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3

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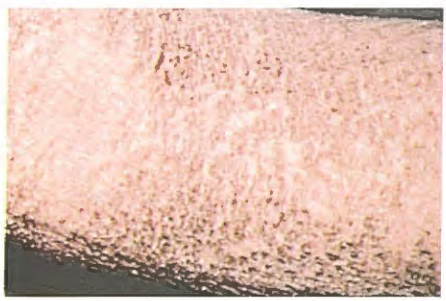

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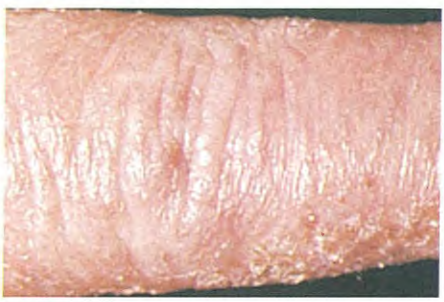

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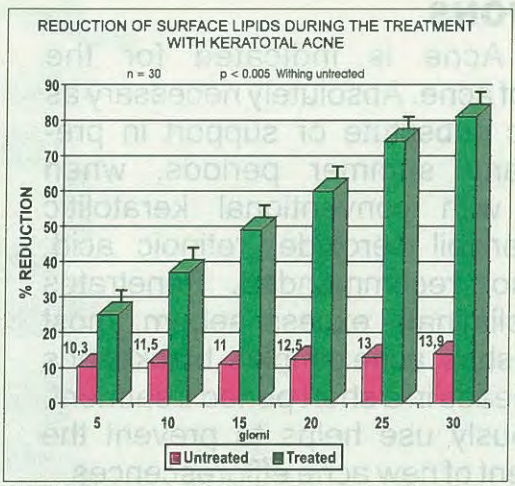

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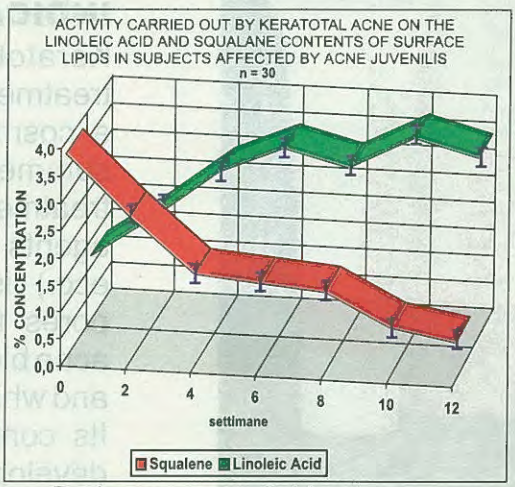

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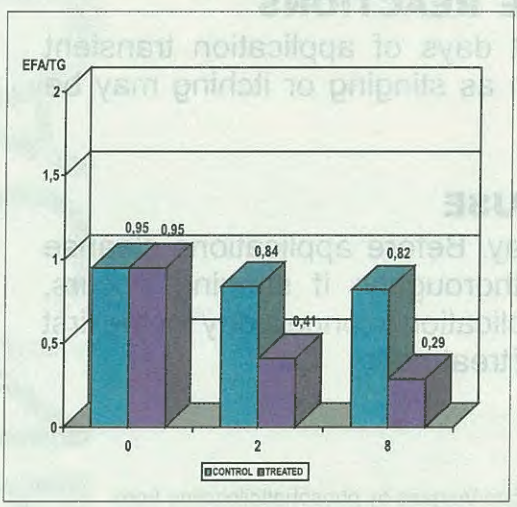

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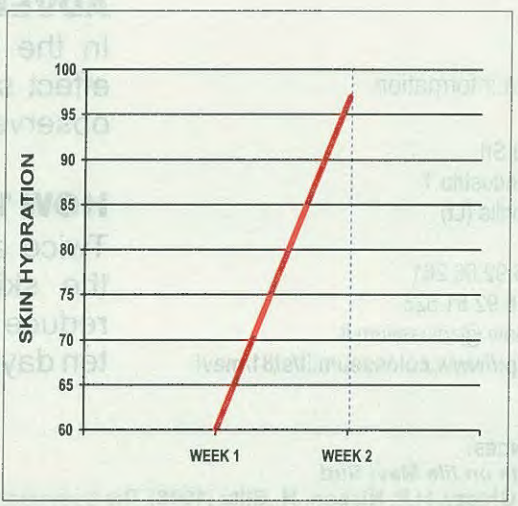

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ADVERSE REACTIONS

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

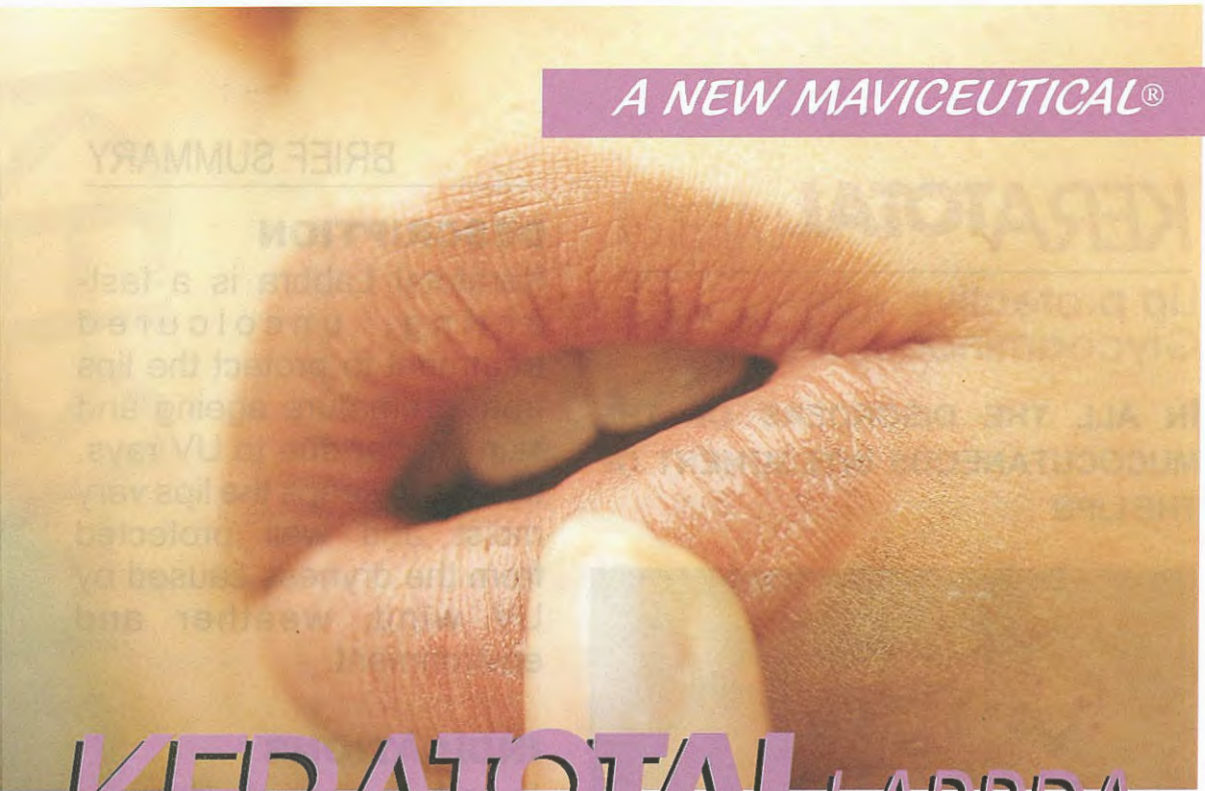
HOW TO USE

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

REFERENCES:

*1,2 - **Data on file Mavi Sud***

3 - **M. Ghiczy, H.P. Nissen, H. Biltz (1996)** The treatment of Acne Vulgaris by phosphatidilcholine from Soybeans, with a high content of linoleic acid. *J. Appl. Cosmetol.* **14**, 137-145



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• Environmental pollutants	Angular cheilitis

HOW TO USE

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

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

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DESCRIPTION

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INDICATIONS

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ADVERSE REACTIONS

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HOW TO USE

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what?
when?
where?
why?

why not?

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what?

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when?

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where?

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- acts within the synthesis of proteins, particularly keratins, thanks to its optimum, balanced content of folic acid, magnesium, omega-6 and omega-3 (DHA) in 1:4 ratio.

why?

Before, during and after pregnancy, to prevent deficiencies due to poor intake, absorption and use of FOLIC ACID, OMEGA-6, OMEGA-3 and MAGNESIUM

and to reduce the risk of

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- uterine hypercontractility;
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journal of applied cosmetology

Trimestrale di Dermatologia Cosmetologica
Quarterly Review of Cosmetic Dermatology

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3) Ebling FJ, Rook (1972) Ciclic activity of the follicle. In: Textbook of dermatology 11, Blackwell, Oxford, p. 1567-1573.

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Trimestrale di Dermatologia Cosmetologica
Quarterly Review of Cosmetic Dermatology

Contents

General Articles

J. Appl. Cosmetol. **15**, 77-83 (July-September 1997)

TOPICAL TRETINOIN FOR PHOTOAGING. THE CHINA EXPERIENCE.

Xinghua Gao[1], Hong-Duo Chen[1], Albert M. Kligman[2]

[1] Professor and Chairman - Department of Dermatology - No. 1 Hospital of China Medical University - Shenyang, 110001, China

[2] University of Pennsylvania - School of Medicine - Department of Dermatology - Philadelphia, PA 19104

Received: *May 10, 1997*

Key words: tretinoin, photodamage, chinese, premature aging, face.

Synopsis

Topical tretinoin (Retin-A®, Ortho Pharmaceuticals) was applied for 6-9 months to photoaged faces of 27 Chinese, mostly women, living in North China where climatic conditions are very unfavorable. Summers are hot and sunny, predisposing to photodamage. The weather is also frequently dry and windy predisposing to dryness.

The results were similar to those previously described on Orientals in Thailand, Phillippines and S.E. Asia. Based on global estimates, most subjects showed a satisfactory degree of improvement in over-all appearance.

Beneficial effects were noteworthy for the following features.

1) Dyspigmentation: Hyper-pigmented spots and blotches faded. Skin color became more uniform.

2) Wrinkles: Fine wrinkles, especially those around the eyes, were effaced.

3) Sallowness: Yellow-tinged skin was replaced by a rosy glow, which added greatly to attractiveness.

4) Dry-roughness: The surface became smoother and less scaly.

A control group treated with a moisturizer.

Moisturizer failed to show appreciable benefits in any of the above features.

Irritant reactions, consisting mainly of burning, scaling and erythema were more common than in Caucasoids. Almost a third of the participants dropped out bacause of these adverse changes.

This experience corresponds to a widespread belief by physicians that Oriental skin is more "sensitive" to potentially irritating substances.

Riassunto

La tretinoina topica (Retin-A®, Ortho Pharmaceuticals) è stata applicata per 6-9 mesi al volto di 27 cinesi, principalmente donne, affette da foto-invecchiamento e provenienti dal Nord della Cina dove le condizioni climatiche sono molto sfavorevoli.

I risultati sono simili a quelli descritti in precedenza per gli orientali della Thailandia, delle Filippine e del Sud/Est Asiatico. Al controllo la maggior parte dei soggetti ha mostrato un soddisfacente grado di miglioramento sull'apparenza globale, dopo un controllo di tutti i parametri presi in considerazione. Effetti migliorativi si sono verificati nei seguenti parametri:

(1) Depigmentazione: nelle zone cutanee iperpigmentate il colore delle cute è divenuto più uniforme.

(2) Rughe: le rughe sottili si sono ridotte soprattutto nella zona del contorno occhi.

(3) Colorito giallastro: le aree cutanee di aspetto giallastro sono state sostituite da aree rosate che hanno migliorato notevolmente il grado di attrazione individuale.
(4) Secchezza-rugosità: la superficie cutanea è diventata più liscia e meno rugosa. Un gruppo di controllo trattato con un'idratante non medicata non ha mostrato apprezzabili miglioramenti sui parametri descritti.
Le reazioni irritative, quali l'arrossamento evidente e le fessurazioni cutanee, sono state più comuni rispetto ai Caucasici.
Quasi un terzo dei partecipanti hanno interrotto il trattamento a causa di questi effetti collaterali. Questa esperienza convalida quanto sostenuto dai medici che la cute degli orientali è più "sensibile" a sostanze potenzialmente irritanti.

INTRODUCTION

Numerous reports all over the world have documented the beneficial effects of topical vitamin A acid (tretinoin) in improving the appearance of photodamaged skin (1,2,3,4).
Clinically, fine wrinkles become effaced, dyspigmentations fade, the rough-dry surface becomes smoother, laxity gives way to firmer more elastic skin, uneven texture becomes uniform, yellow sallowness is replaced by a rosy glow. The over-all effect is toward a more youthful and more physically attractive skin.
Histologically, epidermal atrophy and atypia are completely corrected accompanied by a normal pattern of differentiation. Keratinocytes become hypertrophic with evidence of increased metabolic activity, associated with an increase in epidermal turnovers, dense clusters of hyper-melanized pigment granules become dispersed and individual melanosomes contain much less melanin. New collagen is laid down sub-epidermally and new small vessels are generated (angiogenesis) (5,6,7).
Physiologic and immunologic functions are also enhanced.

PHOTOAGING IN ORIENTALS

Reports from Thailand, the Phillipines and Asia have demonstrated that the capacity of tretinoin to reverse the clinical and histologic signs of photodamage in Oriental skin is similar to that of Caucasoids (7,8,9,10).
In Orientals, dyspigmentations and uneven texture are not only more frequent than in Caucasoids but are far more distressing psychologically. Hyperpigmentations such as melasma and lentigos are genuinely dreaded and are viewed as serious cosmetic defects which compromise social activities. Huge sums of money are spent on bleaching agents, some of which are toxic, while many others are only marginally effective.
Wrinkles, hated and feared in Western societies, are not only less frequent in Orientals but are less stigmitized as a sign of aging. Still, as the population ages, wrinkles become coarser and more despoiling of appearance, especially in sunny areas. Thus, wrinkles are becoming a highly visible cosmetic defect, which are less likely to be viewed as a proud badge of age.
China is a huge country. In the South, climatic conditions are comparable to countries of Southeast Asia. In Northern China, where this study was carried out, meteorologic circumstances are quite different.
The weather is frequently dry and windy, predisposing to dryness.
Summers are hot and sunny, resulting in marked signs of photodamage in middle-aged adults, most of whom make no effort to protect themselves from the ravages of sunlight.
Changing social mores are also making people more conscious of their appearance as they become older.
It is against this background that we decided to evaluate topical tretinoin for the treatment of photoaged skin.
We attained results which compare favorably to those reported for other ethnic populations.

MATERIALS AND METHODS

We recruited 27 volunteers, ages 35 to 67, of whom 23 were women.
All gave a history of excessive sun-exposure in childhood and in their teens. Working in the fields was a common background.
Each showed typical signs of moderate to severe heliodermatitis, namely, peri-orbial and perioral wrinkles, rough dry skin, dyspigmentations of various sorts and degrees, very spotty mottling, lentigos and blotches, loose, inelastic skin and uneven, coarse texture.
The subjects considered themselves handicapped cosmetically and were eager to participate in the study.
Treatment consisted of the nightly application of 0.05% tretinoin cream (Retin A®, Ortho Pharmaceuticals) for six to nine months, according to the specifications in the package insert and

comparable to usage in prior reports in other countries. Purpose Cream (Ortho Pharmaceuticals) was applied liberally in the mornings to combat dry, windy weather and to moderate the scaling and stinging often induced by tretinoin. The entire face was treated. Additionally, one hand and dorsal forearm was similarly treated once daily with 0.05% tretinoin cream.

The duration of treatment was six to nine months. One hand and forearm was treated with Purpose cream, as a non-medicated control.

Global evaluations of improvement were made monthly, along with assessments of adverse effects.

RESULTS

Adverse Events

Attention was specifically paid to the following changes; erythema, scaling, dryness, burning and itching. These were present to varying degrees in nearly all patients, generally making their appearance in the first few days, persisting over the next few weeks and slowly declining thereafter as the skin became accommodated. These reactions were more frequent and more intense on the face as composed to the hands and forearms.

Tightness was also a frequent complaint. Dryness and scaling tended to persist longer than other signs and symptoms, especially when the weather was windy.

After 4 to 8 weeks of trying to comply with the nightly tretinoin regimen, 8 of the 27 panelists decided to quit the study because of persistent side effects.

Burning and dryness were the most frequent and distressing complaints.

All the others completed the study, with minor difficulties exempt for occasional bouts of scaling for which we advised stopping treatment for a few days, using Purpose Cream generously in the drug-free periods.

Clinical Evaluating of Improved Appearance

Beneficial effects were obtained more quickly and more markedly on the face as compared to the hands and forearms.

Fine wrinkles, especially around the eyes, were either completely effaced or appreciably diminished in the majority of subjects. Coarse deep wrinkles on the other hand, especially in older subjects, were mostly resistant to treatment, in the view of both the patients and the physician.

Fading and bleaching of dyspigmentations, along with a more uniform skin tone was perhaps the most successful aspect of this trial. Nearly every subject experienced a definite

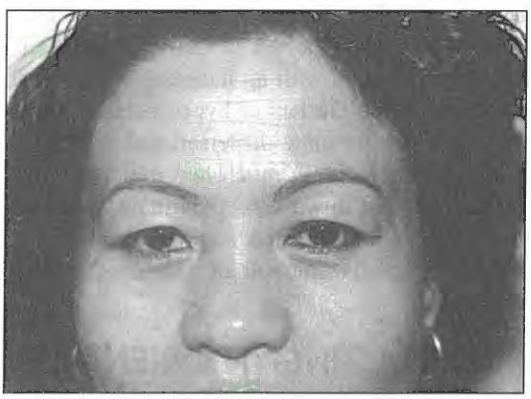

Fig. 1a. Pretreatment photo showing fine lines, dyspigmentation, coarse surface and small bags under eyes.

Fig. 1b. After 24 weeks of treatments the surface is smooth, pigmentation has faded, texture is more uniform and bags under eyes are no longer evident.

lightening of hyperpigmentation, noticeable to friends and relatives as well as to the physician. However in several subjects who initially showed hyper-pigmented elevated seborrheic keratoses, no improvement occurred either in the degree of pigmentation or the lesion itself.

Another effect which most subjects found particularly pleasing, was replacement of a sallow, yellow complextion by a rosier color. This change in coloration was also apparent to the physician, though difficult to score.

In nearly every case, too, the texture and tone of the surface became smoother and softer to the touch. These are subjective assessments which the volunteers are better able to perceive than outside observers. While we did not have the subjects fill out detailed questionnaires for each cosmetic feature, they were asked to volunteer as much information as their experience allowed, whether positive or negative.

We concluded from listening and looking that the volunteers were particularly impressed by the manifest benefits in lightening hyperpigmentations, effacement of fine wrinkles, improved tone and coloration, and more uniform texture ans smoothness.

Hands and Forearms

Except for some improvement of dryness and roughness, Purpose Cream by itself had no appreciable effect on any subject.

On the other hand, lightening of pigmented spots were, as on the face, was perceivalbe to be the most noticeable cosmetic benefit of Retin-A®. The rate of bleaching of dyspigmentations was slow and in a few cases non-apparent, even after 9 months. Overall the bleaching effect was real, but only modest.

Most experienced some improvement in surface smoothness and uniformity of skin tone. This too was noticeable but not dramatic.

Adverse side reactions were much less frequent and of lesser magnitude on the forearms as compared to the face.

So, while tretinoin was better tolerated on the hands and forearms, it was also less effective.

DISCUSSION

Our results confirm that topical tretinoin can improve the appearance of photodamaged skin of Chinese persons.

Applied daily to the face for 6 to 9 months, we found substantial lightening of hyperpigmentations (mottling, blotches, lentigos), effacement of fine wrinkles, improved surface texture and smoothness and replacement of sallow skin with a rosier complextion.

On the hands and forearms, the cosmetic benefits were slower to develop and less impressive.

The dry, windy metereologic conditions in North China pose a challenge for the daily use of tretinoin, which by itself can provoke dryness, burning and erythema. These discomforts occurred in nearly all subjects. Eight of 27 panelists were unable to accommodate to these adverse events and dropped out of the study after one to two months.

In the rest, these signs and symptoms abated after a month or so, enabling completion of the study without undue difficulty.

As regards tolerance to potentially irritating substances, Japanese dermatologists have come to believe through clinical experience, that Oriental skin is more "sensitive" than that of Caucasoids. They emphasize that neurosensory purely subjective reactions such as itching, burning and stinging are more frequent and intense in Oriental skin. Though rigorous proof is not at hand, our drop-out rate is at least consistent with the notion that Oriental skin is more reactive to chemicals.

People in North China are not yet fully aware of the hazards of excessive exposure to sunlight and accordingly take no protective measures. Thus, heliodermatitis is very common in middle-aged adults and is destined to become much worse as the population ages. Although it is widely believed that melanin affords protection

against photodamage, most of our patients had a prematurely aged appearance with advanced signs of photodamage, namely, numerous deep wrinkles, laxity, multiple dyspigmentations and a sallow complection. Whatever protection may be conferred by melanin, was certainly overwhelmed in our hot, dry windy climate with intensive solar radiation in summer.

Despite the difficulties in conducting this trial, we were able to show that topical tretinoin had the same beneficial effects in photoaged chinese skin that have been noted in many other studies in other cultures and climates.

REFERENCES

1) **Kligman AM, Grove GL, Hirose R, Leyden JJ. (1986)** Topical tretinoin for photoaged skin. *J Am Acad Dermatol.* **15**:836-59.
2) **Weiss JS, Ellis CN, Headington JT et al. (1988)** Topical tretinoin improves photoaged skin. *JAMA*; **259**:527-32.
3) **Weiss JS, Ellis CN, Headington JT, Voorhees JJ. (1988)** Topical tretinoin in the treatment of aging skin. *J Am Acad Dermatol*; **19**:169-75.
4) **Voorhees JJ. (1990)** Clinical effects of long-term therapy with topical tretinoin and cellular mode of action. *J Int Med Res*; **18** (Suppl. 3):26-8C.
5) **Ellis CN, Weiss JS, Voorhees JJ. (1988)** Tretinoin: its use in repair of photodamage. *J Cutan Aging Cosmet Dermatol*; **1**:33-9.
6) **Leyden JJ, Grove GJ, Grove MJ et al. (1989)** Treatment of photodamaged facial skin with topical tretinoin. *J Am Acad Dermatol*; **21**:638-44.
7) **Kotrajaras R, Kligman AM. (1993)** The effect of topical tretinoin on photodamaged facial skin: the Thai experience. *Brit J Derm* **129**:302-309.
8) **Goh JH. (1990)** The treatment of visible signs of senescence: the Asian experience. *Br J Dermatol*;**122** (Suppl. 35):105-9.
9) **Leung RSC. (1990)** Photoaged skin and tretinoin: Hong Kong. *Excerpta Medica. Asia Pacific Congress Series*; **106**:13-14.
10) **Juang MY. (1990)** Treatment of visible signs of senescence. The Taiwan experience. *Excerpta Medica. Asia Pacific Congress Series*;**106**:11-12.

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HUMAN SKIN FIBROBLAST CULTURE TO TEST GLYCOLIC AND LACTIC ACID SOLUTIONS.

M. Mattioli Belmonte[1], MG. Tucci[2], E. Vellucci[3], R. Solmi[4], G.Ricotti[5], O. Talassi[4],G. Biagini[1], P. Morganti[3]

[1] Istituto di Morfologia Umana Normale, Università degli Studi di Ancona - Italy

[2] Dipartimento Ricerca, I.N.R.C.A, Ancona - Italy

[3] Research and Development Mavi sud, Aprilia (LT) - Italy

[4] Istituto di Istologia ed Embriologia Generale, Università degli Studi di Bologna - Italy

[5] Unità Operativa di Dermatologia e Chirurgia Plastica, I.N.R.C.A, Ancona - Italy

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Key words: human skin fibroblasts, glycolic acid, lactic acid, experimental study.

Synopsis

Alphahydroxy acids (AHAs) have become quite popular as skin rejuvenating agents. The action of AHAs on the skin is affected by the contact time, the concentration and the pH of the solution used. In a previous in vitro study we showed that by using salified derivatives of glycolic and lactic acid one may prolong the contact time for longer than with the pure form. In the present work we test the effects of different concentration of natural glycolic and lactic acid at a pH between 4.5 and 5.5, combined with a special protective mixture formed of gelatine-glycine-aminoacids at the same pH. The investigations performed brought to light no significant differences among the various solution employed. Further study are necessary in order to obtain in vitro results matching the in vivo clinical experience.

Riassunto

Negli ultimi anni gli AHA hanno assunto un ruolo sempre più importante tra i trattamenti anti-ageing. L'effetto dell'applicazione degli AHA sulla cute dipende dal tempo di contatto, dalla concentrazione e dal pH delle soluzioni utilizzate. In un precedente studio eseguito in vitro, abbiamo dimostrato che utilizzando soluzioni salificate di acido glicolico e di acido lattico, anziché le soluzioni pure, il tempo di contatto può essere prolungato. Nel presente lavoro abbiamo testato gli effetti di differenti concentrazioni di acido lattico e acido glicolico ad un pH compreso tra 4.5 e 5.5, combinando questi acidi con una particolare miscela di aminoacidi (gelatina-glicina). Lo studio condotto non ha evidenziato particolari differenze tra le varie soluzioni utilizzate.

INTRODUCTION

Alphahydroxy acids (AHAs) have become quite popular as skin rejuvenating agents. They are a class of compounds that are derived from food sources (e.g., glycolic acid is found in sugar cane while lactic acid is present in sour milk) reported to be effective in the treatment of a condition predisposing to dry, rough skin (1). Although a large number of these compounds are available, glycolic acid has been the most widely used. Therapeutic benefits have been reported with not only dry skin but also in the treatment of acne, keratosis, both seborrhoic and actinic, warts and problems related to ageing such as dyschromia and wrinkling (2). Among the AHAs glycolic acid has a low molecular weight and size and hence a high penetrative capacity (3). Alphahydroxyacid compounds cause disadhesion of keratinocytes, and at high concentration epidermolysis (4) as well as increasing water absorption by varying the kind of lipids and enzyme systems produced by Odland's lamellar bodies (3).

The action of AHAs on the skin is affected not only by the contact time but by the concentration and the pH of the solution used (5). There is always some stinging and burning sensation for the patients.

In a previous in vitro study we showed that by using salified derivatives of glycolic and lactic acid one may prolong the contact time for longer than with the pure form, since the pH is more physiological (6). That study extended the investigation to lactic acid since, despite having a higher molecular weight than glycolic, this acid has the biological property of converting into chetonic form (pyruvic acid), and this proves far more active in reducing the cohesion of corneocytes (7).

In vivo studies have recently shown that the use of partially salified AHAs does not detract from their clinical effectiveness, and may even improve the penetration (8,9).

The present work thus sets out to test the effects of various different concentration of natural glycolic and lactic acid at a pH between 4.5 and 5.5, combined with a special protective mixture formed of gelatine-glycine-aminoacids at the same pH. Cutaneous fibroblast cultures were used since such cells are sensitive to treatment with glycolic and lactic acid (10). Investigations consisted in analysis of cell proliferation and assessment of cell morphology by phase contrast light microscopy and scanning electron microscopy.

MATERIALS AND METHODS

Materials

The glycolic acid and lactic acid solutions used in this study are reported in table I. Concentration tested for each solution were 1.2 mM, 4.6 mM e 9.2 mM for 100 gr. of culture medium.

TABLE I.
DETAILS OF AHA SOLUTIONS.

	COMPOSITION	pH
C	Glycolic acid 70%	4-5.5
C1	Glycolic acid 70% with an 8% (w/w) protective admixture *	4-5.5
D	Lactic acid al 70%	4-5.5
D1	Lactic acid 70% with an 8% (w/w) protective admixture *	4-5.5

** protective admixture = Natural gelatin-glycin-aminoacids.*

Cell culture

Human skin fibroblasts, obtained from a lower forearm biopsy of a 20 years old healthy donor, were used to test the above mentioned solutions. Cells were subcultured at the first passage, in a logarithmic growth phase, were used for these experiments.

The fibroblasts were grown at 37° C, in humidified air with 5% CO^2, in 25 cm^2 flask containing Iscove's modified Dulbecco's medium (Gibco, Grand Island, NY,USA), supplemented with 0.2% sodium bicarbonate (Sigma,St.Louis, Mo,USA), 10% foetal bo-

vine serum (Bio Whittaker, Verviers, Belgium), 200 U/ml penicillin, 200 µg/ml streptomycin (Gibco). Fibroblasts were checked for mycoplasma contamination (PBI International, Milano, Italy). Twenty-four hours after plating cell vitality (90 -95%) was measured by Trypan Blue exclusion (Gibco).

Cell proliferation

Fibroblasts were plated on wells (Nunc,Roskilde, Denmark) in a complete medium. After 24 hours the medium was exchanged for serum free IMDM plus 0,1% BSA (Sigma). After 12 hours the fibroblasts were treated with different glycolic and lactic acid solutions diluted in the same serum free culture medium plus 0,1% BSA. Evaluations were performed after 24, 48 and 72 hours.

Haemocytometric Chamber Counts: Fibroblasts were plated in 24 well plates at number/concentration of 20,000 cells/well. Fibroblasts were removed from the substrate by incubation in 200 ml of 0.25% w/v trypsin (Gibco) for 5 min at 37° C and then counted using a haemocytometric chamber.

Tritiated thymidine Incorporation: Fibroblasts were plated, in sextuplet, in flat bottomed 96 well tissue culture plates at a density of 10,000 cells per well. At each time (24, 48 and 72 hours), the cells were exposed to 3H-thymidine (Amersham, Buckinghamshire, U.K.) (0.5 mCi per well) for 6 hours and then stored at - 20° C. Afterwards, the cutaneous fibroblasts were collected on glass microfibre filters using a multiple automated sample harvester, the wells and the filters were wasbed with distilled water to release all cells from the plates and remove unbound nucleotides. Filters were placed into vials and 0.1 ml Soluene 350 (Camberra Packard, Meriden, Ct, USA) was added, followed after 40 minutes by 4.5 ml of scintillation liquid (Beckman, Fullerton, Ca,USA). Six hours later, the fiberglass filters were counted using a liquid scintillation counter (LKB WALLAC).

Cell morphology

Phase contrast microscopy: Plated fibroblasts were examined by phase contrast microscopy (Leitz-Labovert F8) and photographed 24, 48 and 72 hours after contact with solutions. In order to study fibroblasts behaviour on first contact with the substances, before and after the cells had time to adapt and organize themselves in terms of culture conditions, we decided to examine the samples over the first 72 hours of contact with solutions. Semiquantitative evaluations were performed by the Kiellstrand method (11), taking 4 images for each samples at 800 magnification phase contrast light microscopy and seeking zone representative of the whole well. Values from 0 to 10 were set for each image, the parameters assessed being: cell morphology, number and distribution of cell present. Totals for these evaluations are plotted in graph against observation times.

Scanning electron microscopy: After 72 hours of contact with solutions, the cells were fixed with 2.5% v/v glutaraldehyde in a 0.1 M cacodylate buffer, post-fixed with 2% w/v osmium tetroxide in 0.1 M cacodylate buffer, dehydrated in an ascending ethanol gradient and critical point dried (CPD). Specimens were then mounted on aluminium holders, gold film metalized and observed with a SEM Philips XL20 at 25 kV.

Statistical analysis

The proliferation data are reported as a mean ± standard deviation. Analysis of variance (ANOVA) was used to evaluate the influence of the compounds, their concentration and their incubation time on cell proliferation.

RESULTS

Cell proliferation

Counts in Haemocytometric Chamber: Investigations of cell proliferation revealed no significant differences between controls and fibroblasts cultured in the various solutions at the diffe-

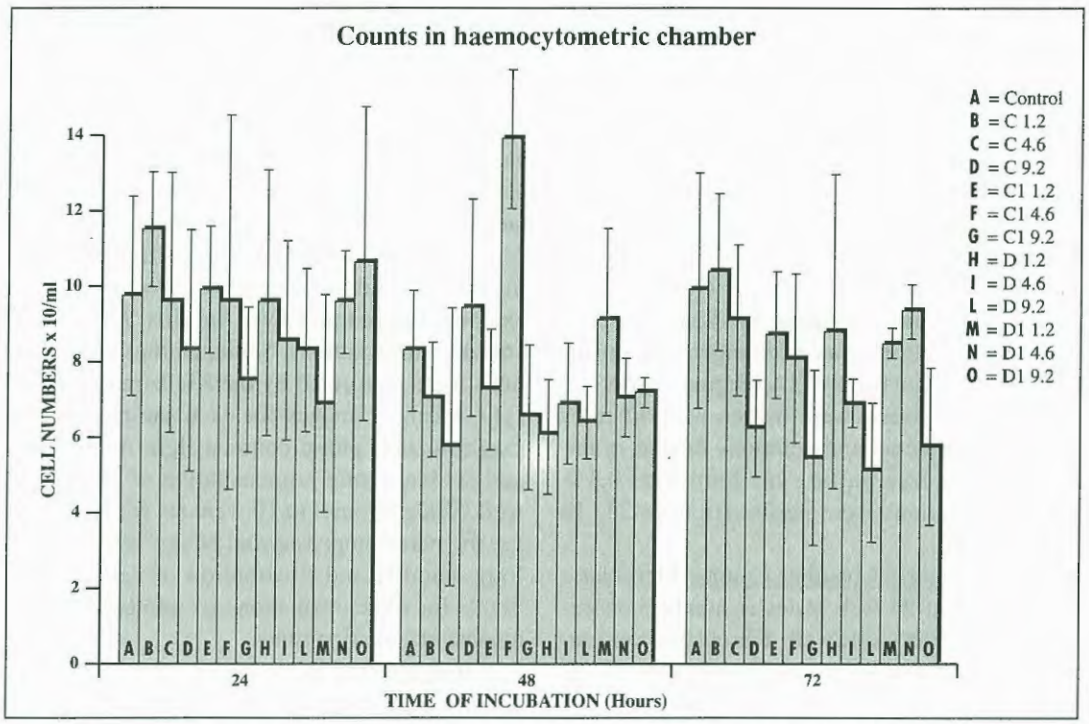

Fig. 1 Evaluation of cell proliferation using a haemocytometric chamber. Mean Values ± standard deviation are reported.

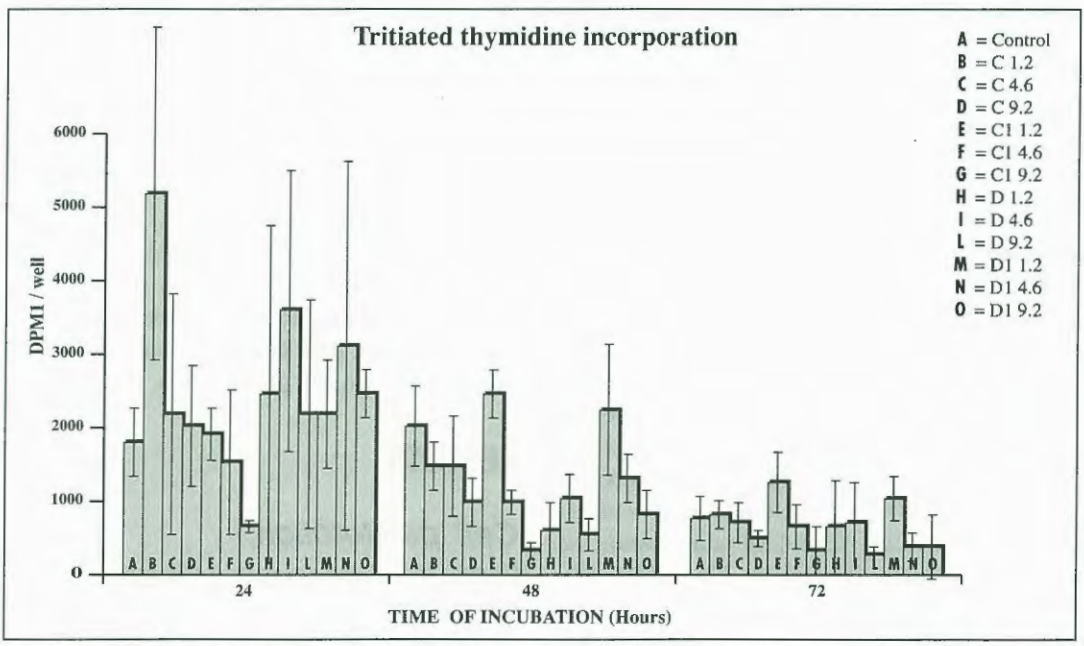

Fig. 2 Evaluation of cell proliferation by incorporation of H^3-thymidine. Mean Values ± standard deviation are reported.DPM1=disintegration per minute

rent contact times. A slight reduction in cell number may nonetheless be detected as the contact time increases (Fig.1).

Comparison of glycolic with lactic acid as culture solutions shows a lower cell count with the latter, though not significantly so (Fig 1). In general, all solution and contact times confirm that the use of high concentration (9.2mM) involves a grater reduction in cell count. The only exception to this was when lactic and glycolic acid contained a protective admixture, at 24 and 48 hours respectively (Fig.1) .

Tritiated thymidine Incorporation: A more sensitive test than the foregoing, it confirms that there are no significant differences between controls and fibroblasts cultured with the various solutions where DNA duplication is concerned. The only exception was low concentration (1.2 mM) glycolic acid at 24 hours. A marked decrease in DNA duplication is seen at the contact time increases (Fig.2). Comparison between glycolic and lactic acid shows there to be less DNA duplication with the latter, tough not significantly (Fig.2). A similar picture emerges between the solutions with and without the protective admixture. High concentration (9.2 mM) had consistently lower scores than the other concentrations (Fig.2).

Cell morphology

Phase contrast microscopy: As the graph shows (Fig.3), there were no significant differences between the various samples and controls, whether at the same time intervals or comparing across intervals. Fig.4 reports the morphology of a) control cells against low concentration (1.2 mM) of b) glycolic and c) lactic acid. Non confluent cells shows numerous cell prominences in both controls and the glycolic acid samples, unlike that with lactic acid where the cell shape itself is more elongated. Fig. 5 shows the normal cell morphology of samples treated with a) glycolic and b) lactic acid at a medium (4.6 mM) concentration. The morphologic appearance of cells cultured with a high (9.2 mM) con-

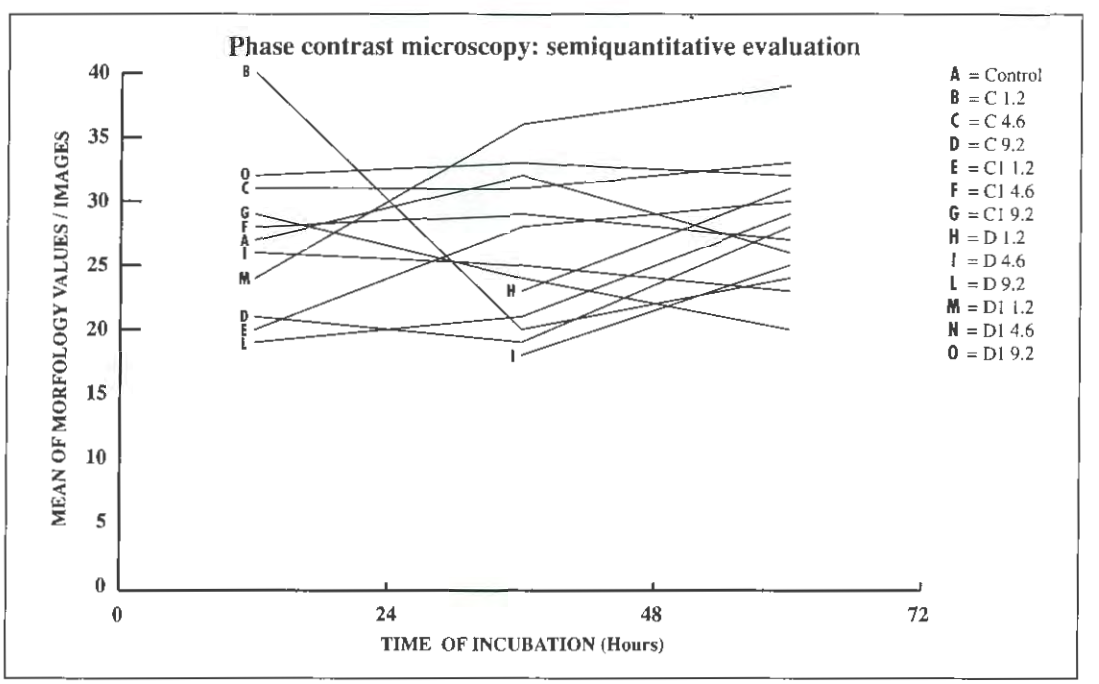

Fig. 3 Graph of the semiquantitative evaluation of cell growth by phase contrast microscopy.

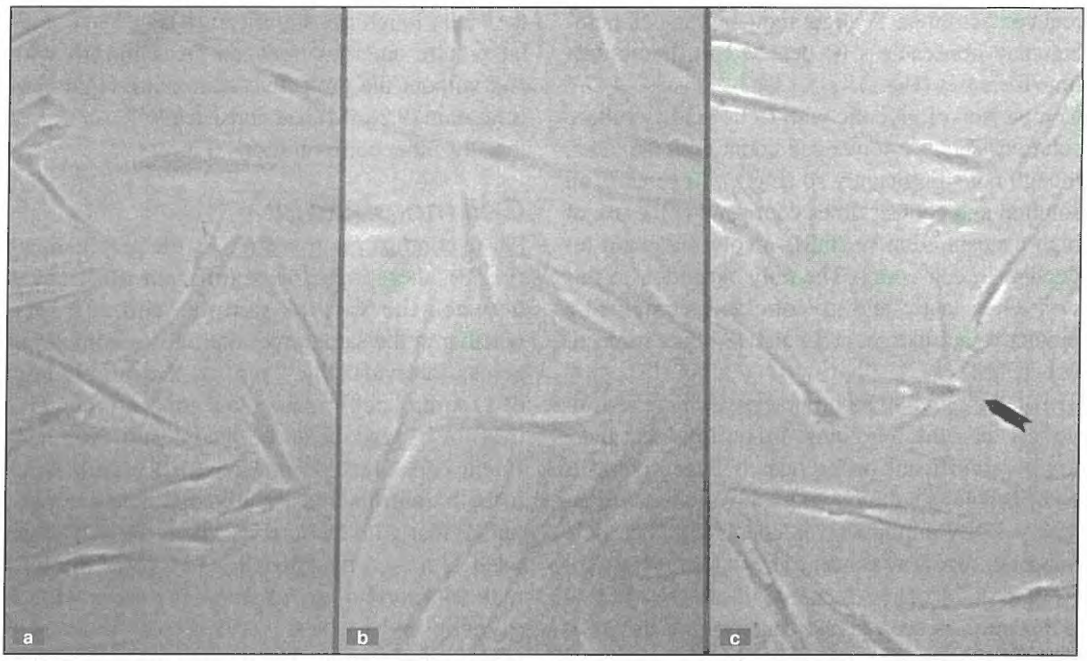

Fig.4 Fibroblast cultures of a) controls vs cultures treated with a low concentration (1.2 mM) of b) glycolic acid and c) lactic acid, observed by phase contrast light microscopy (x250). N.B. The elongated appearance connected with lactic acid (➡).

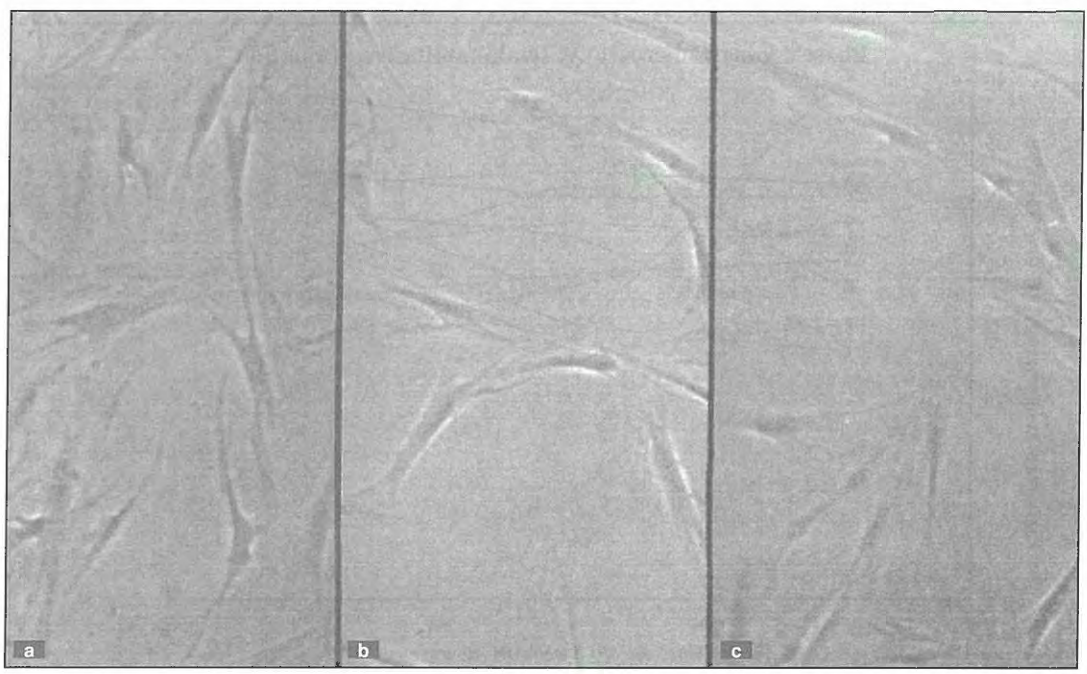

Fig.5 Appearance under phase contrast light microscope of fibroblast cultured a) with glycolic acid at an intermediate concentration (4.6 mM) and b) with intermediate (4.6 mM) and c) high concentration (9.2 mM) lactic acid (x250).

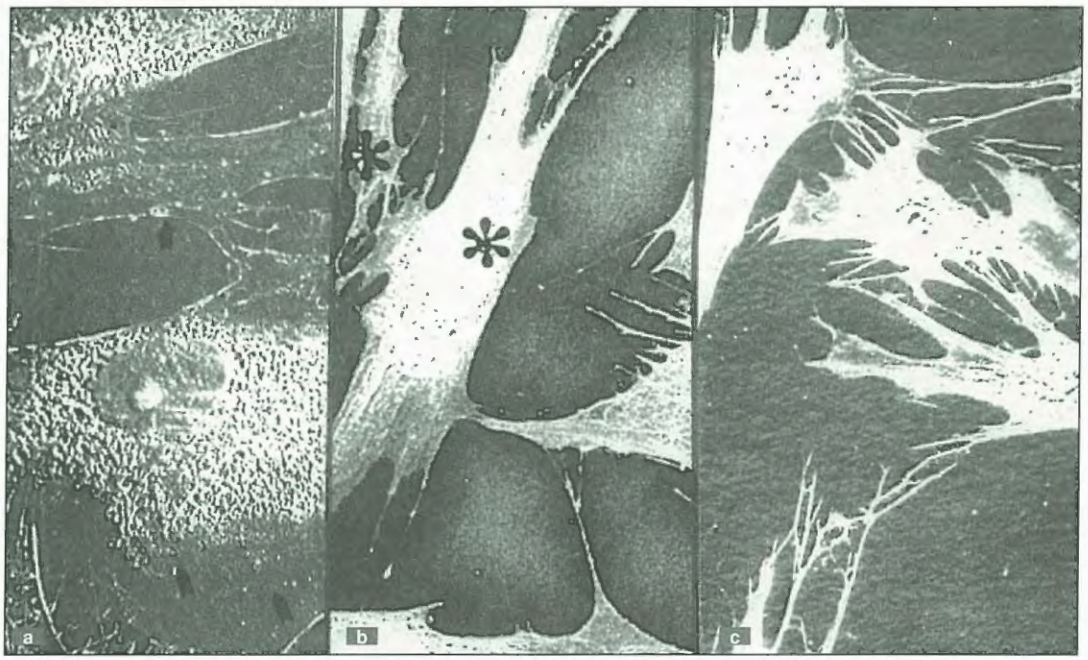

Fig.6 Scanning electron microscope photograph of a) control fibroblast (SEM x700), as well as fibroblasts cultured with b) glycolic acid (SEM x400) and c) lactic acid (SEM x400) at a low concentration (1.2 mM).N.B. The flattened appearance (➡) of control fibroblast and the marked cell spreading (✻) with glycolic acid.

centration of lactic acid is somewhat altered, on the other hand, the cytoplasm being very diaphanous and a great number of thin prominences present.

Scanning electron microscopy (SEM): Cell growth among controls seemed good, the cells being mainly arranged round the outer edge of the well. Cells were generally spindle-shaped and no signs of degradation were noted (Fig.6a).

After the various treatment, morphological investigations of cultured fibroblasts revealed the tendency of cells to take an increasingly stellate shape as the glycolic acid concentrations rose (Fig.6b; Fig.7c). At high concentrations there was extremely pronounced cell spreading as well as many signs of cell degeneration (Fig.8a). No significant differences could bee seen between those with and without the protective admixture. At both low (Fig.6a) and high (Fig.8b) concentrations, whether "protected" or not, lactic acid cultures were comparable with glycolic. A contrast to this was observed at the intermediate concentration, where the pure solution yielded a cell appearance similar to controls (Fig.7), while considerable degeneration was observed where the protecting solution had been used.

DISCUSSION

The recent literature on AHAs treatment has been rich in vivo trials (12-15) but undersupplied with in vitro experiments on cells. In vivo studies confirmed that the pH, and hence salification level, of AHAs is a factor of the highest importance. With salified solutions at a pH between 4 and 5.5 one notes a reduction in the side effects of AHA application, such as stinging and burning (8). In a previous study we showed, in vitro, that salified derivatives of AHAs have better biotolerance than pure solu-

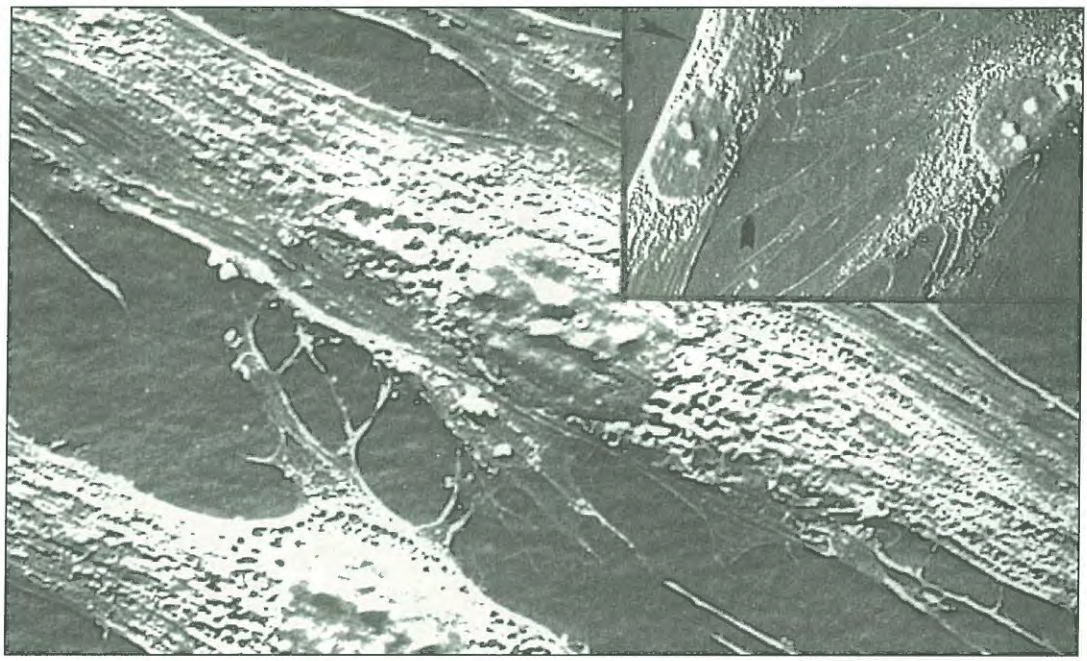

Fig.7 Scanning electron microphotograph of fibroblasts cultured with an intermediate concentration (4.6 mM) of lactic acid (SEM x1300). Insertion : fibroblasts (➢) cultured with glycolic acid (SEM x700) at an intermediate concentration (4.6 mM) showing cell spreading (➡).

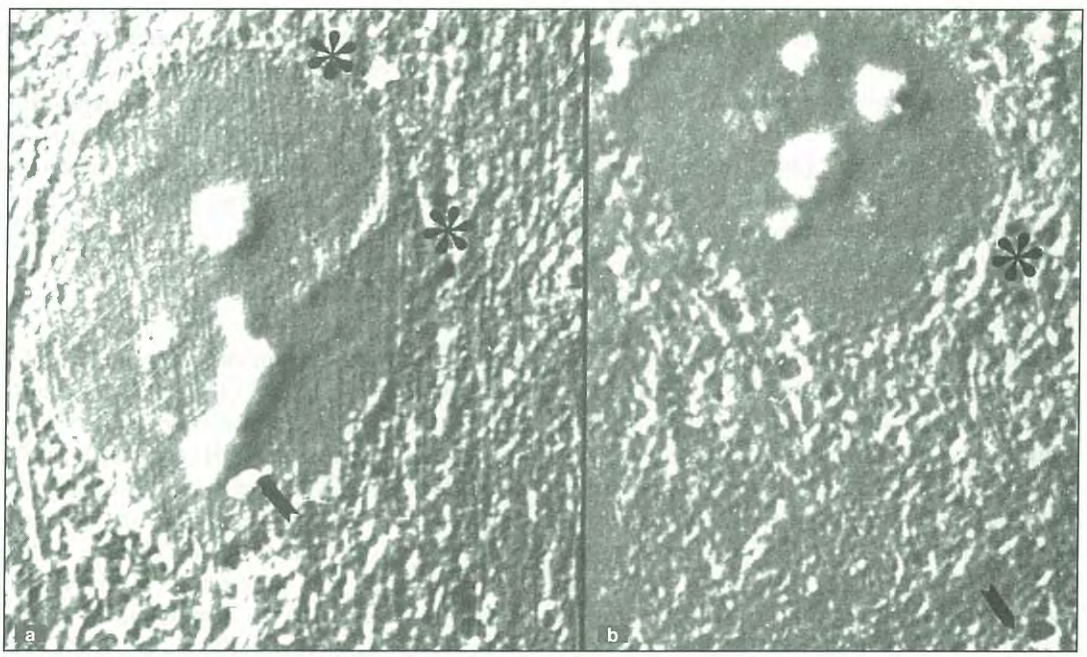

Fig.8 Scanning electron microphotograph of fibroblasts cultured with a) glycolic (SEM x2000) and lactic (SEM x2000) acid at high concentration (9.2 mM). Cell spreading (✳) is most marked and cell degeneration evident

tions (6). The present study does not show any significant differences among pure glycolic or pure lactic acid treated cultures, or those with protective admixtures, or controls.; there is simply a tendency for lactic acid solution to be less compatible. Evidently the solution brought into contact with cells do not significantly alter the microenvironment, and the fibroblasts preserve a stable state similar to controls throughout the experiment. One other decisive factor proves to be the concentration of AHAs used: as this rises, so does the compatibility to fall.

Since investigations brought to light no differences among the various solution employed, it is reasonable to assume that the buffer mixture was no suitable either in quality or in quantity in order to obtain in vitro results matching the in vivo clinical experience (8). Optimising the buffer mixture will be attempted in subsequent studies designed to test the correlation between in vitro predictive models and in vivo clinical results.

REFERENCES

1) **Van Scott EJ, Yu RJ. (1982)** Substances that modify the stratum corneum by modulating its formation. *In: Frost P. Horowitz SN, eds. Principles of Cosmetics for the dermatologist St. Luis; CV Mosby*; 70-94
2) **Van Scott EJ, Yu RJ. (1986)** Alpha hydroxyacids procedures for use in clinical practice. *Cutis*; **43**: 222-9.
3) **Morganti P. (1996)** Alpha hydroxyacids in cosmetic dermatology. *J Appl Cosmetol*; **14**: 35-41.
4) **Moy LS, Murad H, Moy RL. (1993)** Glycolic acid peels for treatment of wrinkles and photoageing. Surg Oncol; **19**: 243-6
5) **Wehr FR. (1992)** A controlled comparative efficay study of 5% ammonium lactate lotions vs an emollient control lotion in treatment of moderate xerosis. *J Am Acad Dermatol*; **25**: 849-51.
6) **Talassi O, Vellucci E, Mattioli Belmoute M, Tucci MG, Solmi R, Ricotti G, Biagini G, Morganti P.** In vitro study for alphahydroxy acids (AHAs) and derivatives assessment
7) **Elson ML. (1993)** The regular structure of glycolic acid and its importance in dermatology. Cosmet Dermatol; **6**: 31-5
8) **Morganti P, Randazzo SD, Fabrizi G, Bruno C. (1996)** Decreasing the stinging capacity and improving the antiageing activity of AHAs. *J Appl Cosmetol*;**14**: 77-91
9) **P. Morganti (1996)** Cosmetic composition for the treatment of dry skin and/or hyperkeratosis. *Patent Application*. D.N. 1264- SP 138 P
10) **Moy LS, Howe K, Moy RL. (1996)** Glycolic acid modulation of collagen production in human skin fibroblast cultures in vitro. *Dermatol Surg*; **22**: 439-41
11) **Kjellstrand P, Okmark P, Odselius R, Thysell H, Riede G, Holmquist B. (1991)** Adherence of blood cells to dialyzer membranes as a measure of biocompatibility. Int J Artif Organs;**14**: 698-702.
12) **Stiller MJ, Bartolone J, Stern R, Smith S, Kollias N, Gillies R, Drake LA (1996 Jun)** Topical 8% glycolic acid and 8% L-lactic acid creams for the treatment of photodamaged skin. A double-blind vehicle-controlled clinical trial. *Arch-Dermatol.*; **132**(6): 631-6
13) **Effendy I, Kwangsukstith C, Lee JY, Maibach HI (1995 Nov)** Functional changes in human stratum corneum induced by topical glycolic acid: comparison with all-trans retinoic acid. *Acta-Derm-Venereol.*; **75**(6): 455-8
14) **Ditre CM, Griffin TD, Murphy GF, Sueki H, Telegan B, Johnson WC, Yu RJ, Van Scott EJ (1996 Feb)** Effects of alpha-hydroxy acids on photoaged skin: a pilot clinical, histologic, and ultrastructural study. *J-Am-Acad-Dermatol.*; **34** (2 Pt 1): 187-95
15) **Becker FF, Langford FP, Rubin MG, Speelman P. (1996 May)** A histological comparison of 50% and 70% glycolic acid peels using solutions with various pHs. *Dermatol-Surg.*; **22**(5): 463-5

Correspondence to:
P. Morganti, Ph.D. - Research and Development
MAVI sud srl - Via dell'Industria 1,
04011 Aprilia (LT), ITALY
Phone: + 39 06 9286261
Fax: + 39 06 9281523
E-mail=mavi@colosseum.it
URL=http://www.colosseum.it/st81/mavi

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THE USE OF A CAPACITANCE DEVICE TO EVALUATE THE HYDRATION OF HUMAN SKIN.

V. Zuang[1], C. Rona[2], F. Distante[2], E. Berardesca[2]

[1] ECVAM, JRC Environment Institute, 21020 Ispra, Italy; and Department of Human Anatomy & Cell Biology, University of Nottingham, Medical School, Nottingham NG7 2UH, UK.

[2] Dept. of Dermatology, University of Pavia, IRCCS Policlinico S. Matteo, 27100 Pavia, Italy.

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Key words: Corneometer® CM 820, human skin, hydration, non-invasive, capacitance, bioengineering instrument.

Synopsis

Directive 93/35/EEC on the testing of cosmetics requires that evidence is provided to support the efficacy claims made for marketed products. In order to fulfil this requirement without resorting to the use of animals, non-invasive skin bioengineering techniques are now being employed with human volunteers. These techniques provide quantitative and objective data, if the measurements are performed under rigorously standardised conditions. In this study, a non-invasive instrument, the Corneometer® CM 820, which measures skin capacitance, has been used to evaluate the short-term effects of three commercially available moisturisers, by monitoring the water content of the stratum corneum at different treated test sites of human skin (inner forearm) in comparison with that at an untreated site. The three products, respective leaders in the perfumery, pharmacy and supermarket distribution, have been confronted with a standard reference material (20% glycerol in distilled water), so that the results can be compared between laboratories and to avoid differences relating to instrumentation and methodologies. Measurements with the Corneometer® CM 820 were taken at the baseline visit, and at 1, 3 and 6 hours post-application, at each test and control site. The results show that all of the test products have a hydrating effect on skin, but that the effect is significantly different between the products at each timepoint, except 3 hours post-treatment, when there was no significant difference between one of the products and the standard reference. If used properly and according to appropriate protocols, the electrical methods for the evaluation of skin hydration, have proved to be useful for the non-invasive evaluation of the efficacy of cosmetic products on human volunteers, and consequently, for the reduction of animal tests.

Riassunto

La Direttiva 93/35/EEC sui test dei prodotti cosmetici dispone che debbano essere fornite delle prove obbiettive per sostenere le rivendicazioni di efficacia fatte per i prodotti venduti sul mercato. Al fine di soddisfare questo requisito senza il ricorso all'utilizzazione di animali, si stanno attualmente impiegando volontari umani e tecniche non-invasive di bioingegneria cutanea. Queste tecniche, se

le misurazioni sono eseguite in condizioni rigorosamente standardizzate, forniscono dati quantitativi e oggettivi. In questo studio e' stato utilizzato uno strumento non-invasivo che misura la capacitanza cutanea, il Corneometer® CM 820, al fine di valutare gli effetti a breve termine di tre idratanti disponibili in commercio, monitorando il contenuto idrico dello strato corneo in differenti sedi cutanee selezionate sull' avambraccio. I tre prodotti, rispettivi leader nella distribuzione nelle profumerie, farmacie e mercato di massa, sono stati confrontati con un riferimento standard (glicerolo 20% in acqua distillata), in modo da poter comparare i risultati con altri ottenuti utilizzando la stessa tecnologia, ma in diversi laboratori. Le misurazioni sono state effettuate alla visita basale, e dopo 1, 3 e 6 ore dall' applicazione. Differenze significative sono state rilevate tra i vari prodotti confermando differenti caratteristiche funzionali dei prodotti testati. I metodi elettrici per la valutazione del contenuto idrico cutaneo, quando opportunamente utilizzati, ed in presenza di protocolli corretti, permettono di essere utili nel valutare l'efficacia dei prodotti cosmetici in modo non invasivo e nel ridurre la necessità di tests sugli animali.

INTRODUCTION

Bioengineering instruments, already widely used in experimental dermatology, have been introduced into cosmetology in order to, *inter alia*, substantiate efficacy claims on cosmetic products, as required by *Directive 93/35/EEC* on the regulation of cosmetic products [1]. These instruments have the advantage of being non-invasive, providing quantifiable and objective information on the mechanical and physiological properties of the skin. However, precautions should be taken in the design and conduct of the study, and in the interpretation of the test results in order to obtain accurate, reproducible and reliable data. Among the various claims made for cosmetics, a very common one is the capacity of the product to restore the water content in the superficial layer of the skin, i.e. the stratum corneum. The Corneometer® CM 820 has been employed in this study to measure the short-term improvement of skin hydration after the application of three commercially available moisturisers at different sites on the skin of the inner forearms of a group of selected human volunteers.

PURPOSE OF THE STUDY

This study has been undertaken in order to investigate if the Corneometer® CM 820 was a valid and reliable instrument to establish a claim on skin hydration and, being used non-invasively and *in vivo* on human skin, if it was contributing to the replacement of animals for cosmetic testing as required by *Directive 93/35/EEC*. Furthermore, it was examined if the instrument was able to discriminate between different moisturisers.

BIOPHYSICAL BASIS

The change in the electrical properties of the stratum corneum induced by hydration involves at least three different types of electroconductive elements [2]:

- conduction by electrons and holes
- conduction exchange of protons all along the H-bonded network of water molecules
- conduction by ions larger than protons ("large ions").

Conduction by electrons and holes is only significant in an abnormally dry skin, and conduction by large ions only if the stimulating frequency is below the MHz range. In consequence, protonic conduction is thought to be the predominant event [3]. Water molecules bound by hydrogen links can mutually exchange protons, which migrate within the network of hydrogen bonds. For that reason, electrical measurements are highly dependent on the water-keratin interaction and hence on the water content of the stratum corneum. The water absorption isotherm describing the quantity of water binding to the stratum corneum at a given temperature has shown that the water-keratin interaction follows the Brunauer-Emmet-Taylor (BET) model [4] and that it is possible to distinguish three types of water according to the type of interaction; "tightly-bound water" for water contents from 0 to around 7%, "bound water" between about 7% and 35%, and "free water" beyond 35% [4,5]. Microcalorimetric measurements have shown the respective adsorption energies which increase with the strength of the binding [4]. Because of the variation in water binding strength, there is no direct proportionality between total water content and electrical conductance. Substances or treatments which interact with the keratin-water network may therefore modify conductance without changing the total water content at the test site [3].

INSTRUMENT

The Corneometer® CM 820 measures the skin capacitance at low frequency (40-75 KHz). It

should be noted that measurements of skin capacitance are frequency-dependent, unlike the electrical capacitance of non-biological materials. The Corneometer consists of a main recording device which displays the hydration values, and a measuring probe. The probe is formed of an interdigital grid of gold-covered electrodes, arranged closely in parallel and functioning as capacitor [6,7,8,9]. The electric scatterfield formed at the edges of the gold lines decreases in proportion to increasing distance between them. The active part of the electrodes has a surface of 7 x 7 mm. The electrodes are 50 µm wide, with an interdigital spacing of 75 µm, covered with a low dielectric vitrified material of 20 µm thickness, to avoid a direct contact between the gold-plated electrodes and the skin surface. No current passes through the skin, but an electric field of variable frequency is formed in the upper part of the skin, i.e. in the stratum corneum and the underlying layer of the epidermis. The depth and the arrangement of the electric field formed in the skin depends on the geometry of the electrodes and the dielectric material covering the electrodes (constant capacitance), and on the capacitance of the biomaterial in contact with the electrodes (variable capacitance). The approximative skin depth measured is 30µm according to the manufacturer [8]. Other authors have reported a depth of about 60-100 µm [10,11]. The total capacitance is only influenced by variations in the dielectric constant of the biomaterial in contact with the electrodes [7]. A dry stratum corneum is a dielectric medium. However, when the stratum corneum is hydrated, a significant change in its dielectrical properties occurs [2]. Increasing the water content of the stratum corneum will increase its relative permittivity. Consequently, the capacitance of the probe in contact with the stratum corneum is increased. A resonating system in the instrument measures the shift in frequency (40 to 75 kHz) of the oscillating probe which results from the changes in the total capacity.

MEASUREMENTS

The capacity of the skin surface is measured by applying the probe (surface area 0.95 cm^2) with a constant pressure of 3.5 N [3] on the skin for about 1.5 s. A graduated spring system is incorporated in the probe to facilitate the measurements and to assure a reproducible pressure. The switch inside the probe turns on when the correct pressure has been applied. A "H" for hydration appears on the screen, to inform the operator that the instrument has been activated. A microprocessor in the device reads the measuring times, compares the value with the stored calibrated data and displays digitally a converted value (arbitrary units of skin hydration) of the variable total capacitance on screen [7,8]. A sound indicates the end of the measurement. The hydration value remains on display for two minutes, after which the instrument automatically switches off after three audible signals.
The instrument is able to measure values from 0-150 arbitrary units (a.u.). In practice, the values of hydration vary from 30-60 for very dry skin, from 60-70 for dry skin, from 70-90 for hydrated skin, and lie above 90 for very moist skin [7].

STUDY MATERIAL

The three test materials used in the clinical investigation were current commercial moisturisers. Product A is an oil-free serum enriched with ceramides, hydrating agents and vitamins. Products B and C are both oil-in-water (O/W) emulsions, enriched, respectively, with Jojoba oil and Lecithin (product B) and by a moisture collector (product C).

STUDY DESIGN

The clinical trial was conducted on twenty healthy Caucasian human volunteers, including

females and males. They were chosen according to precise inclusion and exclusion criteria. One week before starting the study, the subjects were asked to sign an informed consent form and to provide details of their medical histories. At the same time, the skin test sites were visually examined by the dermatologist in order to confirm subject eligibility.

The subjects were instructed to discontinue the use of all topical products on their forearms, including moisturisers and medicated cleansers three days before the study, but were asked to continue their normal cleansing routine.

Each of the subjects' forearms was divided into four sites of 4x5 cm each, plus a fifth site on both upper forearms, by using a template. They were all treated with a single application of each product, with one site serving as the control. The site on the upper forearm served as the standard reference and was treated with an aqueous solution containing 20% glycerol. The standard reference is used to compare results within the same laboratory and between different laboratories, avoiding differences related to instrumentation, environmental conditions and methodologies. Test products were applied symmetrically on each forearm, according to a randomisation table, at a rate of $2\mu l/cm^2$, as small, evenly spaced blobs within the delineated areas. Different fingers were used for applying different products.

After the baseline measurement, readings were taken at 1, 3 and 6 hours post-application. Instrumental assessment was performed under standardised environmental conditions (at a room temperature of 19-21° C, with 40-50% relative bumidity), following a period of relaxation and acclimatisation for the human volunteers of at least 15 min. At each timepoint, four readings were taken at different areas of each test site to provide a meaningful value. Any cream remaining on the probe was removed at every change of site.

Each untreated site value (the control) was subtracted from the treated site values for the respective forearms at each timepoint (A1-control1=A1norm, B1-control1=B1norm, etc...). This has been carried out to normalise the data. The mean value of the four measures at each of the treated test sites was calculated. The three products plus the control were assigned to the different test sites on each human volunteer at random, so that any differences among the sites were minimal.

Left and right forearms of each human volunteer have been treated the same way, to allow left-right comparisons.

The normally distributed data were analysed by using analysis of variance (ANOVA) to compare the hydration values of the different products at the same time interval. Multiple subgroup comparisons were calculated by using the Neumann-Keuls test to detect where the differences between the products lay. The software Statistica 3.0 for Macintosh was used to analyse the data.

RESULTS

The results are presented in Table I and Fig.1.

Table I

Products[1]	Mean ± SD
A1	16 ± 9.4
B1	7.7 ± 10.9
C1	21.6 ± 9.5
Gly1	19.1 ± 10.4
A3	13.6 ± 9.6
B3	4.7 ± 11.3
C3	16.2 ± 10.9
Gly3	16.3 ± 10.1
A6	10.4 ± 10.1
B6	3.1 ± 11
C6	12.4 ± 10.6
Gly6	14.5 ± 8.6

Table I: [1]normalised hydration values of the products at 1, 3 and 6 hours post-application (mean and standard deviation)

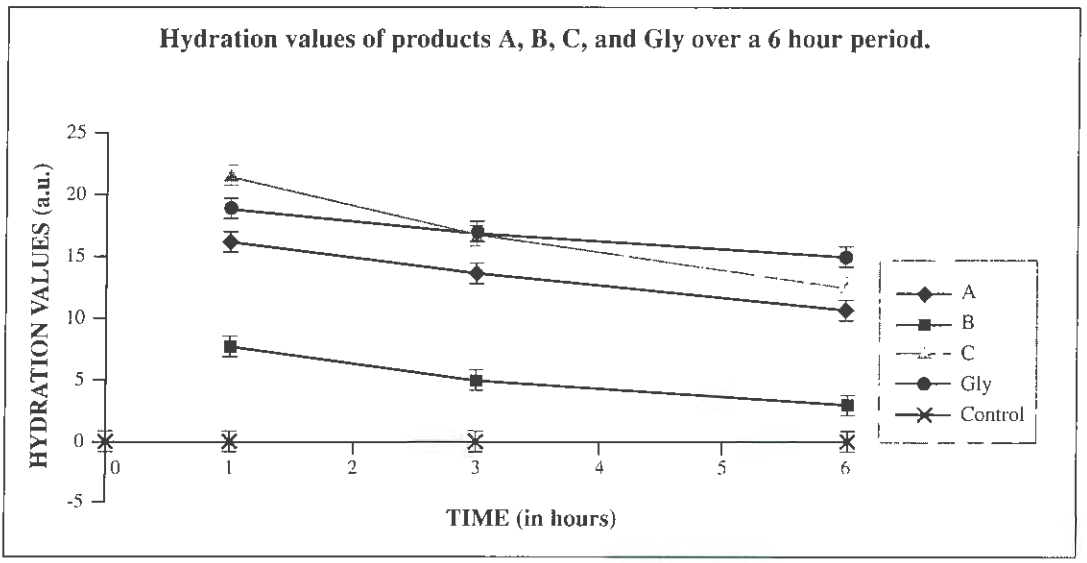

Fig. 1: The normalised hydration values are all greater than zero, i.e. each treated test site gives higher hydration values (in arbitrary units) than the control site at the respective timepoint. All the products show a maximum hydration effect one hour post-application. Product C and glycerol in water give the best results and have a very similar effect at three hours post-application.

It has been found that:

- All 4 products have a hydrating effect at each timepoint (Fig. 1). This has been checked by calculating the mean ± 2x (standard error). All such confidence intervals are strictly greater than zero
- Some treatments are better than others. This is confirmed by time-by-time analysis of variance (ANOVA).

The significance level of the test was set at 0.05. By comparing products A, B, C and glycerol at 1, 3 and 6 hours post-treatment, significant differences between the products were found ($p<0.01$). Follow-up comparisons with the Neumann-Keuls test showed where such differences lay. The effects of all the products were significantly different ($p < 0.05$), except at 3 hours post-treatment, when there was no significant difference between product C and glycerol ($p=0.88$).

DISCUSSION

In general, product C and glycerol had the highest hydrating effects. Product B had the least effect. The standardised conditions in the laboratory avoided a rapid evaporation of the glycerol. Furthermore, the cosmetic properties (smell, feeling, aspect) of glycerol are unpleasant and would not be appreciated by the consumer.

The usual hydration profile of a cosmetic moisturiser depends on whether it is an oil-in-water (O/W) emulsion or a water-in-oil (W/O) emulsion. Capacitance measurements for an O/W emulsion show an immediate and significant increase in hydration (capacitance values) after application of the product [13]. This increase in capacitance is thought to be due to the water content of the product, which itself is dependent on the nature and the quantity of humectant which keeps the water in the formulation. After 10 or 15 minutes, a rapid decrease in hydration follows, due to the evaporation of excess water from the skin surface [3, 7, 14]. After a certain

time, the capacitance values are maintained at an increased level in relation to the control, even for several hours, depending on the efficacy of the cosmetic product [7].

The control has been subtracted from each value to increase the sensitivity of the study by avoiding individual-related differences and allowing a more precise comparison of the products (smaller standard deviation).

A short-term study was chosen rather than a long-term study, to avoid the influence of changes in the environment (e.g. changes of climate or season during the experiment) and to permit better discrimination between the products tested [3]. However, because the products were applied in a single application on the inner forearm, a useful subjective evaluation of their effects by the human volunteers themselves was not possible.

CONCLUSION

In this study, the Corneometer® CM 820 has been shown to be a sensitive and useful tool, able to quantify skin hydration in a rapid and inexpensive way. The study has been designed in such a manner as to avoid as much as possible the limitations of the instrument. However, even then the results have to be interpreted with caution, bearing in mind that the instrument only gives relative information on the water content of the stratum corneum and not absolute values [3].

REFERENCES

1) **Anon. (1993)** Council Directive 93/35/EEC amending for the sixth time Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetics products. *Official Journal of the European Communities*, **L 151**, 32-35.
2) **Levêque J.L., de Rigal J. (1983)** Impedance methods for studying skin moisturisation. *J. Soc. Cosmet. Chem.*, **34**, 419-428.
3) **Berardesca E. et al. (1996)** European Group for Efficacy Measurements on Cosmetics and Other Topical Products (EEMCO). *EEMCO Guidance for the Assessment of Stratum Corneum Hydration: Electrical Methods. In press.*
4) **Levêque J.L., Escoubez M., and Rassneur L. (1987)** Water-keratin interaction in human stratum corneum, *Bioeng. Skin*, **3**, 227-242.
5) **Salter DC. (1981)** Studies in the measurement, form and interpretation of some electrical properties of normal and pathological skin *in vivo. Doctor of Philosophy (D. Phil.) thesis, University of Oxford*, (3 volumes, 694 pages).
6) **Tagami H., Ohi M., Iwatsaki K., Kanamaru Y., Yamada M., Ichijo B. (1980)** Evaluation of skin surface hydration in vivo by electrical measurements, *J. Invest. Dermatol.*, **75**, 500-507.
7) **Barel A.O., Clarys P. (1995)** Measurement of Epidermal Capacitance. *In Handbook of Non-invasive Methods and the Skin.* (Eds. Serup J., Jemec, G.B.E.) pp. 159-164. CRC Press, Boca Raton.
8) **Courage W. (1994)** Hardware and Measuring Principle: Corneometer. In *Bioengineering of the Skin*: Water and the Stratum Corneum. (Eds. Elsner P., Berardesca E., Maibach H.I.) pp. 171-175. CRC Press, Boca Raton.
9) **Distante F., Berardesca E. (1995)** Hydration. In *Bioengineering of the Skin*: Methods and Instrumentation. (Eds. Beradesca E., Elsner P., Wilhelm K.-P., Maibach H.I.) pp. 5-11. CRC Press, Boca Raton.
10) **Blichmann C.W., Serup J. (1988)** Assessment of skin moisture: measurement of electrical conductance, capacitance and transepidermal water loss. *Acta. Derm. Venereol.* (Stockholm), **68**, 284-290.
11) **Barel A.O., Clarys P., Wessels B., de Romsée A. (1991)** Non-invasive electrical measurement for evaluating the water content of the horny layer: comparison between the capacitance and the conductance measurements. In *Prediction of Percutaneous Penetration* - Methods, Measurements, Modelling, (Eds. Scott, R.C., Guy, R.H., Hadgraft, J., Boddé, H.E.) pp. 238-247. IBC Technical Services, London.
12) **Prem S. Mann. (1995)** Introductory Statistics. 2nd edition. John Wiley and Sons, Inc., pp. 606-619, New York.
13) **Tagami H. (1995)** Measurement of Electrical Conductance and Impedance. *In Handbook of non-invasive Methods and the Skin.* (Eds. Serup J., Jemec, G.B.E.) pp.159-164. CRC Press, Boca Raton.
14) **Tagami H. (1989)** Impedance measurements for evaluation of the hydration state of the skin surface. *In Cutaneous Investigation in Health and Disease, Noninvasive Methods and Instrumentation*, (Ed. Lévêque, J.-L.), pp. 79-111. Marcel Dekker, New York.

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CLINICAL EVALUATION OF TWO BABY WIPES IN A CROSS-OVER STUDY.

Feliciani C*, Di Carlantonio M§., Bellucci R§., Guarracino M§. and Amerio P.*
* Department of Dermatology, University "G.D'Annunzio", Chieti, Italy
§ Centro Ricerche Fater P&G SpA, Sambuceto, Italy.

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Synopsis

To investigate the safety of a new moisturized wipe we performed a double blind study evaluating several clinical and laboratory parameters. 55 babies, wearing diapers, (56% males, 44% females) were divided in two groups, both randomly stratified for age and sex. Age ranged from 1 month to 2 years. First group has been cleaned with a new baby wipe during the test phases and the second group with a commercially available baby wipe. Three visits have been made. During the calibration phase (between visit 1 and 2) the babies were cleaned with water and a uniform soap. During the test phase (between visit 2 and 3) the two baby wipes were exclusively used. At the beginning and at the end of the test phase a questionnaire for parents, a clinical evaluation of a dermatologist, skin pH measurement and skin microbiology were carried out. The use of the new baby wipe is safe and does not alter skin parameters compared to control product.

Riassunto

E' stato affrontato uno studio a doppio cieco per valutare, attraverso esami biologici e clinici, la sicurezza nell'uso di un nuovo detergente idratante.
55 bambini, affetti da eritema da pannolino (56% maschi e 44% femmine) sono stati suddivisi a doppio cieco in due gruppi, per età e sesso. L'età variava da 1 mese a 2 anni.
Mentre il primo gruppo è stato deterso con il nuovo fazzoletto detergente (durante la fase-test), il secondo gruppo veniva deterso con un fazzoletto detergente già in commercio.
Sono stati effettuati 3 controlli clinici.
Durante la fase di calibrazione (tra la 1 e la 2 visita) i bambini sono stati detersi in modo uniforme con acqua e sapone. Durante la fase-test (tra la 2 e la 3 visita) sono stati utilizzati soltanto i due tipi di fazzoletti detergenti.
All'inizio e alla fine della fase-test è stato realizzato un questionario per i genitori, una valutazione clinica da parte del dermatologo assieme ad un controllo del ph e della carica batterica cutanea.
L'uso del nuovo fazzoletto detergente è sicuro e non altera i parametri cutanei se paragonato al prodotto di controllo.

INTRODUCTION

The use of wipes is gradually increasing with modern habits that lead to the use of new products in baby toiletries.

Wipes are very practical tools in baby cleaning because of their thinness, moisture, small space they occupy, and their easy use. Wipes are designed to have the right moisture and a pleasant smell. Wipes must not be too wet so as to dilute the excrements or to leave the skin humid, neither too dry so as not to clean or to irritate the diaper area. It must be kept in mind that the wipes are used at every diaper change, more than a normal cleaning habit for the face or other areas of the baby's body. Wipes should be formulated to provide a minimum of interference with the functions of normal skin which is an important property in preventing skin infections (1-2). Continuous and excessive use of soap could be aggressive to the hydro-lipidic mantle and reduce the stratum corneum which is the first defence of the skin and could change skin pH (3-5). The aim of our study was to investigate that the regular use of baby wipes is safe and effective for skin cleaning during the change of diapers for the test phase (2 weeks).

MATERIALS AND METHODS

The study was carried out on 55 children,56% of the subjects were male, 44% were female. Two randomly groups stratified according to the nine criteria below were formed by a personal computer, one of which was made of 28 subjects (Group 1, Test group), the other of 27 (Group 2, control group, with a commercially available product).

A pre-phase visit was made and the following parameters have been analyzed:

1)**Sex and Mobility.** The percent of males was 51% in the group 1 (49% of females) and 53% in the group 2 (47% of females).
The percent of subjects who walked without help was 61% in group 1 and 52% in group 2 .

2) **Breast feeding status.** 93% of the subjects in group 1 were breast-fed, 85% in group 2.

3) **Changes of the diet** during the test. 4% of the subjects in group 1 changed their diet, 11% in group 2.

4) **Teething.** During testing none was observed in group 1, 11% in group 2.

5) **Health condition.** 19% of the subjects in group 1 and 8% in group 2 were affected by several illnesses (e.g. flu or cold) but none of them were severe enough to stop the test.

6) **Regular use of drugs.** In both groups none of the subjects have used drugs during the test period.

7) **Use of systemic drugs before starting the test.** 18% of the subjects made use of drugs in group 1, 4% in group 2, during the week before the product was tested.

8) **Use of topically applied drugs in the diapered area.** None of the subjects used oinments in the diaper area in group 1, 4% in group 2.

9) Presence of **atopic dermatitis and seborrheic dermatitis** (6) 4% of seborrheic dermatitis was found in group 1, none in group 2. No presence of atopic dermatitis was recordered in both groups.

DIAPERS

Babies, during the study, used uniform diapers provided by us. At the beginning of the test phase the wipes were provided in unlabelled packages and the mothers were trained in their use.

WIPES

TEST PRODUCT: it was made of a soft, thick air laid with 100% cellulose fibers substrate. The lotion was an oil in water emulsion. The oil phase was composed of emollient and spreading substances, in the water phase the preservative system was present.

CONTROL PRODUCT: in the control wipes the same substrate was present, as described above for the test product. The lotion was made of a water based solution with surfactant, emollient ingredients and the preservative system. Control product has been merchandise for many years.

CALIBRATION PHASE

With the aim of having the babies in the same skin conditions the babies' toiletries were carried out with a soap for 15 days before the beginning of the test (calibration phase, visit 1 to visit 2).

TEST PHASE (visit 2 to visit 3)

At the end of the calibration phase a baseline clinical evaluation was performed using several skin parameters (visit 2): clinical skin inspection with erithema evaluation ranging from 0 to 4 (0=absent, 1=slight, 2=moderate, 3=extended, 4=severe) and presence or absence of papules, vescicles and desquamation in the diapered area, pH measurement and microbiological culture. A questionnaire for the parents of the babies was also made. After 15 days use of the wipes the same parameters were evaluated (visit 3). visit 2 and visit 3 were performed by a dermatologist without the knowledge of which product was assigned to the baby. A standard pH-Meter (Beckman, Fullerton, CA, USA) was used and skin pH measurement was determined twice, after opening the diaper and after cleaning, on three sites inside the diaper area (symphisis pubis, right hip, buttocks) and one site outside the diaper area (thigh).

The measurements were performed only if the sites were not fecally soiled. Specimens for the microbiological test were taken on the buttocks with a swab (Cultiplast LP ITALIANA SPA, Milan, Italy). Microbiological analysis were carried out in an external laboratory. At the beginning of the test phase a questionnaire was given to the mothers.

Questions were about practical, useful and pleasant aspects (or compliances) of the wipes. Questionnares were given back at visit 3 with a personal comment about the products.

For statistical analysis, all the values inside the diaper area were defined as the average of the sites values for symphisis pubis and right hip, and the net value were defined as the difference of the value inside the diaper area minus the value outside the diaper area. Furthermore an analysis has been performed for the buttocks pH values. Buttocks net values were defined as the difference of the value of the buttocks minus the value outside the diaper area.

The changes between visit 2 and visit 3 were the criteria for statistical analysis. Non-parametric test methods were used for the analysis of medical rash evaluation (Mann-Whitney-U test and Chi-square). Non-parametric and parametric test methods were used for the analysis of pH measurement (Mann-Whitney-U test and Student's t test). Values of $p<0.05$ were considered statistically significant. Summarizing (see table 1) three visits were performed. visit 1: calibration phase of 15 days during which the babies were cleaned with soap and general clinical conditions were evaluated; visit 2: test phase with baseline clinical evaluation and use of wipes for 15 days; visit 3: final clinical evaluation on the last day of use of the product.

Table I

Clinical design of the study.

	WEEKS				
	0	1	2	3	4
Visit 1	..... Calibration phase ..... (Use of soap/water)				
Visit 2			..... Test phase ..... (Use of test products)		
Visit 3					Final clinical evaluation

RESULTS

Clinical evaluation

The medical rash grades for erythema has been analysed evaluating the grade obtained at visit 3 (after use of the products tested) compared to the grade at baseline, visit 2 (before the use of the products tested) for each product group. Results are summarized on table 2.

Product group 1 (new baby wipe).

The erythema was absent in the 89% of the subjects at both visit 2 and visit 3. 12% (3 cases) of the subjects presented slight erythema at visit 2; at visit 3, 8%(2 cases) of the subjects showed slight erythema and 4% (1 case) extended erythema. However, the differences between visit 2 and visit 3 were not statistically significant. Papules were noted in 4% (1 case) of subjects in both visit 2 and 3, no presence of vescicles or edema were recorded. 1 case (4%) of desquamation was present at visit 2, none at visit 3.

Product group 2 (control group).

Erythema was absent in 81% of the subjects at visit 2, in the 89% of the subjects at visit 3. At visit 2, 20% (5 cases) of the subjects presented slight erithema; at visit 3, 4% (1 case) of the subjects presented slight erythema, 4% extended erythema, 4% severe erythema. The differences between visit 2 and visit 3 were not statistically significant. The presence of papules was registered in the 12% (3 cases) of the subjects in both visits 2 and 3. No presence of papules, vesicles and desquamation was diagnosed. 1 case (4%) of desquamation was registered at visit 2, no case at visit 3. In conclusion no influence of the product tested exists on the medical rash grading. Comparison between visit 2 and visit 3 for the medical rash grading didn't give evidence of any statistical significant differences in both product groups. In the only case with strong erythema, a patch test with the control product and the new product was done. Occlusive patches were applied on the left arm for 24 hours. The patches were removed and showed an extended erythema and slight vescicles only in the control product. The patch of the new product did not show any skin reaction.

Analysis of skin pH measurements

pH values - Product group 1

The average difference between the inside diaper area and outside diaper area was 0.07 in visit 2, 0.17 in visit 3, which is not statistically significantly different. The difference between the buttocks values and the external diaper site value (thigh) was 0.2 in visit 2, 0.26 in visit 3, which is not statistically significantly different. The same comparison for pH value after the cleaning of the same sites has been performed. No difference existed for both the average net values and the buttocks net

Table II

Clinical examination: comparison between before the use (visit 2) and after (visit 3). In brachets the number of patients is indicated.

		erythema grade							
		0	1	2	3	4	Papules	Vescicles	Desquamation
Test product (28)	visit 2	(25)	(3)	-	-	-	(1)	-	(1)
	visit 3	(25)	(2)	-	(1)	-	(1)	-	-
Control Product (27)	visit 2	(21)	(5)	-	-	-	(3)	-	(1)
	visit 3	(23)	(1)	-	(1)	(1)	(3)	-	-

values between visit 2 and visit 3. In conclusion no influence of both the products tested existed for the pH values. The comparison between the average net values and the buttocks net values of visit 2 and visit 3 didn't give evidence of any statistically significant difference.

pH values - Product group 2

The average difference between the inside diaper area and outside diaper area was 0.12 in visit 2, 0.04 in visit 3, which is not statistically different. The difference between the buttocks values and the external diaper site value (thigh) was 0.23 in visit 2, 0.14 in visit 3, which is not statistically significantly different. The same comparison for pH value after the cleaning of the same sites has been performed. No difference exists for both the average net values and the buttocks net values between visit 2 and visit 3.

In conclusion no influence of both the products tested exists on the pH values. The comparison between the average net values and the buttocks net values of visit 2 and visit 3 didn't give evidence of any statistically significant difference.

Skin microbiology evaluation

The following elements have been investigated: Total Microbic Count (TMC), Proteus, Streptococcus Faecalis, Staphilococcus, Pseudomonas, Esherichia Coli, Fungi colture. Microbiological samples have been taken on the buttock area (4 cm^2). The presence of microrganisms was qualitatively expressed by symbol "+" = growth of a small number of microorganisms; "++" = growth of a discrete number of microorganisms; "+++" = growth of a large number of microorganisms; "-" = negative.

Both groups gave the same results before and after product use (table 3). In conclusion very low TMC has been found after the use of wipes and products don't affect skin microbiology.

Table III

Microbiological test before and after the use of wipes.

	BEFORE	AFTER
Total Microbic Count (TMC)	+++	+
Proteus	-	-
Streptococcus Faecalis	++	-/+
Staphilococcus	+	-
Fungi	-	-
Pseudomonas	-	-
Escherichia Coli	-	-

DISCUSSION

The continuous use of detergents on a sensitive skin such as a baby's skin could influence the skin barrier functions and defences against external agents (e.g. bacteria and fungi) (7-8).

Wipes are practical tools in baby cleaning in the modern times. The aim of this study was to assess whether the use of a new baby wipe could influence the skin conditions leading to skin irritative phenomena in comparison with the control group. Both products didn't show any statistical differences in laboratory analysis and clinical evaluation (except for a case of contact dermatitis). Clinically the skin showed a normal appearance with no increment of rashes or other lesions in both groups. pH measurements demonstrated slight differences not statistically significant. Microbiological specimens showed a low presence of total microbic count with a majority of Streptococcus Faecalis.

At the end of the study the skin was cleaner with very few microrganisms. From the questionnaire it resulted that the new baby wipe was best accepted for its moisture, smell and resistance. In conclusion both products were positively accepted by parents of the babies, and gave good results in cleaning with no alterations in phisical characteristics of the epidermis.

Author address: Dr. Claudio Feliciani
Clin. Dermatologica - Pol."SS Annunziata"
Via dei Vestini, 66013 Chieti - Italy
phone +39-871-552310-551057
fax +39-871-551057
e-mail: felician@unich.it

REFERENCES

1) **K. Abrams, J.D. Harvell, D. Shriner, P. Wertz, H. Maibach, H.I. Maibech, S.J. Rehfeld. (1993)** Effect of organic solvents on in vitro human skin water barrier function. *J.Invest.Dermatol.* **101,** 609-613.
2) **R.J. Scheuplein, I.J. Blank. (1971)** Permeability of the skin. *Physiol.Rev.* **51,** 702-747.
3) **R.J. Scheuplein and L. Ross. (1970)** Effects of surfactants and solvents on the permeability of epidermis *J. Soc. Cosmet. Chem.* **21,** 853-873.
4) **P. Schmid and E. Hunter. (1971)** Extraction and purification of lipids. I. Solubility of lipids in biologically important solvents. *Physiol. Chem. Phys.* **3,** 98-102.
5) **G. Savermann and A Doerschner. (1986)** Comparative study of the skin care efficacy on in-use properties of soap and surfactant bars *J. Soc. Cosm. Chem.* **37,** 309-327
6) **B.H. Keswick, J.L. Symour, M.C. Milligan. (1987)** Diaper area skin microflora of normal children and children with atopic dermatitis. *J.Clin Microbiol.* **25,** 216-220.
7) **H.J. Yardley, R. Summerly. (1981)** Lipid composition and metabolism in normal and diseased epidermis. *Pharmacol. Ther.* **13,** 357-383.
8) **P.W. Wertz, D.C. Swartzendruber, W. Abraham, K. Madison, D.T. Downing. (1987)** Essential fatty acids and epidermal integrity. *Arch. Dermatol.* 123, 1381-1384.

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A NEW PELOID MASK OF ETRUSCAN ORIGIN

G. Fabrizi *, P.Morganti**

*Department of Dermatology, Catholic University of Rome Italy

** Secretary General, International Society of Cosmetic Dermatology
President, Director Research & Development - MAVI SUD S.r.l. Aprilia (LT), Italy
Department of Dermatology, Dermatologists Training School, II University of Naples, Italy

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Synopsis

Cosmetics can be labelled as "natural" and environmentally friendly if their components are biocompatible and do not cause problems of environmental impact.
We tried to develop a cosmetic product answering to the above requirements.
A natural peloid of Italian origin has been used as sole raw material. This peloid has "sui generis" characteristcs, that allow its classification as an High-moor Peat or a Peat Mud.
This mud can be defined "natural" according to Pisani.
The mud is a completely water-soluble product of sea origin. It settled about 30.000 years ago during the last ice age. It does not contain pollen, nor vegetable traces of its formation. It has a buttery consistency and can be easily spread on the skin and conditioned trough the use of several mineral waters. A double blind test was conducted with 20 female volunteers (aged between 18 and 45 years) with medium degree cellulitis variously located on the anterior-lateral surface of thighs.
Obtained data have shown a statistically important reduction ($p<0.01$) of the hypoderm thickeness at the end of this brief treatment, and have therefore proven the validity of this new natural cosmetic.

Riassunto

Un cosmetico può essere definito "naturale" ed ecologico se la totalità dei suoi componenti è biocompatibile e non causa problemi di impatto ambientale.
Abbiamo cercato di mettere a punto un prodotto rispondente a questi requisiti.
Un peloide naturale di origine italiana è stato utilizzato come esclusiva materia prima.
Il peloide ha delle caratteristiche "sui generis" che permettono la sua classificazione come Torba Alta o Torba Umica.
Questo fango può essere definito "naturale" secondo Pisani.
Il fango è un prodotto completamente solubile in acqua, di origine marina. Si è depositato circa 30.000 anni fa durante l'ultima glaciazione. Non contiene pollini, né tracce vegetali della sua formazione. Ha una consistenza burrosa e può essere spalmato facilmente sulla pelle e condizionato attraverso l'uso di diverse acque minerali.
Un test doppio cieco è stato condotto su 20 donne volontarie (di età compresa tra i 18 e i 45 anni) con una cellulite media presente in modo sparso sulla superficie antero-laterale delle cosce.
I dati ottenuti hanno dimostrato una riduzione statisticamente significativa ($p<0.01$) dello spessore dell'ipoderma alla fine di questo breve trattamento, ed hanno quindi provato la validità di questo nuovo cosmetico naturale.

In our opinion cosmetics can be labelled as "natural" and environmentally friendly if their components are biocompatible and do not cause problems of environmental impact.

We tried to develop a cosmetic product answering to the above requirements.
A natural peloid of Italian origin has been used as sole raw material. This peloid has "sui generis" characteristcs, that allow its classification as an High-moor Peat or a Peat Mud.

This mud can be defined "natural" according to Pisani (1) since thanks to its unique characteristics, it can be used as it is after having undergone a simple industrial process that does not change its essential chemical and physical characteristics.

PEAT MUD CHARACTERISTICS

The mud is a completely water-soluble product of sea origin. It settled about 30.000 years ago during the last ice age. It does not contain pollen, nor vegetable traces of its formation. It has a buttery consistency and can be easily spread on the skin and conditioned trough the use of several mineral waters (Tab I, II).
To better catalogue this Peat Mud the ether-methanol-acetonic extract of the dried mask has been checked in a 40°C stove as per Curri-Agostini method (2,3).
From the respective extracts, sterols have been separated by columns and GC, such as sitosterol, ergosterol, cholesterol, etc. (Tab. III) and fatty acids such as myristic, myristoleic, palaistic, stearic, oleic, linoleic, linolenic, arachidic, behenic and lignoceric (Tab. IV).
Furthermore the presence of a sugar fraction composed by glucose, fructose, rhamnose, allose, xylose and arabinose has been verified(4).

Table I

Chemical and physical characteristics

pH of centrifuged liquid	5,1
Dry residue at 105°	10,36%
Ashes	14,58% on dry residue
Ammonia on centrifuged liquid	traces
Nitrites on centrifuged liquid	traces
Phosphates on centrifuged liquid	absent
Bisulphides on centrifuged liquid	absent
Chloroformic extract	0,59% on dry residue
Organic nitrogen as per Kjedal	1,85% on dry residue
Proteins and aminoaeids (Bradford)	7,35% on dry residue

MATHERIALS AND METHODS

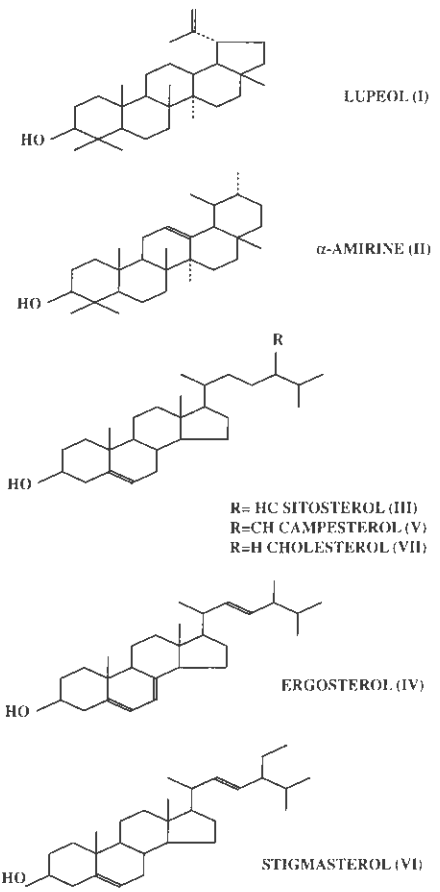
Having been refined by cylinders, the mud has been used for the topical treatment of cutaneous areas affected by cellulitis.

Table II

Chemical and physical characteristics
Peat Mud Ashes' analysis

Na_2O	1,15%	equal to	Na^+	0,85%	(0,37 meq/g)
K_2O	0,24%	equal to	K^+	0,20%	(0,05 meq/g)
CaO	29,10%	equal to	Ca^{++}	20,80%	(10,40 meq/g)
MgO	2,22%	equal to	Mg^{++}	1,34%	(1,10 meq/g)
SrO	0,08%	equal to	Sr^{++}	0,07%	(0,01 meq/g)
Fe_2O_3	5,04%	equal to	Fe^{+++}	3,53%	(1,89 meq/g)
MnO	0,10%	equal to	Mn^{++}	0,08%	(0,03 meq/g)
Ni^{++}	12ppm				
Zn^{++}	39ppm				
Cr^{+3}	2,8ppm				
Pb^{++}	0,2ppm				
Cu^{++}	17,0ppm				
Cd^{++}	traces				
Li^+	traces				
Al^{+3}	traces				
As^{+3}	0,60ppm				
Hg^{++}	0,07ppm				
Se^{+4}	traces				
Cl^-	0,77%				(0,21 meq/g)
SO^3	34,70%	equal to	SO_4^{-2}	41,70%	(8,68 meq/g)
P_2O_5	0,02%	equal to	PO_4^{-3}	0,03%	(0,009 meq/g)
N_2O_5	0,48%	equal to	NO_3^-	0,56%	(0,09 meq/g)
SiO_2	14,00%				

Table III



Sterol's content

SUBJECTS AND DESIGN OF THE STUDY

A double blind test was conducted with 20 female volunteers (aged between 18 and 45 years) with medium degree cellulitis variously located on the anterior-lateral surface of thighs.

The mud was applied in a homogeneous way (about 0.2 cm thick) on the affected area (9 cm^2) of the right or left thigh, while on the other one a black coloured placebo mask was applied (mask B).

The treatment was carried out in an environment of controlled temperature and humidity 20 consecutive days (at 10 a.m. during February 1997) and, in order to eliminate possible external influences, 50% of women were treated on the right thigh with the mask A (active) and the other 50% treated with A on the left thigh. The selected subjects were not taking drugs for systemic administration or anti-cellulitis topic products and, moreover, had not undergone specific diets before and during the clinical trial's period.

Before the beginning of the treatment three points on the surface to be examined in each subject and for both thighs were located. At the level of these points the ultrasonographic measurements were carried out before and after the treatment using the Dermoscan A((Cortex Technology, Denmark) already used by our group in former studies (5).

Through the use of Dermoscan A the tickness

Table IV

Fatty acids content of the Peat Mud

	Systematic name	Trivial name/abbreviation	Shorthand notation
Saturated	eicosanoic	arachidic	(20:0)
	hexadecanoic	palmitic	(16:0)
	octadecanoic	stearic	(18:0)
	tetracosanoic	lignoceric	(24:0)
Unsaturated	9-octadecenoic	oleic	(18:1, n-9)
	9, 12-octadecadienoic	linoleic	(18:2, n-6)
	9,12,15-octadecatrienoic	linolenic	(18:3, n-6)

evaluation of the subcutaneous tissue is made possible.
When a beam of ultrasounds passes through structuraly different portions of a tissue different echos are produced depending on the acoustic characteristics of the examined areas; those echos are recorded by the instrument and visualized on the screen as peaks (A-Scan). By measuring the distance among the peaks it is possible to determine the thickness of the different structural components of the skin and/or the subcutaneous tissue.
The statistically relevant ($p<0.01$) obtained results are shown in Fig. 1.

RESULTS AND COMMENTS

As already proven by other authors the Scan ultrasound technique seems to be the best methodology feasible for exactly measuring the variations of the cellulitic layers during an anti-cellulitis treatment (6,11). Through the use of this methodology we wanted to verify if this particular natural mud was capable of acting on the cutaneous disorder commonly named cellulitis.
Obtained data (Fig. 1) have shown a statistically important reduction ($p<0.01$) of the hypoderm thickenss at the end of this brief treatment, and have therefore proven the validity of this cosmetic treatment.
Supported by these positive results we are continuing our studies in order to study in detail the potential other cosmetic uses that this interesting natural mud seems to have.

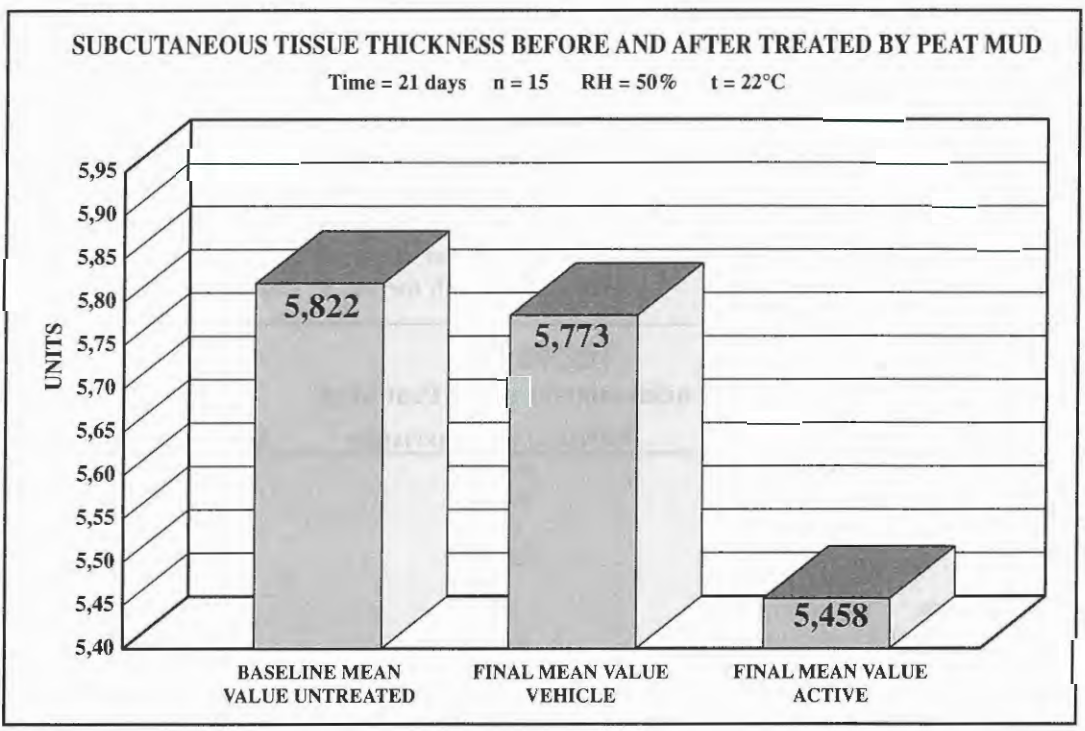


Fig. 1

REFERENCES

1) **Pisani S. (1951)**, I peloidi, in : (M.Messini Ed.) Trattato di idroclimatologia clinica, *Cappelli Ed.*, p. 6
2) **Curri S.B., P.Gonzato (1963)**, Analisi cromatografica su frazioni lipidiche e sterotiche peloidee, *Biochm. Biol. Sper.*, **3** :3, 1963
3) **Agostini G., Fedeli E., Cortesi N., Martinelli M., Curri S.B. (1983)**, Costituenti lipidici di una torba italiana, *La clin. Term.*, **36**: 135:1983
4) **Morelli 1, Piccioli I, Agostini G. (1986)**, Caratteristiche chimico-fisiche di estratti di torba del lago di Massaciuccoli, in : Proceedings VI Giornate mondiali del termalismo, Montecatini luglio 4-7, p. 61
5) **Morganti P, Celleno L, Vasselli A (1995)**, A new trandermal delivery cosmetic system to control the skin localized lipodystrophy, *J. Appl. Cosmetol.* **13,** 15:20
6) **Edwards C. (1988)** Non invasive in vivo measurement of dimensions and properties of stratum corneum and epidermis by A-Scan ultrasound, *15th IFCC International Congress*, **4**, 263-4
7) **Pugliese P.T. (1989)** Use of ultrasound in evaluation of skin care products, *Cosm Toil.* **104** : 61-76
8) **Armengol R., Girones E., Belles A. et al (1992)**, Quantitative evaluation of the anti-cellulitis efficacy by scan-ultrasound and its relation with clinic studies, *17th IFCC Int. Congr*, **Vol. 3**, 1218-1240
9) **Seidenari S. (1995)** Ultrasound B-mode imagine and in-vivo structure analysis, in : Non invasive methods and the skin (Serup and Jemec Ed.), *CRC press Inc., Bocaraton, Florida*, p. 257
10) **Fornage B.D. and Anderson M.D. (1995)** Ultrasound examination of the skin and subcutaneous tissues at 7.5 to 10 MHz, in : Non-invasive methods and the skin (Serup and Jemec Ed.), *CRC press Inc., Bocaraton, Florida*, p. 259
11) **Agner T. (1995)**, Ultrasound A-mode measurement of skin thickness, in : Non-invasive methods and the skin (Serup and Jemec Ed.), *CRC press Inc., Bocaraton, Florida*, p. 289

Correspondence to:
P. Morganti, Ph.D.
Research and Development
MAVI sud srl - Via dell'Industria 1,
04011 Aprilia (LT), ITALY
Phone: + 39 06 9286261
Fax: + 39 06 9281523
E-mail=mavi@colosseum.it
URL=http://www.colosseum.it/st81/mavi

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