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DECREASING THE STINGING CAPACITY AND IMPROVING THE ANTIAGING ACTIVITY OF AHAs

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Synopsis

The AHAs ability to affect wrinkling, specially increasing the rate of skin cell renewal, seems to be strictly correlated to the plasticization effect and to some degree of irritation. The purpose of this study was to determine wheteher some aminoacids, used to buffer AHAs, can reduce the stinging and burning sensation caused by the use of Alphahydroxyacid containing formulation, enhancing the skin penetration and maintaining, its antiaging activity.
The obtained results seem to demonstrate that the aminoacids used to buffer glycolic acid (gelatin-glycine enriched with arginine), markedly reduces the irritating side effects of AHAs-containing emulsions potencing their antiageing activity. Conversely, AHAs depigmenting activity on age-spots, as well as their control on free radicals, are enhanced.

Riassunto

La capacità degli AHAs di agire sulle rughe, soprattutto aumentando il tasso di rinnovamento delle cellule della pelle, sembra essere strettamente correlata all'effetto plastificante e ad un certo livello di irritazione. Lo scopo di questo studio era di determinare se alcuni aminoacidi, usati per tamponare gli AHAs, potessero ridurre la sensazione pungente e di bruciore causata dall'uso di formulazioni contenenti alfaidrossiacidi, aumentandone la penetrazione attraverso la pelle e mantenendone l'attività anti-invecchiamento. I risultati ottenuti sembrano dimostrare che gli aminoacidi usati per tamponare l'acido glicolico (la gelatina-glicina arricchita con arginina) riducono considerevolmente gli effetti collaterali irritanti delle emulsioni contenenti AHAs, potenziandone l'attività anti-invecchiamento. Viceversa risulta potenziata sia l'azione schiarente degli AHAs nei confronti delle iperpigmentazioni cutanee provocate dall'età, che il loro controllo sui radicali liberi.

Introduction

As well known AHAs when applied topically, have been demostrated an interesting efficacy in the treatment of conditions predisposing to dry, rough skin, including ichthyosis. Therapeutic benefits have been reported also in the treatment of acne, keratoses, and problems related to aging, such as dyscromia and wrinkling (1-5)
The AHAs ability to affect wrinkling, specially increasing the rate of skin cell renewal, seems to be strictly correlated to the plasticization effect and to some degree of irritation. (6-8) The purpose of this study was to determine whetеher some aminoacids, used to buffer AHAs, can reduce the stinging and burning sensation caused by the use of Alpha hydroxyacid containing formulations, enhancing the skin penetration and maintaining, its antiaging activity. All the study was carried out during the months of September through December 1995. Each caucasian subject was used, when possible, as her or his own control; the tests and comparative formulations being, on randomized basis, to bilateral symmetrical areas.

Materials and methods

MATERIALS

TREATMENT A

Day cream
Vehicle + glycolic acid, gelatin, glycine, arginine. pH 5.5.

Night cream
Vehicle + glycolic acid, gelatin, glycine, arginine. pH 5.5.

TREATMENT B

Day cream
Vehicle + glycolic acid, gelatin, glycine, lysine. pH 5.5.

Night cream
Vehicle + glycolic acid, gelatin, glycine, lysine. pH 5.5.

TREATMENT C

Day vehicle
Day cream - Water, stearyl heptanoate, ceteareth-10, beeswax, cetearyl octanoate, cetyl esters, myrystyl alcohol, dimethicone copolyol, mineral oil, isopropyl lanolate, dihydroxycetyl phosphate, isopropyl hydroxycetyl ether, cetearyl alcohol, titanium dioxide, desamidocollagen, glycerin, linoleic acid, linolenic acid, sodium PCA, tocopheryl acetate, allantoin, disodium EDTA, imidazolidinyl urea, methylparaben, fragrance - pH 5.5.

Night vehicle
Night cream - Water, sodium PCA, caprylic/capric triglyceride octyl stearate, cyclomethicone, glycerin, cetyl dimethicone copolyol, isopropyl lanolate, cetearyl octanoate, squalane, linoleic acid, linolenic acid, retynil palmitate, tocopheryl acetate, magnesium sulfate, BHT, imidazolidinyl urea, methylparaben, propylparaben, fragrance - pH 5.5.

TREATMENT D

Day cream
Vehicle + glycolic acid. pH 5.5.

Night cream
Vehicle + glycolic acid. pH 5.5.

Selection of stingers
We selected 40 women (between age 27/35) with light complexions and personal hystory of easy sunburning. According to Frosh and Kligman (9) the stinging activity was evaluated using a 5% aqueous solution of lactic acid rubbed briskly over the nasolabial fold and check after sweating induced by a 15 minutes stream. Those who experience sharp stinging at least 3 to 5 minutes were identified as stingers.

METHODS

1st. STUDY

Stinging test

The first experimental study was carried out on preselected volunteers individuals (40 women between age 27/35) classified s "stingers" according to Frosh and Kligman metodology. (9)
Stinging test was evaluated out on four groups of 10 stingers.

- 1 group treatment formulation A (day cream) on right nasolabial fold and check vehicle A on left side
- 2 group treatment formulation A (day cream) on left nasolabial fold and check vehicle A on right side
- 3 group treatment formulation B (day cream) on left nasolabial fold and check vebicle B on right side
- 4 group treatment formulation B (day cream) on right side and fold vehicle B on left side

Each subject placed the face directly into a steam stream (40°C) for at least 10/15 minutes. When sweating was brisk, each cream and vehicle were randomly rubbed over one side of nasolablial fold and check (right or left). The other side serving as control . Stinging was evaluated immediately after application of the cream (10 sec) and at 2.5, 5.0 and 8.0 min. on a 4 point scale:

0 = no stinging
1 = slight stinging
2 = moderate stinging
3 = severe stinging

The obtained results are reported at **fig. 1**. The mean of the three readings at 2.5, 5.0 and 8.0 min was considered as "delayed stinging" score.
The obtained results are reported on **fig. 2**.

Stinging capacity

As is known, the topical use of glycolic acid and/or lactic acid based emulsions at concentrations of between 5 and 14% can give rise to transitory forms of erythema and to widespread sense

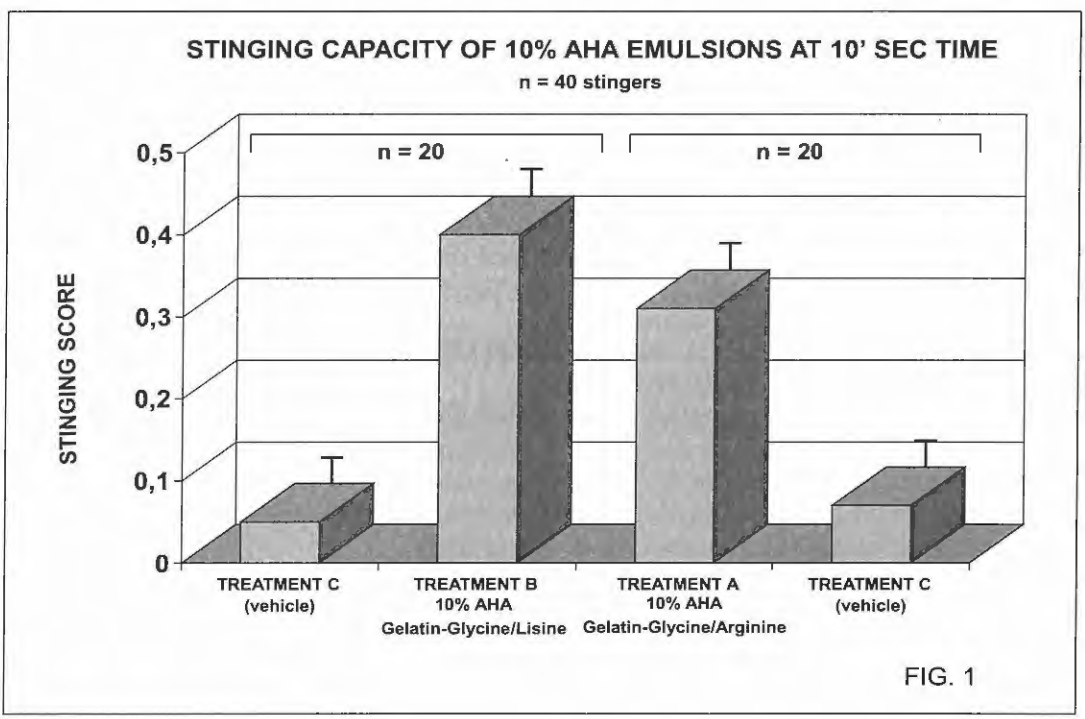

FIG. 1

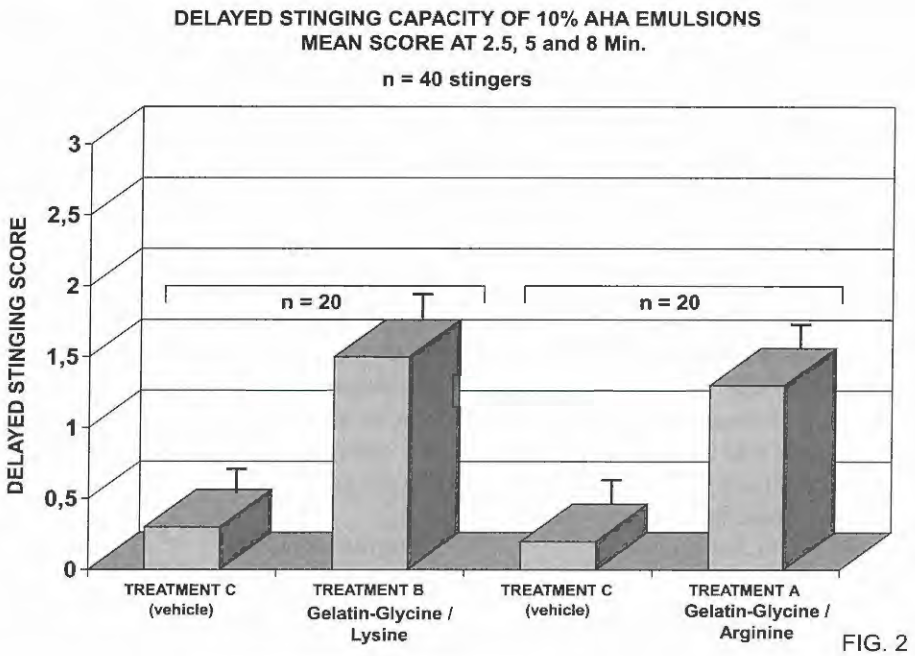

FIG. 2

of burning. By partially neutralising these acids with the gelatin-glycine-lysine or arginine mixtures, the erythemogenous and reddening action is practically eliminated (10). This unpleassant sensation is no longer noted by the user, while the stimulant activity on cell turnover remains unaltered. To better control this in use recovery,erythema was induced by washing forearms of 3 groups of 10 volunteers women (between age 18-25) with a solution of acetone/ether for 15 minutes (day-1). The different tested emulsions (A,B and C/0,8ml each) were applied twice a day (morning/day-cream and evening/night-cream) from day zero on one arm of each group of 10 volunteers, the others, untreated arm, served as control. Immediately after the second application, were evaluated in both the arms the level of redness or skin roughness using a scale:

3 intense erythema and extreme skin dryness
2 medium intensity erythema and evident dryness
1 barely perceptible erythema and very little dryness
0 no erythema and skin with normal appearance.

The recovery was carried out on the same day before the erythema was provoked (day-1), after provocation of the erythema and after 1,2 and 3 days from application of the emulsions, which were applied on day zero. The results are given in **fig. 3.**

2nd STUDY

Depigmenting Activity

The depigmenting activity is often required for the treatment of some hyperpigmentary disorders such as melasma, lentigo solaris or the so called Age-Spots.

Age-Spots

To control the depigmenting activity of the studied treatment A and B in relation of the treatment D (10% glycolic acid alone), each emulsion was ap-

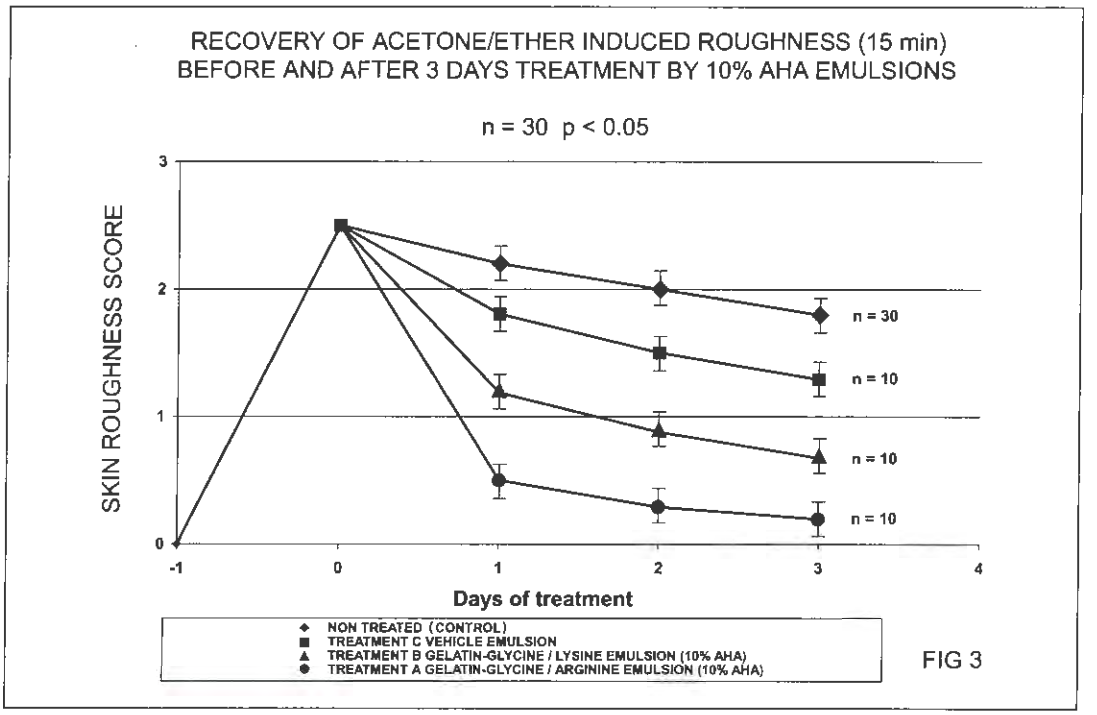

FIG 3

plied twice daily (day-cream morning and night-cream evening) for 3 months on 3 groups of 10 women (between age 65-68 and selected. from the residents of nursing home for the elderly).

The tested products were applied to the back of one hand of each group affected by hyperpigmented lentigo, (dark skin spots), the other hand served as untreated control.

Other peoples, unaffected by lentigo, served as normal control.

The intensity of the color was measured with a Minolta Chromameter CR 200, that is a lightweight and compact tristimulus color analyzer for measuring reflected object color (11).

Chromameter CR 200 provides practical numerical basis for quantifyng the perceived color of the skin's surface. This method assures also good accurancy and reproducibility (measuring error < 1%).

Each parameter L, a and b related to skin colour, were measured, six times, at the beginning and at the end of the treatment period. The statistical analysis between untreated and treated hands, showed that all the active emulsions (A,B and D) significantly lightened the age spots ($p < 0,005$). The average results obtained are given in **fig.4**. As can be clearly seen from fig. 4, positive results were obtained ($p < 0,005$) both using the emulsion based on 10% glycolic acid (treatment D) and using same neutralized with gelatin-glycine/lysine ($p < 0,005$) (treatment B) and gelatin-glycine/arginine ($p < 0,005$) (treatment A). However, the most noticeable depigmentation was obtained using the emulsion based on the use of gelatin-glycine/lysine and gelatin-glycine/arginine, which showed a depigmenting activity almost double that of glycolic acid alone.

Skin Elastic Properties

The skin firmness was evaluated by the use of the Twistometer (12) after two months treatment twice a day (day cream morning and night cream evening) on 30 volunteer women (between age 65 - 68).

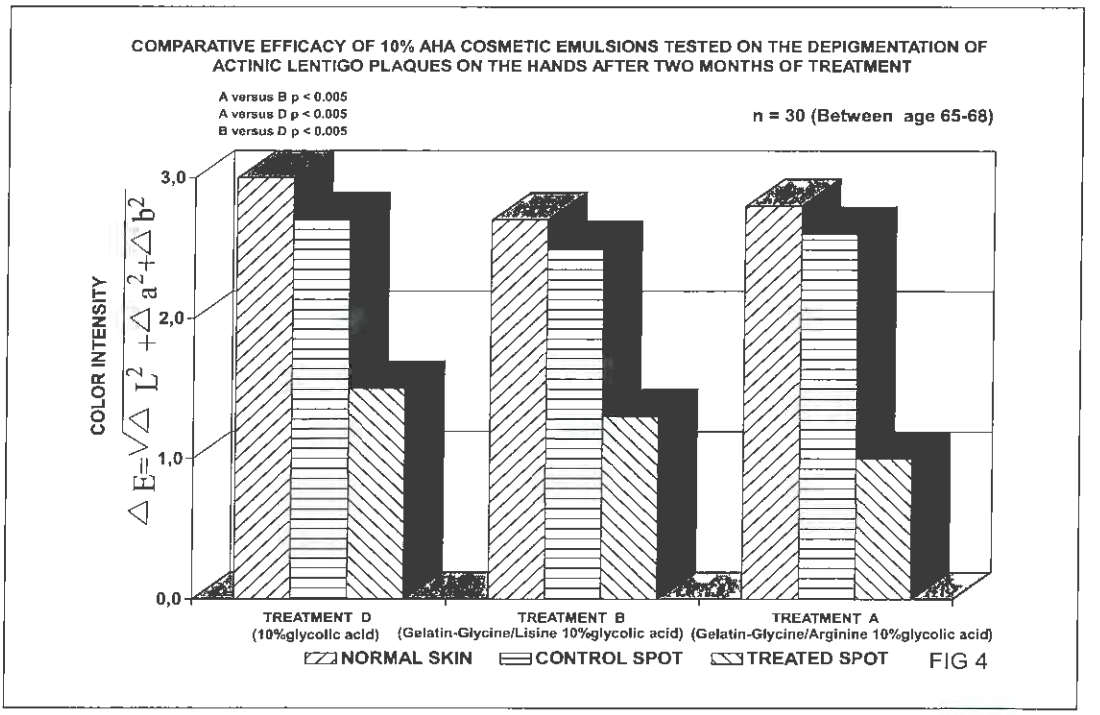
COMPARATIVE EFFICACY OF 10% AHA COSMETIC EMULSIONS TESTED ON THE DEPIGMENTATION OF ACTINIC LENTIGO PLAQUES ON THE HANDS AFTER TWO MONTHS OF TREATMENT
A versus B p < 0.005
A versus D p < 0.005
B versus D p < 0.005
n = 30 (Between age 65-68)
COLOR INTENSITY
$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$
3,0
2,0
1,0
0,0
TREATMENT D (10%glycolic acid)
TREATMENT B (Gelatin-Glycine/Lisine 10%glycolic acid)
TREATMENT A (Gelatin-Glycine/Arginine 10%glycolic acid)
NORMAL SKIN
CONTROL SPOT
TREATED SPOT

FIG 4

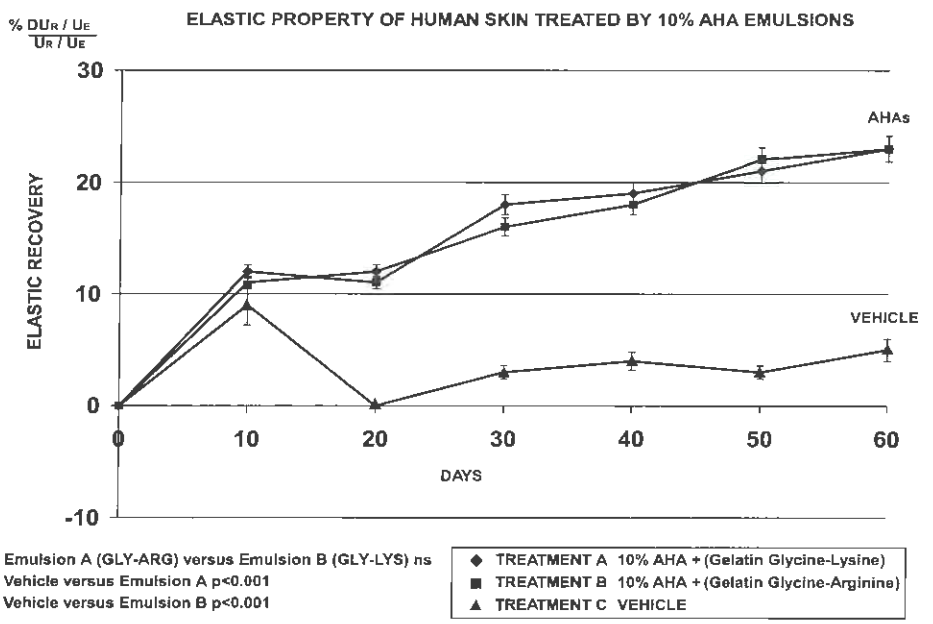
% DUR / UE / UR / UE
ELASTIC PROPERTY OF HUMAN SKIN TREATED BY 10% AHA EMULSIONS
ELASTIC RECOVERY
30
20
10
0
-10
0
10
20
30
40
50
60
DAYS
AHAs
VEHICLE
Emulsion A (GLY-ARG) versus Emulsion B (GLY-LYS) ns
Vehicle versus Emulsion A p<0.001
Vehicle versus Emulsion B p<0.001
TREATMENT A 10% AHA + (Gelatin Glycine-Lysine)
TREATMENT B 10% AHA + (Gelatin Glycine-Arginine)
TREATMENT C VEHICLE

FIG. 5

The tested emulsions were applied for 60 days to one arm the other served as untreated control (only vehicle). The obtained elastic recovery controlled each 10 days is reported on **fig. 5**. The figure shows the results obtained after a continous treatment twice a day for 60 days relative to the untreated controlateral arm in term of elastic recovery (UR/UE) ($p<0,001$).

Measurement of skin hydroperoxides

Skin, serving as a barrier against the external attacks, is the potential target organ of environmental oxidative stress, the major cause of skin aging . The greater the amount of peroxides at skin level, the faster the ageing.

A topical treatment which neutralizes peroxides seems, thus, able to perform an antiageing activity.

The tested emulsions (1 mg each for each cm2) were applied twice a day (day cream morning and night cream evening) to one arm (forearm) of 30 volunteers women (between 65-68) the other, treated by the vehicle, served as control. Skin lipids were extracted from the skin by acetone. According to the methodology of Pugliese (13). A glass cylinder measuring 5 cm in diameter was placed on the skin and held snugly, extracting the lipids by two different aliquots of 5 ml portions of acetone.

The two added portions of acetone were dried under a nitrogen stream.

The lipid residue was emulsified with 0,2 ml of 8% sodium dodecyl sulfate, 1,5 ml of 20% acetic acid and 0,5% of thiobarbituric acid solution making up the final volume to 4 ml with water. Finally the concentration of peroxides determined as MDA precursors on supernatant extracted with 4 ml of n-butanol, was read at 531 nm.

The obtained results are reported on **fig 6**.

3rd STUDY

Measurement of skin hydration and trans epidermal water loss (TEWL)

It is known that the electrical properties of the skin and its hydration are related to the water content of the stratum corneum (SC). SC hydration state may be detected by the capacitance method (14).

It is also known that TEWL provides an assessment of the integrity of the SC rather than its moisture content. A stratum corneum that is damaged, as happened in psoriasis, will result in an high TEWL and low hydration.

TEWL

Quantitative measurements of TEWL were performed using the 3C System DERMOTECH (15). TEWL was expressed as the amount of water evaporated per unit of surface in 1 hour gr/m2 /h . The instrument probe has held perpendicular to the skin surface (forearm) and allowed to equilibrate for 20 seconds. The computeryzed 3C System collects up 10/15 measurements over 25 second sampling period and records the mean value automatically standardizing the environmental conditions.

The study control was made on 30 volunteers patients (15 men and 15 women) aged between 8 and 18 years and suffering from atopic dermatitis. 10 normal volunteers (5 women and 5 men) acted as controls. For this study was used the Treatment A only (gelatin-glycine-arginine) twice a day (day cream morning and night cream evening) for 30 days.

As is known, skin affected by atopic dermatitis has an high TEWL when compared with normal individuals, and low capacitance levels (skin hydration) (**fig.7**) As can be seen from **figures 7** and **8**, after 30 days of twice-a-day treatment (active A), the atopic skin gives TEWL and Capacitance values more or less the same as those found in the skin of normal individuals. The vehicle dont' has the same activity.

Hydration activity

The hydration activity was controlled on a group of 30 volunteers women aged between 35 and 48 years suffering from psoriasis on restricted areas of both the forearms, at least 25 cm. apart.

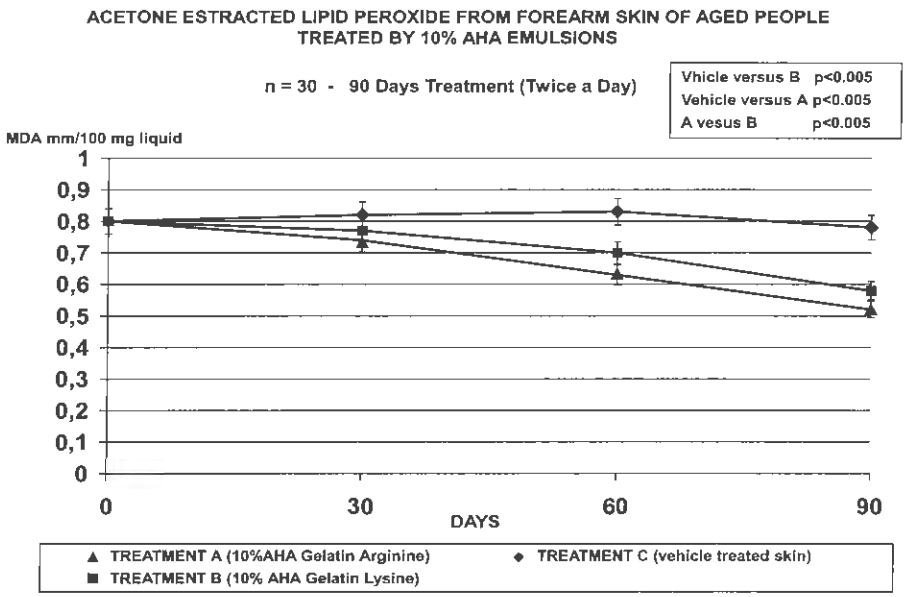
ACETONE ESTRACTED LIPID PEROXIDE FROM FOREARM SKIN OF AGED PEOPLE
TREATED BY 10% AHA EMULSIONS
n = 30 - 90 Days Treatment (Twice a Day)
Vhicle versus B p<0.005
Vehicle versus A p<0.005
A vesus B p<0.005
MDA mm/100 mg liquid
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
0
30
60
90
DAYS
TREATMENT A (10%AHA Gelatin Arginine)
TREATMENT B (10% AHA Gelatin Lysine)
TREATMENT C (vehicle treated skin)

FIG. 6

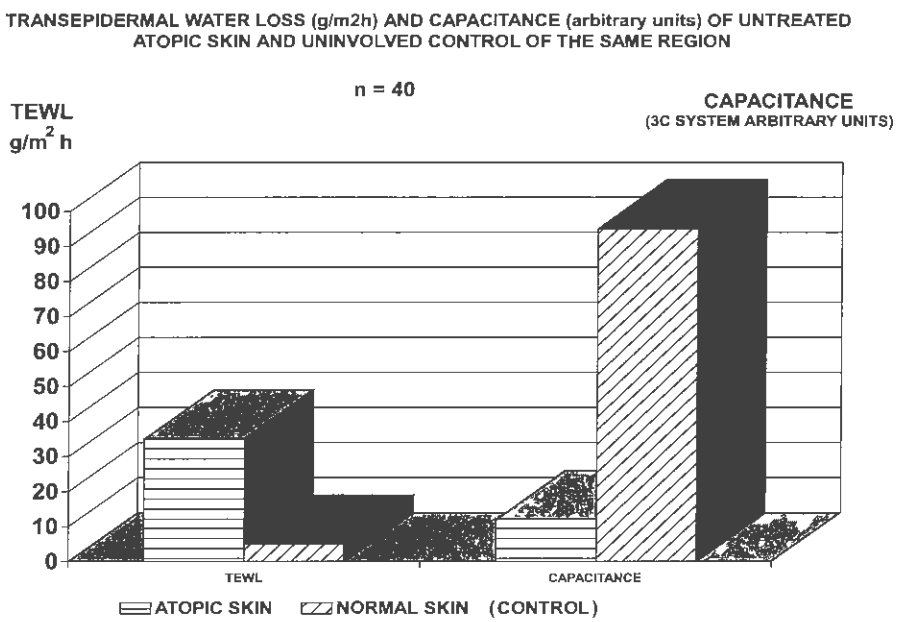
TRANSEPIDERMAL WATER LOSS (g/m2h) AND CAPACITANCE (arbitrary units) OF UNTREATED
ATOPIC SKIN AND UNINVOLVED CONTROL OF THE SAME REGION
n = 40
TEWL
g/m2 h
CAPACITANCE
(3C SYSTEM ARBITRARY UNITS)
100
90
80
70
60
50
40
30
20
10
0
TEWL
CAPACITANCE
ATOPIC SKIN
NORMAL SKIN (CONTROL)

FIG 7

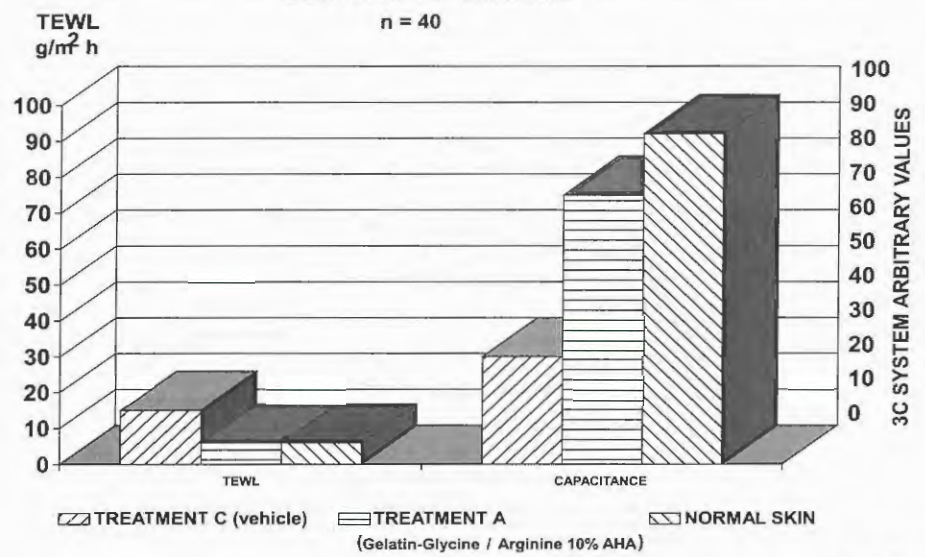

FIG 8

Experimental design

No volunteers had used other topical treatments within two weeks or systemic drugs or dietetics within 4 weeks prior to commencement of the study.

Study products were packaged in identical container (150 ml. tube) identified by study number, women number and side administered (right or left).

The experimental design used a randomized, douhle-blind and controlateral comparison (vehicle only).

Women were randomely divided into two groups, one of which contained 12 people, who were instructed to use the treatment A and C (right or left forearm) ; the other contained 13 people who were instructed to use the treatment B and C. 5 people served as untreated control.

After the forearms were washed with a non-medicated soap (MAVIGEN® SAPONE) and patted dry with a soft towel, they applied the assigned product treatment. Depending of the group, one site on the ventral forearm of each people was treated with treatment A or B (1 ml.) twice a day (day cream morning and night cream evening) for 35 days. The other site, on the ventral forearm, was treated with treatment C.

A randomized schedule was used to determinate which was used on which ventral forearm.

The average values recorded, which represent the average of five simultaneous measurements taken on the same area of the skin, are given in **fig. 9**.

Hydration measurements were taken every five days using the 3C SYSTEM DERMOTECH (15).

4th STUDY

Consumer complaints: skin redness

A degree of transitory stinging, burning or moderate irritation is a common side effect of glycolic acid preparation especially at the beginning of the treatment (first 10/15 days). When the cosmetic formulations are buffered at the right way closer to pH 5.5, they are generally, less irritative. Mo-

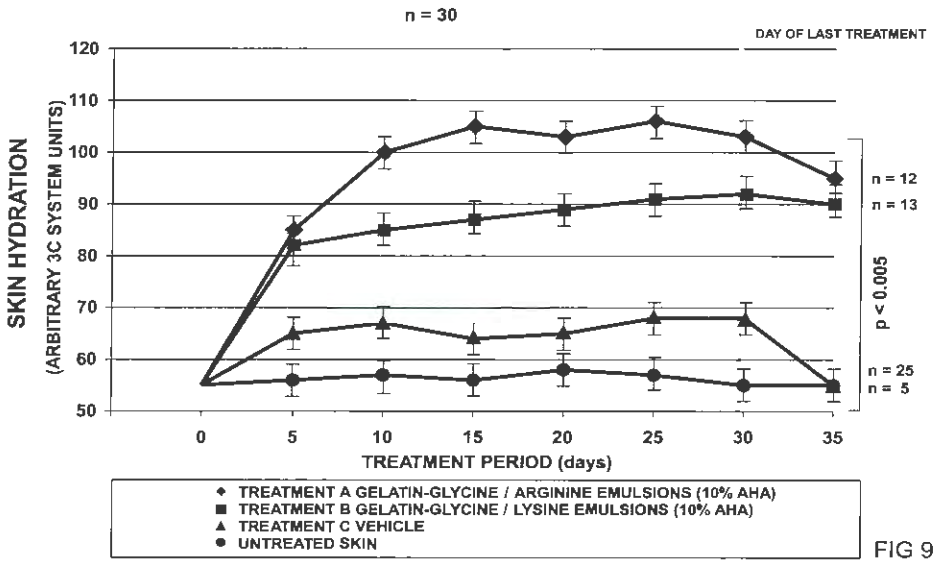

FIG 9

reover the degree of skin irritation and skin redness seems to be strictly correlate to the buffer-compound and to the kind of vehicle used. By this last study it was recorded the stinging and redness score over the first 2 weeks of product use.

Thirthy women with normal to dry and slightly sensitive skin (between age 20-27) divided in 3 groups of 10 peoples were supplied with two AHAs containing treatments. Each treatment was coded as A,B,C or D including day and night creams. Each product was packaged in 150 ml. tube and labeled left and right, day and night, to avoid panelist confusion.

Therefore each panelist received 4 tubes, A (day and night) + C (day and night); or B (day and night) + C (day and night); or D (day and night) + C (day and night) treatments, which were randomly applied (2 mg./cm2) to the left of right side of their face twice a day (day cream morning and night cream evening).

One week before the study all subjects were refrained from using any kind of cosmetics products.

On days 0,1,2,3,4,5,6,7 and again at 15, and 7 all the subjects were controlled by a trained clinician at the Test Center in the morning (prior the day-cream application) to evaluate score redness on a 4 point scale:

0 no redness
1 slight redness
2 moderate redness
3 severe redness

The obtained results are reported in **figure 10.**

RESULTS AND COMMENTS

These first experimental data seem to show clearly that some balanced mixtures of aminoacids (gelatin-glycine), especially enriched with lysine and arginine, reduce the stinging and burning sensation which are usually a side-effect of emulsions containing glycolic acid (**Figs. 1, 2 and 10**). In fact, the emulsions containing gelatin-

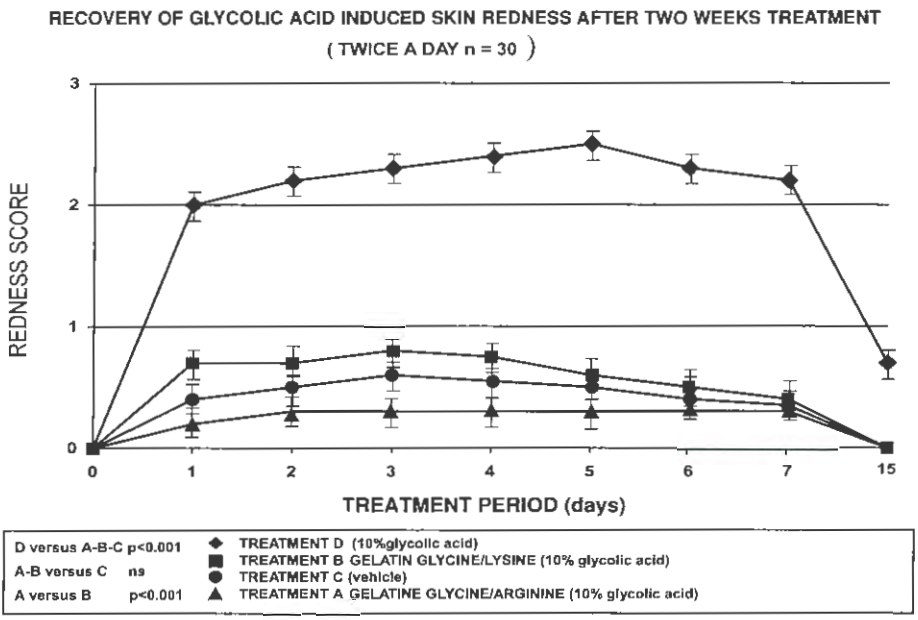

FIG 10

glycine/lysine and specially gelatin-glycine/arginine noticeably reduce the severity and duration of the stinging effects provoked by glycolic acid (**Figs. 1 and 2**). Thus, the above aminoacids seem to have not only an anti-irritative activity but also a rehydrating and normalizing effect on the surface lipid film.

The skin delipidized with solvents such as acetone - a well known solvent of ceramides too - rapidly gets back to normal, especially when emulsions are added with these aminoacids (**Fig. 3**). It is mostly arginine that seems to speed up thehydration and the normalization of the lipids arrangement in the skin, be it healthy (**Fig.3**) or affected with diseases including atopic dermatitis (**Figs. 7 and 8**) and psoriasis (**Fig. 9**). These two particular diseases are also characterized by markedly reduced ceramide-1 at the level of keratin-filled cells (17) and a greater lack of free fatty acids and cholesterol esters (18) respectively.

As we know, the molecular substitution of one lipid with another may alter the structure and functional properties, such as fluidity and water permeability, of the membranes in the stratum corneum. In addition, it may affect the action of non-lipid components.

Upon the re-arrangement of lipids, which are interlocked among keratin-filled cells, both the skin moisture level and TEWL (transepidermal water loss) get back to normal (**Figs. 8 and 9**). This rebalancing is important to keep and make the skin look younger, as shown also by the marked improvement in skin elasticity and the noticeable depigmentation obtained on hyperpigmented lentigo just after rispectively 30 or 60 days continuous treatment (**Figs. 4 and 5**).

The reason why adding an aminoacid, such as arginine, brings about an increase of water, re-arranging the lipid enriched intercellular skin matrix is still unknown. However, it is possible to propose an hypothesis, without necessarily limiting ourselves to it. It is suggested that arginine, when added to the AHA formulations with gela-

tin/glycine, may turn into urea at skin level and enhance the skin rehydrating activity of gelatin/glycine (18-19).
In fact, urea is known to turn bonded water into free water, thus rehydrating the skin tissue and emiliorating its tone and appearance.
To sum up, we can state that buffering glycolic acid in a balanced way (10) with a mixture of aminoacids, such as gelatin-glycine, especially enriched with arginine, markedly reduces the irritating side effects of AHAs-containing emulsions (**Fig.10**) potencing their antiageing activity. Conversely, AHAs depigmenting action on age-spots (**Fig. 4**), as well as their control on free radicals (**Fig. 6**), are enhanced.

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References:

1. **Van Scott E.J., Yu R.J. (1974)** Control of keratinization with alpha hydroxy acids and related compounds, 1. Topical treatment of ichthyotic disorders, *Arch. Dermatol.*, **110**:586-590.
2. **Van Scott E.J. (1988)** The unfolding therapeutic uses of alpha hydroxy acids, in: *Med. Guide to Dermatol.*, **3**:1-5.
3. **Van Scott E.J., Yu R.J. (1989)** Alpha hydroxy acids: therapeutic potentials, *Canadian J. Dermatol.*, **1**:108-112.
4. **Murad H., Shamban A.T., Moy L.S., et al. (1992)** Study shows that acne amproves with glycolic acid regimen, *Cosm. Dermatol.*, **5**:32-35.
5. **Hermitte R. (1992)** Aged skin, retinoids and alpha hydroxy acids, *Cosmet. Toilet.*, **107**:63-66.
6. **Alderson S.G., Barratt M.D., Black J.G. (1984)** Effect of 2, hydroxy acids on guinea-pig footpaad stratum corneum, *Int. J. Cosmet.*, SCI **6**:91-100.
7. **Hill J.C., White R.H., Barratt M.D., Mignini E. (1988)** The skin plasticization effect of a medium chain 2, hydroxy acid and the use of potentiators, *J.Appl. Cosmetol.*, **6**:53-68.
8. **Smith W.P., Smith W. (1993)** Hydroxy acids and skin aging, *Soap Cosmet. Chem. Special.*, **69**:(n9):91.
9. **Frosch P.J., Kligman A.M. (1981)** A method for appraising the stinging capacity of topically applied substances, *J. Soc. Cosmet. Chem.*, 28, 197, 247.
10. **Morganti P. (1996)** USA patent application.
11. **Elsner P. (1994)** Chromametry: hardware, measuring principles and standardization of measurements, in Bioengineering of the skin, Cutaneous Blood Floward Erythema, Berardesca,Elsner and Maibach Eds CRC. Press, Boca Raton, FL.
12. **Léveque J.L., De Rigal J. (1985)** *In vivo* measurement of the stratum corneum elasticity, *Bioeng. Skin.*, **1**:13-23.
13. **Pugliese P. (1990)** Asssessment of antiaging products in: *Clinical Sufety and Efficacy Testing of Cosmetics*, WC Wogger ed. Marcel Dekker Inc. N.Y. **1**:306.
14. **Blichman C.W., Serup J. (1988)** Assessment of skin moisture. Measurements of electrical conductance, capacitance, and trans epidermal water loss, *Acta Dermatol. Venerol. (Stockholm)*, **68**:284.
15. **Cardillo A., Morganti P. (1994)** A fast, non invasive method for skin hydration control, *J. Appl. Cosmetol.*, **12**:11.
16. **Imokawa G., Abe A., Jin Kumi, Higaki Y., Kawashima M., Hidano A. (1991)** Decreased level of ceramides in stratum corneum of atopic dermatitis, *J. Invest. Dermatol.*, **96**: 523-526.
17. **Wilkinson D.I., Farber E.M. (1967)** Fatty acids of surface lipids from lipids from uninvolved skin in psoriasis, *J. Invest. Dermatol.*, **48**:248-251.
18. **Morganti P., Randazzo S.D. (1987)** Enriched gelatin as skin hydration enhancer, *J.Appl. Cosmetol.*, **5**:105-120.
19. **Morganti P., James B., Randazzo S.D. (1990)** The effect of gelatin-glycine on skin hydration, *J. Appl. Cosmetol.*, **8**:81-88.