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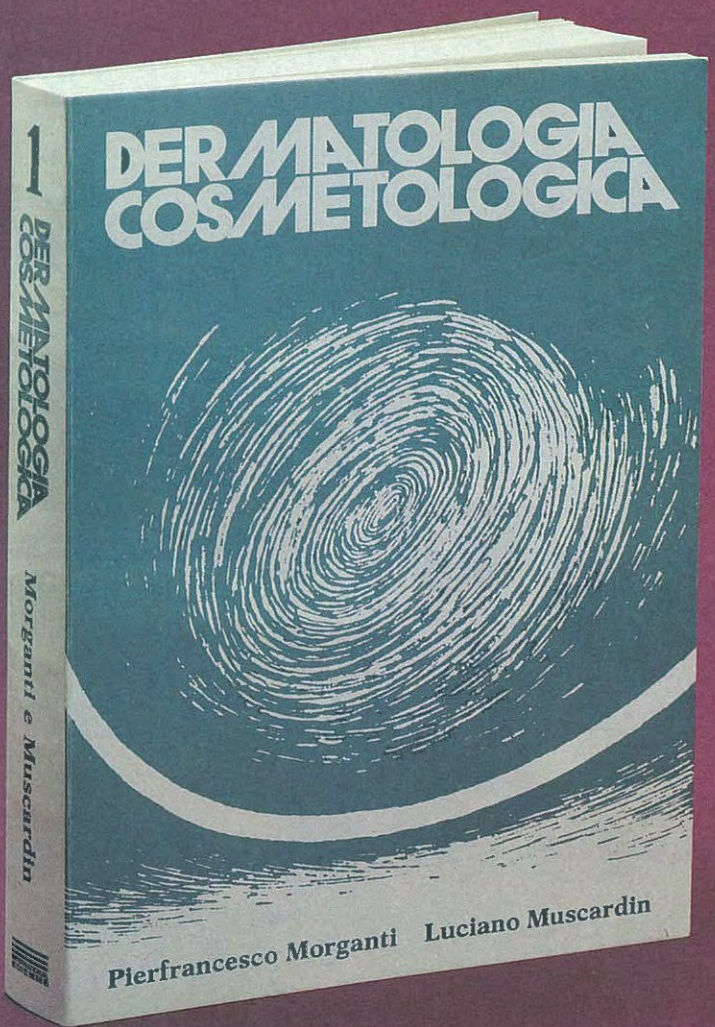

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General Articles

J. Appl. Cosmetol. 14, 35-41 (April-June 1996)

ALPHA HYDROXY ACIDS IN COSMETIC DERMATOLOGY

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Received: *February 22nd, 1996*

Key words: *AHAs; Gelatin-Glycine, Gelatin/Arginine, Gelatin/Lysine, Skin Peeling, Glicolic Acid, Hyperkeratosis, Skin Turnover.*

Synopsis

AHAs, the latest class of cosmetic active ingredients, are the focus of extensive research and many expectations. This paper deals with their chemistry, physiological activity and properties and possible therapeutic effectiveness in Cosmetic Dermatology.

Riassunto

Ultima classe di principi attivi di uso cosmetico, gli alfaidrossiacidi sono al centro di molte ricerche e di molte attese. Ne viene descritta la composizione chimica, il probabile meccanismo d'azione e le varie possibilità di impiego nella Dermatologia Cosmetologica.

Much has been said about alpha hydroxy acids (AHAs). The latest class of active ingredients currently used in the cosmetic industry is the focus of many expectations and extensive research. That is the reason why they deserve to be dealt with in a detailed manner, so that dermatologists, plastic surgeries and beauticians can give their customers expert advice on this matter. AHAs are used both in dermatology and in cosmetic surgery as a supplement to standard medical therapy. In addition, their capacity to facilitate cell turnover is by now widely accepted. Let's see how they act in patients with xerosis, hyperkeratosis, acne and dehydrated skin. (1-10)

Keratinization consists in the malpighian epithelium undergoing changes. This results in the transformation of keratinocytes into corneocytes, external cells which are removed from the skin surface due to desquamation.

Before being removed, corneocytes carry out a protective and elastic action, which consists in making the epidermis compact, semi-permeable, flexible and soft. (11)

In patients with keratogenesis disorders, dermatologic events occur which lead to hyperkeratinization. Skin dryness, ichthyosis, keratosis and comedones are different disorders of similar nature: they are all associated with hyperkeratinization caused by increased corneocyte cohesion with a subsequent decrease in interstitial water. Increased corneocyte cohesion, which is linked also to the presence of intercellular lipids, leads to a greater number of more external cell layers. (12)

The resulting presence or lack of water, a main intercellular lubricant, as well as qualititative and quantitative lipid changes affect not only the physical properties, but also the scaling capacity of the stratum corneum. (13)

This pluricellular layer is generally characterized by a high capacity to absorb water, as in natural sponges. In addition, it can bind and retain water through NMFs and polar lipids, both of which are found in intercellular spaces.

Thus, corneocyte cohesion can be reduced by ensuring that both water in the stratum corneum and intercellular lamellar lipids, which control and regulate water diffusion, are constantly present. In order to treat hyperkeratinized skin, it is necessary to re-hydrate the stratum corneum using active ingredients which are able to bind water over long periods and subsequently reduce corneocyte cohesion. (14-19)

In fact, corneocyte cohesion depends on the moisture level, that is on water.

This decreased cohesion may also be due to a different arrangement of ionic bonds. The resulting biochemical process might be linked to the non-formation of the cross-linking extracellular matrix proteins, which usually trigger the events leading to keratinocyte and corneocyte cohesion. (1-2)

According to another hypothesis by Ziboh, it seems possible that an elevation of AHAs by dietary or topical means could suppress the cutaneous inflammatory reactions elicited by excessive generations of prostaglandins and leukotriens. (20)

In fact, the skin is characterized by an active metabolism of polyunsaturated fatty acids, the lack of which is known to cause dermatosis and skin barrier changes. Thus, a link is supposed to exist among AHAs, polyunsaturated fatty acids and skin cell metabolism. (20)

The concurrent therapeutic use of the gamma linolenic acid both topically and systemically is interesting also for the above reasons. (21)

AHAs: CHEMISTRY AND ACTIVITY

Alpha hydroxy acids (AHAs), also known as alpha hydroxyenoic acids, are used in Cosmetic Dermatology. They are a peculiar group of biologically ubiquitary organic acids, since they play a specific role in the cycle of carbohydrates and other metabolic pathways (Table 1).

Among the many active ingredients currently known, alpha hydroxyenoic acids undoubtedly

have proved the most effective regulators of corneocyte cohesion. They facilitate water absorption among cells at the outer layers and reduce corneocyte cohesion at the lower layers of the stratum corneum, changing lipid typology and the enzymatic systems produced by Odland's lamellar bodies. As a result, a higher skin moisture level, improved cell turnover and normalization of keratoses and pigmentary abnormalities (Fig.1 a/b).

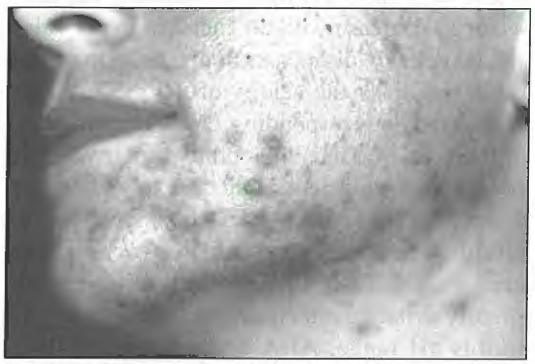


***Fig. 1a** - Before glycolic acid treatment*

***Fig. 1b** - After one month treatment (by courtesy of Dr A. Corbo - Rome)*

The most biologically significant AHAs are usually found in nature and are regularly taken through food. Glycolic acid is contained in cane sugar, lactic acid in milk, malic acid in apples, citric acid in many citrus fruits, and tartaric acid in grape wine.

As can be noticed (Tab.1), AHAs belong to a family of chemical derivatives with different molecular weights, due to the varying length of their carbon chain.

Glycolic acid is made up of two carbons only, while, for example, citric acid contains six of them. We can easily understand why glycolic acid has a higher penetration degree than other alpha hydroxy acids with greater weight and size. However, we should not be misled by this. For example, the carbon lactic acid has also the biological capacity to convert into its ketonic form, namely piruvic acid, which is as much active in reducing corneocyte cohesion. (22)

There are added problems, such as the possible salification of these acids, which is often useful or even necessary to reduce their stinging or burning action on the skin and their reactivity to other molecules in cosmetics. As regards active ingredients, we should not forget that their skin penetration speed and ways depend not only on their molecular size, but also on their chemical and physical characteristics, on the formulation type, and thus on the vehicle which contains them and from which they should be released. It was shown why some AHAs mixtures act more rapidly than single compounds in both reducing corneocyte cohesion and improving the skin hydration state. The optimum pH value, at which they appear extremely active, was shown to range from 4 to 5.5. (23-26)

In addition, standard moisturizers, such as collagen, appeared to be active over some hours only, while AHAs administered topically and gelatin-glycine administered both topically and

Tab 1

Glycolic acid	$\underset{CH_2COOH}{\overset{OH}{}}$	mw 76,05
Lactic acid	$\underset{OH}{CH_2CHCOOH}$	mw 90,08
Malic acid	$HOOC\text{-}\underset{OH}{CH}\text{-}CH_2\text{-}COOH$	mw 134,09
Tartaric acid	$HOOC\text{-}\underset{OH\ OH}{CH\text{-}CH}\text{-}COOH$	mw 150,09
Mandelic acid		mw 152,14
Citric acid	$\underset{HOOC\text{-}CH_2\text{-}\underset{OH}{C}\text{-}CH_2\text{-}COOH}{\overset{COOH}{}}$	mw 192,12

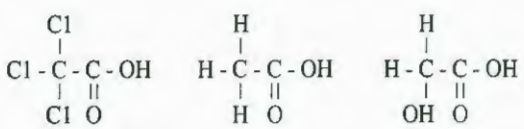
systemically, act over long periods (days), even when they are temporarily discontinued. Finally, the action of AHAs was shown to be directly proportional to their concentration, which depends on the vehicles used. (27-28)

Although its mean of action is still unknown, current data seem to indicate that AHAs activity consists in decreasing corneocyte cohesion through a modification of ionic bonds.

When applied locally at low concentrations (10-12%) using suitable vehicles, AHAs gradually make hyperkeratinized stratum corneum less tough by reducing corneocyte cohesion at the lower level of stratum corneum without affecting the upper cells level, directly in contact with the outside.

When applied at high concentrations as, for example, in peeling treatments (70% glycolic acid), they cause no disaggregation of corneocytes in the outer layers of the stratum corneum, as happens when using other chemical agents such as strong acids and alkali, including trichloroacetic acid, thiols or other denaturants (urea and lithium salts at high concentrations).

Unlike what happens with the above actual keratolitics, AHAs affect the cohesion of newly-formed corneocytes in the lower layers. In fact, glycolic acid is structurally similar to both trichloroacetic acid and acetic acid:



Trichloroacetic acid — Acetic acid — Glycolic acid

Glycolic acid shows an intermediate acidity with respect to the other two acids, due to the presence of the -OH hydroxyl group in its molecule. Thanks to its small molecular size, it penetrates the epidermis and probably also the dermis. Due to its acidity, it brings about an intraepithelial chemical peeling, reducing the cohesion power that bind corneocyte together. (29) This peculiar way of acting suggests that their dynamic pharmacological action is carried out only at a specific stage of the keratinogenic process.

pH VALUE AND CELL TURNOVER

When being in their acid form and at suitable concentrations (5% to 10% approx.), AHAs increase cell turnover: their stimulating capacity seems to decrease with the increase in pH value and completely disappears at pH 7. On the other hand, the lower the acidity of the medium, the stronger the corresponding erythemic property of these acids. Thus, the optimum pH value for AHA-based emulsions is thought to be between 4 and 5.5. In fact, at pH 4.5 the amount of free glycolic acid is still great and, in any case, sufficient to effectively stimulate cell turnover with slightly irritating effects on the skin.

In this pH range, AHA erythemic property is thought to be reduced to its minimum, without affecting the action AHAs seem to carry out on the proteic bonds that desmosomes establish with epithelial cells. The dissolution of these bonds due to a low pH value seems to underlie the increase in DNA synthesis and in the metabolism of the skin cells subject to AHA action. (29)

SYNERGIC SUBSTANCES

According to recent studies, gelatin-glycine and gelatin-arginine seem to be able to synergize the re-moisturizing activity of AHAs. Concurrently using gelatin-glycine both topically and systemically allowed to reduce AHA dosage and to improve AHA re-moisturizing activity, positively effecting "wrinkle lines" due to photoageing. Concurrently using these two active ingredients reduces AHA risk-benefit ratio and prevents the so-called *status cosmeticus* sometimes caused by the low pH value of these acids.(30)

AHA CONTROLLING SKIN HYPERKERATOSIS

Much clinical importance is attached to the laboratory finding that, repeatedly applying AHAs at low concentrations, prevents a thick stratum corneum from re-forming, after this has been eliminated by previous intensive treatment (such as peeling). This is a way to treat skin disorders in a relatively short time. (31)

This enables to effectively use AHAs with periodical follow-up of treatment for xerosis, ichthyosis, acne and ageing-related actinic keratosis (the so-called *age spots*), also in order to keep wrinkle-prone skin smoother and firmer. (26-28, 32-34)

If applied in consulting rooms at high concentrations, AHAs penetrate deeper in the skin with a probably less specific effect. They cause reduced keratinocyte cohesion, complete epidermolysis, epidermis separation and heavy stimulation of the dermis, both papillar and reticular, with resulting deep injuries. Thus, the ideal conditions occur that seem to underlie the synthesis of new collagen. (35-37)

This activity naturally and directly depend on the skin area and type, on the pH and AHA concentrations, on the type of vehicle, the exposure time and the cleansing methodology. The clinical relevance of the changes resulting from the use of AHAs at high concentrations indicates that they should be administered exclusively by dermatologists.

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References

1. **Van Scott EJ, Yu RJ. (1974)** Control of keratinization with alpha hydroxy acids and related compounds: 1.topical treatment of ichtyotic disorders. *Arch. Dermatol.* **110**:586-590.
2. **Van Scott EJ, Yu RJ. (1984)** Substances that modify the stratum corneum by modulating its formation. In: Principles of Cosmetics for the Dermatologists. In: Frost, SN Horwitz, eds. CV Mosby Co., St. Louis, pp.70-74.
3. **Van Scott EJ. (1988)** The unfolding therapeutic uses of alpha hydroxy acids. In: *Med guide to Dermatol.* **3**:1-5.
4. **Van Scott EJ, Yu RJ. (1989)** Alpha hydroxy acids: therapeutic potentials. *Canadian J. Dermatol.* **1**:108-112.
5. **Dahl MV, Dahl AC. (1983)** 12% lactate lotion for the tratment of xerosis. *Arch. Dermatol.* **119**:27-30.
6. **Buxman M, Hickman J, Ragsdale W, et al. (1986)** Therapeutic activity of lactate 12% lotion in the treatment of ichthyosis. Active vehicle and active vs. a petroleum cream. *J. Am. Acad. Dermatol.* **15**:1253-1258.
7. **Van Scott EJ, Yu RJ. (1989)** Alpha hydroxy acids: treatment of the Aging Skin with Glycolic Acid procedures for use in clinical practice. *Cutis.* **43**:122-128.
8. **Moy LS. (1990)** The use of glycolic acid in the treatment of various skin disorders. Communication at the 17th Annual Clinical and Scientific Meeting of the American Society for Dermatologic Surgery. Reported in *Canadian Medical News*, July 27.
9. **Anonimous (1992)** Alpha hydroxy acids help protect skin. *Editorial. Am. Pharm*: pp. 32-36.
10. **Burke KE. (1990)** Facial wrinkles. Prevention and non-surgical correction. *Post. Grad. Med.* **88**:207-210.
11. **Van Scott EJ, Yu RJ. (1984)** Hyperkeratinization, corneocyte cohesion, and alpha hydroxy acids. *J. Am. Acad. Dermatol.* **11**:867-879.
12. **Williams ML. (1991)** Lipids in normal and phatological desquamation. In: advances in lipid research: skin lipids. (Ed. Elias PM) A. Cad. Press. N.Y. pp. 211.
13. **Elias P.M., Brown B.E. (1978)** The mammalian cutaneous permeability barrier: Defective barrier function in essential fatty acid deficiency with abnormal intercellular lipid deposition. *Lab. Invest.* **39**:574-583.
14. **Rawlings A.V., Scott I.R., Hardiong C.R., Bowser P.A. (1994)** Stratum Corneum moisturization at the molecular level. *J. Invest. Dermatol.* **103(N5)**:731-740.
15. **Randazzo S.D., Morganti P. (1990)** Skin and water: an update. *J. Appl. Cosmetol.* **8**:93.
16. **Morganti P., Randazzo S.D. (1990)** Skin hydration control and treatment: recent update. *J. Appl. Cosmetol.* **8**:103.
17. **Morganti P., James B., Randazzo S.D. (1990)** The effect of gelatin-glycine on skin hydration. *J. Appl. Cosmetol.* **8**:81.
18. **Morganti P., Persechino S. (1993)** The effect of new generation cosmetics on epidermal barrier permeability. *J. Appl. Cosmetol.* **11**:41.
19. **Morganti P., Bruno C., Persechino S. (1994)** The protective and hydrating capacity of emollient agents in the antiaging treatment of the skin. *J. Appl. Cosmetol.* **12**:25-30.
20. **Ziboh V.A. (1992)** Prostaglandis, leukotrienes, and hydroxy fatty acids in epidermis. *Sem. Derm.* **11(2)**:114-120.
21. **Randazzo S.D. (1995)** Private communication.

22. **Elson M.L. (1993)** The regular structure of glycolic acid and its importance in dermatology. *Cosmet. Dermatol.* **6**:(7), 31-5.
23. **Hermitte R. (1992)** Aged skin, retinoids and alpha hydroxy acids. *Cosmet. Toilet.* **107**:63-66.
24. **Vilaplana J. (1992)** Clinical and non-invasive evaluation of 12% ammonium lactate emulsion for the treatment of dry skin in atopic and non-atopic Subjects. *Acta Dermatol. Venerol.* **72**:28-33.
25. **Wehr F.R. (1991)** A controlled comparative efficacy study of 5% ammonium lactate lotion vs. an emollient control lotion in treatment moderate xerosis. *J. Am. Acad. Dermatol.* **25**:849-851.
26. **Morganti P., Fabrizi G. and Randazzo S.D. and Bruno C. (1996)** Decreasing the stinging capacity and improving the antiaging activity of AHAs. Presented at IN-COSMETICS Milano, February 28-29, March 1. Conference proceedings, pag. 91.
27. **Morganti p., Persechino S. and Bruno C. (1994)** Effects of topical AHAs on skin xerosis. *J. Appl. Cosmetol.* **12**:85-90.
28. **James B., Morganti P. (1994)** Oral administration of gelatin-glycine for aged skin. *J. Appl. Cosmetol.* **12**:17-23.
29. **Smith W.P., Smith W. (1994)** Hydroxy acids and skin aging. *Cosmet. Toilet.* **109**:(9)41-48.
30. **Morganti P., Randazzo S.D., Bruno C. (1996)** Alpha hydroxy acids in the cosmetic treatment of photo-induced skin aging. *J. Appl. Cosmetol.* **13**:1.
31. **Dial W.F. (1990)** Uses of AHAs add new dimensions to chemical peeling. *Cosmet. Dermatol.* **3**:32-34.
32. **Dial W.F. (1990)** Preparations prescribed in anti-wrinkling therapy. *Cosmet. Dermatol.* **3**:6-7.
33. **Ridge J.M., Siegle R.J., Zuckerman J. (1990)** Use of alpha hydroxy acids in therapy for photoaged skin. *J. Am. Acad. Dermatol.* **23**:932.
34. **Ditre C.M., Griffin T.M., Murphy G.F., Sueki H., Telegan B., Johnson W.C., Yu R.J. and Van Scott E.J. (1996)** Effects of alpha hydroxy acids on photoaged skin: a pilot clinical, histologic and ultrastructural study, *J. Am. Acad. Dermatol.*, **34**:187-195.
35. **Stagnone J.J. (1989)** Superficial peeling. *J. Dermatol. Surg. Oncol.* **9**:924.
36. **Moy L.S. (1991)** Glycolic acid effective in comedonal acne. *Skin and Allergy News* **22, 8**:3 e 25.
37. **Moy L., Murad H., Moy R. (1993)** Glycolic acids peels for the treatment of wrinkles and photoaging. *J.Dermatol. Surg. Oncol.* **19**:243.

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VALIDATION TESTS AND CELL CULTURES IN COSMETOLOGY: THE PRESENT AND PROSPECTS

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Synopsis

The interest increasingly attracted by cosmetics in recent years has led to considerable development in the related technologies. This progress, however, has not been followed up by adequate legislation. The recent 93/95 Directive of the European Economic Community established that from January 1st 1998 the safety of cosmetic products can no longer be tested on animal models, and that alternative methods must be employed, if available. Direct experimentation **in vivo** is prevented by ethical reasons, while animal models are frequently poorly predictive. For these reasons **in vitro** culture is now of outstanding interest in cosmetology. The authors review the cell culture types that can be used in cosmetological studies

Riassunto

Il grande interesse di cui sono stati oggetto i cosmetici negli ultimi anni ha determinato un notevole sviluppo delle tecnologie legate alla loro produzione, uno sviluppo che non è stato tuttavia accompagnato da adeguati interventi legislativi. La recente Direttiva 93/95 della Comunità Economica Europea vieta dall'1 gennaio 1998 l'utilizzo di modelli animali nei test di valutazione della sicurezza dei prodotti cosmetici, che da quella data dovranno essere effettuati con metodi alternativi, se reperibili. Motivi etici impediscono la sperimentazione sulla specie umana, mentre i modelli animali sono spesso scarsamente predittivi. Ecco perché in cosmetologia la coltura in vitro è attualmente di grande interesse. Gli Autori riassumono i principali tipi di colture cellulari che possono essere utilizzati negli studi cosmetologici.

That our skin is the mirror of our organism is a recognised concept that is proved consistently by the difference between our chronological and biological age. In this sense Cosmesis must be recognized as much more than the simple attempt at improving the aesthetic quality of our image. Indeed, today the cosmetic industry devises products that do not only aim at improving but also at protecting the skin - for instance against the damage wrought by sunlight - and more and more frequently at assisting dermatological medical treatment (1). The interest increasingly attracted by cosmetics in recent years has led to considerable development in the related technologies. This progress has however not been followed up by adequate legislation. After formulation and production, cosmetics should be analysed in technically advanced laboratories. The field of the evaluation of the safety and activity of cosmetic products appears to be full of interesting prospects (2, 3). Over the last 20 years, a series of directives of the European Economic Community (EEC) have required the definition of a series of experimental models for the study of the toxic properties of chemical substances, based mainly on animal tests (4). This research has highlighted the limitations of such experimental models. Moreover, it has shown that the results obtained **in vivo** with traditional models can be complemented and improved by data obtained **in vitro** on cell populations, which allows to study also the development of the toxicological processes induced by chemical substances. The recent 93/95 EEC directive establishes that from January 1st 1998 the safety of cosmetic products can no longer be tested on animal models, if the alternative methods are available. Alternative methods must be devised to identify the toxic potential of ingredients. Several laboratories are working to provide cosmetic industries with validated and reproducible tests that do not require experimentation on animals. Directive 76/768 moreover requires that by 1997 every finished cosmetic product marketed in the EEC be accompanied by exhaustive information on the whole history of the product, from its quantitative formula and production method to the toxicological files of every component, the allergologic tests where necessary, and the clinical tests of activity if they are mentioned in the label. The 93/95 directive requires these files to take "into consideration the general toxicological profile, the chemical structure and the exposure level of all ingredients". Direct experimentation in vivo is prevented by ethical reasons. On the other hand, animal models are frequently poorly predictive, nor are they directly applicable to man because of species and individual differences, as well as differences in doses and exposure time.
In vitro culture of the various components of the skin is at present widely used in genetic, immunological, neurological and, last but not least, cosmetological studies (5). Research is now focusing on **in vitro** reconstruction of the fibroblast and keratinocyte differentiation to obtain a predictive model on the toxicity and topic efficacy of the various substances or finished products on human skin.

Cell culture types

Cell cultures have provided and continue to provide interesting data, since they are simplified systems constituted by organized living cells. Their main advantages are their reproducibility, affordable cost, the fact that they yield results faster than animal models, and that they allow to study a sufficient amount of specimens to obtain statistical significance. The main problem can be the difficulty in correlating the active concentrations **in vivo** with those **in vitro**, considering also that the cells' features vary over time (4). There are different types of cell cultures. Those that are utilised more frequently in validation tests are:
- **cultures in suspension**, where cells can live and proliferate without an adhesion surface;
- **monolayer cultures,** where cells grow **in vitro** attaching to the surface of a culture dish. They need to attach to the substrate for their life and proliferation. Their growth results in monolayers,

i.e. confluent surfaces constituted by a single cell layer. Monolayer cultures are usually primary cultures, i.e. they derive directly from cells explanted from donors. This type of culture allows cells to maintain many functional and specialization characteristics observed in vivo (Fig. 1);

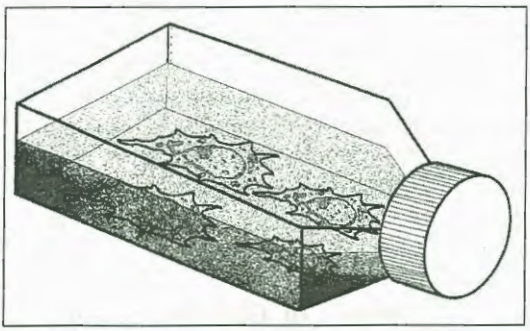


Figure 1: *monolayer culture: cells (fibroblasts) grow in vitro attaching to the surface of culture flask.*

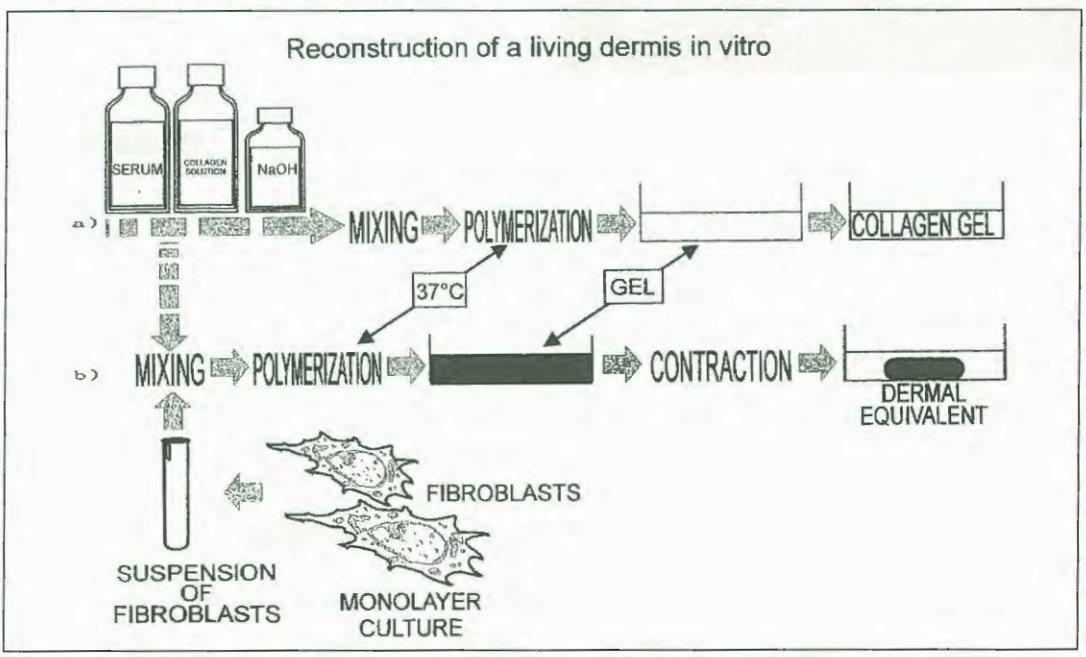
Figure 2:
a) reconstruction of collagen gel in vitro;
b) reconstruction of dermal equivalent in vitro.

- in co-cultures, cells of different types are grown in the same culture dish, for instance fibroblasts and keratinocytes; this allows to reproduce in vitro the complex interactions that occur **in vivo** between different tissues.

a) Monolayer cultures of fibroblasts

In cosmetological research fibroblasts are the cells used more frequently. To reconstruct the dermis in vitro, fibroblasts need to be seeded on a collagen suspension gel that is close to polymerization (dermal equivalent)(6). The fibroblasts attach to the polymerised collagen, contract their fibrils and form the dermal tissue: dermal equivalent (Fig. 2). The three components of this three-dimensional culture model (culture medium, extracellular matrix and fibroblasts) can then be studied separately (6).

Standardization is of central importance in these experiments: donors' age must not exceed a precise range, and fibroblasts must be taken from the same source (skin of the forearm). There are dramatic differences between sites, for instance in enzyme activity (in fibroblasts obtained from



the pubic region the 5 α-reductase enzyme exhibits a five- to six-fold activity as compared to those from the forearm). As for collagen, its origin and extraction must be well characterized and standardized, and working with the same batch for the whole series of experiments is highly recommended. Type I collagen is extracted with acetic acid from rat-tail tendons or from calf skin, while a mixture of types I and III is obtained from human placenta by digestion with pepsin (6). The culture medium is EMEM (Earle's modified Eagles medium), with the addition of 10% foetal calf serum, of pooled AB serum from healthy donors, or serum from patients. Serum greatly influences the life and proliferation of fibroblasts, so the same batch must be used throughout the study. Cultures must be maintained at 37°C in 5% CO_2 /95% air athmosphere. Fibroblasts raised in monolayer cultures divide until they reach confluency, while in dermal equivalents they remain separated (Fig. 3). Their growth rate depends on the density of cells seeded: from $4x10^4$ to 10^5 cells/ml^{-1} proliferation is inversely related to the number of cells seeded. The dermal equivalent can be used as a model of cell growth homeostasis in relation with the external environment. It can also be employed in quantitative studies on collagen fibril condensation by fibroblasts. Rate and extent of contraction are measured by monitoring the diameter decrease of the dermal equivalent. Contraction is directly related to the initial concentration of fibroblasts, and inversely related to collagen concentration. Contraction is very sensitive to pharmacological treatment. The ability of fibroblasts to contract the collagen matrix is inhibited in a linear, dose-dependent manner when dexamethasone or hydrocortisone (2.5 - 1,5 x 10^{-4} M) are added to the culture medium in the presence of an inhibitor of cell division that does not affect the cytoskeleton (1 µl ml^{-1} cytosine arabinoside). Contraction is inbibited by the addition to the culture medium of Acid Fibroblast Growth Factor (a-FGF) in the presence of heparin, which is a factor that stabilises a-FGF (6).

Figure 3: *adherent fibroblasts grown in culture with typical cytoplasmic protrusion and mostly spindle-like features.*

b) Co-cultures of fibroblasts and keratinocytes

To reconstruct in vitro a simplified human skin, an association of dermis and epidermis is necessary as in vivo (5). There are several models, of which we describe the simplest. The dermal equivalent described above is utilized in close

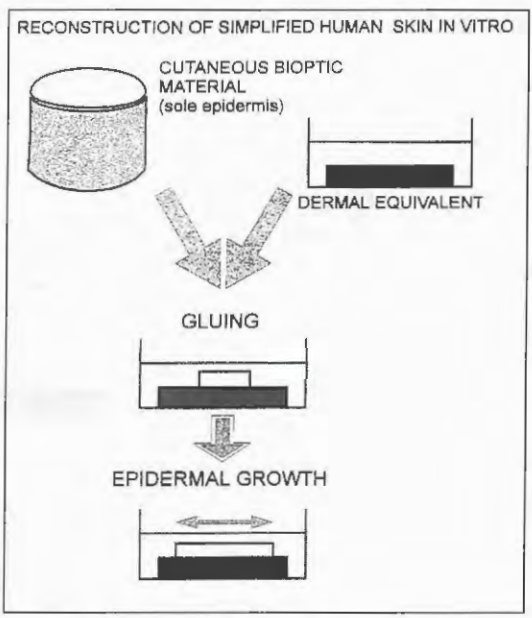


Figure 4: *reconstruction of simplified human skin in vitro.The authors are grateful to Mr G. Forniti of MAVI SUD for providing the drawings*

association with cutaneous bioptic material constituted by the sole epidermis (Fig. 4). It is well known that fibroblasts and collagen affect the growth and differentiation of epithelial cells. A layer of lethally irradiated fibroblasts supports the culture of keratinocytes and increases their lifespan. Collagen provides for their adhesion and for plating efficiency, and is essential in the formation of the basal lamina (6). Keratinocyte sources (donors aged 20 to 40 years) can be superficial cutaneaous biopsies, which are implanted in a dermal layer, or epidermal biopsies, which are glued to the dermis during its fibroblast-mediated formative organization with a drop of soluble collagen. The same source of keratinocytes must be used throughout each study. The donor of keratinocytes differs from that of fibroblasts for practical reasons. No interference is observed between allogenic cells in vitro because there are no cytotoxic cells. Langerhans cells do not appear in the epidermis that is thus formed. Allogenic fibroblasts are not only tolerated when the equivalent epidermis is grafted onto animals, but they are actually integrated structurally. Rejection is not observed when the epidermal equivalent is grafted onto humans, but its tolerance and persistence are yet to be demonstrated (7). Culture conditions are the same as those for the dermal equivalent, except for the addition to the medium of Epidermal Growth Factor (EGF; 10-20 $\mu g\ ml^{-1}$), hydrocortisone (0.4 $\mu g\ ml^{-1}$) and cholera toxin (8.4 $ng\ ml^{-1}$), traditionally used in keratinocyte cultures (6). When there are no specific treatment indications, only EGF is essential, to promote keratinocyte migration and support proliferation as well as the epidermalization of the biopsy. Experiments should however be conducted both with and without EGF (6). The epidermal equivalent can be either completely immersed in the medium, when privileging the study of cell growth, or kept in contact with air when studying prevalently differentiation. The keratinocytes grow and divide, and form an epidermis which spreads throughout the dermal equivalent. This new epidermis can differentiate as in vivo by forming cuboid basal cells, keratohyaline granules, etc (7). This method of epidermalization has several advantages. It allows not only to study differentiation qualitatively in terms of morphology and keratin analysis, but also to study epidermal growth quantitatively in terms of surface area. To study cell proliferation, tritiated thymidine is incorporated and DNA content is evaluated by flow cytometry. DNA content per unit of surface area reflects the subtle balance between proliferating, migrating and differentiating cells. The surface area of the epidermis is visualised by staining the whole culture with Nile blue sulphate before separating the dermis from the epidermis. The surface area can be measured with an image analyser. The mutual influences of the two tissues can be studied by analysing the dermis and epidermis separately. The results obtained from this "organ" reconstructed in vitro permit to identify among the various interactions, those of greater biological significance, and to devise predictive models for the screening of products with pharmacological, toxicological or cosmetic properties.

c) Three-dimensional culture system

SKIN 2 TM (a three-dimensional culture system) is currently used more and more frequently (7) (Fig. 3). It is constituted by a living human three-dimensional tissue substrate. Fibroblasts taken from neonatal foreskin are seeded on a nylon mesh. They attach to this mesh and grow, secreting their matrix protein. Keratinocytes are successively seeded on this dermis and proliferate, forming a multi-layer differentiated epidermis with a well developed horny layer (8). This model has been successfully experimented in the study of collagen deposition, irritancy responses, cytotoxicity and percutaneous absorption (7). Validation studies have demonstrated its reproducibility, while characterization has evidenced its similarity to the human epidermis (9). This model is widely used in the evaluation of phototoxicity and photoprotection

processes. The advantage of utilizing in phototoxicity studies a model providing both the dermis and epidermis lies in the possibility of obtaining a series of specimens in a relatively short time, and of eliminating those species or individual factors that can mask or modify the potential risk. The process of recognition of the validity of this model for the study of phototoxicity has been conducted and approved by the Commission of the European Communities and by the Comité de Liaison des Associations Europeennes des Industries de la Parfumerie, des Produits Cosmetiques et de Toilette (COLIPA). In the framework of this European study, the most promising in vitro photoirritancy tests were selected and their methods standardized to obtain validation.

Conclusions

In vitro techniques can play an important role in the fast acquisition of information that will allow the selection of ingredients characterized by low toxicity and high biological activity, at least in the field of cosmetic products. Cell cultures are probably the most promising biological models, in that they are simpler than the organism in toto, but are constituted by organized living units, thus opening interesting prospects also in toxicological studies. Their characteristic features and intrinsic limitations must however be taken into consideration when programming experiments or interpreting their results. Some studies have demonstrated the suitability of cell cultures in studying the activity of melanocytes (10), the basal membrane in all its components (11), and skin metabolism and permeability (12). In view of the need to standardize validation techniques and related tests, a series of research bodies have been set up at national level, and subdivided in committees to deal with specific fields. The most important of these, and the main national bodies in this field are: in the USA the American Society for Testing and Materials, the Health Industry Manufacturers Association, the American Dental Association, the Food and Drug Administration; in the UK the British Standard Institute; in France the Association Francaise de Normalisation; in Germany the Deutsches Institut fur Normung, in Italy the Ente Nazionale Italiano di Unificazione. At international level, the main regulatory body is the International Organization for Standardization (ISO), constituted by the research units on standardization of the most industrialized countries, also organized in technical committees. The EEC has issued directives setting the minimum standards for safety, reliability and for every aspect of the medical sector, in part already enacted by the Italian Parliament. The European Committee for Standardization (ECS) on behalf of the EEC is currently dealing with the harmonization of a series of basic technical rules and regulations in line with the requirements of EEC directives. In parallel with harmonization in the EEC, an attempt at "global" harmonization is being made by Japan, the USA and Europe for a wide series of rules, tests and certification systems.

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References

1. **Morganti P. (1996)** Cosmetic dermatology: an italian speciality, *Cosmet. Toilet. Manufacture Worldwide*, **4**:220.
2. **Anonymous.** Council directive 76/768/EEC of July 27,1976, On the approximation of the laws of the member states relating to cosmetic products O.J.E.C. n. L262/169,27-09-1976 and following amendaments.
3. **Anonymous.** Council directive 93/35/EEC of June 14,1993,Amending for the sixth tome directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products O.J.E.C. n. L151/32, 23-06-1993.
4. **Bianchi V., Zucco F. (1994)** Potenzialità ed uso delle colture cellulari in tossicologia. Bianchi V., Zucco F. In *Colture cellulari in tossicologia*. Lombardo Editore, Roma.
5. **Marinovich M. (1994)** Irritazione cutanea ed oculare. In *Colture cellulari in tossicologia*. F. Zucco, V. Bianchi, Lombardo editore , Roma.
6. **Marks R., Gerd Plewing G. (1986)** Skin models. Models to study function and disease of skin: Edited Springer- Verlag Berlin Heidelberg .
7. **Edwards S.M. , Donnelly T.A., Sayra R.M., Rheins L.A. (1994)** Quantitative *in vitro* assessement of phototoxicity using a human skin model, Skin 2TM. *Photodermatol Photoinmunol Photomed.*, **10**:111-117.
8. **Slivka S.R., Landeen L.K., Ziegler F., Zimber M.P., Bartel R.L. (1993)** Characterization, barrier function, and drug metabolism of an in vitro skin model. *J Invest Dermatol* ; **100 (1)**:40-46.
9. **Triglia D., Kidd I., De Wever B., Rooman R. (1992),** Interlaboratory validation study of the Advanced Tisseues Sciences' skin 2TM dermal model and MTT cytoxicity assay kits. *AATEX*; **1**:142-147.
10. **Imokawa G., Motegi I. (1993).** Skin organ culture model for examining epidermal melanization. *Invest Dermatol.*; **100 (1)**:47-54.
11. **Contard P., Bartel R.L. et Al. (1993)** Culturing Keratinocytes and fibroblasts in a three-dimensional mesh results in epidermal differentiation and formation of basal lamina - anchoring zone. *Invest Dermatol*; **100 (1)**:35-39.
12. **Sandra R. Silvka, Landeen L.K., Zimber M.P., R.L. Bartel. (1993)** Characterization, barrier function, and drug metabolism of an in vitro skin model. *Invest Dermatol*; **100 (1)**: 40-46.

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ROLE OF EPIDERMAL CERAMIDES IN BARRIER FUNCTION

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Synopsis

Lipids are considered to play an important role in the structure, differentiation and function of the epidermis.
During the process of keratinisation and epidermal differentiation the lipid composition of the skin changes dramatically. These changes are consistent with the requirement for cutaneous waterproofing. In this view, many reports have shown the importance of sphingolipids in maintaining the optimal mammalian epidermal permeability barrier function.
Stratum corneum lipids major constituent, ceramide, has been shown to be associated to both water retaining capacity of the skin and permeability barrier; ceramide is thought to derive primarily from glucosylceramide which is pratically absent in the exterior layer of the stratum corneum.
Altogether these data lead to the hypothesis that the hydrolysis of glucosylceramide to ceramide, mediated by a lysosomal-like β-glucosylceramidase, plays an important role in the formation and maintenance of epidermal permeability barrier.
These evidences will be critically evaluated in the light of our experimental data on ceramide and glucosylceramide content in the skin of patients with Gaucher's disease. This sphingolipidosis, due to the deficiency of lysosomal β-glucosylceramidase, is often associated with cutaneous abnormalities.

Riassunto

È definitivamente accertato che i lipidi giochino un ruolo molto importante nella organizzazione strutturale e funzionale dell'epidermide e dei processi di differenziamento ad essa correlati.
Durante il processo di cheratizzazione e differenziamento la composizione in lipidi della cute si modofica sostanzialmente nell'intento di mantenere una ottimale omeostasi del contenuto d'acqua dell'epidermide. In questa ottica un ruolo molto importante è svolto dai glicosfingolipidi; infatti il principale costituente lipidico dello strato corneo, il ceramide, è coinvolto sia nella ritenzione d'acqua sia nella funzione barriera svolta dall'epidermide. Si ritiene comunemente che questo sfingolipide derivi per idrolisi catalizzata da un enzima specifico, β-glucosilceramidosi, dal glucosilceramide presente nei corpi lamellari. Questi dati verranno criticamente valutati sulla base dei risultati ottenuti nel laboratorio dei relatori in pazienti affetti da malattia di Gaucher, sfingolipidosi caratterizzata dall'assenza, geneticamente determinata, dell'enzima β-glucosilceramidasi.

1. Keratinization process and lipid synthesis

Keratinocytes are the predominant epidermal cell-type: they produce the keratins, a family of related proteins responsible for specific skin functions including resistence against environmental attacks and impermeability to substances which get in touch with the skin.
Epidermis is made of different cell layers referred as to basal, spinous, granular and horny layers. Keratinocytes move from the former to the latter, changing their morphology, shape and metabolic activity. In the basal layer cells show intense metabolic activity due to their rapid division; in the spinous layer cells develop organelles necessary for the synthesis of a keratin precursor protein, keratohyaline, which is converted to keratin by specific enzymes; moreover they produce keratinosomes or Odland bodies containing lipid compounds in the form of bistratified discs stacked one on top to the other. Moving up the cell becomes flat, loses its regular shape, the nucleous is expelled. The cell is so transformed in a flattened envelope which internal walls are covered with lipids. In the granular layer the content of Odland bodies are poured into the intercellular spaces when they form the leaves of lipoid material found between the dead cells (corneocytes) of the horny layer. In the most external portion of this layer the corneocytes gradually lose their cohesion and are desquamated. They will be then replaced by keranocytes pushing up from the underlying layers. (1, 2)
This cyclic process is know as keratinization. The term refers either to the life of epidermal cells, which lasts about 28 days, or to the physiological processes, schematically described above, which occur during this period; as above indicated one of this process accounts for the production of epidermal lipids which are indispensable for the maintenance of epidermal integrity.
The hydrofobic material synthetised in the Odland bodies have been biochemically characterized and it is composed mainly of glucosylceramide and ceramide. Though other substances such as sphingosine, cholesterol esters and fatty acids are also present, it as been clearly demonstrated that only the ceramides play an essential role in barrier function.

2. Sebum: its composition and functions (3)

Production of sebum is not constant, and changes with different stages of life. In the foetus the sebaceous glands begin to secrete sebum during the fourth month. Later when the newborn baby is no longer receiving the maternal hormones which stimulate the glands, sebocytes enter a state of quiescence which only ends when the child reaches puberty; at this stage the sexual glands have developed and start to secrete sex hormones - predominantly androgens in the male and estrogens in the female. In females there is a reduction in sebum production after the menopause caused by the progressive inactivation of the ovaries. On synthesis, sebum has a different composition from that when it reaches the skin surface.

The composition of sebum on the skin surface is reported in the following table:

free fatty acids	20%
triglycerides	40%
squalene	15%
wax	20%
sterol, glyco and phospholipid	5%

Sebum is fundamental in all mammals as it represents the best protection for their fur and, in man, for the skin.
By emulsifying itself, that is, mixing with the water derived from sweating or traspiration, sebum forms a film over the skin which is able, within certain limits, to protect the epidermis

from harmful chemical substances and from the action of pathogenic micro-organism.

3. The hydrolipid film (4)

The hydrolipid film is produced by the sebaceous glands, sweat and transpiration; its importance in the maintenance of the skin's emollience and defence against pathogenic bacteria is well established. Once produced the film can flow into the grooves from one region to neighbouring ones; differences in the distribution density of sweat and sebaceous glands mean that not all the areas of the skin are equally covered by the hydrolipid film.
Regions which are poorly supplied will have less natural emollience and, retaining less water, will have a higher tendency to desquamation. Furthermore, in some individuals the sebaceous glands produce hydrolipid film which is qualitatively altered and tends to irritate the skin itself.
The hydrolipid film is essentially composed of:
- sebum components
- cellular waste (keratinocyte residues)
- bacterial substances (derived from bacteria normally present on the skin)
- water (sweating and transpiration)
- exogenous substances (cosmetics, dirt)

An important point to remember regarding the hydrolipid film is that it constitutes part of the normal dirt that we remove daily from our skin by cleansing. The excessive removal of the hydrolipid film strips the epidermis of its protection and exposes the skin itself to environmental attack and dehydration.

4. Skin hydration (5)

Hydration represents the most important parameter for the health of the skin. Numerous factors determine the water content of the skin, although, overall it is directly related to the ambient humidity and the skin can only retain adeguate concentration of water at a relative humidity of 60%.

The skin's factors which govern the maintenance of its hydration are:
- epidermal lipid concentration and composition
- surface hydrolipid film distribution
- natural moisturizing factor (NMF) occurence
- horny layer physiologically normal architecture
- presence of organic substances (salts, amino acids, jaluronic acid)

Epidermal lipids have a fundamental role both in binding water and in occluding the intercellular spaces. Their action is correlated with the correct structure of the lamellae in the horny layer which otherswise would not be able to retain water.
The hydrolipid film covers the epidermis with a thin protective layer which softens and defends the epidermis itself against both chemical and bacterio-micotic agents. Moreover it slows down the desquamation rate. Removal of the film by excessive cleansing, for example, leads to damage of the horny layer which desquamates and loses intercellular lipids, so opening the barrier for the entry of germs and harmful substances.
The NMF are a series of substances produced by epidermal cells and by sweating which function to bind water both intracellularly and extracellularly toghether with the intercellular lipids. Of the NMFs the most important are PCA (pirrolidon carboxylic acid) and urea, but many other organic substances and mineral salts are part of the group.

EPIDERMAL LIPIDS (1, 6, 7)

The skin protects the body from excessive water loss and at the same time hinders the entrance of chemical substances, microorganisms and antigens. From a physiological and biochemical point of view the skin must ensur firmness, resistance and flexibility, production of keratinocytes, appropriate desquamation rate, rapid exchange of dead cells and formation of a semipermeable barrier which dispels water in a con-

trolled way. As previously indicated the most external level of defence is the surface hydrolipid film, produced by the skin itself; in fact it is able to exert a selective antimicrobial action, thanks to the presence of medium-chain fatty acids and acidic pH and to increase the water retention capacity (WRC) of the horny layer.

On the basis of composition and origin, the surface lipids can be divided into two groups: those derived from the sebaceous glands and those derived from the epidermis. The latter are essential for the WRC of the horny layer, the hydratation state, the functioning of the semipermeable skin barrier (8) and in the light of recent knowledge, also in the control of the processes of maturation and desquamation of the horny layer. In this view the sphingosine derived from the hydrolysis of ceramides is thought to stimulate keratinocyte production and differentiation. (9,10)

Morphologically speaking the epidermal lipids originate from the Odland bodies, appear in the intracellular spaces at the upper limit of the granular layer and are found as laminar structures between the corneocytes.(11) The horny layer is a bi-compartmental system: cells rich in proteins and without lipids embedded in intracellular spaces packed with lipids.

Two different groups of epidermal lipids can be distinguished on the basis of their functions:

- lipids not containing sphingosine
- sphingolipids.

The former are: glycerophospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine), triglycerides, free fatty acids (oleic, palmitic, palmitoleic, linoleic), sterols (cholesterol, squalene, cholesterol esters) and n-alcans. The sphingolipids are: glycosphingolipids, sphingomyelin and six classes of ceramides. (12)

Epidermal cholesterol is a product of keratinocyte metabolism; it is synthesized in the lower regions of the epidermis and subsequently converted into cholesterol sulphate (which is found in highest concentrations in the granular layer) by a sulphotransferase. Steroid sulphatase finally reconstitutes cholesterol so that cholesterol sulphate is not present in the desquamated horny lamellae. (13,14,15)

Based ont the most recent findings, cholesterol is thought to be fundamental in the cohesion - desquamation rate of the corneocytes. (15)

The free or esterified fatty acids found in man have a length varying between C-14 and C-24, the majority of molecules being C-16 and C-18. In normal skin the ratio of saturated fatty acids to unsaturated is approximately one, while in dry skin a decrease in linoleic acid and an increase in palmitoleic acid was shown. The application of emulsions rich in linoleic acid restablishes a distribution of fatty acids which is similar to that of normal skin. (16)

Since the discovery of ceramides, the modifications induced by the lack of essential fatty acids in the skin have been explained as alterations in the ceramide backbone, of which one of the essential constituent is linoleic acid. (17)

Phospholipids are essential to maintain the lipid bi- layer of many cell membranes. They are present in large quantities in the germinative layer of the epidermis; on the contrary in the outer layers, the horny one for example, the phospholipid subclasses (phosphatidylcholine and phosphatidylethanolamine) which were present in the germinative layer have virtually disappeared and new classes of lipids, like the ceramides, appear. (18)

The barrier function of the skin depends mostly on the structural organisation of the lipids rather then the quantity of any single class of lipids present in the horny layer. (19)

SPECIFIC FUNCTIONS OF EPIDERMAL GLICOLIPIDS AND CERAMIDES.

As already mentioned lipids are considered to play an important role in the structure, differentiation and function of the epidermis.
During the process of keratinisation and epidermal differentiation the lipid composition of the skin changes dramatically. (20,21) These changes are consistent with the requirement for cutaneous waterproofing. (21, 22, 23, 24)
In this view, many reports have shown the importance of sphingolipids in maintaining the optimal mammalian epidermal permeability barrier function, (25, 26, 27, 28) and solvent extraction studies have shown that the progressive removal of sphingolipids, rather than non polar lipids, is associated with proportional abnormalities in barrier function. (28)
Stratum corneum lipids major constituent, ceramides, has been shown to be associated to both water retaining capacity of the skin and permeability barrier; ceramides are thought to derive primarily from glucosylceramides which are pratically absent in the exterior layer of the stratum corneum. (29) This suggests the presence in the horny layer of hydrolytic enzymes; in fact, data from the literature show that a number of catabolic enzyme, such as sphingomyelinase, triacylglycerol hydrolase (30), phospholipase A (31), steroid sulphatase (15), and β-glycosidase (32, 33) are localised in the lower part of the horny layer. It was suggested that the last enzyme (E.C.: 3.2.1.45 β-D-glucosyl-N-acylsphingosine glycohydrolase) should have similar characteristic with the lysosomal isoform, even though their topology is different. This suggestion is further supported by the ascertained presence in the lamellar bodies of a proton pump to secure the acidic conditions for the optimum of the activity of the enzyme. (34)
Due to these similarities we investigated the glycolipids and ceramide distribution in the stratum corneum of Gaucher's patients, whose dry skin might have been due to a "storage" of glucosylceramide, compaired with the same parameters in healthy donors. Gaucher's disease is a lysosomal storage disease, characterized by the absence, genetically determined, of β-glucosylceramide glycohydrolase.
Our results indicate that the glucosidase present in the lamellar bodies of Gaucher's patients stratum corneum failed to hydrolyze the glucosylceramide to ceramide, as shown by the high percentage in the pathological samples of the former (range: 48-69% of the total glycolipids), and the complete absence of the latter. (35)
We are evaluating the usefulness of topical therapy (in addition to the replacement therapy which became recently availabe for the treatment of Gaucher's patients) with a lotion containing commercially available 3 hydroxy ceramide, which fatty acids composition is closely related to that of the main ceramide present in human skin. (10) The results so far obtained seem to be very promising.
Altogether these data lead to the concept that the hydrolysis of glucosylceramide to ceramide plays an important role in the formation and maintenance of epidermal permeability barrier.
This prospect introduces new roles for functional cosmetics, which should be made not only by inert substances but also by molecules suitable to interact with the skin and to contribute to the restoration of its correct physiology, biochemystry and structure.

References

1. **Berra B., Rapelli S. (1994)** Biochemistry of the skin. Structural and metabolic aspects of carbohydrates, proteins and lipids. In: *The skin as a dynamic barrier and the function of cosmetics*. RTC Acta, vol. **3**, pp.49-67.
2. **Elias P.M. (1981)** Epidermal lipid membranes and keratinization. *Int. J. Derm.*, **20**, 1-12.
3. **Wheatley V.R. (1986)** The Sebaceous Glands. In: *The physiology and pathophysiology of the skin*. A. Jarret (Ed) vol. 9 Academic Press.
4. **Celleno L. (1994)** Structure and function of the skin. In: *The skin as a dynamic barrier and the function of cosmetics*. RTC Acta, vol. **3**, pp.41-42.
5. **Celleno L. (1994)** Structure and function of the skin. In: *The skin as a dynamic barrier and the function of cosmetics*. RTC Acta, vol. **3**, pp.42-43.
6. **Berra B., Rapelli S. (1991)** Role of lipids in cellular membrane and skin biology. Proc. Intern. Congress: "Progress in Cosmetic Dermatology: Science and Safety.", Rome October 30 - Nov. 2, 1991.
7. **Giannetti A., Di Nardo A. (1994)** The skin as a barrier in dynamic equilibrium. In: *The skin as a dynamic barrier and the function of cosmetics*. RTC Acta, vol. **3**, pp.77-81.
8. **Schurer N.J., Elias P.M. (1991)** The biochemistry and Function of Stratum Corneum Lipids. In: *Advances in Lipid Research*, vol. 24 Skin Lipids P.M. Elias (Ed) Academic Press Inc., pp. 27-56.
9. **Uchida Y., Iwamori M., Nagai Y. (1990)** Activation of keratinization of keratinocytes from fetal rat skin with N-(O- Linoleolyl) w-Hydroxy Fatty Acyl Sphingosyl Glucose (Lipokeratinogenoside) as a marker of epidermis. *Biochem. Biophys. Res. Commun.* **170**, pp. 162-168.
10. **Uchida Y., Ogawa T., Iwamori M., Nagai Y. (1991)** Enhancement of keratin synthesis induced by lipokeratinogenoside, N-(O- Linoleolyl) w-Hydroxy Fatty Acyl Sphingosyl Glucose, in association with alteration of the intracellular Ca2+ content and protein kinase in cultured keratonocytes (FRSK). *J. Biochem.* **109**, pp. 462-465.
11. **Wertz P.W., Downing D.T., Freinkel R.K. and Traczyk T.N. (1985)** Sphingolipids of the stratum corneum and lamellar granules of fetal rat epidermis. *J. Invest. Dermat.*, **83**, pp.193-195.
12. **Lampe M.A., Burlingame A.L., Whitney J., Williams M.1., Brown H.E., Roitman E., Elias P.M. (1983)**. Human stratum corneum lipids: characterization and regional variations. *J. Lipid Res.*, **24**, pp.120-130.
13. **Proksch E., Elias P.M., Feingold K.R. (1990)** Regulation of 3-Hydroxy-3-methylglutaryl-CoA reductase activity in murine epidermis: modulation of enzyme content and activation state by barrier requirements. *J. Clin. Invest.*, **85**, pp. 874-882.
14. **Feingold K.R., Man M.Q., Menon G.K., Cho S.S., Brown B.E., Elias P.M. (1990)** Cholesterol synthesis is required for cutaneous barrier function in mice. *J. Clin. Invest.*, **86**, pp. 1738-1745.
15. **P.M. Elias, M.L. William, M.E. Maloney, J.A. Bonifas, B.E. Brown, S. Grayson, E.H. Epstein Jr. (1984)** Stratum corneum lipids in disorders of cornification: steroid sulfatase and cholesterol sulfate in normal desquamation and the pathogenesis of recessive X-linked ichthyosis. *J. Clin. Invest.*, **74**, pp. 1414-1421.
16. **M. Press, P.J. Hartop, C. Prottey (1974)** Correction of essential fatty acid deficiency in man by the cutaneous application of sun-flower-seed oil. *Lancet*, **1**, pp. 597-599.
17. **S. Hamanaka, C. Asagami, M. Suzuki, F. Inagaki, A. Suzuki (1989)** Structure determination of glucosyl-β1-N- (w-o-linoleoyl)-acylsphingosines of human epidermis. *J. Biochem.* (Tokyo), **105**, pp. 684-690.

18. **L.A. Goldshmidt (1983)** *Biochemistry and Physiology of the Skin*. Oxford Univ. Press, Oxford.
19. **G. Grubauer, K.R. Feingold, R.M. Harris, P.M. Elias. (1989)** Lipid content and lipid type as determinant of the epidermis permeability barrier. *J. Lipid Res.*, **30**, pp. 89-96.
20. **G.M. Gray and H.J. Yardley (1975)** Different populations of pig epidermal cells: isolation and lipid composition. *J. Lipid Res.*, **16**, pp. 441-447.
21. **P.M. Elias, B.E. Brown, P. Fritch, J. Goerke, G.M. Gray and J. White (1979)** Localization and composition of lipids in neonatal mouse stratum granulosum and stratum corneum. *J. Invest. Dermatol.*, **73**, pp. 339-348.
22. **P. M. Elias. (1983)** Epidermal lipids, barrier function, and desquamation. *J. Invest. Dermatol.*, **80,** pp. 40-49.
23. **M.L. Williams and P.M. Elias. (1987)** The extracellular matrix of stratum corneum: role of lipids in normal and pathological function. *Crit. Rev. Ther. Drug Carrier Syst.*, **3**, pp. 95-122.
24. **H.J. Yardley and R. Summerly (1981)** Lipid composition and metabolism in normal and diseased epidermis. *Pharmacol. Ther.*, **13**, pp. 357-383.
25. **P.W. Wertz, D.T. Downing (1982)** Glycolipids in mammalian epidermis: structure and function in the water barrier. *Science* (Wash. DC), **217**, pp.1261-1262.
26. **W.M. Holleran, K.R. Feringold, M. Mao-Qiang, W.N. Gao, J.M. Lee and P.M. Elias. (1991)** Regulation of epidermal sphingolipid synthesis by permeability barrier function. *J. Lipid Res.*, **32**, pp. 1151-1158.
27. **W.M. Holleran, M.Mao Qiang, G.K. Menon, P.M. Elias and K.R. Feingold. (1991)** Sphingolipids are required for mammalian barrier function: inhibition of sphingolipid synthesis delays barrier recevery after acute perturbation. *J.Clin. Invest.*, **88**, pp. 1338-1345.
28. **G. Grubaner, K.R. Feingold and P.M. Elias (1989)** Lipid content and lipid type as determinants of the epidermal permeability barrier. *J. Lipid Res.*, **30**, pp. 89-96.
29. **W.M. Holleran, Y. Takagi, G.K. Menon, G. Legler, K.R. Feingold, P.M. Elias. (1993)** Processing of epidermal glycosylceramides is required for optimal mammalian cutaneous permeability barrier function. *J.Clin. Invest.*, **91**, pp. 1656-1664.
30. **G.K. Menon, S. Grayson and P.M. Elias. (1986)** Cytochemical and biochemical localization of lipase and sphingomyelinase activity in mammalian epidermis. *J. Invest. Dermatol.*, **86**, pp. 591-597.
31. **P.M. Elias, G.K. Menon, S. Grayson and B.E. Brown.(1988)** Membrane structural alterations in murine stratum corneum: relationship to the localization of polar lipids and phospholipases. *J. Invest. Dermatol.*, **91**, pp. 3-10.
32. **M.K. Nemanic, J.S. Whitehead and P.M. Elias (1983)** Alterations in membrane sugars during epidermal differentiation: visualization with lectins and role of glycosidase. *J. Histochem. Cytochem.*, **31**, pp. 887-897.
33. **W.M. Holleran, Y. Takagi, G. Imokawa, S. Jackson, K.R. Feingold and P.M. Elias. (1992)** β-Glucocerebrosidase activity in murine epidermis: characterization and localization in relationship to differentiation. *J. Lipid Res.*, **33**, pp. 1201-1209.
34. **S.J. Chapman, A. Walsh. (1989)** Membrane-coating granules are acidic organelles which possess proton pumps. *J. Invest. Dermatol.*, **93**, pp. 466-470.
35. **L. Adorni, S. Rapelli, R. Ciuffo, B. Berra. (1995)** Skin lipid abnormalities in Gaucher's disease. Submitted for pubblication.

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TRANSDERMAL DRUG DELIVERY BY IONTOPHORESIS. I. FUNDAMENTALS AND THEORETICAL ASPECTS.

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Synopsis

Although the skin can represent an useful pathway for the local and systemic administration of drugs, its barrier properties strongly limit the efficacy of conventional topical delivery systems. Electrically-enhanced drug release, or iontophoresis, offers a valid mean for the transdermal delivery of charged and hydrophilic, or high-molecular weight substances at a controlled rate. This technique consists of the application of a known current field between two electrodes placed on the skin surface. A drug solution is layered beneath one of the electrodes and, by controlling the duration and intensity of current flow, a predetermined amount of the drug can be released into epidermis and dermal tissues.
In this review, the theories underlaying this technique of drug administration are analyzed, along with a brief description of skin structure and the effects of iontophoretic therapy on skin integrity.

Riassunto

La cute rappresenta una utile via per la somministrazione locale e sistemica di molti farmaci, per i diversi vantaggi che può fornire, rispetto ad altre possibili vie di somministrazione tradizionali; tuttavia, le eccellenti proprietà di barriera che la pelle offre, limitano l'efficacia delle formulazioni dermatologiche tradizionali a poche molecole lipofile e dotate di una elevata attività.
Tra i diversi approcci terapeutici proposti, la iontoforesi si è dimostrata un'attraente alternativa, in quanto è in grado di permettere il rilascio transdermico di farmaci carichi e altamente idrofili, come pure di molecole ad alto peso molecolare (polipetidi e proteine). Tramite l'applicazione di due elettrodi sulla superficie della pelle, al di sotto di uno dei quali viene posta una soluzione contenente un farmaco, viene creato un campo elettrico di intensità nota; in questo modo, controllando la durata e l'intensità del flusso elettrico, è possibile assicurare il rilascio di quantità predeterminate di farmaco nell'epidermide e nei tessuti sottostanti.
In questa prima parte di una rassegna sulle tecniche iontoforetiche finora sviluppate, vengono presentate alcune considerazioni teoriche sulle quali tale metodica e le sue applicazioni pratiche si basano. Per una migliore discussione del processo di trasporto transdermico indotto dall'applicazione di corrente, viene rapidamente discussa l'importanza dei vari costituenti della pelle sulle sue proprietà di permeazione, nonché gli effetti provocati dalla iontoforesi sull'integrità della pelle stessa.

Introduction

The penetration of compounds through the skin has both pharmacological and toxicological significance. The possibility of using the intact skin as the pathway for drug administration has been investigated for many decades: in fact, transdermal delivery of drugs shows some advantages over other administration pathways, in particular the avoidance of gastrointestinal incompatibility and liver "first-pass" effect. On the other hand, the skin represents a very efficacious barrier to the transport of many substances, and the conventional topical delivery devices are thereby limited to drugs with a local action or to highly potent, small and lipophilic molecules for a systemic effect. Contrarely, it is very difficult to transport charged or at least hydrophilic compounds as well as high-molecular weight molecules through the skin.
Among the various therapeutical approaches recognized to overcome such limitations, including chemical enhancers (1), transdermal drug delivery devices (2), use of ultrasonic and thermal energy (3), or application of ointments containing skin delipidizing compounds (3), iontophoresis can represent a valid method for the transdermal delivery of many substances at a controlled rate. Iontophoresis (or ion transfer) is defined as the migration of ions when an electric current is passed through a solution containing ionic species. In particular, the applied electric field imposes a force on ionized drugs which adds to the "diffusion force" or concentration gradient; this additional force drives the ion through the membrane into the body, more efficiently than in the case of pure diffusion or "passive" transdermal drug delivery. Since the preliminary studies of Leduc in early 1900 (4), iontophoresis has been used to transport drugs across several biological membranes including the skin (5), tooth mucosa (5, 6), the eyes (8-12), mucosae (13, 14), and cervix (15-17), in many pathological conditions. Recent extensive collections of drugs used by this administration tecnique have been reported by Yoshida and Roberts (18), Singh and Roberts (19), and Gangarosa and Hill (7). Barner (20) have described an iontophoretic device for the introduction into the skin of pigments for tattooing.
In this first part of a review upon iontophoresis, the theories underlaying such a procedure of drug administration will be discussed, along with a brief description of skin structure and the effects of iontophoretic therapy on skin integrity. In the forthcoming papers, the experimental procedures and the in vitro-in vivo models described in the literature will be reviewed, together with the present clinical applications and the future possibilities offered by this tecnique.

Structure of human skin and passive diffusion of topically applied compounds

Anatomical and physiological properties of skin have to be accurately considered to better understand and then conveniently exploit the potential of iontophoresis as a drug delivery device.
Mammalian skin is a multi-layered epithelium, supported by connective tissue and containing complex structures as eccrine and apocrine sweat glands, hair follicles with sebaceous glands, and nails, all of them referred to as skin appendages. Three main histological layers compose the skin: epidermis, dermis, and subcutaneous tissue (Fig. 1). The epidermis is further subdivided into several strata, which are characterized by hystological and functional differentiation of keratinocytes, as they move up from the lowest stratum basale to the outer surface, where they form the stratum corneum (SC), which consists of some layers of keratin-filled dead cells (corneocytes), that are anucleate, dehydrated, flattened and compacted (Fig. 1). Corneocytes are surrounded by lipid lamellae, mainly consisting of a mixture of cholesterol and its esters, fatty acids, phospholipids, glycerol and sphingosine esters and, in particular, ceramides, which represent about 50% of the SC

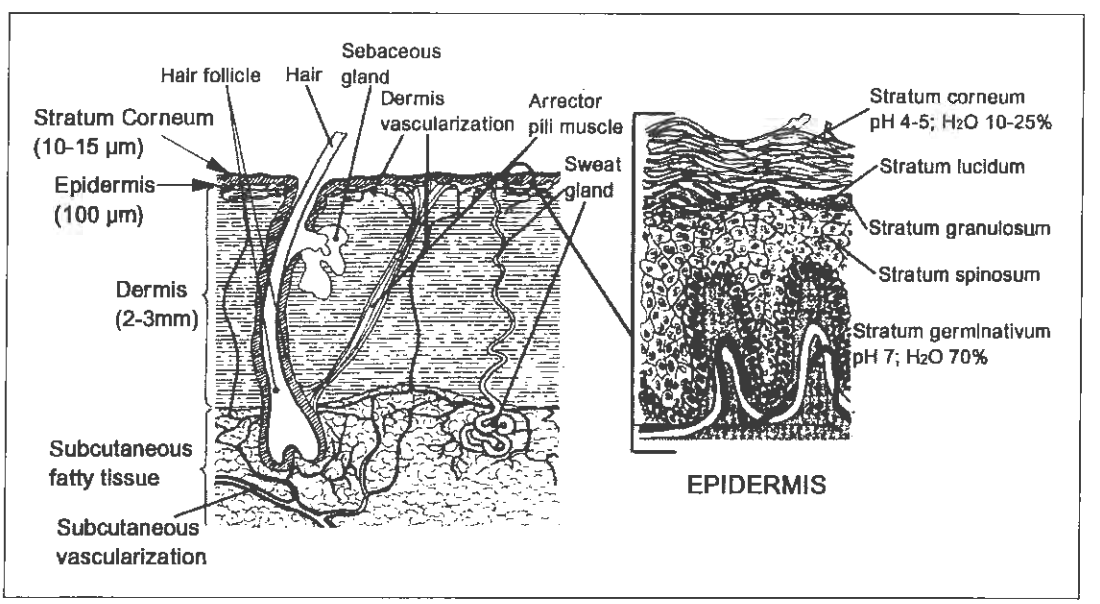
lipids; this lipid matrix forms a continuous medium through the SC and represents the primary barrier to the permeation of water and other hydrophilic substances, as well as the pathway for penetration of lipophilic compounds (21-23); moreover, these lipids are able to form multiple lipid bilayers and become important for the mechanical properties and desquamatory process of the SC (24). Corneocytes are connected together by desmosomes, proteinaceous structures which ensures an excellent mechanical protection for the underlaying, more sensitive viable tissues. The presence of these thight junctions suggest the possibility of a transcellular pathway of penetration through the SC (25).

The stratum corneum has a water content of only 20% as compared to 70% of other viable skin layers. Its mean thickness is around 10-50 µm, but significative differences are observable as a function of the rate of hydration of SC and between different areas of the body (17). The pH of the skin surface is between 3 and 4, which is about the isoelectric point of keratin in the SC (26); namely, skin surface has a positive charge below pH 3 and a negative one above pH 4. This aquires a great importance in predicting the permeation behaviour of basic or acidic substances.

The four layers situated under the SC (lucidum, granulosum, spinosum and germinativum, from the upper toward dermis), constitute the viable epidermis; this is an aqueous solution of proteins encapsulated into cellular compartments by thin cell membranes, fused together by tonofibrils. Epidermis has a thickness of about 100-150 µm and is avascular, performing all nutrition and waste exchanges by diffusion with the capillary beds inside the papillary layers of the dermis (Fig. 1). The convolute interface between the two layers enhances the contact area with dermal capillary loops, then facilitating epidermal metabolism.

The dermis consists of connective tissue and contains the inner portions of skin appendages, all of them highly vascularized. Dermis is 2-3 mm thick and is made of a fibrous protein matrix, mainly formed by collagen, elastin, and reticolin, embebbed in an amorphous colloidal polysacharidic ground substance. A papillary layer in contact with epidermis and a deeper

Figure 1: Sectional structure of the skin and, in particular, of epidermis components.



coarse reticular layer (the main structural layer of the skin) form the dermis (27). The dermis also contains blood vessels, sensory nerves and lymphatic structures. At its basis, a subcutaneous fat layer protects the body from shock and injuries (mainly heat), provides flexibility and strenght, acts as a barrier to infection and as a water-storage tissue (28). It has no effect on drug percutaneous permeation, since it is placed below the dermal vascular system.

Skin appendages

Human skin contains hair follicles and sebaceous glands as well as eccrine, apocrine and apoeccrine sweat glands (29). Skin surface contains, on the average, 40-70 hair follicles and 200-500 sweat ducts per square centimeter (17). Thermoregulatory eccrine glands are distributed in humans all over the body surface but are particularly concentrated in the hand palms and feet soles; the sweat secretion of the apocrine glands is limited to the axillae, breast areola and genital perianal areas. It is noteworthy that no completely homologous animal model exists for human skin: porcine and hairless rodent skin lacks eccrine sweat glands, while other mammals, including primates, show them only in the palmar and plantar regions.

Vellus or terminal hairs are present over the entire body, except for the red part of the lips, the palmar and plantar surfaces, and parts of sex organs. The follicles of terminal hairs can extend deep to the subcutaneous fatty tissue, while vellus follicles may only reach the upper dermis.

Sebaceous glands are more numerous on the face, forehead, in the ear, on the midline of the back, and in the perianal area. They produce an oily secretion, known as sebum, deriving from cell disintegration and acting as a skin lubrificant and a source of SC plasticizing lipids and maintains the acidic conditions on the skin's outer surface (27).

Passive diffusion of topically applied compounds

The process of percutaneous absorption can be viewed as the movement of a substance from the skin surface into the systemic circulation. It requires the penetration into the SC, diffusion through each layer of the skin, uptake by the capillary network at the interface between epidermis and dermis, and finally transport by the blood circulation to the action sites.

For a substance dissolved in the vehicle, a modification of Fick's law describes the passage through the SC:

$$J = \frac{dQ}{dt} = \frac{P C_v D A \alpha}{h}$$

(Eqn. 1)

where dQ/dt is the amount of drug appearing in the viable epidermis (i.e., the flux J), P is the partition coefficient of the drug between vehicle and skin surface, D is the diffusion coefficient of the drug, i.e., the ability of penetrate the SC, A and h the surface area and thickness of this latter, the fraction of drug which is in a non-ionized (liposoluble) form, and Cv the bulk concentration of drug in the vehicle.

The percutaneous absorption can occur by means of two different pathways, transfollicular (hair follicles, sweat ducts and secretory glands) and transepidermal (across the SC, intracellularly or intercellularly) routes (Fig. 2). The occurrence of percutaneous absorption through human skin is attributed to the passive diffusion of the drugs from a vehicle placed on the skin surface. Such a simple diffusion, regulated by a chemical (concentration) gradient, is strongly influenced by a number of factors which have to be accurately considered before executing a transdermal permeation study or application. These factors includes both physico-chemical characteristics of the drug (concentration, solubility, relative affinity for skin and vehicle, ionization rate, molecular weight and size) and of the vehicle or carrier (viscosity, solvent properties, affinity for skin

surface) as well as a number of physiologic or pathologic conditions of the skin, like individual or species-related variability in skin permeability and sensitivity to the systems applied (17).

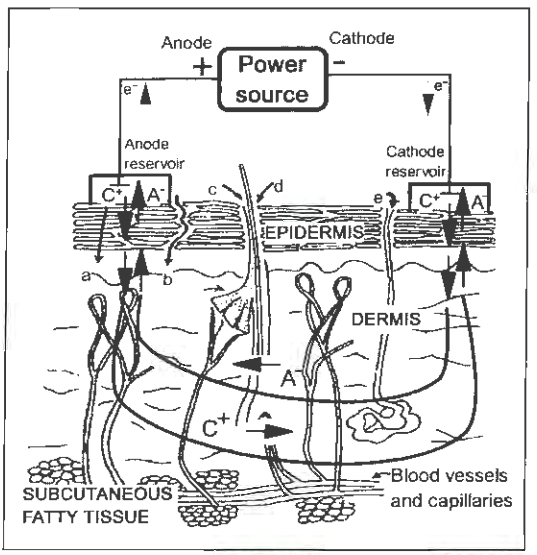


Figure 2: A schematic representation of charged species flow in an iontophoretic circuit, along with the possible routes of penetration through the skin: a) transcellular; b) diffusion between intercellular pores; c) through sebaceous gland duct; d) transfollicular; e) through sweat gland ducts. For simplicity, it is assumed that both aqueous solutions under the electrodes (reservoir) contains only one positively charged drug (C+) and one negatively charged counterion (A-), respectively.

There are some but discordant data in the literature showing that hair follicles and sebaceous glands act as routes (pores) for the passive absorption of polar substances (30). The use of appendage-free (hairless rat skin regrown after scalding and newborn rat skin) as models for both neutral (hydrocortisone and caffeine) and ionized drugs (niflumic acid and p-aminobenzoic acid) (31, 32) would suggest that the presence of hair follicles positively influence the permeation. However, because of the fact that all the skin shunts (follicles plus sweat glands) represent only 0.1% of the total human skin surface area (33), it is generally assumed that they normally give only a minor contribution to the passive flux of substances through intact skin after topical application.

Iontophoresis: definition and advantages

Although the impermeability of skin to ionic and polar species, it would seem that pathways ('pores') for the transport of small ions exist within the skin, particularly via the transfollicular and transappendageal routes. Paracellular transport also takes place and all of these forms of transport can be enhanced by the application of an exogenous transdermal potential.

When an electrical potential gradient is imposed across the skin, ions and charged compounds will move along the pathways of lowest electrical resistance. By repulsion of ions at the active electrode, they are driven into skin tissues: negative ions are delivered by cathode [cathodal (-) iontophoresis] and positive ions by anode [anodal (+) iontophoresis] (34) (Fig. 2).

Practically, a battery is used to create an electric field; the electrodes are placed at two adiacent sites on the skin: battery anode is at the highest potential, while the cathode is the lowest potential point. The electric field induces a migration of electrons toward the anode and the contemporary movement of ions within the ionic solution portion of the circuit, which consisted of the skin surface under the anode reservoir, the hydrated skin tissues between the two electrodes and the surface under the cathode. Positive ions (C^+) migrate toward the cathode and negative ions (A^-) toward the anode. If the solution in the anode reservoir contains a cationic drug, it will move in the direction of the cathode across the circuit and, hence, into the skin. Before it reaches the cathode, it is partitioned into the different skin tissues and/or removed by dermal blood circulation away from the site of permeation. Similarly behaves a drug which is an anion, when delivered by the cathodic reservoir (Fig. 2).

Many evidences indicate that the transport of ionic substances takes place both via a paracellular (along interconnections among cells) and skin pore routes. Cullander and Guy used confocal microscopy to visualize that two fluorescent

ions, calcein and NBD-diethanolamine, after iontophoresis into mouse skin, are distributed both in the follicular spaces and between cells, whereas no transcellular diffusion was observed (30). Similarly, mercuric chloride iontophoresis both in nude mouse, pig and human skin, indicated that the compound permeates through the intact SC via an intercellular route and was mainly localizated in the extracellular spaces (30, 35).

Other experiments clearly demonstrated that many ionic species cross the outer skin layers through pores, which are usually, but not necessarily, associated with appendages (30, 36). Iontophoresis of pilocarpine is routinely used to induce sweating in the diagnosis of cystic fibrosis (37); Abramson et al. (38, 39) and Grimnes (40) employed charged dyes to individuate the position of sweat glands within the epidermis. Experiments with the more sensitive vibrating probe electrodes (30, 41) confirmed that appendages, and particularly sweat pores behave as the highest conductive pathways in the skin. As highlighted by Cullander (30), this could begin a limitation of the efficacy of iontophoresis, when the administered drug has to act within the epidermis, since the rapid transappendageal transport can drive it far from its region of action.

Interestingly, Abrahamson and Gorin (42) have hypotesized that the flux of drugs through sweat glands is limited under normal conditions, because they are not completely filled with fluids. The drug flux is enhanced by the application of an electric current across the skin, which causes a filling of the gland duct, as a consequence of the electro-osmotic phenomenon (see below).

Several reports in the literature (7, 18, 19) indicate the efficacy of iontophoresis in topical and systemic administration of drugs, in particular for polar compounds that can not be applied to other non-iontophoretic transdermal systems. Many studies of model cationic and anionic drugs evidenced a clear increase in transdermal permeation by means of iontophoresis, with respect to passive diffusion. In Fig. 3, the examples of sotalol hydrochloride and sodium salicylate are reported (43).

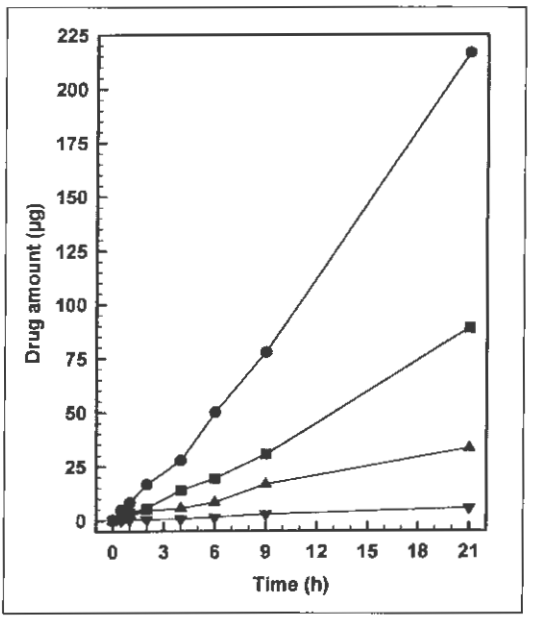


Figure 3: *Passive and iontophoretic in vitro fluxes of two model drugs through excised human skin. Anionic drug (sodium salicilate): passive ▼ and iontophoresis ■ ; cationic drug (sotalol hydrochloride): passive ▲ and iontophoresis ● (adapted from ref. 43).*

This non-invasive method lows the risk of trauma, infections and damage to the wounds. With respect to the oral route, it permits to avoid drug (e.g., peptides and proteins) chemical and/or enzymatic degradation in the gastrointestinal tract and the hepatic first-pass metabolism, and also represents a valid alternative to parenteral route, expecially because a better patient compliance can be achieved.

An aspect of iontophoresis that can be considered as an advantage or a side effect, is the so-called 'epidermal reservoir' phenomenon (42). Different Authors have observed that iontophoresis induces a certain retention of some drugs within skin areas close to the delivery electrode. This fact results in a pharmacological effect limited to the administration site, as described for pilocarpine (37) and bretylium tosylate (44), or a 'depot' effect that allows to prolong or repeat the delivery of the drug during time, as described with insulin (45, 46), histamine (42) and so-

me amino acids (47). However, the accumulation of a drug at the delivery site could also represent a risk, since the prolonged residence time can enlarge its eventual local irritative or toxic effects.

The limits of iontophoresis

The use of iontophoresis has often been associated with more or less severe skin damages (17, 48, 49), although comprehensive studies in this field lack and many Authors tend to underestimate such aspects. Common side effects observed with iontophoresis are erythema, edema and burns produced on the skin. Pain is not generated up to the higher current intensity commonly used (0.5 mA/cm^2). In general, findings showed that a higher skin damage and longer recovery times were observed when lower voltages were applied for longer times to obtain the same decrease in skin electrical resistance than higher voltages at shorter times (50). Burns are caused without any sensation of pain and tend to heal slowly (48, 51, 52). The generation of pain and burns has often been related to the electrochemically induced modification in the pH of the skin surface area under the electrodes. In fact, with many commonly used electrodes, OH^- and H^+ ions are generated at the cathode and anode, respectively, as a consequence of the electrochemical induced hydrolysis of water. As demonstrated by Molitor and Fernandez (53), the contemporary passage of the current through the resulting alkaline and acid layers causes the observed skin damages. Studies of Schwartz et al. (54) confirmed that the lowering of pH is the principal responsible for these effects. In fact, the application upon the skin of 0.1 M HCl without current for 5 min caused no sensation, while the application of 0.01 M HCl with current (2 mA/cm^2) caused pain and blistering within 2 min. More diluted HCl solutions were innocuous. Also with other buffer solutions, pain sensation was not reported until pH went down to 1-2, whereas pH 8.0 solutions did not give pain or blisters beneath the anode.

The problem of avoiding the effects of pH changes during iontophoresis has received much attention, and an useful solution has been attained by modifying the nature of electrodes used; such an argument will be discussed in a forthcoming part of this review.

Erythema has also frequently been observed as a reaction to iontophoresis, probably as a consequence of a mild irritation due to the drug used or to localized cellular damage, which could trigger the stimulation of C-fiber nociceptors and the following release of the usual mediators of inflammation (48). However, irritation and erythema are usually of little duration and do not cause any permanent damage of the skin.

Inada et al. (50) have investigated the effects of current voltage (250-4000 mV) and duration of its application on human epidermis and they found that the observed increase in skin permeability can be referred to the hystological alterations induced by the electrical field, that causes formation of pores.

An experimental approach to measure the effects of iontophoresis upon the skin has recently been described by Oh and Guy (55). They registered the resistance changes of the skin during application of different currents (10, 50 or 100 $\mu A/cm^2$) for increasing times; at the end of current passage, the recovery time of initial resistance was monitored. While skin resistance dropped rapidly (within the first 10 seconds of application) at all the current densities used, the time required for recovery of skin resistance is greater by increasing both time of current application and current density. Thereby, Authors concluded that in vivo measurement of skin impedance, and hence of resistance, can provide a valid assessment of the effects of iontophoresis upon skin surface (55).

Two other potentially limiting aspects must be considered: (i) drug solubility, and (ii) drug stability. The drug solution should have a sufficient concentration to carry the current necessary for permeation through the skin; thus, water solubi-

lity of the drug can be a critical parameter affecting the efficiency of the entire process. The second aspect, is represented by the stability of the drug to the electrical current, since hydrolysis, protein denaturation, racemization and other degradative processes can take place following ionic motion and local temperature raise which occur under iontophoretic application.

Theoretical considerations

The electrically induced transport of an ion across a membrane can be considered as the sum of three factors: simple diffusion, related to a chemical potential gradient; electrical mobility, due to an electric potential gradient; and solute transfer, due to convective solvent flow (electroosmosis) (56):

$$J = Jp + Je + Jc$$

(Eqn.2)

where J is the total flux of the drug, Jp the passive diffusion flux, Je the electrical flux, and Jc the convective flux.

During iontophoresis, the primary contribution to drug transport across the skin derives from electromigration, but electroosmotic flow has been recently reviewed as an important factor affecting the efficiency of iontophoresis (57). Electroosmosis occurs when an electric voltage is applied across a charged porous membrane, like skin, and has to be considered as a bulk fluid flow or 'volume flow'. This flow is not a diffusion: it is a movement of the fluid without concentration gradients; the direction of the flow may be with the current field or against the current flow, depending on the nature of the membrane. Generally, electroosmotic flow occurs in the direction of counterion flow (57): a counterion is an ion with an opposite (in sign) charge to the immobile charges of the membrane. Human skin is negatively charged above pH 4, due to the presence of ionized carboxyl groups. Therefore, electroosmotic flow occurs in the direction of the current, from anode to cathode: thus, anodic delivery is assisted by electroosmosis, while cathodic iontophoresis is hindered (57-59). The main physico-chemical factors which determine the size of electro-osmotic flow are the net charge density of the membrane, the density of the current applied, and the ionic strength and viscosity of the electrolyte (60). Although the role of electroosmotic flux is less significant than ionic transport for small positive ions, it may be dominant when large ions, like polypeptides and proteins, or small negative charged species are iontophoresed (36, 57, 61, 62). However, the effect of convective solvent flow on the iontophoretic flux of a drug is inversely related to the molecular size (diffusion coefficient) of the permeant (60).

Recently, Delgado-Charro et al. (63, 64) have observed that anodic iontophoresis of nafarelin, a cationic peptide, alters the extent and direction of the convective flow, by neutralizing the negative fixed charge on the skin and leading to a reversal (from the anode-to-cathode to the cathode-to-anode direction) of the electroosmotic flow itself. As a consequence, nafarelin transport by electroosmosis is suppressed and an anomalous flux/concentration profile is observed (64).

The migration of ions through the skin under an applied electric field, can be expressed as an ionic current (Eqn. 3). The fraction of current carried by each ion, as a function of its cationic or anionic nature and charge, is called the *transference or transport number*: i.e., a transport number of 0.6 means that the selected ion carries the 60% of the current through the membrane. Importance of such transport number in iontophoresis is analogous to that of permeability coefficient in transdermal passive diffusion (65).

$$J = \frac{Tl}{Z\mathcal{F}}$$

(Eqn. 3)

where J and Z are the flux and the valence (or molecular charge) of the ion, t its transport num-

ber, I the current density resulting from the migration of the ion and $\mathcal{F}$ the Faraday constant (96,500 coulomb mol^{-1}). This equation predicts that the ion flux is linearly dependent on the applied current (66) and, in effects, in most cases iontophoretic penetration of drugs was found to be related to the used current intensity (18, 67). Several laws and theories have been adopetd to optimize the prediction of the experimental iontophoretic behaviour of drugs (68). Among them, the equation proposed by Masada et al. (69) (Eqn. 4) resulted in good agreement with *in vitro* experimental permeation data when a low voltage iontophoresis (0-0.25 V) was performed:

$$E = Y/Y_O = (FZ\Delta V)\ \{RT[\exp-(FZ\Delta V/RT-1)]\}^{-1}$$

(Eqn. 4)

where E is the flux enhancement ratio, that is the ratio between the flux with (Y) and without (Y_O) the electric field, ΔV the potential drop, R the universal gas constant, and T the absolute temperature.

More accurate predictions can be made by using the Nernst-Plank and Poisson equations or also their simplifed approximations (36, 68). Nernst-Plank equation (Eqn. 5) is the most common starting point for the description of the flux of an ion through aqueous pores (J^w), under the influence of both a concentration gradient and an electric field. When the potential gradient is zero, the equation reduces to Fick's passive diffusion law (Eqn. 1).

$$J^w = -D\frac{dc}{dx} - \frac{Z\mathcal{F}}{RT}cD\frac{d\Psi}{dx} + vc$$

(Eqn 5)

Ψ is Galvani's potential at any point x in the membrane, v is the average velocity of the solvent, and vc indicates the transport of ion due to the convective solvent flow: its value is positive for a positively charged permeant, while it is a negative term for negatively charged species. The potential gradient $d\Psi/dx$ can be also approximated with its ohmic resistivity (i/k, where i is the current density and k the conductivity).

Sims and Higuchi (70) developed the following equation for the permeability coefficient of a weak electrolyte under iontophoresis (Eqn. 6):

$$P_T = X_S P_L + X_S P_P + E(1 - X_S)P_P$$

(Eqn. 6)

where P_T is the total permeability coefficient, X_S the fraction of undissociated species, P_L the permeability coefficient for the lipoidal pathway, and P_P the permeability coefficient for the aqueous pore route.

More recently, Srinivasan and Higuchi (60) have proposed an extension of Nernst-Plank model for iontophoresis, including a term for the effect of electrosmotic flow based on the Peclet number Pe:

$$dC/dX + [dV/dX - Pe]\ C = -M$$

(Eqn. 7)

In this equation dimensionless variables are used: the dimensionless distance $X = (x/h)$, h being the membrane thickness; the dimensionless electric potential $V = (Z\Psi/RT)$, and a dimensionless concentration $C = (c/c_d)$, where c is the drug bulk concentration and c_d is the concentration of the permeant at the membrane-donor side interface. The coefficients are: $M = (Jh/Dc_d)$ and Peclet number $Pe = (vh/D)$, where D is the diffusion coefficient of the permeant, and v is the average velocity of the solvent. The same Authors also proposed a method for decoupling and comparing the relative contributions of the applied electric field and induced electroosmotic flow on the observed overall iontophoretic flux. Moreover, rearrangement of such equation allows to obtain the enhancement factor E, the ratio between iontophoretic to passive flux for an ion; it gives a direct impression of the efficacy of iontophoretic flux for a charged species at the applied voltage $\Delta\Psi$. For a positively ion, E is:

$$E = - K[1 - (Pe/K)]/[1 - \exp \{K(1 - Pe/K)\}]$$

(Eqn. 8)

while, for a negatively charged one:

$$E = - K[1 + (Pe/K)]/[1 - \exp \{K(1 + Pe/K)\}]$$

(Eqn. 9)

where $K = (Z\mathcal{F}\Delta\Psi/RT)$.
These two latter equations give the iontophoretic flux enhancement due to both electric potential across the membrane and electroosmotic flow (60). Obviously, none of the mathematical models developed can completely describe all the phenomena experimentally observed during iontophoresis, including convective (electroosmotic) flow and characteristics of the delivery device (thickness of skin or membranes, area of electrodes, pH and concentration of drug solution), but each allows to make good predictive calculations.
In a forthcoming part of this review, the experimental and operational aspects of iontophoresis will be analyzed, along with the description of in vitro and in vivo models (human and animal skin) to date described.

References

1. **Walters K.A. (1989)** Transdermal Drug Delivery, Development Issues and Research Initiatives, Hadgraft J, Guy RH, Eds, Dekker, New York, pp. 197-246.
2. **Chien Y.W. (1990)** New development in drug delivery systems, *Med. Res. Rev.* **10:**477-504
3. **Lee P.I., Good W.R. (1987)** Controlled Release Technology, *Pharmaceutical Application*, ACS, Washington, DC, p. 281.
4. **Leduc C. (1900)** Introduction of medicinal substances into the depth of tissues by electric current, *Ann. Electrobiol.*, **3:**545-560.
5. **Harris P.R. (1982)** Iontophoresis: clinical research in musculoskeletal inflammatory conditions, *J. Orth. Sport. Phys. Ther.*, **4:**109-112.
6. **Fujii K., Matsui T., Kobayashi T., Nihei K., Maruyama T., Oohashi M., et al. (1993)** The application of iontophoresis in dental local anestesia. 3. Influence of the local anestetic, *Nippon Shika Masui Gakkai Zasshi*, **21:**320-324; *cfr Chem. Abstr.* **119:**131-344.
7. **Gangarosa L.P. Sr, Hill J.M. (1995)** Modern iontophoresis for local drug delivery, *Int. J. Pharm. Sci.*, **123:**159-171.
8. **Hill J.M., Shimomura Y., Kwon B.S., Gangarosa L.F. (1985)** Iontophoresis of epinephrine isomers to rabbit eyes induced HSV-1 ocular shedding, *Invest. Ophtalmol. Vis. Sci.*, **26:**1299-1303.
9. **Hill J.M., O'Callaghan R.J., Hobden J.A. (1993)** Ocular iontophoresis. *Drugs Pharm. Sci.*, **58:**331-354.
10. **Callegan M.C., Hobden J.A., O'Callaghan R.J., Hill J.M. (1995)** Ocular drug delivery: a comparison of transcorneal iontophoresis to corneal collagen shields, *Int. J. Pharm.* **123:**173-179.
11. **Lam T.T., Fu J., Chu R., Stojack K., Siew E., Tso MOM (1994)** Intravitreal delivery of ganciclovir in rabbits by transscleral iontophoresis, *J. Ocul. Pharmacol.*, **10:**571-575.
12. **Sarraf D., Lee D.A. (1994)** The role of iontophoresis in ocular drug delivery, *J. Ocul. Pharmacol.*, **10:**69-81.
13. **Murthy K.S., Tailim S.T., Singh I. (1973)** A comparative evaluation of topical application and iontophoresis of sodium fluoride for desensitization of hypersensitive dentin, *Oral. Surg. Oral. Med. Oral. Pathol.*, **36:**448-452.
14. **Gangarosa L.P. (1974)** Iontophoresis for surface anesthesia, *J. Am. Dent. Assoc.*, **88:**125-128.
15. **Riar S.S., Sawhney R.C., Bardhan J., Thomas P., Jain R.K., Jain A.K. (1982)** *Andrologia*, **14:**481-491.
16. **Schaeffer M.L., Bixler D., Yu P. (1971)** The effectiveness of iontophoresis in reducing cervical hypersensitivity, *J. Periodontal*, **42:**695-700.
17. **Singh S., Singh J. (1993)** Transdermal drug delivery by passive diffusion and iontophoresis: a review, *Med. Res. Rev.*, **13:**569-621.
18. **Yoshida N.H., Roberts M.S. (1992)** Structure-transport relationships in transdermal iontophoresis. *Adv. Drug. Delivery. Rev.*, **9:**239-264.
19. **Singh J., Roberts M.S. (1989)** Transdermal delivery of drugs by iontophoresis: a review, *Drug. Des. Delivery.*, **4:**1-12.
20. **Barner H.B. (1961)** Cataphoresis in dermabrasion tattoing, *Plast. Reconstr. Surg.*, **27:**613-617.
21. **Grayson S., Elias P.M. (1982)** Isolation and lipid biochemical characterization of stratum corneum membrane complexes: implications for the cutaneous permeability barrier,

J. Invest. Dermatol., **78:**128-135.

22. **Williams M.L., Elias P.M. (1975)** The extracellular matrix of stratum corneum: role of lipids in normal and pathologic function, *Crit. Rev. Ther. Drug. Carrier. Syst.*, **3:**95-112.
23. **Forslind B. (1994)** A new look at the skin barrier. A biophysical and mechanical model for barrier function, *J. Appl. Cosmetol.*, **12:**63-72.
24. **Rawlings A.V., Scott I.R., Harding C.R., Bowser P. (1994)** Stratum corneum moisturization at the molecular level, *Prog. Dermatol.*, **28:**1-12.
25. **Scheuplein R.J. (1972)** Properties of the skin as a membrane.
In: Montagna R, Scott EJ, Stoughton RB (Eds),
Pharmacology and the Skin, **Vol. 12**, Appleton-Century-Crofts, New York, p. 125-152
26. **Schade H., Marchionini A. (1927)** *Munchen Med Wchnschr*, **74:**1435.
27. **Flynn G.L. (1979)** In: Banker GS, Rhodes CJ (Eds). *Modern Pharmaceutics*, Dekker, New York, **Chap. 8**, p. 263.
28. **Katz M., Poulsen B.J. (1971)** In: Brodie BB, Gillete JR (Eds) *Handbook of Experimental Pharmacology*, Springer Verlag, New York, **Vol. 28, Part 1, Chap. 7**, p. 103.
29. **Urmacher C. (1990)** Histology of normal skin, *Am. J. Surg. Pathol.* **14:**671-686.
30. **Cullander C. (1992)** What are the pathways of iontophoretic current flow through mammalian skin? *Adv. Drug. Delivery. Rev.*, **9:**119-135.
31. **Illel B., Schaefer H. (1988)** Transfollicular percutaneous absorption. Skin model for quantitative studies, *Acta. Derm. Venereol. (Stockh)*, **68:**427-430.
32. **Illel B., Schaefer H., Wepierre J., Doucet O. (1991)** Follicles play an important role in percutaneous absorption, *J. Pharm. Sci.*, **80:**424-427.
33. **Scheuplein R.J., Blank I.H. (1971)** Permeability of the skin, *Physiol. Rev.*, **51:**702-747.
34. **Harris R. (1967)** Iontophoresis. In: Licht S (Ed) *Therapeutic electricity and ultraviolet radiation*, 2nd Edn, Williams & Wilkins, Baltimore, p. 156.
35. **Monteiro-Riviere N.A., Inman A.O., Riviere J.E. (1994)** Identification of the pathway of iontophoretic drug delivery: light and ultrastructural studies using mercuric chloride in pigs, *Pharm. Res.*, **11:**251 -256.
36. **Burnette R.R., Marrero D. (1986)** Comparison between the iontophoretic and passive transport of thyrotropin releasing hormone across excised nude mouse skin,
J. Pharm. Sci. **8:**738-743.
37. **Gibson L.E., Cooke R.E. (1959)** A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis, *Pediatrics (Evanston)* **23:**545-549.
38. **Abramson H.A., Engel M.G. (1941)** Skin reactions. XII. Patterns produced in the skin by electrophoresis of dyes, *Arch. Dermatol. Syphilol.*, **44:**190-200.
39. **Abramson H.A., Gorin H.H. (1940)** Skin reactions. IX. The electrophoretic demonstration of the latent pores of the living human skin; its relation to the charge of the skin,
J. Phys. Chem., **44:**1094-1102.
40. **Grimnes S. (1984)** Pathways of ionic flow through human skin in vivo,
Acta. Derm. Venereol. (Stockh), **64**:93-98.
41. **Cullander C., Guy R.H. (1991)** Sites of iontophoretic current flow into the skin: identification and characterization with the vibrating probe electrode, *J. Invest. Dermatol.*, **97:**55-64.
42. **Abramson H.A., Gorin H.H. (1939)** Skin reactions. VII. Relationship of skin permeability to electrophoresis of biologically active materials into the human skin,
J. Phys. Chem., **43:**335-346.

43. **Hirvonen J., Kontturi K., Murtomaki L., Paronen P., Urtti A. (1993)** Transdermal iontophoresis of sotalol and salicylate; the effect of skin charge and penetration enhancers, *J. Controlled. Release*, **17:**109-117.
44. **Kellogg D.L., Johnson J.M., Kosiba W.A. (1989)** Selective abolition of adrenergic vasoconstrictor responses in skin by local iontophoresis of bretylium, *Am. J. Physiol.*, **257**:H1599-H 1606.
45. **Kari B. (1986)** Control of blood glucose levels in alloxan-diabetic rabbits by iontophoresis of insulin, *Diabetes*, **35:**217-221.
46. **Siddiqui O., Sun Y., Liu J.C., Chien Y.W. (1987)** Facilitated transdermal transport of insulin, *J. Pharm. Sci.*, **76:**341-345.
47. **Wearley L.L., Tojo K., Chien Y.W. (1990)** A numerical approach to study the effect of binding on the iontophoretic trasnport of a series of amino acids, *J. Pharm. Sci.*, **79:**992-998.
48. **Ledger P.W. (1992)** Skin biological issues in electrically enhanced transdermal delivery, *Adv. Drug. Del. Rev.*, **9:**289-307.
49. **Wang Y., Allen L.V. Jr, Li L.C., Tu Y.H. (1993)** Iontophoresis of hydrocortisone across hairless mouse skin: investigation of skin alteration, *J. Pharm. Sci.*, **82:**1140-1144.
50. **Inada H., Ghanem A.H., Higuchi W.I. (1994)** Studies on the effects of applied voltage and duration on human epidermal membrane alteration/recovery and the resultant effects upon iontophoresis, *Pharm. Res.*, **11:**687-697.
51. **Kovacs J. (1935)** Electrotherapy and light therapy, Lea & Febiger, Philadelphia
52. **Monteiro-Riviere N.A. (1990)** Altered epidermal morphology secondary to lidocaine iontophoresis: in vivo and in vitro studies in porcine skin, *Fundam. Appl. Toxicol.* **15:**175-185.
53. **Molitor H., Fernandez L. (1939)** Studies on iontophoresis. 1. Experimental studies on the causes and prevention of iontophoretic burns, *Am. J. Med. Sci.*, **198:**778-785.
54. **Schwarz V., Sutcliffe C.H., Style P.P. (1968)** Some hazards of the sweat test, *Arch. Dis. Childh.*, **43:**695-701.
55. **Oh S.Y., Guy R.H. (1995)** Effects of iontophoresis on the electrical properties of human skin in vivo, *Int. J. Pharm.*, **124:**137-142.
56. **Burnette R.R. (1989)** Iontophoresis. In: Hadgraft J, Guy RH (Eds) *Transdermal Drug Delivery: Developmental Issues and Research Initiatives*, Dekker, New York, p. 247-291.
57. **Pikal M.J. (1992)** The role of electroosmotic flow in transdermal iontophoresis, *Adv. Drug. Del. Rev.*, **9:**201 -237.
58. **Sims S.M., Higuchi W.I., Srinivasan V. (1991)** Interaction of electric field and electroosmotic effects in determining iontophoretic enhancement of anions and cations, *Int. J. Pharm.* **77:**107-118.
59. **Kim A., Green P.G., Rao G., Guy R.H. (1993)** Convective solvent flow across the skin during iontophoresis, *Pharm. Res.*, **10:**1315-1320.
60. **Srinivasan V., Higuchi W.I. (1990)** A model for iontophoresis incorporating the effect of convective solvent flow, *Int. J. Pharm.*, **60:**133-138.
61. **Gangarosa L., Park N., Wiggins C., Hill J. (1980)** Increased penetration of non-electrolytes into mouse skin during iontophoretic water transport (iontohydrokinesis), *J. Pharmacol. Exp. Ther.*, **212**:377-381.
62. **Burnette R., Ongipattanakul B. (1987)** Characterization of the permselective properties of excised human skin during iontophoresis, *J. Pharm. Sci.*, **76:**765-773.
63. **Delgado-Charro M.B., Guy R.H. (1995)** Iontophoretic delivery of nafarelin across the skin,

Int. J. Pharm., **117:**165-172.

64. **Delgado-Charro M.B., Rodrlguez-Bayon A.M., Guy R.H. (1995)** Iontophoresis of nafarelin: effects of current density and concentration on electrotransport in vitro, *J. Contr. Rel.*, **35:**35-40.
65. **Roberts M.S., Singh J., Yoshida N., Curries K.I. (1990)** Iontophoretic transport of selected solutes through human epidermis. In: Scott RC, Guy RH, Hadgraft J (Eds) *Prediction of percutaneous absorption*, IBC Technical Services, London, p. 231-241.
66. **Phipps J.B., Gyory J.R. (1992)** Transdermal ion migration, *Adv. Drug. Del. Rev.*, **9:**137-176.
67. **Leławongs P., Liu J.C., Chien Y.W. (1990)** Transdermal iontophoretic delivery of arginine-vasopressin (II): Evaluation of electrical and operational factors, *Int. J. Pharm.* **61:**179-188.
68. **Kasting G.B. (1992)** Theoretical models for iontophoretic delivery, *Adv. Drug. Del. Rev.*, **9:**177-199.
69. **Masada T., Rohr U., Higuchi WI., Fox J., Behl C., Malick W., Goldberg A.H., Pons S. (1985)** In: 39th National Meeting of the Academy of Pharmaceutical Sciences Abstracts, Minneapolis, **Vol. 15(2)**, p. 73.
70. **Sims S.M., Higuchi W.I. (1990)** Baseline studies on iontophoretic transport in hairless mouse skin: the effect of applied voltage drop and pH on the iontophoresis of a model weak electrolyte, *J. Membr. Sci.*, **49:**305-320.

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IS THERE ANY RISK OF ALLERGIC REACTION IN CRENO-COSMETOLOGY?

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Key words: *Creno-cosmetology, mineral waters, muds, allergological risks.*

Synopsis

High content of salts, which prevents the growth of pathogen microorganisms, is the main hallmark of mineral waters. This feature gives mineral waters and muds useful, long-duration self-preservative properties which allow for their utilization in both cosmetology and dermatology (as vehicles of pharmacological agents). As concerns cosmetology, broad possibilities of application of mineral waters have long been recognized, which are based on the eudermic properties of salts, the radioactive power of solutions and the bio-activating charge of suspendend colloidal substances.
It is well know that the overall incidence of untoward effects from cosmetics is low and a thorough literature review did not detected any report of irritant/allergic reactions to mineral water-derived cosmetics. Considering the ever-increasing use of 'creno-cosmetics', these circumstances might be explained as follows:
1) so far, the problem has not aroused medical concern; or
2) cases have been overlooked or misdiagnosed; or
3) creno-cosmetics have no irritant/sensitizing potential.

Riassunto

Dopo la sempre più ampia e promettente applicazione delle acque minerali e dei loro derivati in campo medico, peraltro da molti secoli già conosciuta e sfruttata, tali prodotti hanno più recentemente trovato impiego anche in campo cosmetologico.
L'applicazione sulla cute di qualsiasi sostanza reca sempre in sé un più o meno rilevante rischio di produrre reazioni allergiche, o più genericamente indesiderate. Per quanto più specificamente attiene i cosidetti creno-cosmetici, nessuna informazione in argomento pare finora essere stata consegnata alla letteratura. Tale circostanza potrebbe dipendere o dallo scarso interesse finora suscitato dal problema, o dal mancato riconoscimento dei casi, oppure da una intrinsecamente bassa capacità dei creno-cosmetici di indurre irritazione e/o sensibilizzazione. Gli Autori propendono per quest'ultima ipotesi e ne discutono le possibili basi farmacologiche.
Parole chiave: Creno- cosmetologia, acque minerali, fanghi, rischio allergologico.

The possibility of employing mineral waters for medical purposes has very ancient traditions in the Old World. Crenotherapy, as a whole, is based on the more or less demonstrated "bio-activity" of mineral waters and has been added to other therapeutic tools in a variety of skin disorders *(see table I)*. In some instances, quite well codified regimens have been conceived (to cite an example, the so-called "Tomesa therapy" for psoriasis, which consists of the association of ultraviolet light with baths containing salts from the Dead Sea). In others, the crenotherapeutic approach is rather more empiric, but may represent a proposable alternative in the management of a large number of disease (1).

Also in cosmetology broad possibilities of application of mineral waters have long been recognized which are based on the eudermic properties of salts, the radioactive power of solutions and the bio-activating charge of suspended colloidal substances. Moreover, the benefit properties of mineral waters have found additional fields of application in a variety of cosmetological methodologies, including:

- **Baths in carbonic waters**, from which benefits derive from the stimulating effect of nascent carbon dioxide. This method is widely used in beauty farms, in which an artificial carbon dioxide supply is often provided to common water;
- **Plankton-cosmesis**, which employs a rich organic matrix, named 'plankton', consisting of unicellular algae that are collected from the water overhanging mature thermal muds;
- **Peloido-cosmesis**, in which the prolonged contact of virgin muds with thermal waters gives raise to development of a micro and macroflora producing bioactive substances that stimulate cutaneous metabolism;
- **Fucoido-cosmesis**, which employs organic substances (including carotenoids, phospholipids, aminolipids and polysaccharides) produced by algae developing in mature muds (1).

After these preliminary outlines, we will now begin to deal with the allergological aspects of creno-cosmetology or, more comprehensively, with overall untoward effects of creno-cosmetics.

As a matter of fact, a thorough literature review did not enable us to find any data regarding this issue. In our opinion, this may be due to the fact that:

1) so far, the problem has not aroused medical concern; or

2) cases have been overlooked or misdiagnosed; or

3) creno-cosmetics have no irritant/sensitizing potential.

Table 1

SKIN DISORDERS IN WHICH A CRENO-THERAPEUTIC APPROACH CAN BE PROPOSED

Psoriasis	Peripheral vasculopathic diseases
Contact dermatitis	Distrophic dermatoses
Atopic dermatitis	Ichthyosis
Pompholyx	Cutis laxa
Pruritus	Lymphoedema
Seborrheic dermatitis	Acrocyanosis
Acne	Vaginitis
Rosacea	Lichen ruber planus

Actually, considering the results of our enquiry, the third hypothesis is more likely to be the true. However, before presenting any possibile argument in favour of this point of view, some premises would be necessary.

No allergological survey can be perfomed if information is lacking on the ingredients of a cosmetical product. On the basis of their mineral content (in terms of both absolute and relative concentration of ions) thermal waters are classified as sulfurous, iodic, bromic, cloric, carbonic, and radioactive.

One of the most represented active ingredients of mineral waters is sulfur, a nonmetallic element weighing nearly 36,000. Sulphuric waters contain variable amounts of H_2S, ranging from 1 to more than 100 milligramms per litre. It is well know that sulfur preparations (in precipitated, sublimed and colloidal forms) are widely used in medicine due to germicidal actions and keratolytic properties, and that they have a well established, dose-dependent irritant potential; by contrast, they are only very rarely responsible for allergy (2). Thus, the risk related to the use of sulfur-containing creno-cosmetical products is likely to be practically negligible. Moreover, while in medical preparations sulfur content is considerable (2% or more), in crenocosmetology much lower concentrations of this element are supposed to be supplied and this may well provide further explanation of the absence of documented allergic as well as irritative reactions. Actually, when employed for cosmetological purposes, sulfur does retain its full effect despite of its dilution. In fact, it can be assumed that the small size of sulfur particles in water solutions increases the area available for sulfur-cutaneous interaction and sulfur efficacy as well. Moreover, it may be supposed that high dilution of salts in water results in an increase of corneum hydratation state and, consequently, in a paradoxical enhancement of bio-availability and biological effect of soluted molecules.

However, we believe that, apart from high dilution and low allergenicity per sé, further reasons for safery and tolerability of sulfur may lie also on antioxidant and immunosuppressive properties. In fact, in vitro studies have demonstrated that sulfur is able to inhibit T-lymphocyte proliferation and to down-regulate both production and release of lymphokines. Moreover, sulfur-containing thermal waters have been shown to inhibit antigen-presenting-cells activity (3). Finally, as concerns antioxidant properties of sulfur, some circustance should be briefly discussed: mineral waters contain free H_2S, a highly instable molecule which reacts with oxigen giving origin to more stable reduced products. In this way, H_2S-containing mineral waters may be considered a good antioxidant agent and antioxidants have been demonstrated to be effective downregulators of irritant and allergic contact reactions (4, 5, 6). On the basis of all these premises (namely, antioxidant charge and immunomodulating effects) allergy to sulfur might be restained or masked (in the same way as cyclosporin or corticosteroids do).

As for the other components of mineral waters, including bromine, jodine, calcium, potassium, sodium, chlorine, magnesium and so forth, no sensitizing potential has been acknowledged so far to any of them.

Considering all these circumstances, it would seem that there is no allergological risk upon contact with active ingredients of creno-cosmetics. On the other hand, a possible risk of allergy should be sought in bases employed for incorporation of mineral components. In this view, as shown in previous surveys (7), perfumed compounds and preservatives are likely to play a major role. However, it's well know that the overall incidence of untoward effects from cosmetics is very low, and no special risk of allergy seems to be borne by crenocosmetics.

Moreover, the following detail should be incidentally considered: one of the main hallmarks of mineral waters and muds is high content of salts. Such condition is likely to decrease or abolish the need of addition of flavouring and

preservative agents as it could directly prevent the growth of patogen microorganism, giving useful self-preservative properties to creno-cosmetics.

Certainly, all these points of view remain highly speculative and, in order to asses a safety profile of creno-cosmetological formulations, more information should be provided by the manufactures on the ingredients of products that have been put on the market.

In conclusion, safety of creno-cosmetics will be confirmed only when they will be used on a larger scale and wide pre- and post- market skin testings will have carried out among consumers.

References

1. **Vena G.A., Mastrolonardo M., Angelini G. (1992)** Dermatologia termale, *La mia salute*, **4**:31-34.
2. **Lin A.N., Reimer R.J., Carter D.M. (1988)** Sulfur revisted, *J. Am. Acad. Dermatol*, **18**:553-558.
3. **Simonelli C. (1994)** Come le acque sulfuree modulano il sistema immunitario, *Sulphurea*, **1(suppl.)**:13-15.
4. **Ikeda M., Schroeder K.K., Mosher L.B., Woods C.W., Akeson A.L. (1994)** Suppressive effect of antioxidant on Intercellular Adhesion Molecule-1 (ICAM-1) expression in human epidermal keratinocytes, *J. Invest. Dermatol*, **103**:791-796.
5. **Memon A.A., Molokhia M.M., Friedmann P.S. (1994)** The inhibitory effects of topical chelating agents and antioxidant on nickel induced hypersensitivity reactions, *J.Am. Acad. Dermatol.*, **30**:560-565.
6. **Vena G.A., Foti C., Loconsole F., Marrella A.L. (1994)** Valutazione dell'efficacia clinica di una crema a base di furalglucitolo (AR-GB11) nella terapia della dermatite da contatto, *Boll. Dermat. Allerg., Prof.* **9**:93-101.
7. **Angelini G., Vena G.A., Giglio G., Fiordalisi F., Meneghini C.L. (1985)** Contact dermatitis due to cosmetics, *J. Appl. Cosmetol.*, **3**:223-236.

HAIR TRANSPLANTATION

Third edition Expanded, 1995
Edited by Walter P. Unger - 848 pages bound illustrated
ISCN O-8247-9363-3, DEKKER INC. N.Y.

It's rare to come across any medical-surgical publication which is versatile and acts as a useful reference for both newly-postgraduated young physicians and eminent, experienced surgeons.
In my opinion, versatility is the distinguishing feature of this book, which obviously entails that topics are examined in exceptionally exhaustive detail.
The history of baldness surgery is dealt with briefly and pleasantly, but no mention is made of artificial fiber implantation. This ruinous method should be absolutely abolished, but not forgotten, so that young physicians do not venture into surgical fields which are bound to carry iatrogenic consequences. The physiologicoanatomic features of the scalp are described exhaustively. This acts as an introduction to the complex pathophysiologic aspects of androgenetic alopecia involving major genetic, chemical and hygienic factors.
After a brief description of the relevant medical treatments, surgical anatomy is dealt with and baldness degrees are topographically classified as a basis for choosing the right surgical treatment.
A careful analysis is made of surgical instruments and pre and postoperative graphic documentation methods, while a whole chapter deals with patients' first examination in an extremely pleasant, useful and exhaustive way.Very good!
The various aspects of surgical options are exhaustively described and dealt with through excellents photographs and drawings which make understanding the text easier.
Surgical techniques were basically divided into two groups including graft-based procedures and the various options in skin flap use respectively.
The autologous graft methodology for baldness treatment is described down to the smallest details. This makes it possible to rapidly and clearly understand the guidelines for a correct transplant planning and the application of punch graft, micrograft and minigraft techniques.
Two chapters examine the postoperative details which are known to play a major role in a successful surgical treatment.
There follow the chapters dealing with graft use in baldness-reducing techniques and in the techniques for moving skin flaps from donor areas with a considerable hair density to bald host areas.
Here we go into proper surgery: the relevant issues are dealt with in a clear and well-arranged way by means of good photographs.
Though that's no easy task, the following chapters succeed in describing and clearly differentiating the current graft techniques, namely conventional transplantation and microsurgery.
In my opinion, complications are dealt with inadequately in these chapters. This issue certainly deserves to be examined more exhaustively and through a greater number of photographs.
Finally, I must say this book is a valuable one and thoroughly examines all the aspects of scalp surgery. I believe the title does not convey the exact educational significance of this essay, which gives a great contribution to Scalp Surgery, rather than hair transplantation.

Dr. Jorge de Antueno
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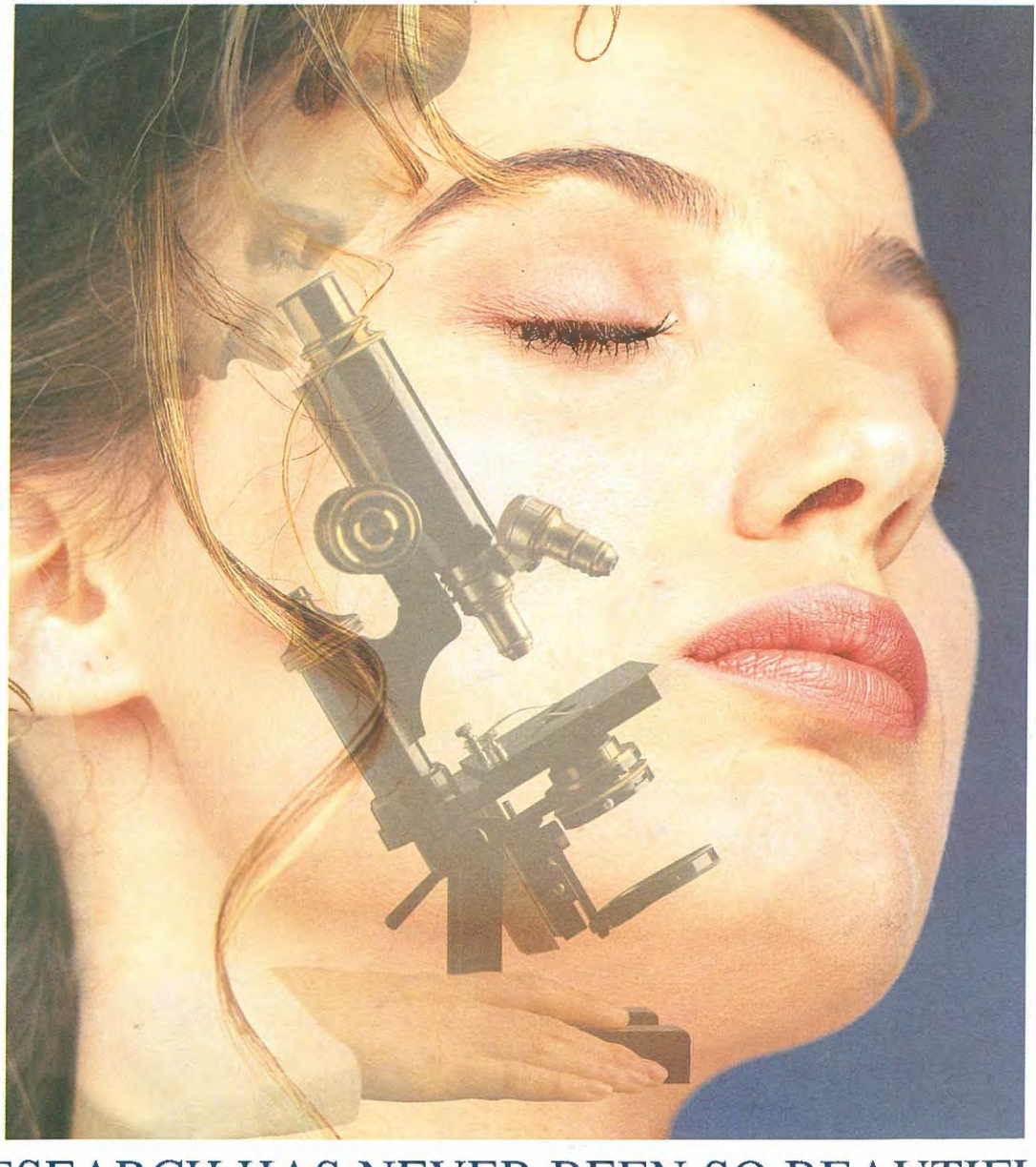

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