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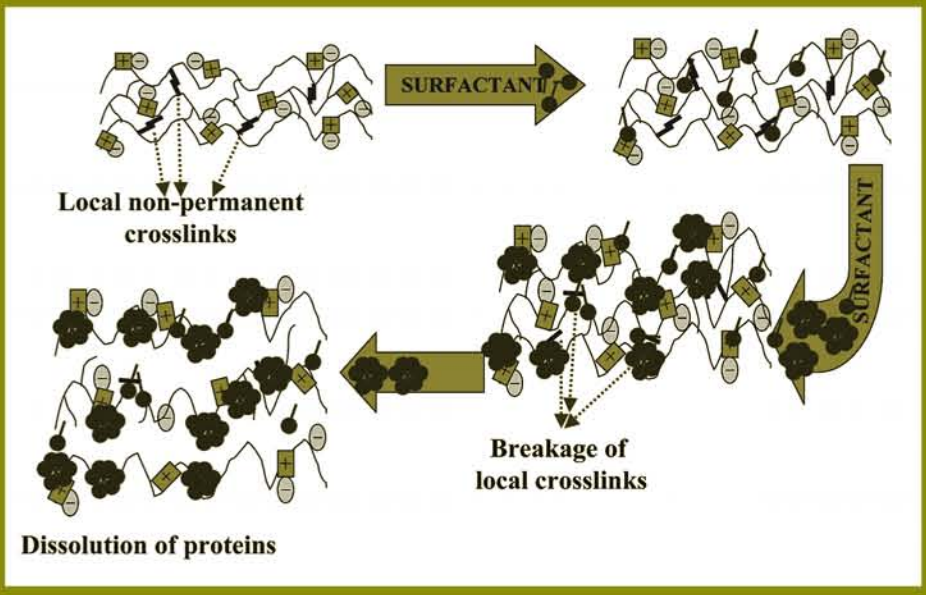
SURFACTANTS IN PERSONAL CARE PRODUCTS AND DECORATIVE COSMETICS

Third Edition



Edited by

Linda D. Rhein
Anthony O'Lenick
Mitchell Schlossman
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SURFACTANTS IN PERSONAL CARE PRODUCTS AND DECORATIVE COSMETICS

THIRD EDITION

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THIRD EDITION

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Preface

This is the third edition of *Surfactants in Cosmetics*. The first edition focused on such topics as the types of surfactants used in cosmetics, why they are needed, what functions the different structures serve, and the problems associated with their use in personal care products. The second edition covered fundamental physical chemical principles of surfactants in cosmetic emulsions, introducing multiple emulsions, phase inversion emulsions, microemulsions, vesicles, liposomes, solubilization in emulsions, and emulsion stability. The chemistry of interaction of surfactants with the substrates — skin and hair — and strategies to provide milder formulations or optimal cleansing were also provided in the second edition.

This edition now focuses, for the first time, on the use of surfactants in decorative cosmetics. The first few chapters cover fundamental aspects of the use of surfactants in personal care products and decorative cosmetics, discussing surfactant solution properties, surfactant emulsions, nanotechnology, cleanser/conditioner systems, and pigment dispersions. A review of fundamental skin science, including the rapidly advancing area of skin lipids, is included. Additionally, the measurement of skin color and the use of state-of-the-art noninvasive instrumental technology to measure efficacy of skin care cosmetic products are reviewed. Also provided is a chapter detailing strategies to assess consumer acceptability of cosmetic formulations.

The second part of this edition covers the role of surfactants in pigmented products such as nail enamel, lipsticks, makeup/foundations, sunscreens, self-tanners, and hair care products. The final section covers the use of specific surfactants with application to the formulation of decorative cosmetics and personal care products.

The editors thank the authors for their contributions and apologize for the significant delay in obtaining all the submissions. We also thank Taylor & Francis for inviting us to edit this new edition. We hope the readers find the edition valuable in their quest for providing more consumer-acceptable technologies for decorative cosmetics in the marketplace.

Editors

Linda D. Rhein, Ph.D., received her M.S. and Ph.D. in biochemistry and neurobiology from the University of Maryland and completed postdoctoral training at the University of Pennsylvania. She has worked in the cosmetic and OTC pharmaceutical industries for many companies for 25 years. She has been serving as editor of *Surfactants in Cosmetics*, part of the Surfactant Science Series. She has served as editor of the *Journal of Cosmetic Science* and as monograph editor for the society for several years. Currently, she is president-elect of the Society of Cosmetic Chemists and has served on their Committee on Scientific Affairs for numerous years. She was the recipient of the Women in Industry Award in 1987, as well as several awards from the Society of Cosmetic Chemists: the merit award in 2004, two best paper awards in 1985 and 1999, and the literature award in 1999. She is a member of the New York Academy of Sciences, American Association for the Advancement of Science, and the Scientific Society of Sigma Xi. Currently, she is an adjunct professor for the master's degree in cosmetic science program at Fairleigh Dickinson University, teaching biochemistry of skin, hair, and nails. She is best known for her innovative research contributions in skin lipid biophysical structure and its relevance to barrier function, and also mechanisms of surfactant damage to skin, prevention, and repair. Her research has resulted in more than 50 publications in scientific journals, and she has given over 25 invited lectures in her field.

Mitchell L. Schlossman received his B.A. from New York University and his M.A. from Kean University. Mitch was cofounder of Kobo Products, Inc. He has been part owner of Presperse, Inc., Tevco, Inc., Emery Industries, Inc., Pfizer, Inc., and Revlon, Inc. He is a member of the Society of Cosmetic Chemists (fellow) and past chairman of the New York Chapter of SCC (fellow). He has served as past director–east merit awardee and is also a member of the American Chemical Society, AIC (fellow), Chemist's Club, CIBS, and AAAS. He has served as editor of *Chemistry and Manufacture of Cosmetics*, third edition, has coauthored several books in the field of cosmetic chemistry, including publishing articles in numerous professional journals and trade magazines, and is the patentee of various U.S. and foreign patents.

P. Somasundaran, Ph.D., received his M.S. and Ph.D. from the University of California at Berkeley. Initially employed by the International Minerals and Chemical Corporation and Reynolds Industries, he was then appointed in 1983 as the first La von Duddleson Krumb Professor in the Columbia University School of Engineering and Applied Science. In 1987, he became the first director of the Langmuir Center for Colloids and Interfaces, and in 1998, the founding director

of the National Science Foundation Industry/University Cooperative Center for Advanced Studies in Novel Surfactants. He was also elected chairman of the Henry Krumb School at Columbia University in 1988 and 1991, and chair of the chemical engineering, material science, and mineral engineering department in 1992 and 1995. He was inducted in 1985 into the National Academy of Engineering, the highest professional distinction that can be conferred to an engineer at that time, in 1998 to the Chinese National Academy of Engineering, in 1999 to the Indian National Academy of Engineering and in 2000 to the Russian Academy of Natural Sciences. He is the recipient of the Antoine M. Gaudin Award (1982), the Mill Man of Distinction Award (1983), the Publication Board Award (1980), the Robert H. Richards Award (1987), the Arthur F. Taggart Award for best paper (1987), and Henry Krumb Lecturer of the Year (1989) and is a distinguished member (1983) of AIME. He is the recipient of the "Most Distinguished Achievement in Engineering" Award from AINA (1980). In addition, he was awarded the "Ellis Island Medal of Honor" in 1990 and the Engineering Foundation's 1992 Frank F. Aplan Award. This year he won the AIME Education Award (2006). He is the author/editor of 15 books and over 500 scientific publications and patents. He is the honorary editor-in-chief of the international journal *Colloids and Surfaces*. He has served on many international, national, and professional committees and National Research Council panels. He served on the Congress's 28th Environmental Advisory Committee, several NSF research panels, and Engineering Research Center review panels. He was the chairman of the board of the Engineering Foundation (1993 to 1995). He was member of the Committee on Scientific Affairs (COSA) of the Society of Cosmetic Chemists from 2003 to 2005. He has served on the board of the SME/AIME (1982 to 1985). His research interests are in surface and colloid chemistry, nanogel particles, liposomes, polymer/surfactant/protein systems, environmental engineering, molecular interactions at surfaces using advanced spectroscopy, flocculation, and nanotechnology.

Anthony J. O'Lenick, Jr., received his B.S. and M.S. in chemistry at Rutgers University. Tony O'Lenick is president of Siltech LLC, a silicone and surfactant specialty company he founded in 1989. Prior to that, Tony held technical and executive positions at Alkaril Chemicals, Inc., Henkel Corporation, and Mona Industries. He has been involved in the personal care market for over 30 years. Tony is the author of *Surfactants Chemistry and Properties*, published in 1999, and *Silicones for Personal Care*, published in 2003. He teaches continuing education courses in silicones and patent law for the SCC. He has also published over 45 technical articles in trade journals, contributed chapters to 5 books, and is the inventor on over 250 patents. He has received a number of awards for his work in chemistry, including the 1996 Samuel Rosen Award given by the American Oil Chemists' Society, the 1997, Innovative Use of Fatty Acids Award given by the Soap and Detergents Association, and the Partnership to the Personal Care Award given by the Advanced Technology Group. Tony was a member of the Committee on Scientific Affairs of the Society of Cosmetic Chemists.

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Contents

Part I

ASSESSMENT OF PERSONAL CARE AND DECORATIVE COSMETICS

1 Review of Skin Structure and Function with Special Focus on Stratum Corneum Lipid

Linda D. Rhein, Ph.D. and Svetlana Babajanyan, M.D.
Fairleigh Dickinson University

CONTENTS

1.1 INTRODUCTION

This chapter provides a review of the structure and function of skin. It will begin with a detailed discussion of the general structure of the epidermis and dermis, followed by the stratum corneum as a specialized part of the epidermis. Keratinization and epidermal renewel as an outcome of differentiation are detailed. After this, the primary focus is on lipids in the stratum corneum, namely, as a component of the protective membrane on the surface, i.e., the barrier that protects and retains moisture and interacts with topical products of interest to the cosmetic and pharmaceutical industry.

1.2 OVERVIEW OF THE STRUCTURE AND FUNCTION OF SKIN

Skin is a formidable physical barrier that protects us from the environment. It has become particularly adapted to withstand desiccation, allowing us to live in a nonaqueous environment, and is essential in thermal regulation of body temperature.

The skin is a continuous membrane or sheet covering the entire body surface. It is composed of two main layers:[1,2]

- Epidermis
- Dermis

The relative and total thickness of the two layers varies over different regions of the body.[1] The epidermis is thickest on the palms and soles of the feet. The dermis is thickest on the back and thinnest on the palms. The various layers of skin are shown in the histological cross section taken from a biopsy of skin in Figure 1.1.

The epidermis is the uppermost layer, and its purpose is to generate the stratum corneum, the so-called horny layer, which is the most superficial layer of dead cells and is the protective layer for the entire body — from dehydration and damage from foreign substances. The epidermis also interacts with stimuli from both the blood and the outside and is programmed to respond in various ways to the stimuli in an effort to preserve the protective function of the layer. It contains three main living cell types: keratinocytes, Langerhans cells, and melanocytes. Keratinocytes are the major cell type and eventually are converted by programmed cell death to corneocytes that make up the dead upper layer. The Langerhans cells are also called dendritic cells and provide the immune function in the epidermis, and the melanocytes provide the color of skin. These will be discussed later.

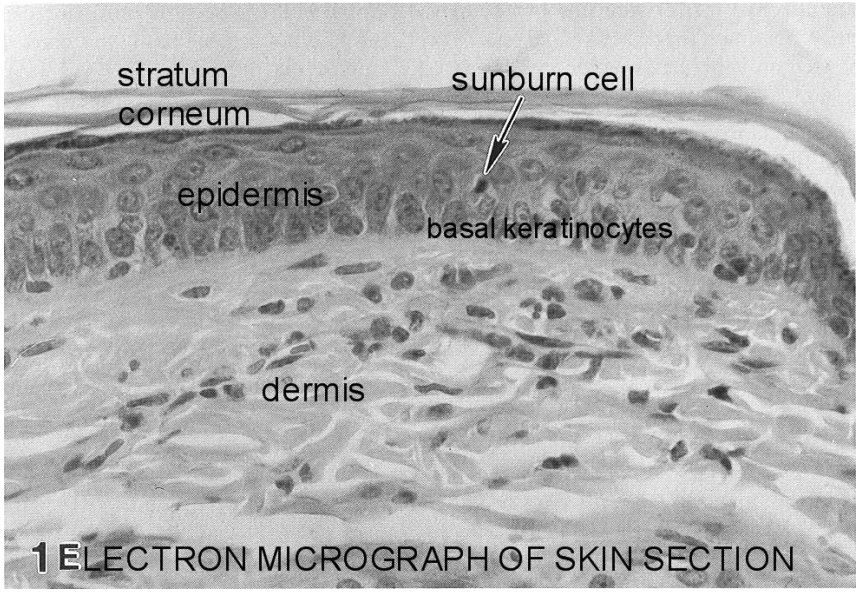


FIGURE 1.1 Cross section of the epidermis and dermis. The epidermis is a self-renewing stratified epithelium composed mostly of keratinocytes. It takes about 1 month from the time a basal cell leaves the bottom layer until it is desquamated or sloughed off from the surface. Differentiation is a genetically programmed event (apoptosis) that begins with a postmitotic keratinocyte and terminates with dead cells. The keratinocytes are organized into various layers that represent different stages of differentiation, as illustrated in the schematic. The events of cell differentiation include: appearance of new organelles, reorganization of existing organelles, and loss of organelles; synthesis and modification of structural proteins, especially keratins; change in cell size and shape; specialization of cellular metabolism; changes in the properties of cell membranes; dehydration; and formation of the barrier. (From Kligman, *J. Soc. Cosmet. Sci.* 47, 135, 1996. With Permission).

The dermis is the thick, fibrous layer beneath the epidermis, as shown in Figure 1.1. It provides structure to the membrane such that the membrane can cover the organs underneath and protect them from damage — mechanical or other. The dermis contains nerves and blood vessels and has one main living cell type: fibroblasts that generate the fibrous material. It contains several connective tissue proteins: collagens, elastin, and proteoglycans. Collagen is the rigid scaffold that covers the body. Elastin provides elasticity and strength, and proteoglycans are involved in damage repair.

The focus for the purposes of this chapter will be on the epidermis.

1.2.1 The Epidermis

The epidermis is the surface layer of the skin. It has a ridged and patterned surface — you can clearly see on your fingertips. The structure of the epidermis is complex. Cells in the epidermis form a multilayered system. These cells change morphologically as they migrate across the epidermis to fulfill their functions, i.e., form a barrier. The epidermis does not contain blood vessels, nerves, or sweat glands.

The epidermis is in a continual state of renewal as the keratinocytes, the major cell type within the epidermis, are formed, mature, and die.[1] It is estimated that the epidermis completely renews itself every 45 to 75 days.[1]

Keratinocytes are so called because they contain large amounts of the protein keratin.[1] Keratin is a tough, insoluble protein that provides physical protection and rigidity and strength to the cells. Keratin is the same substance that makes up the bulk of hair and nails, as well as animal claws and horns, hence the origin of the term *horny layer.*

Keratinocytes are formed by cell division from the stem cells in the basal layer of the epidermis, shown in Figure 1.1.[2] The newly formed keratinocytes move upward through the epidermis, continually maturing and changing structure via a process called differentiation (Figure 1.2).[1] This process is actually one of regulated cell death or apoptosis. This culminates in the formation of dead cells called corneocytes in the uppermost layer — the stratum corneum. The various differentiated states can be clearly seen when you look at a cross section schematic of the epidermis, such as that in Figure 1.2.[3]

The epidermis is composed of four distinct layers, as shown in Figure 1.1 and Figure 1.2:

- Stratum corneum — horny layer
- Stratum granulosum — granular layer
- Stratum spinosum — spiny layer
- Stratum basale — basal layer

Each layer represents a progressive stage in the life cycle of a keratinocyte. The histological cross section taken from skin can be seen in Figure 1.1, and the schematic in Figure 1.2 depicts the various cell types present in each layer.

As newly formed keratinocytes move upward, they flatten, lose their nucleus, and die. They also begin to produce increasing amounts of keratin, so that cells in the uppermost layer, the stratum corneum, are completely filled with keratins. This process of cell maturation and increased keratin production is known as *keratinization*.[2] The terminal differentiation of epidermal keratinocytes is characterized by development-specific gene expression. For example, the proliferating relatively undifferentiated basal keratinocytes (found in the stratum basale) express keratin proteins K5 (58 kD) and K14 (50 kD), whereas the keratin filaments specifically expressed in suprabasal keratinocytes — the upper, differentiated layers — are K1 (67 kD) and K10 (56.5 kD), along with K2 and K11,

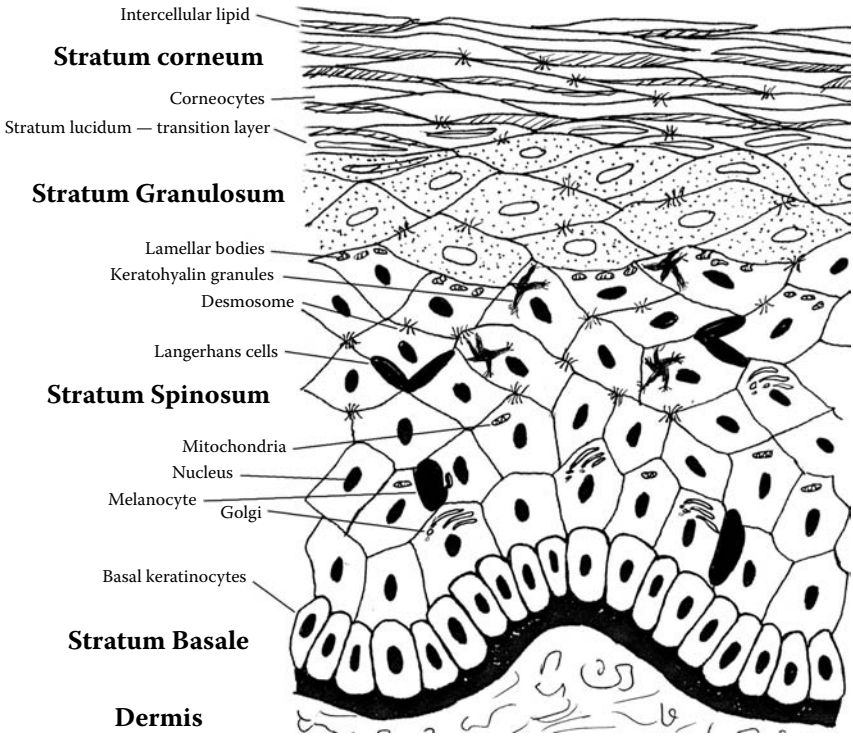


FIGURE 1.2 Fixed cross section of the outer layer of the skin, the epidermis, a stratified squamous epithelium, and the closely apposed sublayer, the dermis. It is from the malar eminence of a 20-year-old white woman. The majority of cells are keratinocytes, which are organized into layers. The layers are named for either their function or their structure. Cells interspersed among the keratinocytes may be lymphocytes (white blood cells), Langerhans cells (immune cells), melanocytes (pigment cells), or Merkel cells. The basal keratinocytes have cytoplasmic rootlets (serrations) that extend into the papillary dermis. In the malpighian layer, the keratinocytes gradually become flattened and are parallel to the surface. Keratohyalin granules develop in this layer. In the two to three layers of the stratum granulosum, the keratinocytes are stretched horizontally. The stratum lucidum is compact, and the stratum corneum appears lacy in its upper part. The stratum corneum has lipid in the intercellular space between proteinaceous corneocytes.

as well as retaining the basal keratinocyte-derived keratins K5 and K14.[4–6] The keratinocytes in the stratum granulosum are characterized by keratohyalin granules containing abundant quantities of profilaggrin and loricrin proteins.[5,6] As the terminally differentiated keratinocytes develop into corneocytes, their plasma membrane is transformed into the cornified envelope as a result of extensive cross-linking of involucrin and loricrin and other proline-rich structural proteins by the epidermal transglutaminases 1, 2, and 3 enzymes.[7] This calcium-dependent enzyme cross-links the ε-amino group of lysine on one chain to the γ-glytamyl

residue of glutamic acid on another chain. These cornified envelopes with extensive cross-linking render the stratum corneum resistant to degradation. Corneocytes are anuclear, postapoptotic (dead) keratinocytes that provide strength and rigidity to the stratum corneum. Figure 1.2 further exemplifies these structures and the subcellular structures in the epidermis.

Filaggrin is derived from keratohyalin granules, as are other keratinaceous proteins, shown in Figure 1.2. Filaggrin helps aggregate or stack the keratin fibers[8] and is ultimately degraded in the upper layers to form amino acids, pyrrolidone carboxylic acid, and lactic acid in the stratum corneum. These are thought to function as a natural moisturizing factor, an old concept in skin moisturization. The precursor to filaggrin is profilaggrin, which contains many filaggrin chains joined by tyrosine-rich linker regions; it is highly phosphorylated on the serine residue. Each repeating unit of profilaggrin has 10 to 20 phosphates that prevent its interaction with keratin. When profilaggrin is processed to filaggrin, all the phosphates are removed and the linker regions cleaved. After the keratin is aggregated by filaggrin, the arginine of filaggrin is converted to the uncharged citrulline, thereby dissociating it from the keratin. Then it is digested by proteolytic enzymes to the natural moisturizing factors mentioned above.[9]

The cells in the outermost layer of the stratum corneum flake off and are replaced by new cells from below.[1] It is estimated that a new cell takes 28 days[1] to differentiate and reach the beginning or bottom of the stratum corneum (recall from previous discussions that it takes 45 to 75 days for a new cell to reach the top of the stratum corneum). It is also estimated that each day, one layer of the stratum corneum is shed and one is synthesized from the stratum granulosum below.[10] The entire stratum corneum is shed in approximately 14 days. The rate of addition of new keratinocytes to the stratum corneum is in balance with the rate of loss of dead keratinocytes from its surface. It is this balance in the rate of proliferation and differentiation that is thought to maintain a healthy skin barrier. When this balance is disturbed, such as in diseases like psoriasis, where cells proliferate much too fast,[11] the barrier becomes severely compromised and exhibits excessive scaling, itching, and ultimately inflammation.

This balance between proliferation and differentiation is thought to be regulated by nuclear hormone receptors such as the retinoid receptor, vitamin D receptor, peroxisome proliferator-activated receptor (PPAR), and others, along with the T-lymphocyte-derived cytokine balance. The details of this regulation are still not well understood.[12–14] Nuclear hormone receptors are transcription factors that regulate cellular functions, including differentiation and proliferation. Nuclear hormone receptors that heterodimerize with the retinoid receptor and the vitamin D receptor regulate expression of differentiation-specific genes coding for enzymes, proteins, and lipids that support differentiation and development of a healthy stratum corneum barrier. Some of these are transglutaminases, involucrin, loricrin, and the enzymes that form the barrier lipids. Agonists of these nuclear hormone receptors alter barrier formation.

Studies[12–14] have demonstrated that ligands that bind to PPAR receptors stimulate differentiation and inhibit proliferation in cultured human keratinocytes and

accelerate epidermal development and permeability barrier formation in fetal rat skin explants. Topically applied PPARalpha ligands regulate keratinocyte differentiation in mouse epidermis *in vivo*. For example, topical treatment with PPARalpha activators resulted in decreased epidermal thickness. Furthermore, topically applied PPARalpha activators also decreased cell proliferation and accelerated recovery of barrier function following acute barrier abrogation. Expression of differentiation-specific structural proteins of the upper spinous/granular layers (involucrin, profilaggrin–filaggrin, loricrin) also increased. Studies with PPARalpha–/– knockout mice showed that these effects are specifically mediated via PPARalpha.[12] Additionally, in cultured human keratinocytes, this group has demonstrated that PPARalpha activators induce an increase in involucrin mRNA and an increase in gene expression that requires an intact AP-1 DNA response element. All studies support the role of PPARalpha in potentiating keratinocyte/epidermal differentiation and inhibiting proliferation.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that regulate the expression of target genes involved in many cellular functions, including cell proliferation, differentiation, and immune/inflammation response.[12–14] The PPAR subfamily consists of three isotypes: PPARalpha, PPARbeta/delta, and PPARgamma, which have all been identified in keratinocytes. PPARbeta/delta is the predominant subtype in human keratinocytes, whereas PPARalpha and PPARgamma are expressed at much lower levels and increase significantly upon keratinocyte differentiation. PPARbeta/delta is not linked to differentiation, but is significantly upregulated upon various conditions that result in keratinocyte proliferation, and during skin wound healing. *In vitro* and *in vivo* evidence suggest that PPARs appear to play an important role in skin barrier permeability, inhibiting epidermal cell growth, promoting epidermal terminal differentiation, and regulating skin inflammatory response by diverse mechanisms. These properties are pointing in the direction of PPARs being key regulators of skin conditions characterized by hyperproliferation, inflammatory infiltrates, and aberrant differentiation, such as psoriasis, but may also have clinical implications in inflammatory skin disease (e.g., atopic dermatitis), proliferative skin disease, wound healing, and acne.

It has been known for decades that the level of calcium *in situ* regulates growth vs. differentiation of epidermal keratinocytes. Mammalian epidermis normally displays a distinctive calcium gradient, with low levels in the basal/spinous layers and high levels in the stratum granulosum.[15] Changes in stratum granulosum calcium gradients regulate the lamellar body secretory response to permeability barrier alterations as well as the expression of differentiation-specific proteins *in vivo*. Elias et al.[15] has shown that acute barrier perturbations reduce calcium levels in stratum granulosum. Further studies showed that mouse epidermal differentiation can be regulated after loss of calcium due to acute barrier disruption by exposure of such acutely perturbed skin to either low (0.03 *M*) or high (1.8 *M*) calcium. For example, a few hours after acute barrier disruption, coincident with reduced calcium, there is ultrastructural evidence of accelerated lamellar body secretion and decreased mRNA levels for loricrin, profilaggrin, and involucrin in

the outer epidermis. Moreover, exposure of disrupted skin to low-calcium solutions sustained the reduction in mRNA levels, whereas exposure to high-calcium solutions restored normal mRNA. Finally, with prolonged exposure to a low (<10%) relative humidity, calcium levels increased, but at high (>80%) relative humidity, calcium levels increased and then declined. Accordingly, mRNA and protein levels of the differentiation-specific markers increased at low and decreased at high relative humidities, respectively. These results provide direct evidence that acute and sustained fluctuations in epidermal calcium regulate expression of differentiation-specific proteins *in vivo*, and demonstrate that modulations in epidermal calcium coordinately regulate events late in epidermal differentiation that together form the barrier.

The roles of calcium and vitamin D are synergistic in the regulation of keratinocyte differentiation as reviewed by Bikle et al.[16] Both calcium and 1,25(OH) vitamin D promote the differentiation of keratinocytes *in vitro*. As discussed above, it is known that keratinocytes cultured in low calcium grow or proliferate, while keratinocytes grown in high calcium cease to grow and differentiate *in situ*. The production of 1,25(OH) vitamin D by keratinocytes, combined with the role of the epidermal calcium gradient in regulating keratinocyte differentiation *in vivo*, suggests the physiologic importance of this interaction. Calcium and 1,25(OH) vitamin D synergistically induce the differentiation-specific protein involucrin found in the cornified envelope. The involucrin promoter gene contains an AP-1 site essential for calcium and 1,25(OH)(2) vitamin D induction and an adjacent vitamin D receptor element essential for 1,25(OH)(2) vitamin D but not calcium induction. Calcium regulates coactivator complexes that bind to the vitamin D receptor. *In vivo* models support the importance of 1,25(OH) vitamin D–calcium interactions in epidermal differentiation. The epidermis of 1alphaOHase null mice fails to form a normal calcium gradient, has a reduced expression of proteins critical for barrier function, and shows little recovery of the permeability barrier when disrupted. Thus, *in vivo* and *in vitro*, calcium and 1,25(OH) vitamin D interact at multiple levels to regulate epidermal differentiation.

Retinoic acid receptors play a crucial role in tissue homeostasis, lipid metabolism, cellular differentiation, and proliferation.[17,18] These functions are in part controlled through the retinoid signaling pathway. Retinoids are compounds with pleiotropic functions and a relatively selective targeting of certain skin structures. They are vitamins, because retinol (vitamin A) is not synthesized in the body and must be derived from the diet, but also hormones with intracrine activity, because retinol is transformed into molecules that bind to nuclear receptors, exhibit their activity, and are subsequently inactivated. Retinoids are also therapeutically effective in the treatment of skin diseases (acne, psoriasis, and photoaging) and of some cancers.[17–22] Most of these effects are the consequences of retinoic acid activation of a series of nuclear hormone receptors, including RAR and RXR, which trigger transcriptional events leading to either transcriptional activation or suppression of retinoid-controlled genes. Synthetic ligands are able to mimic part of the biological effects of the natural retinoic acid receptors, all-trans retinoic acid. Therefore, retinoic acid receptors are valuable therapeutic targets, and

limiting unwanted secondary effects due to retinoid treatment requires a molecular knowledge of retinoic acid receptors' biology.

For example, in the case of photodamage, ultraviolet (UV) irradiation of human skin sets in motion a complex sequence of events that causes damage to the dermal matrix.[19,20] When topical tretinoin is applied to human skin, any collagen deficiency existing in photoaged skin is remedied at least partially, and skin metabolic activity is primed to prevent further dermal matrix degradation induced by UV radiation. Production of procollagen is increased to augment the formation of types I and III collagen, and retinoids block expression of mediators of inflammation. Retinoids have therefore become essential in the treatment and prevention of photoaging.

Retinoids also play a vital role in the treatment of acne because they act on the primary preacne lesion, the microcomedo.[21,22] Several retinoid compounds are used for treating acne, in either topical or systemic form, for example, tretinoin (all-trans retinoic acid), isotretinoin (13-cis-retinoic acid), adapalene (derived from naphthoic acid), and tazarotene (acetylenic retinoid). They act mainly as comedolytics, but anti-inflammatory actions have also been recently discovered. The retinoids have great beneficial effects, but also some adverse effects, the main one being teratogenicity (causing birth defects). It is preferrable not to use them in topical form for pregnant women, although a pregnancy test is only compulsory for Accutane® and tazarotene. Only isotretinoin is used in systemic (oral tablet) form: Accutane. Isotretinoin acts on most of the factors causing acne (over active sebaceous glands, inflammation, and blocked pilosebaceous units) and offers long remissions, and sometimes complete cures. Precautions must be taken for women of childbearing age due to its teratogenicity. It is also important to be aware of its other adverse effects, explain them to the patient, and, if possible, deal with them in advance.

Although keratinocytes are the major cells within the epidermis, melanocytes (Figure 1.1 and Figure 1.2) also play an important role in skin barrier function and specifically confer skin color. There is approximately 1 melanocyte for every 36 keratinocytes in the human epidermis. Together these form an epidermal melanin unit.[23] Melanocytes synthesize melanin, a compound that absorbs UV light and is responsible for substantial protection against UV radiation.[23] Melanin is secreted by the melanocytes into the associated keratinocytes in the melanin unit. Melanocyte function is able to determine skin color. It is not dependent on melanocyte number (this is constant across racial groups), but upon the organization of the epidermal melanin unit.[23]

Of the four epidermal layers, the one of most importance in terms of topical treatment for skin disorders and enhancing appearance of skin is the stratum corneum.

1.2.2 The Stratum Corneum[10]

The stratum corneum is the outermost layer of the epidermis. It is made up of approximately 15 layers of flat, dead cells called corneocytes that are completely

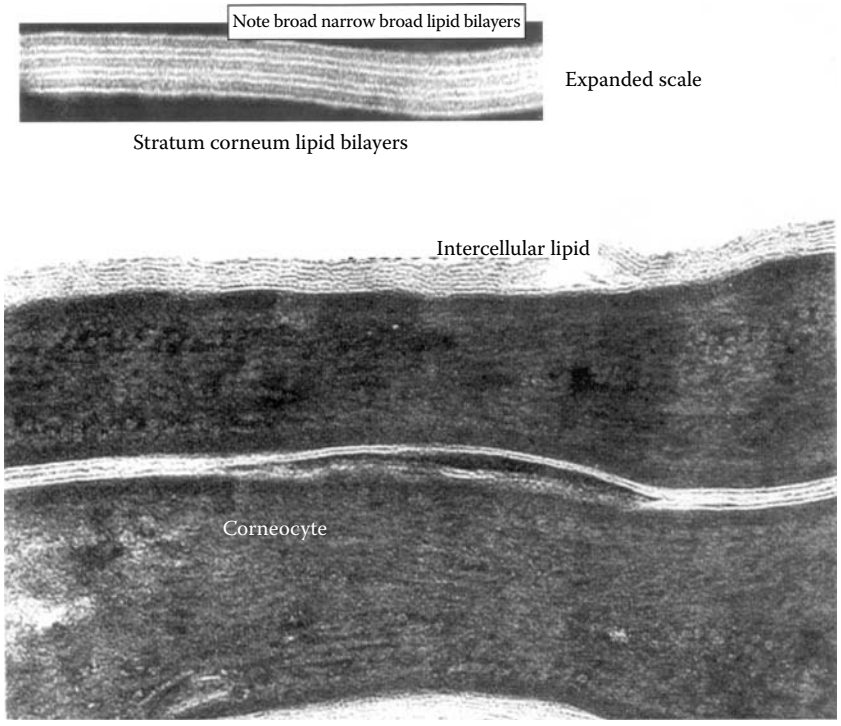


FIGURE 1.3 Transmission electron micrographs of stratum corneum of mammalian species depicting the intercellular lamellae. Note the multilayered repeat units (arrows). Postfixation is with ruthenium tetroxide to show lipid. (Modified from Rawlings, A.V., *J. Cosmet. Sci.* 47, 32, 1996. With permission.)

filled with keratin (Figure 1.3). For this reason, it is sometimes called the horny layer. The highly keratinized cells of the stratum corneum are packed tightly together and are surrounded by a multilayered lipid structure.[10] This tightly packed structure is often compared to bricks and mortar — providing structural strength through the keratin-filled bricks and waterproofing through the layer of lipid mortar (Figure 1.3).

The cells of the horny layer are constantly worn away and replaced by newly keratinized cells, in a continual cycle. The stratum corneum itself is replaced about every 14 days; that is, it takes about 14 days for a dead cell to move from the bottom of the stratum corneum to the surface of the membrane. This cycle of keratinization, cell loss, and replacement is important because a variety of skin problems, including ichthyosis, psoriasis, atopic eczema, and acne, involve disturbances of the normal sequence. The cycle may be disturbed by a variety of factors, including exposure to substances such as cosmetics, low relative humidity, cold weather, vitamin A, steroid hormones or drugs, as well as chemical messengers produced by the cells themselves or a disease, e.g., psoriasis, that is characterized by accelerated cell growth or proliferation.[11]

The stratum corneum is composed of lipids and proteins. Some of the proteins that originate from the differentiating keratinocyte and the keratohyalin granules visible in Figure 1.2 were discussed in the last section, but a detailed discussion of the lipids found in the stratum corneum is in order for the purposes of this chapter. Lipids constitute about ~10% of the wet weight or <30% of the dry weight of the stratum corneum. Lipids in the stratum corneum originate from the lamellar bodies shown in Figure 1.2. Lamellar bodies are secretory granules originating from the Golgi. The lamellar body attaches to the cell membrane of the differentiated keratinocytes, and their lipid contents are extruded into the intercellular spaces around the corneocytes (dead keratinocytes) in the upper part. They are then modified by enzymes present in the intercellular spaces to produce the intercellular lipids surrounding the corneocytes found in the upper layers of the stratum corneum.[24–26] Lipids in the upper layers contain predominantly cholesterol, ceramides, and free fatty acids in a ratio of approximately 25:50:15, as weight percent, with 10% other minor lipids.[24,25] They are unique because they do not contain phospholipids, which are typically found in other biological membranes. During the process of modification to produce the stratum corneum lipids, phospholipids present in the lamellar body secretions are completely abolished and converted to free fatty acids; glucosyl ceramides also present in the secretions are converted to ceramides, and cholesterol sulfate is converted to free cholesterol.[24,25]

Table 1.1 exemplifies this lipid modification process in the various layers of the epidermis.

Also, it is important to note that on the skin surface, the sebaceous lipids originating from the sebaceous glands will become an important entity, along with the interactions and mixing of sebaceous lipids with the epidermal lipids. Sebaceous lipids are enriched in triglycerides, wax esters, cholesterol, and free fatty acids; the latter can vary, depending on the enrichment of microorganisms on the skin surface that convert triglycerides to free fatty acids, primarily *Propionibacterium acnes*.[27–30]

TABLE 1.1
Lipid Composition in Different Epidermal Layers (as Weight Percent)

Lipid Class	Basal/Spinous Layers	Granular Layer	Stratum Corneum
Phosopholipid	63	25	0
Glucosyl ceramide	7	10	0
Ceramides	0	15	50
Cholesterol	10	21	25
Free fatty acids	7	17	15
Other	13	12	10

Source: Review by Abraham, W., in *Surfactants in Cosmetics*, Rieger, M. and Rhein, L., Eds., Marcel Dekker, New York, 1997, chap. 20.

The lipids are organized in a multilayered structure (see Figure 1.3). The lipid bilayers are attached hydrophobically to the corneocyte envelope on the surface of the corneocyte; this envelope contains covalently attached omega-hydroxy ceramide and fatty acids, lipids that serve as a template for the lipid bilayer attachment (see review by Abraham[24]). The carbon chain length of the acyl ceramides has been extensively studied by Wertz and Downing[26] and is on average 24 carbons, compared to the average chain length of 16 to 18 for phospholipids typically found in other biological membranes, again suggesting that stratum corneum lipids are unique. The need for increased hydrophobicity of this membrane is thus evident from the structure. Additionally, the fatty acids, both free and esterified to sphingolipids are highly enriched in saturated species (>70%), although linoleic acid is also a major fatty acid in ceramide (~14% and more). Details on their exact compositions can be found in additional references.[31,32] The enrichment of longer chains and saturation both attest to the hydrophobic nature of the barrier. More details on the lipids in skin can be found in a review by Wertz.[25]

In healthy skin, the stratum corneum is an effective barrier against UV light, heat or cold, bacteria, mechanical disruption, and many chemicals.[2] Some oils and alcohols, however, penetrate the stratum corneum layer quite well. This is why many skin preparations contain or are based on these components, to help carry the active ingredients into the deeper layers of cells. The stratum corneum is responsible for preventing both water loss from and environmental insult to the skin.[2]

A more definitive role of stratum corneum lipids in maintaining a healthy barrier is discussed in the next section.

1.3 ORIGIN AND FUNCTION OF STRATUM CORNEUM LIPIDS

Elias and Feingold[33] very effectively reviewed the origin of current concepts of stratum corneum and lipid structure. The old perception of mammalian stratum corneum, based on histological studies, was that of a loosely bound layer in various stages of disorganization and sloughing off. Table 1.2 displays the stages of understanding of the characteristics of this membrane.

This misleading image of a disorganized structure was refuted by physical-chemical, frozen-section, and freeze–fracture studies. The histological fixation

TABLE 1.2
Stages in the Understanding of Stratum Corneum Structure

Concepts	Approximate Date Observed
1. Disorganized, nonfunctional	Through 1950
2. Homogenous film	Up to the 1970s
3. Lipid–protein compartmentalization	1975–present
4. Metabolically active	1984–present

technique had resulted in destruction of the membrane. Thus, the ultrastructural preparations obscured further advances until the 1970s, therefore confusing recognition of the actual structure. Initially these studies showed a membrane film covering the stratum granulosum and led to the hypothesis that the stratum corneum was a homogeneous film. The freeze–fracture studies later refuted this and established that the layer was composed of tightly arrayed polyhedral structures in vertical interlocking columns. The initial awareness of lipid–protein segregation to specific tissue compartments came from freeze–fracture replication studies, which revealed the presence of multiple broad lamellations in the interstices of several types of mammalian keratinizing epithelia. X-ray diffraction studies previously demonstrated a highly ordered lipid structure in the stratum corneum that could account for the lamellations observed in freeze–fracture.

Definitive evidence for the compartmentalization of lipids came from the isolation of stratum corneum membrane "sandwiches" containing trapped intercellular lipids, reviewed by Elias and Feingold.[33] These structures that became visible with better staining techniques, i.e., ruthenium tetroxide postfixation, contained the same broad lamellae found in the interstices of the whole stratum structure (Figure 1.3). This came to be commonly referred to as the brick-and-mortar hypothesis for the stratum corneum structure, with the bricks being dead corneocytes and the mortar the intercellular lipid lamellae; this was discussed briefly earlier. Researchers from the Alza Corporation simultaneously reported the existence of lipophilic vs. hydrophilic pathways of percutaneous absorption,[34] which supported the idea of a protein pathway and an intercellular lipid pathway.

That the lipid part of the stratum corneum is metabolically active has also now been demonstrated by Elias and Feingold.[33] Co-localization of lipid catabolic enzymes within the intercellular lipid by both ultrastructural cytochemistry and enzyme biochemistry is further evidence for the structural heterogeneity of the stratum corneum. The fact that the phospholipids disappear in the stratum corneum and neutral lipids and sphingolipids (primarily ceramides) appear in abundance is now common knowledge (Table 1.1). The metabolic enzymes responsible for these changes are secreted along with the lipids into the intercellular spaces by the lamellar bodies (Figure 1.2 and Figure 1.4).[33,35] These enzymes are lipases, including acid lipase, phospholipase A, sphingomyelinase, steroid sulfatase, and acid phosphatase, and also proteases, like cathepsins and carboxypepidase. Figure 1.4 shows how the probarrier enzymes (phospholipase A, sphingomyelinase, and glucosidase) found both in the lamellar granules (except steroid sulfatase) and in the intercellular spaces work together to form the optimal intercellular lipids of the stratum corneum. Catabolic enzymes like acid phosphatase, proteases, other lipases, and glycosidases now work together to mediate normal desquamation of the stratum corneum.

Certain striking features of the modulation suggest that these alterations are necessary for optimal barrier function. Sphingolipids, in particular, have been cited as a critical molecule for barrier function because (1) they are the most abundant class of lipids, (2) they possess a long-chain hydrophobic base, (3) they have very long chain, highly saturated N-acylated fatty acids (such long chains

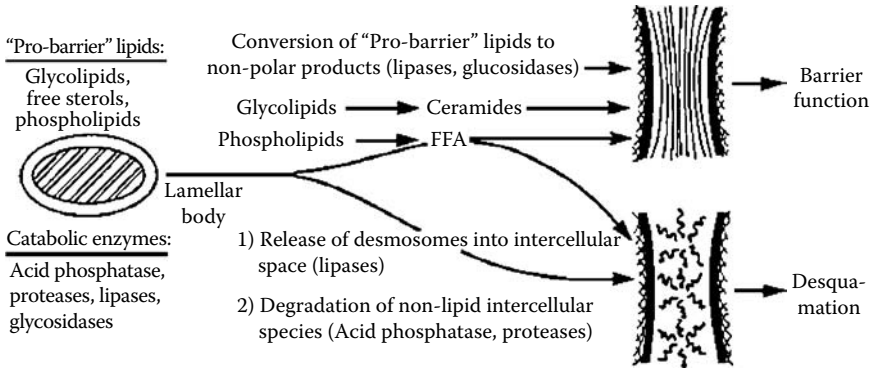


FIGURE 1.4 Scheme showing secretion of probarrier lipids and enzymes involved in lipid modulations and desquamation originating from lamellar body secretions.

have been postulated to span the bilayer), and (4) some species are the most abundant repository for an ester-linked linoleate residue (oleate substitution for linoleate is associated with a defective barrier in essential fatty acid deficiency).[24,36–38] The enrichment of longer chains and saturation both attest to the hydrophobic nature of the barrier. More details on the lipids in skin can be found in a review by Wertz.[25]

While these results allude to the importance of the sphingolipids in barrier function, they do not attest to the role of the neutral lipids, in particular free fatty acids and cholesterol. To establish a more definitive role of the lipids to barrier function, Elias took a more physiological approach. Elias studied hairless mice and damaged their skin barrier with either acetone or tape stripping. He tracked transepidermal water loss and the rate of its recovery along with the activity of enzymes involved in the synthesis of the three classes of stratum corneum lipids. After barrier abrogation, lipid synthesis increased rapidly, within 1 to 2 h. This paralleled the increase in activity of HMG-CoA reductase (rate-limiting enzyme in cholesterol biosynthesis), acetyl CoA carboxylase, and fatty acid synthase (the latter two are involved in fatty acid biosynthesis).[35,39] In contrast, the increase in sphingolipid synthesis and the activity of serine palmitoyl transferase (the first committed enzyme in sphingolipid biosynthesis) are delayed approximately 6 h after barrier disruption. Other studies have demonstrated that mRNA levels for HMG-CoA reductase, HMG-CoA synthase, farnesyl diphosphate synthase, squalene synthase, acetyl CoA carboxylate, and fatty acid synthase all increase soon after barrier abrogation. In contrast, sphingolipid synthesis and serine palmitoyl transferase, the first committed enzyme in sphingolipid biosynthesis, increase just a few hours later, suggesting regulation by a different mechanism.[35] As the mRNA levels for these proteins increase after either tape stripping or acetone treatment, these changes appear to be independent of the method of barrier perturbation. Moreover, the increase in mRNA levels for these enzymes is prevented by immediate occlusion

with a plastic wrap, which provides an artificial permeability barrier. These data indicate that the increase in mRNA levels for these enzymes is regulated by barrier function rather than simply representing a response to injury.

The findings also suggest that actives can be identified that would alter the expression or activity of the barrier-forming enzymes to reconstruct a healthy barrier. Tanno et al.[40] studied the action of nicotinamide, one of the B vitamins, on the barrier. Their group studied the effects of nicotinamide on biosynthesis of sphingolipids, including ceramides and other stratum corneum lipids, in cultured normal human keratinocytes, and on the epidermal permeability barrier *in vivo*. The rate of sphingolipid biosynthesis was measured by the incorporation of [14C]-serine into sphingolipids. They found that the rate of ceramide biosynthesis was increased dose dependently by 4.1- to 5.5-fold on the sixth day compared with the control. Nicotinamide also increased the synthesis of glucosyl ceramide (sevenfold) and sphingomyelin (threefold) in the same concentration range effective for ceramide synthesis. Furthermore, the activity of serine palmitoyl transferase (SPT), the rate-limiting enzyme in sphingolipid synthesis, was increased in nicotinamide-treated cells. Nicotinamide increased the levels of human LCB1 and LCB2 mRNA, both of which encode subunits of SPT. This suggested that the increase in SPT activity was due to an increase in SPT mRNA. Nicotinamide increased not only ceramide synthesis, but also free fatty acid (2.3-fold) and cholesterol (1.5-fold) synthesis. Topical application of nicotinamide increased ceramide and free fatty acid levels in the stratum corneum and decreased transepidermal water loss in dry skin. Thus, nicotinamide improved the permeability barrier by stimulating *de novo* synthesis of ceramides, with upregulation of SPT and other intercellular lipids.

Below is summarized the evidence that barrier function regulates epidermal lipogenesis (Elias and Feingold[33]), and Table 1.3 summarizes support that epidermal lipids regulate barrier function.

- Dietary or solvent-induced barrier disruption stimulates epidermal lipogenesis
- Extent of lipid biosynthesis rates parallels severity of barrier defect
- Normalization of lipid biosynthesis rates parallels barrier recovery
- Occlusion with impermeable, but not vapor-permeable membrane after barrier disruption blocks acceleration of lipid biosynthesis
- Application of inhibitors of enzymes that synthesize all three classes of barrier lipids delays recovery of barrier after barrier disruption

Thus, Elias's research has demonstrated the importance of all three lipid classes to optimal barrier function, not just the sphingolipids. He has also shown that regulation of epidermal lipogenesis is controlled by conditions at the surface of the skin; i.e., the presence of plastic wrap on top can stop lipogenesis, and damage to the lipids by solvent extraction potentiates lipogenesis. Table 1.3 builds on this and summarizes evidence that it is the lipids that mediate epidermal barrier function.

TABLE 1.3
Evidence That Lipids Mediate Mammalian Epidermal Barrier Function

Solvent/Detergent Treatment
Removal of stratum corneum lipids by either organic solvents or detergents perturbs barrier function
The extent of the solvent-induced defect in barrier function correlates with both the amount and type of lipid removal
Recovery of barrier function correlates with return of lipid or artificial application of the natural lipid
Essential Fatty Acid Deficiency
The barrier defect in essential fatty acid-deficient epidermis is associated with loss of intercellular lipids
The barrier defect is corrected by topical application of natural and synthetic lipids that fix barrier function independent of eicosanoid generation
Topographic Differences in Barrier Function
The barrier to water transport across the stratum corneum of different body sites is inversely proportional to the lipid content of those sites
Dry vs. Moist Keratinizing Epithelia
Epidermal lipids are more polar, and constituent fatty acids are more unsaturated in marine mammals than in terrestrial mammals
The lipids of oral mucosa are more polar, and these compositional changes reflect permeability differences at these sites
Metabolic
Inhibitors of barrier lipid biosynthetic enzymes delay recovery of barrier function

Source: Modified from Elias, P.M. and Feingold, K., *Ann. N.Y. Acad. Sci.*, 548, 4–13, 1988.

That the stratum corneum is the main barrier to water transport was shown very elegantly by Smith et al.[41] Fragments of stratum corneum were reduced to single cells by grinding with a motor and pestle, followed by exhaustive extraction of the intercellular lipids with either ether or 2:1 chloroform:methanol and with the aid of a ground glass homogenizer. They found that >95% of the lipids were removed. When single cells were placed on water as a thin film in chloroform:methanol and the solvent left to evaporate, the cells showed no evidence of reaggregation into a sheet and provided no barrier to water transport. Upon addition of the extracted lipid, the single cells aggregated into a sheet and provided a barrier to water permeability as measured by its diffusional resistance. This was also dependent on the amount of lipid added. At the optimal lipid-to-cell ratio of greater than 4% lipid (wt/wt) the diffusional resistance was 1.0 mg/cm-h. This is close to the value for intact stratum corneum. The ability of cells to reaggregate was eliminated if the cells were trypsinized, suggesting certain proteins are involved in the aggregation, and we speculate that these are the desmosomes

found in the intercellular spaces or the corneocyte envelopes that anchor the lipids' layers. Miriam Brysk et al. later found that triton X-100 also prevented reaggregation, and this may be caused by its binding to the covalently bound cell envelope lipid (omega-hydroxy ceramide) blocking the template upon which the bilayers are built.[42] Plantar cells were not as responsive to lipid reaggregation and offered a very poor barrier to water transport. Plantar skin is known for its excessive sweating, functionally supporting these findings and suggesting site differences.

Several authors also examined the water-holding capacity and skin condition before and after extraction of stratum corneum lipids from healthy adults (forearms) with organic solvents. Imokawa et al.[43] showed that removal of lipids increased with length of time of extraction with acetone ether and a dry scaly condition appeared that endured for more than 4 days. Shorter extraction times removed sebaceous lipids, and longer times removed stratum corneum lipids. The skin conductance went down, indicative of reduced water content. Application of extracted lipids from sebum (mostly wax esters and triglycerides) did not affect the conductance values, but application of the total stratum corneum epidermal lipid fraction or individual lipids (sterol ester, free fatty acid, cholesterol, ceramide, or glycolipids) significantly increased skin conductance, indicating an increase in water content of the membrane. Scaling also improved. Similar studies by Grubauer et al.[44] used hairless mice as the model and transepidermal water loss (TEWL) as the measure of barrier damage and came to similar conclusions regarding the importance of lipids to the water-holding capacity of the stratum corneum.

Using a structure activity study of 38 different ceramide derivatives, Imokawa et al.[45] showed that the presence of saturated straight alkyl chains with the absence of unsaturation or methyl branches in the ceramide species was optimal for water-retaining capacity after application to skin *in vivo*, as is characteristic of native stratum corneum ceramides. However, the preferred alkyl chains of C14 to C18 showed the greatest improvement in water-retaining capacity (measured by skin conductance); this differs from native ceramides, which tend to have very long chain lengths. Of course, one could argue that the barrier serves additional functions beyond water-holding capacity, i.e., normal desquamation, and it is possible that the longer chains are necessary for optimizing this function of the membrane.

1.4 SKIN LIPID MACROMOLECULAR STRUCTURE AND PUTATIVE ORGANIZATION

Friberg and colleagues[46–49] have studied the macromolecular structure of the stratum corneum lipids. Friberg combined all of the lipids found in the surface layers of stratum corneum with 32% water and obtained a milky mixture of multiple phases. Upon adjusting the pH to be between 4.5 and 5.5, i.e., in the range of stratum corneum pH (done by neutralizing 41% of the fatty acid), a liquid crystalline phase emerged. This was verified by photomicroscopy and by

small-angle x-ray diffraction. Diffraction patterns using small-angle measurements of the lipid model exhibited a broad reflection band with a maximum reflectance at 65 to 75 Å and were similar to that of intact isolated human stratum corneum. The microscopy patterns showed Maltese crosses and the typical light reflectance of a liquid crystal. Based on this effect, Friberg has proposed that the fatty acid/soap structure is the basis of the multilamellar structure. The layered structure formed even in the absence of all other lipids except the fatty acid/soap, but not with any of the other lipids alone, supporting the fatty acid/soap as the basis of the bilayer. However, this model does not contain the stratum corneum proteinaceous corneocytes with their covalently attached omega-hydroxy ceramide lipid template and the desmosomes and enzymes. Others have found a somewhat different pattern for murine stratum corneum; this will be discussed later.[36]

Friberg et al.[46–49] studied the effect of individually adding each of the lipids of stratum surface layers to the fatty acid/soap mixture on the initial interlayer spacing of the small-angle x-ray diffraction patterns generated by the fatty acid/soap. Changes in the interlayer spacing can provide information on the possible location of the specific lipids in the bilayer. They also varied the water content and tracked changes in the diffraction patterns upon dehydration of the lipid. Figure 1.5 shows the outcome of these experiments.

The lipids with polar head groups are postulated to be located between the chains, with the polar head group adjacent to the hydrophillic layer, e.g., fatty acid soaps and ceramides. Cholesterol also associates between the chains, and in fact, the data suggest that as more water is added to the bilayer, the cholesterol pushes some of the fatty acids out from between the chains to the hydrophobic methyl layer. This results in an increase in the interlayer spacing with added incremental water beyond what can be attributed only to the added water. Triglycerides and wax esters, surface lipids of mostly sebaceous origin, will exist in the hydrophobic layer along with squalene, and will therefore also increase the interlayer spacing upon their addition. The results shown in Figure 1.6 and Figure 1.7 for ceramides demonstrate its unique dependency on water content in

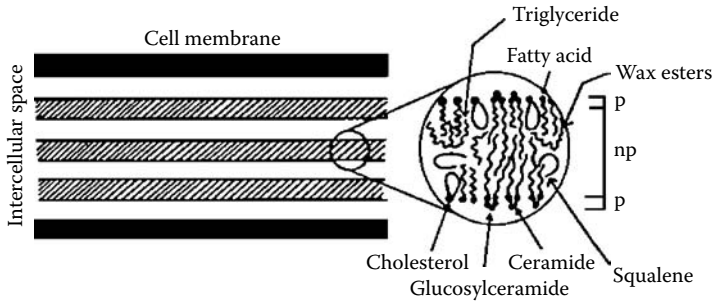


FIGURE 1.5 Schematic showing the putative location of stratum corneum lipids in the bilayer structure as predicted from x-ray diffraction studies.

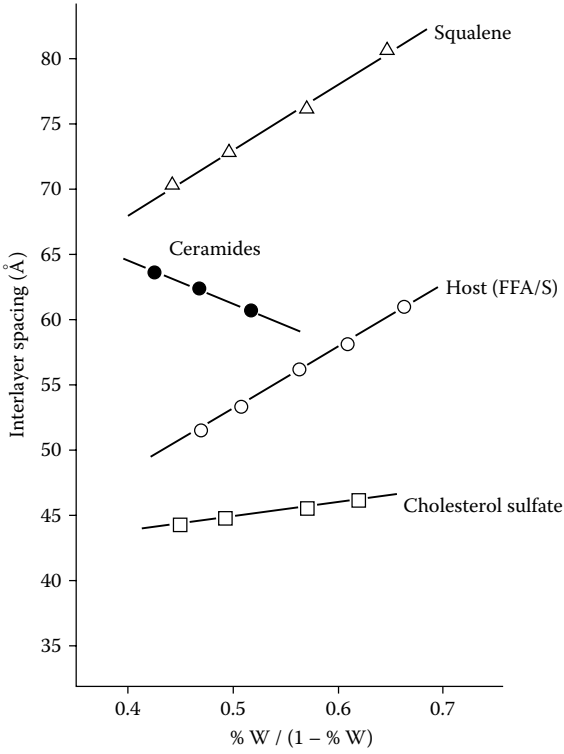


FIGURE 1.6 Addition of ceramide to the host fatty acid/soap lipid model; variations in interlayer spacing with increasing water content of the model lipid.

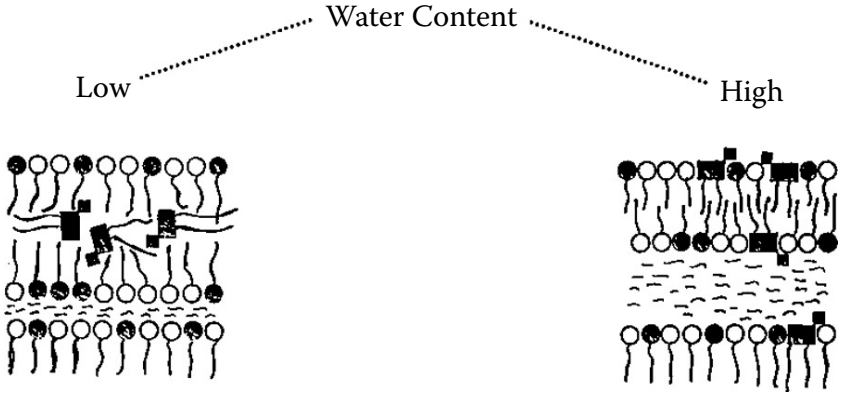


FIGURE 1.7 Illustration showing that the location of ceramides in the layered structure depends on moisture content. Note that at high moisture, ceramides move to the region between the chains. This will decrease the volume and concomitantly the interlayer spacing seen in Figure 1.6.

the layered structure. Figure 1.6 shows what happens to the interlayer spacing when ceramide is added to the fatty acid/soap model. The interlayer spacing decreases as the water content of the model lipid increases.

At low-moisture ceramide tended to reside in the hydrophobic methyl layer, giving an interlayer spacing of 65 Å, and at higher water content, it exists mostly between the chains of the palisade layer, resulting in a decrease in the interlayer spacing. Changes in moisture content can thus have a dramatic effect on the location of lipids in the ordered structure. This is illustrated schematically in Figure 1.7.

The other important learning from this research is that the lipids in at least part of the layered structure are dynamic, not static, and can move around in response to added compounds or environmental conditions, such as low relative humidity. The putative location of the lipids in the layered structure (Figure 1.5) is supported by the stratum corneum lipid extraction studies of Imokawa and Hattori discussed earlier.[51] They showed that with acetone extraction, triglycerides, waxes, and any hydrocarbons were removed in 30 sec, suggesting loose bonding by dispersion forces, while fatty acids, ceramides, and cholesterol continued to be removed after 15 min, suggesting they are more tightly bound by polar forces and hydrogen bonds and are partially ordered.

Accommodation of saturated fatty acids is problematic for the layered structure.[47] When saturated fatty acids were added to the model with only unsaturated fatty acid/soap, a phase change was observed. The unsaturated fatty acid/soap forms a liquid crystalline structure. Their combination with saturated fatty acids formed crystals suggestive of crystalline (orthorombic) character. The presence of sufficient crystals in the lipid structure perturbs the structure such that the barrier to water permeation is compromised. In fact, saturated fatty acids/soap alone offer no barrier to water permeation. Yet the stratum corneum barrier lipid is quite enriched in saturated fatty acids. Saturated fatty acids add rigidity and order to the layered structure. Extended studies show that once the other lipids are incorporated into the layered structure, the saturation becomes a nonissue. For example, addition of cholesterol to the unsaturated–saturated fatty acid/soap mixture returned the structure to the liquid crystalline form.[47] Similarly, ceramides, especially certain ceramides enriched in unsaturated fatty acids, will fluidize the structure. This is because cholesterol and unsaturated ceramides sufficiently disturb the order to fluidize the rigid structure. This research demonstrates the importance of the key lipids to the layered structure. It also shows the resulting delicate balance to help maintain the rigidity of the lipid macromolecular structure, yet ensures that it will be fluid enough to support mobility, permeability, and a water barrier function.

Friberg and colleagues also studied the effectiveness of various liquid crystalline structures at providing a barrier to water transport when reaggregated with delipidated corneocytes.[52] Table 1.4 shows the results for these various structures.

The data in Table 1.4 show that any of the liquid crystals establish a barrier to water transport through the reaggregated discs. Thus, it is the macromolecular structure that is essential for establishing a barrier to water transport, not the

TABLE 1.4
Permeability Constants (Kp)[a] of Reconstituted Stratum Corneum Samples Using Lyotropic Liquid Crystals, Model Lipid, Fatty Acid/Soap Model, and Natural Lipid

Aggregation Element (with Delipidated Corneocytes) (Structure Type)	Kp × 10^3 cm-h^{-1}
% relative to corneocytes	
25% natural lipid	4.7 ± 0.2
25% lecithin:water (lamellar liquid crystal)	7.9 ± 0.07
25% SDS:$C_{12}(EO)_2$:water (lamellar liquid crystal)	7.6 ± 0.07
25% SDS:decanol:water (lamellar liquid crystal)	6.3 ± 0.21
25% Tween:water (hexagonal liquid crystal)	6.2 ± 0.49
20% model lipid (lamellar liquid crystal)	4.1 ± 0.6
20% unsaturated/saturated fatty acid mixture (lamellar liquid crystal)	3.9 ± 0.5
6% unsaturated/saturated fatty acid mixture (lamellar liquid crystal)	4.5 ± 0.6
Intact stratum corneum	0.7 ± 0.11

[a] Permeability constant determined at 93% RH and 31°C. Constants were determined for water vapor penetrating through five different reconstituted discs, and the values are means ± standard deviations.

Source: Taken and modified from Kayali, I. et al., *J. Pharm. Sci.*, 80, 428–431, 1991; Friberg, S.E. et al., *J. Invest. Dermatol.*, 94, 377–380, 1990.

composition or organization of the specific lipids. However, the natural lipid with the polar as well as the nonpolar components provided the better barrier to water transport compared to the surfactant:water liquid crystals, second only to intact stratum corneum. In the case of the unsaturated:saturated fatty acid mixture, which also provided an excellent barrier, the fatty acid is the nonpolar lipid and the soap is the polar species; this supports the role of fatty acid/soaps in creating the permeability barrier. However, the presence of unsaturated fatty acids like linoleic acid also fluidizes the barrier to get around the rigidity that may create a leaky barrier. The studies also show that one does not need a lamellar liquid crystal to provide a barrier to water transport in the reconstituted system; the hexagonal liquid crystal was also very effective.

Earlier studies showed that lipid crystals do not provide a barrier to water transport because a slurry of lipid crystal aggregates gave a barrier equal to an unprotected water surface, whereas liquid crystalline structures gave a barrier to

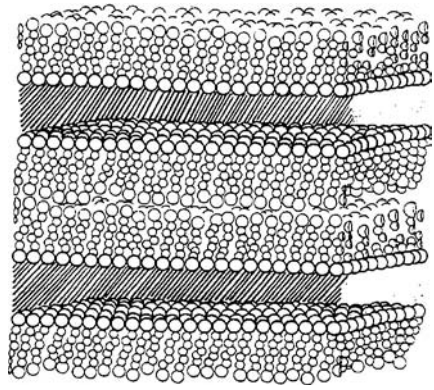
water transport several orders of magnitude smaller.[54] Saturated fatty acids of 16 to 18 carbon chain lengths alone or partially saponified fatty acids in the soap form do not form liquid crystals.[47] However, in combination with the unsaturated species, like linoleic acid, they exist as a lamellar liquid crystal. That unsaturates provide this function is verified in essential fatty acid deficiency, where a scaly barrier persists and can be eliminated with topical treatment with linoleic acid, but not saturated fatty acids.

1.5 POLYMORPHISM IN THE STRATUM CORNEUM LIPID STRUCTURE

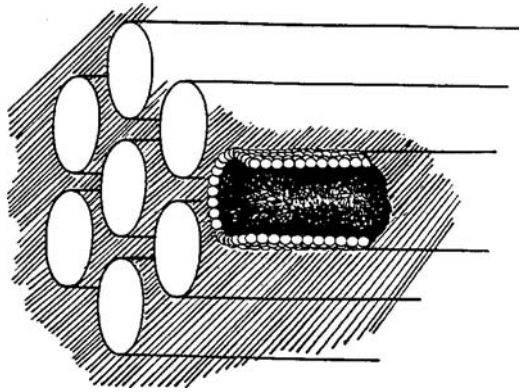
Other authors have examined the macromolecular structure of stratum corneum lipid further. Abraham and Downing[55] have used nuclear magnetic resonance (NMR) techniques and found polymorphism in the structure of the lipid. For example, thermal transitions were noted from lamellar to a hexagonal liquid crystal phase starting at 60 through 70°C. Schematic drawings of both lamellar and hexagonal phases are shown in Figure 1.8. The hexagonal phase of inverted micelles has a much more highly disordered hydrocarbon environment, but according to Friberg's research discussed above,[52] such phases do provide an effective barrier to water transport, and in some cases, it was as good as a lamellar phase. Downing also noticed that increasing ceramide and decreasing cholesterol lowers the energy barrier for the transition from lamellar to hexagonal phases to around 60°C, and also increases the disorder in the hexagonal phase; thus, the transition can be effected by changes in composition. Downing has suggested that the transitions to the hexagonal phase may have important implications in the reassembly of membrane discs extruded from the lamellar granules. During this stage there is a dramatic change in the composition of the lipids, predominantly catabolism of phospholipids and glucoceramides to free fatty acids and ceramides. There is also reduced water content and loss of polar head groups. These factors could regulate the structural preference of the lipids causing the transformation. Lipid molecules in membrane bilayers undergo momentary departure from bilayer organization at the point of contact and form intermediary structures such as inverted micelles during membrane fusion. Thus, the ability of stratum corneum lipids to form hexagonal phases establishes a plausible pathway for the reassembly of the membranous discs into extended lamellar sheets by membrane fusion processes involving transient nonlamellar intermediates, as mentioned by Abraham and Downing.[55]

1.5.1 Additional Small-Angle X-Ray Diffraction Studies Suggesting Polymorphism

As discussed previously, Friberg studied the small-angle x-ray diffraction patterns of human stratum corneum and found a broad reflectance band that exhibited a maximum reflectance between 55 and 80 Å.[47] White et al.[50] reported studies of the x-ray diffraction patterns of isolated hairless mouse skin. He studied the



(a)



(b)

FIGURE 1.8 Schematics of lamellar (A) and hexagonal (B) lipid phases.

lamellar lipid domains and corneocyte envelopes. The diffraction pattern with the sample at 25°C showed a Bragg spacing of 131 Å for the repeat unit. The small-angle x-ray patterns of extracted lipids gave a repeat unit of 60 Å. Bouwstra et al.[56] also reported a low-angle diffraction spacing of 65 Å in human stratum corneum, but later reported that she was unsure if this was a first-order diffraction. She later reported a possible spacing of 130 Å in agreement with White above and concluded the huge background of the 65 Å peak may have obscured the first-order band. Upon heating, the pattern of intact stratum corneum became amorphous, but when cooled down, the repeat pattern returned, and thus the order returned. This suggests another component is present in the intercellular space that helps maintain the lamellar organization. It may in fact be attributed to the desmosomes that interconnect the corneocytes, and their degradation is key to desquamation or sloughing of the outer layers of corneocytes. It could also be

some type of solid lipid structure, but it is not known. As discussed in the following sections, other authors are finding evidence for orthorhombic/gel lipid domains in the stratum corneum structure.

Hou et al.[57] reviewed and summarized the x-ray diffraction studies done and information that supports the findings. Cross-sectional images of the intercellular regions of mouse stratum corneum (in thin sections fixed with ruthenium tetroxide) are filled with lipid lamellae (Figure 1.3), and these images revealed a pattern with a periodicity of 128 Å, supporting White et al.'s findings[50] on murine stratum corneum. All the x-ray data reveal that the reflections occur in multiple orders of a given spacing due to stacking of repetitive lamellar units, supporting Friberg's original proposal of a multilayered intercellular lipid structure, also identified by the freeze–fracture studies.[33]

In the superficial layer of skin, the stratum corneum (SC), the lipids thus appear to form two crystalline lamellar phases with periodicities of 64 Å or 6.4 nm and 134 Å or 13.4 nm (long periodicity phase). The main lipid classes in SC are ceramides, free fatty acids, and cholesterol. Studies with mixtures prepared with isolated ceramides revealed that cholesterol and ceramides are very important for the formation of the lamellar phases, and of the nine ceramides in the stratum corneum, the presence of ceramide 1 is crucial for the formation of the long periodicity phase. This observation and the broad–narrow–broad sequence of lipid layers in the 13.4-nm phase led Bouwstra et al.[58] to propose a molecular model for this phase. This consists of one narrow central lipid layer with fluid domains on both sides of a broad layer with a crystalline structure. This is referred to as the sandwich model. While the presence of free fatty acids does not substantially affect the lipid lamellar organization, it is crucial for the formation of the orthorhombic sublattice, since the addition of free fatty acids to cholesterol/ceramide mixtures resulted in transition from a hexagonal to a crystalline lipid phase.

1.5.2 Studies with High-Angle X-Ray Diffraction and Other Techniques Supporting Polymorphism

Hou et al.[57] reviewed that in biological membranes lateral packing of hydrocarbon chains of lipids also gives rise to high-angle reflections. Two spacings are commonly found: a sharp reflection at 4.0 to 4.2 Å indicative of crystalline packing and a broad diffuse reflection at 4.5 to 4.8 Å indicative of fluid-like packing. Longer-chain lipids in the solid state are known to give spacings of 4.2 and 3.8 Å. Stratum corneum lipid domains exhibit reflectance bands around 4.2 and 3.8 Å. In the liquid state, a reflectance band of 4.6 Å is also seen. The appearance or disappearance of these reflections is dependent on the transition temperatures. Table 1.5 and Table 1.6 (modified from their review paper) summarizes what is known about the reflection bands of stratum corneum in relation to temperature. The 4.2- and 3.8-Å bands disappear above 40°, indicating the crystalline structure has liquified. The 4.6-Å band persists as in Table 1.6; this is due to the liquid alkyl chains. Upon extraction of stratum corneum with solvent, the shorter bands

TABLE 1.5
Summary of Thermal Behavior of Lipid and Protein Domains in Stratum Corneum Using Wide-Angle X-Ray Diffraction: Assignment of Reflection Bands

Stratum Corneum Source	Assignment	Interlayer Spacing (Å) and Behavior as a Function of Temperature			
		25°C	40°C	69–70°C	80–90°C
Neonatal rat	Lipid	3.7	Absent		
		4.2	4.2	Absent	
	Protein	4.6	No change up to 77°C		
		9.8	No change up to 77°C		
Human	Lipid	3.7	Absent	Absent	Absent
		4.2	4.2	4.2	Absent
		4.6	4.6	4.6	Absent
	Protein	4.6	No change up to 77°C		
		9.8	No change up to 77°C		

Source: Assimilated from Hou, S.Y. et al., *Adv. Lipid Res.*, 24, 141–171, 1991.

TABLE 1.6
High-Angle Spacing (Å) Observed in Stratum Corneum as a Function of Temperature

Temperature			
25°C	45°C	75°C	Interpretation
9.4s	9.4s	9.4s	Both of the sharp lines, 9.4 and 4.6, originate from the protein in the corneocyte envelope
4.6s	4.6s	4.6s	
4.6b	4.6b	4.6b	Liquid alkyl chains
4.16s	Absent	Absent	Both 4.16 and 3.75 Å spacings are due to the crystalline alkyl chains organized as an orthorhombic perpendicular subcell; there may be a distribution of alkyl chains in the gel state because the 4.16 line is wide
Absent	4.12s	Absent	The 4.12-Å spacing is due to gel state alkyl chains organized as a hexagonal subcell — transition from crystalline state at 25°C (the 4.16- and 3.75-Å bands)
3.75s	Absent	Absent	Both 4.16- and 3.75-Å spacings are due to the crystalline alkyl chains organized as an orthorhombic perpendicular subcell

Note: s = sharp; b = broad, referring to the width of the reflection.

Source: Assimilated from Hou, S.Y. et al., *Adv. Lipid Res.*, 24, 141–171, 1991.

disappear; thus, they are attributed to the lipid part of the stratum corneum oriented perpendicular to the axis of the keratin fibers. Lipids in bilayers would have their hydorcarbon chains oriented perpendicular to the keratin. A halo present at 4.6 Å is attributed to protein along with the 9.8-Å band.

White et al.[50] obtained high-angle x-ray diffraction patterns (Table 1.6) of lipid extracts and intact stratum corneum at several temperatures and saw similar patterns in extracts and intact mouse stratum corneum; the patterns were consistent with both liquid and solidified alkyl chains present at 25 and 45°, while at 75° the chains are all in the liquid state. They report that at 25° the sharp lines appearing at 3.75 and 4.16 Å are crystalline alkyl chains characteristic of orthorhombic perpendicular subcells. By 45° these lines are replaced by a single line at 4.12 Å, which is expected of a gel state as alkyl chains ordered into hexagonal subcells. This band disappeared at 75° and was interpreted to be a gel-to-liquid crystalline transition. White's findings support coexistence of various macromolecular domains of the lipid.

Still, other authors[59–61] have used small-angle x-ray to study stratum corneum lipid domains and have reported the presence of two lamellar phases with periodicities of 13.4 and 6.4 nm, or same as 134 and 65 Å, as others found (above), and that may vary between species. The lateral lipid organization in stratum corneum has been studied by wide-angle x-ray diffraction as above by still others,[62–64] and spacings at 0.417 and 0.412/0.375 nm have been detected in humans by Garson[64] and Bouwstra et al.,[63] which they attributed to hexagonal (gel) and orthorhombic (crystalline) lipid lattices, respectively.

It seems that low- and high-angle x-ray diffraction can lead to somewhat different conclusions regarding the actual structure of the lamellar phases; the structures range from liquid crystal of the lamellar or hexagonal type to crystalline orthorhombic phases. It may depend on where in the stratum corneum structure one is looking. At lower levels in the stratum corneum, the structure may mirror the phospholipid-rich lamellar body secretions, while at the surface of the stratum corneum the sebum lipids may become intercalated into the membrane lipid and alter the structure. This is supported by recent studies investigating the role of ceramides in the phase behavior of stratum corneum. Deuterated NMR demonstrated that substitution of sphingomyelin for ceramide has dramatic effects on the physical properties of the model stratum corneum.[65,66] This result is of physiological significance, as ceramide in the stratum corneum is derived from the enzymatic cleavage of sphingomyelins at the lower levels and in lamellar granules. Further reports support this contention. Pilgram et al.[67] used the technique of cryoelectron diffraction to obtain local information about mixtures prepared from isolated pig ceramides, cholesterol, and long-chain fatty acids. It appeared that the addition of free fatty acids caused a transition from a hexagonal to an orthorhombic packing, and that electron diffraction can be applied to very nicely distinguish lattices. One comment is that no effort was make to study the fatty acid soap form of palmitic acid that would exist at a physiological pH of 4.5 of the stratum corneum. Of course, the pH will be closer to neutral at the lowest layers, near the living epidermis, and more acidic at the surface. Thus, as lipids are modified, such as by conversion of phospholipid to free fatty acids, as they

approach the skin surface and prepare for desquamation, likely changes in the lipid domains may result. Bouwstra et al.[56] showed how incorporation of an azone completely altered the macromolecular structure of the lipid. So, the structure may be somewhat sensitive to the appearance of different lipid species during barrier development or artificially from exposure to substances on the outside. This technique can be used to track such regionally defined changes.

In fact, Pilgram et al.[68] have gone further and studied the relationship of lipid organization to depth in the stratum corneum using cryoelectron diffraction. Stratum corneum tape strips were prepared from native skin *in vivo* and *ex vivo*. They found that the lipid packing in samples prepared at room temperature is predominantly orthorhombic. In samples prepared at 32°C (the temperature of the outer layers of skin), the presence of a hexagonal packing is more pronounced in the outer layers of the stratum corneum. Gradually increasing the specimen temperature from 30 to 40°C (the inner temperature of skin is 37°C) induced a further transition from an orthorhombic to a hexagonal sublattice. At 90°C, all lipids were present in a fluid phase. These results are in good agreement with previously reported wide-angle x-ray diffraction and Fourier-transformed infrared spectroscopy studies. Thus, at the temperature of human skin, the hexagonal liquid crystal seems to predominate in the tape strip studies.

The research of Moore et al.[69,70] and Bouwstra et al.[71] examined artificial lipid combinations mirroring stratum corneum lipids. Both groups studied the role of certain ceramides in stratum corneum lipid organization. At least six to nine ceramides exist in the stratum corneum differing in either head group architecture or fatty acid chain length and degree of saturation. The fatty acid esterified to the sphingosine base can be either alpha-hydroxy or nonhydroxy acid. The chain length varies from C16 for ceramide 5 to C30 for ceramide 1. Furthermore, ceramide 1, assumed to be crucial for the characteristic organization of stratum corneum lipids, contains linoleic acid linked to the omega-hydroxy fatty acid. Linoleic acid is an essential fatty acid known to be crucial for normal barrier function, as shown in the condition of essential fatty deficiency discussed earlier. These structural lipids were reviewed previously (Wertz[25]).

To continue, Bouwstra et al.[71] studied the role of ceramide 1 and 2 further. They showed that to obtain the two lamellar phases reported for intact stratum corneum lipids at physiological amounts of cholesterol and ceramides, all of the ceramides together work much better. The complete ceramide mixture with lower amounts of cholesterol (ratio of 0.4 for cholesterol to ceramides) gave the two lamellar phases at x-ray periodicities of 5.6 and 12 nm. With cholesterol combinations with ceramide 1 and 2 alone, the ratio had to be greater than 1:1 cholesterol:ceramides 1 and 2. Solubility of the mixture of cholesterol with ceramides increased with ceramide 1 addition, suggesting its possible role. Incorporation of fatty acid (palmitic acid) in a physiological ratio of 1:1:1 cholesterol:ceramides 1 and 2:palmitic acid exhibited phase behavior less similar to that in intact stratum corneum than the cholesterol/ceramide 1 and 2 mixtures. An additional strong peak was found at 3.77 nm that was never seen before in stratum corneum and can be ascribed to the appearance of an

additional phase. It is likely that the presence of only short-chain fatty acids like palmitic induces phase separation.

Moore et al.[70] used Fourier-transformed infrared (FTIR) spectroscopy to study the interactions of deuterated palmitic acid in the cholesterol/ceramide/palmitic acid model at pH 5.5 and hydrated. At physiologic temperatures, the CD2 scissoring mode of the palmitic acid and the rocking mode of the ceramide methylenes are each split into two components, suggesting that the components exist in separate conformationally ordered phases, probably orthorhombic perpendicular subcells. The magnitude of the splitting indicates that the domains are at least 100 chains in size. Overall, the FTIR observations suggest the following: Thermotropic studies at different temperatures show that from 10 to 40° a significant fraction of the palmitic acid in this model exists in a separate conformationally ordered orthorhombic phase of domain size greater than 100 chains, as stated above. This is consistent with the NMR studies of Kitson[65,66] showing, that 80% of the palmitic acid is in the solid phase at physiological temperatures. Between 40 and 50° the palmitic chains progressively disorder, but ceramide remains solid and there is significant phase separation; this is also consistent with the NMR studies above. Between 60 and 70° the ceramide disorders. The breadth of the phase transition suggests palmitic is mixing with cholesterol as it melts between 40 and 50°. Thus, the Fourier-transformed infrared scan shows a split in the CD2 peaks of the deuterated palmitic acid below 44°C. A similar split occurred for the CH2 methylene of ceramide. These findings suggest separate conformationally ordered phases or domains for the lipids. Thus, the most important result from this work is the detection of orthorhombic domains of palmitic acid and ceramide 3 at skin temperature. This was supported by the melt transition temperatures. The existence of more than a single lipid domain is thus supported by Moore's research. This work supports the fact that there may be distinct lipid domains in model stratum corneum, reinforcing the domain mosaic model of Forslind, discussed next.

A model proposed by Forslind called the domain mosaic model warrants some discussion.[72] It has been accepted that the lipids in the stratum are organized in some sort of multilayered structure. It has also been assumed that the lipids are at best randomly distributed in the bilayers. Forslind has hypothesized that this assumption is probably not the case as the lipids with very long chain fatty acids would tend to exist in the crystalline state. He feels that to satisfy all the functions of the stratum corneum, the lipids will have to be segregated into their own domains. Lipids with long-chain fatty acids segregate and exist in the gel or crystalline state, and this would provide the best barrier to water transport, but would also be a very rigid structure and would not address the mechanical requirements of the stratum corneum, i.e., flexibility. To provide this feature, lipids with shorter-chain fatty acids associate at the grain borders, where the lipid here is in the liquid crystalline state. These regions would allow some water to permeate the barrier and mix with the corneocytes to keep them pliable, as well as providing flexibility to the barrier, and allow hydrophobic substances to permeate into the barrier through this region. Some support for this model comes from the