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TECNOLOGÍA COSMÉTICA

CONTENIDOS

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MATERIAL DE APOYO

13

Skin Anatomy and Physiology

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Summary

If plastic surgeons are to provide state-of-the-art care in the techniques of skin rejuvenation and minimally invasive treatments for aging, a thorough understanding of skin, anatomy, and physiology is required. With the changing population demographics, the plastic surgeon must also be knowledgeable about ethnic variations in skin anatomy and physiology. The changes that occur with aging skin, inherent developmental disorders, and development of skin cancers are complex. This chapter provides a practical guide to the understanding of skin and its application to clinical conditions.

Skin

The average thickness of skin is between 2 and 3 mm. The thickness of the epidermis and dermis at the sacrum is significantly greater than that of the abdominal wall, groin, lateral gluteal area, and gluteal fold areas.[10] In the face, an area of particular interest to plastic surgeons, the epidermis is relatively constant in thickness measuring approximately 150 μm. However, dermal thickness varies considerably. For example, the dermal thickness in the periorbital area is approximately 200–250 μm, whereas the dermal thickness of the lip and forehead regions is 900–1,000 μm[8] (Figure 13.1). This variability has significant consequences when skin resurfacing is performed.[8] An area of skin that measures about 6 cm^2 can contain upwards of 20 blood vessels, 650 sweat glands, 60,000 melanocytes, and thousands of nerve endings.

Other functions include excretion of sweat and piloerection for temperature regulation. Skin provides sensation, helping us determine hot from cold, pressure, injury, vibration, and light touch. The use of local anesthetics is targeted to inhibit the sodium pumps in these sensory fibers to effect afferent signals to the brain to painful stimuli. The skin can also act as an interface for the diffusion of substances into the body including gases (CO_2, O_2, and N_2 in minute amounts). Delivery of topical medications is also based on the absorption and diffusion from the skin.

Skin Embryology

Skin is derived from both ectoderm and mesoderm. The epithelial layers are formed from the ectoderm. The various skin appendages including the pilosebaceous glands, sweat glands, and hair follicles are ectodermal elements. Specialized cells including melanocytes and neural elements are derived from the neuroectoderm. The cells of the dermal layers that include the fibroblasts, mast cells, blood vessels, lymphatic channels, and the adipocytes are derivatives of the mesoderm. Macrophages, Langerhan's cells (LCs), and Merkel cells are also derived from the mesoderm

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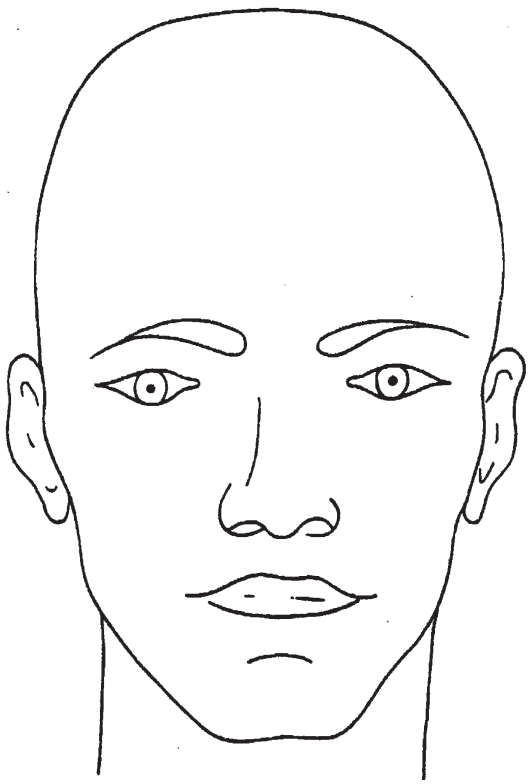

Figure 13.1. Variation of the epidermis and dermis about the face. While the epidermis is relatively uniform from location to location, the thickness of the dermis varies significantly. (Adapted from Gonzalez-Ulloa et al.[8]).

as are the components of the dermal layer. There are many inherent processes that are coordinated during embryogenesis for successful formation of the skin covering. Any alterations in these processes can lead to abnormal skin formation. Such congenital skin diseases include cutis laxa and Ehlers–Danlos syndrome.

Components of Skin

There are three distinct layers of the skin: the epidermis, the dermis, and the hypodermis (Figures 13.2 and 13.3). The epidermis is the primary defense layer against organic elements

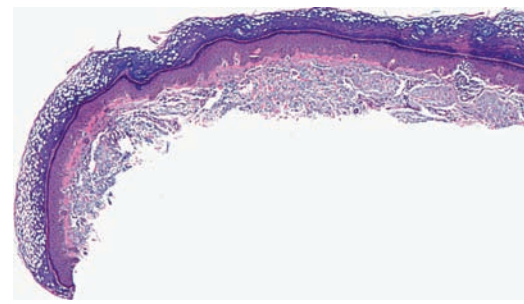

Figure 13.2. Histology of aging skin of the face. Note that solar elastosis and the loss of rete pegs are clearly visible.

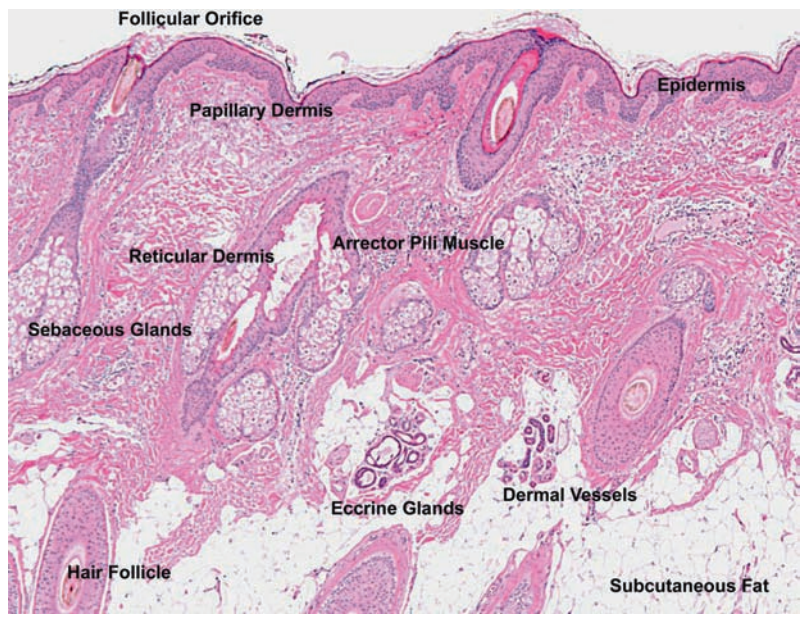

Figure 13.3. Skin histology of the scalp.

such as bacteria, viruses, parasites, and other organisms. In addition, the presence of a thick and tough stratum corneum (the keratin layer) in conjunction with the melanocytes provides protection from photodamage. The epidermal layer of skin also protects us from harsh elements in the external environment. The epithelial cells of the epidermis, also known as keratinocytes, sit on an underlying basal lamina. This layer of skin contains no blood vessels. The nourishment for the keratinocytes comes from a process of diffusion from the underlying dermis. In addition to keratinocytes, melanocytes, Langerhans', and Merkel cells are also present in the epidermal layer of the skin.

The thickness of the epidermis can vary in different regions of the body. It is approximately 150 μm in the eyelids and almost 1.5 mm in the soles of the feet. Regional variations in epidermal thickness result from the different number of keratinocytes and the length of the rete pegs. Such anatomical changes should be kept in mind when handling tissues. Areas where skin is quite thin are particularly prone to surgical injury when roughly handled.

Layers of the Epidermis

There are five distinct cellular layers of the epidermis. These layers include (from superficial to deep) the stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum germinativum. During mitosis, cells from the stratum germinativum migrate superficially to populate the more superficial layers of the epidermis. The deeper layers of cells are columnar in structure, but as they migrate toward the surface, they become flatter in appearance as cellular differentiation takes place. As keratinocytes migrate upwards, they mature and fill with keratin and lipids. Keratinocytes undergo apoptosis in the stratum granulosum and subsequently are shed to make way for newer cells once they reach the stratum corneum. This process, known as keratinization, happens continuously throughout life.

The stratum corneum is sometimes described as the "horny" layer. Composed mainly of dead keratinocytes, the stratum corneum is the final stop for skin cells before they are shed. The cells in this layer contain mainly keratin and lipids. The protein keratin is of vital importance, because it keeps the human body hydrated by minimizing evaporative water loss from the skin. While preventing dehydration, keratin can also absorb outside moisture. The thickness of the stratum corneum varies in different parts of the body. Skin that covers body parts exposed to constant wear and tear, such as the hands and feet, have thicker stratum corneum than other parts of the body. Also skin that is located on pressure points and joint surfaces such as the sacrum, elbows, and knees is much thicker. Beneath the stratum corneum is the stratum lucidum, so named for its translucent appearance. The stratum lucidum is a thin layer of dead cells that contain Eledin, a clear intermediate form of keratin that contributes to its "lucid" appearance. Usually, thicker parts of the body, that is, palms and soles, contain the stratum lucidum.

The stratum granulosum is found just below the stratum lucidum and is present in all regions. Characterized by small basophilic granules in the cytoplasm of squamous cells, the stratum granulosum is the outermost layer where living cells are found. The granules in these cells contain phosphorylated histidine-rich and other cystine-rich proteins through which keratin bundles traverse. The stratum granulosum also helps bundle keratin through a protein called filagrin. "Membrane-coated" lamellar granules are also present and contain lipids. The lamellar granules are exocytosed in this layer to generate a waterproof barrier. This physical barrier to evaporative water loss also prevents life-sustaining nutrient diffusion for these cells and thus leads to the characteristic cell death of the outer layers of keratinized epithelium.[7,11]

The next layer of the epidermis is the stratum spinosum. Sitting underneath the stratum granulosum, the stratum spinosum consists of cuboidal cells in a multilayered fashion. The keratinocytes flatten out as they progress through the stratum spinosum. When these cells shrink during staining, they sometimes look spiny from the desmosomes that connect them together, hence, the name "spinosum." Cells of the stratum spinosum actively synthesize intermediate filaments called cytokeratins. These intermediate filaments are anchored to the desmosomes joining adjacent cells to provide structural support, helping the skin resist abrasion.[13]

The basal layer of the epidermis is the stratum germinativum. This layer is composed of columnar cells that undergo mitosis to populate the epidermis and gradually migrate superficially.

This layer of cells lies directly on the basal lamina of the skin and forms the dermal/epidermal junction.

A desmosome or macula adherens functions in cell-to-cell adhesion. Found in the cell membrane between keratinocytes, desmosomes are important in controlling shearing forces in the epithelium by anchoring cells to each other. As complexes of proteins, desmosomes help link cell surface proteins to intracellular keratin cytoskeletal filaments. An autoimmune disease known as pemphigus vulgaris is caused by auto antibodies to desmosomes. Such an aberrant process leads to acantholysis or separation of the adherent cell layers and results in the characteristic sloughing of skin and formation of painful blisters.

Melanocytes are cells found in the stratum germinativum of the epidermis and in the middle layer of the eye. Melanogenesis, which is the production of melanin by melanocytes, can be altered by various stimuli. The production process is not completely understood at this time, but isobutylmethylxanthine, retinoids, melanocyte-stimulating hormone (Melanotan), metabolites of vitamin D, cholera toxin, forskolin, UV light, ACTH, and diacylglycerol all stimulate the process of melanogenesis.[14] In addition, DNA damaged by UV radiation can lead to the formation of thymidine dinucleotide (pTpT) fragments that can stimulate melanogenesis. Once made, melanin is moved along dendrites in a special container called a melanosome. Melanosomes are organized into a cap and protect the DNA in the nucleus of the keratinocyte from ultraviolet light. One keratinocyte provides melanin for 4 to 10 keratinocytes.[6] Melanin provides pigment to the skin. The skin helps protect against harmful UV irradiation and contains enzymes to help repair DNA damage from UV light.

Differences in skin pigmentation between races can be directly associated with the latitude of various continents. More melanin provides greater protection against increased UV radiation near the equator and gives individuals a darker pigmentation. Data show that skin tone is independent of geographic origins of a human race, being derived from ancestral pigmentation.[12]

The Langerhans' cells, also located in the epidermis, play a vital role in the immune response. Langerhans' cells are classified as dendritic cells or antigen trapping dendrites. After capturing the antigens, these cells travel from the epidermis to local and regional lymph nodes. While in transit, the Langerhans' cells (LCs) become activated and expose the captured antigens to circulating T cells. Although the main function of LCs is to aid in host protection, dysfunction of these cells can lead to neoplastic changes. Langerhans' cell tumors are a result of cellular atypia.

Dermis

The second major layer of the skin is the dermis composed of collagen, elastin, salts, water, and a gel of glycosamin proteoglycans. All these proteins and molecules give significant density to the dermal layer of the skin. The dermis varies considerably in thickness from location to location. It can be as thin as 200 μm in the eyelid and as thick as 3 mm on the back skin. The dermis provides protection from stress and strain by providing a cushion. Hair follicles, sweat glands, sebaceous glands, apocrine glands, and blood vessels all partially exist within the dermis and exit through the dermis. Nourishment and waste removal of the dermis are dependent on the blood vessels that exist in the vicinity.

Dermal fibroblasts help control the production and maintenance of the dominant structural components of the dermis. Fibroblasts make up the majority of cells in the dermis along with interspersed mast cells and tissue macrophages.

The tensile strength of the dermis comes from collagen, which accounts for a significant amount of the fat-free dry weight of skin. The majority of collagen in the dermis is Type I collagen and constitutes up to 80% of the collagen in skin. Type III collagen constitutes about 15%, while Type V and VI account for the remainder. The typical ratio of Type I collagen to Type III collagen is 4:1. This ratio is maintained even in scars after wound healing.

Collagen is the most abundant protein in mammals and is found mainly in connective tissue. As a long fibrous structural protein, bundles of collagen or collagen fibers are the major constituent of the extracellular matrix. The collagen fibers provide support for tissue and cell structures. Collagen has significant tensile strength and is found in the fascia, ligaments, tendons, bone, teeth, and cartilage. Collagen maintains skin elasticity and strength in a synergistic manner with elastin. Tissue development is also aided by collagen because

of the support it gives to blood vessels. With decreased production and turnover of collagen, the signs of aging including rhytids, loss of skin elasticity, and thinning of skin become apparent. In conjunction with collagen, elastin fibers help maintain structure and allow for flexibility of the skin.

Papillary Dermis

The dermis comprises two layers: pars papillaris and pars reticularis. The papillary dermis is the thinner and more superficial layer of the dermis zepidermis and dermis are contoured by ridges and folds of papillae that arise from the papillary layer of the dermis, which give integrity and increase the surface area of the dermal/epidermal junction. The folds and ridges are referred to as rete pegs. With aging, these rete pegs diminish and subsequently lead to a decrease in the surface area of the dermal–epidermal junction. This phenomenon can lead to epidermal gliding and shearing. Papillae are most prominent in the hands (palms and fingers) and the feet (soles and toes) as these areas must withstand the greatest frictional forces. Rete pegs are also known as friction ridges, because the exaggerated rete pattern gives the hands and feet the ability to grasp objects through friction.

Elastin and collagen fibers are dispersed more widely and are arranged in a more haphazard fashion in the papillary dermis than in the reticular dermis. However, the papillary dermis has a higher amount of ground substance and connective tissue cells. Blood vessels and lymphatic plexuses are found in the papillary region directly below the dermal papillary ridges.

Reticular Dermis

Directly below the papillary dermis is the reticular dermis. Thicker than its more superficial counterpart, the reticular dermis is avascular and acellular, but contains a high amount of collagenous and elastic tissue. Type III and V collagen fibers are seen mostly in the epidermal–dermal junction. However, they are also found in both the papillary and reticular dermis as sheaths for epithelial appendage structures, vessels, and nerves.

These collagen fibers are arranged in an interwoven, crisscross pattern in a plane parallel with the skin. The arrangement of the collagen fibers was first observed by Langer (1861) who described the arrangement as a "lattice-like network with much extended rhomboidal meshes." The pattern performs important functions especially with regard to skin extensibility. An anatomic feature in this layer of the dermis is the even distribution in thickness of the collagen and elastin fibers.

Dermal Ground Substance

An important component of the dermal connective tissue is what is known as ground substance. This substance is composed of a broad class of anionic polysaccharides or glycosaminoglycans, which comprise the milieu for cells of the dermis.[5] Hyaluronates, dermatan sulfate, chondroitin-4-sulfate, and heparin sulfate constitute the ground substance in the skin and are regulated by fibroblasts and mast cells. Existing as a viscoelastic solgel of hydrophilic polymers, the ground substance in the dermis has complex interactions involving water binding, flow resistance, collagen, and other glycosaminoglycans.

Clinical Correlation: Skin Expansion

When there is a shortage of skin because of injury or skin needs replacement because of tumor resection, plastic surgeons may take advantage of the viscoelastic nature of the dermis. This can be accomplished by expansion of the residual skin. Skin expanders are placed subcutaneously and are gradually inflated to stretch the skin taking advantage of the process called "creep." The collagen and elastin fibers located in the dermis are stretched and the ground substance located in the dermis is displaced out of the area of expansion allowing for skin expansion. As a result, the dermis gradually thins and the epidermis thickens. The underlying subcutaneous tissues and muscle also undergo some degree of atrophy and thinning. Any underlying bone will also undergo some resorption or remodeling. Such expansion is ideally performed over months and is done in areas adjacent to the area in need of reconstruction. Once the skin is expanded and used in reconstruction, it gradually undergoes a reversal of the changes noted prior to expansion and can return close it its normal anatomic and physiologic composition (Figure 13.4).

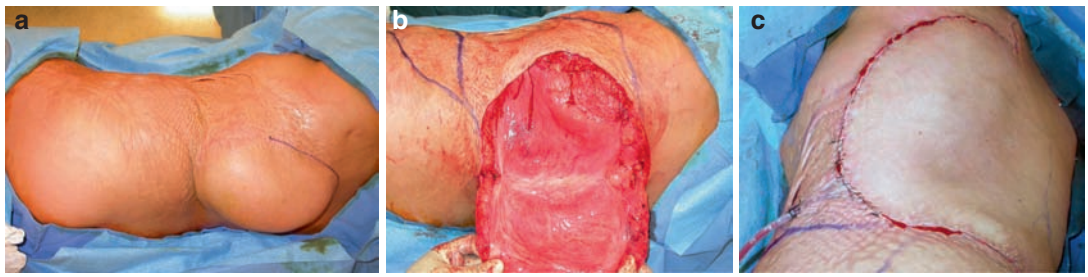

Figure 13.4. (**a**) Skin expander in the lateral back. Healed burn with skin contracture noted. (**b**) Expander removed with the expanded skin shown. (**c**) The reconstructed skin contracture with the expanded skin.

Skin Circulation

Cutaneous branches of the musculocutaneous arteries are the main suppliers of blood for the skin. Branches from these cutaneous arteries form a small vessel plexus within the dermis. Some of these branches protrude outward as perforating arteries morphing into arterioles as they reach the papillary dermis layer of the skin. The small arterioles and venules constitute the microvasculature/microcirculation of the skin. The circulation in the skin plays a vital role in maintaining the body temperature in concert with the temperature-regulating control centers in the hypothalamus. Chronic changes that occur from diabetes, smoking, and other vascular disorders can lead to changes in skin circulation and can cause significant soft tissue injury. Some of the autoimmune disorders such as Raynaud's lead to decreased circulation in the skin of the digits and result in pain and discoloration and cold intolerance.

Nerve

Within the deep dermal plexus, branches of myelinated sensory nerves lie parallel to the skin surface. These branches project upward into the dermis to form a web in the superficial dermal plexus. These nerves convey sensations from the skin to the brain through specialized receptors for touch, pressure, temperature, and pain. These nerves also carry autonomic fibers that innervate the smooth muscles of the cutaneous blood vessels, pilomotor units in the hair follicles, and the sweat and sebaceous glands.[4] Cholinergic fibers use acetylcholine, adrenergic fibers use norepinephrine, and the purinergic terminals use ATP as a neurotransmitter.

Clinical Correlation

The use of botulinum toxin for hyperhydrosis of skin is specifically targeted to inhibit the cholinergic fibers that innervate the sweat glands. Excellent results can be obtained with appropriate use of this medication in patients with hyperhydrosis. The appearance of rhytids or wrinkles as a result of the action of the underlying facial mimetic musculature can also be improved with selective weakening of the mimetic muscles with botulinum toxin. This minimally invasive procedure involves injection of small quantities of the toxin directly into the muscles of interest that results in improvement in rhytids caused by the facial mimetic muscles (Figure13.6).

Hair Follicles

Hair follicles in the skin contain lanceolate terminals, Merkel cell–neurite complexes, and Ruffini corpuscles. The lanceolate terminal is composed of axon endings and Schwann cell membranes located over the hair bulb on the sheath. For fine body hair, the terminals encircle the whole shaft, whereas in terminal hairs, they are less evenly distributed. Hair pigmentation is dependent on the amount of melanin in the hair follicle. The melanin present produces eumelanin and pheomelanin giving hair a specific color. It has

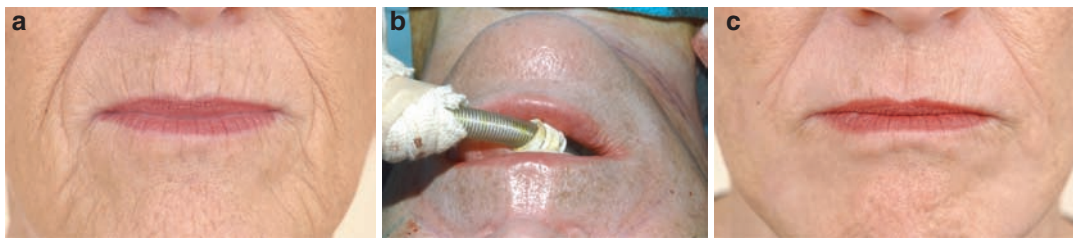

Figure 13.5. Skin resurfacing using phenol-croton oil peeling. (**a**) Preoperative. (**b**) Intraoperative view after application of peel. (**c**) Eight-month postoperative result.

been shown that genetics plays an important role in hair color.[17] Production of melanin decreases with age. The new hairs that grow in the elderly grow without melanin, which results in grey hairs. This process is not limited to the elderly but can present as early as adolescence in some individuals. Stem cells responsible for the maintenance of the melanin are reduced with increasing age, resulting in a decrease in melanin production.[15]

Clinical Correlation

Another important function of the hair follicle is its involvement in the regenerative capacity of the epidermis. Progenitor epithelial cells located along the hair bulb and shaft migrate outward to areas of de-epithelialization gradually repopulating it with new keratinocytes. This regenerative capacity of skin is of vital importance in burns and other trauma. This principle applies in the use of split-thickness skin grafts as well. Preservation of these skin appendages is crucial in the proper execution of skin resurfacing procedures as well. Loss of these appendages from trauma, burn, or iatrogenic causes will lead to wound healing with delayed re-epithelialization. If re-epithelialization is significantly delayed, hypertrophic scarring becomes more likely. In situations of full-thickness skin loss, it is important to recognize the extent of the injury early and perform reconstructive surgery to prevent hypertrophic scarring and possible scar contractures. This is especially true in preservation of the functional anatomic areas, such as the joints, hands, feet, neck, and face.

Any skin resurfacing procedures should be done with precise knowledge of the desired depth of skin injury. Laser resurfacing or deep chemical peeling procedures must extend deep enough to eradicate wrinkles but not so deep as to destroy epidermal progenitor cells in the reticular dermis if healing is to proceed without hypertrophic scarring. This depth is controlled by limiting the fluence (energy) and/or the number of passes with an ablative laser. Similarly, the number of coats of any chemical peeling agent should be limited and the appearance of skin and the resultant desired changes with application should be understood to prevent unwanted injury to skin (Figure 13.5).

Eccrine Glands

Eccrine sweat glands are distributed over the entire body surface but are particularly abundant on the palms of hands, soles of feet, and the forehead. Eccrine glands play an integral role in temperature regulation of the body. Eccrine sweat glands are coiled tubular glands derived from squamous epithelium that extends into the dermis. The sweat glands are controlled by Sympathetic cholinergic nerves, which are controlled by a center in the hypothalamus. The hypothalamus senses core temperature directly via thermoreceptors in close proximity to circulating blood and also has input from temperature receptors in the skin. The hypothalamus maintains homeostasis by modifying sweat output, along with other thermoregulatory processes.

Apocrine Glands

Apocrine glands develop during early to mid puberty. They help regulate sweat production. Apocrine glands are mainly found in hair-bearing areas such as the axillae and genitalia. The sweat produced by these glands can have an odor due to the bacteria that break down the organic compounds in the sweat. Emotional stress increases the production of sweat from the apocrine glands.

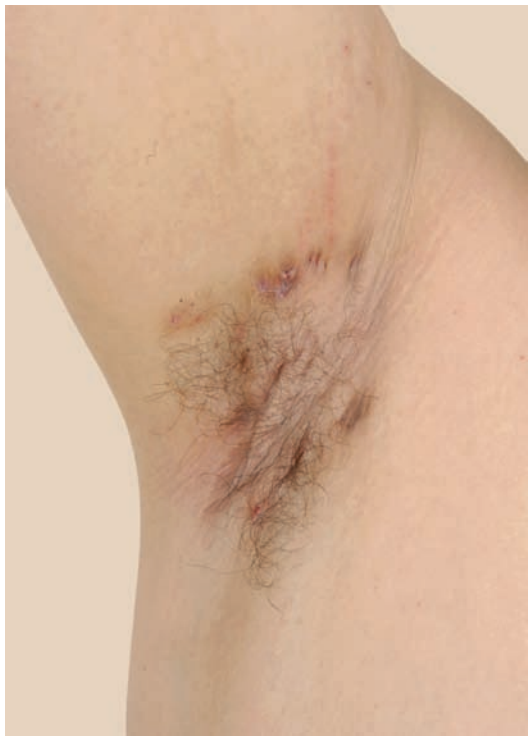

Figure 13.6. Hidradenitis suppurativa of the axilla. Note areas of folliculitis.

Clinical Correlation

Hidradenitis suppuritiva, which results in multiple abscesses, is a disease of the apocrine sweat glands and occurring predominantly in hair-bearing areas. An alternative mechanism of this condition is thought to occur from acne-like follicular obstruction and hence is referred to as acne inverse by some dermatologists and dermatopathologists. It is associated with repeated bouts of inflammation, infection, and abscess formation. Treatment usually consists of multiple courses of antibiotics. Severe cases not responsive to conservative treatment require radical debridement and secondary wound healing or reconstructive procedures (Figure 13.6).

Acne and Inflammatory Conditions of the Skin

Early in the teenage years acne can cause physical and emotional stress. There have been many products and procedures recommended for its treatment. However, none has proved to be uniformly successful. This condition is caused by the production of excess sebum associated with follicular hyperkeratinization. This leads to the formation of a keratin plug that obstructs the follicular opening and is clinically seen as a comedo. Subsequently, local inflammation and secondary contamination by skin flora lead to the formation of papules, pustules, and skin nodules. This is thought to be heavily influenced by the hormonal changes that occur during the teenage years. The general treatment is to target the various processes that are affected. Benzyl peroxide is used as an antibacterial agent and as a comedolytic agent. Topical antibiotics such as clindamycin are used to target the secondary bacterial overgrowth. Retinoids are the mainstay of acne treatment. They target the initial events in acne formation – follicular hyperkeratinization, excessive sebum production, and inflammation. They also improve the vascularity and turnover of the dermal components. Such retinoids include tretinoin in a topical format and isotretinoin given in a pill format. Isotretinoin should be avoided in patients with liver disease or lipid abnormalities. It is a potent teratogen. Women of child-bearing potential must use two forms of birth control and be closely monitored with monthly pregnancy tests while taking the medication. Systemic retinoids should also be avoided before any skin resurfacing procedures as they interfere with the re-epithelialization of the skin. Some agents such as vitamin C and plant extracts may be used as antioxidants. Various peels that use alpha hydroxyls, such as glycolic acid, kojic acid, and salicylic acid, are used to exfoliate the superficial dead layers of skin, thus clearing obstructed pores. More invasive peels using potent chemicals that penetrate deeper can be combined with the more superficial alpha hydroxyl agents to resurface the skin.

Skin Aging

Skin aging is a product of a variety of factors.[2] With age, the dermis changes anatomically and physiologically. Vital functions such as vitamin D production, sensory perception, wound healing, and other functions have been shown to decline.[3,9] The most evident component of skin change is dermal atrophy resulting from decreased production and turnover of collagen. Enzymes associated with post-translational processing of collagen decrease with age.

Hydroxylation and glycosylation of the collagen proteins also decline and cross-linking between collagen molecules tends to decrease with age. Fibroblast numbers that produce collagen and blood vessels decrease with age.[3] In women, collagen decline can be reduced with exogenous estrogen as there is a direct correlation between skin collagen production and estrogen.[16] It has been shown that estrogen has a positive effect on wound healing as estrogen increases the production of TGF-β secretion.

Skin thickness also changes with age. These changes can be evident as early as age 20 and continue into late adulthood. In women, decline in skin thickness can reach up to 1.13% per year. The thickness of skin is directly related to and dependent on the components of the dermis, including collagen, elastin, and the ground substance.

Wound healing can also be impaired with the aging process. Proper wound healing is dependent on neovascularization, granulation, collagen deposition, and re-epithelialization, which all decrease in efficiency with age.

Clinical Correlation

Treatments exist to help slow down the signs of skin aging and in some instances reverse the aging process. These treatments target various processes in the skin and include sunscreen to reduce the harmful ultraviolet radiation of the sun preventing photodamage to skin, antioxidants (vitamin C, vitamin E, coenzyme q10, lipoate) to combat the oxygen-free radicals, DNA repair agents (niacin, folate, reemergence) to help repair damaged cells, cell turnover stimulants such as retinoic acid and niacin, and epidermal barrier enhancing agents (niacin). The newest of these agents are topical prodrugs aimed at delivery of molecules that penetrate the skin and function as precursors at various stages to improve skin health. Other agents target the loss of the ground substance and the decrease in the collagen content of the skin. Such products include the various fillers used to augment the soft tissues (hyaluronic acid fillers, collagen-based fillers, hydroxyapatite fillers, PMMA fillers, and other agents). The plastic surgery and dermatology literature is replete with articles that describe results with these various agents.

The use of hyaluronic acid for soft tissue augmentation is a popular treatment gaining widespread acceptance in plastic surgery and dermatology. This product is associated with minimal side affects and requires no skin testing. Maintenance of volume enhancement can be seen with time as the product is hydrophilic, recruiting water molecules and maintaining the augmentation of the soft tissues. A new calcium hydroxyapatite treatment mixes calcium hydroxyapatite with a polysaccharide gel for soft tissue augmentation use. This treatment serves as a base for collagen growth and has been shown to be safe in humans.

The use of dermal fillers is rapidly increasing. Recent estimates by the American Society of Plastic Surgeons estimate that more than 5,00,000 patients were injected with hyaluronic acid in 2006.[1] It should be noted that these fillers are FDA approved for nasolabial fold correction only. Any other use is *off-label*.

Classification of Skin Types

Often in plastic surgery and dermatology, skin is classified in terms of its response to sun exposure and the resultant changes that occur with photodamage. This is termed Fitzpatrick classification (Figure 13.7). The resultant pigmentation/tanning changes that occur with sun exposure are due to alterations in the production of melanin. There is a paucity of melanin in the lighter skin types and is usually only located in the basal layer. In the darker skin types, there is an abundance of melanin and it is also present in more superficial layers of the skin. The presence of melanin plays a protective role in minimizing photodamage to skin.

Clinical Correlation

A thorough knowledge of skin type and the ability to correctly assign a skin classification to a patient are important when skin resurfacing or nonablative laser or light-based treatment is being considered. Such classification can help a plastic surgeon decide on the appropriate skin care or rejuvenation procedure. Inappropriate selection of skin rejuvenation techniques performed in skin types IV through VI, including chemical peels, laser resurfacing, or dermabrasion, can result in hypopigmentation or hyperpigmentation (Figure 13.7). In general, melanin suppression for a minimum of 6 weeks using bleaching agents such as hydroquinone 4% should be used before superficial, intermediate, or deep peeling in darker skinned individuals (Fitzpatrick IV–VI).

Figure 13.7. The Fitzpatrick skin classifications. I (never tan, always burns) to VI (dark, African-American skin type).

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Stratum Corneum: The Role of Lipids and Ceramides

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KEY WORDS: *stratum corneum, intercellular lipids, ceramides, sphingolipids, sphingomyelins, glycosylceramides, barrier function*

ABSTRACT: *This paper reviews recent findings about the structure of the stratum corneum and the different models developed to better understand its function and behavior. The roles of intercellular lipids and ceramides as its key constituents are discussed.*

The skin exhibits several essential functions, the major one being the containment function. It provides protection from various external insults that include: microorganisms, chemicals, electricity, mechanical pressure and heat. The skin also filters about 70% of UVB radiation and plays a key role in the regulation of body temperature. It contains several subtissues that differ in structure and function.

The stratum corneum (SC) is part of the epidermis and is the outermost upper layer of the skin. It provides the rate-limiting barrier for penetration into and through the skin. The dermis lies beneath the epidermis and under it, the subcutaneous tissue. The skin also includes sebaceous glands and hair follicles.

In order to allow a desired interaction between a compound and the skin, a manipulation of this heterogeneous barrier structure is required. When applied to the skin, a compound may partition into the SC, diffuse and possibly penetrate. Its partition and diffusion profiles can vary depending on its interaction with the subtissue. The compound's pathway of removal or clearance from the skin will be highly dependent upon its level of penetration. The amount of the compound remaining on the surface of the skin will be removed eventually with the dead corneocytes during the desquamation process. The amount penetrated to the living epidermis and absorbed through blood vessels will be cleared through the circulation and is prone to metabolism.

Stratum Corneum Structure

The SC is basically a two-compartment system, composed of vertically stacked cells called corneocytes surrounded by a matrix of lipid membranes.[1] Corneocytes are dead, flat and thin squamous cells that are filled with keratin protein. The matrix between corneocytes is composed of lipids arranged in numerous lamellar sheets, along with intercellular adhesion complexes called corneodesmosomes. Much of the barrier function of the skin and its water loss prevention capability is provided by the cornified cell envelope of cross-linked proteins and lipids, as well as by the lamellar sheets between the cells.

The formation of the epidermis is a coordinated process of cell proliferation, differentiation and death that allows for a continuous renewal of this tissue. When keratinocytes end their proliferation process they start to differentiate into corneocytes and migrate to the SC. The basal keratinocytes are capable of proliferating and differentiating as they progress from the basal layer to the skin surface. This turnover can be accelerated in different diseases such as in psoriasis.

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The differentiating cells synthesize keratohyalin granules, composed of several proteins that play a significant role in final stages of epidermal differentiation.

The major proteins formed within keratinocytes are keratins, which are intermediate filament proteins that form the cytoskeleton of keratinocytes. There are numerous types of keratin, and different keratins are associated with skin, hair and nail formation.[2] An important process that leads to desquamation is the degradation of cholesterol sulfate by sterol sulfatase. Its degradation permits proteases to attack corneodesmosomes. If the enzyme sterol sulfatase that under normal conditions will degrade cholesterol sulfate is defective, it will lead to a recessive X-linked ichthyosis disorder manifested by markedly dry, thickened, scaly skin and leads to permeability barrier dysfunction.[3]

In the final stages of the keratinization process, the protein profilaggrin is dephosphorylated and proteolytically clipped to produce filaggrin. The granular cells lose their nucleus and die. The dead cornified cells become the major part of the SC and together with the intercellular lipids provide the skin barrier. A disorder in any of the steps of the process can cause skin barrier abnormalities.

This integrity of the SC is therefore a factor of several elements that are all a result of the keratinocytes' differentiation: the physicochemical quality of the corneocytes, the mechanical strength of the junctions connecting these cells, the composition and organization of the lipid "mortar" in the intercellular spaces. Modification in composition and structure of SC lipids may result in changes in proteolytic enzyme activity. Consequently, this can influence epidermal desquamation that is regulated by extracellular enzyme's access to corneodesomosmes.[4]

Formation of the Stratum Corneum

Scientists have found various ways to describe the SC barrier and its manner of formation. They have used various lipid combinations to mimic the intracellular lipid structure to create an appropriate model. The model often dictates the analytical test methods that allow for tracing the interaction of compounds with the model. Among the test methods used are molecular dynamic simulations (MD), Fourier Transform Infrared (FTIR), X-ray diffraction and nuclear magnetic resonance (NMR).

Molecular dynamic simulation provides a computerized calculation of the time-dependent behavior of molecular systems. It permits the study of complicated dynamic processes that occur in biological systems. These processes include: protein stability and conformational changes as well as ion transport across biological membrane systems and molecular recognition phenomena. It can, for example, model a pattern of distribution of different filament densities in the epidermis.[5]

FTIR spectroscopy is an important technique in organic chemistry. It is a relatively easy way to identify the presence of certain functional groups in a molecule. In addition, one can use the collection of pronounced absorption bands provided to confirm the identity of a pure compound or to detect the presence of a specific impurity. X-ray diffraction is a scattering of X-rays by crystal atoms to produce a diffraction pattern that yields information about a structure or a crystal, or the identity of a crystalline substance. This technique is used by Bouwstra and her group to investigate lipid organization in the SC.[6]

NMR uses the absorption of electromagnetic radiation of a specific frequency by an atomic nucleus that is placed in a strong magnetic field. This technique is used especially in spectroscopic studies of molecular structure. By using deuterium NMR, Kitson and Thewalt[7] learned the relationships between lipid composition and phase behavior. They further established the effects of temperature and composition dependencies of model SC membrane structure and phase behavior, and they specifically examined the effect of ceramide on phosphatidylcholine membranes.[8] Since some of these techniques involve certain modifications of the specimen to allow analysis, it is always recommended to use more then one technique to substantiate observations and acquire information to support structure theories.

Development of Models

Based on their research findings, scientists have developed different models of the SC structure and its generation. Early in 1984, Landmann[9] suggested that the intercellular lamellar structures of the SC are derived from the extruded contents of membrane-coating granules through a membrane fusion process. Landmann tested various artificial lipid systems that were close in composition to the SC and the stratum granulosum that lies under it. These systems generated lamellar forms that showed organizational properties similar to those found in the membrane-coating granules and the intercellular spaces. He concluded that the lipid composition has a major role in the molecular lipid arrangement that is responsible for the epidermal barrier.

Among the interesting models for the barrier and its formation that have been developed are the "single gel-phase model" and "the membrane-folding model" proposed by Norlén in 2001.[10] These models suggest that the skin barrier exists as a single and coherent lamellar gel phase. In the upper layers of the SC there may be crystalline segregation and phase separation that will occur as a result of the desquamation process. The generation of such structure takes place via a dynamic process in which membranes are unfolding and crystallizing simultaneously with the creation of the multilamellar lipid structure.

While Landmann's model considers changes in membrane topology, here the topology is thought to be constant during skin barrier formation. Norlen claims that his model for barrier formation requires low energy, conserves membrane continuity with no membrane fusion events and is faster to generate. Moreover, this model claims to explain the water permeability properties of the SC and its particular lipid composition at the intercellular spaces.

Another model composed of bilayers, including either free fatty acid or fatty acid/cholesterol mixtures, was developed in order to understand the effect of cholesterol on skin structure and dynamics.[11] It was found that cholesterol strongly influences the phase behavior of the model. Cholesterol demonstrated a smoothing effect on the rigid fatty acid phase, enlargement of the area per molecule and reduction in SC thickness. Cholesterol was described further as a molecule that either fluidizes membrane domains or contributes to their rigidity. This will depend upon its concentration and other compounds included in the model.[12]

Bouwstra's group chose to define the SC structure as a sandwich model.[13] This model was developed based on the observation that the intercellular lipid bilayers are organized into broad-narrow-broad lamellae in which crystalline and liquid domains coexist. The broad-narrow-broad trilaminar units are thought to reflect crystalline-liquid-crystalline lamellar units with an overall dimension of 13.4 nm. Although the crystalline state predominates, a subpopulation of lipids in a liquid state was found to coexist. The outer broad lamellae are said to be crystalline, while the central narrow lamellae is the more fluid liquid phase. This model was challenged later on by Hill and Wertz, and this will be discussed in the next section.

Trying to explain skin permeability to certain molecules, a hypothesis was developed in which the epidermal barrier was represented as a porous medium.[14] Similarly, and in support to Bouwstra's

model, this model describes the epidermis as being composed of permeable and impermeable regions. The corneocyte envelope is classified as impermeable. The intercellular lamellae exist in solid impermeable and liquid-crystalline permeable states. Hill and Wertz suggest[15] that this ordered-fluid-ordered structure can be an advantage in minimizing destruction to the skin, since when a toxic substance partitions into the tissue it would be diluted effectively by lateral diffusion in the fluid phase domains.

Intercellular Lipids

The intercellular lipids of the SC comprise 10–15% of dry weight of the tissue. This lipid is composed mainly of fatty acids (10%), cholesterol (25%) and ceramides (50%). There are also smaller amounts of cholesterol esters, cholesterol sulfate and glucosylceramides. When progressing from the basal layer of the epidermis toward the SC, phospholipids are present initially. They are degraded as cells enter the SC. In parallel, the cholesterol and fatty acid contents are elevated gradually. The aliphatic chains in the ceramides and the free fatty acids are mostly straight, long-chain and saturated compounds.

The melting points of the SC lipids are high, and the polar head groups are small. At physiological temperatures the lipid chains are present mostly in their solid crystalline or gel state. This state permits only slow lateral diffusion and is less permeable then the state of liquid crystalline membranes that are present at higher temperatures. The lateral packing of the aliphatic chains of lipids is described to have either hexagonal- or orthorhombic-shaped lattices. The hexagonal chain packing is more fluid than the orthorhombic. These lamellar structures are derived from the content of the lamellar granules that are synthesized in the viable epidermis layers in the keratinocytes. The granules are exocytosed from the keratinocytes before the final steps of terminal differentiation to form corneocytes.

The extruded granule contents are thought to be flattened lipid vesicles that fuse in an edge-to-edge manner to form the sheet-like lamellae that exists in the SC.[16] At the time of exocytosis, the lipid mixture in the granules consists mainly of phospholipids, glycolipids and cholesterol. The granules also deliver the enzymes that hydrolyze the phospholipids and glycolipids to produce the mixture of ceramides, fatty acids and cholesterol. It is believed that the formation of highly organized, mostly gel-phase membrane domains is due to the cylindrical or rod-like shape of the ceramides and fatty acids.[12]

The intercellular lipids and their state of organization are partially responsible for skin hydration homeostasis. Other factors that regulate the hydration are water-binding substances within the corneocytes and the protein-lipid structure of the SC. Disruption of the barrier may result in changes in hydration state and can trigger a cascade of events that include an increase in epidermal lipid synthesis for the creation of new lamellar bodies. The secretion of the lipid contents from lamellar bodies results in re-accumulation of lipids in the intercellular spaces of the SC and the recovery of normal barrier function.[17]

As previously discussed, intercellular spaces in the SC were shown to contain 13 nm tri-lamellar structures. These lamellae were thought to be of unequal thickness alternating 5-3-5 nm in dimension; however, Hill and Wertz claim that the actual lamella are of uniform 4.3 nm in width and that the misleading impression that the lamellae differ in thickness is a result of the asymmetric deposition of stain from ruthenium tetroxide used as a post-fixative for transmission electron microscopy. It is theorized that linoleate chains from acylceramides in the central lamelle reduce more ruthenium than the reactive groups in the outer lamellae. This process leads to an asymmetric distribution of the stain.[15] The lamellar structure was shown to be organized in a zipper-like interdigitated structure. This structure, in addition to the cohesion provided by desmosomes contributes to the strength of the SC.[12]

Role of Ceramides

The involvement of ceramides in essential structures such as the SC and different processes of cell signaling turned ceramides into attractive molecules for research in molecular biology. Ceramides and glucosylceramides are pro-vital molecules in multiple biological processes such as apoptosis, the process by which a cell actively "commits suicide;" signal transduction, the cascade of processes by which an extracellular signals interact with a receptor on the cell surface; and mitogenesis, the process of stimulating transit through the cell cycle.

Although accumulation of ceramides has been reported in stress- and receptor-induced apoptosis in the nervous system, the role of ceramides in apoptosis signaling remains unclear. In fact, ceramides that are produced by the hydrolysis of sphingomyelin activate a cycle that is followed by three different cellular responses: down regulation of cell proliferation, induction of cell differentiation and apoptosis.

> Cholesterol was described further as a molecule that either fluidizes membrane domains or contributes to their rigidity.

The generation of intracellular ceramide also may provide a link between an extracellular signal and the induction of an apoptotic program for the elimination of damaged cells. Differences in chain length and type and extent of hydroxylation or saturation provide for heterogeneity among the epidermal sphingolipids (see Figure 1). The effect of long-chain ceramides in a model membrane (16–24 C atoms) was found to increase bilayer permeability to aqueous solutes. This effect was not supported in the presence of short-chain ceramides (2-6 C atoms).[18]

At least nine classes of ceramides have been identified and have been designated as CER1 to CER9.[19] In 1993, when investigating ceramide composition of psoriatic scale, Motta et al. suggested a simple system of nomenclature of ceramides that is based on ceramide structure. They initially divided ceramides into five classes. In this system, the amide-linked fatty acid is designated by one letter: N, normal fatty acid; A, alpha hydroxy acid; O, omega hydroxy acid. Likewise, S designates the long-chain base component for sphin-

gosine; and H for 6-hydroxysphingosine or P for phytosphingosine. Thus, what is commonly known as ceramide 2 can be unambiguously named ceramide NS. The presence of an ester-linked fatty acid in acylceramides is indicated by a prefix E.

This nomenclature system, because of its simplicity and unambiguous indication of structure, is being used with increasing frequency by the scientific community.[20] Ceramides are a major lipid constituent of the intercellular lamellar sheets, accounting for 40% to 50% of the SC lipids by weight. They play a key role in preventing transepidermal water loss (TEWL) and maintaining the water permeability barrier function of the skin. They also are partly responsible for the cohesion of corneocytes and therefore play a key role in the establishment of the SC barrier function for water evaporation. Ceramides structurally are heterogeneous. They are a complexed group of sphingolipids, containing derivatives of sphingosine bases in amide linkage with a variety of fatty acids.[21]

The *o*-acylceramides have distinctive structures not seen in other tissues. Their unique structures allow for their functionality in the epidermal permeability barrier.

The ester-linked fatty acids in each of the three *o*-acylceramides (EOS, EOP, EOH) in normal healthy epidermis are predominantly linoleic acid. In essential fatty acid deficiency, linoleate is replaced by oleate. This creates disorder in the intercellular lamellae resulting in increased TEWL.

Extracellular glycosylceramides and sphingomyelins are the precursors for SC ceramides. The enzymes beta-glucosylceramidase and sphingomyelinase are responsible for the hydrolysis and generation of ceramides from these precursors. Both precursors and enzymes are responsible for the epidermal permeability barrier homeostasis. Desquamation of skin cells is dependent on the transformation of cholesterol sulfate into cholesterol.

Uchida Y. and his group[22] claim that all major subfractions of SC ceramides are derived from lamellar bodies containing glucosylceramides. This hypothesis is based on reported chemical structures of epidermal glucosylceramides and SC ceramides. It also is known that sphingomyelin-derived ceramides are essential for normal barrier function homeostasis.

Two epidermal sphigomyelins SM-1 and SM-3 were found to be important precursors of two corresponding ceramides in the SC: ceramide NS (ceramide 2) and ceramide AS (ceramide 5), respectively. Other ceramide species, including the omega-hydroxyceramide species, which exhibit a different saturation level, are not derived from sphingomyelin.

Other Roles for Ceramides

In addition to their role in determining barrier properties, ceramides may play roles in other biochemical events in the skin. These functions are not clear and yet to be explored. Ceramides were found to be present at significant levels both in the basal cells and in the upper epidermis. In basal epidermal cells and fibroblasts, ceramides were found at the cell nuclear envelope, the inner and outer mitochondrial membranes, at the golgi, the endoplasmic reticulum and

at the plasma membrane. In the upper epidermis, ceramides were localized in the lamellar bodies, cornified envelope and, as already explained, within the intercellular space of the SC.[23]

Skin barrier disruption by organic solvents results in an increase in synthesis of free fatty acids, sphingolipids and cholesterol in the living layers of the epidermis leading to barrier repair. Cholesterol synthesis will begin 90 minutes after barrier disruption.[17] The enzyme HMG CoA reductase regulates this synthesis. Sphingolipid synthesis, which is regulated by serine palmitoyl transferase, will start about six hours after barrier disruption. If the barrier disorder is chronic, it also will stimulate DNA synthesis and cell division.

The link between skin disorders and changes in barrier lipid composition, especially in ceramides, has been difficult to prove because of the many variables involved. However, most skin disorders that have diminished barrier function also show a decrease in total ceramide content with some differences in ceramide pattern. There is, however, a debate about the pronunciation of ceramides content and profile in atopic dermatitis and in psoriasis. While the differences in ceramides content in lesional skin are clear, it is unclear whether those differences are significant in nonlesional skin when compared to healthy skin.[24]

Preparations containing lipids that are close in structure to lipids preset in the SC and in particular ceramides, could improve skin conditions.[21] However, it was demonstrated that partial lipids compositions may result in an abnormal lamellar bodies contents and hence may create disorder in the intercellular lamellae. Complete lipid mixtures result in normal lamellar bodies and generate intercellular repair. An alternative strategy to improve barrier function is to enhance the natural lipid synthesis in the epidermis through topical delivery of lipid precursors.

Xerosis, which is a condition of pathologic dryness of the skin associated with aging, results from a decrease in dermal collagen levels and SC ceramide content. Topically applied formulations containing lipids that are similar in composition to those in the skin, and in particular ceramides, were shown to improve this disorder.[25]

Ceramides also are being used in skin moisturizing formulas to reduce irritation from cationic surfactants and in combination with alpha hydroxy acids to assist with skin firming. Complete lipid combinations that include the three major components of the intercellular lipids (fatty acids, cholesterol and ceramides) were shown to result in the generation of normal lamellar bodies to support and restore intercellular bilayers.

New Forms of Therapy

Understanding the ratios and combinations of the different lipids can be the basis for developing new forms of topical therapy for different skin disorders that are related to SC and epidermal damage.[26] For example, when ceramide NP (ceramide 3) was applied topically in an emollient cream and compared to a control, it was shown to significantly reduce skin erythema and TEWL.[27]

In an abnormal skin condition, such as atopic dermatitis, and also with aging, there is a change in ceramide content and in the relative proportions of the different ceramides in the SC. Imokawa et al[28] described two types of deacylases that hydrolyze the amide linkage in sphingomyelin and glucosylceramides. These enzymes were found to exist in both lesional and nonlesional SC obtained from patients with atopic dermatitis. They are not expressed in normal healthy epidermis. It is theorized that these enzymes are at least partially responsible for the reduced levels of ceramides in SC of subjects with atopic dermatitis.

Analysis of the whole epidermis in chronologically aged skin revealed that although total lipid content is reduced by 30%, the proportions of the different ceramides remains the same.[29] The skin becomes dry and more permeable. It was discovered that the skin microorganism flora is one of the factors responsible for ceramide deficiency in the SC.

The enzyme ceramidase cleaves ceramide into sphingosine and fatty acid. This enzyme was found to be secreted in excess from bacterial flora in atopic dermatitis patients when compared to healthy subjects. This situation may increase skin's hypersensitivity by impairing the permeability barrier.[30]

Ascorbic acid (vitamin C) and its derivatives were found to effectively increase the synthesis of epidermal ceramides, and hence improve the skin barrier function, moisture content and surface appearance.[31] A synergistic effect was found when ascorbic acid was used in combination with its esters. This combination especially contributed to the generation of ceramides AP and AH (ceramides 6 and 7).

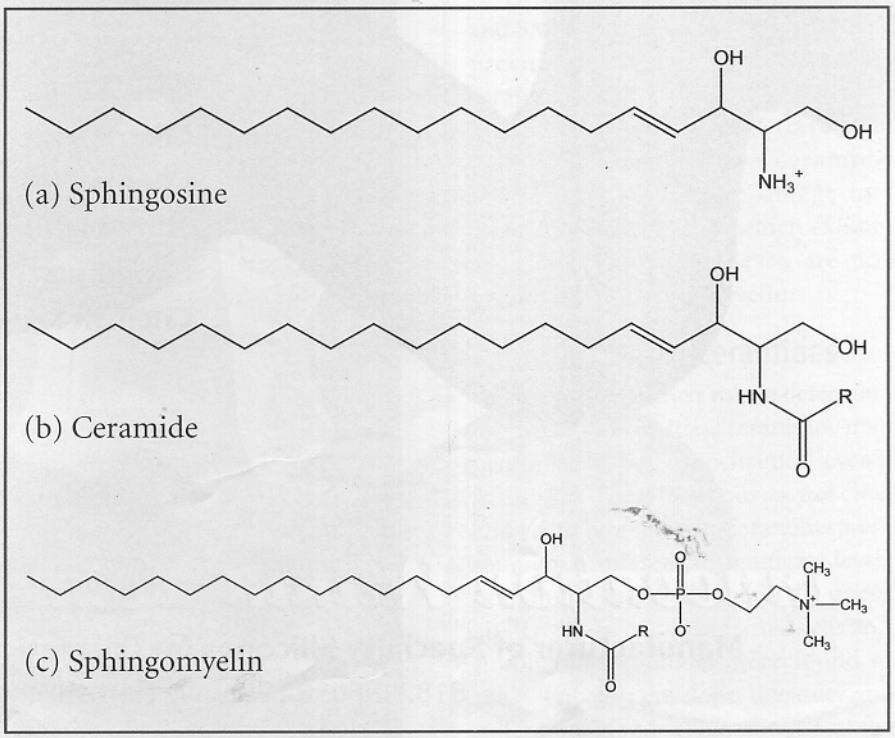

Figure 1: Chemical structures of (a) sphingosine, (b) ceramide and (c) sphingomyelin

Another compound that was shown to increase ceramide content in the skin is ursolic acid. This is a plant-derived triterpenoid that demonstrated anti-inflammatory properties in laboratory animals. When tested in vitro in cultured normal human epidermal keratinocytes, ursolic acid generated an increase both in ceramide and collagen content. When tested clinically, ursolic acid was shown to elevate ceramide content in the skin over an 11-day period.[32]

The effect of nicotinamide on ceramide content was tested both in cell cultures and topically. Nicotinamide was shown to significantly increase levels of glucosylceramides and sphingomyelin and to enhance the activity of serine palmitoyltransferase, the rate-limiting enzyme in the sphingolipid synthesis. Nicotinamide also increased synthesis of free fatty acids and cholesterol. Overall, nicotinamide improved the skin permeability barrier by stimulating de novo synthesis of ceramides and up regulating serine palmitoyltransferase.[33]

Summary

Being the outmost layer of the skin, the SC plays a major role in the creation of a healthy, youthful appearance. It is a highly dynamic organ in which many metabolic biochemical cascades occur and yet has to serve as an ideal barrier from the surrounding environment. The more attempts made to understand its biochemistry, microstructure and content, the better the realization that its appearance is being controlled from within, i.e., the living epidermis and dermis.

In order to develop sophisticated formulations for topical application, the role of the components that are responsible for the barrier formation should be researched. The generation and metabolism of intercellular lipids in general, and ceramides in particular, play a crucial role in the creation and maintenance of the barrier. When treating the skin with such compositions, their path of application, penetration (if permitted) and metabolism should be followed, and the correlation between pronounced clinical results to actual biochemical changes understood.

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Pathways for Skin Penetration

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KEY WORDS: *stratum corneum, penetration pathways, intercellular lipids, ceramides, ceramide 2, enzymes, vesicular systems, penetration enhancers, delivery systems*

ABSTRACT: *This paper reviews recent findings about three skin penet
pathway") and four types of penetration enhancers (enzymes, vesicular systems, ceramides and chemical enhancers).*

At the very early stages of understanding the roles of the body's organs, we understood that the skin functions as a barrier between our body and our surroundings. Therefore it was clear that the skin would challenge penetration of compounds and repel outside insults. Having such a nature it was not surprising to discover that skin's upper layer, the stratum corneum (SC), is a subtissue that very efficiently limits penetration of compounds.

Over the years scientists have attempted to find compounds or systems that will allow overcoming this barrier and interaction with deeper subtissues or tolerating permeation to the circulation system. After years of research, it is now clear that there are ways to allow permeation of compounds to and through the skin. The focus has shifted toward understanding the microstructure of the skin, as well as the mechanism of action of these enhancers.

Because skin penetration enhancers provoke structural changes in the SC, they often trigger undesired immune system reactions such as irritation, allergy, or inflammation. Most of the enhancers are not specific and will allow penetration of any compound that is small and lipophilic enough to penetrate. When dealing with cosmetic formulations this means that compounds such as fragrance components and preservatives will penetrate in conjunction with the active compound. Moreover, the skin is a very viable tissue. It includes many metabolic systems that were originally aimed to drive biochemical processes such as desquamation, creation of extracellular lamellar sheets, programmed cell death (apoptosis) and sebum or sweat secretion. These enzymes may attack active or inactive compounds as they penetrate, and convert them into an inactive, active or toxic form.

It is therefore believed that by understanding the details of the interaction of penetration enhancers with the skin, one can find ways to prevent their drawbacks or overcome their limitations.

This article will review possible ways for compounds to penetrate the skin and interact with it. Four families of enhancers will be discussed, along with their possible mechanisms of action and limitations. This is written in an attempt to trigger creative thinking about possible research work for better understanding the skin and the design of formulations to treat and protect it.

Penetration between SC corneocytes is the pathway by which most compounds penetrate the skin.

When designing a formulation for topical application, one must understand the possible interactions between the formulation and the skin. This article attempts to provide an initial understanding of this matter. Skin penetration differs from skin permeation. The former describes the passage of an ingredient into the skin, we hope, to the target skin layer. The latter describes the passage of an ingredient through the skin, to the circulatory system such as in

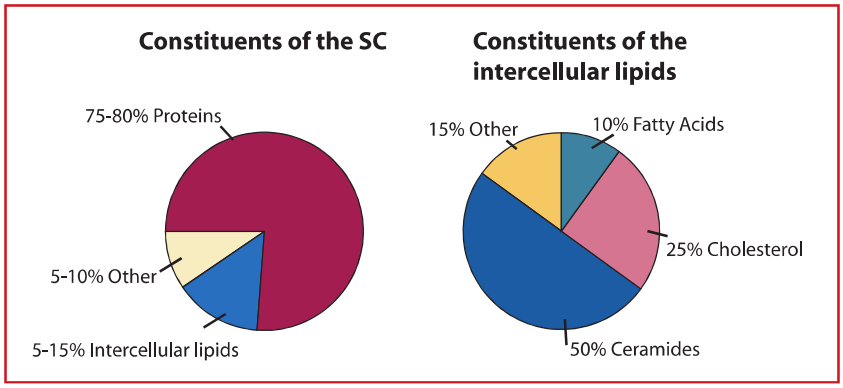

Figure 1. Percentage (by dry weight) of ceramides within the composition of the SC

pharmaceutical transdermal systems. It is however difficult to differentiate between the 2 because once a compound passed the limiting step of the SC and reached to the living epidermis it is almost impossible to prevent its partitioning into skin subtissues, including the blood vessels that lead to the circulation. Both terms will be encountered in this article, and used according to these definitions.

Factors Affecting Skin Penetration

The SC is a heterogeneous structure. Microscopically, the upper cells of the epidermis, the corneocytes, are arranged in pillars to form clusters. The SC consists of alternating cell and lipid layers and is only 6-10 μm thick, which is equivalent to about 14-18 cellular layers. Corneocytes are typically 200-300 nm thick, 30-50 μm in diameter and hexagonal or polygonal in shape. The barrier nature of the SC is governed by its constituents: 75%-80% are proteins, 5%-15% lipids, and 5%-10% unidentified elements (Figure 1). Such a composition favors the absorption of certain lipid-soluble compounds by the skin.[1]

In order to simplify the calculation of a compound's permeation profile through skin, scientists have adopted models for simple diffusion to explain the flux of compounds through SC lipid domains. When using this model one is assuming that interaction between a given compound and the skin is physicochemical in nature, with the multi-layer structures of the corneocytes within the SC organized horizontally. The majority of molecules that cross the epidermis will permeate between the cells via the intercellular route. To penetrate, a compound must partition into the SC before diffusing across the viable epidermis. Therefore, the major pathway for a compound is highly dependent upon its partition coefficient. Hydrophilic compounds may preferably partition into the intracellular domains, while lipophilic ones may cross the SC through the intercellular route. In fact, although the nature of the compound may dictate its preferred route of penetration, most molecules penetrate this layer through both pathways simultaneously.

Factors that may affect penetration include the size of the molecule, its affinity to the surface of the skin, and its compatibility with the intercellular lipids. Also of significance for penetration are general skin condition, moisture content, temperature, thickness of the SC (that can differ between races and body parts), and physical integrity. SC integrity can be affected by age, exposure to solvents, skin care routine, health condition and environmental factors.

Often a correlation can be made between the value obtained for a compound partitioning between octanol and water (i.e., partition coefficient) and its postulated route of penetration through skin. Study results suggested that while mannitol, for example, permeated via a polar route, hydrocortisone permeated mainly through the lipid route and progesterone via a lipid pathway with the aqueous layers affecting its permeation rate. These results correlated with the compound's octanol-water partition coefficient.[2]

> Human skin appendages, hair follicles, and sebaceous glands, constitute a significant route for penetration of steroids and thus probably for other chemicals of similar molecular properties.

When considering polar and non-polar pathways for penetration, it is usually assumed that polar compounds will penetrate through polar routes, while non-polar compounds will favor lipophilic routes.

The intercellular pathway: Penetration between SC corneocytes is the pathway by which most compounds penetrate the skin. Since corneocytes are not stacked parallel to one another in the layers, when penetrating between them, a compound has a sinuous way to pass. This pathway is considered to enable free volume diffusion through lipid bilayers present between the cells.

Most skin penetration enhancers were found to affect the intercellular lipid bilayers of which this route consists. Skin penetration accelerators such as dimethylsulphoxide (DMSO), laurocapram (Azone), glycols and surfactants enhance penetration into the skin by reversibly decreasing the diffusional resistance of its intercellular lipid bilayers.[3] These enhancers may not only act as solvents that solubilize the intercellular lipids but can also affect intercellular desmosomal connections or interfere with metabolic activity necessary for creation of an intact barrier. In normal skin conditions the effect on lipid bilayer structure is reversible. The decrease in the lipid bilayers' resistance to penetration can be due to a thermodynamic effect of fluidization (decrease in lipid's transition temperature) or due to phase separation of lipids in the intercellular spaces.

The intrafollicular pathway: The amount of sebaceous glands on the total skin surface represents not more than 0.1%. Therefore there are scientists who believe that this route is not a significant penetration pathway for most molecules. Others claim that the appendages can bypass the low diffusivity of the SC and may act as diffusional shunts. When the follicle is the site of action, such as in acne, scientists find ways to target a compound to this site, by developing delivery systems with specific physicochemical properties.[4]

When considering these openings as a possible route for penetration, it is important to understand the variations in follicle distribution among different body locations.[4] The highest hair follicle density and percentage of follicular orifices were found on the forehead. The highest average size of the follicular orifices was measured in the calf region. The forehead and the calf regions were also found to exhibit the highest infundibular volume and therefore the highest potential for creation of a reservoir. The lowest values for all parameters were found on the forearm. The plantar and palmar regions of the skin are the only sites completely devoid of sebaceous glands.

It is difficult to study penetration via follicles because of the lack of suitable animal models. A technique was developed to differentiate between penetration through a shunt route and bulk trans-epidermal permeation. This method compares delivery through the epidermis as a membrane versus delivery through a sandwich of SC and epidermis.

Follicles are present in layers and only rarely are superimposed. This model assumes the possibility of follicles to be superimposed is diminutive, and therefore the bottom of the skin's top layer blocks the openings of the follicles. It therefore allows one to distinguish between the 2 ways of penetration. If penetration through follicles is dominant, the passage through the sandwich model will be significantly reduced in comparison to passage through the epidermis membrane alone.[5]

Another method to evaluate penetration through shunts was based on the comparison between scarred and normal skin. The main modifications observed in scarred skin include the absence of hair follicles and sebaceous glands and thinning of the collagenous fibers.[6] In this study, the percutaneous absorption of 4 steroids through scarred skin and normal skin was observed. Results of the experiments demonstrated that human skin appendages, hair follicles and sebaceous glands, constitute a significant route for penetration of steroids and thus probably for other chemicals of similar molecular properties. The results also showed that steroids tend to create larger reservoirs in the SC of an appendage-free scarred skin in comparison to normal skin. Steroids concentrations appearing in the epidermis and dermis were greater in normal skin, where openings played a role in allowing deeper penetration.

Particulate delivery systems may play a role in targeting molecules to follicles. Depending on the formulation and the compound's intrinsic properties, certain compounds can enter faster into shunts than through a different route in the SC. It was demonstrated, for example, that microspheres with an optimal size of around 1.5 µm showed 55% penetration into hair follicles.[7]

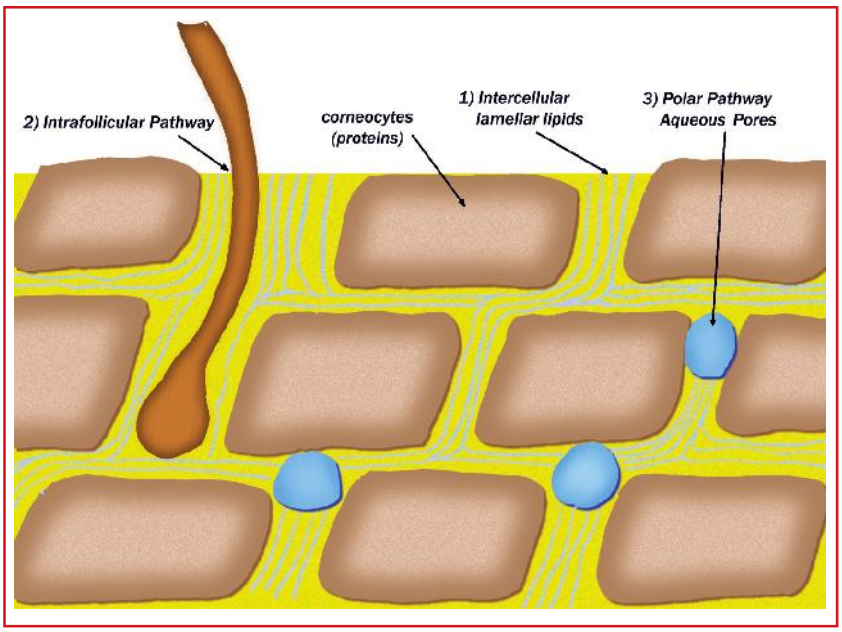

Figure 2. The "Polar Pathway"

Two types of liposomes (classic multilamellar vesicles and flexible ultradeformable liposomes) were found to contribute significantly to penetration through shunt routes.[8]

The "polar pathway": This route is believed to be hydrophilic in nature. It is composed of aqueous regions surrounded by polar lipids that create the walls of microchannels (see Figure 2). It is known to have a high penetration resistance to lipophilic compounds but low resistance to hydrophilic compounds. It is also thought to be the route by which water evaporates through the skin. The localization of the hydrophilic pores is unclear. While some scientists claim compounds permeating through this route will penetrate between the corneocytes clusters through imperfections that create openings comprising of water, others think the intracellular keratin provides this pathway.[1] It may be that both pathways exist and that the preferred route for penetration is a function of molecular properties and the test model used.

> Different enzymes, depending on their biological function, can affect metabolism and biochemical cascades in the skin that eventually allow penetration of compounds.

Supporting the first theory, a study conducted on the percutaneous penetration of baclofen through cadaver skin suggested that the polar pathway is intercellular and is made up of aqueous regions surrounded by polar lipids.[9]

A technique developed using fluorescent ultra deformable lipid vesicles with confocal laser microscopy allowed for visualization of the penetration pathway in intact skin.[1] It was observed that 3-10 corneocytes create "columns" that form a cluster. Corneocytes edges inside each cluster were found to intercalate extensively, but neighboring clusters were separated by gaps of a few micrometers. Lipid packing in the inter-cluster region showed less regularity in comparison to the packing within clusters. This technique also allowed the quantification of 2 different hydrophilic pathways: the inter-cluster route with low penetration resistance made up of around 1% of the total or about 20% of the pathway area of the skin. The inter-corneocyte pathway that showed higher penetration resistance exhibited about 3% of the skin, or 80% of the pathway area. This later route was

found to be twisted as it goes between the corneocytes in a cluster and traces the irregularities between the intercellular lipid lamellae and/or the adjacent corneocytes envelopes that may act as virtual channels in the skin.

Most research indicates that classic multi-layered phospholipid liposomes create a skin reservoir and would not be the carrier of choice for transdermal delivery.

Ways to Enhance Skin Penetration

There is more than one way to differentiate and classify skin penetration enhancers. They can be classified according to their ability to carry different molecules or according to their mechanism of enhancement. Another differentiation would be their preferred route of penetration. This classification divides penetration enhancers into three main categories:[2]

- Solvents that enhance penetration through both polar and non-polar pathways such as 2-pyrrolidone, N-methyl pyrrolidone, N-methylformamide and propylene glycol in combination with azone
- Enhancers that preferentially affect the polar route, such as propylene glycol, in combination with decylmethylsulfoxide, and
- Enhancers that mainly modify the non-polar route, such as propylene glycol and oleic acid, propylene glycol alone and to limited extent water.

Penetration enhancers enhance transport of polar molecules by three major mechanisms:[9]

- Extracting the SC lipids
- Inducing a relaxation of the polymeric structure of the cytoplasmic matrix in keratinocytes, and
- Changing the solvent properties of the SC.

Surfactants, for example, were shown to enhance transport of polar molecules by solubilization and removal of intercellular lipids and binding to keratin filaments of the intracellular matrix. This resulted in cell order disruption. Non-ionic surfactants were shown to demonstrate fluidizing effect on the SC. Skin pretreated with gels containing various non-ionic surfactants showed a loosely layered SC and wide intercellular spaces.[10]

Skin penetration enhancers differ in structure, properties and mechanism of action, as will be shown in the following discussion of enzymes, chemical enhancers, vesicular systems, and ceramides.

Enzymes: The approach of using enzymes to affect the barrier properties of the SC refers to either affecting activity of enzymes present in the skin, or to enzymes applied to it from the outside.

Each of the three key lipid classes in the SC (fatty acids, cholesterol and ceramides) is required for normal barrier

function. Inhibition of the generation of any of these components can delay the barrier recovery after barrier insult.[11] Inhibition of fatty acid synthesis (by 5-(tetradecyloxy)-2- furancarboxylic acid), or cholesterol (by fluvastatin) resulted in increased percutaneous absorption of lidocaine. Modulation of epidermal lipid biosynthesis, following application of conventional chemical penetration enhancers, can cause further increase in a compound's delivery across the skin creating a simultaneous effect of interference with barrier homeostasis and thermodynamics.

The same effect of creating a controlled metabolic interference provides the rationale for the application of enzymes onto the skin. Different enzymes, depending on their biological function, can affect metabolism and biochemical cascades in the skin that eventually allow penetration of compounds. For example, an application of papain, a proteolytic enzyme that was conjugated to SC-glucan revealed an enhancement of percutaneous absorption. This application triggered structural changes in the SC that led to an increase in thickness of the SC and the living epidermis. Application of the enzyme also induced subtissues phase separation, lamellar bodies formation and created disorder in lamellar structures in the SC. It is hypothesized that these structural changes were induced by the hydrolysis of the cross-linkage between corneocyte envelopes and intracellular proteins. The SC-glucan-papaine conjugate showed no irritation when applied to the skin.[12]

> It was concluded that branching of the alkyl chain reduces the ability of the enhancer to affect lipid fluidization in the SC lipid lamellae at the target site.

Chemical enhancers: This is probably the largest and most studied group of permeation enhancers mostly to allow transdermal delivery. Most chemical enhancers affect the intercellular lipid bilayers in the SC. They lead to a reversible deformation in the bilayer structure that allows the creation of various types of "openings" in the bilayers. The nature of these "openings" can vary. It can be triggering of a thermodynamic imbalance within the lipid domains leading

CERAMIDE NS

Ceramide NS, commonly referred to as ceramide 2, and its analogues are being used in formulations for topical application to treat dry, flaky skin. It is also claimed to slow down skin aging. Formulations containing ceramide NS are believed to support and reinforce the cutaneous barrier properties. All major sub-fractions of stratum corneum ceramides are to some extent generated from lamellar body derived glucosylceramides. Yet, it was shown that sphingomyelin-derived ceramides are required for normal barrier homeostasis. Moreover, sphingomyelin can be available for the generation of ceramides from two sources: the plasma membrane and the lamellar bodies. The epidermal sphingomyelin SM-1 (Figure 3) is an important precursor for ceramide NS.[22]

Ceramide NS was shown to inhibit cell proliferation and induce apoptosis. The hydrolysis of sphingomyelin was shown to lead to the generation of ceramide NS that is responsible for the formation of apoptotic bodies. Apoptosis is an active process of programmed cell death. It requires metabolic activity by the dying cell, and is often characterized by cleavage of the DNA into fragments. Induction of apoptosis is necessary when mature cells reach the end of their life cycle. Since the skin is a continuously renewing tissue, it is essential to maintain a "birth-death" cycle to clear the way for new, fresh cells.[23]

A synthetic analogue of ceramide NS, pseudo-ceramide 2 (N-stearoyl-DL-erythro-sphinganine), was shown to significantly decrease TEWL values when applied after stripping or treatment with sodium lauryl sulfate. The analogue was applied at 0.5% or 1% levels in a cream. The results of this study suggest that these NS ceramide analogues participate in the restructuring of the stratum corneum.[24]

It is known that while cleansing the skin, detergents may remove valuable skin lipids, hence, disrupting the epidermal barrier function and elevating TEWL.[25] Reduction in TEWL following an application of monoglycerides, squalene, cholesterol ester and pseudo-ceramide 2 was tested after the application of 5% sodium lauryl sulfate. Among the compounds tested, monoglyceride and ceramide 2 demonstrated the best results in restoring barrier function. It was concluded that the addition of ceramide NS into skin cleanser formulations could have a beneficial effect in the prevention of detergent induced barrier disruption. When applied to the skin, ceramide NS can be enzymatically converted to other ceramides and hence, may provide enhanced barrier properties. To achieve effective enzymatic recognition, it is crucial that ceramide NS will be in its pure optically active form.

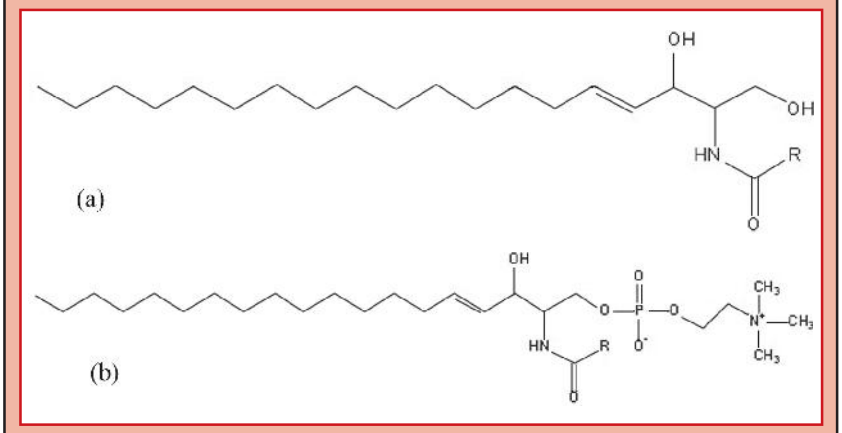

Figure 3. Chemical structures of ceramide (a) and sphingomyelin (b)

to increased lipid fluidity or creation of actual microscopically visual pores.

Various esters and fatty alcohols are known to enhance skin penetration. Examples are isopropyl myristate and isopropyl alcohol. Both were shown to increase permeation through skin. When applied together, they demonstrated a synergetic enhancement of permeation. Their mechanisms of action were found to be different. While isopropyl myristate generated disordered bilayers in the corneocyte-bound lipids, isopropyl alcohol resulted in fluidization and disorder in the free bilayer structures of the intercellular lipids.[13]

When combining 2 or more skin penetration enhancers to improve skin permeation, it is recommended to choose enhancers with different mechanisms of action. This will prevent competition at the site of action and has a better probability of not provoking irritation.

Oleic acid was found to increase epidermal permeability through a mechanism involving the perturbation of the SC lipid bilayers.[14] A technique using electron microscopy with osmium or ruthenium tetroxide allowed for ultra structural examination of the SC after the application of oleic acid. It was observed that marked alteration in the SC occurred specifically in the intercellular spaces.

Vesicular systems: Vesicles are microscopic spheres, usually composed of amphiphilic molecules. Classic liposomes are composed of phospholipids. Topically applied vesicles can either mix with the SC lipid matrix or penetrate the SC by using the lipid-water interface of the intercellular matrix. A major force driving vesicle penetration through the skin may be the water gradient across the epidermis.[15]

When comparing the effect of classic lecithin-derived vesicles with SC derived vesicles, scientists claim 2 different opposing effects. Most research indicates that classic multi-layered phospholipid liposomes create a skin reservoir and would not be the carrier of choice for transdermal delivery.[16] SC lipid-based liposomes, on the other hand, were found to deliver a greater amount of radiolabeled marker to the deep layers of the skin (epidermis and dermis) when compared to classic lecithin liposomes. Classic liposomes were also shown to significantly reduce systemic absorption of the marker and reduced organ distribution. Liposome size was found to be a crucial factor in penetration. The larger the mean size, the poorer the penetration to SC layers.[17]

Contradicting evidence was found in a study were liposomes formed from phospholipids were compared with liposomes composed of lipids imitating the SC (ceramides, cholesterol, palmitic acid and cholesteryl sulfate).[18] In this study, phospholipids-composed liposomes were found to increase SC lipid bilayer disorder and increase percutaneous permeation. SC lipid-derived liposomes demonstrated high affinity to the SC and generated a reservoir. These liposomes were also observed to increase the order of lipids in the intercellular lamellae.

Ceramides: SC ceramides are fundamental to maintaining the skin barrier. Most ceramides and their analogues contribute to repair of a disturbed skin barrier and hence decrease skin permeability (see sidebar on ceramide NS).

The understanding of the effect created by application of ceramides to the skin is crucial to achieve desired results. While application of ceramides, in specific concentrations, and especially in combination with fatty acids and cholesterol, can contribute to an improvement in skin barrier resistance, certain ceramides, in certain concentrations, were shown to create an imbalance in the intercellular lamellar organization and to enhance skin penetration.

In a study conducted to understand the correlation between the chemical structure of enhancers and their enhancement efficacy, it was found that in some cases the polar head of an enhancer is responsible for the penetration and anchoring of a molecule into the SC.[19] This study, conducted with branched-chain alcohols as skin permeation enhancers, suggested that the branched-chain alcohols have lower enhancement capability than the primary alcohols of the same molecular weight. It was concluded that branching of the alkyl chain reduces the ability of the enhancer to affect lipid fluidization in the SC lamellae at the target site.

A similar study conducted with a series of ceramide analogues further supports this finding.[20] These analogues included different polar head groups and different chain lengths. Here, ceramides having the same chain length exhibited enhanced activity that is only dependent on their permeability coefficients. It was found that the hydrogen bonding ability of the ceramide is inversely related to the enhancement capacity. The same group of scientists also studied transdermal L-serine- and glycine-based ceramide analogues as permeation enhancers that are related to SC ceramides. The glycine-based ceramide analogue was shown to significantly enhance skin permeation of theophylline through human skin in vitro.[21]

Summary and Future Research

Although, as described in this paper, one can distinguish between different routes for penetration with different properties, most compounds applied to the skin will permeate through more than one pathway. A certain compound, however, depending on its characteristics and its vehicle may exhibit a preferred route of penetration. Still, there is a continuous need for models that mimic the SC layer and techniques that will enable the tracking of compounds applied to it.

When studying the penetration properties of a compound it is important to understand its possible penetration pathways. Changing its molecular properties, choosing a delivery system or a carrier formula, can alter its preferred route of penetration. Understanding the possible routes for penetration can provide tools for the design of an appropriate system that will deliver the molecule to the desired target of action.

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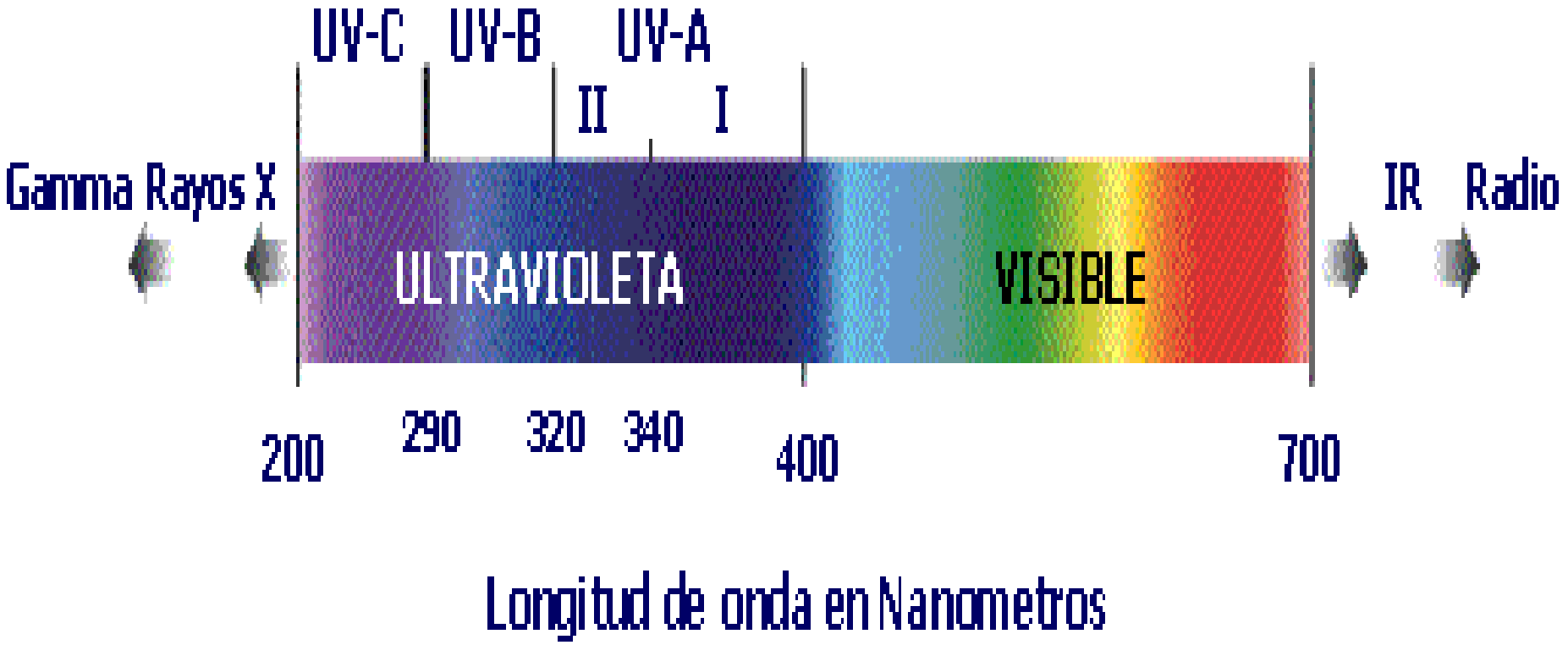
PRODUCTOS DE PROTECCIÓN SOLAR

PROF. OLOSMIRA CORREA


UV-C
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ULTRAVIOLETA
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Longitud de onda en Nanometros

RADIACIONES SOLARES

- La radiación UV-C (200-290 nm)
- tóxica y letal para microorganismos y plantas.
- Es filtrada casi totalmente por la capa de ozono.

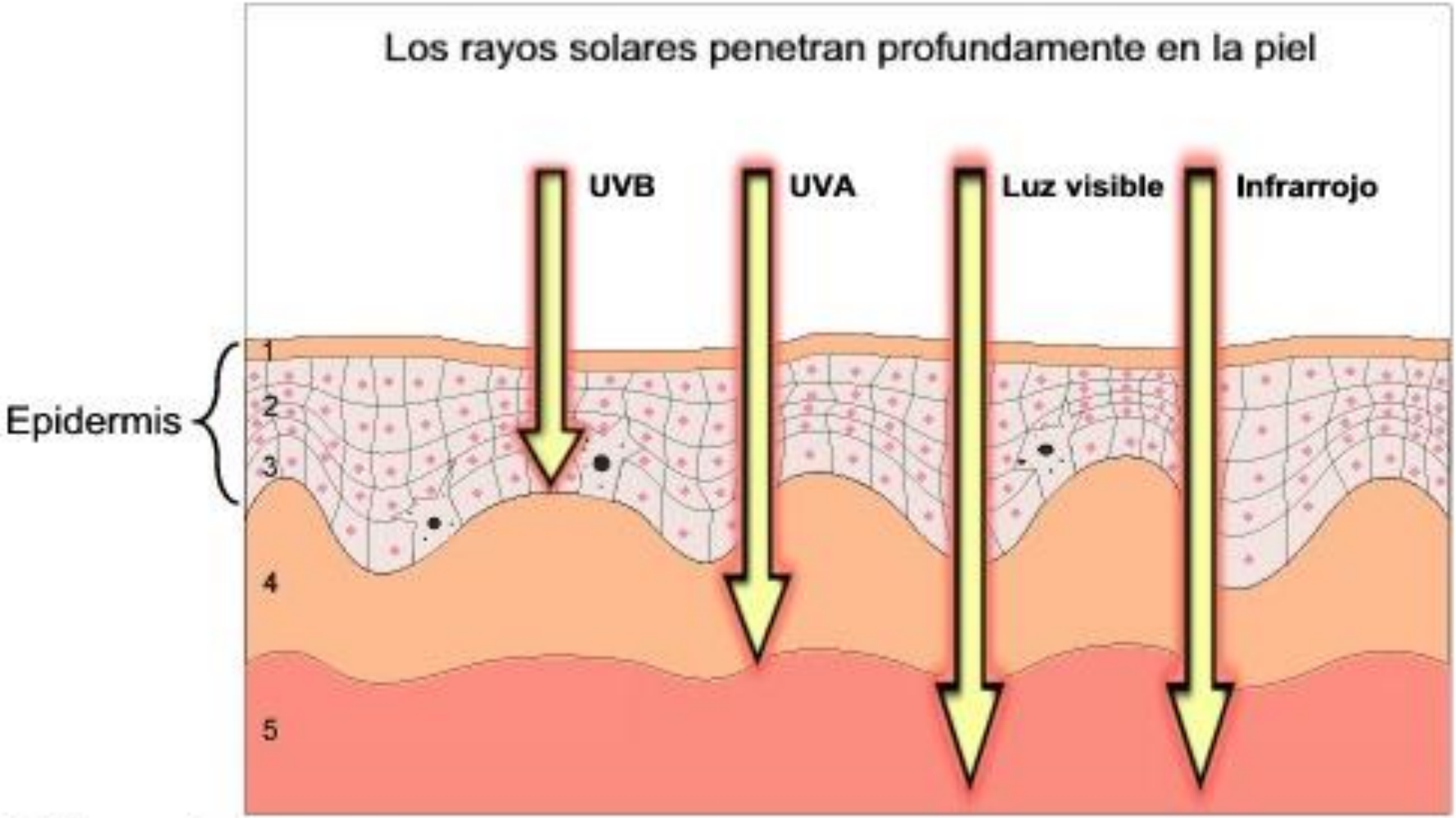
Energía es inversamente proporcional a longitud de onda

- UV-B (290-320 nm)
- causa de quemaduras solares (eritematógena)
- y de la pigmentación indirecta.
- transformación del ergosterol epidérmico en vitamina D
- penetra Epidermis

- La radiación UV-A (320-400nm)

no produce eritema pero posee poder pigmentógeno (melanina).

- Es responsable de la pigmentación directa (bronceado).
- Es la causa del fotoenvejecimiento
- relacionada con la fotosensibilidad de medicamentos o enfermedades.
- <u>Llega a la Dermis</u>



1 Capa córnea
2 Capa spino-celular
3 Capa basal y melanocitos
4 Dermis
5 Tejido subcutáneo

- Daño solar es acumulativo.
- 80% del daño solar de una persona ocurre antes de los 18 años.
- Los efectos nocivos del sol sobrevienen luego de cada exposición no protegida del sol.

MECANISMOS DE DEFENSA FRENTE AL UV

- **Síntesis de melanina**
- **Acido urocánico: Filtro natural**
- **Hiperqueratosis**

EFECTOS AGUDOS DE LA RADIACION SOLAR EXAGERADA

- Bronceado
- Quemadura solar
- Agravamiento de enfermedades dermatológicas
- Reacciones de fotosensibilidad a medicamentos

CLASIFICACION DE TIPOS DE PIEL Fitzpatrick

- **TIPO I** Piel Blanca que se quema con facilidad y NO se broncea.

TIPO II Piel Blanca que se quema con facilidad y se broncea minimamente

TIPO III Piel ligeramente Morena que se quema moderadamente y se broncea gradualmente

TIPO IV Piel Morena que se quema minimamente y se broncea bien

- **TIPO V** Piel muy Morena que dificilmente se quema y se broncea intensamente.

- **TIPO VI** Piel Negra que no se quema y de profunda pigmentacion.

FACTORES AUMENTAN EL RIESGO DE QUEMADURAS SOLARES

- **Hora del día**
- **Altitud** : El riesgo de quemaduras se incrementa con la altura.
- **Lugar geográfico**
- **Estación del año**
- **Agua, nieve, arena**

PRODUCTOS DE PROTECCIÓN SOLAR

- **Bronceador: Contienen filtros solares que absorben solo UV-B.**

- **PRODUCTOS DE PROTECCIÓN SOLAR:**

  Decreto:
  Artículo 1º.- Introdúcense las siguientes modificaciones al decreto supremo Nº 239 de 2002,
  "l) **Producto de Protección Solar o Protectores Solares**: Aquellos destinados a ser aplicados sobre la piel con la finalidad exclusiva o principal de protegerla de la radiación **ultravioleta A y/o B, ya sea absorbiéndola, dispersándola o reflejándola**.

FILTROS UV FÍSICOS (PANTALLAS)

Ventajas	Desventajas
○ Bajo riesgo de reacciones de sensibilización ○ Estables y seguros (únicos usados en pediatría)	○ Textura poco elegante al aplicar producto (efecto fantasma o mimo)

EJEMPLOS DE FILTROS SOLARES QUÍMICOS

Absorben UV - A	Absorben UV - B
Dioxibenzona (**benzofenona 8)** Metil antranilato Oxibenzona **(benzofenona 3**) Sulisobenzona (**benzofenona 4)**	Homosalato (homometil salicilato) Octocrileno **Metoxicinamato de octilo** Salicilato de octilo **Padimato O (Octil dimetil PABA)** Acido sulfónico Fenilbenzaimidazona Salicilato de TEA Metoxicinamato DEA

LISTADO DE FILTROS SOLARES PERMITIDOS EN COSMÉTICOS (CHILE)

Número	Ingredientes/INCI INCI Name	Concentración máxima autorizada
1	PABA	5%
2	Camphor Benzalkonium Methosulfate	6%
3	Homosalate	10 %
4	Benzofenone-3	10 %
5	-Potassium phenylbenzimidazole sulfonate -Sodium phenylbenzimidazole sulfonate -TEA-Phenylbenzimidazole sulfonate	8% (en ácido)
6	Terephthalydene dicamphor sulfonic acid	10% (en ácido)
7	Butyl methoxy Dibenzoiylmethano	5%
8	Benzylidene camphor sulfonic acid and salts	6%(en ácido)
9	Octocrylene	10% (como ácido)

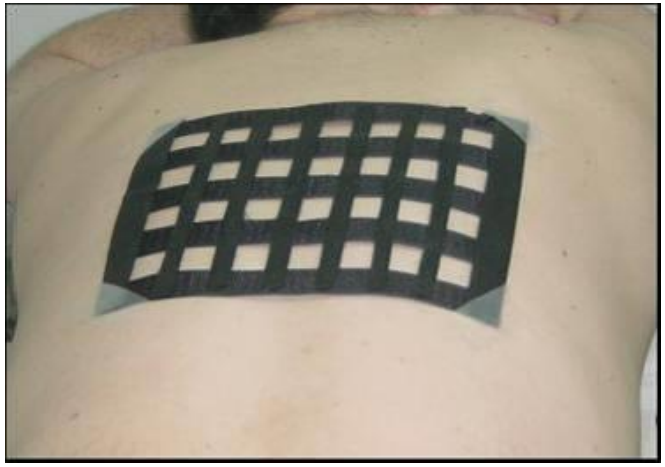
- 2.- Reemplázase el artículo 27° por el siguiente:
- b) Los productos cosméticos que deseen anunciarse **como protectores solares, cosméticos hipoalergénicos o cosméticos infantiles** deberán adjuntar un **resumen de los estudios técnicos que permitan avalar sus propiedades,** declarando que se mantendrán a disposición de la autoridad sanitaria, en todo momento, los estudios completos.

MÉTODOS PARA EVALUAR LA EFICACIA DE UN PROTECTOR SOLAR

- ***Métodos in vivo (SPF, PPD) solo estos son aceptados por el ISP***
- Métodos *in vitro (UTILIZAN EQUIPOS DE MEDICION ESPECTROFOTOMÉTRICA)*
- Métodos *in sillico (SE UTILIZAN PROGRAMAS COMPUTACIONALES Ej. Simulador empresa BASF on line)*

FACTOR DE PROTECCIÓN SOLAR (SPF)

MÉTODO *IN VIVO*



DIFERENTES METODOLOGÍAS PARA DETERMINAR FPS O SPF

- **COLIPA** o método europeo.
- Es el más ampliamente utilizado en la actualidad.

FACTOR DE PROTECCIÓN SOLAR

$$\text{FPS o SPF} = \frac{\text{DEM SOBRE PIEL PROTEGIDA}}{\text{DEM SOBRE PIEL } \underline{\textbf{NO}} \text{ PROTEGIDA}}$$

*DEM: Dosis eritematosa mínima es la **cantidad de energía que produce eritema** (Joules por m2) requerida para producir la primera reacción de enrojecimiento perceptible con bordes claramente definidos"*

FACTOR DE PROTECCIÓN SOLAR (SPF O FPS)

Ej: DEM sin protección = 6 minutos

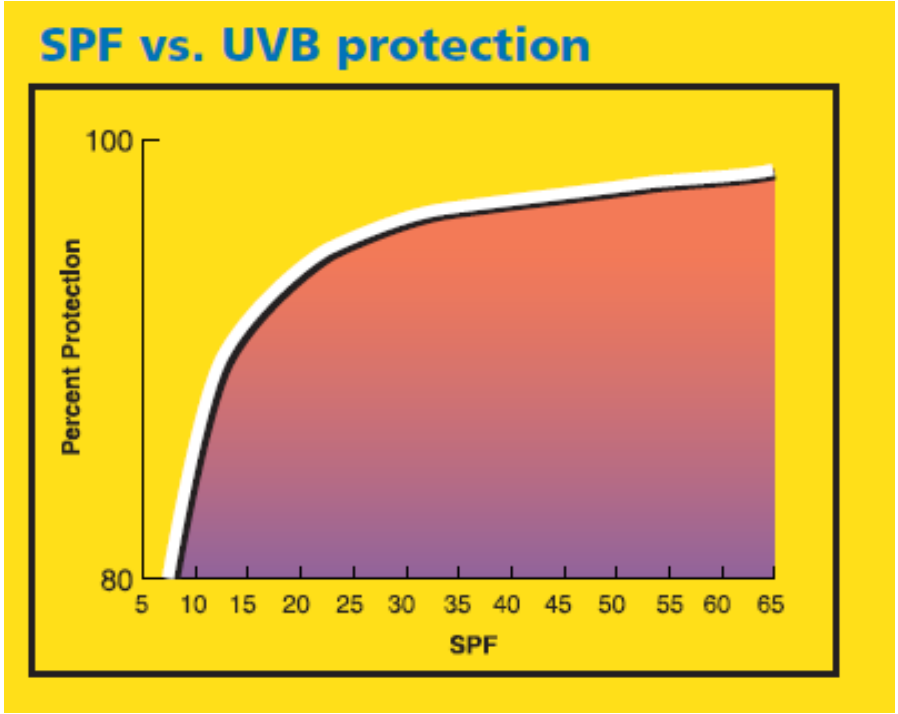
DEM con protección= 180 min

SPF = 180/6 $\Rightarrow$ SPF= 30

Proteje la piel durante tres horas ,

entonces debe aplicarse cada 3 hrs.

SPF	Proporción de UVB filtrada
SPF 10	90,0%
SPF 20	95,0%
SPF 30	96,7%
SPF 60	98,3%



SE RECOMIENDA SOBRE SPF 30

DETERMINACIÓN DE LA RESISTENCIA AL AGUA

Productos “resistentes al agua”	Productos “a prueba de agua”
Deben mantener su SPF luego de 40 minutos de inmersión, como se detalla: **Se aplica el producto incluyendo tiempo de espera de test SPF** **20 min. de actividad moderada en agua.** **20 min. de descanso.** **20 min. de actividad moderada en agua.** **Se seca la zona y se procede a determinar SPF.**	**Deben mantener su SPF luego de 80 minutos de inmersión, como se detalla:** **Se aplica el producto incluyendo tiempo de espera de test SPF** **20 min. de actividad moderada en agua.** **20 min. de descanso.** **20 min. de actividad moderada en agua.** **20 min. de descanso** **20 min. de actividad moderada en agua.** **20 min. de descanso** **20 min. de actividad moderada en agua.** **Se seca la zona y se procede a determinar SPF.**

Se utiliza una piscina o jacuzzi mantenido entre 23 y 32ºC.

MÉTODO PPD (PERSISTENT PERMANENT DARKENING) *IN VIVO*

- No es un método oficial para evaluar protección UVA pero es el que se ve con mayor proyección.

- Se realiza irradiando la piel con radiación UV-A, obteniendo como respuesta de la piel la formación de melanina (inicio del bronceado).

MÉTODO PPD

$$\mathbf{PPD} = \frac{\textit{Dosis mínima de radiación para oscurecer la piel con protector}}{\textit{Dosis mínima de radiación para oscurecer la piel sin protector}}$$

FACTORES QUE INFLUENCIAN LA EFICACIA DE LOS FOTOPROTECTORES

- **Solubilidad en el vehículo**
- **Espectro de absorción**
- **Cantidad y método de aplicación:**
  2 mg/cm^2 y 30 minutos antes de exponerse al sol.
- **Sustantividad**
- **Fotoestabilidad**

CONSIDERACIONES SOBRE LOS VEHÍCULOS EN PROTECTORES SOLARES

Para alcanzar factores de protección altos:

1. Depositar película de protector solar uniforme, gruesa y a prueba de agua.
2. Además se debe considerar una buena solubilidad del filtro en el vehículo.

CONSIDERACIONES SOBRE LOS VEHÍCULOS EN PROTECTORES SOLARES

Para obtener formulaciones resistentes al agua (waterproof) se debe usar:

- **Filtros solares insolubles en agua.**
- **Porcentaje alto de fase oleosa.**
- **Resinas resistentes al agua.**
- **Niveles bajos de emulgentes hidrofílicos.**

ROTULACIÓN FPS

Categoría a indicar en la etiqueta	Factor deprotección solar (FPS) a indicar en la etiqueta	Factor de protección solar medido
Protección Baja	6 10	6-9.9 10 - 14.9
Protección Media	15 20	15-19.9 20-24.9
Protección Alta	30 50	30-49.9 50-59.9
Protección Muy -Alta	50	+ Igual o mayor a 60