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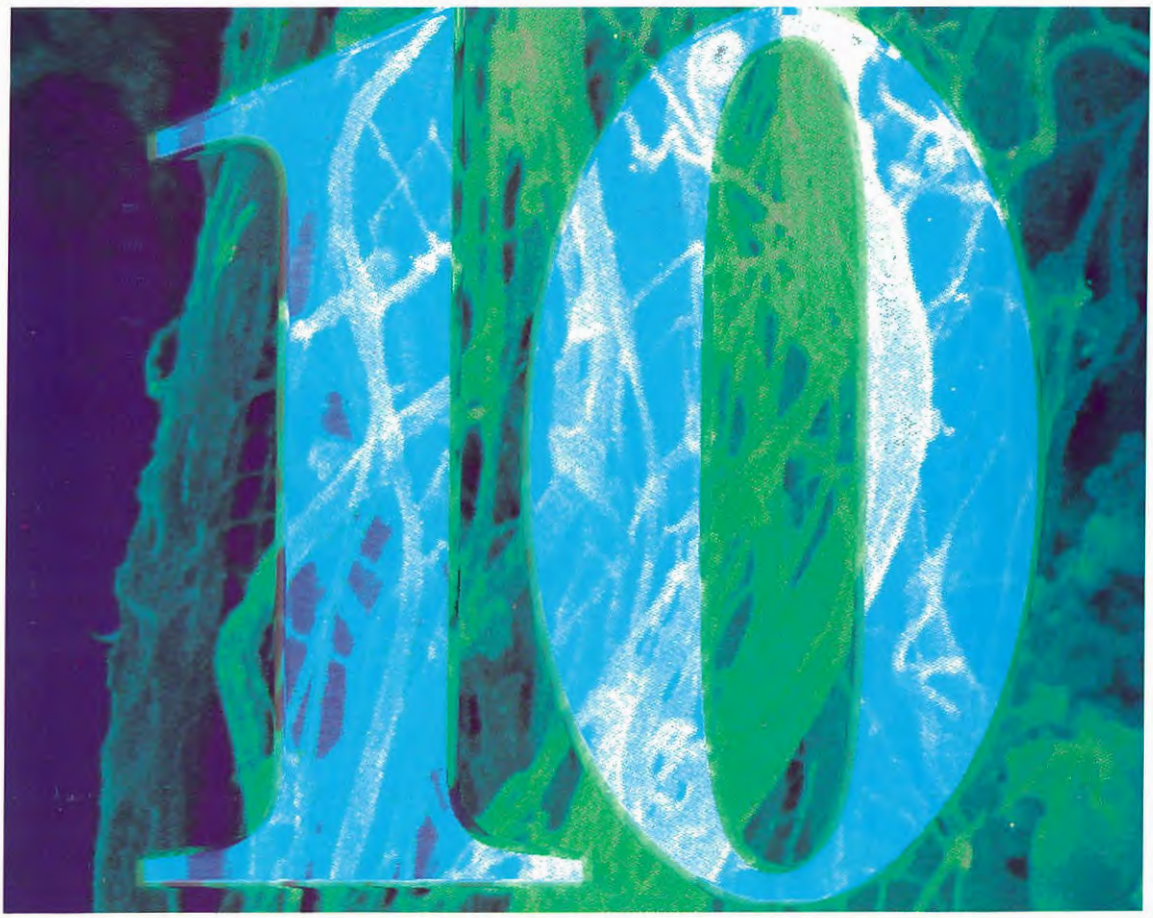


1983 - 1992: 10 years

Official Journal of International Society of Cosmetic Dermatology

INTERNATIONAL EDIEMME

Volume 10 - Number 4
October/December 1992
ISSN 0392-8543 Sped. abb. post. IV°70

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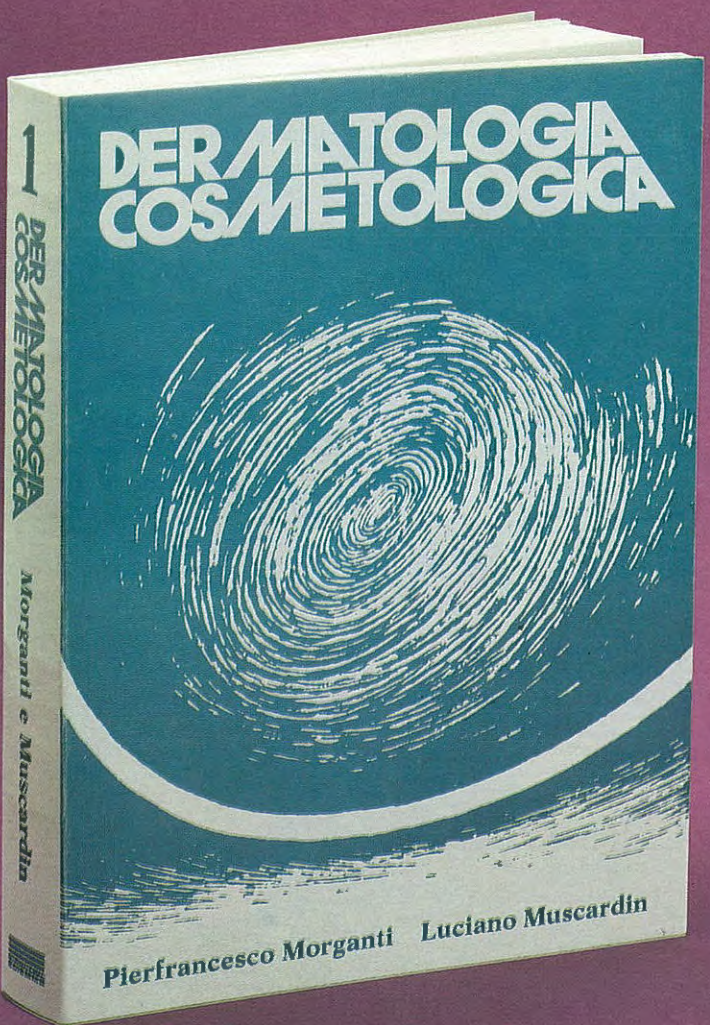


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A cura di P. Morganti e L. Muscardin
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*It is with sorrow that we report the unexpected and untimely death of our dear friend and colleague Professor Luciano Muscardin. We all knew Dr. Muscardin for his outstanding professional and human credentials. He was a very known dermatologist in the specific fiels of Cosmetic Dermatology, and an expert member of many scientific and legal committees for italian and european government authorities. He has written extensively on Cosmetology editing one book and many scientific papers.*

*However, this does not measure the man, and this is important, because, above all Dr. Muscardin was a warm, caring, and happy human. His high professional standing was a small part of his presence. Of greater importance were his companionship with those near to him, his ready smile, his warmth, his dependable faith in his fellow man.*

*Luciano Muscardin's qualities were those which earn the title "Physician". He was loved by his patients and respected by his peers. Our society is priveleged to have many great names on its rosters. Perhaps, because of this, we find the loss of any member to be noteworthy. The loss of Dr. Muscardin is deeply felt by us all.*

*Our sympathy is sent to all who share in the sorrow.*

journal of applied cosmetology

# Trimestrale di Dermatologia Cosmetologica
# Quarterly Review of Cosmetic Dermatology

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New York City - U.S.A. October 7-9, 1993

J. Appl. Cosmetol. **10**, 81-89 (October-December 1992)

# ROLE OF LIPIDS IN CELLULAR MEMBRANES AND SKIN BIOLOGY.

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***Received:*** *October 30, 1991. Presented at the IV International Congress on Cosmetic Dermatology "Progress in Cosmetic Dermatology: Science and Safety" Roma (Italy) October 31 - November 2, 1991.*

***Key words:*** *Membrane Fluidity; Cellular Membrane; Biologicol Membrane; Glycoproteins; Glycolipids; Phospholipid Bilayer; Glycoshingolipids; Liposomes; Lipidbilayers; Stratum Corneum Lipids.*

## Synopsis

A barrier that separates cellular contents from the environment is an absolute condition for life. This separation is provided by the plasma membrane also called the cell membrane. All biological membranes, whether from eucaryotic or procaryotic cells, have the same classes of chemical components, a similarity in structural organization and a number of properties in common. Biological membrane consist of a continuous double layer of lipid molecules in which proteins are embedded. This lipid bilayer is fluid, with individual lipid molecules able to diffuse rapidly within their own monolayer hut usually not from on monolayer to the other.
The three major lipid components of membranes are phosphoglycerides, sphingolipids and cholesterol; their percentage varies significantly in the different membranes and is related to the specific roles of the individual membranes. Although lipid molecules display considerable structural diversity, all of them share one important structural property: they are amphipatic, so they can assemble spontaneously into hilayer when placed in water, forming sealed compartments that reseal if torn. The functional significance of the lipid compositions of different membranes will be discussed and focused mainly on the lipid lamellar structure of stratum corneum.

## Riassunto

La barriera che separa dall'ambiente il contenuto delle cellule rappresenta la condizione indispensabile per la vita. Questa separazione è mantenuta dalla membrana plasmatica chiamata anche membrana cellulare. Tutte le membrane biologiche sia che provengano da cellule eucariotiche che precariotiche, sono formate dagli stessi componenti chimici e posseggono una uguale struttura organizzata. La membrana biologica è formata da un doppio strato continuo di molecole lipidiche attraversate da qualche molecola proteica.
Il doppio strato lipidico è fluido e permette soltanto l'interscambio tra i diversi lipidi di uno stesso strato. I tre principali componenti delle membrane sono i fosfogliceridi, gli sfingolipidi ed il colesterolo. La loro percentuale varia in un modo significativo tra le diverse membrane ed è legato al loro specifico ruolo. Anche se i diversi lipidi posseggono considerevoli diversità

strutturali, sono tutte molecole anfipatiche che formano spontaneamente strati bimolecolari se inserite in un ambiente acquoso.
Il significato funzionale della composizione lipidica delle diverse membrane verrà discusso in modo da mettere a fuoco soprattutto la struttura lamellare lipidica dello strato corneo.

## Basic structural features of cellular membranes.

Biological membranes, both from eucaryotic and procaryotic cells are built up from the same classes of chemical compounds - lipids, proteins and in smaller quantity, carbohydrates, however there are important qualitative and quantitative differences within any given class of compounds.

Biological membranes divide the intracellular milieu from the external environment. In contrast to the procaryotes, eucaryotic cells have a well defined membrane surrounding a central nucleus and a variety of intracellular structures and organelles. The intracellular membranous system establishes a number of distinct subcellular specialization. By means of this compartmentalization of the cell, different chemical reactions, requiring different environments, can occur simultaneously.

As it will be discussed later in more detail, membranes are lipid in nature and many substances that are soluble in a hydrophobic environment will concentrate in cellular membranes, where many specific biochemical reactions can occur; the extensive membrane system of eucaryotic cells create an additional environment that the cell can use for its different functions.

The mean thickness of a typical plasma membrane is 75-90 Å, but this value is lower for intracellular membranes. All of them, however, constitute a dynamic structure in which lipids are present in a fluid state and make up a non aqueous compartment where the different compounds can move and possibly interact.

Biological membranes, and particularly the plasma membranes, share unique properties: they are impermeable to a great variety of substances and within the membranes itself there are selective transport systems that allow strict control of the flux of substrates, cofactors and ions from one compartment to the others. In this way the concentration of the different substances and consequently the different metabolic pathways can be modulated.

The plasma membrane of eucaryotic cells also plays an important role in cell-cell recognition and interaction and represents the target of many hormones and metabolic regulatory factors which are important for the signal transmission and transduction to intracellular mediators.

As already mentioned, lipids and proteins are the main components of biological membranes; their amount varies considerably in different membranes according to the type and the function of a given cell. A smaller amount of carbohydrates is also present, not in free form, but linked to glycoproteins and glycolipids.

Based on evidence from physicochemical, biochemical and electron microscope investigations knowledge of the structure of biological membranes has evolved. Proteins can be divided, roughly, into two main categories on the basis of their extractibility from the membrane: 1 - peripheral (or extrinsic) localized on the membrane surface, to which they are loosely bound and from where they can be easily removed. They account for approximately 30% of the total and in many instance have enzymatic activity. 2 - integral (or intrinsic) which span the phospholipid bilayer (see also below), with portions protruding on each side. Hydrophobic interactions between the lipids and the hydrophobic domains of these integral proteins prevent them from being readily removed, and their extraction leads to disruption of the membrane.

In general membrane proteins play diversified roles and are involved in many functions: transport of small molecules; recognition and adhesion phenomena; transduction of signals from external ligands; they can act as enzymes and receptors, besides their structural role.

Membrane lipids belong to three main classes: phospholipids, sphingoglycolipids and cholesterol. Their percentage in different membranes varies significantly and it is quite clearly related to the cell and/or organelle functions.

The phospholipids (or glycerophospholipids) are polar, ionic lipids composed of 1,2 diacylglycerol with a phosphodiester bridge that links the

glycerol backbone to a specific base, usually a nitrogenous one, such as choline, serine, ethanolamine. Phosphatidylcholine (PC) contains mostly palmitic (C16:0) in the sn-1 position of glycerol and primarily the unsaturated 18-carbon fatty acids - oleic, linoleic or linolenic - in the sn-2 position. Phosphatidylethanolamine (PE) has the same saturated fatty acids as PC at the sn-1 position but contains more of the long-chain polyunsatured fatty acids, namely 18:2, 20:4, 22:6 - at the sn-2 position.

PC, PE and phosphatidylserine (PS) are the most abundant phospholipids in human tissue. At physiological pH PC and PE have no net charge and exist as a dipolar zwitterions, whereas PS has a net charge of -1, i.e. it is an acid phospholipid.

There are two other compounds with this characteristic: phosphatidyl inositol and phosphatidylglycerol; the first one occurs in mammalian membranes and it is rather unusual because it often contains almost exclusively stearic acid at the sn-1 position and arachidonic acid (C20:4) at the sn-2 hydroxygroup. Phosphatidylglycerol occurs in relatively large amount in mitochondrial membranes and it is a precursor of cardiolipin. Most phospholipids contain more than one kind of fatty acids per molecule, so that a given class of these compounds actually represents a family of different molecular species.

Sphingolipids are complex lipids whose core structure is provided by the long-chain aminoalcohol sphingosine. There is a close similarity between carbons 1,2 and 3 of sphingosine and glycerol mainly because both of them have nucleophilic groups, hydroxyl or amino, at these carbon atoms. Sphingosine as such with its free amino group does not occur naturally.

The fundamental building block or core structure of natural sphingolipids is ceramide, the long-chain fatty acid amide derivative of sphingosine. Therefore there are two long-chain hydrocarbon domains in the ceramide molecule and these hydrophobic regions are responsible for the lipoidal character of sphingolipids.

Free ceramide is not a component of membrane lipids but rather it is an intermediate in the biosynthesis and catabolism of glycosphingolipids and sphingomyelin.

In sphingomyelin the primary alcohol group at C-1 of sphingosine is esterified to choline through a phosphodiester bridge of the kind that occurs in the acyl glycero-phospholipid; the amino group of sphingosine is attached to a long-chain fatty acid by means of an amide bond. Sphingosine is therefore a ceramide phosphocholine. It contains one negative and one positive charge so that it is neutral at physiological pH.

As indicated above, in plasma membranes there are also carbohydrate-containing sphingolipids, which include cerebrosides, sulfatides, globosides and gangliosides. In this compounds the polar head group is attached to sphingosine via the glycosidic linkage of a sugar molecule rather than a phosphate ester bond, as in the case of the phospholipids.

In other words the glycosphingolipids do not contain phosphate and have a sugar attached by a ß-glycosidic linkage to the 1-hydroxyl group of the sphingosine in a ceramide. One subgroup is the cerebrosides, which contain either a glucose or a galactose attached to ceramide and are referred to as glucocerebrosides or galactocerebrosides, respectively. Cerebrosides are neutral compounds. Galactocerebrosides are found predominantly in brain and nervous tissues, whereas the small quantities of cerebrosides in non-neutral tissues usually contain glucose. Galactocerebrosides may contain a sulfate group esterified on the 3 position of the sugar. They are called sulfatides. Cerebrosides and sulfatides usually contain very long-chain fatty acids with 22 to 26 carbon atoms.

In place of one monosaccharide, neutral glycosphingolipids often have 2 (dihexosides), 3 (trihexosides), 4 (tetrahexosides) sugar residues attached to the 1-hydroxyl group of sphingosine. D-glucose, d-galactose, N-acetylglucosamine, and N-acetylgalactosamine are the usual sugars.

The most complex group of glycosphingolipids

is represented by gangliosides, which contain oligosaccharide head groups with one or more residues of sialic acid; these are amphipathic compounds with a negative charge at pH 7.0. Specific gangliosides and glycolipids are found in the skin either in normal or pathological conditions.
The third major lipid present in membranes is cholesterol that contains four fused rings - which makes it a planar structure - a polar hydroxyl group at carbon-3, and an eight-member branched hydrocarbon chain attached to the D ring at position 17. Cholesterol is a compact hydrophobic molecule; however the hydroxyl group, that can be esterified, gives, when it is free, a little hydrophilia to the molecule. In a given membrane cholesterol is located between the phospholipid molecules, oriented in a way which allows it to interact with the other lipid components, modulating the fluidity and, consequently, the permeability of the membrane itself.

## Micelles, lipid bilayers and liposomes

The physicochemical properties of the lipid components just described dictate the basic structural characteristic of their organization in living cells.
All amphipathic compounds, with a hydrophilic head and a hydrophobic tail, react in a unique fashion in an aqueous system because of their low solubility in water. Under proper conditions, these lipid molecules will come together to form spheres, termed micelles, with the hydrophobic tails interacting to exclude water and with the charged polar head groups on the outside. Micelles with a single lipid or a mixture of lipids can be made. The formation of the micelle depends also on the temperature of the system and, if a mixture of lipids is used, on the ratio of concentrations of the different lipids in the mixture. The micelle structure is very stable because of the hydrophobic interaction of the hydrocarbon chains and the attraction of the polar groups to water. Depending upon the condition, amphipathic lipids will interact to form a bimolecular leaf structure with two layers of lipid in which the polar head groups are at the interface between the aqueous medium and the lipid while the hydrophobic tails interact in a second way to form an environment that excludes water. This bilayer conformation is the basic lipid structure of all biological membranes as described later in more detail.
Lipid bilayers are extremely stable structures held together by noncovalent interactions of the hydrocarbon chains of the acyl groups and the ionic interactions of the charged groups with water. Hydrophobic interactions of the hydrocarbon chains lead to the smallest possible area for water to be in contact with the chains, and water is essentially excluded from the interior of the bilayer.
A lipid bilayer may close in on itself, forming a spherical vesicle separating the external space from an internal compartment. These vesicles are termed liposomes.
Individual phospholipid molecules can readily exchange place with the neighbouring molecules, which leads to rapid lateral diffusion in the plane of the membrane. In addition, the fatty acyl chains can rotate around the carbon-carbon bonds; in fact there is a greater degree of rotation nearer the methyl end, leading to greater motion at the center of the lipid bilayer. Individual lipid molecules cannot migrate readily from one monolayer to the other, a process termed flip-flop. Thus the lipid bilayer has not only an inherent stability but also a fluidity in which individual molecules can move rapidly in their own monolayer but do not exchange with the adjoining monolayer.
Artificial membrane systems have been studied extensively as a mean to determine the properties of biological membranes. A variety of techniques are available to prepare liposomes, using synthetic phospholipids and lipids extracted from natural membranes. Depending on the procedure, unilamellar vesicles and multilamellar vesicles (vesicles within vesicles) of various

size (20nm-1μm in diameter) can be prepared. The interior of the vesicle is an aqueous environment, and it is possible to prepare liposomes with different substances entrapped. Thus the external and internal environments of the liposome can be manipulated.

## Biological membranes

The basic structure is a bimolecular leaf arrangement of lipids in which phosphoglycerides, sphingolipids, and cholesterol are oriented so that the hydrophobic portions of the molecules interact to minimize their interactions with water or other polar groups. The polar head groups of the amphipathic compounds are at the interface with the aqueous environment. A number of models for the structure of biological membranes have been suggested dating back to one by H. Davson and J. Danielli in 1935, which was refined in later years by J.D. Robertson. In early 1970s, G.L. Nicolson and S.J. Singer proposed the mosaic model for membranes in which it was suggested that proteins are on the surface as well as in the lipid bilayer. Some proteins could span the lipid bilayer with their polar groups in contact with the aqueous surroundings on both sides and the hydrophobic portions interacting with the lipids in the interior of the membrane. This model has been extensively refined and now is referred to as the fluid mosaic model to indicate the movement of both lipids and proteins in the membrane. The proposed structure accounts for many properties of mammalian membranes, but it continues to undergo modifications and refinements.

These properties, including fluidity, flexibility, which allow changes of shape and form, self-sealing features and impermeability for many substances are mainly related to the physicochemical characteristics of the lipid bilayer.

It is now recognized that the membrane lipids may not be randomly distributed in the monolayers, but that there are islands of lipids associated together or in contact with a specific protein. The individual lipids are not, however, immobilized in these islands but rapidly exchange with molecules in the surrounding area of the membrane.

An important difference between the Nicolson-Singer model and earlier models is that the lipid bilayer is discontinuous, with proteins embedded in the hydrophobic portion of the bilayer. Many membrane proteins span the bilayer, with portions protruding on each side. Hydrophobic interaction between the lipid and the hydrophobic domains of these integral proteins prevents these proteins from being readily removed, and their extraction leads to disruption of the membrane. Protein can also be loosely bound to the membrane by interaction between charges on the protein and these peripheral proteins are easily removed by mild treatment with little damage to the membrane.

Even though the model would suggest that proteins are randomly distributed throughout and on the membrane, evidence from a variety of sources supports a high degree of functional organization with definite restriction on the localization of some proteins. Membrane proteins also have a definite orientation within and across the membrane. Integral proteins with enzymatic activity may have their catalytic site on either the inner or outer surface. This orientation is established during the biosynthesis of the membrane and remains unchanged. Another restriction is that specific peripheral proteins are bound to only one side.

## Asymmetry of the Membrane

In contrast to the random distribution of lipids between the outer and inner lipid monolayer of liposomes, there is an asymmetric distribution of lipid components across biological membranes.

Each layer of the bilayer has a different composition with respect to individual phosphoglycerides and sphingolipids.

Sphingomyelin and phosphatidylcholine are predominantly in the outer layer, whereas phosphatidylethanolamine, phosphatidylserine and

phosphoinositides are predominantly in the inner lipid layer. In contrast, cholesterol is equally distributed on both sides of the membrane.

## Membrane fluidity

The interaction among the different lipids and between lipids and proteins are complex and dynamic. There is a fluidity in the lipid portion of the membrane in which both the lipids and proteins move. The degree of fluidity is dependent on the temperature and the composition of the membane. At low temperature the lipids are in a gel-crystalline state, with the lipids restricted in their mobility. As the temperature is increased, there is a phase transition into a liquid-crystalline state, with an increase in fluidity. With liposomes prepared from a single pure phospholipid, the phase transition temperature, Tm, is rather precise; but with liposomes prepared from a mixture of lipids, the Tm becomes less precise because individual clusters of lipids may be in either the gel-crystalline or the liquid-crystalline state. The Tm is not precise for biological membranes because of their heterogeneous chemical composition. Interactions between specific lipids and between lipids and proteins also lead to variations in the gel-liquid state throughout the membrane and to differences in the fluidity of different areas of the membrane.

The specific composition of the individual biological membranes leads to differences in fluidity. Phosphoglycerides containing short-chain fatty acids will increase the fluidity as does an increase in unsaturation of the fatty acyl groups. The cis double bond in an unsaturated fatty acid of phospholipid leads to a kink in the hydrocarbon chain, preventing the tight packing of the chains, and creates pockets in tbe hydrophobic areas. It is assumed that these spaces, which will also be mobile due to the mobility of the hydrocarbon chains, are filled with water molecules and small ions. Cholesterol with its flat stiff ring structure reduces the coiling of the fatty acid chains and decreases fluidity. Ca2+ directly decreases the fluidity of a number of membranes because of its interaction with the negatively charged phospholipids, which, in turn, reduces repulsion between the polar groups and increases the packing of lipid molecules.

Ca2+ causes aggregation of lipids into clusters, which also reduces membrane fluidity. Fluidity at different levels within the membrane also varies. The hydrocarbon chains of the lipids have a motion, which produces a fluidity in the hydrophobic core. The central area of the bilayer is occupied by the ends of the hydrocarbon chains and is more fluid than the areas closer to the two surfaces, where there are more constraints due to the stiffer portions of the hydrocarbon chains. Cholesterol makes the membrane more rigid toward the periphery because it does not reach into the central core of the membrane.

Individual lipids and proteins can move rapidly in a lateral motion along the surface of the membrane. However, electrostatic interactions of polar head groups, hydrophobic interactions of cholesterol with selected phospholipids or glycolipids, and protein-lipid interactions all lead to constraints on the movement. Thus there may be lipid domains in which lipids move together, such as islands floating in a sea of lipids. Integral membrane proteins also move in the lipid environment, even if their movements are slower than that of lipids.

There is very little transverse motion of proteins in membranes. Movement of membrane proteins may be restricted by other membrane proteins, matrix proteins, or cellular structural elements such as microtubules or microfilaments to which they may be attached.

Evidence is accumulating that the fluidity of cellular membranes can change in response to changes in diet or physiological state. Their content of fatty acid and cholesterol is modified by a variety of factors. In addition, pbarmacological agents may have a direct effect on membrane fluidity (e.g. anaesthetics).

Thus cellular membranes are in a constantly changing state, with not only movement of pro-

teins and lipids laterally on the membrane but with molecules moving into and out of the membrane. A variety of forces, including hydrophobic and electrostatic interactions are involved in maintaining the basic structural characteristics.

## Stratum corneum lipids as bound-water modulators and their importance for water uptake

In the skin of all vertebrates a membrane is present to function as a homeostatic barrier which regulates water diffusion throughout the skin itself; this membrane is constituted by the horny layer of which the main components - proteins, lipids, water - are assembled in a very ordered structure.
Lipids derive from desquamated keratinocytes and from the sebaceous secretion.
In the horny layer the intercellular spaces include a super imposed lamellar structure, derived by exocytosis from granules localized in the lower granular layer.
The basic unit of lamellar structures that are found in the end-phase of keratinocytes differentiation consists of a lipid bilayer similar to that found in myelin and rods of the retina. In common with other biological membranes the lipid distribution shows asymmetry; however a characteristic feature is that this asymmetry is not due to phospholipids but to glucosylceramides and ceramides. Altogether these compounds are prevalently localized in the external leaflet. The less polar species have an unusual structure in which the fatty acid linked to sphingosine is represented by a long chain alpha-hydroxyacid esterified in turn mainly with linoleic acid. In some glycosylceramides this last one esterifies the glucose moiety in the 3' position.
Thus glycolipids have the function of a "reservoir" of essential fatty acids normally accomplished in biological membranes by phospholipids. These last molecules are present only in the living cells of the basal and spinous layer and are rapidly degraded when keratinogenesis starts.
The hydrolyzed fatty acids from phospholipids contribute to the pool of free acyls present in the horny layer together with those derived from sebaceous secretion; the sphingosine moiety is recycled for the synthesis of glucosylceramides or ceramides.
The remainder of the lipid component of the horny layer is represented by free cholesterol (20%), and free fatty acids.
Lamellar granules, in which the bilayer is organized in a disk-shape configuration, assemble to give the final structure of the layer under the influence of: 1- acylglucosylceramides which during the "dispersion" of lamellar disks undergo a deglycosilation process; 2- lysophospholipids formation, catalyzed by a Ca-dependent phospholipase that acts on phospholipids of the granular layer; 3- the parallel increase of free fatty acids in the area between the granular and horny layers.
A role which is not only structural but also functional is played by the cholesterol esters localized in the stratum corneum; the exfoliation of horny layer cells, which constitutes the last stage of epidermal differentiation, is accomplished by the hydrolysis of cholesterylsulfate.
In conclusion, stratum corneum lipids that form the lamellar structure serve as a water modulator. This water-holding function of the stratum corneum is thought to be completely distinct from the water permeability barrier, although the same intercellular lipids play a crucial role and pathological (delipidated) skin generally suffers a defect in both functions. Recent data from the literature suggest that although ceramides are the main determinant of both functions, ceramides with relatively short, non-branched, and saturated alkyl chain lengths are mainly associated with the water-holding function, whereas acylceramides with linoleic acid or ceramides with long alkyl chain lengths serve as a permeability barrier.

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J. Appl. Cosmetol. **10**, 91-99 (October-December 1992)

# ADVANCES IN PERCUTANEOUS ABSORPTION

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***Received:*** *October 30, 1991. Presented at the IV International Congress on Cosmetic Dermatology "Progress in Cosmetic Dermatology: Science and Safety" Roma (Italy) October 31 - November 2, 1991.*

***Key words:*** *Percutaneous Absorption, Skin Penetration; Iontophoresis; Skin Metabolism.*

## Synopsis

The follicular route plays a major role in the early permeation phase. Later on topically applied material held in the follicle provides an additional reservoir. Both factors are of uppermost importance for the short contact (minutes) therapy which seems to be applicable to a broad range of substances. On the other hand, it is still a matter of debate whether the follicular route is a preferential pathway for penetration in the late phase. Recent results have improved our knowledge about the influence of age, body region, massage, washing, UV irradiation and diseases on percutaneous absorption. Iontophoresis and ultrasound enhance the permeation of topical agents, the former not only by direct action on topically applied charged compounds incl. drugs and peptides, but also by electroosmosis of endogenous electrolytes. The usefulness of artificial membranes, or of the skin of the marmoset and the hairless guinea pig, or of shed snake skin as models for tbe permeability of human skin awaits further confirmation. Skin metabolism can be used for design of drug precursors, but it has also to be taken into account for toxification and detoxification. In this respect, it is important to note that chemical agents are metabolized differently by the skin compared to other organs and the sex differences in skin metabolism and bioavailability after topical application can be observed.

## Riassunto

La via follicolare gioca un ruolo fondamentale nella prima fase della permeazione delle sostanze applicate topicamente. In una fase successiva il materiale che si lega al follicolo giuoca un ruolo di riserva. Entrambi questi fattori risultano molto importanti per le terapie rapide di contatto (minuti) adatte per l'uso di molti principi attivi. D'altra parte è ancora materia di discussione se la via follicolare possa essere considerata quale via preferenziale di penetrazione in una fase successiva. Recenti risultati hanno migliorato la nostra conoscenza sull'influenza esercitata sull'assorbimento percutaneo dall'età, dalla regione del corpo, dal massaggio, dal lavaggio, dall'irradiazione con UV e dagli stati patologici. Sia la ionoforesi che gli ultrasuoni facilitano la penetrazione di agenti topici: il primo soprattutto non soltanto per l'azione diretta esercitata sulla carica elettrica dei principi attivi quali farmaci e peptidi, ma anche per effetto di fenomeni elettrosmotici ed elettrolitici. L'utilità dell'uso delle membrane artificiali, o della pelle dell'ùstiti e della cavia senza pelo, o del serpente come modello per simulare l'assorbimento percutaneo della cute umana deve ancora ottenere ulteriori conferme. Il metabolismo della pelle può essere utilizzato per studiare precursori di farmaci, ma può anche servire per studi di tossicità e detossicazione. A tal riguardo è importante

ricordare che gli agenti chimici sono metabolizzati in modo diverso dalla pelle rispetto ad altri organi. Inoltre sono da sottolineare anche le differenze sessuali e la diversa biodisponibilità dei diversi prodotti applicati per via topica.

# Introduction

Recent research and new developments have increased insight into skin permeability, the structure and location of the diffusion barriers against hydrophilic and lipophilic compounds and percutaneous absorption for systemic treatment. Two workshops sponsored by the FDA and other organisations tried to summarize current knowledge in the light of regulatory requirements and to initiate further research to improve and standardize the various methods currently used. The first was focused on percutaneous penetration in vitro (Skelly et al. 1987), the second on absorption in vivo (Shah et al. 1991). This article will concentrate on some of these matters. For more details the reader is referred to recent reviews (Brandau & Lippold, 1982: Bronaugh & Maibach, 1985: Schaefer et al. 1982: Schalla & Schaefer, 1987: Scheuplein, 1978: Scott & Dugard 1990: Shroot & Schaefer, 1987).

## *Follicular Route of Penetration and Short Contact (Minute) Therapy*

Some 12 years ago, we compared normal and diseased skin and found in a number of studies: that the entry of drugs is not only increased, but also faster in skin with disturbed barrier function (review in Schaefer et al. 1982). Washing off the excess should therefore reduce the drug concentration in the viable layers of diseased (parakeratotic) skin relatively more than in the surrounding uninvolved skin. Indeed, we were able to observe a pronounced drop of anthralin concentration in normal human skin compared to skin from whicb the horny layer has been removed by tape stripping (fig. 1) leading to only a slightly weaker antipsoriatic efficacy when excess of the drug was washed off as early as 10 min. after application (Schaefer at al. 1980; Schalla, 1981, 1989).

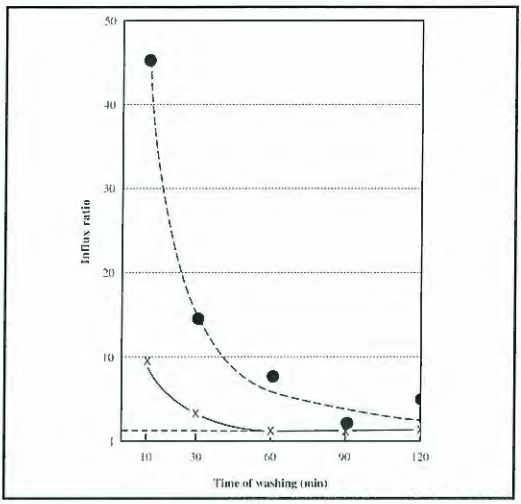


***Fig. 1** - Ratio of the skin influx, non-influenced, to that influenced by washing off the excess drug at different times with intact (- - -) and damaged (——) barrier function, of 0.1% tritium-labelled anthralin: total penetration period always 1000 min: in vitro (Schalla et al: 1981)*

Others confirmed our clinical results, and it was shown that the application period could be further reduced to only 4 min. These very short applications could not anymore be explained on the base of differences in skin permeability between involved and uninvolved skin. In addition, the reduction of side effects on the surrounding uninvolved psoriatic skin was not reduced to the extent we had expected. Therefore additional phenomena had to play a role.

One candidate was the follicular route of penetration. Scheuplein (1978) concluded from his results using a series of alcohols that this pathway plays a predominant role in the early penetration phase which is then taken over by interfollicular "bulk" diffusion into the viable skin. Using histoautoradiography we could already find huge amounts of various dermatological topicals within the follicular channel some minutes after application. Whereas these findings confirmed the results of Scheuplein we were unable to observe a faster clearance of the follicular input compartment, and only a small accumulation of drug could be found in the perifollicular dermis 4 days after application in vitro, with none in vivo (fig. 2).

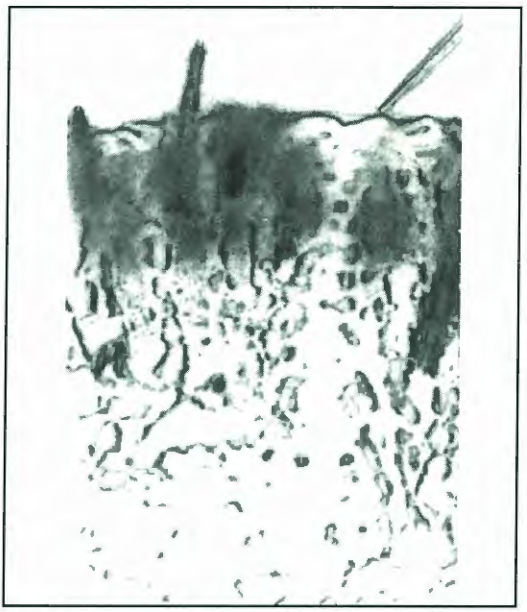

*Fig. 2 - Histoautoradiography 96 h after a single application of 1% anthralin to intact skin (the horny layer was removed just before sectioning the biopsy to allow longer exposure of the photoemulsion for better visualization of the follicular and dermal content)*

The reason could be either the insensitivity of the histoautoradiographic techniques which is at the most semiquantitative or that the barrier function of the follicular epithelium is comparable with that of the interfollicular epidermis, i.e. the follicular channel serves more as an additional reservoir for the topicals applied than as a preferential route of penetration - at least for the more lipophilic drugs most often used in dermatology.

Interestingly, Behl et al. (1985) found a lower permeability coefficient for hydrocortisone in the hairless mouse in their very first days of life than in adult mice, followed by an increased permeability in young animals. These changes coincided with the development of hair follicles from the 5th day of life.

Illel (1989, 1991) removed the follicular and horny layer content by repeatedly cementing cyanoacrylate to the skin surface and tearing it off after polymerisation. By stripping another part of the same skin specimen with adhesive tape which removes the horny layer, but not the follicular content, she was able to calculate the amount of a drug in the follicular channel. The results obtained after different penetration periods are shown in fig. 3.

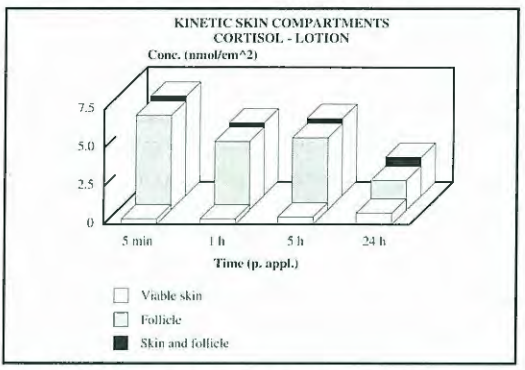


*Fig. 3 - Amount of drug 5 min, 1 h, 5 h and 24 h after topical application of 1% 3H-hydrocortisone in acetone calculated by the radioactivity found in the viable skin layers vs the marker content within the follicular channel vs the total (Illel, 1989)*

By far the highest concentration of the marker molecule $^{3}$H-hydrocortisone was found within the follicular channel 5 min after application, giving a 35-fold difference in drug concentration between the follicle and the viable skin layer. The follicular concentration is more than 5-fold higher at 24h, serving still as the driving force for a net diffusion.

The total follicular surface is smaller that the total interfollicular skin area (rough estimates assume 1% (up to 10%)) thereby restricting the importance of that penetration pathway. Nevertheless, by increasing the driving force, e.g. by increasing the concentration of anthralin in its vehicle antipsoriatic efficacy could be achieved which was comparable to conventional therapy by washing off the interfollicular drug after a few minutes.

## Environment

**Clothes** may produce uneven absorption due to both 'rubbing-effects' and skin temperature changes and sweating. Intentional massage did not, however, augment percutaneous absorption of corti-

sol, neither in the guinea pig nor in the rhesus monkey (MacMaster et al. 1985). We found a marked increase in one series of experiments using young hairless rats, but could not confirm these results in a second set (Lamaud & Schalla, unpublished). The discrepancy is probably explained by the fact that the 'hairless' rats used in the first series were in their phase of hair growth, early in life.

**Washing** after prolonged application may have the opposite effect. A washing in effect could be observed by washes with detergents and solvents 24 h after application of hydrocortisone or a herbicide (Wester & Maibach, 1985).

Soaps and detergents increased the transepidermal water loss (TEWL) under exaggerated experimental conditions. This increase seems to be related to irritation and/or the rough dry aspect induced by various soaps and detergents (Frosch & Kligman, 1979; Leveque, 1989). Repeated daily washings progressively disturb the permeability barrier measured by TEWL (fig. 4) and by urinary excretion of 14C-malathion (Marty, 1985).

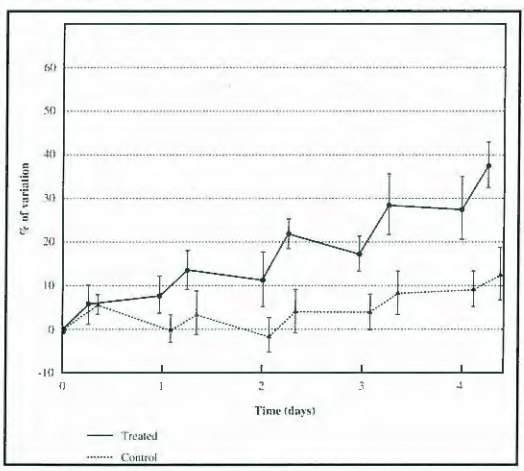


*Fig. 4 - Transepidermal water loss (TEWL) in vivo during chronic exposure of the forearm to soap (one forearm was washed 4x/day for 5 days ) (de Rigal & Leveque presented in Leveque, 1990).*

**Acute UV exposure** induces a sharp dose-dependent increase in skin permeability starting 2 days after irradiation (Fig. 5).

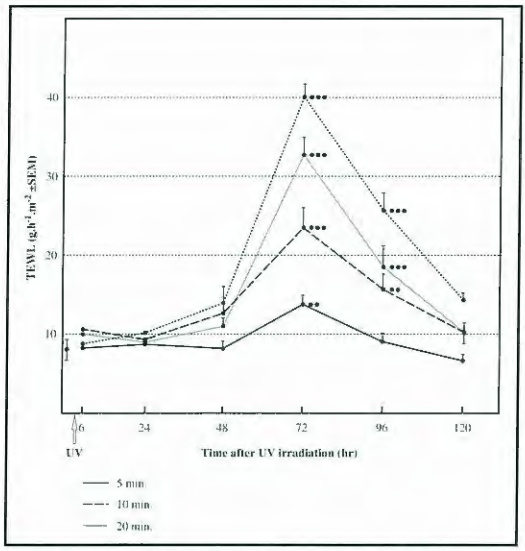


*Fig. 5 - Transepidermal water loss (TEWL) (x- ± S.E.M.) through hairless rat skin following irradiation by different UV doses (Lamaud & Schalla, 1984)*

There was a linear relationship between the logarithm of the UV dose and the TEWL (r=0.929) (Lamaud & Schalla, 1984). Nevertheless, the horny layer does not completely loose its barrier function by a moderate sunburn. The TEWL (as a measure of hydrophilic compounds) was increased from 5 ± 1g*m$^{-2}$*h$^{-1}$ to 33 ± 6 g*m$^{-2}$*h$^{-1}$ in UV - irradiated hairless rats, but removal of the horny layer by stripping produced an increase to 89 ± 5 g*m$^{-2}$*h$^{-1}$. That this also holds true for percutaneous absorption of more lipophilic compounds such as cortisol (log P= 1.5 - 1.6) could be shown by the urinary excretion after topical application of the $^{3}$H - labelled drug (fig. 6).

## Age and Body Region

The permeability of infant skin is well-known to be higher not only in preterm babies, but also to a lesser extent during the first years of life, indicating a maturation process of the barrier. The systemic bioavailability after topical application is further augmented in infancy by the higher skin surface to body weight ratio.

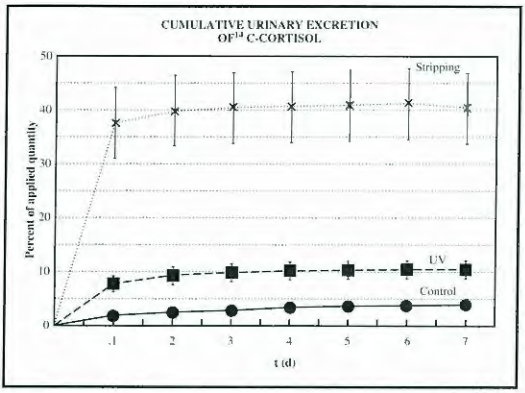


*Fig. 6 - Urinary excretion of drug and its metabolites following topical application of a 1% 3H-hydrocortisone lotion to intact, stripped and UV-irradiated hairless rat skin (Lambrey & Schalla, unpublished).*

In fact, these features are combined in particular in preterm babies leading to a considerably higher risk of toxicity via percutaneous absorption. Initiated by a demand from pediatricians some years ago we calculated whether, on the other hand, this increase would be sufficient to envisage percutaneous nutrition of premature babies. The intention was to avoid the high risk of thrombo(phlebi-)tic complications by intravenous feeding with fat emulsions. Based on a number of in vitro experiments with neutral lipids and essential fatty acids using preterm skin of different gestational age, we concluded that the caloric intake by the topical route was far below the needs of premature babies.

We also showed that the caloric intake via the topical route does not play any role in adult skin, i.e. adults cannot become obese by normal or even excessive use of cosmetics.

In the elderly, investigations are sparse and the results somewhat contradictory. The topical bioavailability may be increased because of delayed clearance from the skin. The extent to which penetration is augmented by age, and whether this is indeed due to age rather than sun damage remains unclear.

It is well-known that percutaneous absorption varies according to the body region. Rougier et al (1988) suggested that such differences may be related to the corneocyte size. The larger the corneocyte surface area, the lower was the permeability for $^{14}$C-benzoic acid. This makes sense since the distance for a molecule to pass through the horny layer by the intercellular pathway will be greater for the larger corneocytes. Nevertheless these results have to be confirmed by others and should be extended to other body regions.

## Skin Diseases

Dermatoses may roughly be divided into hyperkeratotic, orthokeratotic and parakeratotic disorders. The latter are associated with increased skin permeability as shown for psoriasis and eczema in many studies. In hyperkeratotic conditions, changes in percutaneous absorption depend upon the nature of the disturbances. Details are found in the reviews cited in the introduction.

## Iontophoresis

Iontophoresis and ultrasound enhance the permeation of topical agents, the former not only by direct action on topically applied charged compounds incl. drugs and peptides, but also by electro-osmosis of endogenous electrolytes.
Recent experimental and theoretical approaches (review in Pratzel, 1987) have underlined that iontophoresis induces a facilitated diffusion through the main barrier, i.e. charged and polar molecules are directed by the electric field along the shortest path through the horny layer instead of net diffusion provoked by random migration within a concentration gradient.

The iontophoretic current were found to be primarily through the appendages and certain appendages (e.g. small hairs) appeared to carry most of it (Cullander & Guy, 1991), i.e. the follicular route seems to be even more important

under these conditions than for the normal diffusion pathways discussed earlier.

The number of small, very mobile electrolytes in the viable skin layers is much more than the minute amount coming out of the barrier. Therefore, iontophoresis does not continue to play a major role in these deeper compartments.

## Animal and membrane models for studying percutaneous absorption

The pig (and probably also the mini and micro pig) remains the animal species of choice. Most data for skin permeability are quite comparable to those for man, but recent results indicate some differences (Scott & Dugard, 1990). The Rhesus monkey is somewhat inferior to pig. The hairless rat seems to be the best rodent species already giving higher values relative to man. The value of marmoset skin and shed snake skin as well as of skin of newer varieties of species such as the hairless guinea pig and the hairless Mexican dog remains to be established, but first results are promising. All other species (hairless mouse, all hairy rodents, including guinea pigs and rabbits) are rather poor predictors of percutaneous absorption of topicals and of the effect of penetration enhancers in man.

The usefulness of artificial membranes and, more interestingly, of biological skin substitutes by modern cell culture techniques awaits further confirmation. The same holds true for newer mathematical models.

## Skin Metabolism

Skin metabolism can be used for design of drug precursors, but has also been taken into account for toxification and detoxification (review in Bouclier & Shroot, 1987; Kappus, 1989; Shroot & Schaefer, 1987).

It is important to note that chemical agents are metabolized differently by the skin than by other organs and that sex differences in skin metabolism and bioavailability after topical application can be observed.

Uprooted human scalp hairs - a technique used in clinical dermatology for hair analysis - have been grown in culture and found to be excellent for studying skin metabolism linked to carcinogenesis (Merk et al. 1987). They also proved to be applicable, to other metabolic pathways. The use of cell cultures has also become increasingly popular. The relevance of these models to human skin in vivo has yet to be established.

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J. Appl. Cosmetol. **10**, 101-109 (October-December 1992)

# ACTIVITY OF VEHICLES AND DIFFUSION THROUGH THE HORNY LAYER

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***Received:*** *October 30, 1991. Presented at the IV International Congress on Cosmetic Dermatology "Progress in Cosmetic Dermatology: Science ond Safety" Roma (Italy) October 31 - November 2, 1991.*

***Key words:*** *Horny Layer; Skin Permeability; Skin Penetration Enhancers; Percutaneous Absorption; Vehicles and Carriers; Liposomes, Niosomes; Transdermal Delivery; Penetration Blockers.*

## Synopsis

The interest in transdermal delivery for extracutaneous treatments has stimulated a better understanding of percutaneous absorption, mainly in trying to increase skin permeability and controlling the release of drugs or their precursors.
Permeation can be increased by various types of enhancers such as fatty alcohols and acids, their esters, Azone, monoglycerides, pyrrolidone derivatives, cyclic monoterpenes, methyl sulfoxides and urea. There is evidence that they act by different modes of action: disruption of the intercellular lipid structures, altering their composition or the cohesiveness between cells, effects on the cell membrane or on intracellular components. Enhancement by these agents does not correlate with their irritancy, and it is not identical between man and a number of animal species.
Additional vehicle effects are caused by modifying the solubility and partitioning of the active compounds. Liposomes and niosomes are just examples. The ratio between the interfollicular and the follicular penetration pathways can also be altered. Solutions seem to favour the former, particles the latter.
On the other hand, there is also an increasing need for penetration blockers in view of the risk of toxic and allergic reactions to the chemicals used in our private and occupational life.
Whether for enhancement, or blocking of penetration, it has to be taken into consideration that cosmetic and dermatological topical applications act differently on diseased skin from normal skin.

## Riassunto

L'incrementato interesse per i trattamenti topici ha provocato una migliore conoscenza sull'assorbimento percutaneo. Si è cercato così di incrementare la permeabilità cutanea controllando meglio la cessione sia dei farmaci che dei loro precursori.
il grado di permeazione può essere incrementato da vari tipi di acceleranti l'assorbimento quali gli alcooli e gli acidi grassi, i loro esteri, l'azone, i monogliceridi, i derivati del pirrolidone, i monoterpeni ciclici, i metil sulfossidi e l'urea. È stato dimostrato che queste sostanze agiscono con diversi meccanismi di azione: scompaginamento delle sostanze lipidiche intercellulari, alterazione della lo-

ro composizione o della coesione delle cellule, effetti sulle membrane cellulari e sui componenti intracellulari. L'accelerazione nel grado di assorbimento di questi agenti non è correlato con il loro potere irritativo e non è uguale tra l'uomo e le diverse specie animali. Inoltre, modificando il grado di solubilità e il coefficiente di ripartizione dei composti attivi si possono ottenere dai veicoli ulteriori effetti. Ne sono alcuni esempi i liposomi ed i niosomi.

Può anche essere alterato il rapporto tra penetrazione interfollicolare e penetrazione follicolare. Le soluzioni sembrano favorire la prima via di penetrazione mentre le particelle (emulsioni) favoriscono la seconda.

D'altra parte si sente anche la necessità di incrementare lo studio sulle sostanze che riducono o impediscono l'assorbimento percutaneo soprattutto ridurre il rischio di reazioni tossiche o allergiche provocate da inquinanti ambientali con cui si viene in contatto durante la vita di relazione o nell'ambiente di lavoro.

Nell'usare accelleranti o bloccanti l'assorbimento bisogna tener anche conto che la cute sana e la cute patologica reagiscono in modo diverso all'applicazione topica di farmaci dermatologici o di cosmetici.

# Introduction

This paper will focus on some aspects of the horny layer in relation to its barrier function and modifications induced by vehicles including additives such as penetration enhancers. The following reviews give more details: Brandau & Lippold, 1981: Bronaugh & Maibach, 1985: Elias, 1983; Landmann, 1988; Maltoltsy, 1986; Schaefer et al. 1982; Schalla, 1989; Schalla & Schaefer, 1987; Scheuplein, 1978: Scott & Dugard 1989; Shroot & Schaefer, 1987.

The reader should bear in mind that vehicles also influence other features of the horny layer, such as smoothness, appearance, subjective feelings etc., which are not within the scope of this article.

## *Composition of the Horny Layer*

It is generally agreed that the horny layer is the main barrier against diffusion. This layer is composed of cells which die during their terminal differentiation, with consequent reduction of the water content which in turn leads to a tightly packed structure of filaments and matrix resistant to many liquids and solvents. This cellular content is encased in an even more resistant cornified envelope. The intercellular space between these flattened cells is composed of lamellar structures formed by multiple bilayers of the hydrophobic and lipophilic regions of fatty acid esters, ceramides, cholesterol sulphate and others (reviewed by Elias, 1983; Elias et al. 1987; Landmann, 1988; Matoltsy , 1986). The lamellae tightly pack the structures and completely fill the intercellular space in the innermost horny layer, whereas they form lacunae in between the now non interrupted lamellae in the mid-portion and outer stratum corneum (Hou et al. 1991).

There is an inverse linear correlation between the horny layer thickness and the transepidermal water loss (TEWL) as a simple measure of overall skin permeability for hydrophilic compounds (Leveque, 1989; fig. 1).

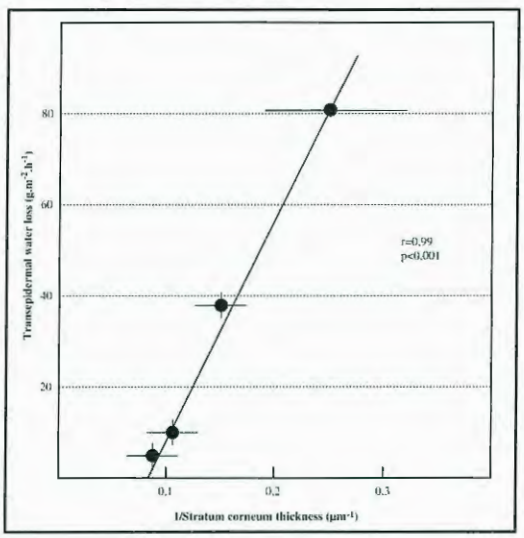


*Fig. 1 - inverse linear correlation between transepidermal water loss and horny layer thickness (Leveque, 1989)*

The horny layer thickness in vitro and in vivo depends on the relative humidity (RH). Up to 85% RH, the thickness is increased to 125% relative to 0% RH. A steep increase was calculated for RH > 98% leading to 65µm thickness at 100% RH compared to 20 µm in vivo at 0% RH (Blank, 1984).

Most of the fascinating contributions in the last two decades have focussed on the essential role of the intercellular domain for percutaneous absorption, i.e. the intercellular pathway through the horny layer. Nonetheless, other routes such as the transcellular pathway should not be ruled out. The latter route is much shorter. 'Vertical' gates in the direction of the viable epidermal layers can hardly be seen between neighboured corneocytes in fig. 2 - not surprisingly, since the mean diameter of these hexagonal cells is in the range of 30 µm whereas their thickness is less than 1 µm. The distance for a molecule to diffuse by the intercel-

lular pathway is therefore extremely long relative to the transcellular route.

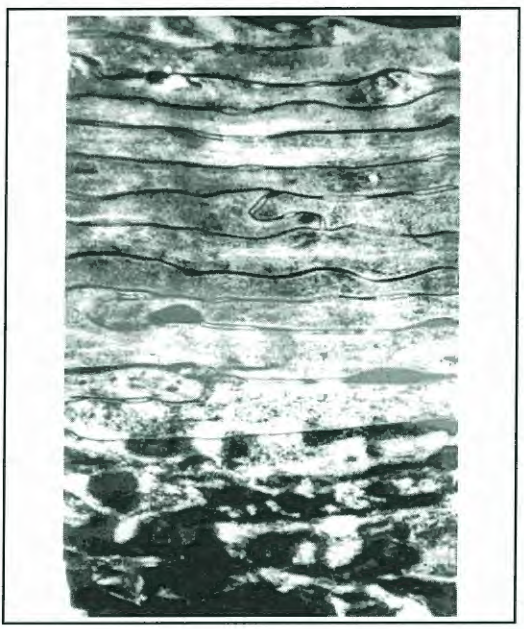

*Fig. 2 - Packing of corneocytes.*
*Note that just a few cell membranes can be seen in the vertical direction in this field of view trasmission electron microscopy) indicating that the intercellular pathway is very much longer than the transcellular route, contradictory to what is suggested in most of the schemes used for comparing the penetration pathways.*

Moreover, detergent-induced cell dissociation and desquamation can be stimulated by EDTA and inhibited by aprotinin, a proteinase inhibitor, underlining the importance of proteinaceous structures for the morphological changes observed during terminal differentiation within this barrier layer (Egelrud & Lundstrom, 1990). The corneocyte attachment was found to be correlated with the number and distribution of corneosomes (modified desmosomes in the horny layer). The inner stratum compactum was tightly packed and corneosomes were numerous, whereas cohesion were mainly peripheral in the stratum disjunction. In this superficial part of the horny layer, corneosomes were restricted to corneocyte edges (Chapman & Walsh, 1990). On the other band, extracellular lipids derived from membrane-coating granules seem to play a significant anti-cohesive role preventing apposition of corneocytes, indicating that lipid envelopes are unlikely to mediate cohesion in normal stratum corneum (Chapman et al. 1991).

The follicular route was considered to be important only in the early phase of penetration, whereas the major part of any given compound permeates later on via the interfollicular epidermis. Based on a few experiments this phenomenon was explained by a reduced barrier of the follicular epithelium on the one hand and the approximately 100 fold larger surface area of the interfollicular epidermis on the other hand. This point of view needs to be modified in the light of recent results (see the article "Advances in Percutaneous Absorption" in this monograph). As Brian Barry (1987) pointed out: "We can postulate that for most penetrants of intermediate polarity, a variety of routes will come into play dependent on skin condition and diffusional time, but for simplicity investigators often consider only the predominant route for any particular drug. However it is important not to concentrate solely on one theoretical pathway and to ignore other possibilities".

The interest in transdermal delivery for extracutaneous treatments has further stimulated the ef-forts to better understand percutaneous absorption, mainly in trying to increase skin permeability and controlling the release of drugs or prodrugs.

## Penetration Enhancers

Permeation can be increased by various types of enhancers such as fatty alcohols and acids, their esters, in particular monoglycerides, Azone, pyrrolidone derivatives, cyclic monoterpenes, methyl sulfoxides, salicylic acid and urea. There is evidence that their modes of action differ.

Disruption of the intercellular lipid structures seems to be one of the most common mechanism for enhancers, including Azone, oleic acid and dimethyl sulphoxide. The enhancers could be put into three main categories: those which promoted permeation through both polar and lipid routes of the intercellular space, accelerants which preferentially affected the lipid route, and enhancers which mainly modified polar pathways (fig. 3) (Barry, 1987).

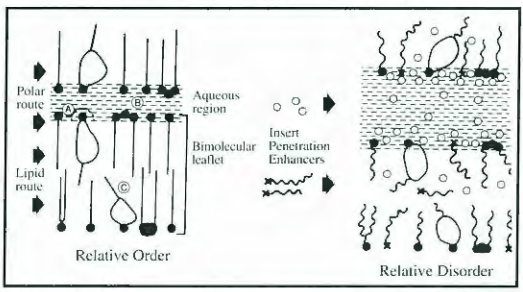


*Fig. 3 - A composite diagram representing postulated sites for penetration enhancers to act in the intercellular domain. The figure illustrates the change from relative order to relative disorder after the insertion of accelerants. Small circles represent polar accelerants such as the pyrrolidones, propylene glycol, ethanol and aprotic solvents: linear chain molecules represent Azone and bent molecules correspond to cis-unsaturated long-chain compounds such as oleic acid and oleyl alcohol (Barry, 1987).*

Other modes of action of penetration enhancers involve altering the composition of the intercellular space and/or the cohesiveness between cells as well as effects on the cell membrane or intracellular components. The enhancement by these agents does not correlate with their irritancy, and it is not identical between man and a number of animal species.

## Vehicles as Carriers of Active Ingredients

Other components of vehicles do not interact as much as the penetration enhancers with horny layer structures. Rather, they modify the solubility and partitioning of active compounds in a given vehicle and thereby the permeation.

Liposomes and niosomes are newer developments in this direction. Such bilayered and multilayered vesicles to some extent mimick the intercellular lamellar structure in the stratum corneum and can serve as carriers for active ingredients. In this respect, they probably act more as an additional reservoir rather than as a penetration enhancer. Results suggesting that liposome entrapped molecules have increased skin permeability have hampered by that fact that the vehicles used were far from optimal for the given agents. In other words: it is easy to improve skin permeability by every method if a poorly releasing vehicle is used as reference. We could for example not enhance the penetration of hydrocortisone by incorporating it into niosomes when we used for comparison a vehicle which we had found to release the drug very well to the skin.

The ratio between the interfollicular and the follicular penetration pathway can also be altered. Solvents seem to favour the former, penetration as particles, washing off the remaining excess of a topical from the skin surface and probably also entrapping into liposomes or niosomes, the latter.

## Reduction of Penetration

The most common approach used for reducing the activity is dilution of the active agent in the preparation, e.g. by mixing a drug containing topical with its vehicle without any drug. However, it was observed that some such diluted preparations were as active as the undiluted ones (Gibson et al. 1984). One reason for this phenomenon is that quite often suspensions are used containing solubilized as well as unsolubilized drug. Only the former can immediately be liberated to the skin. Crystals have first to be dissolved within the vehicle or at least within the stratum disjunctum of the horny layer before they can penetrate into the skin. Dilution if such su-

spensions diminished the unsolubilized portion, but not the solubilized concentration because crystals will again dissolve up to drug saturation in the given vehicle.

Another point to be stressed in respect to decreased penetration is the following:

Reduction of the active agent at the target site is not automatically followed by a decreased activity, as holds true for the opposite, i.e. enhanced penetration may not always improve the efficacy of cosmetic, dermatological or transdermal treatment. It has to be born in mind that the dose response relationship is usually saturable and that there is a maximal response which cannot be further augmented by increasing the concentration. The need for use of penetration enhancers (or reducers) has therefore to be checked individually.

There is also an increasing need for broad penetration blockers in view of the risk of toxic and allergic reactions from the chemicals used in our domestic and occupational lives. The barrier creams, including topicals containing ion exchangers, silicone, tanning agents etc., are at present far from being ideal for most of their practical uses. Therefore, the best protection still remains the avoidance of direct contact with such chemicals by instrumental aids or by wearing gloves.

## Altered Horny Layer Structures

The stratum corneum is modified by environmental factors, skin diseases and their treatment and so on. Acute sunburn is followed by a sharp, short-lasting increase in TEWL (see "Advances in Percutaneous Absorption" in this monograph); chronic UV exposure induces a thickening of the horny layer. Thickening of the horny layer is not automatically accompanied by a stronger barrier function since it depends on the type: ortho-hyperkeratosis such as in callosities strengthens the barrier, pathological hyperkeratoses in most ichthyotic conditions diminish the barrier function, as in parakeratotic diseases such as psoriasis and acute and subacute eczema (fig. 4) (review in Schalla, 1987, 1989).

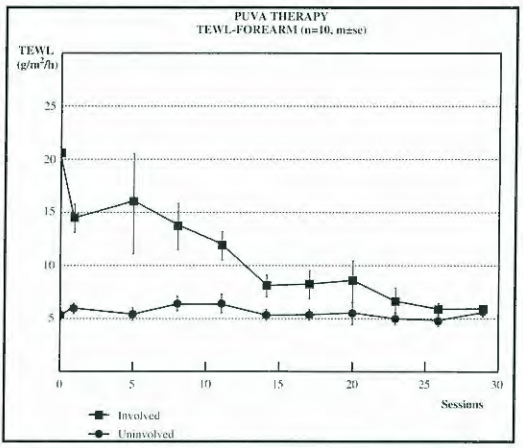


*Fig. 4 - Time course of the transepidermal water loss (TEWL) of involved and uninvolved psoriatic skin under PUVA therapy.*

Treatments may modify the horny layer in several ways:

Occlusion is followed by maximal hydration of the horny layer. As already pointed out, the thickness increases by a factor of about 2.5. The barrier function is usually reduced by occlusion as shown in a number of studies (reviewed in Brandau & Lippold, 1982; Bronaugh & Maibach, 1985; Schaefer et al. 1982). The increased skin permeability is usually accompanied by an increase in the topical as well as the systemic bioavailability. We have found as an exception of this rule that occlusion only augmented the systemic bioavailability (Schalla et al. 1987).

Bathing also induces a maximal hydration of the horny layer, but it is of shorter duration. Interestingly, the reservoir function of the horny layer seems to be reduced by bath application of active agents. Fischer (1976) compared the time courses of photosensitivity by a trioxsalen bath to those from trioxsalen painting and methoxypsoralen tablets (fig. 5).

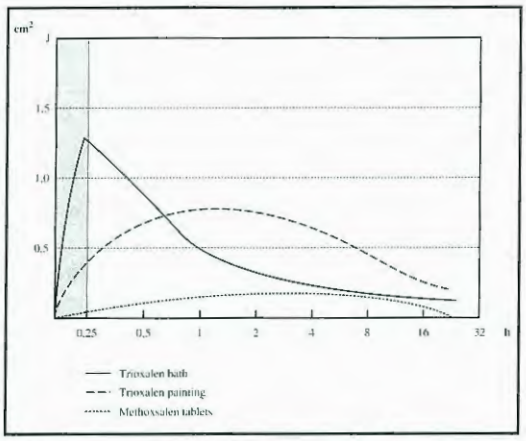


***Fig. 5** - Time course of the erythema reaction after different application procedures of psoralens. Shadowed area: Duration of bathing (Fisher, 1976)*

The photosensitivity dropped immediately after termination of a bath for 15 min, a far shorter time than that after painting whichdid not reach its maximum within the first hour. We were able to confirm this and in addition found no detectable serum levels, in contrast to painting, indicating that the kinetics after bathing are completely different from those of topical application. The kinetics are in fact superimposable on those for iontophoresis, but at a lower level (fig. 6).

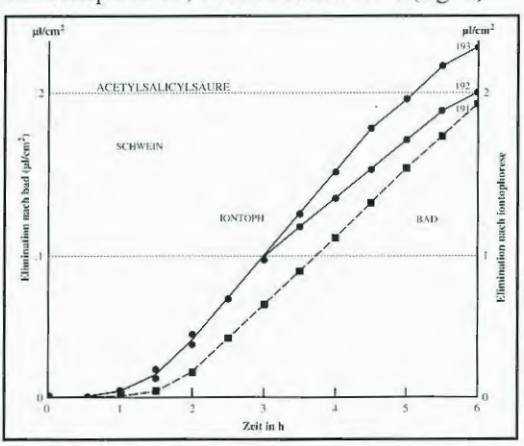


***Fig. 6** - Elimination kinetics of acetylsalicylic acid after iontophoretic and bath applications, resp. (Pratzel, 1987).*

In either of those particular applications, the horny layer reservoir is diminished and effective drug concentrations in the skin are reached earlier. If short application periods (rough estimate < 30 min) and highes drug concentrations in the same vehicle are used the topical bioavailability is often higher than that by conventional application, but the systemic bioavailability is lower. Hence, the benefit/risk ratio is better when such short applications are used.

Corticoids cause skin atrophy and thinning of the horny layer (Wendt & Frosch. 1982). One can assume that the barrier function is reduced and that this in turn may reinforce any side effects.

Retinoids also alter the structure of the horny layer. Serum levels normally reach a steady state after 1 - 2 weeks of repeated dosing to the intact horny layer. As shown in fig. 7, a plateau is, indeed, achieved within the 2nd week, but this is followed by another increase. This indicates that the barrier function is disturbed by chronic exposure to retinoids which thus after 2 weeks facilitate their own penetration.

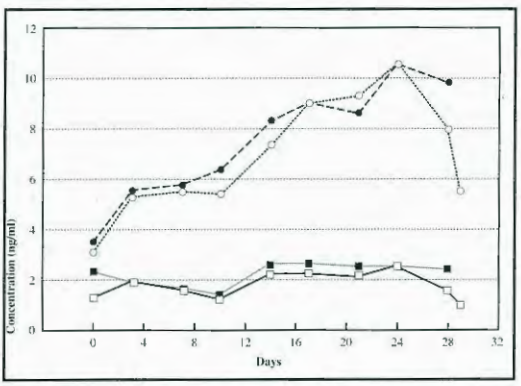


***Fig. 7** - Serum levels of a retinoid and its metabolite after once daily application for 4 weeks.*

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# Author index

# Subject index

Announcement

# 5th WORLD CONGRESS OF THE INTERNATIONAL SOCIETY OF COSMETIC DERMATOLOGY

# PROGRESS IN COSMETIC DERMATOLOGY: BASIC SCIENCE AND TECHNOLOGY

**OCTOBER 7-9, 1993**
**Sheraton New York Hotel and Towers**
**New York, New York U.S.A.**

This meeting presents the first opportunity in the USA to meet with dermatologists, cosmetic chemists, pharmacologist, physiologist, toxicologist, and other experts with an identified interest in the maintenance of normal skin as well as the therapy of unaesthetic conditions.

## Program

- Basic and Advanced science
- Component technology
- Test modalities
- Instrumentation
- Regulatory affairs
- Aging skin
- Skin nutrition & metabolism
- Dermocosmetic Therapy and Management of Special Skin Conditions
- Cosmetic crenodermatology

The meeting will further promote:
- Understanding and cooperation among the various disciplines which have special interest in protecting and preserving the skin.
- Studies concerning experimental and clinical cosmetic dermatology within institutes and hospitals.
- The highest standards for patient care through education.

## Call for abstracts

Scientific contributions will be considered for inclusion in the program based upon merit and educational interest. Abstracts must be submitted on the official Congress Abstract Reproduction Form and received before September 1, 1993.

Abstract form and Submission Guidelines are available from the 5th World Congress Secretariat Office at:
ISCD
875 Kings Highway, Suite 200
West Deptford, New Jersey 08096 USA
Tel. 609-845-1720 Fax 609-853-0411

## Registration

Advance registration is requested and will help avoid delays at the time of the Congress. Those who remit the appropriate fee with the official form prior to September 10, 1993, quality for a reduced registration fee. Please use the Advance Registration Form included in this announcement.

Advance registration will not be accepted after September 10, 1993. If you cannot pre-register at the time of the Congress. The Congress Registration Desk located at the Sheraton New York & Towers will be open daily.

Registration fees are payable in U.S. funds only, drawn on a U.S. Bank, and must be accompanied by the official registration form. Make checks payable to: ISCD.

The registration fees for members and non-members, includes admission to the scientific session with the exception of the Pre and Post Meeting Workshops; Exhibits, opening and closing ceremonies; luncheon; and all Congress publications to include final program and abstracts.

## Accomodation

Hotel rooms have been reserved at the Sheraton New York Hotel & Towers. The special, reduced room rate for the Congress is $150.00 for a single room and $170.00 for a double room plus applicable State and Local Taxes. Room reservations at the specially reduced rate will be made on a first-came; first-served basis. The ISCD room block will be held until September 15, 1993. Reservations received after this date will be accepted on a space available basis and will be subject to the rates prevailing at the time of the reservation. The special Con,gress room rates cannot be guaranteed to be available after September 15, 1993. **Rooms will be held only until 6:00 pm unless late arrival is advised and payment for the first nights accomodations is guaranteed.**

Reservations may be made directly with the hotel at the following address:
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Please note that your registration to the Congress will include a voucher worth $100 US which may be used towards accomodation expenses at the Sheraton New York Hotel & Towers during the official dates of the meeting.

## Acknowledgement of receipt of fees

Upon receipt of your completed registration form and payment of fees, the Secretariat will send you an acknowledgement which should be presented at the Advance Registration Desk at the Sheraton New York & Towers, where you will be given your badge, tickets and all Congress documents.

## Cancellation and refunds

A full refund of registration fees will be made for cancellations received befor September 10, 1993. An administrative charge of $100 US will apply to cancellations receveid between September 10 and October 1, 1993. No refunds will be made for cancellations received after october 1, 1993.

## Entry requirements

A valid passport is reqired for all visitors entering the United States. Visas are required for most nationals entering the country. Before finalizing your travel plans, please consult the US Embassy or Consular Office in your country of residence for details regarding visas and entry requirements.

## Insurance

The 5th International Meeting on Cosmetic Dermatology cannot accept liability for injures or losses sustained from accidents or other occurrences that may arise in connection with the Congress. We recommend that participants subscribe to appropriate travel and health insurance.

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New York City Offers a tremendous variety of unique and exciting activities for visitors. Theatres, museums, historic sites and an exotic array of restaurants abound. World-renowned attractions include the Statue of Liberty, Broadway, Rockfeller Center, the United Nations, Lincoln Center, the Empire State Building, and much more.

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Communications regarding housing registration, abstract submission, and request for general information should be directed to.

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Communications regarding committee activities and program participation should be directed to.

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## Important dates

| | |
|---|---|
| **July 1, 1993** | **Deadline date for receipt of abstracts** |
| **September 10, 1993** | **Advance registration deadline** |
| **September 10, 1993** | **Hotel reservation deadline** |

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# OFFICIAL ADVANCE REGISTRATION FORM

5th International Meeting of Cosmetic Dermatology
October 7-9, 1993, Sheraton New York & Towers, New York, USA

**To qualify for the reduced advance registration fee, registration must be postmarked no later than September 10, 1993.** If you cannot register by september 10, 1993 you may register at the time of the Meeting in New York (at thehigher on-site fee as detailed on this form).

Name and Mailing Address of Registration (Please Print or Type)

Name: _____

Family Name | First name | Middle initial | Degree

_____

Title | Institution/Affiliation

Address: _____

City _____ State/Province _____

Country _____ Postal code _____

Telephone number: _____ Fax number: _____

Please indicate any disability which will require special assistance:

_____

Unless the ISCD is advised prior to the meeting of any special requirements due to disability, we cannot guarantee that service will be available if requested on site.

## B. Congress advance registration fees

☐ ISCD Members $ 460 US $ _____ ☐ Non ISCD Members $ 570 US $ _____

## C. Accompanying persons registration form

Accompanying persons _____ $175.00 U.S. per registrant
Names of accompanying persons:

| Family name | First name |
|---|---|
| _____ | _____ |
| _____ | _____ |

**Total congress registration fees $ _____**

The registration fees for members and non-members, includes admission to the scientific session with the exception of the Pre and Post Meeting Workshops; exhibits; opening and closing ceremonies; luncheon; and all Congress publications to include final program, and abstracts. All registrants will receive a voucher worth $100 US for use towards accomodation expenses at the **Sheraton New York Hotel & Towers.** This voucher is non trasferable and may only be used at the Sheraton New York towards accomodation expenses over the dates of the V International Meeting on Cosmetic Dermatology.
***Note: Do not mail your registration after September 10, 1993-bring it with your payment to the Congress. Your registration will be processed on site.**

A full refund of registration fees will be made for cancellations received before September 10, 1993. An administrative charge of $100 US will apply to cancellations received between September 10 and October 1, 1993. **No refunds will be made for cancellations received after october 1, 1993.**

**Total registration fees for accompanying persons $_____**

**Registration fees include admission to the exhibits, luncheon, hospitality suite, and opening ceremonies.**
**For office use only Date _____ MOP _____ Amount _____ D# _____**

## D. Registration Summary

Congress Registration Fee $ _____ Accompanying Persons Fees $ _____

**total of all fees $ _____ US**

All registrants: To aid us in processing your registration correctly, please indicate how payment will be made. Registrant name must appear on all bank documents.

☐ Credit card (Visa or Mastercard only) Circle one: Visa Mastercard
Your name as it appears on credit card: _____

Account number: Expiration date: _____

Signature: _____

Please note: individuals are responsible for all bank charges. Check with your bank before processing payment.

☐ Payment is enclosed in US dollars by check and issued by a US correspondent bank. Enclose check make payable to: ISCD (foreign check will be returned to sender).

☐ *Payment is being processed separately by: (Name of your bank)

_____

☐ *My bank advised that payment to the Congress will be made by:
(U.S. bank name) _____

(City/State) _____

☐ Wire transfer of Funds. Transfer funds to:
ISCD
The Bank of Gloucester County
1100 Old Broadway
Woodbury, NJ 08096 USA
ABA# 031207636 Acct# 16022448-12

*Registration will not be processed until transferred funds have been received.*

- Please make a copy of this form for your records.
- Mail completed form with payment, or a copy of the trascontinental bank transfer information, to:
  ISCD
  875 Kings Highway, Suite 200
  West Deptford, New Jersey 08096 USA

To qualify for the reduced advance registration fee, registration must be postmarked no later than September 10, 1993. After this date you may register at the time of the congress in New York Cty; where the registration fees will be as follows:

ISCD Members $570 Non ISCD Members $650 US

**Chiuso in tipografia: 31 Marzo 1993**

Journal of Applied Cosmetology published quarterly by INTERNATIONAL EDIEMME, Via Innocenzo XI, 41 00165 Roma Italy. Direttore responsabile P. Morganti. Direzione, Redazione ed Amministrazione Via Innocenzo XI, 41 - 00165 Roma Italy. Stampa Grafica Flaminia Roma. Progetto grafico ed impaginazione STYLOgrafica Roma. Spedizione in abbonamento postale gruppo IV/70. Aut. del Trib. di Roma n. 3173/83 del 8-7-83

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