Copie n.............................. del Volume n. 1

☐ Invio in contrassegno

☐ Accettare assegno n......................................................

 Nome

 Indirizzo

TIMBRO E FIRMA

Specificare condizioni di pagamento e fornire N° Codice Fiscale se è richiesta fattura.
Cosmetic Dermatology

Series Editor: P. Morganti

Volume 2
Every day Problems in Dermatology:
The Cosmetic Connection

Editors: P. Morganti, F.J.G. Ebling

Every day Problems in Dermatology:
The Cosmetic Connection is the second addition to the Cosmetic Dermatology Series

This book is comprised of 41 previously unpublished papers dealing with research in various fields of cosmetic dermatology. The main themes covered are: inter-relationship between drugs and cosmetic in the skin; the efficacy of, and the reaction to, cosmetics; cosmetics in sports and work; cosmetics in relation to sexuality and pregnancy; and finally, the interconnection existing between cosmetics and diet. By so comprehensively covering the science of cosmetics, this text is indispensable to those involved in research and development for the cosmetics, toiletries and pharmaceutical industries. It will also be a great benefit to university and hospital pharmacists and health care professionals entrusted with any aspect of skin care.

CONTENTS (Main Chapters)
Psychological aspects of every day cosmetic dermatology (E. Panconesi)
Cosmetic, drugs and common skin disorder (W. Raab)
Percutaneous absorption and lipids of the elderly skin (J. Wepierre)
Mechanism of solar erythema (E. Quencez, P. Agache)
The skin plasticisation effect of a medium chain alpha-hydroxy acid and the use of potentiators (J.C. Hill, R.J. White, M.D. Barrat, E. Mignini)
Analytical problems of cosmetic evaluation resulting from EEC Italian regulatory procedures (L. Gagliardi, A. Amato)
Kathon C.G.: risk of sensitization (A.C. De Groot)
Methods for evaluating irritant - erythematogenic activity ircosmetics (A. Sertoli, S. Gioragni, C. Martinelli, M.C. Melli)
Social problems related to perspiration: the cosmetic connection (C. Jacobson)
Barriers creams (L.C. Parish)
Evaluation of a new skin barrier providing water and solvent protection (P. Morganti, S.D. Randazzo)
Cosmetology and sexuality in the history of gynaecology (G. Forleo, M. Fraticelli)
Metabolism of steroids in human skin (A. Lanzone, A.M. Fulghesu, F.P. Bellante, A. Caruso, S. Mancuso)
The structure and permeability of the oral mucosa (A. Jarret)
Oral mucosa and dental care problems (E. Benagian)
Vitamins and mineral nutrition in the skin (B. Berra, S. Zoppi, S. Rapelli)
Good manufacturing and quality control practices in the cosmetic industry (F. Pocechiari)
Cosmetology and public health (L. Toti)

<00 pages about - Hard-bound
Price: U.S. $ 90.00 / in Italy L. 120.000
Trimestrale di Dermatologia Cosmetologica
Quarterly Review of Cosmetic Dermatology

EDITOR
P. MORGANTI
Ph.D.
SECRETARY GENERAL
INTERNATIONAL SOCIETY OF COSMETIC DERMATOLOGY
Via Innocenzo XI, 41 - 00165 Roma (Italy) - Fax +39-6-633,95309

ASSOCIATE EDITOR
S.D. RANDAZZO
M.D.
Professor of DERMATOLOGY
UNIVERSITY OF CATANIA
Via Iacopi, 7 - 95124 Catania (Italy) - Fax +39-95-719884

ASSISTANT EDITOR
M.B. JAMES
M.D.
PROGRAM DIRECTOR
INTERNATIONAL SOCIETY OF COSMETIC DERMATOLOGY
JAMES CLINIC
Suite 1078 Temple Lane Canberra, Majic 02045 USA - Fax 001-407.9972137

SECRETARY EDITOR
M. BASCOLI
Via Innocenzo XI, 41 - 00165 Roma (Italy) - Fax +39-6-9281.523

EDITORIAL ADVISORY BOARD
P. AGACHE MD, Prof. of Dermat. Centro Hosp. Regional de Béziers (F)
G. BELLOMONTE CChem, Prof. of Chem., Food Deapt. Ist. Sup. Santità - Rome (I)
W.E. BERGFIELD MD, FACP Cleveland Clinic Ohio (USA)
B. BERRA DSc. Prof. of Biol. Chem. Univ. of Milano (I)
R. CAPUTO MD, Prof. and Chairman, Dept.of Dermat. Univ. of Milano (I)
G. CARLESIMO MD, Prof. and Chairman, Dept.of Dermat. Univ. of Rome (I)
D. CERMELE MD, Prof. and Chairman, Dept. of Dermat. Catholic Univ. of Rome (I)
E. CHIACCHIERINI CChem, Prof. and Chairman, Dept. of Commence Univ. of Rome (I)
J. COTTE DSc. Prof. of Cosmet. IPL. Lyon (F)
M. DINIA MD, Prof. and Chairman, Dept. of Pharmac, Anat. Catholic Univ. of Rome (I)
G. FASIRI MD, Ass. Prof. of Paediatric Dermatologist. Catholic Univ. of Rome (I)
A. FIDANZA DSc. Prof. and Chairman, Dept. of Physical Univ. of Rome (I)
D. GRANETTER PhD, Inst. for Clinical and Exp. Medicine Parigi (CSI)
J.A. GRAHAM B.Sc, PhD. Dept. Dermatology, Univ. of Pennsylvania (USA)
L. GAGLIEARDI Chairman, Dept.of Pharmac. Chem. Ist. Sup. Santità of Rome (I)
B. GUARNIERI MD, Prof. and Chairman, Dept.of Dermat. Univ. of Messina (I)
A.J. JOUBAR M.D.MRSC (Beaconsfield) (GB)
F.H. KEMPERS MD, Enquiries Food, Pharmaco logy & Toxicology. Univ. Munich (D)
A.M. KILMAN MD, PhD, Prof. of Dermatol. Univ.of Pennsylvania, Philadelphia (USA)
N. LOPRIENO DSc. Prof. of Genetics Univ. of Pisa (I)
G. PUGLISI CChem, Dept. of Pharmacol. and Tox. Univ. of Catania (I)
C.I. NENZORINI MD, Prof. and Chairman, Dept. of Dermat. Univ. of Bari (I)
F.L. MUSCARDIN MD, Emeritus Prof. of Dermat. Centro Hosp. Regional, Idi Roma (I)
O. ORENREICH MD, Chair. Prof. of Dermat. New York, (USA)
E. PANCONEI MD, Prof. and Chairman, Dept. of Dermat. Univ. of Firenze (I)
R. PADGETTI MD, Prof. and Chairman, Dept. of Pharmacol. and Tox. Univ. of Milano (I)
W.E. PARISH MA, PhD, BVS. Head of Environmental Safety Division, Unilever Research Schen brook (GB)
L. PUGLISI DSc. Prof. of Pharmacognosy. Univ. of Milano (I)
W. RAAB MD, Prof. and Chairman, Dept. of Dermat. Univ. of Wies (A)
G. RABBINI MD, Prof. and Chairman, Dept. of Dermat. Univ. of Pavia (I)
A. REBORA MD, Prof. and Chairman, Dept. of Dermat. Univ. of Genova (I)
V. RIZZA PhD, Prof. of Biol. Chem. Univ. of Catania (I)
G. SALVATORE CChem, Dept. of Toxicol. Ist. Sup. Santità of Rome (I)
A. SANNI MD, Prof. and Chairman, Dept. of Microbiol. Catholic Univ. of Rome (I)
P. SANTORO MD, Prof. and Chairman, Dept. of Dermat. Univ. of Napoli (I)
H. SCHAEFER Ph.D., Prof. and Scientific Director L’Oreal, Paris (F)
F. SERRI MD, Emeritus Prof. , Dept. of Dermat. Catholic Univ. of Rome (I)
A. SERTOLI MD, Assoc. Prof. of Allergo and Occupational Dermat. Univ. of Firenze (I)
A. STAMMATI DSC, Dept. of Toxicol. Ist. Sup. Santità of Rome (I)
L. TADDI B.Sc., Prof. and Chairman, Dept. of Pharmacol. Science Univ. of Siena (I)
H. TRONNIX MD, Emeritus Prof., Dermatology.. Univ. Witten-Herdecke (D)
V. VALKOVIC Ph.D. Prof. of Physic Ruder Boskovic Inst. of Zagreb (CRO)
GENERAL INFORMATION

The **JOURNAL OF APPLIED COSMETOLOGY** is an international journal devoted to publishing original papers, reviews and other material which represent a useful contribution to research on the skin and on cosmetics.

It is aimed at cosmetic chemists, dermatologists, microbiologists, pharmacists, experimental biologists, toxicologists, plastic surgeons, and all other scientists working on products which will come into contact with the skin and its appendages.

The Journal is published quarterly in English. It is distributed to cosmetic chemists, dermatologists, plastic surgeons, medical and pharmaceutical schools, medical libraries, selected hospitals and research institutions throughout the world, and by subscription to any other interested individuals or organizations. Statements and opinions expressed are personal to the respective contributors and are not necessarily endorsed by the Editor(s), Advisers, Publishers of Distributors of this Journal.

COPYRIGHT

Submitted material must be the original work of the author(s) and must not have been submitted for publication elsewhere.

By submitting a manuscript, the authors agree that the copyright for their articles is transferred to the publisher if and when the article is accepted for publication. None of the content of this publication may be reproduced in whole or in part, translated, stored in a retrieval system, or transmitted or distributed in any form or by any means (electronic, mechanical, photocopy, recording or otherwise) without the prior written permission of the Publishers.

Sections of Journal

The following sections will be features of the Journal:

*Original Laboratory Studies*: descriptions of original investigative laboratory research in cosmetics and related areas.

*Special Reports*: items of special interest to the readers, including reports on meetings, societies, legislation, etc.

*General Articles*: scientific articles of general interest to our readers will be considered for publication. These articles should be concerned with newer developments in such related fields as dermatology, biology, toxicology, etc.

*Short Communications*: the length should not exceed 5 typewritten pages with not more than 3 figures included. Headings (“Materials”, “Discussion”, etc.) as well as Summaries are to be omitted. If accepted, these submission will appear in print in a very short time.

*Letter to the Editor*: comments on Journal articles are invited as well as brief contributions on any aspects of cosmetic science. Letters may include figures, and/or references, but brevity is necessary.

*Guest Editorials*: concise, authoritative, substantiated commentary on specific topics of contemporary interest.

*Book Reviews*: book and monographs (domestic and foreign) will be reviewed depending on their interest and value to subscribers. Send material for review to the Editor, Dr. P. Morganti. No such material will be returned.

Address: all papers should be submitted to:

Dr. P. Morganti
INTERNATIONAL EDIEMME
Via Innocenzo X1, 41
00165 Rome - Italy
Tel. 0039/6/393.78.788
Fax. 0039/6/63.80.839
INFORMATION FOR AUTHORS

Papers must be submitted in English. Authors whose mother tongue is not English should arrange for their manuscripts to be written in proper English prior to submission.

Procedure of Submission of Manuscripts: submit three copies of both the manuscript and all illustrative material to the above address.

Organization of the Manuscript: investigative studies should be organized as follow: title, abstract page, introduction, material and methods, results, discussion, acknowledgments, references, legend for figures, tables. All pages should be numbered consecutively starting with the abstract. The entire manuscript is to be typewritten, double–spaced, and with 3 cm margins.

Trade names must be capitalized: the common name for compounds may be used if the formal chemical name as established by international convention is given after the first use. Any abbreviations other than those which are generally accepted must be defined. In the text, references to dual authors will use both surnames throughout. For multiple authors, use the surnames of all authors at the first reference and only the first author followed by “et al.” thereafter. Please mark in the margin of the manuscript the desired position of the figures and tables. To allow faster publication only set of proofs will be furnished to the author including the figures and tables in their final position.

Title page: list the title, name(s) and degree(s) of author(s), department(s) and institution(s) at which the work was done, city, state, and postal code. Any preliminary report or abstract of the work should be referred to as a footnote to the title.

Summary: each paper must be headed by an English language title of not over 70 characters (including spaces) suitable for use as a running head and must also be preceded by an English summary not exceeding 300 words typed double–spaced. The summary will include statements of the problem, method of study, results, and conclusions. Since this summary will be used by abstracting journals, it must be self-explanatory and should not include abbreviations, footnotes, and references.

Footnotes: should be listed consecutively at the bottom of the page on which they fall, designated by the following symbols in order *, +, –, §, ‡, etc.

Key Words: key words for computerised storage and retrieval of information should be incorporated in the summary.

References: the references have to be abbreviated as listed in the Index Medicus. The style of the references must conform to the examples given below:

Illustrations: figures should be numbered consecutively using Arabic numerals Tables should be numbered consecutively, using Roman numerals. All photographs should be black and white, glossy and unmounted. The number and size of illustration should be restricted to the minimum needed to clarify the text. Authors requiring extra space for illustrations will be charge accordingly. This is also the case for color illustrations. All figures, photographs, graphs, or diagrams should be submitted on separate sheets.

Animal Experiments: descriptions of animal experiments should include full details of the types of animal used (inbred, etc.) and the conditions under which they were kept (standard diet, etc.)

Trade Names: all common cosmetic ingredients should be referred to by their generic names, as indicated in the latest edition of CTFA Cosmetic Ingredient Dictionary, and the European Pharmacopeia. If a materials is not listed, then the trademarked name can be used, with the chemical composition given in footnotes.
INFORMAZIONI PER L'ABBONAMENTO

L'abbonamento annuale comprende quattro numeri. È possibile ottenere abbonamenti a prezzo ridotto da parte dei ricercatori che lavorano presso Istituti che abbiano sottoscritto almeno un abbonamento a prezzo normale.
L'Editore potrà fornire a richiesta notizie più dettagliate. Le sottoscrizioni di abbonamento possono essere effettuate mediante assegni postali, bancari, di conto corrente o per contanti indirizzandoli a:

INTERNATIONAL EDIEMME - Via Innocenzo XI, 41, 00165 ROMA - ITALIA
c/c bancario n. 3184/51 Banca di Roma Ag. 1 - Aprilia (LT) - ITALIA

L'IVA è a carico dell'editore, non detraibile dall'abbonato a norma art. 74 lett. C DPR 633/72

SOTTOSCRIZIONI ANNUALI

Italia L. 125.000 - Altre Nazioni $ 80

Numero singolo L. 50.000

Numero arretrato L. 60.000

SUBSCRIPTION INFORMATION

Subscriptions are entered on a calendar years basis only and include four regular quarterly issues. Half-price subscriptions are available to research scientists whose institutions already subscribe at full rate. Details on application from publisher.
Payment must be made in U.S. dollars using bank draft, international postal money order only. Italian residents only may pay by personal check:

INTERNATIONAL EDIEMME - Via Innocenzo XI, 41, 00165 ROMA - ITALY
c/c bancario n. 3184/51 Banca di Roma Ag. 1 - Aprilia (LT) - ITALY

ANNUAL SUBSCRIPTION RATE:

Italy, Lit. 125.000 - Other Countries, $ 80
ISCD Members Free of Charge

Statements and opinions expressed in the articles and communications herein are those of the author(s) and not necessarily those of the Editor(s), or publisher. The Editor(s) and publisher, disclaim any responsibility or liability for such material and do not guarantee, warrant, or endorse any product or service advertised in this publication nor do guarantee any claim made by the manufacturer of such product or service.
Informazioni per l'Abbonamento

L'abbonamento annuale comprende quattro numeri. È possibile ottenere abbonamenti a prezzi ridotti da parte dei ricercatori che lavorano presso istituti che abbiano sostenuto almeno un abbonamento a prezzo normale.

L'Editore può fornire a richiesta notizie più dettagliate. Le iscrizioni di abbonamento possono essere effettuate mediante assegni postali, bancari, di conto corrente o per contanti indirizzando a:

INTERNATIONAL EDIEMME - Via Innocenzo XI, 41 - 00165 Roma

Istruzioni per l'abbonato:

☐ desidero abbonarmi a questa rivista per l'anno in corso
☐ rinnovo automaticamente il mio abbonamento per gli anni futuri (questa forma di abbonamento può essere comunque disdetta in ogni momento).
☐ desidero ricevere le norme editoriali per eventuali collaborazioni (Scrivere in stampatello)

Nome

Indirizzo

Città CAP Nazione

Abbonamento JOURNAL OF APPLIED COSMETOLOGY

Italia L. 125.000 - Altre Nazioni $ 80

Ordine Form JOURNAL OF APPLIED COSMETOLOGY

Annual subscription rate: Italy, L. 125.000 - Other Countries $ 80

Please Check

☐ 1 Year subscription
☐ Renew my subscription automatically in future years (this continuation order is intended for subscriber's convenience only and my be cancelled at any time).
☐ Send me a copy of information for Authors.
☐ Please charge this order to my credit card (All order subject to credit approval). Delete as necessary:

☐ AMERICAN EXPRESS ☐ DINERS CLUB - Card Number

Expiration date

(Please Print)

Name

Address

City Postal Code Country
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. Roveglio

137 The treatment of Acne Vulgaris by Phosphatidylcholine from Soybeans, with a high content of Linoleic Acid
M. Ghyczy, H-P. Nissen, H. Blitz

147 On the heavy metals content in cosmetic formulation: an atomic absorption spectroscopy investigation
Marcelo E. Conti, Francesco Botrè, Franco Mazzei

155 Application of a film method for microbial monitoring of cosmetic raw materials
L. Piu, C. Juliano, G. Pirsino, 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:
THE I.S.C.D. DAY
"Quality & safety aspects scientific backgrounds"
"Experimental and Dermatological Approaches"
Düsseldorf (Germany) - May 6, 1997

THE NINTH ANNUAL
Symposium on Aesthetic Surgery Featuring live surgery
San Francisco (California) - March 20-22, 1997
VACUUM SKIN-ABRASION VERSUS GLYCOLIC ACID PEELING IN THE TREATMENT OF ATROPHIC ACNE SCARS

S. Jurassich, A. Lo Schiavo, F. Pinto, M. Nacca
2nd University of Naples - School of Medicine and Surgery - Department of Dermatology - Naples, Italy

Received: May 21st, 1996

Key words: Glycolic acid peeling and microabrasion.

Synopsis

Glycolic acid, an alphahydroxyacid, 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 cornum and facilitates the brushing off of the superficial cells, since it weakens the cohesion of corneocytes.

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

Riassunto

L'acido glicolico, un alfa-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 velutata.

Gli effetti dell'acido lattico sull'epidermide sono soprattutto cheratoplastici ed esfolianti dal momento che riduce le 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 percent 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)\(^3\).

The atrophic scars have a hollow, whitish and persistent appearance which make the skin appear irregular. These unsightly lesions have induced dermatologists to seek more than one method (table I) to alleviate the problem\(^4\). Glycolic acid peeling and vacuum skin microabrasion 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\(^3,7\).

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

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>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAIN TREATMENTS USED FOR POSTACNE ATROPHIC SCARS</td>
</tr>
<tr>
<td>Intralesional therapy</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Surgery</td>
</tr>
<tr>
<td>Physical treatments</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Chemical peeling</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Bleeding is necessary because it indicates that the epithelial proliferative layer has been reached, and must not be surpassed to avoid new scarring. 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. 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. 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 antiinflammatory 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 fieldmeter. 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

<table>
<thead>
<tr>
<th>Action</th>
<th>Preoperative treatment</th>
<th>Square area treated each time</th>
<th>Time for each treatment</th>
<th>Interval between treatments</th>
<th>Postoperative treatment</th>
<th>Post-treatment clinical features</th>
<th>Clinical improvement (means)</th>
<th>Number</th>
<th>Depth</th>
<th>Diameter</th>
<th>Manegeability</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin-abrader (microabrasion)</td>
<td>mechanical</td>
<td>not needed</td>
<td>&lt; 10 cm²</td>
<td>10' - 15'</td>
<td>10' - 15 days</td>
<td>abrasive detergent</td>
<td>erythema</td>
<td>exudation</td>
<td>bleeding</td>
<td>pain</td>
<td>moderate oedema</td>
<td>crusting</td>
</tr>
<tr>
<td>79,16%</td>
<td>75,24%</td>
<td>80,15%</td>
<td>82,10%</td>
<td>good</td>
<td>considerable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70% glycolic acid peeling</td>
<td>chemical</td>
<td>topic keratolytic for 15 days</td>
<td>whole face</td>
<td>3'-5'</td>
<td>10' -15 days</td>
<td>gentle topical remover</td>
<td>erythema</td>
<td>slight exudation</td>
<td>frost</td>
<td>burning</td>
<td>possible crusting</td>
<td></td>
</tr>
<tr>
<td>75,70%</td>
<td>70,80%</td>
<td>77,60%</td>
<td>78,70%</td>
<td>excellent</td>
<td>low</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Furthemore 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 epidemolysis 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 sterile 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. When scars are old they call for longer treatment schedules, while those of recent onset require fewer application. Moreover, since IV and V skin phototypes have more frequent hyperpigmentations, we dealt with Fitzpatrick type III subjects.

**Conclusions**

As previously described, microabrader is a handy tool for the operator. Furthemore, its closed circuit does not damage the environment. The throw away heads and inherent 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. Furthemore patients can interrupt of 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.

Address:
Stefano Jurassich, Ada Lo Schiavo
Clinica Dermosifilopatica - Policlinico
Seconda Università degli studi di Napoli
Via S. Pansini, 5 - 80131 Napoli, Italy.
Vacuum skin-abrasion versus glycolic acid peeling in the treatment of atrophic acne scars

References:

A VIDEO-MICROSCOPE STUDY OF "HAIR KNOTS"

R. Strumia*, C. Roveggio*
*Clinica Dermatologica, Università di Ferrara - Direttore: Prof. A. Califano - Italy

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 entangled 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.
A video-microscope study of "hair knots"

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 cuniculi) 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” TTL 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 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 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.

**Author address:**
Renata Strumia - Clinica Dermatologica Università Via Savonarola, 9 - 44100 Ferrara - Italy
Tel. (0532) 205825 - Fax (0532) 206791
References:

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

M. Ghyczy*, H-P. Nissen**, H. Biltz**
*Nattermann Phospholipid GmbH, Nattermannallee 1, D-50792 Cologne
**Derma Consult, Kirchgasse 19, D-53347 Alfter/Bonn

Received: September 25th, 1996 - Presented at the ISCD V World Congress, October 26/29, 1995 - Montecatini Terme (PT) Italy

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 soy lecithin 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 [4,5].

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 LTB₄ by human neutrophiles [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 neutrophiles [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 LTB₄.
3. Linoleic acid itself or a not yet characterized reaction product, inhibits phagocytosis and the production of aggressive oxygen metabolites by neutrophile 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.
Evaluations: 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:
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 differentiation 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-
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.

Author address:
M. Ghyczy
Nattermann Phospholipid GmbH,
Nattermannallee 1, D-50792 Cologne.
The Treatment of Acne Vulgaris by Phosphotidylcholine from Soybeans, with a High Content of...

References:

19. EP 0 582 239 A1, published 09.02.94, to Rhone-Poulenc Rorer GmbH, Cologne (DE).
ON THE HEAVY METALS CONTENT IN COSMETIC FORMULATIONS: AN ATOMIC ABSORPTION SPECTROSCOPY INVESTIGATION

Marcelo E. Conti¹, Francesco Botrè¹, Franco Mazzei²
¹ Istituto di Merceologia, Università “La Sapienza”, Via del Castro Laurenziano, 9 - 00161 Roma, ITALY
² Dipartimento di Studi di Chimica e Tecnologia delle Sostanze Biologicamente Attive, Università “La Sapienza”, Piazzale Aldo Moro, 5 - 00185 Roma, ITALY

Received: May 27th, 1996

Key words: Heavy metals, atomic absorption spectroscopy, cosmetic formulations.

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 hand 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 interdisciplinar 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 pharmacists.

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 S.p.A., 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 weighing bottles;
2. Transfer of samples in the quartz weighing bottles and determination of the humid weight;
3. Dissipation of the sample in oven (T = 105 °C; time of treatment: 12 hours);
4. Slow thermal treatment (∆T/Δt = 50 °C/hour); then
5. Calcination at T = 450 °C ± 10 °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 °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 HNO₃/H₂SO₄ (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 HNO₃ (Merck, Darmstadt, Germany). All reagents were analytical grade. Cadmium, chromium, lead and mercury standards were prepared by dilution with 2% HNO₃ from stock standard solutions of their respective nitric salts (1000 ± 2 ppm in 0.5 M HNO₃), 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**

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
<th>Slit width (nm)</th>
<th>Matrix modifier</th>
<th>Graphite tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>283.3</td>
<td>0.7</td>
<td>0.2 mg/10 µl NH₄H₂PO₄</td>
<td>pyrolytic/wall</td>
</tr>
<tr>
<td>Cd</td>
<td>228.8</td>
<td>0.7</td>
<td>0.2 mg/10 µl NH₄H₂PO₄</td>
<td>pyrolytic/wall</td>
</tr>
<tr>
<td>Cr</td>
<td>357.9</td>
<td>0.7</td>
<td>0.05 mg/10 µl NH₄H₂PO₄</td>
<td>pyrolytic/wall</td>
</tr>
<tr>
<td>Hg</td>
<td>253.7</td>
<td>0.5</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>
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 thermal 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 µl) | | | |
| Step | Temperature (°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 µl) | | | |
| Step | Temperature (°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)

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature (°C)</th>
<th>Ramp (s)</th>
<th>Hold (s)</th>
<th>Argon flow (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>120</td>
<td>20</td>
<td>10</td>
<td>300</td>
</tr>
<tr>
<td>2</td>
<td>300</td>
<td>10</td>
<td>20</td>
<td>300</td>
</tr>
<tr>
<td>3</td>
<td>450</td>
<td>10</td>
<td>20</td>
<td>300</td>
</tr>
<tr>
<td>4</td>
<td>1650</td>
<td>10</td>
<td>20</td>
<td>300</td>
</tr>
<tr>
<td>5</td>
<td>2500</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>2700</td>
<td>1</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>5</td>
<td>5</td>
<td>300</td>
</tr>
</tbody>
</table>

Table 5

RECOVERY TESTS

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

Table 6

PRECISION TESTS

<table>
<thead>
<tr>
<th>Element</th>
<th>N. of tests</th>
<th>Concentration (µg/L)</th>
<th>Variation coefficient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>10</td>
<td>4.2</td>
<td>6.1</td>
</tr>
<tr>
<td>Cd</td>
<td>10</td>
<td>1.0</td>
<td>3.6</td>
</tr>
<tr>
<td>Cr</td>
<td>10</td>
<td>8.2</td>
<td>4.7</td>
</tr>
<tr>
<td>Hg</td>
<td>8</td>
<td>1.5</td>
<td>8.4</td>
</tr>
</tbody>
</table>

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 formulations 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),
On the heavy metals content in cosmetic formulations: an atomic absorption spectroscopy...

### Table 7

CONCENTRATION OF HEAVY METALS ON SAMPLES OF COSMETIC FORMULATIONS. RESULTS (MEAN OF FIVE INDEPENDENT SAMPLE PREPARATIONS ±SD) ARE EXPRESSED IN µg/100 g OF SAMPLE.

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

n.d.: not detectable ([Pb] < 5 µg/L; [Cd] < 0.1 µg/L; [Cr] < 4 µg/L; [Hg] < 1 µg/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 270.7 ppb. 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 intimate 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.

Address for correspondence:
Dr. Marcello E. Conti
Istituto di Merceologia, Università “La Sapienza”
Via del Castro Laurentiano, 9
00161 Roma - ITALY
Tel.: (+39-6)-49766516
FAX: (+39-6)-4941621
E-Mail: CONTIM@AXRMA.UNIROMA1.IT
References:

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

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

*Dipartimento di Scienze del Farmaco-Università degli Studi di Sassari. Via Muroni, 23/A - 07100 Sassari
**Istituto di Chimica Farmaceutica-Università degli Studi di Milano. Viale Abruzzi, 42 - 20131 Milano.

Received: September 18th, 1996

Key words: Microbial Monitoring, Dry Rehydratable Film, Cosmetic Products.

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

È 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 prolonged.

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 microorganisms.

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 convenient 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

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

#### GLYCOLIC EXTRACTS

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

<table>
<thead>
<tr>
<th>n°</th>
<th>Main constituent</th>
<th>Preservative</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>Not declared</td>
<td>Not declared</td>
</tr>
<tr>
<td>27</td>
<td>Jojoba oil-almond oil</td>
<td>Not declared</td>
</tr>
<tr>
<td>28</td>
<td>Glycerin</td>
<td>Not declared</td>
</tr>
<tr>
<td>29</td>
<td>Macadamia nut oil</td>
<td>Not declared</td>
</tr>
<tr>
<td>30</td>
<td>Tocopherol</td>
<td>Not declared</td>
</tr>
<tr>
<td>31</td>
<td>Vegetables extracts</td>
<td>Not declared</td>
</tr>
<tr>
<td>32</td>
<td>Vegetables extracts</td>
<td>Not declared</td>
</tr>
</tbody>
</table>
Table 2

MICROBIAL COUNTS (mean ± SE x 10^3 cfu/ml) AFTER SPECIFIC CONTAMINATION OF PRESERVATIVE-FREE RAW MATERIALS (Standard inocula of 10^3 to 10^6 /ml)

<table>
<thead>
<tr>
<th>CONTAMINANT</th>
<th>PRODUCT (n°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>E.COLI</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>TSA</td>
<td>4.8 ± 0.6</td>
</tr>
<tr>
<td>PETRIFILM® SM</td>
<td>6.4 ± 0.7</td>
</tr>
<tr>
<td>S.AUREUS</td>
<td>0.71 ± 0.5</td>
</tr>
<tr>
<td>PETRIFILM® YM</td>
<td>0.73 ± 2.4</td>
</tr>
</tbody>
</table>

*SStatistically 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 monoooleate (Tweein 80, Atlas Europol), 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 1). 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, Dulbecco "A" Oxoid; pH 7.3) was used to prepare the samples. The plates for the total aerobic microbial count were incubated at 37 ± 1°C for 5 days, and those for the fungal count at 25 ± 1°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 ± 1°C or at 25 ± 1°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 10⁴ to 10⁶ micro-organisms/ml. Samples characterized by a self-preservation activity of solvent such as glycolic extracts were contaminated by an inoculum of about 10¹ to 10⁴ 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 60 cfu/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>
<thead>
<tr>
<th>Prod. (n°)</th>
<th>TSA</th>
<th>PETRIFILM® SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>3.3 ± 0.2*</td>
<td>5.2 ± 0.4*</td>
</tr>
<tr>
<td>20</td>
<td>0.29 ± 0.1</td>
<td>0.33 ± 0.7</td>
</tr>
<tr>
<td>21</td>
<td>3.8 ± 0.2</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>22</td>
<td>0.32 ± 0.1</td>
<td>0.37 ± 0.9</td>
</tr>
<tr>
<td>23</td>
<td>0.45 ± 1</td>
<td>0.51 ± 1.1</td>
</tr>
<tr>
<td>24</td>
<td>3.8 ± 0.3</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>25</td>
<td>3.2 ± 0.5</td>
<td>2.6 ± 0.4</td>
</tr>
</tbody>
</table>

*(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 ± SE x 10^5 cfu/ml) AFTER SPECIFIC CONTAMINATION OF PRESERVED RAW MATERIALS (Standard inocula of 10^4 to 10^6/ml)

<table>
<thead>
<tr>
<th>CONTAMINANT</th>
<th>PRODUCT (n°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>E.COLI</td>
<td></td>
</tr>
<tr>
<td>TSA</td>
<td>4.1 ± 0.8</td>
</tr>
<tr>
<td>PETRIFILM® SM</td>
<td>4.1 ± 0.7</td>
</tr>
<tr>
<td>C.ALBICANS</td>
<td></td>
</tr>
<tr>
<td>SDA+CAF</td>
<td>42 ± 0.2</td>
</tr>
<tr>
<td>PETRIFILM® YM</td>
<td>43 ± 0.7</td>
</tr>
</tbody>
</table>

Table 5

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

<table>
<thead>
<tr>
<th>CONTAMINANT</th>
<th>PRODUCT (n°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>26</td>
</tr>
<tr>
<td>S.AUREUS</td>
<td></td>
</tr>
<tr>
<td>TSA</td>
<td>6.8 ± 1.3</td>
</tr>
<tr>
<td>PETRIFILM® SM</td>
<td>7.5 ± 1.4</td>
</tr>
<tr>
<td>P.AERUGINOSA</td>
<td></td>
</tr>
<tr>
<td>TSA</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>PETRIFILM® SM</td>
<td>27 ± 1</td>
</tr>
</tbody>
</table>

*statistically significant differences for p=0.01

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.

Author to whom correspondence should be addressed. Tel: 079/228735; Fax: 079/228733.
References:

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 - 1st note
L. Armino - P. Morganti
15

Enhanced antiinflammatory activity of Diclofenac in jojoba oil submicron emulsion cream
J. S. Schwarz, M. R. Weissapir, A. Shani and S. Amsleman
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
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. Ghì, 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. Mastrodonato, 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 colla-
gen: 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 - 1st 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
Ghi B., The Treatment of Acne Vulgaris by Phosphatidylcholine from Soybeans, with a High Content of Linoleic Acid, 137
Jacò L., see Rodrigues L., 93
Jirillo R., see Ghi A., 25
Juliano C., see Piu L., 155
Jurassic S., Vacuum skin-abrasion versus glycolic acid Peeling in the treatment of atrophic acne scars, 127
Lo Schiavo A., see Jurassic S., 127
Mastrodonardo 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 I., 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 AHA's, 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 Jurassic S., 127
Nissen H-P., see Ghyczy M., 93
Paulli R., see Morrison D.S., 111
Pereira L.M., see Rodrigues L., 93
Pinto F., see Jurassic S., 127
Pirisino G., see Piu L., 155
Piu L., Application of a film method for microbiological 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 videomicroscope 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
Weisspapir 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 phosphatidyl-
Acne vulgaris, 137
Age-spots, 1; and AHAs, 80
Aging, 1; 79
AHAs, 1; How to use, 35; 80; chemistry and ac-
tivity, 36; and pH, 38; alpha hydroxyenoic
acids, 36; how synergize the activity of, 38; de-
pigmenting 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 lin-
oleic 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; sy-
nergizing activity of, 38;
Corneocyte, cohesion, 36; 38; disaggregation, 38
Cosmetic, classification, 26; labelling, 32; sup-
ply, 29; principal companies, 31; market seg-
ment, 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; VIIth modification
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

Ichthyosis, 36

Ichthyosis, 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

NMF, 53

Odland bodies, 52

Onychoschizia, 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 cosmetics, 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
Tattoing, 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
IN-COSMETICS Conference 1997
Düsseldorf, May 5-7

"MODERN CHALLENGES TO THE COSMETIC FORMULATION"

**CALL FOR PAPERS**

Consumer demands and behavior as well as economic, regulatory and ecological changes present new challenges to the cosmetic and toiletries industry. To investigate in the newest developments of the cosmetic science and to keep up-to-date with them and to discuss and communicate with the leading scientists in the field is a necessity for everyone involved in the industry.

The IN-COSMETICS Exhibition and Conference is the leading event that meets these demands.

1997 the IN-COSMETICS Conference is organized for the first time by the "Verlag für chemische Industrie, H. Ziolkowsky GmbH", publisher of the "SÖFW Journal" and other scientific publications for the personal care industry. The conference will show tomorrow's technology and address the key problems of the industry. Leading dermatologists, regulatory specialists, consultants and research personnel will be invited to present their latest findings.

A board of influential specialists guarantees the topicality and scientific quality of the program.

If you are interested in giving a paper or if you have a suggestion for an interesting topic please contact:

**Organizing secretariat:**
Mr Robert Fischer

Verlag für chemische Industrie • H.Ziolkowsky GmbH
POSTBOX 10 25 65, D-86015 Augsburg/Germany
Tel. +49 821 51 93 45/46 • Fax +49 821 - 51 79 53
IN-COSMETICS Conference 1997
“MODERN CHALLENGES TO THE COSMETIC FORMULATION”

In cooperation with:
Colipa (The European Cosmetic, Toiletry and Perfumery Association),
ISCD (International Society of Cosmetic Dermatology)
IASC (International Aloe Science Council)

Sponsored by SÖFW Journal

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.

Simultaneously the IASC will hold its scientific workshop on Aloe vera. “What quality standards exist?”, “How can Aloe successfully improve formulations etc.”, “What is the efficacy of Aloe vera and how can it be tested?”. For all who are interested in this unique raw material the IASC work shop offers many interesting features.

DAY 2 of the IN-COSMETICS Conference 1997 is the ISCD Day. The ISCD is a multidisciplinary association of dermatologists, pharmacologists, cosmetic biologists and chemists. Founded in 1986 the goal of ISCD is to encourage a more continuous and stable exchange between the medical community, dermatologists, and cosmetic chemists, pharmacologists, physiologists, toxicologists and experts of all other disciplines related to Cosmetic Dermatology. It provides a forum for the exchange of ideas and methodologies pertinent to the related disciplines and Cosmetic Dermatology.

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 pigmented disorders
- Alternatives to animal experiments.

DAY 3 is devoted to “Green actives for cosmetics” and “Modern formulation concepts”. Leading Research & Development specialists explain the latest developments in new raw materials and formulation concepts.

The IN-COSMETICS Conference 1997 in a must for all involved in cosmetic, pharmacologic and dermatologic science. Some of the most important issues of the industry are discussed. With a visit you will be able to converse with leading scientists and your individual questions and problems could be answered. At the same time a visit of the IN-COSMETICS Exhibition in Düsseldorf gives you the unparalleled opportunity to see and speak to leading manufacturers and suppliers of raw materials and ingredients.

Organizing secretariat:
Mr Robert Fischer

Verlag für chemische Industrie • H.Ziolkowsky GmbH
POSTBOX 10 25 65, D-86015 Augsburg/Germany
Tel. + 49 821 51 93 45/46 • Fax + 49 821 - 51 79 53
In close cooperation with IN-COSMETICS

THE I.S.C.D. DAY
Düsseldorf - 6 May 1997

President: Prof. Dr. Rodolfo Paoletti,
President of European Federation of Farmacologists (EPHAR)

SECOND ANNOUNCEMENT
THE DOSSIER - Quality & Safety Aspects
Scientific Backgrounds
Chairs: P. Morganti, C.E. Orf anos

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);
Prof. Dr. Benvenuto GIANNOTTI (Italy); Prof. Dr. Constantine D. KOUSKOUKIS (Greece)
Prof. Dr. Constantin E. ORFANOS and Dr. Ulrike BLUME (Germany);
Prof. Dr. Pietriancesco MORGANTI (Italy); Prof. Dr. Fritz H. KEMPER (Germany);
Prof. Luis RODRIGUES (Portugal); Prof. Coleman JACOBSON (USA); Prof. Brodie JAMES (USA).

CALL FOR POSTERS

Organizing secretariat:
Mr Robert Fischer - P. Morganti, Ph.D.

Verlag für chemische Industrie • H.Ziolkowsky GmbH
Beethovenstr. 16, D-86015 Augsburg/Germany
Tel. + 49 821 51 93 45/46 • Fax + 49 821 - 51 79 53
E-Mail:mavi@colosseum.it
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 pigmented disorders  

11,30  
Prof. Dr. Constantine E. KOUSKOUKIS, Greece  
Allergic reactions to cosmetics
Announcement

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

Organizing secretariat:
Mr Robert Fischer - P. Morganti, Ph.D.

Verlag für chemische Industrie • H.Ziolkowsky GmbH
Beethovenstr. 16, D-86015 Augsburg/Germany
Tel. + 49 821 51 93 45/46 • Fax + 49 821 - 51 79 53
E-Mail: mavi@colosseum.it
The Division of Plastic Surgery
University of California School of Medicine San Francisco, California
and Davies Medical Center, San Francisco
present

THE NINTH ANNUAL

SYMPOSIUM ON
AESTHETIC SURGERY
FEATURING LIVE SURGERY

Endorsed by the American Society of Aesthetic Plastic Surgery (ASAPA)

March 20-22, 1997
San Francisco - California

Symposium Chairman: John Q. Owsley, M.D.
Programm Chairman: Bernard S. Alpert, M.D.
Televised Surgery Coordinator: Issa Eshima, M.D.
Chiuso in tipografia: 15 dicembre 1996

THE ANTIAGING LINE
GLYCOLIC ACID ACTIVATED BY GELATIN - GLYCINE®
TO NORMALIZE THE SKIN TURNOVER

LA LINEA ANTIAGING
CON ACIDO GLICOLICO "ATTIVATO"
PER NORMALIZZARE
IL TURNOVER CUTANEO

THE EVOLUTION IN COSMETIC SCIENCE
HYPOALLERGENIC COSMETIC PRODUCTS

LA RICERCA NON È MAI STATA COSÌ BELLA

LA RECHERCHE N'A JAMAIS ÉTÉ AUSSI BELLE

RESEARCH HAS NEVER BEEN SO BEAUTIFUL