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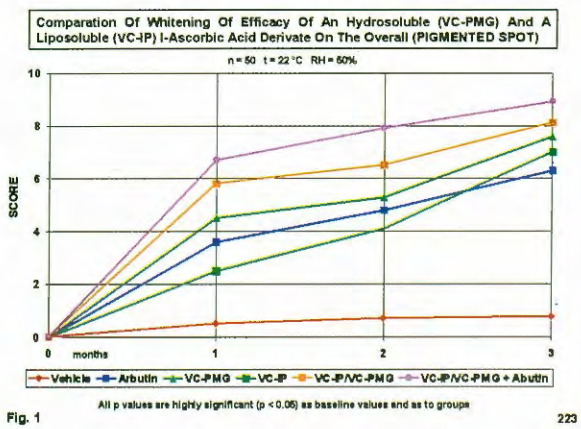

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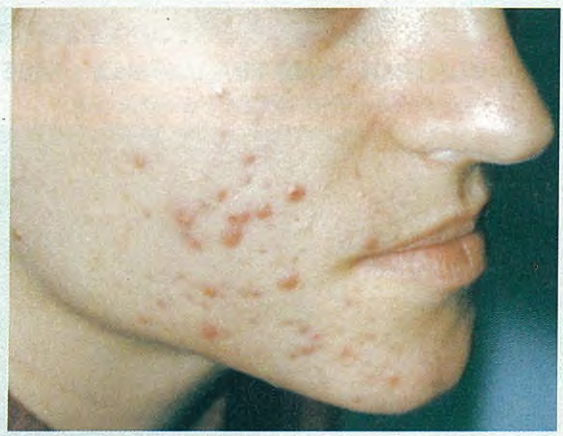

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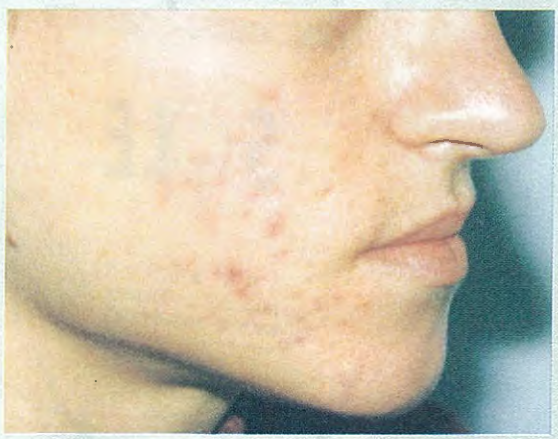

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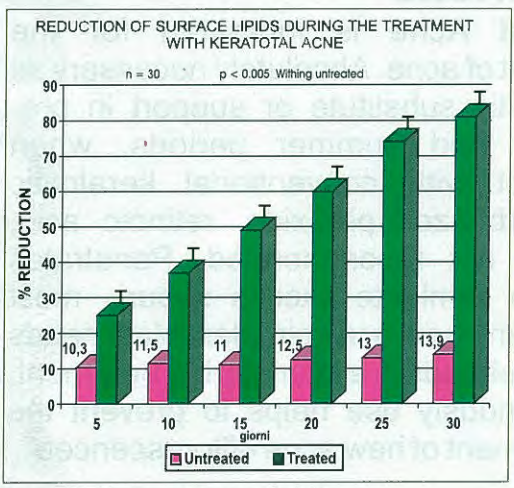

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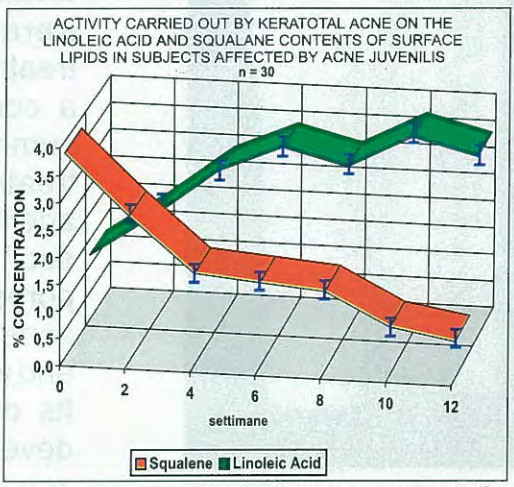

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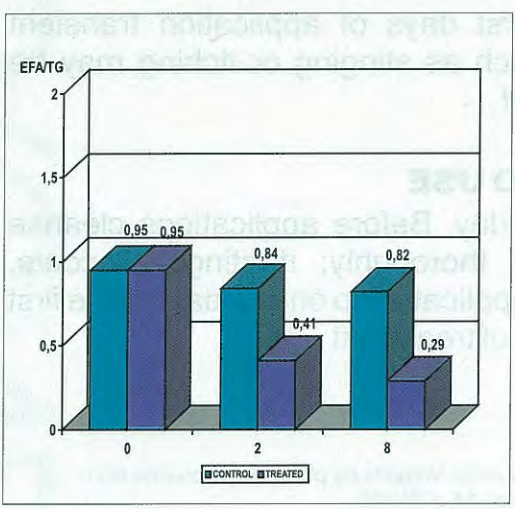

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Twice a day. Before applications cleanse the skin thoroughly; if stinging occurs, reduce application to once a day for the first ten days of treatment

REFERENCES:
*1,2 - **Data on file Mavi Sud***
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J. Appl. Cosmetol. **19**, 1-10 (January/March 2001)

EYELID SKIN EXPLANTS UNDER NEURO-INFLAMMATORY STRESS: SYNERGISTIC PROTECTION BY ESCINE AND DEXTRAN SULFATE

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Received: *December 2000.*

Key words: *Eyelid, Stress, Inflammation, Escine, Dextran Sulfate, Substance P, TNFα.*

Summary

Human eyelid skin explants can be maintained in an appropriate culture medium in vitro for several days while retaining most of the histological features of normal skin. It thus represents a valuable tool to investigate the potentially damaging effects of biological, chemical or even physical stress that might alter their integrity. It is also a useful model to study the ability of various ingredients and products to prevent such damages and/or improve the morphological integrity of the skin. We observed here that a multi-stress inducing preparation including Substance P, Arachidonic Acid and Tumor Necrosis Factor could alter eyelid skin morphology. Dilation of superficial plexus microvasculature of the epidermis and an increase in water retention between collagen bundles of tbe extracellular matrix were the most reproducible histological alterations observed. We furthermore evidenced that topical application of escine and dextran sulfate synergistically protected eyelid skin explants in culture against the neuro-inflammatory stress preparation when applied prior to neuro-inflammatory stress conditions. It is thus predictable that not only these two ingredients will be well tolerated in vivo but also that they may, to some extent, protect eye skin outline against the major environmental external insults encountered day to day.

Riassunto

La cute prelevata della palpebra può essere mantenuta in vitro in appropriato mezzo di coltura per parecchi giorni mantenendo le caratteristiche istologiche di una pelle normale.

Questo metodo rappresenta, quindi, un valido mezzo per verificare gli eventuali danni biologici, chimici e psicofisici da stress che possono alterare l'integrità del tessuto perioculare. Inoltre rappresenta anche un utile modello per studiare e verificare come e quando i diversi ingredienti e prodotti possano prevenire determinati danni o migliorare l'integrità morfologica della pelle.

Abbiamo osservato che uno stress multiplo indotto su una preparazione contente sostanza P, acido

arachidonico e TNF (Tumor Necrosis Factor) altera la morfologia della cute delle palpebre. E' stata osservata una dilatazione del microcircolo cutaneo ed un incremento della capacità di trattenere acqua da parte delle fibre di collagene della matrice extracellulare a livello della quale si verificano la maggior parte delle alterazioni istologiche.
Inoltre, abbiamo potuto evidenziare che l'applicazione topica di escina e di destrano solfato proteggono in modo sinergico l'espianto della cute delle palpebre in coltura dallo stress neuro-infiammatorio, se applicati prima che si verifichino le condizioni di stress sperimentali. Sembrerebbe, perciò, che questi due ingredienti non siano soltanto ben tollerati in vivo ma che possono proteggere in qualche modo la zona cutanea delle palpebre contro le aggressioni ambientali a cui è sottoposta tutti i giorni.

INTRODUCTION

Eyelid skin is of particular interest in both dermatological and cosmetic research. It is highly innervated mostly by sensitive c-fibers that goes up to the upper layers of the epidermis (1, 2). In addition, it undergoes frequent stress, either physical (UV radiations), chemical (pollutants), or even mechanical (as being frequently rubbed). Due to this high level of stimulation combined with dense innervation, it thus represents an alert part of the body, the appearance of which reflects the extent of sustained stress. Thus eyelid biopsies maintained in a survival medium represent a sensitive « skin alert tool » that might help to predict both deleterious or protective effects of any topically applied product .We investigated the effects of a multi-stress inducing preparation on this model in order to evaluate to what extent skin morphology might be affected. This stress "cocktail" was designed so that it could mimic both a neurogenic agression (Substance P, SP) and two distinct inflammatory pathways (Arachidonic Acid ; AA) as a lipidic mediator and Tumor Necrosis Factor-a (TNFa) as a classical pro-inflammatory cytokine mediator of stress. Under those conditions, as previously reported for SP alone (1), we observed that two representative histological parameters were reproducibly affected by such a stress. These are a vasodilation of the superficial microvasculature of the skin and water retention within extracellular matrix. Then, in a second time, we attempted to protect the skin from the above stress–induced effects using a combination of escine and dextran sulfate. Furthermore, with a view to test the combination when formulated as a cosmetic product, it was evaluated using Lucas Spring water as a vehicle since it was previously shown to have a protective effect against inflammation induced by SP alone on surviving skin (3).

MATERIALS AND METHODS

Cosmetic ingredients: dextran sulfate (MW 10000) was obtained from Pharmacia Biotech (Upsala, Sweden) and escine 3030000 was obtained from INDENA Co (Italie). Lucas Spring Water was obtained from a 50 ml cosmetic Spray " EAU THERMALE/ THERMAL SPA WATER" marketed by Laboratoires Vichy, Vichy-France.

Human eyelid skin explant culture: normal human eyelid skin samples were obtained after informed consent from volonteers undergoing plastic surgery (caucasian women from 25 to 35 years old). The method for growing human eyelid skin ex vivo was adapted from previously reported work by Boisnic et al. (4) on normal skin. Briefly, skin fragments were washed three times with antibiotics. Subcutaneous fat and lower dermis were mechanically removed under a stereomicroscope using a surgical scalpel and skin samples were cut into 0.5 cm2 full thickness pieces. Dissected fragments were then placed with the epithelium uppermost and maintained at the air-liquid interface on culture inserts (filter pore size 12µm; Costar, Poly Labo Paul Block, France). These inserts were set on 12 well plates (Costar) for 24 hours at 37°c in an humidified incubator with 5% CO2. Dulbecco's minimal essential medium supplemented with antibiotics (100U/ml penicillin ; 100µg/ml steptomycin), L-Glutamin (200µg/ml) , bovine pituitary extract, growth factors (Gibco BRL, USA) and fetal calf serum (DAP, France) was added to the culture wells so that the surface of the medium was level with the filter as previously described; (5-8). Cohesion between skin and insert was obtained with polysiloxane vinyl seal so that neither skin retraction nor direct lateral passage of topically applied product towards the dermis could be possible (4).

Application of an irritant stress cocktail to the explants: a multi-stress reaction was induced for 2 hours by applying 25µl of a combination of substance P (SP ; 5µM solution; Bachem, France) together with Arachidonic Acid (A.A.;

40mg/ml solution; SIGMA, France) and Tumor Necrosis Factor-a (TNF-a; final concentration of 50ng /ml; Valbiotech, France) to the surface of normal eyelid pieces.

Application of products: to assess their protective potential, both escine and dextran sulfate were applied topically, either alone or in combination at the indicated concentrations (see legend for figures), just before applying the « stress cocktail » to the surface of eyelid explant,. Moreover, they were tested after dilution in Lucas Spring water.

Histological evaluation: after 2 hours of application, the stressed skin samples were fixed in Bouin's liquid and embedded in paraffin prior to histological analysis (4). 5µm thick sections were stained with hematoxylin and eosine. Histological evaluation was performed on papillary dermis and on the upper part of reticula dermis. Sections were double-blind evaluated and histologically scored by two distinct operators using an Olympus light photomicroscope with a 10X objective and they were photographed using an Ektachrome 64t Kodak film.

Histological criteria were scored as follows:

a) superficial skin microvasculature vasodilation: the evaluation was made on luminal areas of blood capillaries which are easily detected by their endothelial cells (see fig 1). 0-no vasodilation; 1-slight vasodilation; 2-moderate vasodilation; 3-marked vasodilation; 4-severe vasodilation

b) Water retention in the intercellular spaces:

0-no retention; 1-slight retention; 2-moderate retention; 3-marked retention; 4-severe retention.

Intermediate values were attributed when the observed parameters were not homogeneous whithin the same section. For each sample of skin (3 different donors per condition), 4 slides were evaluated and scored. The results are expressed as the mean and SEM of these scores for each of those parameters analyzed.

Statistics: the Student's t-test was used to analyze paired data. Significance was calculated with stressed eyelid skin as a reference and $p<0.05$ was considered as a statistically significant score.

RESULTS

Histological alterations following application of a « stress cocktail » on eyelid skin in culture ex vivo : the "stress cocktail" was designed so that it could mimic at the same time a neurogenic stimulation (SP), a lipid mediator stimulation (AA) and a pro-inflammatory cytokine stimulation (TNFa).

Figure 1a shows a normal eyelid skin explant incubated in culture medium. Vessels can be seen with a flat lumen and normal endothelial cells nuclei (see arrows) indicating the absence of any spontaneous vasodilation. In the dermis, collagen's bundles are dense and homogeneous with no space between collagen fibers. No lymphocytes can be seen, indicating no evident sign of irritation of the skin in vitro (9).

By contrast, after 2 hours of incubation with the "stress cocktail", one can clearly see a severe

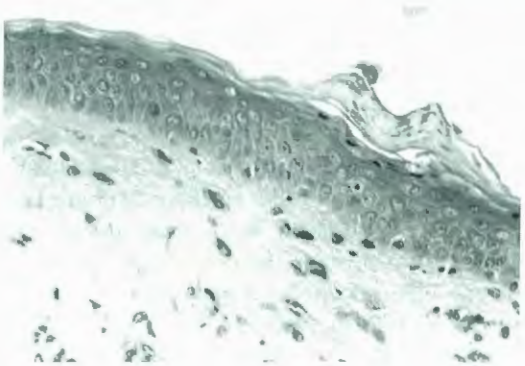

Fig. 1a

vasodilation (see fig.1b) as well as a severe increase of white spaces between collagen fibers (fig. 1c). These observations suggest a swelling of the skin isolated ex vivo, resembling an in vivo edematous reaction. As shown in table I, there is indeed a significant change in the scores of

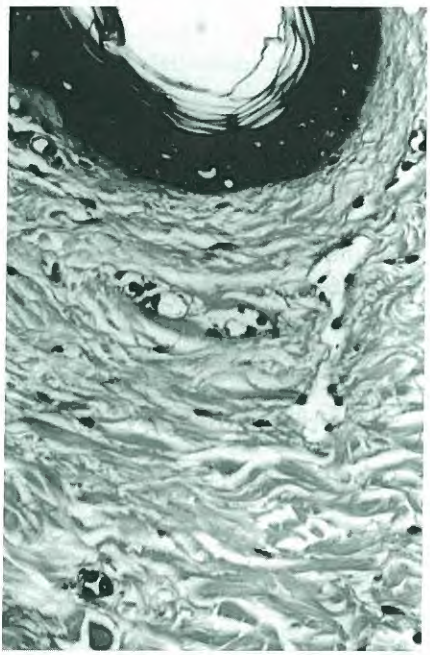

Fig. 1b

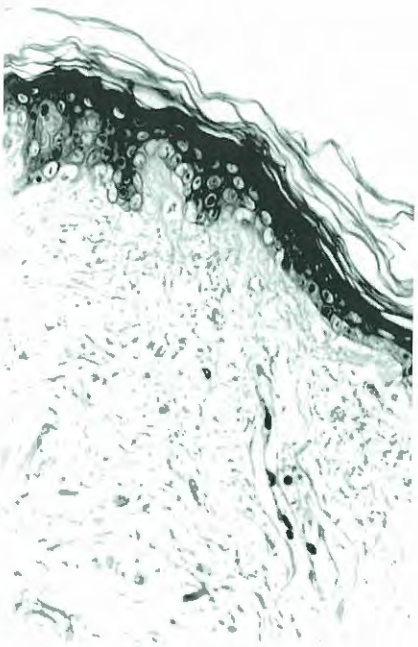

Fig. 1c

vasodilation ranging from 0.37 (absent to slight vasodilation) for normal eyelid skin to 2.2 (moderate to marked vasodilation) for stressed skin. Similarly, the scores of water retention moved from 0.75 (absent to slight retention) in normal conditions to 3.0 (marked retention) under stress conditions (Exp.1 ; table I).

This experiment was performed on another set of 3 different donors (Exp.2; table I) and

Table I

Scoring of the effects of the stress cocktail (SP+AA+TNFα) on human eyelids biopsies in culture in vitro.

	EXP.1	EXP.1	EXP.2	EXP.2
	Vasodilation	Interstitial retention	Vasodilation	Interstitial retention
Control eyelid	0.37 +/- 0.47	0.62 +/- 0.63	0.5+/-0.57	0.75+/-0.64
Stressed eyelid	2.2 +/- 0.57	2.8 +/- 0.76	2.5+/-1.0	3.0+/-0.61

showed again an increase in water retention from 0.62 for normal skin (absent to slight) to 2.8 (moderate to marked) for stressed skin. In both experiments, the difference between control skin and stressed skin was statistically significant ($p<0.05$). Similarly, vasodilation increased from 0.5 (absent to slight) to 2.5 (moderate to marked) after applying the "stress cocktail" suggesting a good reproducibility of the scoring method (table I).

Protective effect on the dilation score: As shown in figure 2, Dextran Sulfate alone could not protect eyelid skin from vasodilating in response to the "stress cocktail" treatment. Histological scores of stressed eyelid skin were ranging from 2.2 (+/- 0.57) without Dextran Sulfate to 1.8 (+/- 1.04) after pre-treatment with Dextran Sulfate. The difference between the two experimental treatments was indeed statistically not significant. Similarly, escine, despite showing a clear tendency to protect eyelid skin from dilation 1.5 (+/- 0.5) without escine versus 2.2 (+/- 0.57) after escine pretreatment to could not give a statistically significantly protection to the eyelid skin in response to the "stress cocktail". However, it was only when the two ingredients were combined that a significant protection was obtained (0.50 +/-0.50 versus 2.2 +/- 0.57 for unprotected skin ; $p<0.05$).

Protective effects on the interstitial water retention score:

As shown in figure 2, as seen in the vasodilation score, dextran sulfate alone could not provide a statistically significant protection of the eyelid skin against interstitial water retention increase in response to the "stress cocktail". The score were 2.8 (+/- 0.76) without dextran sulfate and 2.1 (+/- 1.04) after pre-treatment with dextran sulfate). By contrast, escine albeit inefficient to significantly protect against vasodilation increase showed a significant protective effect against interstitial water retention increase 1.17 (+/- 0.77) versus 2.8 +/- 0.76 in stressed unprotected skin ($p<0.05$). When combined together, dextran sulfate and escine produced even an enhanced protection compared to escine Alone (0.43 +/- 0.51 versus 2.8+/- 0.76 ; $p<0.05$).

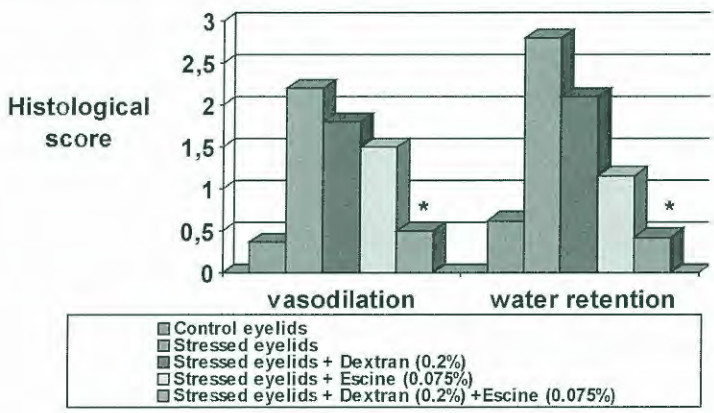

Fig. 2

Dose-effect study for optimal protection: As shown in figure 3, in an attempt to define a minimal level of concentration of both ingredients for which a synergistic and significant activity could be still observed after topical application on the eyelid skin explant, we evaluated the protective activity of serial dilutions (in distilled water) of a concentrated solution prepared in

25% Vichy Water (Lucas Spring; Vichy, France). The experiment was performed using Vichy Water for two other reasons i) check whether Vichy water might interfere with the synergistic protective effect of the two combined ingredients . ii) determine the lowest concentrations still showing a significant protective effect.

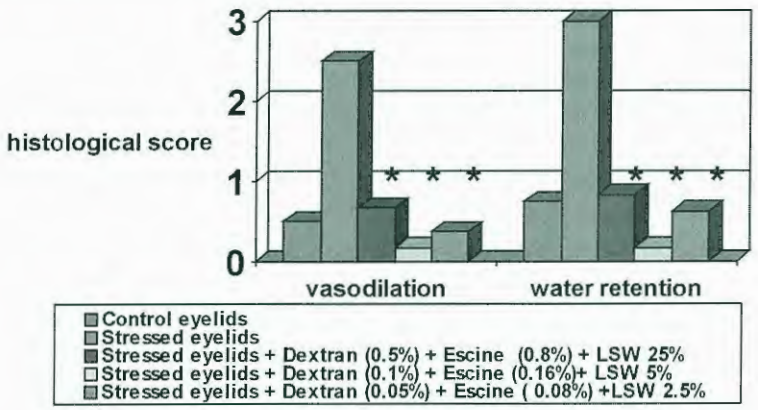

Fig. 3

The results indicate that the lowest concentration showing a protective effect on both vasodilation and interstitial water retention scores is 0.05% escine , 0.08% dextran sulfate and 2 .5% Vichy Water. The best efficiency on both histological scores was however obtained with 0.1% Escine, 0.16% Dextran Sulfate and 5% Vichy Water. In both cases, the protective effect was statistically significant ($p<0.05$) on the two endpoints.

DISCUSSION

Our results show that eyelid explants can be maintained ex vivo using conditions culture similar to those used for normal skin explants from other parts of the body (1,3-8). Furthermore, eyelid skin explants in culture medium are responsive to a "stress cocktail" containing both a neurogenic stimulator (SP), a precursor of the inflammatory and chemotactic lipid mediators (AA) and a pro-inflammatory cytokine (TNF-a). Each of those factors has distinct but sometimes overlapping function in the skin. SP receptors are expressed on cutaneous Mastocytes, Langerhans Cells and keratinocytes (10). In response to SP, keratinocytes produce the pro-inflammatory cytokines IL-1 and TNFa (1,3, 10, 11). In response to SP, an increase in IL-1 production has been recently evidenced in human skin explants in culture (1,3). TNFa is an other pro-inflammatory cytokine which, together with IL-1 (12, 13), but through distinct receptors, can directly induce the expression of several inflammatory genes by keratinocytes among which those of the so-called inflammatory cytokines such as Interleukin-8 or MCAF (13). The latter inflammatory secondary cytokines belong to the large family of chemokines, responsible for specific attraction of blood cells infiltrates into the epidermis (14). Apart from the cytokine cascade, Arachidonic Acid (AA) which is the common precursor for both Prostaglandins and Leukotrienes, and is commonly generated through the activity of cell membrane phospholipase A2 (15) represents a non-specific branch of the inflammatory process responsible (among other phenomenons) for vasodilation (prostaglandins) and even for non specific chimiotacti-

sm (leukotrienes); (16). Altogether, these factors contribute to the so-called "inflammatory cascade" in the skin.

It is thus expected that the combination of the above stress factors might, to some extent, recapitulate most of the biological pathways that a normal skin is supposed to go through during usual stress condition. The cocktail was adjusted so that the response of the skin could not be histologically quoted as severe but rather displayed a moderate to marked stress which may model the reaction of normal skin under non-pathological life-conditions.

Using this stress model, we could evidence that some cosmetic ingredients, when applied topically on the eyelid skin in culture ex vivo could protect the skin, to some extent, from morphological changes induced by multiple stress. Synergistic combinations were found to provide a significant protection from multi-stress alterations ranging from moderate to marked (but never severe) on a wide range of concentrations. Vichy water from Lucas spring, which had previously been reported to inhibit the histological alterations induced by SP in a similar model (3) was used as a vehicle. It was shown not to interfere with the synergistic protective activity of escine and dextran sulfate in a range of concentration from 2.5% and 25%. Whether the combination product is active in vivo remains now to be established which is currently under investigation in our research laboratory.

If so, the ex vivo model that we developed for these studies would be confirmed as a valuable and sophisticated biological tool which could represent a good alternative to in vivo evaluation.

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ANTINFLAMMATORY, ANTIMICROBIAL, COMEDOLYTIC EFFECTS OF A TOPICAL PLANT COMPLEX TREATMENT IN ACNE VULGARIS: A CLINICAL TRIAL.

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Summary

Acne is a disease of the pilosebaceous unit and it may be characterized by non inflammatory lesions, such as comedones and cysts and/or by inflammatory lesions such as erythematous papules, pustules, nodules. The lesions may or may not resolve with scars. Three major factors are involved in the pathogenesis of acne: increased sebum production, an abnormality of the microbial flora, cornification of the infundibula duct. These three components represent the target of different plant extracts, which may play an important role in inducing comedolytic, antimicrobial effects and also in improving pitted scars.

This study was aimed at evaluating the efficacy of a topical plant complex treatment in acne vulgaris in 30 volunteers. These volunteers were divided in two groups: 15 received only the topical treatment and 15 received topical treatment plus oral placebo capsules. The instrumental investigation evaluated the following parameters during 90 days: skin hydration, transepidermal water loss, erythema, skin brightness, sebum on skin surface, scars.

The statistical analysis of data at the end of this clinical trial underlined that the topical plant complex product improves the clinical appearance of acne.

Riassunto

L'acne è una malattia dell'unità pilosebacea e può essere caratterizzata da lesioni non infiammatorie, come comedoni e cisti e/o da lesioni infiammatorie come papule eritematose, pustole, noduli. Le lesioni in alcuni casi possono risolversi con cicatrici. Sono tre i maggiori fattori implicati nella patogenesi dell'acne: ipersecrezione sebacea, alterazione della normale flora microbica, ipercheratosi infundibolare. Questi tre fattori rappresentano l'obiettivo di differenti estratti vegetali, che possono svolgere un importante ruolo nella comedolisi, possono avere effetti antimicrobici e migliorare le cicatrici più superficiali.

Lo scopo di questo studio è stato quello di valutare l'efficacia di un complesso vegetale topico nel trattamento dell'acne vulgaris in 30 volontari. Questi soggetti sono stati suddivisi in 2 gruppi: 15

hanno ricevuto solo il trattamento topico e 15 il trattamento topico più le capsule placebo. L'indagine strumentale consisteva nel valutare l'andamento, durante 90 giorni, dei seguenti parametri: idratazione della pelle, TEWL (transepidermal water loss), eritema, luminosità, sebo sulla superficie della pelle, repliche (impronte) cutanee.
L'analisi statistica dei risultati alla fine dello studio clinico ha indicato che questo prodotto migliora l'aspetto clinico dell'acne.

INTRODUCTION

Acne is characterized by polymorphic noninflamed (comedones) or inflamed lesions (papules, pustules, nodules), which occur predominantly on the face, but also on the back and chest. Although acne is present mainly during teenage years, it may continue as a clinical problem into twenties and older.

Typical lesions of mild to moderately severe acne vulgaris (comedones, small papules, seborrehoea) in adolescents come and go for several years, sometimes resolving with residua, i.e., pitted scars and patulous follicle on the nose and malar region.

There is no single cause of acne, and several factors seem to play important pathogenetic role. Active sebaceous glands are a prerequisite for the development of acne; in fact acne patients excrete abnormally high levels of sebum that may result from an high androgen production or increased availability of free androgen. Anyway there is an amplified target response, i.e. sebum production. Moreover acne patients show ductal hypercornification which clinically presents as comedones. The presence of abnormal environment (sebum excretion rate and ductal cornification) causes an alteration of the microbial flora (1-7).

On the basis of these main etiologic factors, treatments should be carried out through substances that are both effective in reducing the excessive release of the sebum and in regulating cornification of the infundibula and in improving the cutaneous microflora and facilitating healing of lesions.

The purpose of this study is to evaluate the efficacy of a topical treatment in acne vulgaris in a group of 30 selected volunteers with 90-day clinical trial.

In particular, a formulation containing the following active principles, Prepared by Medestea Internationan Laboratories, based in Torino, Italy, has been tested: lauric acid, standardized lipophilic extract of Krameria trianda Ruiz, 18-á glycyrrhetic acid in phytosome form, standardized lipophilic extract of Serenoa repens and Centella asiatica (pure triterpenic fraction).

The instrumental investigation measured basal (prior to treatment), intermediate (after 7-30-60 days) and final (after 90 days) values of the following biophysical cutaneous parameters: skin hydration, Transepidermal water loss, erythema and skin brightness, sebum and imprint of skin surface (8- 17).

MATERIALS

Thirty subjects (8 male and 22 female, average age 24.62 years) presenting different degrees of facial acne (slight, moderate, severe) were included in this study.

The volunteers were divided into two groups in which one half received only the topical treatment and the other half received the topical treatment associated with an orally administered product (placebo). Volunteers were subjected to a clinical evaluation before (T0) and after treatment (T1) (Tab. I). During the trial, volunteers did not use other anti-acne products.

Degree of skin hydration was evaluated by a cornometer CM 820 PC (Courage & Kazhaka®, Koln, Germany). The instrument measures the dielectric constant of the stratum corneum and is constituted by a cylindrical sensor (measuring probe) connected by a spiral coil. Measurements are taken by placing the measuring head on the skin: after about 8 seconds, the water content is measured with the simultaneous display of the hydration value.

An evaporimeter (evaporimeter EP 1 Servomed®, Sweeden) is used to assess Transepidermal water loss (TEWL). This instrument measures the flow of water vapor through a given surface (unit), by means of the variations of water concentration in the atmosphere near the stratum corneum. Measurements are taken 30' after positioning the probe on skin surface.

Table I

Clinical criteria for evaluating five degrees of acne severity in the two groups of volunteers.

Topical treatment	T0	T1
level	n° subjects	n° subjects
none	0	0
very slight	0	8
slight	5	6
moderate	9	0
severe	1	0

Topical + oral treatment	T0	T1
level	n° subjects	n° subjects
none	0	4
very slight	0	5
slight	2	5
moderate	10	0
severe	3	0

Index of erythema and skin brightness was evaluated through the use of a colorimeter (Minolta Chroma Meter Cr-200®), which uses a geometry with diffuse illumination with an observation angle at 0° to obtain a reading correlated to the surface examined. The illumination chamber of the instrument is a cylinder with a conical opening of 8 mm in diameter directed towards the skin surface. The vertically reflected light on the measurement surface is captured by a optic fiber cable for the analysis of the color.

The sebumetric evaluation is performed by a sebumeter Sm810 Pc® (Courage and Khazaka, Koln-West Germany). An opaque synthetic band is applied on the area of skin to be tested for 30 sec. The surface of the band of about 64 mm2 becomes more transparent as the sebum deposits. This variation of transparency to the light represents the value of the skin sebum and is measured by means of a photometer. The values obtained are expressed in sebometric units which are convertible in micrograms per surface unit.

The imprint of skin surface, obtained by skin replica using silicone (Silflo®), was studied by an image analyzer Philips NMS 8280. Then, the images were elaborated by a computer which gives values about microrelief and deep furrows.

At the end of the trial, the data were analyzed by paired and unpaired t-student test.

RESULTS

The values of hydration (Fig. 1) of the stratum corneum in subjects only using topical products were significantly increased by 16.94%, after 3 months of treatment (t-test = 0.013), ranging from a basal value of 67.52 to a final value of 78.96.

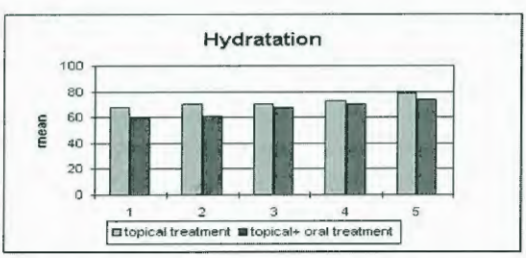

Fig. 1: *Graphic presentation of the cutaneous hydration in the two groups of volunteers.*

An increase of the hydration parameter, equal to 23.63%, was found also in the volunteers who had associated the oral product to the topical product. In this case, there was an increase from a basal value of 59.32 to a final average value of 73.34, with significant variations already after 1 month of treatment (t-test bas/1 month = 0.004; t-test bas/2 months = 0.003; t-test Bas/3 months = 0.0001).

The Transepidermal Water Loss (TEWL) (Fig.2) was significantly decreased by 37.81% (t-test= 0.039) in the first group after 3 months of treatment (average basal value = 15.89, average final value = 9.88). A significant reduction was recorded also after two months of treatment (t-test = 0.048; average value after 2 months = 10.27).

A significant decrease (t-test = 0.002) equal to 19.54% was also recorded in the second group after three months of treatment (basal average value = 10.54; final average value = 8.48).

Nevertheless, it is important to note a significant reduction in TEWL already after 1 or 2 months of treatment (bas/ 1 month t-test = 0.035, average value after 1 month = 9.05; bas/2 months t-test = 0.003 average value after 2 months = (8.77).

The values relative to the erythema index (Fig.3) of the skin recorded at the end of the treatment, remain unchanged.

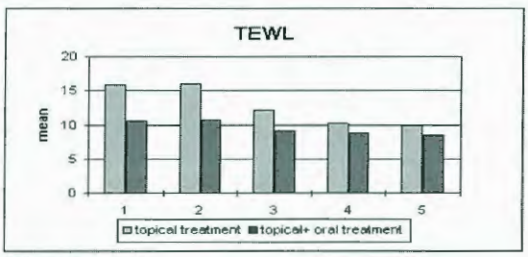

Fig. 2: *Graphic presentation of the TEWL in the two groups of volunteers.*

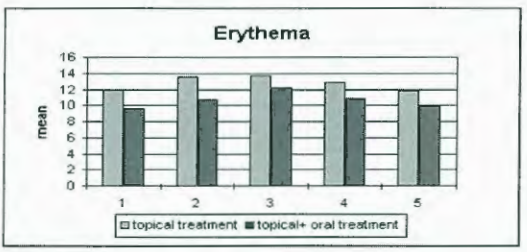

Fig. 3: *Graphic presentation of the erythema in the two groups of volunteers.*

The values relative to brightness (Fig.4) recorded in the first group revealed a increase of 0.47%, which was not statistically significant at the end of the treatment (t-test = 0.766) going from a basal average value of 65.29 to a final average value of 65.6). Similar results were obtained in the second group of volunteers, who had an L* parameter increased by 1.94% which was not statistically significant (t-test = 0.6618).

The sebum values (Fig.5) were significantly decreased by 30.92% (t-test= 0.0001) after 3

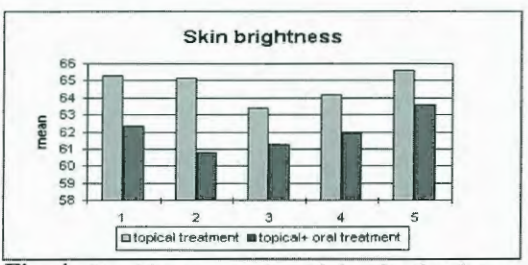

Fig. 4: *Graphic presentation of the skin brightness in the two groups of volunteers.*

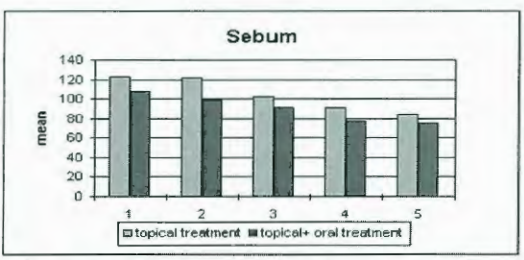

Fig. 5: *Graphic presentation of the sebum in the two groups of volunteers.*

months of treatment (basal average value = 108.36, final average value = 74.86) in the first group of volunteers. Nevertheless, significant reductions were also recorded after one and two months of treatment (t-test = 0.003; t-Test= 0.0001).

The same results were obtained in the second group of volunteers with highly statistically significant decrease (t-test = 0.0001) at the end of treatment. There were also significant reductions of sebum in intermediate measurements (bas/1 week t-Test = 0.005; bas/1 month = 0.004; bas/ 2 months t-test = 0.0001).

Values of deep furrows (Fig.6) recorded in the first group after 3 months of treatment indicated a statistically significant reduction of 5.25% (t-test = 0.003), of the depth of the acne-related lesions going from a basal average value of 103.56 to a final average value of 98.11. As for the surface microrelief (Fig. 7), a statistically significant decrease was recorded equal to 1.18% (t-test = 0.003), going from basal average values of 50.14 to final average values of 49.55 at the end of treatment.

Significant variations in deep furrows and microrelief were also recorded in the volunteers who had used both the topical and oral products. Deep furrows measurements (Fig.6) after 3

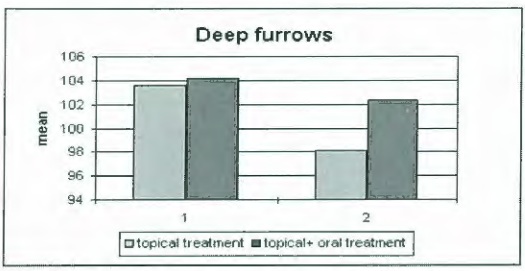

Fig. 6: *Graphic presentation of the deep furrows in the two groups of volunteers.*

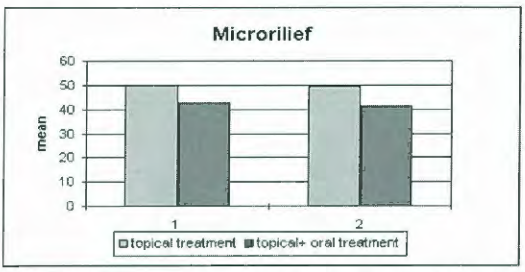

Fig. 7: *Graphic presentation of the microrilief in the two groups of volunteers.*

months of treatment decreased, a statistically significant finding (t-test = 0.003), going from a basal average value of 104.17 to a final average value of 102.35. Measurements of variations in microrelief (Fig.7) showed a statistically significant reduction (t-test = 0.0287), going from basal average values of 42.62 to final average values of 41.39 at the end of the treatment.

In Fig. 8 (a- b) and Fig. 9 (a- b) it is possible to observe the clinical acne improvement in two

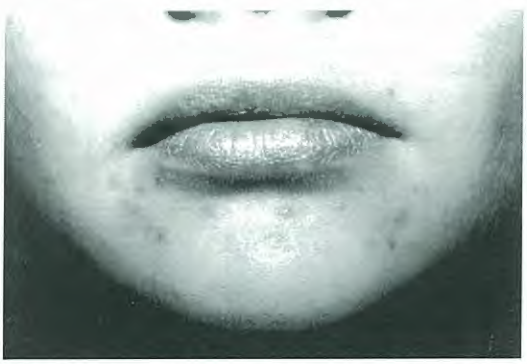

Fig. 8a: *Clinical picture of a subject before topical treatment: it is possible to observe inflammatory and non inflammatory acne lesions.*

Fig. 8b: *The same subject after the treatment: improvement of acne lesions.*

Fig. 9a: *Clinical aspect of a volunteer before topical treatment plus oral placebo: many papules and pustules are evident.*

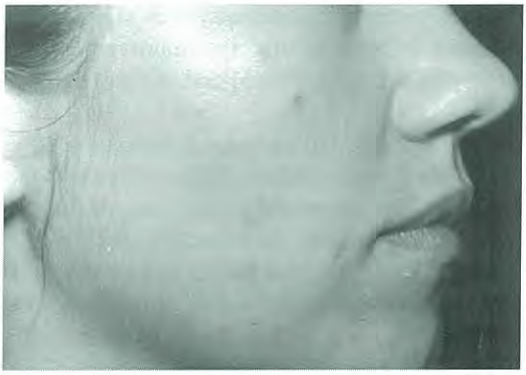

Fig. 9b: *After the treatment it is present a sensible reduction of acne lesions.*

patients representing the two different groups (topical treatment and topical plus oral placebo). Two volunteers dropped out of the study after 10 days of treatment because we observed the presence of slight erythema.

DISCUSSION

Both topical anti-acne cream and topical anti-acne plus placebo had a positive therapeutic effect on acne after the evaluated 90 days of application.

Recent studies showed the effectiveness of natural active principles present in this acne treatment (18). Lauric acid and standardised lipophilic extract of Serenoa repens are able to oppose the excessive production of sebum by the pilo-sebaceous unit . In particular, the standardised lipophilic extract of Serenoa repens inhibits the 5-reductase which is involved in testosterone metabolism and response for a series of androgen-mediated disturbances such as acne and greasy skin.

Standardized lipophilic extract of Krameria trianda Ruiz re-establishes the normal flora by preventing the proliferation of the microorganisms such as Propionilbacterium acnes, Streptococcus pyogenes or Staphylococcus aureus, present in acne lesions (19, 20). Moreover the astringency of extract of Krameria trianda was mainly due to proanthocyanidis that have been isolated from this rhatany root.

The 18-á glycyrrhetic acid in phytosome form and standardised lipophilic extract of Serenoa repens, determine an effective local anti-inflammatory response (21, 22).

In particular, the mechanism of action of 18-á glycyrrhetic acid is apparently due to its inhibition of 11-á-hydroxy-steroid dehydrogenase, an enzyme that reduces the activity of endogenous cortisol produced in response to the release of inflammation mediators. The standardized lipophilic extract of Serenoa repens inhibits the cyclo-oxidase, an enzyme involved in local inflammation and responsible for the release of some inflammation mediators.

Centella asiatica stimulates the production of collagen and correct tissue cicatrization (23). Centella's active substances are called its "triterpenic fraction". By interacting with fibroblasts, their main target, they accelerate the uptake and metabolism of lysine and proline (two amino acids need for the final structure of collagen), increase the synthesis and release of tropocollagen and stimulate the turnover of acid mucopolysaccharides in connective tissue.

The statistical analysis of data in this clinical trial indicate an improvement of acne.

In fact, overall acne severity was significantly reduced in both groups: the improvement was recorded both on inflamed and non inflamed lesions.

The product tested produce a hydrating effect reducing dryness of the stratum corneum, thereby inducing improved functioning of the skin barrier expressed by the reduction in TEWL, as well as decreasing the production of sebum in subjects with acne lesions of varying severity and finally are effective in smoothing the skin, notably decreasing the anti-esthetic scars.

The erythema index recorded showed a modest, although significant erythematogenic effect of the products tested. In fact we must recall that two volunteers dropped out of the study after 10 days of treatment following a slight erythema. However a moderate burning sensation that was reported by some volunteers at the beginning of the study, decreased or disappeared during the treatment.

In conclusion, satisfying results were obtained at the end of two treatments (topical treatment, and topical + oral placebo), underlying the role of this topical plant complex treatment in improving acne lesions.

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THE COSMETIC USE OF AN ANCIENT PEAT OF THERMAL ORIGIN

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Summary

Used in ancient times, clays and natural peats, but mostly vegetal origin peats, are still used today in therapeutic treatments of real pathologies as well as for simple cutaneous alterations, depending on their qualitative/quantitative mineralization and composition.

For these reasons, we wanted to control the activity performed by a thermal bio-peloid of natural origin, of dark aspect, and vegetal derivation (over 90%), particularly rich in proteins, sterols, trace-elements, lipids, that we previously valued carefully for its peculiar characteristics.

The study, a double blind treatment, was carried out for a two month period, on 60 healthy volunteers (32 women and 28 men, range age 32-45) with a minimum xerosis severity of grade 6, according to the grading scale of Dahl and Dahl.

The mask was applied on skin and/or on hair as a thin layer 3 times a week for 24 total applications leaving it to act 15 minutes.

Soon after, it was removed and cleaned with a cotton pad soaked always with the same bath oil supplied (Keratotal Bath oil) and abundant water rinsing. Then, on the treated skin of face and legs was only applied a vitamin C phospholipidic cream (Kera C).

It was controlled skin hydration, surface lipids and skin firmness by 3C System, and hair resistance to traction by the Instron Tenside Tester®; hair elasticity, comb-ability and shyness was evaluated by the users.

After the two month treatment, the hydration both of skin face and legs increased of 24% ($p<0.01$) and lipids of about 22% ($p<0.05$).

Also the skin elasticity had a light improvement because of the vitamin C based cream, but in this case, the mask did not cause any improvement.

From these first results, we can say that the set up of natural or thermal "cosmeceuticals" is possible only if using adapt raw materials in those percentages allowing them to perform the cosmetic activity required.

Riassunto

I fanghi di origine minerale o vegetale usati fin dall'antichità, costituiscono ancora oggi un trattamento terapeutico per molte patologie e anche gravi in rapporto al loro grado di mineralizzazione

quali-quantitativa.
Per questi motivi si è voluto controllare l'attività svolta da un bio-peloide di origine naturale, di aspetto scuro e di origine vegetale (90%), particolarmente ricco di proteine, steroli, elementi traccia, e lipidi di cui erano già state verificate le caratteristiche chimico-fisiche.
Lo studio a doppio ceco è stato condotto per un periodo di due mesi su 60 volontari (32 donne e 28 uomini, di età compresa tra 32 e 45 anni) affetti da una leggera xerosi di grado 6 secondo la scala di Dahl e Dahl.
La maschera è stata applicata sulla cute e/o sui capelli in leggero strato 3 volte a settimana per 15 minuti per un totale di 24 applicazioni. Subito dopo è stata rimossa e l'area trattata è stata detersa con del cotone imbibito sempre con lo stesso olio (Keratotal Bath Oil) e con abbondante acqua. Sulla cute del viso e delle gambe trattate è stata applicata una crema fosfolipidica a base di vitamina C (Kera C).
E' stata quindi controllata l'idratazione cutanea, i lipidi di superficie e la consistenza della cute mediante l'utilizzo del 3C System.
La resistenza del capello è stata controllata mediante l' Instron Tenside Tester®, mentre la pettinabilità, la lucentezza e l'aspetto generale sono state controllate direttamente dagli stessi volontari.
Dopo due mesi di trattamento, si è notato che mentre l'idratazione ed i lipidi di superficie aumentavano rispettivamente del 24% ($p<0,01$) e del 22% ($p<0,05$) sia sul viso che sulle gambe, l'elasticità cutanea veniva incrementata soltanto dall'uso della crema, mentre non aveva effetto la maschera.
Dai risultati raggiunti, si può affermare che i cosmetici di origine termale, svolgono una loro specifica attività soltanto se si utilizzano materie prime adatte e nella percentuale richiesta da un trattamento cosmetico.

INTRODUCTION

Used in ancient times, clays and natural peats, but mostly vegetal origin peats, are still used today in therapeutic treatments of real pathologies as well as for simple cutaneous alterations, depending on their qualitative/quantitative mineralization and composition (1-7).
Basically natural peats, which are special peloids of vegetable origin, consist of the following three matrices:

- a solid inorganic one: clay and mineral salts
- a liquid one: mineral water
- a solid, organic one: bacteria, diatoms protozoa, arthropods, etc.

All the above mentioned matrices have a heterogeneous and multiphasic nature: the diluted component, be it inorganic or organic, may be of a varied nature, while the diluting component, vs water, may be differently organized, depending on its capacity to interact with the solid components and the presence or absence of bioactive compounds (8). Important to remember is that the organic matrix usually prevails in a natural peat or biopeloid (Biomud) and its final composition substantially changes. These changes depend on the locations from which water originates, on the peat maturation time, on the type of vegetable the peat originates from, and on the geological and morphological conditions under which it formed.
For all the mentioned reasons, we wanted to control the activity performed by a thermal biopeloid of natural origin (Tab.I and II), of dark aspect (fig.1) and vegetal derivation (over 90%), particularly rich in proteins, sterols (Tab III), trace-elements (tab IV), lipids, (Tab.V), that we previously valued carefully for its peculiar characteristics (8-14).

Fig. 1

CLASSIFICATION	
INORGANIC COMPOUNDS (mineral)	
INACTIVE	ACTIVE (in situ)
Common mud Synonyms: lotus, mire, slime, etc.	**Mineral mud** Compound (natural or man made) of clays or other earth-like materials of volcanic origin with thermal water. It is used for medical treatments.
ORGANIC COMPOUNDS (vegetable)	
INACTIVE	ACTIVE
Common peloids Compounds and decomposition of prevalently vegetable organic substances.	**Thermal biopeloids** Natural, thermal, vegetable compounds, which are active also when taken away from the source.

TAB. I

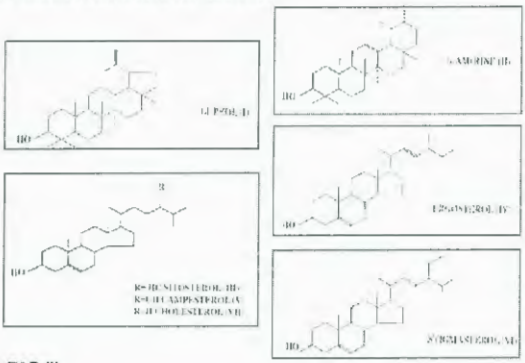
General Characteristics of a 3000 years old Biopeloid	
pH of centrifuged liquid	5,1
Dry residue at 105°	10,36%
Ashes	14,58% on dry resudue
Ammonia on centrifuged liquid	traces
Nitrites on centrifuged liquid	traces
Phosphates on centrifuged liquid	absent
Bisulphides on centrifuged liquid	absent
Chloroformic extract	0,59% on dry residue
Organic nitrogen as per Kjedal	1,85 on dry residue
Proteins and aminoacids (Bradford)	7,35% on dry residue

TAB. II

AIMS

The aim of this study was to verify the efficacy of the bio-mud used as:
1 - active principle of a shampoo studied for dry and weakened hair (product A)

STEROLS CONTENT OF BIOMUD



TAB III

CHEMICAL AND PHYSICAL CHARACTERISTICS PEAT MUD ASHES' ANALYSIS

Na_2O	1,155	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	0,09%	Equal to	Sr++	0,07%	(0,01 meq/g)
Fe_2O_3	5,04%	Equal to	Fe+++	3,53%	(1,09meq/g)
MnO	0,10%	Equal to	Mn++	0,08%	(0,03 meq/g)
Ni++	12ppm				
Zn++	39ppm				
Cr+3	2,6ppm				
Pb++	0,2ppm				
Cu++	17,0ppm				
Cd++	Traces				
Li+	Traces				
Al^{+3}	Traces				
As^{+3}	0,60ppm				
Hg++	0,07ppm				
Se^{-2}	Traces				
Cl-	0,77%				
SO^{3}	34,70%	Equal to	SO4+	41,70%	(8,68meq/g)
P_2O_5	0,02%	Equal to	$PO4^{-1}$	0,03%	(0,009meq/g)
N_2O_5	0,48%	Equal to	NO3-	0,56%	(0,09meq/g)
SiO_2	14,00%				

TAB.IV

Free Fatty Acids Content

	SYSTEMIC NAME	TRIVIAL NAME ABBREVIATION	SHORTH AND NOTATION
SATURATED	eicosanic	arachidic	(20:0)
	hexadecanoic	palmitic	(16:0)
	octadecanoic	stearic	(18:0)
	tetracosanoic	lignoceric	(24:0)
UNSATURATED	9-octedecenoic	oleic	(18:1,n-9)
	9,12-octadecadienoic	linoleic	(18:2,n-6)
	9,12,15-octadecatrienoic	linolenic	(18:3,n-6)

TAB. V

2 - a purifying body mask (product B)
3 - an hydrating mask (product C)
4 - and a nourishing one (product D)

MATERIALS AND METHODS

Put in uncovered tanks in contact with the open air, this mud is mellowed with a selected mineral water for a period of six months. After that period, mud stores the biological characteristic of the mineral water used to mellow it.

SHAMPOO A:
sodium laureth sulfate/decyl glucoside based shampoo in peat water bio-mud
(product A *active*)

SHAMPOO A1:
sodium laureth sulfate/decyl glucoside based shampoo in water (product A1 - *control*)

BODY PURIFYING MASK B:
W/O emulsion + 10% biomud
(product B - active)

BODY PURIFYING MASK B1:
W/O emulsion + 10% clay
(product B1 - *control*)

HYDRATING MASK C:
O/W emulsion + 10% biomud
(product C - *active*)

HYDRATING MASK C1:
O/W emulsion + 10% clay
(product C 1 - *control*)

NOURISHING MASK D:
W/O emulsion + 10% biomud
(product D - *active*)

NOURISHING MASK D1:
W/O emulsion + 10% clay
(product D 1 - *control*)

EXPERIMENTAL DESIGN

The study, a double blind treatment, was carried out for a two month period, on 60 healthy volunteers (32 women and 28 men, range age 32-45) with a minimum xerosis severity of grade 6, according to the grading scale of Dahl and Dahl (15):

0	Normal skin:	no sign of dryness.
1-3	mild xerosis:	ashy appearance and appearance of minute skin flakes
4-6	moderate xerosis:	defined scaling
7-8	severe xerosis:	heavy scaling and deep erythematous fissures, included eczema craquelè

All volunteers were instructed to apply the cosmetic products on the randomly assigned (right or left) skin face and lower legs area (lateral, medial and petibial) right or left for an 8 weeks period.

MASK TREATMENT

The mask was applied on skin and/or on hair as a thin layer 3 times a week for 24 total applications leaving it to act 15 minutes.
Soon after, it was removed and cleaned with a cotton pad soaked always with the same bath oil supplied (Keratotal Bath oil) and abundant water rinsing. Then, on the skin of face and legs was only applied a *Vitamin C phospholipidic cream* (Kera C).
The double-blind treatment was performed after teaching volunteers how to apply the mask C and C1 in the morning or the mask D or D1 in the evening (8 p.m.) at home to the right or left half of the face, together with the mask B or B1 to the right or the left leg.
This way, all the 60 volunteers used the *Hydrating mask* C and C1, and the *Nourishing mask D* e D1. All the 60 volunteers used for their legs purifying mask B and B1. At time 0 (starting) and at 1st, 2,3,4, 5,6,7, and 8th week of treatment, always on the day following the last application, it was controlled skin hydration, surface skin-lipids and pH by the 3C System Methodology (16), and skin elasticity using a torsional equipment (17). During the whole study, the researcher checked also that the mask were regularly applied according to instructions, and that no other cosmetic product was used, except the vitamin C phospholipidic cream and the mask applied. Thirty days before starting, all systemic drugs or diet supplements were discontinued.

SKIN HYDRATION, pH AND SURFACE LIPIDS MEASUREMENTS

pH, quantitative measurements of skin hydration and surface skin lipids were performed by the 3C System methodology (16), always in the morning from 8 to 11 a.m. on skin cleaned the night before.
This computerized method collects up to 10/15 measurements over 25 second sampling period and records the mean value automatically standardizing the environmental conditions, since it is known that rate of water loss and, consequently, skin hydration is affected by environmental conditions.
To alleviate the possibility of the volunteers' physiologic state, the other major factor influencing rate of water loss, it was asked to rest in the testing room for 30 minutes before measurements.
Possible site-to-site variation was eliminated by random selection of treated sites.
Skin hydration was assessed by measuring total capacitance of the horny layer, and the values are expressed in 3C arbitrary units;
skin lipids, absorbed by a special frosted plastic foil, are measured photo-metrically and expres-

sed as mg/cm2.
All the obtained results are expressed as mean values of the measurements performed on four different right or left sites of the face (check, forehead, chin and nose) and /or the legs.
The obtained results are reported on figures 2-7.

Surface Skin Lipids after a Two Month Topical Treatment by a Biomud Mask and a Vitamin C cream
(Face Treatment)
n = 60 t = 22 °C RH = 50%

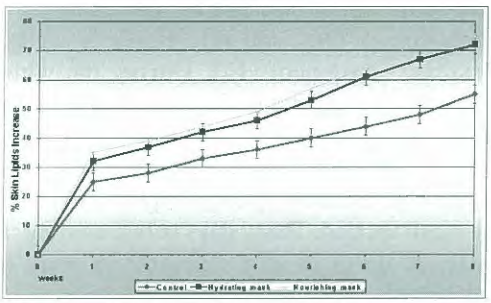


FIG.2 All p values are highly significant (p<0.05) as to control and as to groups

Skin Hydration after a Two Month Topical Treatment by a Biomud Mask and a Vitamin C Cream
(Face Treatment)
n = 60 t = 22 °C RH = 50%

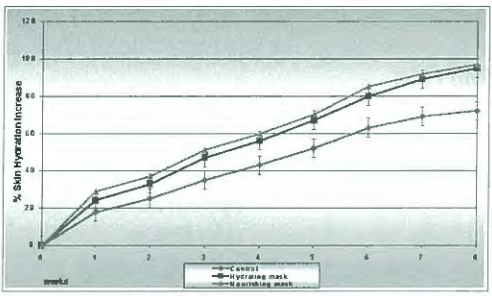


FIG. 3 All p values are highly significant (p<0.01) as control and as to groups

Skin Hydration after a Two-Month Topical Treatment by a Biomud Mask and a Vitamin C cream
Mask (Leg Treatment)
N = 60 t = 22 °C RH = 50%

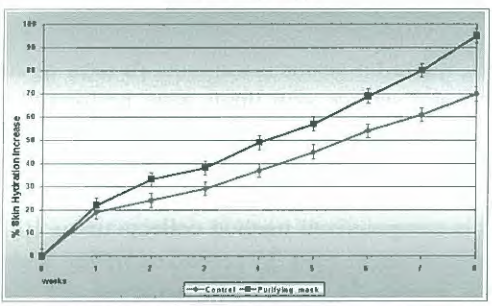


FIG.4 All p values are significant (p<0.01) as controls and as to groups

Surface Skin Lipids after a Two-Month Topical Treatment by a Biomud Mask and a Vitamin C cream
(Leg Treatment)
n = 60 t = 22 °C RH = 50%

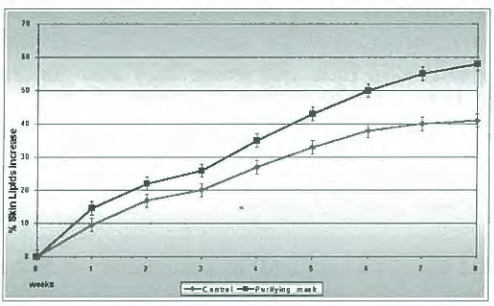


FIG.5 All p values are highly significant (p<0.05) as control and as to groups

Skin pH after a Two-Month Topical Treatment by a Biomud Mask and a Vitamin C cream
(Leg Treatment)
n = 60 t = 22°C RH = 50%

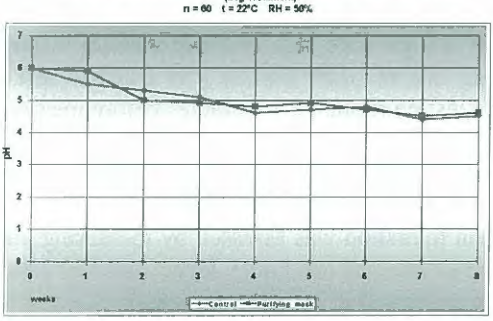


FIG.6 All p values are not significant as control and as to groups

Skin pH after a Two-Month Topical Treatment by a Biomud Mask and a Vitamin C cream
(Face Treatment)
n = 60 t = 22 °C RH = 50%

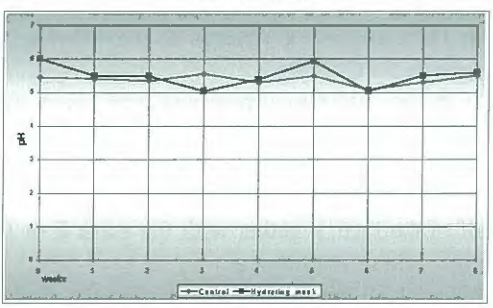


FIG.7 All p values are not significant as to control and as to groups

SKIN FIRMNESS

Skin firmness was evaluated measuring the skin elasticity using a torsional equipment (17) Torsional equipment works through a disk glued to the skin, which is rotated by a motor powered by a controlled voltage, thereby loading the peripheral skin with a torque, the value of which can be adjusted and evaluated.
This test purposed to quantitatively assess the skin changes, which are usually detectable by palpation but not measurable otherwise.
The obtained results are reported on Figure 8.

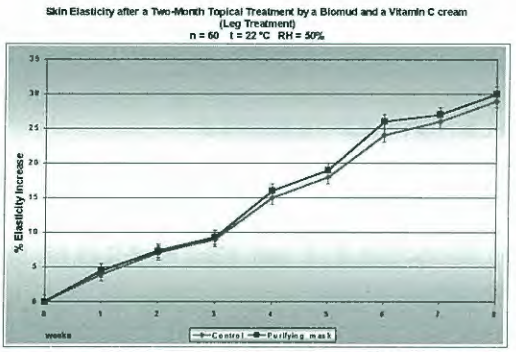


FIG.8

HAIR TREATMENT

Shampoo A or A1 was given in double-blind to all the participants at the experimentation, so that 30 of them used *Shampoo Active 1,* and the other 30 *Control shampoo A1.* Hair washing was effectuated every day for all the treatment period (60 days).
On the same days of the skin controls, it was measured hair resistance to traction, the relative electric charges and combability using l'INSTRON® Tensile Tester (18).
As a matter of fact, in an elastic substance, as hair is, for every deformation (strain) the hair tends to go back to the starting condition (stress). This hair property was verified using l'INSTRON® Tensile Tester, by which it's possible to highlight the hair elasticity variation before and after proper cosmetic treatments.
A specific sensor, linked to an automatic combing system can record the electrostatic charges caused by combing and the eventual anti-static effect given by the product.
Elasticity, combability, shyness, extricability, manageability to the treatment were evaluated directly by the products users, who, before starting the treatment, filled a form using an arbitrary scores scale.
The obtained results are reported on Figure 9 and 10.

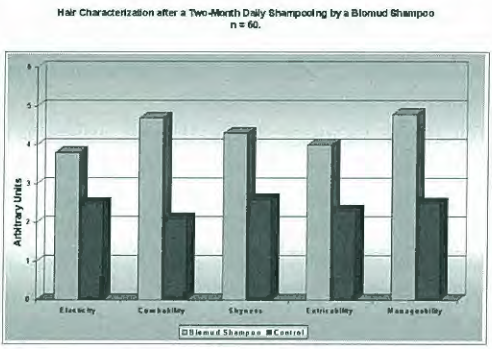


FIG.9

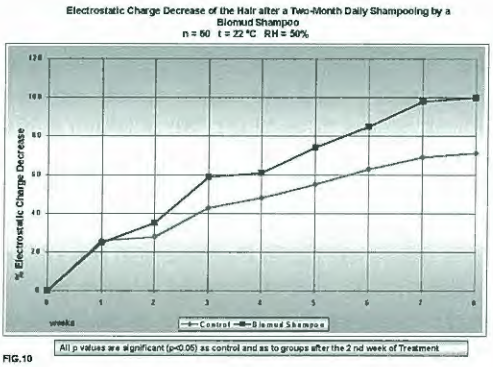


FIG.10

STATISTICAL ANALYSIS

The obtained results are recorded on disks using a micro-calculator TRS 80 (C PU-1284) furnished of two drive disks and an analysis system Advanced Statistical Analysis (ASA) reported on the Radio Schach software (19).

RESULTS AND COMMENTS

As it can he seen from the obtained results (fig. 2-10), the Biomud used shows to have characteristics that make it very useful as raw material of polyvalent cosmetic use.
In fact, adding a high percentage of this peat to the different formulations improves remarkably the cosmetic efficiency characteristics of the studied cosmetic formulations.
Concerning its usage as facial mask, it has to be underlined how the positive activity it performs, both on the hydration and on the surface lipids, it's evident since the first week of treatment. The hydration on skin face and legs, in fact, increases of about 24% ($p<0,01$) (fig. 3 and 4) and the lipids of about 22% ($p< 0,05$) (fig.2 and 5) till reaching respectively positive values of about 95 and 70% after 8 weeks of treatment.
The vitamin C based cream used had a remarkable positive activity towards dry and dehydrated skin of the group studied, activity strengthened when in addition to the twice daily creams application are used the active masks enriched with the peloid (Bio-Mud). We obtained similar results for the legs treatment, with a notable decrease in xerosis found at the beginning of the study and an improving in hydration and surface lipids (Fig.4 and 5). In this case also the vitamin C based cream used performed a better activity, but the contemporaneous usage of the mask gave an increasing of the two parameters controlled.
Concerning the pH, at facial and legs skin level, we did not note any suhstantial change. pH was acid at the beginning of the study and remained the same during the whole period of observation both at face and legs level (fig.6 and 7). Also the elasticity of the skin legs had a light improving because of the vitamin C based cream (Fig.8), but in this case the mask did not cause any improving.
With regards to the activity performed by the peloid included in the hair shampoo, the results were satisfactory. Observing figures 9 and 10 it's possible to see how the shampoo is able to reduce notably the electrostatic charges improving also hair combability and shyness.
From these first results, we can say that the set up of natural or thermal "cosmeceuticals" is possible only if using adapt raw materials in those percentages allowing them to perform the cosmetic activity required.

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IMMUNOFLUORESCENCE IN CLINICAL IMMUNOLOGY A PRIMER AND ATLAS

By Wolf B Storch
2000. 248 pages. Hardcover
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Immunofluorescence, as a powerful method for the microscopic demonstration of antigenes and antibodies, may provide a broad spectrum of possibilities for evidencing the pathogenesis of many diseases in histological or cytological preparations both for routine work and for research purposes.
The large experience of Dr. Storch in this particular field allowed him to write this volume of great interest to the whole medical class for enriching and their knowledge in diagnosis.
Moreover, the large room devoted to the advances in new laser-scanning microscopy and the use of modern fluorescent dyes will surely improve the application of immunofluorescence in clinical immunology, especially in diagnosis for of auto-immune-diseases.
The volume consists of 7 chapters.
The first one introduces the basic principles and techniques of immunofluorescence by a short description of the methodologies and the most important procedures used for direct and indirect Immunofluorescence.
The second chapter describes in a clear way the structure and function of antibodies, such as immunoglobulins, the relative production of antisera, how to isolate immunoglobulins from serum, and the different methodologies to labeling antisera with fluorochroms.
The third chapter is entirely dedicated to the characterization and quality control of antisera and conjugates.
As matter of fact, the use of conjugates, exhibiting the same specificity and having similar properties, is the most important requirement to obtain reproducible results and valid comparisons between laboratories.
At this purpose, different kind of methodologies and procedures to assessing the quality of conjugates (anti-IgGs)-based are reported together with many scientific references and examples of protocol for the simultaneous detection of different kind of antibodies (against mitochondria, ribosomes, cell nuclei etc.).
In the description of the procedures, every phase is described in detailed way, such as the conversion of weight and molar fluorochrome-protein ratios for conjugates and their characterization.
In the fourth and in the fifth chapters, all the actual Immnnofluorescence techniques and the instrumental needs are respectively reported.
Controlled conditions and appropriates apparatus such as confocal laser –scanning fluorescence microscopy, are necessary to obtain successful and reproducible results in the various areas of application. For this reason specific instructions and are given, from the sample setting up to the exact pre-

paration of apparatus and reagents to be used, up to the relevant instructions and the methods to carry out to obtain the best result.
Recommendations are given to the experienced staff about the specificity controls to be done to avoid obtaining false negative as well as false positive results: the best results are obtainable by the better technique used which is the confocal laser-scanning microscopy. This technique permits image processing and storaging by computer, as well as color printing and the scale possibility of manipulation the scale of any past of the obtained image.
After some general advice on analysis of results reported on the sixth chapter, chapter seventh gives an overview of the actual field of application of immunofluorescence in all areas of biology.
This relatively new technique is used in microbiology in preference to other methods for the detection of viruses, bacteria rickettsia, protozoa and fungi as well as for demonstrating antibodies activity against different microorganisms. But in clinical and in experimental pathology and immunology this technique is nseful to demonstrate organ-tissue-or cell-specific antigens in cells or their surface, as well as a histochemical staiming method for making particular proteins visible, using specific antibodies (hormones, enzymes, tumor or foreign antigens).
The seventh chapter describes all the actual applications of immunofluorescence. It is surely the most interesting chapter and represents about one half of the entire book.
It contains many high-quality color photomicrographs, as well as detailed descriptions of new antibodies. All the material is presented in the form of a broad overview, and all the photos used are clear and easy to understand from non-expert people also.
This richly illustrated and carefully analyzed book is a real scientific masterpiece introducing to the technique of immunoflnorescence. It will be a useful tool in clinical and experimental pathology and immunology, to control the antibodies involved in the immune response as "markers" necessary to establish a correct diagnosis in different diseases.
Usefnl for expert pathologists, this atlas-book is suitable also for every day practical work in laboratory, and reports fundamental updated news for all the people interested to know the today existing immunofluorescence techniques used in all areas of biology.

P. MORGANTI
Editor-in-Chief

AUTOLOGOUS FAT TRANSPLANTATION

By Melvin A. Shiffman
January, 2001/ 336 p., / Hardcover
U$ 165.00
ISBN: 0-8247-0484-3
Marcel Dekker Inc.
250 Madison Avenue
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http://www.dekker.com

This interesting volume reports in 28 chapters all the current techniques to use fat as skin filler. The transplantation of fat from one area of the body to another is in fact a safe and effective procedure used by many surgeons.
The volume highlights the guidelines for autologous fat transplantation executed by gentle handling, always maintaining the sterility both of the fat retrieval and of the injection.
The back of standardization of fat collection and transplantation allows a wide range of methods with varied results always operator-dependent and requiring the use of proper instruments and careful selection of recipient sites.
Because the problem of re-sorption of fat with fat transplantation, common to all the substances of biological origin used as fillers, 30-50% over injection is ordinary used.
Those injections have to be always sub-dermal for having long-term results.
Moreover a prerequisite for improving the method would be the transplantation of only intact viable fat cells.
As matter of fact, the presence of blood in the fat injected stimulates macrophage activity to remove the cells. Washing the cells in a physiological solution prior to injection will solve the problem. Another solution is also to centrifuge the adipose tissue to remove blood products and free lipids, thus improving the quality of the fat to be injected.
Candidates for autologous fat transfer would be systematically healthy individuals without specific medical contraindications to surgery or anesthesia.
However controversy remains relative to the ideal manner of harvesting, preparing, and transferring the fat. Although the process of fat transplantation has not been standardized many surgeons acknowledge and report very significant degrees of effectiveness and safety for the procedures.
By the way, fat tissue is aspired through a minor skin incision from such areas as the abdominal panniculus below the umbilicus, the buttocks, or the inner portion of the upper thigh. The section site as well as the point of transplantation are locally anesthetized before the procedure. The aspiration system used consists of a suction cannula, a connective hose, and a pressure pump that is fitted with a tissue filter, which traps any fat cell formation.
After an injection of Klein's solution with hyaluronidase, a 30- to 45 minute delay is taken to wait until the intercellularly binding connective tissues between the fat cells are depolymerized. The hyaluronic solution is given simultaneously with a vasoconstrictor to decrease bleeding.
All the steps to follow by precise methods in dependence of the different plastic surgeries are specifically described, and each chapter quotes in its bibliography the updated scientific literature.
Thanks to its plain language and to the accurate techniques description, this volume represents undoubtedly a valuable tool for the plastic surgeons, the dermatologists and all the medical operators involved in the aesthetic medicine.

P. MORGANTI
Editor-in-Chief

DWI Deutsches Wollforschungsinstit an der RWTH Aache

HairS'01

12th International Hair-Science Symposium

Heidelberg / Germany
5th – 7th September, 2001

1st Circular, Invitation and Call for Papers

About the Meeting

- HairS'01 is the twelvth in a well established series of symposia, organized by **DWI**, to address the scientific and practical aspects of hair care.
A key aspect and primary concern of the symposium is to bring together professionals from different areas of hair care science, technology and practice in order to present and discuss the different facets of fundamental and applied research.

- With this scope, the symposium provides an unrivalled platform for dialogue across industry and academia and between those in hair care products research, development, marketing, and practice.
A broad spectrum of topics will be addressed, placing special emphasis on the various approaches of detecting and evaluating alterations imparted to hair by cosmetic treatments.

- HairS'01 will take place in Heidelberg (Germany) in the Marriott Hotel (www.marriotthotels.com). The hotel is uniquely situated on the banks of the river Neckar, within walking distance of the historic city centre. It offers all facilities to ensure an effective meeting in a relaxed and comfortable atmosphere.
Heidelberg is readily reached by train, car from all directions or by air through Frankfurt or Stuttgart.

Symposium Organizers

- HairS'01 is organized by Prof. F.-J. Wortmann and Dr. B. Küppers of **DWI** (German Wool Research Institute, Aachen) in cooperation with DWI's Hair Science Advisory Committee.

Time Schedule

Wed, Sept. 5th	14.00-18.00	Lectures Evening free
Thurs, Sept. 6th	9.30-12.00 14.30-open	Lectures Social Event and Conference Dinner
Fri, Sept. 7th	9.30-12.00	Lectures

Scientific Programme

- Altogether about 15 lectures, each lasting 30 min (incl. discussion) are planned, covering a wide range of scientific, technological, and practical topics, as related to hair care science.

Call for Papers

- Interested colleagues from industry and academia are cordially invited to submit proposals for lectures together with a short abstract (1 page max.) in English, before April 11th, 2001.
- The authors will be informed about the acceptance of their contribution after the meeting of DWI's Hair Science Advisory Committee in the second half of May 2001. A book of abstracts will be provided to the participants at the beginning of the Symposium.
- The language of the Symposium is English.

General Information

- A ***preliminary registration*** should be made with the enclosed registration form or by E-mail to contact@dwi.rwth-aachen.de by April 11th, 2001. This is also the deadline for the submission of abstracts.
- The ***2nd circular*** with the final registration form will be mailed to interested parties in June 2001.
- The ***symposium fee*** will be around DM 1000 for participants, DM 800 for DWI-member companies, and DM 500 for speakers.
  This fee includes meals, coffee breaks, the social event, the Conference Dinner, and the book of abstracts. Accomodation is extra and approx. DM 240 per night.
- ***Hotel accommodation*** will be arranged for all participants through the symposium venue, the Marriott Hotel in Heidelberg. Booking forms will be provided with the 2nd Circular.
- ***Correspondence address*** for all matters concerning the symposium is:

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PRESENT

NUTRI-COSME-CEUTICALS: A CHALLENGE FOR THE FUTURE
Rome – 6-7-8 February 2002

This international and multidisciplinary Symposium organized to celebrate the XX anniversary of the scientific activity of the International Society of Cosmetic Dermatology, intends to give a complete picture of the progress had over the last twenty years in skin physio-biological activity and in the manufacturing of innovative cosmetics and dietary supplements useful to improve people's health and appearance, and to prevent possible patho-

logies in the early age, in the middle age and in aged people.
For the first time participants could assist to scientific discussions presented by international experts in Physiology and Biology involved in the basic studies on skin and its appendages; by specialized technicians in Cosmetic Chemistry and Dietetics involved in the setting-up and production of cosmetics and dietary supplements; by Dermatologists, Gynecologists, Pediatrics and Dieticians who daily advice and prescribe to their patients these categories of products.
The Symposium will give proper room also to dietary and cosmetic aspects in Alternative and Complementary Medicine, such as Chinese Medicine and Indian Ayurvedic Medicine.
Aesthetic Medicine using medical-surgical devices, such as "filler", or methodologies, such as ionophoresis, ozone therapy or mesotherapy and natural products based on mineral waters and therapeutic muds of thermal origin, will be widely treated.
These topics will try to highlight all the problems concerning the activity performed by the different active principles and by the relevant carriers used for the setting-up of the finished product. The activity performed by both cosmetic and dietary products is, in fact, always in dependence of the chemical physical formulation of the active principles selected, which will be described by the formulators working for the raw materials industries, and, of course, in dependence of the carriers used in the setting-up of the finished product described by the chemists and technicians working for the cosmetics and dietetics industries.
Their real efficacy and the eventual undesirable side effects when coming into contact with the skin areas or the mucous membranes, will be investigated by a huge number of biologists, physiologists and pharmacologists involved in the absorption through the different biologic membranes.
The medical community, comparing their opinions with their colleagues chemists, biologists and physiologists, will discuss why to prescribe and how to use these products.
Because of these considerations, the Symposium will consists of five main sessions:

1. The current knowledge on skin, hair, nail and mucous membranes
2. Percutaneous and mucous absorption: the new control-release carriers
3. Functional food and cosme-ceuticals needs at different age: the-state-of-the-art
4. Botanicals, sea salt and mud in Alternative and Complementary Medicine
5. Innovative medical devices and nutri-cosme-ceuticals in Aesthetic Medicine: present and future market

We have the pleasure to invite you to participate at this happening, as attendee or as a speaker, giving your personal contribnte to its success.

CALL FOR PAPERS

Authors who wish to present a paper for poster or oral presentation according to the Symposium topics are requested to forward their one-page abstract to the Symposium Secretariat.
Abstracts should be written in English, typed single space in Times New Roman font, 11-12 point, in an area of 15 x 23 cm (6 x 9 inches) on a single sheet A4 page.

Authors may either send their abstract by e-mail to iscd@libero.it, as a Word attachment or mail the abstracts on a diskette in Word format to reach the Symposium Secretariat together with a 4 copies. Material should be sent by air mail in a padded envelope that should be marked "do not fold".

Please be advised that authors registering as participants of the Symposium may submit more than one abstract.
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Presenters of abstracts will be informed by a separate letter regarding the status of their abstract whether it was accepted as an oral or poster presentation. Accepted abstracts will be printed in the book of abstracts and distri-

buted at the symposium.

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Please note that all presenting authors must register

EXHIBITION

A trade exhibition will be held in conjunction with the meeting.
Manufacturers and suppliers of cosmetic and pharmaceutical raw material specialties and medical equipment are invited to present their services , products and literature.
For exhibition space, please contact the Organizing Secretariat:

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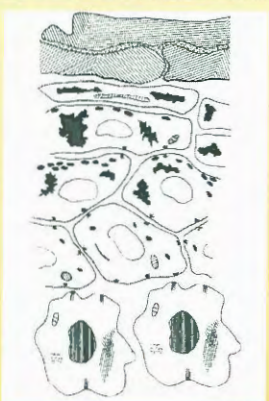

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