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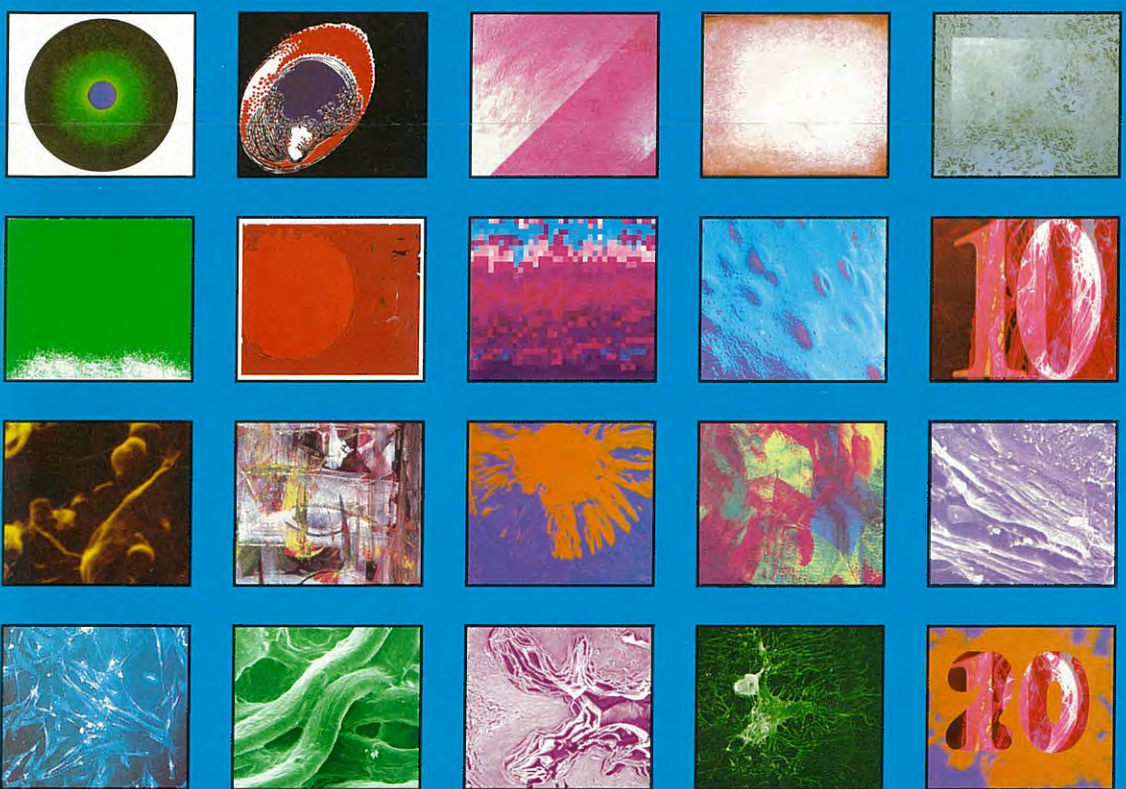

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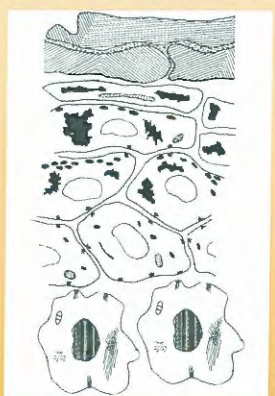

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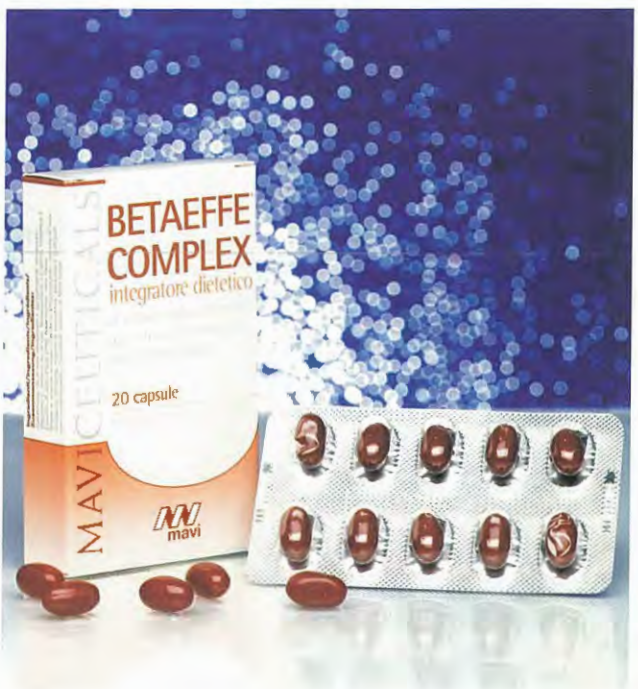

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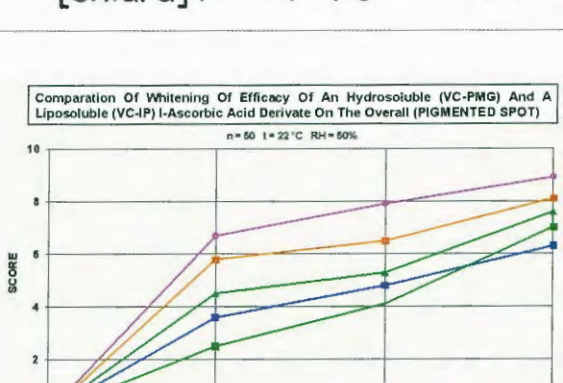

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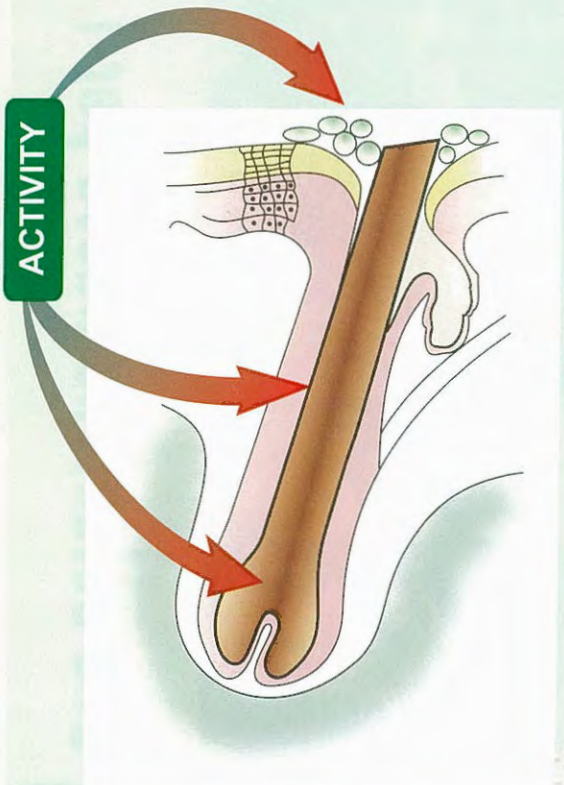

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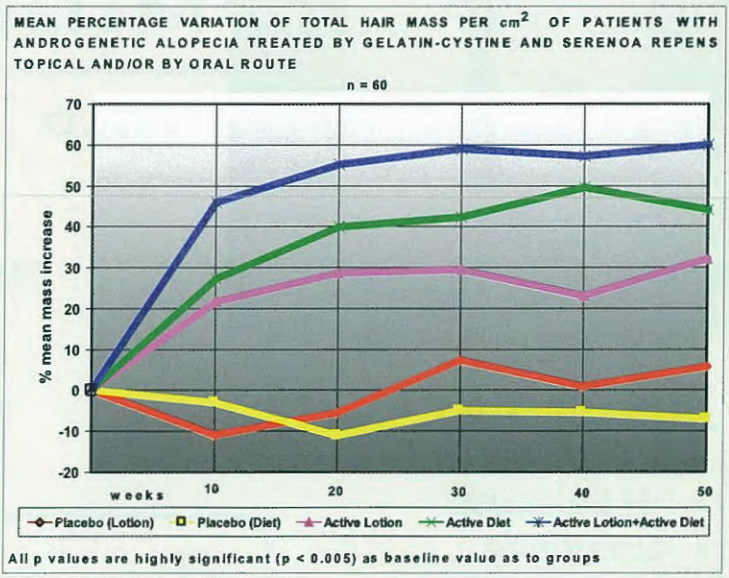

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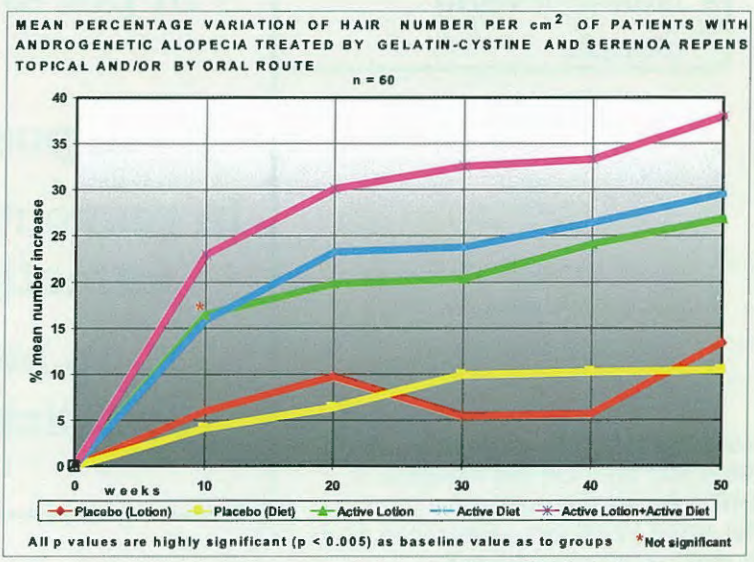

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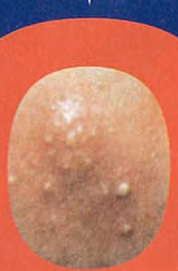

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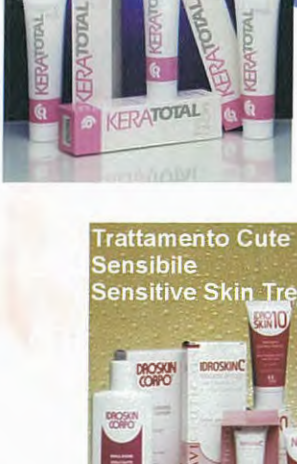

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Contents

Original Laboratory Studies

J. Appl. Cosmetol. **20,** 129-136 (April/June 2002)

Sunscreens, suntan and anti-sun-burn preparations today

Paolo U. Giacomoni
Clinique Laboratories, Inc125 Pinelawn Road, Melville, New York 11747

Received: *February, 2002*

Key words: *Sunscreens, DNA repair, anti-oxidants, tan, anti-UV strategies*

Summary

Until recently, the only strategy affording the protection of the skin against the harmful solar radiation was to reduce the number of impinging photons with fabrics, umbrellas, hats, sunglasses or sunscreens.
Diverting impinging photons is the first step in sun protection. It is not the only one. Non-diverted photons enter the skin and provoke molecular damages to DNA, RNA, proteins, lipids, vitamins etc. often by indirect oxidative reactions.
It is important to perform a second step and scavenge the reactive species, which provoke these residual reactions, by using antioxidants against different reactive oxygen species such as superoxide, hydroxyl radicals and singlet oxygen.
The third step in sun protection is to stimulate the endogenous self-defense responses. As of today it is possible to stimulate DNA repair, energetic metabolism, heat shock proteins, immune response, etc.

Riassunto

Oggi come oggi, la sola strategia che permetta di proteggere la pelle contro i danni del sole è quella di ridurre il numero dei fotoni incidenti. Questo può essere ottenuto usando parasole, cappelli, vestiti, occhiali da sole ed anche mediaute l'applicazione di preparati che assorbono o riflettono la radiazione solare, i cosiddetti schermi o filtri solari.
Contrariamente a quanto comunemente si crede, è stato recentemente portato all'attenzione dei ricercatori come un'abbronzatura naturale fornisca solo un piccolo fattore di protezione. In termini di Sun-Protection-Factor (SPF), l'abbronzatura è equivalente a un filtro solare con un SPF = 3 o 4. È quindi imperativo tener presente, quando ci si espone agli ultravioletti, non solo che il processo di abbronzatura si accompagna comunque alla produzione di danni molecolari, ma anche che l'abbronzatura stessa non offre protezioni particolari e che, quindi, l'uso dei filtri solari non deve essere abbandonato mai, neanche ad abbronzatura ottenuta.
Deviare o assorbire i fotoni incidenti è solo il primo passo di una moderna strategia per la protezione solare. Non è l'unico! I fotoni che non sono riflessi o assorbiti penetrano nella pelle e provocano danni agli acidi nucleici (DNA e RNA), alle proteine ed ai lipidi. È necessario rimuovere questi danni perchè essi provocano la morte cellulare e la conseguente reazione infiammatoria, che, come è noto, accelera il processo di invecchiamento cutaneo, inducendo mutazioni anche troppo spesso

sottovalutate.
È da poco tempo possibile fornire preparati in cui il rilascio di anti-ossidanti è ritardato nel tempo, e che contengono enzimi di riparazione del DNA. È anche possibile fornire prodotti che provocano un'abbronzatura artificiale con piccola ma misurabile capacità di protezione. Permettendo di mantenere il livello di danni molecolari al minimo, tali preparati riducono l'intensità dell'eritema solare ed i danni secondari associati alla risposta infiammatoria.
I risultati scientifici degli ultimi anni lasciano ben sperare su un progresso tecnologico che permetterà l'aumento del numero delle armi di difesa anti-solare per usufruire di un'abbronzatura senza danni. Gli induttori della sintesi della melanina e delle proteine da choc termico, gli stimolatori del metabolismo energetico e della risposta immunitaria (che è parzialmente ridotta dall'esposizione agli UV) sono in corso di studio approfondito. E la possibilità di ottenere la protezione mediante somministrazione di farmaci per via orale è anch'essa allo studio.

Abstract

Until recently, the only strategy affording the protection of the skin against the harmful solar radiation was to reduce the number of impinging photons with fabrics, umbrellas, hats, sunglasses or sunscreens.
Diverting impinging photons is the first step in sun protection. It is not the only one. Non-diverted photons enter the skin and provoke molecular damages to DNA, RNA, proteins, lipids, vitamins etc. often by indirect oxidative reactions.
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The third step in sun protection is to stimulate the endogenous self-defense responses. As of today it is possible to stimulate DNA repair, energetic metabolism, heat shock proteins, immune response, etc.

INTRODUCTION

It is a truism that solar radiation is one of the major environmental factors on earth. Another major environmental factor is oxygen. Oxygen and light are essential for several life forms, and specifically for vertebrates and higher plants. Until the middle of the twentieth century, solar radiation was known to be the cause of erythema, but was substantially believed to be harmless. The discovery of the biological role of DNA in 1944 and the use of UV-C to generate mutations attracted the attention of the scientists on the importance of avoiding radiations which might be absorbed by DNA. It turned out that sunscreens produced to avoid the erythema in summertime sunbathers were able to filter off the UV-B part of solar radiation, which is both erythemogenic and absorbed by DNA.
In the second half of the twentieth century it was observed that skin cancers in humans, such as squamous and basal cell carcinomas, occurred much more frequently in the skin of zones exposed to UV (hands, face, neck) than in non-exposed regions. Work with laboratory rodents pointed out that UV-B is a complete carcinogen, and UV-A was found out to be a cancer promoter. Humans exposing themselves to solar radiation with UV-B protection experienced sagging of the skin and this led to the understanding that UVA can be harmful.
In the last decade of the twentieth century it was observed that, in the presence of oxygen, UVA damages biological macromolecules, such as DNA, lipid and proteins (1, 2, 3, 4). It was realized that UV from solar radiation can be directly absorbed by biological molecules and provoke direct damages, or generate reactive species, such as singlet oxygen or other oxygen-containing free radicals, which can provoke oxidative damages to these same macromolecules. It was therefore proposed to associate anti-oxidants to sunscreens, in order to avoid the oxidative damages generated by those photons, which happen to reach the skin notwithstanding the presence of sunscreens.

STRATEGIES TO ACHIEVE SUN PROTECTION

The biochemical analysis of organisms exposed to UV radiation led to the understanding of the mechanisms of DNA repair (5). Other repair and/or defense systems were uncovered in the skin of vertebrates. The induction of heat shock proteins, was originally thought to be elicited only by a sudden increase of the temperature. Later it was observed that cells under a variety of stresses were also induced to synthesize heat shock proteins. In recent years it was observed that the accumulation of mRNA of a major heat shock protein was associated to exposure to UVB (6) and UVA has been shown to provoke the synthesis of heat shock proteins in human skin (7). It was also observed that UV radiation from a solar simulator (UVA + UVB) provokes the degradation of NAD, the arrest of glycolysis

and cell death (8). All these and other observation led to the conclusion that the capability to simulate the responses of skin to solar radiation might be helpful in the overall protection against sun exposure.

A reasonable strategy to protect against solar radiation can therefore rest on the following actions:

1-avert impinging photons
2-scavenge reactive species
3-stimulate the endogenous mechanisms of repair

1- Avert impinging photons

Impinging photons can be averted by several artificial devices such as umbrellas, hats, clothes, sunglasses etc. It has to be pointed out that umbrellas and fabrics have a limited capability to filter off impinging photons. In some instances, wearing a shirt will still allow 10% of the impinging photons to reach the skin (9) and it is well known that, because of the reverberation of sand, being at the beach under an umbrella might well be insufficient to avoid an erythema (10).

It has been longtime believed that natural tan constitutes, per se, a great protection against solar radiation, and that, once a tan is achieved, the skin is fully protected against UV. This is not true. It has been recently shown in a cohort of several dozen individuals, by comparing the amount of DNA damage elicited by a same UVB dose in tanned and non-tanned skin of the same individual (11), that a natural tan reduces the damages by only about 50%-60%.

For people desiring or needing to be undressed under the sun, sunscreens in creams for topical application are at hand. Great progress has been made in the conception and in the cosmetic formulation of sunscreens and there are now a dozen or so which offer broad spectrum protection (UV-B and/or UV-A), are photo-stable and do not generate photo-irritations or photosensitizations (12). To offer protection against UV-B or UV-A, a molecule has to scatter or to absorb the radiation at these wavelengths. Because of the limited molar extinction coefficient of any molecule, the fraction of absorbed photons by a sunscreen topically applied in a cream with limited thickness will always be less than 100%. Moreover, when a photon is absorbed, an electron goes in an excited state and the chances are that the excitation energy be transferred to molecular Oxygen to generate a very reactive species called singlet oxygen (see 3, 4 and 12). This is true also for the so-called "physical filters" such as TiO2 and ZnO which do indeed not only scatter, but also absorb UV-A and UV-B.

It appears therefore not only that a fraction of the impinging photons does reach the skin even in the presence of sunscreens, but also that the energy of a fraction of the photons absorbed by sunscreens is used to generate singlet oxygen.

2-Scavenge reactive species

Non-absorbed photons can be directly absorbed by macromolecules such as DNA and proteins and can also strip hydrogen atoms from lipids and trigger the peroxidative cascade. Singlet oxygen can be generated ubiquitously and react everywhere within the cell or in the extra-cellular matrix. It is therefore mandatory to supplement sunscreens with ingredients able to interrupt the peroxidative cascade and to scavenge reactive oxygen species such as superoxide, hydroxyl radical and singlet oxygen. Vitamin E and Vitamin C together with Butylated Hydroxy-Toluene and Nor-Dihydro-Guaiaretic Acid and histidine are known to efficiently counteract those reactive species. Hydrogen peroxide is generated during the oxidative burst associated to the inflammatory reaction foolowing cell damage, and catalase-like molecule will be welcome to join the pano-

ply of the anti-oxidants.
There has been a considerable amount of research conducted about the possibility to induce protection by oral administration of specific anti-oxidants such as β-carotene or vitamin E, or by specific diets. The oral administration of vitamin E and vitamin C for several weeks doubles the value of the minimal erythemal dose (MED) (13). Other anti-oxidants gave deceiving results. On the other hand it is very interesting to note that the oral administration of ω-3 fatty acids seems to have a very positive effect against UV-induced skin cancer (14).

3-Stimulate the endogenous mechanisms of repair

DNA repair

It has been observed that the same dose of UV-A + UV-B originating from a solar simulator elicits different damage-repair patterns in individuals with the same MED (15). These results can be interpreted either as a consequence of the fact that the MED is not linked to DNA damage in a bi-univocal manner (15) or that individuals with the same MED have DNA repair mechanisms of different efficacy (11). It is conceivable to try and increase the efficacy of the mechanisms of the endogenous DNA repair system. The DNA repair systems are crucial: it is indeed known that when these mechanisms are impaired, genetic diseases such as Xeroderma pigmentosum (XP) or Thio Tricho Distrophy (TTD) can follow.
In humans, the expression of DNA repair genes is constitutive, not inducible. The DNA repair enzymes, however, can be protected against UV-linked oxydative damage and therefore their activity can be increased. DNA repair activity can be increased in human cells, by the topical application of liposomes containing exogenous DNA repair enzymes. Liposomes are instrumental in helping the repair enzymes to enter the epidermal cells.
One of these enzymes can be the T4endo V nuclease, which nicks DNA in the close vicinity of a pyrimidine dimer (16). Other DNA repair enzymes can be used, such as DNA photolyase. DNA photolyase binds to pyrimidine dimers in nuclear DNA and utilizes the energy of UVA radiation to undo the dimers and restitute the DNA in the undamaged form. It has been shown on human skin that the application post-UVB of Photolyase under UV-A irradiation for a half an hour removes 50% of the UV-B generated pyrimidine dimers (17). It also restores the inducibility by IFN-γ of the synthesis of ICAM –1, which is inhibited by UV-B (17). This is to say that repairing DNA restores the immune response depressed by exposure to UV-B, which is known to provoke the migration of Langerhans cells and to practically suppress Contact Hypersensitivity (CHS) and Delayed-Type Hypersensitivity (DTH) (18). Interestingly enough, if UV-B provokes the suppression of CHS and DTH, it does not negatively interfere with the production of antibodies against specific antigens in the course of a vaccination (19).

ENERGETIC METABOLISM

When DNA is damaged by UV, the metabolism of the cell is affected. In particular Nicotinamide Adenosine Dinucleotide (NAD) is split in ADP ribose and nicotinamide, glycolysis is arrested and ATP synthesis impaired. The consequence of this is cell death, with the possible triggering of the arachidonic acid cascade and the inflammatory consequences, of which erythema is but one aspect.
It has been shown that the topical application post-UV, of liposomes containing NAD reduces dramatically the intensity of the erythema (8) as if the addition of NAD could help maintaining electron transport and synthesis of ATP at a le-

vel sufficient for keeping the cells alive. Strategies for maintaining the level of energetic metabolism in UV aggressed cells can be designed and ingredient have been found to boost the synthesis of ATP.

HEAT SHOCK PROTEINS

When a cell is subjected to stress (temperature increase, treatment with heavy metals, exposure to modified amino acids, treatment with prooxydants) it stops the synthesis of all proteins except those belonging to a family which, for historical reasons, has been termed the family of heat shock proteins (hsp).

It has been reported that exposure to solar simulators provokes the accumulation of heat shock mRNA (6) and heat shock proteins (7). The role of these proteins is to help nascent polypeptides to fold in the correct tertiary structure upon leaving the ribosomes where they are synthesized, and also to help refolding those proteins the structure of which has been somehow perturbed by the stress. The synthesis of heat shock proteins requires a few hours after the stress and is a response to an aggression, not a prevention against it. It can be surmised that sun protection might be improved by achieving the prevention against solar aggression by using gratuitous inducers of hsp synthesis.

Gratuitous inducers of hsp synthesis are innocuous substances able to stimulate the response of self-defense without representing, per se, an aggression to the skin. Topical application of gratuitous inducers of heat shock proteins a few hours before exposing one-self to the aggression of sunlight or of other environmental aggressors could be one of the newest and most efficient ways to accompany sunscreens and anti-oxidants to achieve sun protection.

DISCUSSION

The effects of solar radiation, in particular of ultraviolet radiation have been studied for more than a century on a variety of organisms, ranging from bacteria to plants to man. A part of our knowledge on the effects of solar radiation on human skin, hair and eye is summarized in a monograph of the European Society for Photobiology, published in 2001, which I was asked to be the editor of. The majority of information in this paper is described in full detail in chapters of that monograph.

When learning about the effects of an aggression on an organism, as well as when learning about he endogenous reactions of the organisms to those aggressions, it comes spontaneously to the mind how to design a certain amount of strategies to help fighting the aggression.

In this paper I have exposed a Three Step method to achieve sun protection, which consists in averting impinging photons, scavenging reactive species generated by non-averted photons and boosting the endogenous defense mechanisms. This third step might be divided in two sub-steps, i.e. enhancing the self defense (activate DNA repair, induce the synthesis of heat shock proteins) and restoring physiological functions which are impaired by the aggression (boost energetic metabolism, restore immune response).

Active ingredients can be added to cosmetics to achieve the three steps of sun protection. Others will be found in the future, to make protection and repair as complete as possible

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N-3 AND N-6 PUFAS IN HEALTHY AND DISEASED SKIN

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Summary

Western type diets do hardly provide the recommended intakes of certain long-chain polyunsaturated fatty acids (LC-PUFAs), although research revealed their functionality and essentiality that was even honored with a Nobel prize in 1982.
LC-PUFAs exert 2 major biological functions:

• **Structural elements of membranes**

A double layer of phospholipids, containing besides other FA also LC- PUFAs (arachidonic acid, docosahexaenoic acid), is responsible for the particular properties of membranes. They are highly selective permeability barriers that carry metabolic active proteins like the energy conversion process, gap junctions to control the flow of information between cells and receptors for external stimuli. The transdermal waterloss is mostly due to the linoleic acid content of the skin, although γ-linolenic acid might be as important as well.

• **Messengers**

All LC-PUFAs containing 20 carbon atoms are precursors of eicosanoids. This includes

• dihomo-γ-linolenic acid (DGLA): C20:3 ω-6
• Arachidonic acid (AA): C20:4 ω-6
• Eicosapentaenoic acid (EPA): C20:5 ω-3

Eicosanoids act as local hormones interfering with cellular regulation. In the skin, they can evoke pro-inflammatory or anti-inflammatory reactions, depending on the precursor fatty acid. A balanced application of ω-3 and ω-6 PUFAs, either by food or by supplements, may thus alleviate inflammatory skin disorders.
Based on these multiple involvements in our metabolism and their importance for health, the following recommendations have been elaborated by scientific societies and authorities:
Dnring a workshop on the essentiality of ω-6 and ω-3 fatty acids, April 7-9 1999, in Bethesda, a daily intake of about 0.65 g EPA plus DHA /day has been recommended. This is in good agreement with dietary guidelines, revision 2000, by the American Heart Association and with the qualified health claim issued by the FDA on October 31, 2000, and other scientific recommendations. Since this can hardly be achieved by eating fish only by a majority of the population, alternative sources have to be explored.

Riassunto

La cultura occidentale raccomanda con "poco calore" l'inserimento nella dieta di acidi grassi polinsaturi (LC-PUFAs) anche se gli studi ne hanno rivelato la funzionalità e la essenzialità, addirittura con un Nobel nel 1982.
Gli LC-PUFAs svolgono principalmente 2 funzioni biologiche come:

- Elementi strutturali delle membrane

Un doppio strato di fosfolipidi contenente oltre che altri acidi grassi anche LC-PUFA (acido arachidonico e docosaesanoico) è responsabile delle particolari proprietà delle membrane. Queste membrane sono barriere permeabili altamente selettive e necessarie per il trasporto di proteine metabolicamente attive e indispensabili per la conversione del processo energetico; fungono inoltre da messaggeri nel controllo del flusso delle informazioni tra le cellule e i recettori per gli stimoli esterni.
La perspiratio insensibilis è legata essenzialmente alla presenza nella pelle dell'acido linoleico, anche se svolge una funzione importante anche l'acido γ linolenico.

- Messaggeri

Tutti gli LC-PUFAs che contengono 20 atomi di carbonio sono precursori degli eicosanoidi come:
- l'acido diomo-γ-linolenico (DGLA) C20:3 ω-6
- l'acido arachidonico (AA) C20:4 ω-6
- l'acido eicosapentenoico (EPA) C20:5 ω-3

Gli eicosanoidi agiscono come ormoni locali che interferiscono con la regolazione della vita cellulare. Nella pelle possono indurre reazioni pro-infiammatorie o anti-infiammatorie in dipendenza del precursore dell'acido grasso.
Un'assunzione bilanciata di PUFAs ω-3 e ω-6, sia attraverso gli alimenti che attraverso dietetici, può alleviare eventuali processi infiammatori a carattere patologico.
La comunità scientifica e le autorità hanno perciò elaborato le seguenti raccomandazioni nell'uso dei PUFAs. Dati i loro molteplici coinvolgimenti nei nostri processi metabolici e visto il ruolo importante che rivestono per la salute, il 7-9 aprile del 1999 durante un meeting svoltosi a Betesda, è stata raccomandata l'assunzione giornaliera di circa 0,65g di EPA più DHA. Queste raccomandazioni sono in accordo con le linee guida alimentari consigliate sia nel 2000 dall'Associazione Americana per il Cuore che con quanto raccomandato dall'FDA il 31 ottobre, 2000.
Poiché per la maggior parte della popolazione risulta difficile mangiare più frequentemente la carne di pesce, ricca appunto di PUFAs, è necessario trovarne fonti alternative.

N-3 and n-6 PUFAs in healthy and diseased skin

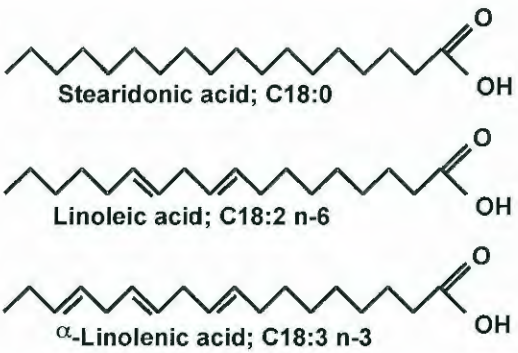
Good health depends on good nutrition. This simple relationship urged US authorities to establish the first recommendations for nutrients in 1943, which are currently being revised for the 11th time. Since their 1st publication, many nutrients have been identified to be critical for an adequate intake. To this list we have to add the long-chain polyunsaturated fatty acids (LC-PUFAs) because their availability in the food chain is limited. In order to bridge the gap between an inadequate intake and requirement, food items can been fortified or the deficiency can be bridged by supplements.

During the 50ies the essentiality of linoleic acid has been recognized since infants fed a linoleic acid deficient formula present a drying and flaking skin[1].

In 1989 only, the essentiality of ω-3 fatty acids has been discussed in the US, but no RDA has been issued due to lack of data[2]. Today, the requirement of α-linolenic acid (ω-3) as well as of linoleic acid (ω-6) is well accepted.

Both, linoleic acid (LA) and α-linolenic acid (ALA) must be elongated and desaturated; the former to dihomo-γ-linolenic acid (DGLA) and arachidonic acid (AA) and the latter to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for full biological activities. Research during the past years revealed that the conversion of ALA to EPA and DHA is rather slow and insufficient in human beings. Therefore, several nutrition societies added EPA and DHA to the list of recommended nutrients taking into account that mankind should have access to these highly unsaturated fatty acids directly and not only via the precursor. Recent studies clearly show a health benefit from the intake of the highly unsaturated PUFAs γ-linolenic acid (GLA, 18:3 ω-6), eicosapentaenoic acid (EPA, 20:5 ω-3) and docosahexaenoic acid (DHA, 22:6 ω-3).

Fatty acids (FA) consist of a carbon chain of up to 24 atoms linked by single or double bonds and an acid group at the end. Double bonds occur either after the 3rd carbon atom leading to the ω-3 or n-3 FA, or after the 6th carbon atom (ω-6 or n-6 FA) (Fig.1). Animals and humans



lack the enzyme that is responsible to introduce the 2nd double bond at the correct position; therefore, for us, the precursor of all functional ω-6 FA, linoleic acid (18:2 ω-6) and the precursor of all functional ω-3 FA, α-linolenic acid (18:3 ω-3), are essential. Between two double bonds there are always two single bonds, which renders the PUFAs vulnerable against oxygen radicals. Furthermore, all double bonds are in the cis-configuration.

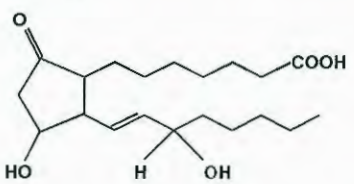
Thus, FA can be classified by following categories:

- Saturated (without double bonds)
- Unsaturated (with one or more double bonds)
- Monoenes (with one double bond) example: oleic acid in, e.g., in olive oil
- Polyenes (with two or more double bonds)
- ω-3 PUFA (1st double bond in position 3) example: EPA and DHA in fish oil
- ω-6 PUFA (1st double bond in position 6) example: γ-linolenic acid in borage oil and arachidonic acid in animal fat.

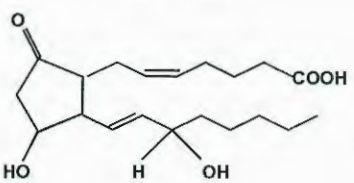
In a healthy diet all these categories have to be balanced against each other for a proper functioning of our metabolism.

Fatty acids exert 3 functions in the metabolism and its regulation:

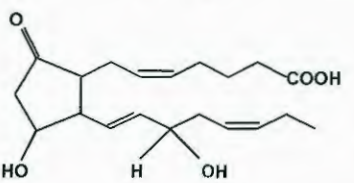
- **Source of energy:**
  Although this concerns mostly medium- and short chain saturated FA it is noteworthy that the FA are an excellent source of energy.
- **Structural elements of membranes**
  FA are the building elements for phospholipids that aggregate to a double layer to form the membranes. They are highly selective permeability barriers that carry other structural elements like proteins. These elements are responsible for metabolic activities like the energy conversion process, control of the information flow between cells, and they contain receptors for external stimuli. LC- PUFAs such as arachidonic acid docosahexaenoic acid gives them the particular physical property.
- **Messengers**
  All LC-PUFAs containing 20 carbon atoms are precursors of eicosanoids (Fig. 2). This includes



PGE_1
(ex DGLA)



PGE_2
(ex AA)



PGE_3
(ex EPA)

- dihomo-γ-linolenic acid (DGLA): C20:3 ω-6
- Arachidonic acid (AA): C20:4 ω-6
- Eicosapentaenoic acid (EPA): C20:5 ω-3

Cyclooxygenase and lipoxygenases convert these fatty acids into the corresponding prostaglandins, leukotrienes, thromboxanes, prostacylins and hydroxy-fatty acids.

Eicosanoids act as local hormones interfering with cellular regulation. They are involved in inflammation, regulation of blood flow, control of ion transport, modulation of synaptic transmission, termination of pregnancy, etc.

Prostaglandins and leukotrienes originating from DGLA or EPA are by far less active as pro-inflammatory agents than the corresponding derivatives of AA[3]. The intake of fish oil derived fatty acids leads to a decrease of AA in the membranes and, thus, to a lower production of the strongly pro-inflammatory eicosanoids, because the enzymes involved in the PUFA metabolism are the same[4].

The human epidermis has a limited capacity to metabolize fatty acids. It lacks the enzymes to introduce new double bonds, e.g. it cannot desaturate LA to GLA or DGLA to AA. However, the elongase is present, which converts GLA to DGLA[5]. This opens the unique opportunity to control the production of the pro-inflammatory eicosanoids by the supplementation of the competing fatty acids, GLA and EPA. Unfortunately, there is no direct source for DGLA, but GLA can be taken instead.

Furthermore, the epidermis expresses only the 15-lipoxygenase, but not the 5-lipoxygenase besides the cyclooxygenase. Therefore, the following set of eicosanoids can be produced by the epidermis:

- Out of arachidonic acid (20:4 w-6), which makes up 9 % of fatty acids in epidermal phospholipids:
  by the cyclooxygenase pathway: PGE_2, $PGF_{2\alpha}$, PGD_2;
  by the 15-lipoxygenase pathway: 15-Hydroxyeicosatetraenoic acid (15-HETE)
- Out of LTA4 derived from stimulated leukocytes:
  by leukotriene A_4 hydrolase: LTB_4
- Out of dihomo-γ-linolenic acid (20:3 ω-6),

which is only a minor constituent of phospholipids:
by the cyclooxygenase pathway: PGE_1;
by the 15-lipoxygenase pathway: 15-Hydroxyeicosatrienoic acid (15-HETrE)

- Out of eicosapentaenoic acid (20:5 ω-3), which is not present in normal epidermis:
by the 15-lipoxygenase pathway: 15-Hydroxyeicosapentaenoic acid (15-HEPE)
- Out of docosahexaenoic acid (22:6 ω-3), which is not present in normal epidermis:
by the 15-lipoxygenase pathway: 17-Hydroxydocosahexaenoic acid (17-HoDHE)

15-Lipoxygenase products inhibit the formation of the chemotactic attractant LTB_4 in a dose dependent manner, the 15-HETrE being the most potent one. The 50 % inhibition of the 5-lipoxygenase occurs at 13 µmol/L 15-HETrE, 26 µmol/L 15-HEPE res. 17-HoDHE and at 40 µmol/L 15-HETE[6].

The significant interaction between the various sources of eicosanoids has been proven in numerous studies. Most of them have in common that AA derived eicosanoids have been counterbalanced by an other precursor given either as supplement or with food.

In a 120 day study 11 healthy men received a standard diet for 30 days followed by either 6 g DHA for the next 90 days (n=7) or LA (n=4). Both diets were supplemented by 20 mg α-tocopherylacetate to assure an adequate intake of vitamin E to protect the LC-PUFAs from oxidation. The DHA concentration in the phospholipids of peripheral blood mononuclear cells (PBMNC) increased from 2.3 wt% to 7.4 wt% in the verum group on the expense of AA that decreased from 19.8 wt% to 10.7 wt%. At the end of the study, lipopolysaccharide stimulation of PBMNC resulted in 60-75% lower release of prostaglandin E_2 and leukotriene B_4[7].

In order to test the health benefit of GLA, 160 patients suffering from atopic eczema received either 3 g borage oil (690 mg GLA) or a placebo for 24 weeks. The reduction of Costa score points was similar in both groups, although improvement of individual symptoms was observed in the verum group. An analysis of a subgroup taking into account the compliance of the patients showed that the volunteers of the verum group used significantly less diflucortolone-21-valerate cream than the participants of the placebo group[8]. This study underlines the usefulness of nutrients as a complementary measure during a normal, standard treatment.

These examples illustrate that membranes carrying mainly AA as LC-PUFA can only synthesize the strongly pro-inflammatory eicosanoids PGE_2, LTB_4 etc. Adding competitive precursor FA, GLA or EPA/DHA, results in a balanced reaction of the immune system.

CONCLUSION

The long-chain fatty acids arachidonic acid, dihomo-γ-linolenic acid and eicosapentaenoic acid are essential for the regulation of the immune system. γ-Linolenic acid can be elongated in the skin to DGLA and is, therefore, a suitable precursor. GLA as well as the fish oil fatty acids EPA/DHA are required for a balanced synthesis of eicosanoids and thus important for skin health. LC-PUFAs are useful for a complementary treatments, they do not replace medical measures. A balanced diet should contain ω-6 and ω-3 LC-PUFAs in a ratio of 5:1. Current recommendations for an intake of EPA/DHA vary between 0.65 g/d (ISSFAL)[9] and 1.1 g/d (BNF)[10]

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Oral N-acetylglucosamine supplementation improves skin conditions of female volunteers: Clinical evaluation by a microscopic three-dimensional skin surface analyzer*

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Summary

Within the skin tissues, acidic mucopolysaccharides such as hyaluronic acid are present in the corium layer and play a large part in water retention and skin resilience. Hyaluronic acid is a polymer composed of dimers containing N-acetylglucosamine and glucuronic acid. Although applications of the use of hyaluronic acid in cosmeceutical food have been reported, the beauty efficacy of orally-ingested hyaluronic acid cannot be predicted adequately because little is known about its digestion and absorption in humans.

The purpose of this study was to investigate the effect of long-term oral N-acetylglucosamine supplementation on skin conditions in females who have a common tendency of xeroderma and rough skin. The subjects (average age: 25.5 ± 10.7) were assigned randomly and double-blind to either a N-acetylglucosamine group (n=11) or a placebo group (n=11), and ingested a daily 1000-mg dose of N-acetylglucosamine or lactose, respectively, for 60 days.

Dermatological examination by doctors suggested that N-acetylglucosamine supplementation favorably affects skin conditions; that is, improvements were observed in the desiccation of facial and whole body skin. After N-acetylglucosamine supplementation for 60 days, the moisture content of the region below the left eye was increased significantly; conversely, a significant decrease in the oil and fat content was observed. In addition, clinical evaluation by a microscopic three-dimensional skin surface analyzer confirmed that oral N-acetylglucosamine supplementation is useful for mitigating the roughness of the skin and the epidermolysis of the corneum. These results indicate that oral N-acetylglucosamine supplementation may be of benefit in enhancing skin hydration. By contrast, no significant improvement was observed in the skin condition of the placebo group, as appraised by either dermatological examination or digital analysis. The beautification effect produced by ingestion of N-acetylglucosamine indicates that this compound may be a potential ingredient for cosmeceutical foodstuffs.

Riassunto

I Mucopolisaccaridi, quali l'acido ialuronico, sono presenti nel derma e svolgono il fondamentale ruolo di trattenere e legare l'acqua a livello della cute.

L'acido ialuronico è un polimero composto di dimeri contenenti N-acetil-glucosamina e acido glucuronico.
Sebbene sia già stato riportato l'uso dell'acido ialuronico quale componente di nutra-cosmeceutici, la sua efficacia orale nel migliorare l'aspetto estetico della cute, è difficile da sostenere essendo poco noti a livello dell'organismo umano sia i processi digestivi che l'assorbimento di questa macromolecola.
Lo scopo di questo studio è stato di verificare l'effetto a lungo termine dell'assunzione orale di un dietetico a base di N-glucosamina sulla cute di donne con tendenza alla xerosi.
Ai soggetti (di età media di 25,5± 10,7) è stata assegnata a doppio ceco ed in maniera casuale la somministrazione orale per 60 giorni sia di confezioni di prodotto a base di N-acetil-glucosamina (1000mg per dose) che di placebo a base di lattosio.
Secondo osservazioni fatte da dermatologi, la sperimentazione con N-acetil-glucosamina sembra migliorare notevolmente l'idratazione cutanea dopo 60 giorni di trattamento, soprattutto a livello dell'area periorbitale. Questi miglioramenti sono stati evidenziati a livello cutaneo anche mediante l'uso del microscopio tridimensionale.
Secondo questi primi risultati l'uso orale della N-acetil-glucosamina può essere utile nel ridurre lo stato di secchezza della pelle e l'epidermolisi dello strato corneo, potenziando l'idratazione cutanea. Al contrario, il placebo non ha modificato l'aspetto della pelle secondo l'osservazione medica e l'analisi digitale.
L'N-acetil-glucosamina può, quindi, essere considerata quale ingrediente cosmetico utile per la formulazione di nutracosme-ceutici.

Abstract

Twenty-two females with a common tendency of xerosis and rough skin (average age: 25.5 ± 10.7) were assigned randomly and double-blind to either an N-acetylglucosamine (NAG) group (n=11) or a placebo group (n=11). The NAG group received supplements of 1000 mg of NAG per day for 60 days, and the placebo group received supplements of an equivalent amount of lactose. Before dermatological examination, all subjects were kept for at least 30 min in a room under controlled temperature (18-20 °C) and humidity (45-60%). Dermatological examination by a doctor suggested that NAG supplementation resulted in a significant improvement in the dessication and flushing of facial skin. After NAG supplementation for 60 days, the moisture content of the region below the left eye was increased significantly, whereas the oil and fat content had decreased significantly. The surface of the skin was analyzed by a microscopic three-dimensional skin surface analyzer, which showed that the smoothness had improved and the degree of dryness of the corneum was reduced in the NAG group, but there were no significant improvements in the placebo group. We have confirmed that oral NAG supplementation may enhance skin hydration and improve in skin roughness; therefore, NAG may be useful as an ingredient of cosmeceutical foodstuffs.

INTRODUCTION

Within the skin tissues, acidic mucopolysaccharides and collagen are present mainly in the corium layer and play a large part in water retention and skin resilience (1, 2). Hyaluronic acid (HA), an acidic mucopolysaccharide, is a polymer composed of dimers containing NAG and glucuronic acid, and has high hydration capacity owing to its hydrophilic groups. It is reported that the HA present in human skin changes structurally with aging (3, 4), and that it alters quantitatively in the dorsal skin of hairless mice exposed to chronic UV irradiation (5). Some studies have demonstrated that there is a distinct decrease of HA in skin during maturation (2, 6-8). Currently, applications of HA have been recognized as an effective means by which to maintain the moisture retention and resilience of skin, thereby diminishing the appearance of rough skin, and fine lines, etc. HA is a good biomaterial, and has been shown by autoradiography to permeate all layers of intact skin in humans (9). A few applications of HA in cosmetics (10) and wound healing material (11) have been reported before now. However, because little is known about the digestion and absorption of orally-ingested high molecular mass compounds such as HA, the beneficial effects cannot be predicted adequately.

On the other hand, a report has indicated that orally-ingested NAG is absorbed rapidly from the intestine and reaches the cutaneous layer (12). Interestingly, it was observed that the NAG in HA and dermatan sulfate was deacetylated and quautitatively decreased in aging human skin (3). Breborowicz et al. demonstrated that exogenous NAG rapidly stimulated the production of HA by human peritoneal mesothelial cells and fibroblasts (13). Accordingly, it is presumed that NAG has a role in maintaining the amount or the chemical structure of HA.

The purpose of this study was to investigate the effect of 60 days of oral NAG supplementation on the skin conditions of females with a common tendency of xerosis and rough skin. The moisture content, oil and fat content, and acidity of skin were measured in addition to the doctor's dermatological examination. Furthermore, the skin conditions (smoothness, dryness, scaliness, and number of skin wrinkles, etc.) were evaluated clinically by a microscopic three-dimensional skin surface analyzer.

For this study, rather than using "synthetic NAG", we used "natural NAG" as the test material; that is, natural NAG obtained by partially hydrolyzing the high molecular mass polysaccharide, chitin, which is found in the shells of

crustacea. By contrast, synthetic NAG is produced by chemical synthesis (N-acetylation) from D-glucosamine hydrochloride, which is prepared by the complete hydrolysis of chitin with concentrated hydrochloric acid. This synthetic NAG is not suitable for use as a food additive, however, because there is little information on its safety in humans. We therefore used the natural form of NAG, which had been manufactured without chemical synthesis.

MATERIAL AND METHODS

Subject

Twenty-two subjects volunteered for this study. The subjects were females with a common tendency of xerosis and rough skin (average age: 25.5 ± 10.7) and the moisture content of their skin on the inside of left upper arm was less than 50 % when analyzed on 17th October 1999 (weather, cloudy; temperature, 20.5 °C; humidity, 51 %). We ascertained that they had not taken any medications and had not received any medical treatments before the beginning of the study. This study was performed according to the Helsinki declaration. All subjects were informed of the purpose of the study before giving their written, informed consent.

Experimental design

Before the investigation, subjects were matched and divided randomly and double-blind into two matched groups: the NAG group (n=11) and the placebo group (n=11). No significant differences between the two groups were noted in age, height, body mass, daily sack duty, or irradiation time of direct sunlight (Table A). The NAG group received supplements of 1000 mg of NAG per day for 60 days, and the placebo group received supplements of an equivalent amount of lactose. The supplementary NAG was not synthesized by the N-acetylation of glucosamine hydrochloride, but instead was a natural form (Marine Sweet®, Yaizu Suisankagaku Industry Co., Ltd, Japan) separated after the partial hydrolysis of chitin. In principle, a dermatological inspection was carried out just before the ingestion period, and then 30 and 60 days after daily ingestion had begun. Before dermatological examination, all subjects remained for at least 30 min in a room under controlled temperature (18-20 °C) and humidity (45-60%). The subjects were asked to come to the laboratory as near to the same hour as possible, wearing a long-sleeved blouse or T-shirt and not a sweater. Furthermore, all subjects were asked to refrain from applying cosmetics to the inspection area of the skin for at least 60 min before the examination started.

INSPECTION PROCESS

1. Dermatological examination and doctor's questions

For the whole body, there were four ranks of evaluation (0, no symptoms; 1, slight; 2, medium; 3, severe) with respect to pruritus, desiccation, flushing, erosion, desquamation, papula, vesicle and tumefaction. For the face, the same four ranks of evaluation were used with respect to cosmetic dermatitis, desiccation, flushing and spread of cosmetics. In addition, the amelioration of overall symptoms, including observations of the whole body and face, was evaluated as a general observation. These evaluations we-

Table A

Physical characteristics of subjects in the NAG group and the placebo group.

	NAG group	*Placebo group*
Age (y)	25.5±10.7	25.5±11.2
Height (cm)	157.4±2.6	158.7±4.2
Body mass (kg)	52.6±5.6	52.9±4.2
Daily sack duty (h/d)	7.2±1.2	6.4±1.1
Irradiation time of direct sunlight (h/d)	1.7±1.4	2.6±2.5

Data represent the mean±standard deviation for each group (n=11). No significant differences were noted between the two groups in any category.

re conducted by several doctors accredited by the Japan Dermatologic Science Society.

2. Moisture content, oil and fat content, and acidity (pH)

The moisture content was measured by a Corneometer CM825 (Courage+Khazaka Electronic Gmbh, Germany), which determines the moisture content of the epidermis by measuring the electrostatic capacity of the corneal layer. The oil and fat content was measured by using a Sebumeter SM810 (Courage+Khazaka Electronic Gmbh, Germany), in which a special tape that absorbs only oil and fat is attached to the measuring region for 30 seconds. The oil and fat content is then estimated by the change in the light transmittance of the tape. The acidity was electrochemically measured by a PH900 (Courage+Khazaka Electronic Gmbh, Germany). The regions for measuring the moisture content and acidity included an area 1 cm below the left eye, the inside of the left upper arm (3 cm above the elbow), and the poll (3 cm below the spinous process of neck region). The oil and fat content was analyzed in the region below the left eye.

3. Analysis by a microscopic three-dimensional skin surface analyzer

This analysis was conducted by using a digital analyzer of the skin surface (VISIONSCAN, Courage+Khazaka Electronic Gmbh, Germany). The skin surface was irradiated with special ultraviolet ray source, and the image was taken by a high performance CCD camera and digitalized for evaluation. As in the measurements of moisture content, etc, described above, the skin areas analyzed included the region below the left eye, the inside of the left upper arm, and the poll. The following factors were used as parameters.

(a) SEsm (skin smoothness): this value is calculated from the average of the width and depth of wrinkles, and is an index that indicates the smoothness of skin. A lower value of SEsm indicates a smoother skin surface.

(b) SEr (skin roughness): this value is obtained by calculating the ratio of points that are darker than the set-up points across the whole image, and is an index that indicates the roughness of skin. A higher value of SEr indicates a rougher skin surface.

(c) SEsc (skin scaliness): scaly areas are identified as brighter than the set-up values in the image. The SEsc value is obtained by calculating the ratio of these brighter areas relative to the whole investigation area, and is an index that indicates the degree of dryness of the corneum (i.e., the amount of scale). A lower value of the SEsc indicates that the skin has a greater moisture content and a lesser amount of scaliness (epidermolysis).

(d) SEw (skin wrinkles): this value shows the number and width of the surface texture or the wrinkles in both the vertical and horizontal direction of the skin. A higher value of SEw indicates that the skin has a greater number of wrinkles, and that the width of these wrinkles is wider.

Statistical analysis

The non-parametric Wilcoxon's rank test was used to analyze differences between pre- and postsupplementation values. Comparison of the characteristics for subjects of the NAG group and the placebo group was evaluated with the non-parametric Mann-Whitney test. A p-value of less than 0.05 was taken as a significant difference.

RESULTS

Doctor's dermatological examination

Table B shows the effect of 30 and 60 days of NAG or placebo supplementation assessed by the doctor's dermatological examination. All data represent the mean of scores evaluated on the basis of four rank standards. A reduction in the score suggests that particular condition of the skin has improved. In the NAG group, when compared with the conditions of presupplementation, the symptoms of 'desiccation' and 'flushing' of facial skin were improved significantly after 30 and 60 days of supplementation. Furthermore, a significant improvement in the 'spread of cosmetics' of the NAG group was observed after 60 days of supplementation. By contrast, no significant improvements were observed for any category in the placebo group.
With respect to symptoms of the whole body, significant improvements were recognized in the category of 'desiccation' in both the NAG group and the placebo group. In the overall assessment, significant improvements were observed in the NAG group, but not in the placebo group.

Table B

Doctor's pre- and postsupplementation dermatological examination for the NAG group and the placebo group

	NAG group				Placebo group			
	Number of subject with a symptom	pre[a]	post[b] (after 30 d)	post[b] (after 60 d)	Number of subject with a symptom	pre[a]	post[b] (after 30 d)	post[b] (after 60 d)
Face								
Cosmetic dermatitis	3	1,00	1,00	1,33	0	—	—	—
Desiccation	11	2,00	1.18**	1.00*	11	2,00	1,73	1,55
Flushing	10	1,80	1.10*	1.10*	10	1,60	1,40	1,30
Spread of cosmetics	6	1,83	1,33	0.83*	6	2,17	1,50	1,67
Whole body								
Pruritus	10	1,40	0,90	0,60	5	1,80	1,60	1,00
Desiccation	11	2,09	1.36*	1.00*	11	2,00	1.55*	1.45*
Flushing	5	1,40	0,80	0,40	5	1,80	1,20	1,00
Erosion	1	2,00	1,00	0,00	1	2,00	1,00	1,00
Desquamation	3	1,33	1,33	1,00	2	1,00	1,00	1,00
Papula	2	1,00	1,50	1,50	2	1,00	1,50	1,00
Vesicle	1	1,00	2,00	2,00	1	1,00	1,00	2,00
Comprehensive observation	11	1,82	1.27*	1.09*	11	1,64	1,18	1,27

Data represent the mean number of subject (s) showing that symptom.
[a]at presupplementation of tested sample. [b]at postsupplementation of tested sample.
*$p<0.05$ from corresponding value at presupplementation. **$p<0.01$ from corresponding value at presupplementation.

Moisture content, oil and fat content, and acidity (pH)

Changes in the moisture content, oil and fat content, and acidity of each region for both groups are shown in Table C. In the NAG group, the moisture content of the region below the left eye increased significantly from 48.0 % to 58.8 % and to 56.2 % after respectively 30 days and 60 days of supplementation. Conversely, the oil and fat content in the same region was decreased significantly from 63.8 % to 40.8 % after 60 days of NAG supplementation. By contrast, there was a significant decrease in the moisture content of this region in the placebo group after 60 days of supplementation. The analysis of the skin acidity showed that there were no significant changes in the pH of any of the measured regions of the NAG group during the supplementation period; however, the pH of the inside of the left upper arm of the placebo group was increased significantly from 5.4 to 5.7 after 30 days of supplementation.

Table C

Moisture content, oil and fat content, and acidity in the NAG group and the placebo group

	NAG group			Placebo group		
	pre[a]	post[b] (after 30 d)	post[b] (after 60 d)	pre[a]	post[b] (after 30 d)	post[b] (after 60 d)
Moisture content (%)						
Region below the left eye	48.0±8.8	58.8±14.2*	56.2±8.4**	58.6±11.7	57.8±10.4	48.1±10.2*
Inside of left upper arm	37.8±7.8	38.7±5.8	36.2±6.8	37.6±9.5	36.5±6.7	32.2±8.5
Poll	51.3±5.6	51.9±5.4	52.1±10.9	51.7±18.9	49.2±14.1	53.6±20.8
Acidity (pH)						
Region below the left eye	6.0±1.0	5.7±0.5	5.8±0.4	5.7±0.6	5.7±0.5	5.8±0.5
Inside of left upper arm	5.5±0.3	5.6±0.3	5.7±0.4	5.4±0.4	5.7±0.4*	5.6±0.5
Poll	5.7±1.1	5.4±0.3	5.3±0.4	5.7±0.7	5.5±0.4	5.6±0.4
Oil and fat content (%)						
Region below the left eye	63.8±42.3	53.0±29.2	40.8±18.7*	37.1±32.2	30.1±20.3	42.6±30.1

Data represent the mean±standard deviation for each group (n=11).
[a]at presupplementation of tested sample. [b]at postsupplementation of tested sample.
*$p<0.05$ from corresponding value at presupplementation. **$p<0.01$ from corresponding value at presupplementation.

Analysis by a microscopic three-dimensional skin surface analyzer

Table D summarizes the changes in each parameter obtained by digital image analysis. In the NAG group, a significant decrease compared with the values before supplementation was observed in the SEsm value and the SEsc value at the poll, whereby it was found that the smoothness of skin was recovered, the dryness of corneum was reduced and the scaliness was decreased. By contrast, no significant improvement was observed in the SEsm value and the SEsc value at any of the investigated regions in the placebo group. With respect to the SEr value and the SEw value, neither the NAG group not the placebo group showed significant changes at any of the investigated regions. The analysis of these parameters showed that oral NAG supplementation is useful for mitigating the roughness of skin and epidermolysis of the corneum.

Table D

Parameters obtained by the microscopic three-dimensional skin surface analyzer in the NAG group and the placebo group

	NAG group			Placebo group		
Parameter[a]	pre[b]	post[c] (after 30 d)	post[c] (after 60 d)	pre[b]	post[c] (after 30 d)	post[c] (after 60 d)
SEsm (desired value: lower)						
Region below the left eye	378,1	337,6	309,4	379,6	398,8	353,7
Inside of left upper arm	384,7	324,7	379,9	335,7	373,1	335,1
Poll	443,8	338.9*	345.2*	387,2	414,1	366,2
SEr (desired value: lower)						
Region below the left eye	0,25	0,26	0,21	0,24	0,23	0,24
Inside of left upper arm	0,30	0,22	0,28	0,10	2,51	0,22
Poll	0,51	0,32	0,40	0,35	0,33	0,44
SEsc (desired value: lower)						
Region below the left eye	238,4	137,0	133,0	166,8	146,7	158,0
Inside of left upper arm	342,9	195,8	202,4	214,1	256,1	246,5
Poll	352,4	201,6	169.3*	231,8	219,7	202,2
SEw (desired value: lower)						
Region below the left eye	33,2	29,0	27,8	29,8	33,6	33,7
Inside of left upper arm	30,2	26,4	31,9	23,7	28,1	24,4
Poll	27,3	23,4	29,5	27,5	26,0	29,3

Data represent the mean number of subjects in each group (n=11).
[a]The parameters are as follows: SEsm, skin smoothness; SEr, skin roughness; SEsc, skin scaliness; SEw, skin wrinkles.
[b]at presupplementation of tested sample. [c]at postsupplementation of tested sample.
*$p<0.05$ from corresponding value at presupplementation.

DISCUSSION

Before now, conventional examinations carried out by dermatologists have been usually applied to appraise clinical studies on the beautification action of sample compounds, including external preparations and cosmetics. Thus, for large-scale examinations, differences in the appraisal methods of each participating doctor has been likely to affect the test results. It is assumed that the credibility of the test results may depend mostly on the skill of the doctor. In this study, therefore, we have introduced a non-human clinical evaluation method using a digital analyzer, in addition to the standard evaluation by the doctor.

The doctor's examination suggested that long-term oral NAG supplementation (1000 mg per day) favorably affects skin conditions; that is, improvements were observed in the desiccation of facial and whole body skin. After NAG supplementation for 60 days, the moisture content of the region below the left eye was increased significantly; conversely, there was a significant decrease in the oil and fat content. Judging from the fact that most females who have oily skin are concerned about make-up deterioration, an ability to decrease in the oil and fat content of facial skin is very desirable. In addition, clinical evaluation by a microscopic three-dimensional skin surface analyzer confirmed that oral NAG supplementation is useful for mitigating the roughness of skin and epidermolysis of the corneum. These results indicate that oral NAG supplementation may be of benefit to enhancing skin hydration. To our knowledge, we have shown for the first time that NAG, a constituent element of HA, has the capacity to improve the skin condition of humans, and we have demonstrated this action per os clinically.

For the placebo group, either no significant improvement was observed in skin condition as appraised by dermatological examinations and digital analysis, or the skin condition got seemingly worse; that is, there was a decrease in the moisture content of the region below the left eye and an increase in the pH of the inside of the left upper arm. It is thought that the pH condition of the placebo group deteriorated slightly because a higher pH value has been reported as a characteristic of disturbances in skin physiology (14). The present study was carried out in autumn (that is, between the end of October and the end of December); therefore, environmental factors accompanying the alteration in climate might influence the results of our study. We consider that, owing to the cold climate and drier air of the autumn environment, the skin conditions of the placebo group showed a tendency toward steady deterioration. We presume that the oral supplementation of lactose (the placebo) did not influence the deterioration of skin conditions, which were caused by climate alteration.

In summary, we have shown that oral NAG supplementation improved skin conditions. Although the mechanism for this beneficial effect of NAG on skin conditions is unknown, we hypothesize that NAG may promote the biosynthesis of mucopolysaccharides, such as HA, leading ultimately to an improvement in skin conditions. Considering its beautification action after ingestion, we conclude that NAG will be potentially useful as an ingredient of cosmeceuticals.

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INFLAMMATORY MECHANISMS IN ALLERGIC DISEASES

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Allergic diseases, as clinical manifestations, are the end result of a complex inflammatory process involving several cellular and humoral participants.
The understanding of these participants' role, still not completely defined, is the objective of this interesting book.
The text is divided in five parts: the introduction, part one (1 chapter), the components in the allergic inflammatory response, part two (10 chapters), The allergic inflammatory mechanisms in clinical disorders, part three (10 chapters), The emerging concepts in the modulation of allergic inflammation, part four (5 chapters), and The overall summary/conclusion, part five (1 chapter).
Mast cells and basophils, that may contribute to innate and acquired immunity, are recognized as the principal cell types to initiate the type I IgE-dependent hypersensitivity reactions.
Mast cells are concentrated at sites in the lamina just around the upper and lower airways, conjunctiva, dermis gastrointestinal mucosa, and perivascular tissues occupying sentinel positions.
Basophils reside in the circulation, but enter tissues at sites of inflammation during the early phase of cell-mediated delayed-type hypersensitivity reactions.
The select participation of basophils and mast cell in various clinical conditions, as well as the duration, intensity and tissue distribution of a particular response, will depend on various characteristics of the agonist immunological sensitivity of the host, the target tissue involved, and any underlying pathology.
These two cell types, basophils and mast cells, are distinguished from one another through pathways for growth, differentiation, and survival. Grow and differentiation, activation and regulation secretion, their mediators and biology and pathobiology of human mast cells and basophils are all well described in the second chapter of part two, where their involvement in human diseases, such as cutaneous allergic reactions, rhinitis, asthma, arthritis and fibrosis, can be addressed.
The physiological and pathological role of eosinophils is described in the chapter three. They form less that 4% of circulating leukocytes, are principally tissue-dwelling cells and their life span is estimated at 13 days from development to death. Eosinophil maturation is T-lymphocyte-dependent and separate from that of the neutrophil and monocyte-macrophage lineages.
Animal models of atopic airway inflammation and human asthma suggest that there is an increase in eosinophil colony-forming activity in allergic diseases. Moreover data from human bone marrow studies suggest that IL-5 Ra expression may be an important step in eosinophil maturation. In particular IL-5 and eotaxin act cooperatively to greatly increase eosinophil release from blood.
However eosinophil emigration from the blood vessel into the tissue matrix is a tightly regulated pro-

cess, involving deadhesion from integrins, with chemo-attractant guiding the cell into the tissue and interaction with tissue matrix proteins, such as fibronectin, laminin.
Release of granule proteins is a major effector limit of eosinophil function.
Major basic protein (MBP) is a single polypeptide chain of 117 aminoacids. It contains 17 arginine residue, which accounts for its basicity, and 9-cysteine residue, which explains its tendency to form disulfide bonds. The reduce form of MBP is as toxic for parasites as the native form, but is less potent at activating leukocytes.
Activation, degranulation, and function of eosinophils and their role in allergic disease are all reported in this interesting chapter.
Neutrophil structure and function is the topic of chapter four. They constitute 50-80% of the circulating blood leukocytes and undergo apoptosis, with subsequent macrophage recognition and phagocytosis of the dying neutrophils. They are motile cells as a reflection of their cytoskeletol apparatus. This motility is increased when neutrophils become activated by various stimuli, including agents that may be released in allergic inflammatory reactions.
Activated neutrophils frequently generate by-products of O2 that can be damaging to microbes and tissue proteins. A second major oxidant-generating pathway relevant to inflammation involves inducible nitric oxide synthetase, which appears to be activated by neutrophils. This enzyme reacts with L-arginine to generate nitric oxide, which is mildly reactive with tyrosine-containing amino acids, sometimes impairing protein function while being converted to the more toxic peroxynitrates. The latter, in turn, can be converted to the OH- radical. Peroxinitrites bind to glutathione, which may inhibit some of their activity. However the role of neutrophils and their pathological mechanism in different allergic diseases merits continued investigation.
The demonstration, provided in the last 15 years, that the IgE antibody production by B cells was regulated by T-helper (Th)-cell-derived interleukins, underlined the central role of lymphocytes in the pathogenesis of allergic reaction. This is the topic of chapter five.
Today allergic inflammation is considered as the final results of a Th2-type T-lymphocyte response to one or more common environmental allergens. The allergen-specific Th2 response represents the triggering event for the recruitment and the involvement not only of IgE-producing B cells, mast cells, and eosinophils, but also of other cell types, as well as, a high number of soluble factors and adhesion molecules, thus giving rise to an inflammatory cascade of unequaled complexity.
Moreover Th2 responses can also account for other hallmarks of allergic inflammation and asthma, such as hyper production of mucus (bx IL-4, IL9 and IL-13) and subendothelial fibrosis (by IL-4 and IL-13).
The question of how Th2 responses are enhanced in atopic patients is also unclear. However, the role of environmental factors together with an inherited disregulation of gene (s) controlling IL-4 expression, has to be considered.
However the new insight in the pathophysiology of T-cell responses in atopic diseases provide exciting opportunities for the development of novel immuno-therapeutic strategies.
These are the concluding remarks of the chapter five.
Leukocyte –Blood Vessel Interactions is the topic of chapter six.
The overall goal of this chapter is to summarize the cell adhesion molecules that function during cellular recruitment in allergic inflammation. As matter of fact inflammation is a complex process-involving interaction between tissue resident and recruited leukocytes.
In allergic diseases, the local generation and display of chemokines and other chemo-attractants,

together with the unique phenotypic pattern of expression of adhesion molecules and chemokine receptors on leukocyte subsets, combine to facilitate the selective emigration and accumulation of eosinophil basophyls, and Th2 lymphocytes at sites of chronic allergic inflammation.
The focus of chapter seven is Extra cellular matrix and in remodeling inflammatory processes associated with interstitial fibrosis and the airway disease asthma.
The epithelium, the largest interface with the environment, has important barrier functions.
In the airway, barrier functions are balanced against the need for cellular trafficking into the airway, and the epithelial basal lamina is adapted to allow regulated passage of inflammatory cells. Elastic fibers and smooth-muscle cells maintain tissue mechanisms over a range of architectures and level distension. Fibrillar collagen provides the ultimate high-tensile modulus to the tissue when fully extended.
Pulmonary fibrosis is a dynamic process, driven by chronic inflammation, that involves matrix degradation, and changes, and ultimately, the accumulation of collagenous matrix that replaces and distorts lung architecture.
This process may be initiated by inhaled particles or gases; by xenobiotics, by systemic autoimmune disease, by the reaction of aeroallergens or by as yet, undefined predisposing genetic factors. In most patients the cause is impossible to determine and the disease is termed idiopathic.
Inhibition of changes in lung architecture is the key future goal in therapy of inflammatory diseases that cause lung remodeling.
Future therapeutic strategies include specific anti-inflammatory agents, inhibition of growth factor synthesis and/or of collagen secretion, the use of agents that inhibit fibroblast proliferation or the expression of collagen genes.
In the chapter eight the regulation of IgE Synthesis is reported the role of cells and cytokines, as well as the molecular mechanisms involved in human immunoglobulin E (IgE) synthesis.
Therefore the role of cells and citokines involved and the molecular events in the induction of IgE synthesis are described together with the perspective for future therapies.
In the chapter nine effector roles of IgE antibodies are reported and well documented.
The role of lipids as mediators in allergic inflammatory diseases is reported on the chapter ten. These mediators are also key participants in the pathogenesis of other allergic diseases. Identification of the enzymes involved in synthesis of these lipid mediators, leucotrienes, prostaglandins and platelet-activating factor has led to the study of specific inhibitors that block their production. Novel receptor blockers and antagonists, such as the CysLT1 receptor antagonists, have recently been developed. These novel therapeutic agents seem to have great potential to modulate the allergic inflammatory response.
With the 11th chapter dedicated to cytokines in allergic inflammation, ends part two of the book.
As matter of fact cytokines and chemokines, are secreted to recruit additional inflammatory cells and augment immune response.
Thus recruitment of inflammatory cells is a complex process that involves the interaction of tissue cells with leukocytes, draining lymph nodes, and bone marrow. Cytokines are, in fact, involved in differentiation and maturation of mast cells, eosinophils and basophils affecting the growth, differentiation, migration and activation of allergic inflammatory cells.
In part three are reported all the allergic inflammatory mechanisms involved in different clinical disorders, such as Allergic Rhinitis; in chapter 12, Nasal Polyps and Sinusitis in chapter 13th Allergic eye disorders and Asthma respectively in chapter 14th and 15th. Because of its localization, the eye

is exposed to several external stimuli eliciting a wide variety of immunological and immunopathological reactions. On the other hand, in view of the wide distribution of blood and lymphatic vessels, ocular tissue also takes part in several systemic allergic reactions and diseases. Accordingly nosological entities grouped under the umbrella term of allergic conjunctivitis are now diagnosed on the basis of clinical criteria, all reported in chapter 14th.

Chapter 16 on atopic dermatitis (AD) is particularly interesting for our readers because this pathology is to be treated by corticosteroids, baths and hydrating emulsions with a high emollient activity. The maintenance of the skin hydration is a master for people affected by Atopic Dermatitis.

Moreover intense pruritis and cutaneous reactivity are also the hallmarks of AD together with bacterial superinfection. Dryness contributes to the irritation and itching of AD and for these reasons, skin should be treated with a continuation of hydration therapy and emollients. The use of appropriate cosmetic emulsions may have the effect to maintain the skin hydration and reduce the application of topical corticosteroids and consequent skin atrophy. The contemporary use of mild cleansing agents, such as special bath-oil, may help the skin to trap water, providing symptomatic relief for the patient affected by AD.

Naturally, the choice of moisturizer should be individualized according to each patient's surrounding humidity and sweat gland function, just to avoid to cause eventually irritation and itching of the skin. Finally treatment of moderate to severe AD may require a multi-drug regimens, reflecting the multifaced nature of allergic inflammation.

Chapter 17 outlines the current state knowledge of the pathophysiology, immunopathology, and molecular basis of the urticarias, highlighting mainly the mechanisms, rather than management.

Chapter 18 and 19 are entirely dedicated respectively to anaphylaxis and inflammation and to non-aphylactic drug disorders Anaphylaxis is a potentially fatal, systemic reaction that results from the release of biologically active mediators.

Clinically it is triggered by antigen exposure and IgE –mediated activation of mast cells or basophils. These cells then release mediators that act on target organs.

On the other hand, non-anaphylactic drug reactions are more common, and they are a greater cause of morbidity, often involved by drug metabolism. For all these reasons this area of research require the collaboration of different groups of investigators-chemists, immunotoxicologists, pharmacologists basic immunologists, and clinical allergists and dermatologists. The basic research of all these specialists will surely lead, in next future, to the development of (1) improved predictive models for drug hypersensitivity, (2) an ability to design and manufacture drugs that are less likely to produce hypersensitivity reaction (3) improved in vitro and in vivo tests for the diagnosis of drug hypersensitivity and (4) superior treatment modalities of hypersensitivity reactions. Reaching some of all of these goals has the potential to greatly decrease iatrogenic morbilidy and motility from the use of medications.

The ultimate seven chapters of part three of the book are respectively dedicated to Vascularitis, Fungal Allergic Respiratory Diseases, to Synthetic and genetically engineered Allergens and to Allergen Trijection Immunotherapy, anti- IgE Antibody Therapy and DNA Immunomodulation of Asthma are also extensively treated.

Although the pharmacological approaches for treatment of allergic diseases has advanced greatly, most of the current therapies are aimed at symptoms, rather than the underlying mechanism. Meanwhile new innovative DNA-based approaches are being developed, including the use of allergen DNA, the complexion of the human genome project will surely open whole new venues of

research useful to know the real mechanisms of activity, for example, of cytokines at the chromosomal level. Understanding why different individuals respond differently to potential antigens and to various therapeutic interventions will be more tangible and future pharmacogenomics will permit the knowledge of new markers of disease for an optimal individualization of innovative therapies. With these hopes ends this book of great importance for all who want to better understand the field of allergy and immunology that affects up to 20% of the individuals seen in general practice clinics. Thanks to the reliable data and the wide bibliography reported in all the 27 chapters this book is useful for all medical doctors, biologists and chemists involved in cosmetic dermatology who directly or indirectly need to know the mechanism and the role of all the components involved in the pathogenesis of allergic diseases.

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CUTANEOUS WOUND HEALING

By Vincent Falanga
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The skin, the largest and most visible organ in the body, is a highly elaborate structure that serves many functions, from protection through perception, and through complex physiological roles. Therefore the understanding and management of acute and chronic skin wound healing represent au important chapter in the fundamental area of biomedical investigation.

This interesting book by 29 chapters and a final well documented Mini Atlas reports the extremely important progress had in developiug sophisticated bioengineered skin constructs and in advanced therapies useful in the treatment of acute and chronic wounds.

As matter of fact, the process of healing in which scarring sometimes evolves slowly and may be accompanied by suppuration and necrosis, requires that lesions be coated and protected and the healing area be stimulated.

The biomaterials, used to dress areas of cutaneous substance loss, interact with the different components of the wound tissue and influence the relative process. Indeed, after the application of non-self materials, the inflammatory reparative tissue response involves resident cells, invading inflammatory cells, extracellular-matrix components, and inflammatory mediators. These interactions may affect the time of tissue repair as well as the morphological and differentiation features of the repaired tissue.

However it is to-day known that optimal epithelialization can be achieved with dressing and with conditions that keep the wound moist. Moreover it is also accepted the concept of slow-release antiseptic agents which can deliver antimicrobial agents in a way that does not prove detrimental to the healing process.

Finally we have addressed major advances in the treatment of wounds by the use of topical growth factors and bioengineered skin. These, together with the prospect of gene therapy, allow us to paint a very bright future for this decade.

With these statements ends the first chapter.

Chapter 2 introduces the reader on the implications for wound healing directly connected with the skin function as barrier.

Stratum corneum, as a biosensor, regulates the epidermal lipid and DNA-metabolic responses to a variety of exogenous insults. Various signaling mechanism-including changes in epidermal ions and/or in levels of epidermal cytokines and growth factors are potential candidates for the mediation of these metabolic responses.

The role of cytokine cascade in initiating cutaneous pathology, including the propagation of hypertrophic scars and keloids, seems, in fact, more certain. Thus, outside-to-inside signaling may account for the pathogenesis of a variety of dermatoses characterized by abnormal barrier function, including hypertrophic scars and keloids.

As matter of fact the disruption of permeability barrier provokes a speed-up of the cytokine cascade, and an increase of lipid and DNA synthesis, with a consequent epidermal hyperplasia. Moreover both environmental humidity and psychological stress influence permeability barrier homeostasis. When barrier perturbation becomes persistent, these processes can escalate and become chronic, where the barrier function remains abnormal long after re-epithelization has occurred.
These conditions can be viewed as an exuberant wound healing response that can be explained by a primary, ongoing barrier abnormality.
Mitigation of the cytokine cascade by normalization of barrier function through application of specific lipid mixtures or selectively permeable membranes should become a standard part of the therapeutic armamentarium.
The vapor-permeable membranes in fact allow restorative process to proceed. Conversely, the ability of occlusive membranes to involute hypertrophic scars and keloids can be explained by their potential damaging effect on subjacent metabolic processes, including the cytokine cascade.
Chapter 3 and 4 describe respectively the cellular and molecular events of wound healing and the keratynocytes involvement in this process, meanwhile chapter 5 focuses on the problem of collagen expression, synthesis and regulation in skin both during development and wound healing.
Humans have to repair wounds rapidly in order to restore the skin's critical protective function and, at the same time, attempt to preserve normal sensation, pliability and cosmesis. The relative importance of processes such as re-epithelialization and granulation tissue formation depends on the degree of tissue loss and the depth of the wound.
Forward migration of keratinocytes across a wound surface is clearly one of the chief components of the tissue repair process. As soon as the wound is covered in a monolayer of keratinocytes, the epidermal cells stop migrating and begin to form a stratified epithelium and an underlying basal lamina from the margins of the wound in a centripetal direction.
Keracinocytes have biological characteristics enabling them to form a co-ordinated multicellular stratified epidermis, with replicating cells at the base becoming committed to a complicated pathway of terminal differentiation and eventual loss. Evidence is accumulating that mesenchymal tissue has a major role in the maintenance of this cellular organization and function, together with epithelial organogenesis and repair.
These dermal-epidermal interactions are achieved by a complicated communication network, which takes place by cell-cell, cell-matrix and cell-diffusible factor connections. Detailed knowledge of these interactions and of their disturbance in diseased states and in wound healing should lead to better understanding of the pathophysiology of the skin and the restoration of skin defects.
Moreover keratin may play a role in providing strength to cells as they are subjected to the forces generated by the contractile actin cytoskeleton both during lamellopodial crawling and during purse-string closure of embryonic wounds. Collagen, a family of 19 molecules produced from 33 genes, is also involved with structure and repair of tissue during wound healing. Cell interaction with different collagens and other extracellular matrix molecules causes changes in cell activity through signals from matrix receptors such as integrins.
In addition, extracellular matrix is a repository for several growth factors influencing cell activity.
The range of cellular responses elicited by growth factors, the relative signal regulations at the level of their receptors and the mechanisms of signal transduction to elicit the specific cellular responses, is the topic of chapter 6. With deeper understanding of the cross talk that occurs within each cell, it may be possible now to fine-tune the use of growth factors for the purpose of accelerating wound

repair and modifying the fibrotic response. This is reported as conclusion of this interesting chapter.
The role of immunocytes, cytokines and angiogenesis in wound healing is focused in chapter 7.
Since wound healing represents a complex multi-step process involving many different cell types, such as keratinocytes, Langerhans cells, melanocytes, fibroblasts, mast cells, etc, it should not be surprising that multiple cytokines, growth factors and chemotactic polypeptides are important and participate in restoration of cutaneous homeostasis. Moreover, complex quantitative and qualitative changes in the angiogenic and angiostatic cytokines occurr in the wound site during the healing phase, confirming the importance of cytokines in wound healing. However this multi-step process still presents many challenges for both clinicians and scientists.
Chronic non-healing wounds such as those viewed in psoriasis, chronic inflammation and ulcers remain as important therapeutic challenges requiring additional study.
Learning more about the pathogenesis of psoriasis will surely provide useful clues to unravel many of the mysteries of normal and abnormal wound healing events in the skin. Therefore it should be necessary to better understand the role to induce keratinocyte hyperplasia for T cells and selected cytokines in the wound healing and in the pathophysiology of psoriasis. For these reasons the future therapeutic progress will depend on further defining the role of immunocytes, cytokines and regulators of angiogenesis in the cutaneous wound healing response.
Chapter 8 provides a focused discussion on various models of wound healing that requires principally the use of animals.
For the complexity of the involved problems the in vitro studies are not sufficient to clarify and address all the specific questions of the healing process, united for example, to the age, ischemia, infection or diabetes. This process requires, in fact, a coordinated response involving multiple systems including the immune and vascular systems, as well as tissue cellular actions, and so is a complex, highly regulated reaction. For all these reasons the utility of animal models for studying wound healing centers on this complexity which can be duplicated by in vitro experimentation.
Different are the in vivo models studied such as Incisional and Burn wounds, Partial-and-full Techniques Wounds, Impaired and Chronic Wound all well documented and described to permit the reader to better understand the specific questions of the healing process, allowing also a tool for improving the treatment of such wounds.
The overall clinical approach to wounds is reported from chapters 9 to 12.
Gene therapy is described as a commonplace new technique moving into the clinical arena. Two are the basic strategies depending on the introduction and expression of foreign DNA into host cells: a stable insertion of the permanent correction of inborn errors of metabolism (gene therapy), and a transient transformation for the short-term expression of a gene product (gene medicine).
All both these therapies may be used to treat cutaneous wounds and other disorders. Understanding the mechanism of action and molecular biology of active peptides in the wound is a critical element for the use of gene therapy in wound healing. It is also necessary to establish dose/response data for all growth factors and others active molecules in the wound, and the effects of combination of these molecules need to be elucidated. Basic principles for the clinical approach to wounds are asepsis, debriment, risk assessment, rapid closure, physical protection, physiologic support and minimization of scar. As matter of fact the most powerful means of removing bacteria wounds of all types is through debridement under clean or sterile conditions. At this purpose washing with disinfectants such as silver sulfadiazine or silver nitrate is reserved for more extreme bacterial contamination, and when primary closure of wounds is not expected.

In presence of burn wound the four main goals are prevention of infection, (by bacterial agents) maintenance of a moist environment (by biological or biosynthetic dressings), protection of the wound, and minimizing scar formations.
Skin grafts are also employed on burns that are expected to take longer than 3 weeks to heal. Substitute skin are also being developed, but to date there are few reported long-term results.
When a wound is not acute but chronic, wound bed preparation seems to be a critical condition to obtain positive results. Therefore clinicians have to reserve more attention to the microenvironment of chronic wounds.
As matter of fact with the increasing number of elderly people in society, the management of chronic medical conditions will be one of the major challenges in the next several decades. Fortunately with the increasing ability to take care of medical problem more efficiently, by expected new therapeutic modalities, the obtainable results on chronic wounds will be surely better than today. At this purpose the role of a multidisciplinary clinic specialist will be fundamental to provide a base-line data for evaluating the effectiveness of care treatments at regularly stated intervals and ensuring that all members of the health care team follow the same protocol. The patients' psychological well being and the control of distressing symptoms should be also other primary objectives.
Therefore, it is essential to harness all available skills to improve our understanding as well as the management of healing and non-healing wounds. At this purpose all the possible laboratory evaluations such as physiological assessment, biochemical tests, histology as well as vascular tests are to be used.
The infected wound is the topic of chapter 14.
The process of tissue infection depends on the number and pathogenecity or virulence of the microflora and can be associated with a delay in healing. Host defense mechanisms play, in fact, a role in protection against the microflora. Infection in a wound reflects, therefore, disturbed host-bacteria equilibrium. Each process involved in healing is affected when bacteria proliferate in a wound and it is only when this equilibrium is in balance that the normal process of wound healing can occur.
Bacteria impair repair processes by producing toxic byproducts and competing with cells for oxygen and nutrients. Debridement of devitalized tissue and wound cleaning are, therefore, the first important components of any wound management protocol. Wound healing cannot take place until necrotic tissue is removed.
At this purpose the use of appropriate dilutions of some antiseptic solutions may be beneficial. The following use of wound dressings is useful to absorb varying amounts of drainage and moist granulating wounds.
And wound dressing is the topic of chapter 15 where the different dressing from the transparent polymeric films, to hydrocolloids, hydrogels and Alginates or Collagen are described.
What all these categories of dressings have in common is that they create or maintain moist wound environment for healing.
Other performance characteristics are their absorptive capacity, hydrating ability, adhesive quality and their different chemical activity and applications.
Chapters 17th to 22nd are all dedicated to the difficult problem of ulcers.
Cutaneous ulcers are, in fact extremely difficult to manage. To better understand this pathology it is necessary to focus not solely on the ulcer but to examine the surrounding skin also. The skin color, the presence or absence of atrophy, edema, and induration are all-important clinical findings. For instance, exaggerated hyperpigmentation and dermatitis usually accompany venous disease.

Compression and wound dressing are the lines of treatments for venous ulcers also. Compression reduces distension of superficial veins and by reducing the vessel area renders incompetent valves competent. Moisture retentive dressings in combination with compression may produce more rapid initial healing rates. Moreover, these dressings have the advantage of promoting a moist environment that enhances autolytic debridement and granulation tissue, relieves pain and lowers the rate of infection. Dressing should be selected according to the characteristics of the wounds.
Hydrocolloids and hydrogels are preferred for mild to moderate drainage, meanwhile alginates and foams are used for moderate to heavy drainage. Finally films are helpful for acute wounds with minimal or no drainage.
Compression may be delivered by a variety of modalities such as graduated compression stockings, elastic and inelastic bandages, orthotic devices and compression pumps.
Which bandage is best?
The type of bandaging used depends largely on geography. North Americans tend to favor Unna's boot or three layers bandages, the British often prefer four layers and continental Europeans are more likely to choose short stretch systems.
Large-scale meta-analyses of published ulcers trials have failed to demonstrate notable differences between various types of compression bandage. Compression bandages are usually used to heal leg ulcers, but the type of bandage used remains controversial. The physiology underlying the development of venous ulceration has not been fully elucidated. Once this is better understood, the future for ulcer healing and prevention may lie manipulation of the ulcer bed or damaged skin.
Until this is the case, compression therapy will remain the gold standard of ulcer treatment.
Chapters 23rd to 28th are dedicated to the more recent topical treatments able to act on different targeted substrates in the wound-healing cascade.
As we have seen, wound healing in the skin is a complex biological cascade of events in which numerous cells, cytokines, growth factors, proteases, and extracellular matrix components act in concert to restore the integrity of injured tissue.
It has become also clear that certain regulatory peptides, such as growth factors, directly regulate many of the processes crucial for normal wound healing. It is obvious that substances that are chemotactic to inflammatory cells, or mitogenic to cells, should benefit wound healing.
With such clear rationale why the enhancing wound healing with topical growth factor application has a limited clinical success?
Probably because the number of growth factors that affect the various processes in the wound healing cascade are considerable and their availability and function is probably sequential.
Another important problem is surely the used delivery system. Therefore it will be of fundamental importance to use adequate carriers to enhance the penetration of growth factors throughout the skin strata.
Together with the clinical use of the growth factors, secreted from the cells, the tissue engineered and the development of bio-engineered skin for wound healing applications have been the focus of many clinical studies of the last ten years.
The goal in the development of bio-engineered skin has been to recreate the beneficial characteristics of skin grafts while eliminating or minimizing their limitations. Two major approaches have been taken to achieve this goal, one is to create a bi-layer skin substitute that is comprised of both dermal aud epidermal layers, the other is to use cultured keratinocyte grafts or dermal substitutes alone as a skin substitute.

All applications require that bio-engineered skin promotes healing of a wound. Products that utilize cell culture technology and/or bio-engineered natural or synthetic materials technology are well described together with their clinical use in treatment of burn wounds, chronic ulcers or surgical wounds.

The more recent advances in burn treatment are also reported and illustrated in a well-documented way. This new technology is undoubtedly the fastest and safest way for optimizing the quality of wound healing, avoiding the formation of keloids and unpleasant scars.

Chapter 29 is the last chapter dedicated to an interesting discussion of how to best deal with chronic wounds in the context of advanced therapeutic agents.

As matter of fact the optimal preparation of wound bed is fundamental to obtain the best results by the use of topically applied growth factors, bio-engineered skin, and systemic new agents.

The goal is to arrive at a stable wound which is characterized by an optimal well-vascularized wound bed and no or minimal exudate.

To achieve this goal it is necessary to remove necrotic/fibrinous tissue, to control edema, to achieve a well-vascularized wound bed, to decrease bacterial burden and to minimize/eliminate wound exudate. This is the starting point to improve the efficacy of new therapeutic agents, which should results in better overall wound care.

The last 15 pages are dedicated to a mini-atlas describing by well-done photos and appropriated explanations many problems of wound skin healing.

This book reports all the updated news on the complicated topic of Cutaneous Wound healing from the basic aspects of wound care to the more sophisticated cascade of events accompanying.

All the more important research done in this area are reported and presented in a very clear and comprehensible way providing an overview for all surgeons, particularly plastic surgeons, dermatologists, cosmetic chemists, biologists and physiologists concerning the new development in the cutaneous wound repair process as well as the proper surgical management and medical approaches necessary to optimize wound care. It also delivers to researchers involved in the technological development which the cutaneous wound healing has undergone in the last ten years, to students willing to learn the basic and the mechanisms of activity that regulate the wound repair process of the skin.

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NITRIC OXIDE AND INFLAMMATION

By D. Salvemini, T.R. Billiar and Y. Vodovotz
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The see-sawing of nitric oxide (NO) between their beneficial and detrimental roles pushed the authors to an overview on the state-of-the-art on NO and inflammation.
As a matter of fact, many studies have demonstrated that NO could lead to apoptosis of certain cell types, meanwhile other research results have described NO as inhibitor of apoptosis of other cell types.
This book through 15 chapters tries to clarify the real role played by nitric oxide.
On the process of inflammation inducible Nitric Oxide synthase (iNOS) catalyzes the conversion of L-arginine, in the presence of oxygen to the free radical NO and L-citrulline. In biological systems, NO is primarily derived from a cationic aminoacid L-arginine and oxygen of NO synthases (NOS). Though L-arginine has been shown to be critical in the antimicrobial actions of macrophages there is also evidence that the citotoxicity of macrophages can be enhanced by L-arginine depletion.
For example, it has long been recognized that tumoricidal actions of activated macrophages are dependent on depletion of environmental L-arginine. L-arginine depletion was also reported in inflammatory sites during macrophage infiltration and wound healing.
NO has a short half-life and is oxidized to the inactive and-products nitrite and nitrate (NO2- and NO3-). Moreover NO play a central regulatory role in variety of physiological and pathological process including inflammation and host defense.
To date NOS isoforms have been identified as neuronal NOS (nNOS, type I), inducible NOS (iNOS, type II), and endothelial NOS (eNOS, type III).
nNOS and eNOS constitutively exist in cell and their activity is mainly regulated by the levels of intracellular free Ca2+. NO generated by eNOS and nNos serves as a signaling molecule participating in various physiological processes ranging from regulation of vascular tone to development of learning and memory.
Too much or too little, NO production in cells and tissues has been implicated in the pathogenesis of a number of diseases including stroke, hypertension atheriosclerosis and heart attack.
In contrast the two constitutive NOS isoforms, iNOS is generally not present in quiescent cells but, with proper stimulation it can be induced in almost any type of cell and tissue. Its expression is often induced by inflammatory substances such as cytokines and microbial endotoxin. Calmodulin is also an essential cofactor for the catalytic function of iNOS. Moreover, while elevated cytosolic Ca2+ combines with calmodulin to form the Ca2+/calmodulin complex, which subsequently activates eNOS and nNOS, activation of iNOS does not require elevation of intracellular Ca2+.
iNOs is also a more potent enzyme with higher L-arginine to NO turnover as comparated with eNOS or nNOS and, for its high enzymatic efficacy, has an important role in host defense and inflammation.

The iNOS gene expression seems to be up regulated, in human by cytokines through the transcriptional factors NF-kB, AP-1 and STAT 1a.
Translational and post-transcriptional regulatory mechanism also play a role in NO expression.
In addition to NO, NOSs have recently been shown to also generate superoxide anion (.O-2). However it is now clear that O2- generation seems to occur from all NOS isoforms. iNOS-derived .O2- and NO interact to form another potent oxidant peroxynitrite (ONOO-). Therefore this free radical –NOS-mediated may contribute to the cytotoxic actions of macrophages in inflammation and immune defense. Modulating both the O2 and ONOO- formation they may provide new approaches to regulate macrophages function in inflammatory process and host defense. Therefore the cytotoxic nature of .O-2 and its associated oxidants not only contributes to the killing of invading microbes, but also causes tissues damage in inflammation. Understanding the molecular mechanism that governs NO synthesis can surely lead to novel therapeutic strategies.
All these interesting topic are reported i the first and the second chapter.
Recent studies over the last decade have demonstrated that NOS is regulated primarily by cytokines. Both cytokines and free radicals, such as ROS and not yet fully defined reactive nitrogen oxide species (RNOS), mediate signals that allow cells to decipher their environment.
The modulation of NO production by cytokines, as well as the possible feedback effects that NO has on expression and activity of various cytokines, is discussed in chapter 3.
NO plays also a major role in the regulation of cyclooxigenase (COX) enzyme, augmenting eicosanoid production by acting at several levels.
Therefore there is an NO-mediated increase in the production of pro-inflammatory prostaglandins (PGs) resulting in an exacerbated inflammatory response.
NO or its by-product peroxinitrite (ONOO-) can increase PGs release by either activating or inducing the COX-2 enzyme. Thus, regulation of COX-2 by NO or peroxynitrite represents a powerful mechanism, which amplifies the course of the inflammatory response.
The challenge in the future to understand the molecular mechanism used by such species in modifying key steps of the cyclooxygenase pathway as this will undoubtedly elucidate important molecular targets for the future pharmacological intervention. With these auspices ends chapter 4.
Nitric oxide regulation of leukocyte adhesion molecule expression and leukocyte recruitment are treated on chapters 6 and 7.
The vascular endothelium serves as an important autocrine/paracrine organ that regulates vascular wall contractile state and cellular composition.
The endothelium interacts with both cellular and hormonal mediators from the blood and the vascular wall. These interactions are of crucial importance in inflammatory reactions. One of the initial events in vascular inflammation is endothelial activation. NO inbibits endothelial adhesion molecule expression by at least two trascritional factor (NF-kB) dependent mechanisms.
First NO induces the protein IkBa, the newly synthesized inhibitor binds tocytoplasmic NF-kB resulting in prevention of further NF-kB translocation to the nucleus. Second NO causes nuclear accumulation of NF-kB.
Weather IkBa translocates into the nucleus by active or passive mechanism remains to be established.
Therefore there is increasing evidence that NO may attenuate adhesion molecule expression on endotbelial cells. Indeed intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1- (VCAM-1) –synthesis by human and rodent endothelial cells is reduced with NO

donors.
In addition endogenously produced NO may downregulate the expression of VCAM-1 both from constitutive and iNOS sources.
In conclusion NO seems to function as an anti-adhesive molecule in inflammatory settings.
The poly- (ADP)-ribosytransferasi (PARS) activity and its role in the regulation and generation of the inflammatory response are the topic of the 5th chapter.
PARS occupy, in fact, a critical position in a self-amplifying cycle of oxidant-driven damage.
Its activation seems to lead to cell death and some have argued that the physiological role of PARS should be to eliminate genetically damaged cells, thereby reducing oncogenic potential. Indeed, PARS inhibition has been shown to facilitate the rapid ligation of DNA excision-repair patches and suppress malignant transformation in cells with DNA damage induced by irradiation and chemical carcinogens.
The effect of NO on lymphocyte function is the argument of chapter 8, where a new concept of activity is reported. NO should play a specific role in the intracellular transduction pathways to facilitate production of the cytokine IFN gamma and perhaps other molecules.
The role of nitricoxide in arthritis, gut inflammation, and disorders of the skin, myocardial ischemia-perfusion injury and in myocarditis is reported from chapters 9 to 13.
All these inflammatory conditions seem to be associated with production of comparatively large amounts of NO, produced by iNOS, with consequent cytotoxic effects.
Factors that appear to dictate the consequences of iNOS expression include the type of insult, the tissue type, the level and duration of iNOS expression, and probably the redox status of the tissue.
Moreover to really understand the biological-activity of NO, it should be necessary to better know its relationship with key oxidants such as O2-.
Nitric oxide is clearly a common mediator in both septic and hemorragic shock but is also a mediator of interleukin-2 induced cardiovascular toxicity and antitumoral activity.
This is the topic of the last chapters 12 and 13.
NO contributes to the pathogenesis of vascular leak syndrome (VLS), hypotension and myocardial depression, which are observed as dose-limiting toxycities following administration of a number of cytokines, such as IL-1, il-2, IL-12 and TNFa.
But NO can also inhibit lymphokine activated killer (LAK cell) induction by IL-2.
On the other hand, NO also can inhibit cellular respiration and DNA synthesis in tumor cells, triggering programmed cell death.
A better understanding of these possibilities seems important since pharmacological interventions can be designed to either block NO synthesis or to enhance NO or perhaps more selectively target NO synthesis within tumors.
This book makes a useful reading for biologists, pharmacists, dermatologists, allergologists and students in medicine and chemistry willing to learn the basis and the mechanism of activity that regulate all the inflammatory processes modulated and effected by NO and free radicals.

P. MORGANTI
Editor-in-Chief

COSMETIC LIPIDS AND SKIN BARRIER

By Thomas Förster
December 2001. 376 pages Hardcover
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This book divided in four parts for a total of 11 chapters describes the most important developments in understanding the interaction between cosmetic lipids and the skin barrier over the past ten years. Part one reports the chemistry and structure of skin lipids, Part two the biological function of skin lipids, part three the analysis of skin lipids and their effects on skin, and the final part IV the lipids in cosmetic products.

Chapter 1 is completely dedicated to the chemistry of natural and synthetic skin barrier lipids.

According with the last studies the cornified cells, with their surrounding lipid matrix, represent the so-called two-compartment model of the Stratum Corneum (SC). This two component system formed by dead cells and lipid -lamellae constitute the skin barrier against the environment. These studies have also demonstrated the loss of phospholipids and the emergence of neutral lipids, ceramides, and cholesterol in the SC during barrier formation.

The ceramides are credited with playing an important role in the lamellar lipid structure and in barrier functions providing new insights into the pathogenesis of both disorders of cornification and diseases associated with barrier abnormalities. Nevertheless free fatty acids, cholesterol together with the ceramides that occur mainly in equimolar ratios seem to be important for the formation of stable lamellar liquid crystalline lipid structures at 4.5 - 6.0 pH.

Both cholesterol and ceramides, acting also as antagonists, are able to modulate and stabilize the lamellar crystalline gel structure of SC lipids. The dose combination of horny cells and lipids lamellae modulate and regulate transepidermal water loss (TEWL) and water-binding capacity of the skin. At this purpose nature-identical or analogues ceramide are today produced by biotechnology for cosmetic and dermatological use.

The term nature - identical ceramides covers natural-identical ceramides and their racemates, whereas ceramides analogues are usually compounds based on stereoisomerically pure sphyngamine with a different acyl chain length or with a similar skeletal structure.

Pseudo-ceramides generally have structures that differ more clearly from the basic structure and are often designed for cosmetic applications. The structure of all these new chemical compounds, their physiological functions and their dermatological properties are well described in this interesting chapter.

Chapter 2 reports the Structure of SC lipid layers and their interactions with lipid liposomes. This chapter focused on the lipid composition and organization in the SC and the change involved in this lipid lamellar organization as a consequence of topical application of drugs and cosmetics. The lipid lamellae oriented along the surfaces of cells are designed to keep many substances outside the body and act therefore as permeability barriers. Furthermore these lipids, mainly organized in a crystalline sublattice with an unusual long periodicity of the structure being approximately of 13 mm, increa-

se their mobility by the increase of water content.
Considering all the studies reported the drug transport can be adjusted on demand by association of the drug with liposome vesicles with the right size and lamellarity.
Using rational liposome, intact vesicles transport across SC does not occur.
However drug transport increases when single-chain surfactants are intercalated in the vesicles. These single-chain surfactants can act as penetration enhancers.
Molecular dynamics (MD) is a modeling technique that can be used to simulate the thermal motion of a structure as a function of time, using the forces acting on the atoms to drive the motion.
This is the interesting topic of chapter 3, reporting data obtained cutting out molecular dynamics simulations of three different simple stratum corneum lipid models.
According with the conclusion of the study by the molecular simulation of skin lipid composition, cholesterol is able to fluidizing the rigid gel phase, meanwhile ceramides seem to consolidate the lipid assembly playing also an important role in the coherence of the lamellar bilayer.
Therefore this computer modeling method, providing a detailed picture of molecular interactions, can offer useful information for validating or refining the interpretation of experimental data.
Role of lipids in skin barrier function is the topic of chapter 4.
As we know the skin barrier has the important role to protect the water-rich internal organs from the dry environment. For these reasons the thickness of SC is enhanced under a low-humidity. Thus skin barrier homeostasis, through its lamellar lipids and corneocytes, is a self-referential and self-organizing system.
Inhibition of lipid synthesis, lipid processing and migration of the lamellar body induces barrier abnormalities. Thus abnormality of cholesterol sulfate processing induces serious skin abnormalities as well as the phospholipids decrease meanwhile topical application of palmitic acid may restore the barrier function.
The best positive results on barrier recovery may be obtained applying topically ceramide, cholesterol, palmit acid, and linoleic acid in the molar ratios 1:1:1:3 or 1:1:3:1. Stearic acid, in place of palmitic acid seems to show also the same effect. Occlusion or moisturization with artificial material could improve skin condition, even if these treatments potentially perturb its homeostasis.
Therefore a better understanding of skin homeostasis is required to develop an ideal skin care system. Skin models may help to better understand the barrier function necessary to recovery the best way to treat an altered skin.
Skin-equivalent model is the topic of chapter 5.
As matter of fact advances in technology and a movement toward reduced animal testing have spurred the development of alternative methods for determining skin irritation, sensitization, and efficacy of cosmetic products.
Epidermis and skin models are three-dimensional systems produced by seeding single cell suspensions of keratinocytes on an appropriate support. The keratinocytes attach to the substrate and proliferate.
Skin-equivalent models consist of a living dermal equivalent associated with an overlaying epidermis. Besides fibroblast-populated collagen gels, three-dimensional dermal equivalent can be generated by synthesis of extracellular matrix by fibroblasts inside a scaffold or an inert filters before keratinocyte seeding. The inclusion of melanocytes in combination with Langerhans and endothelial cells compose the many variations that have been developed.
At this time, no-validated and accepted model can accurately replace animal experimentation, but

human tissue engineered skin equivalents form promising alternatives, because cells are of human origin and closely resemble the tissue of origin.
Soon after these hopes starts chapter 6 on Analytical techniques for skin lipids.
Skin lipid contains a variety of individual lipids differing in chemical structure and polarity.
Individual factors such as age and sex, and environmental variables, such as seasonal variations and exposure to UV light, play a role in SC barrier lipids, affecting also their composition.
At this purpose it is important to know the composition of the intercellular lipids of SC also because qualitative and quantitative characteristics vary between healthy and diseased individuals.
Therefore, the different analytical technique currently applied for the determination of skin lipids has been reported and well described in this chapter. As matter of fact it is important to control in a reproducible and standardized way all SC lipids in order to better understand the relationship being between the various skin conditions and the barrier function alterations.
Chapter 7 focuses on the description of the most popular, easy-to-handle, and frequently used non-invasive biophysical methods.
These methods involve in fact the application of physical principles to study and explain the structure and the function of the skin to assess its various properties. In order to perform these studies a number of general conditions should be met for optimal results.
All examinations should be done in an air-conditioned laboratory that guarantees constant room temperature.
The volunteers should sit in this location at least 30 minutes before to assess the test procedure, being also in a relaxed physiological and psychological state. Finally a suitable body test site has to be selected and the assesser should be skilled in the art and presses a large amount of experience
These recommendations are reported in the chapter together with the biophysical methodologies more used today.
The SC is not a static but instead a dynamic barrier that is continually being formed by the dermis by differentiation of keratinocytes to corneocytes while an average one layer of corneocytes is lost from the upper surface per day. Infrared and Raman spectroscopy reported on chapter 8, are two techniques that have proved especially suited for the investigation of its dynamic structure.
As matter of fact by these two methods has been elucidated the central role of the intercellular lipid domains in SC barrier function. The studies employed to investigate the thermotropic phase behavior of Stratum Corneum lipid multilamellae, the activity of penetration enhances, the determination of skin types and the effects of cosmetic products on the skin surface lipids, are all well described and reported on this interesting chapter.
Chapters 9 focuses on the chemical characteristics and the application of lipids for skin care formulations reviewing also their interaction with the skin.
The lipids, in fact render a characteristic skin feel, give certain occlusivity to the cosmetic product, and can penetrate into the SC interstices to a certain extent. But lipids, used as building blocks for membranes during growth and repair, are also one of four major classes of biomolecules which are essential for life.
However the shared characteristic of the lipids is their hydrophobicity, which is a result of the presence of long chain hydrocarbon residues, which can be linear, brauched or even cyclic. Moreover they directly influence viscosity and skin feel of the cosmetic formulation, even if the dynamics of the structural change of emulsions due to water loss after application to the skin seem to have the major impact on product performance.

of occlusion used, and the chemical-class, sometime can also cause skin irritation, as clearly described in chapter 1 and 2.
Percutaneous absorption in mammals varies depending on the area of body on which the chemical resides. Therefore when considering skin absorption in humans, the site of application is important to better understand both dermato-pharmacology and dermato-toxicology of all the chemicals applied.
The SC is today considered a dynamic skin barrier where the stratum corneum lipid synthesis is regulated by barrier requirements and the by stratum-granulosum-stratum corneum interface throughout the layers of the SC.
Being the complexity of this skin barrier and its particular biophysical properties, a domain mosaic model was developed. This model depicts the bulk of the lipids as segregated into crystalline domains bordered by grain borders where lipids are in the fluid crystalline state.
Although the abnormalities in lipid composition are correlated to altered ultra-structure responsible for reduced barrier function, biochemical, morphological, biological and functional in vivo analyses of neonatal skin are necessary to obtain a global perspective on skin maturation.
This way ends chapter 4 entirely dedicated to the Development of Skin Barrier Function in the Neonate.
Many enzymes are capable of metabolizing cutaneous xenobiotics such as cytochromo P-450. Their role in the production of toxic and irritant metabolic and their dynamic response to inductive and inhibitory stimuli is discussed in chapter 5.
However the factors that influence the metabolism of cutaneous xenobiotics can be dynamic or static, varying according to the physiological and pathological condition of the skin, if dynamic, or being related to the structure of the skin at a particular site, if static.
Passive follicular delivery is another component of topical delivery via the lipoidal domains of the SC. At this purpose specific particulate systems, such as liposomes and synthetic microspheres, have used as drug reservoirs, localizing in follicular and sebaceous areas for compounds with the right physicochemical characteristics for both active principle and vehicle.
Manipulation of particle size and other formulation factors, such as lipid ratios and composition, may be useful in achieving a greater extent of delivery to the follicles and sebaceous glands.
All the considerations for designing the experimental design and using the appropriate methodologies to obtain the right follicular permeation are well described in chapter 6.
The in vivo relationship between percutaneous absorption and transepidermal water loss (TEWL) is the topic of chapter 7.
Between the permeability of the skin to the outward movement of water (TEWL) and the inward uptake of molecules, a linear relationship exists. Thus, the permeability varies widely in relation to the nature of the molecule administered, occurring physicochemical interactions between the molecule, the vehicle, and the SC.
Moreover, it appears that the linear relationship linking TEWL and percutaneous absorption is independent of the physiochemical properties of the compound applied, but dependent of the efficiency of the skin barrier.
Consequently, as with determinations of TEWL, percutaneous absorption measurement provides a good marker of the cutaneous barrier integrity.
Thus, the chemical and physical properties of the topical vehicle and the barrier properties of the living SC determine the initial absorption of compounds into the skin.

The vitality of the living skin will, in part, determine the metabolism, distribution, and excretion of the compounds through the skin and the body.
The skin substantiality of both the vehicle and the active principle is also to be regarded because the mechanism of washing does not often prevent penetration of some chemicals. Thus, the fate of chemicals, both lipophilic and hydrophilic, that form a reservoir in skin during the absorption process is also to be taken into account. The best in vitro and in vivo methodologies with their limitations and topical/systemic bioavailability of the chemicals applied are all reported in chapters from 8 to11 together with the important steps inflnencing/enhancing the percutaneous absorption process.
The dose response interrelationship and the effect of single versus multiple dosing in percutaneous absorption, are reported in chapter 12 and 13.
Increased concentration of an applied chemical on skin increases the body burden, as does increasing the surface area and the time of exposure.
The body burden is also dependent on the frequency of daily application and on possible effects resulting from long-term topical exposure. Dose accountability (mass balance) competes a dose-response study, meanwhile the pharmacokinetic practice is necessary to determine the bioavailability of a studied chemical, generally done with a single-dose regimen.
The duration of application of a compound may considerably influence the total amount absorbed. Moreover, both the substances added to formulation as the excipient and/or the physical form of the active principle affect not only release and absorption, but also its action, increasing or decreasing the quantity of water in the SC.
To control absorption and topical activity of drugs and cosmetics the tape-stripping methods are being to be used as an alternative approach to chemical trials. By this non-invasive method it is possible to study and determine the bioequivalence of topical dermatological products in parallel to the use of concentration-time curves for systemically administered drugs.
In fact, the amount of chemical present in the SC at the end of application may represent the SC vehicle partitioning and could also reflect the rate of penetration of the chemical.
New technical improvement and more reproducible chemical and non-invasive measuring procedures may lead to a wide knowledge in the area of skin pharmacology, which requires accuracy and repeatability. Therefore, according to chapters 14 to 16, the physicochemical properties of the drug, the vehicle, and the SC play an important role in total absorption.
Applied vehicles have, in fact, the potential to either increase or decrease the quantity of water in the SC and, thereby, to increase or decrease penetration such as the physical form of the drug, affect not only its release and absorption, but also its action.
Moreover the skin's acid mantle and key lipids of its SC seem to be important for permeability barrier homeostasis, impacting on the transdermal delivery of drugs molecules.
In chapter 17 are reported the new strategies that could be put in place to enhance the transdermal drug delivery, interfering, with the SC lipid biogenesis. A key aspect of any new drug or cosmetic product is its safety and efficacy, as demonstrated in controlled biological and chemical trials.
When they are topically applied, a pharmacologically active agent must be released from its carrier before it can contact the epidermal surface and be available for penetration in the SC and lower layers of the skin.
For these reasons in vitro and in vivo tests are necessary to determine the bioavailabilty and pharmacokineticy of all the active principles used.
These tests are, of course, formulation-specific and are a property of the different formulations and

their method of manufacture. This interesting topic is reported in chapters from 18 to 21 where the kinetics of chemicals and their percutaneous absorption is discussed.
Transdermal drug delivery offers advantages of avoiding local gastro-intestinal irritation, improving also patient compliance.
A second use of this topical application is to treat the upper skin layers, which is the field of external dermatotherapy. A third use of dermal application is to reach deeper underlying tissues with minimal systemic exposure.
Thus the use of sunscreens should limit the penetration of active ingredients only to the first strata of SC. Conditions like :
- acne require drug targeting of pilosebaceous structures;
- in psoriasis therapeutic drug levels should be obtained in deeper epidermal layers, etc.
- Iontophoresis and phonophoresis are novel non-invasive drug delivery systems used to facilitate transdermal delivery. Iontophoresis facilitates the transport across the skin of charged molecules such as ions and soluble salts under an external applied potential difference. Phonophoresis or sonophoresis enhances penetration and movement of drugs through intact skin under the influence of an ultrasonic perturbation.
Strategies to use both these technologies for enhancing dermal distribution and delivery of diverse substances of wide-ranging molecular size and chemical composition, are outlined in chapters from 22 to 24.
Percutaneous absorption of cosmetic ingredients, including fragrances, can represent a major route of ingredient uptake and subsequent systemic exposure.
Therefore its dermal exposure is a function of the concentration of chemical contacting skin and the duration of skin contact.
On the other side, skin absorption is dependent on many factors, including lipid solubility of the chemical, environmental conditions, occlusion of the dosing area by clothing, surface area of skin application and the age of the individual.
Thus, skin absorption studies should be conduced under conditions that approximate specific product use/abuse conditions, attempting to accurately define the estimating systemic exposure to the chemical used.
In fact, the systemic exposure estimates for a single exposure to a cosmetic ingredient is a function of the amount applied to skin, the concentration of the ingredient in the product, the duration of skin contact, and the extent of percutaneous absorption.
Potential systemic exposure, percutaneous absorption and safety evaluation of cosmetic ingredients, such as Fragrances, Hair dyes and Alpha-hydroxy-acids are reported on chapters from 25 to 28 where interesting experimental studies are carefully discussed.
The last seven chapters are dedicated to special topics.
The methodology to be used for controlling the false-negative or false-positive allergic or irritant response from a chemical applied to skin during a patch testing, is reported on chapter 29.
The bioavailability of the applied chemical is to be considered as important as the same intrinsic activity, when a patch test is performed. As matter of fact, a chemical must penetrate the skin eliciting a response.
Nail penetration is the topic of chapter 30, where the physical and chemical properties of human nail pertaining to topical drug delivery are reported.
Aminoacid composition of human nail is much closer to hair than to the SC, because of the lower

glycine and higher half-cystine content in the nail keratin. For these reasons, the human nail plate does not mimic the behavior of a lipophilic membrane, such as the skin, but is more like a hydrogel membrane in its barrier properties. Once hydrated, a hard nail plate becomes more elastic, hence probably more permeable to a topically applied drug.

Since the newer antifungal agents have shown a better risk-benefits ratio, topical therapy in combination with oral therapy may help increase overall efficacy of the drugs used for onychomycosis.

At this purpose, many are the studies to verify the ability of topical treatments alone to cure onychomycosis. Recent research findings begin to lay a theoretical foundation for successful development of efficacious topical products to treat nail diseases.

Chapter 31 tries to describe the ideal vehicle, as the sum of the ingredients in which the drug is presented to the skin.

In fact, the drug delivery vehicles commonly used today, attempt to achieve a balance between the physiochemical requisites for stability, and preservation against microbial spoilage, and release of the active ingredients to the SC.

Thus, modern pharmaceutical and cosmetic formulation development is based upon the stability and compatibility of excipients and active drug (s) cosmetic or aesthetic user-acceptability of the vehicle and bioavailability of the drug(s).

Due to its complexity, dermatological vehicle treatment may, therefore, remains an art gleaned by personal experience rather than a defined textbook science.

Despite widespread use of sunscreens, chapter 32 is entirely dedicated to their percutaneous absorption.

Sunscreens are, in fact, an important group of products, classified as cosmetics for European law or drugs for the FDA in USA, that provide protection against sunburn and many cumulative, suberythemal forms of skin damage.

Even if only small numbers of adverse reactions to sunscreens have been reported, it is possible to predict some extent of penetration of sunscreen agents and to speculate how penetration may be modulated by various types of vehicle.

Therefore, the influence of formulation vehicle and quantification of penetration rates, and skin distribution using experiments designed to mimic actual product use, seem to be essential prerequisites to obtain realistic risk assessment values.

In fact, the development of colloidal drug carrier systems, such as micelles, mixed micelles, liposomes, and microemulsions play an important role in the activity and delivery through the skin of modern pharmaceutical and cosmetic ingredients.

Notwithstanding our deep knowledge on carriers, each drug or active cosmetic ingredient requires a different, precisely formulated, vehicle for optimized therapy or treatment.

Issues of stability and compatibility of carriers and active agents together with local and systemic safety of all components must be counter-balanced with adequate drug delivery potential and user acceptability. Finally, metamorphosis of the vehicle after application to the skin has to be considered in terms of the drug /active cosmetic ingredient reservoir formed in the SC and in terms of the extent and period of delivery desired.

With these interesting considerations ends this complete volume entirely dedicated to the problems concerning the absorption of dermatological products.

For the accuracy with which the absorption of active ingredients and carriers through the skin layers and its appendages are treated, this volume should be considered as a main textbook for dermatolo-

gists, biologists, cosmetic chemists, pharmacists and students of Medicine and Pharmaceutical Chemistry willing to know the metabolic destiny of those substances which, directly and indirectly, come into contact with the skin.

P. MORGANTI
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SKIN MOISTURIZATION

By J.J.Leyden and A.V.Rawlings
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The skin is the most complex organ of human body that allows both physical and immunological protection from the damages of the external environment. It consists of two district layers (1) the dermis, made up of connective tissue elements, and (2) the epidermis, composed primarily of keratinocytes. The outmost layer of the skin, the Stratum Corneum (SC) as true interface with the environment, is represented as bricks and mortar where bricks are the corneocytes embedded in a matrix of specialized lipid bilayers (the mortar).

The protein matrix of corneocytes contains a specific mix of hygroscopic low molecular weight compounds that keep the skin hydrated. Therefore the main objective of the permeability barrier is to be as tight as possible, except for a minimum leakage of water needed for the hydration of the keratin necessary to plasticize the SC.

As matter of fact, water-holding properties of corneocytes are influenced by the rate of filaggrin break down that leads to formation of amino acids known as Natural Moisturizing Factors (NMF).

Functions and dysfunctions of skin effect not only the physical health, but also the self-esteem of the person. For these reasons repetitive and excessive contact with cleansers and water can be very drying and irritating to skin, tending to remove both the lipids and NMF.

All the cleansers surfactant-based or oil-solvent-based can disturb the skin, dissolving and removing the intercellular lipid bilayers of the SC, and can also interact and damage the protein composition of corneocytes. Of course, the extent to which cleansers may provoke all or some of these damages depends upon the formulation of the cleanser and the duration and frequency of skin contact. For these reasons nurses, plastic surgeons, mothers with a number of infants, and all people that have to wash the hands frequently, have to use more mild cleansers and to limit contact with water to maintain skin in good condition.

Water and cleansers are in fact, the main factors damaging the water-holding mechanisms of the superficial SC, while wind and low humidity are the main environmental factors removing water from damaged regions of superficial SC.

Therefore, when the relative humidity is high, the skin's NMF has little difficulty in holding water in the protein matrix of corneocytes. At low humidity the NMF is unable to hold water against the pull of low partial pressure at the skin surface. If the NMF is depleted, there is nothing to hold water at low relative humidity (RH) so that the skin surface becomes very dry.

For all these reasons NMF is essential for the correct functioning of the SC.

Working together with the interlamellar lipids, it assists in the retention of water within the corneocytes, vital for the barrier and mechanical properties of the SC.

Moreover, over-exposure to solar rays may aggravate the problem of dry skin, interfering both with filaggrin degradation and, therefore, with NMF production, and generating lipid peroxides.

further research will be required to commercialize this new technology.
This part of the book ends with chapter 20 dedicated to the description of a new category of skin cleansers designed to deposit beneficial lipids during wash.
Soap-based normal cleansers have, in fact, the potential to interact with skin proteins and lipids leading to dry skin and irritation. Delivering moisturizing benefits from cleansers is a new technical challenge since this involves actually depositing and delivering skin care materials under wash-off conditions that are normally designed to remove materials from skin.
This trend of providing skin care benefits from wash-off systems is a new area of active research resulting in novel product forms and cosmetic technologies.
Part IV is dedicated to the Evaluation methodologies described in six chapters, from 21 to 24.
Like foods, moisturizers have a dual identity. Their are experienced as functional as well as hedonic-giving substances. Thus more emphasis is being placed on understanding the consumer as a person and not as a marketing object, and many marketing managers are asking who to test, what to test, and how to test.
For these reasons the main responsibility of research and development department (R&D) is to help guide the development chemist's efforts to build products that will delight consumers and induce them to become loyal brand users.
But what is a moisturizer? It is a topically applied substance that overcomes the signs and symptoms of dry skin and, today, the amelioration of symptoms is still the primary benefit required. However dry skin has many aspects, from how it looks to how it feels to how it functions. Determining the ideal course for testing is not always easy, and, for these reasons, a wide array of tools, both visual and instrumental measurement methods are used. Thus instrumental methods are ideal at providing objective data to individual aspects of what the product does for skin.
With expert assessment, a qualified human judge integrates many aspects into measurements of the visual appearance of skin. Subject self-assessment within the context of the chemical trial gives quantitative measure to the perception of skin look and feel.
Clearly, the selection of measurement(s) both in vitro and in vivo must be made appropriately for the products being evaluated and the choice of measurement has to balance the different study needs.
Moreover the instrumental measurements have to be done in a controlled environment and the operator must have a clear understanding of their instruments with it.
Chapters 25 and 26 are both dedicated to the Formulation problems of the cosmetic skin-care (part V), focusing not only on the technology necessary to make and produce the moisturizing products but also on the understanding of the consumer's need.
The final objective is to create an efficacious, stable, safe, and esthetically pleasing product that meets consumer expectation.
All the technical elements that go toward supporting successful product development are reported in these two interesting chapters.
In the last two chapters of the book (27 and 28) respectively the Safety and the Regulatory Assessment of Cosmetic Products are discussed (part VI).
Thus the author has tried to show, in the chapter 27th not only what is involved in the safety assessment of cosmetic products and how to go about the process itself, but also to indicate the central role a safety assessor can play in cosmetic company.
As matter of fact, the safety assessor should be totally involved in the project development planning from the product formula, to control of the ingredients safety, to marketing, advertising labeling,

packaging and finally to customer relations and the eventually crisis management.
If utilized fully, the safety assessor can avert many problems early in product development and so directly contribute to effective use of time and resources.
In chapter 28 are reported all the regulations controlling cosmetics in Europe, in USA and in Japan that are moving in the direction of a harmonized system capable to ensure consumer safety and enable free trade.
The book ends with the hope that industries and the regulators can cooperate in the years to come in order to assure that the products meet the same regulations in all target markets.
This highly esteemed volume deserves a special thank for the accuracy used in treating all the topics always followed by complete and update references.
It is advised to be in the library of medical doctors, biologists, chemists, dermatologists and all the students interested in the skin hydration problems.

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Note

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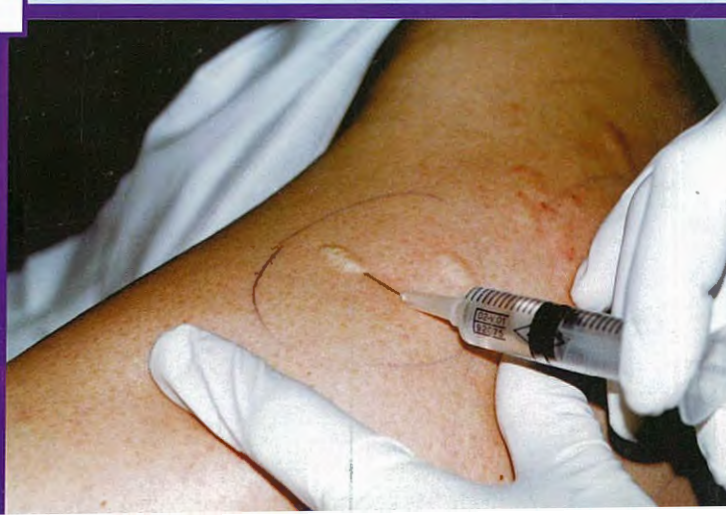

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