Part I

The Skin

Biochemistry and Function

2 The Skin as a Barrier

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CONTENTS

2.1 INTRODUCTION

This chapter will deal with the stratum corneum barrier with a special focus on structure–function relationships. For this reason our approach has been to describe some details of the epidermal physiology that have a bearing on upholding the barrier function. We see it as important that skin barrier function is regarded as part of the dynamic processes of cellular transformation during the differentiation of epidermal keratinocytes, hence dependent on the status of the skin.

It is taken for granted that the skin barrier prevents foreign material from entering the system. But, a deeper insight into the barrier function of the integument makes it clear that the primary function of the barrier is to prevent water loss, and the barrier toward environmental factors is only of secondary importance, albeit very important.[1] The water homeostasis is absolutely necessary for normal physiology, and the role of the kidneys is to maintain that homeostasis. Therefore, the integument should represent a water-impermeable "bag." However, we have to account for the perspiratio insensibilis, which obviously has its origin in the need for a hydration of the corneocytes. Water acts as a plasticizer on the corneocyte keratin, giving the cells the necessary elastic properties. If deprived of water, a dry skin is prone to crack open at mechanical stress. Since the relative humidity of the environment varies enormously, the corneocytes have to be hydrated from a permanent water source, the body. The fact that the perspiratio insensibilis is markedly constant reveals that this water leakage is not a defect in the barrier, but an inbuilt factor with a required function.

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2.2 THE CORNEOCYTES CONSTITUTE A SCAFFOLD FOR THE BARRIER LIPIDS

The entire horny layer, the stratum corneum, can be regarded as the outer barrier of the skin. Although at a closer look there is a differentiation in lipid structure and composition across stratum corneum. The horny layer is continuously exposed to contact with the environment and suffers from the effects of chemical and physical agents, which will cause a continuous loss of material. We can assume that the daily loss of material over the entire body surface ($\sim$1.8 m^2) corresponds to a "film," the thickness of which is at least that of a corneocyte. Assuming that the surface of a corneocyte is $\sim$1000 μm^2, this surface "film" corresponds roughly to $1.8 * 109$ cells. The thickness of a corneocyte is $\sim$0.3 μm, and with a specific weight of 0.75 kg m^{-3} (= protein) these data can be used to calculate a daily loss of about 40 mg of horny cells, most likely an underestimation. Thus, the total amount of material in this turnover is not negligible. This continuous renewal of cells is a prerequisite for keeping the thickness of stratum corneum approximately constant and thus the barrier intact in all its aspects. It has been demonstrated that the control of barrier homeostasis is under strict control. The transepidermal water loss (TEWL) and the Ca^{2+} distribution appear to be important signals controlling the mechanisms involved in the homeostasis of stratum corneum[2–4] such as up-regulation of lipid synthesis. Other important factors are the distribution of sodium and potassium within epidermis and the pH-gradient across stratum corneum.

Through autoradiographic investigations it has been shown that a corneocyte stems from 1 of about 20 basal cells under the projected area of a corneocyte.[5,6] This is the so-called proliferative unit (Figure 2.1). The cells on the basal lamina communicate via gap junctions, and through this means a regulation of cell division is possible within the proliferative unit controlling the progeny

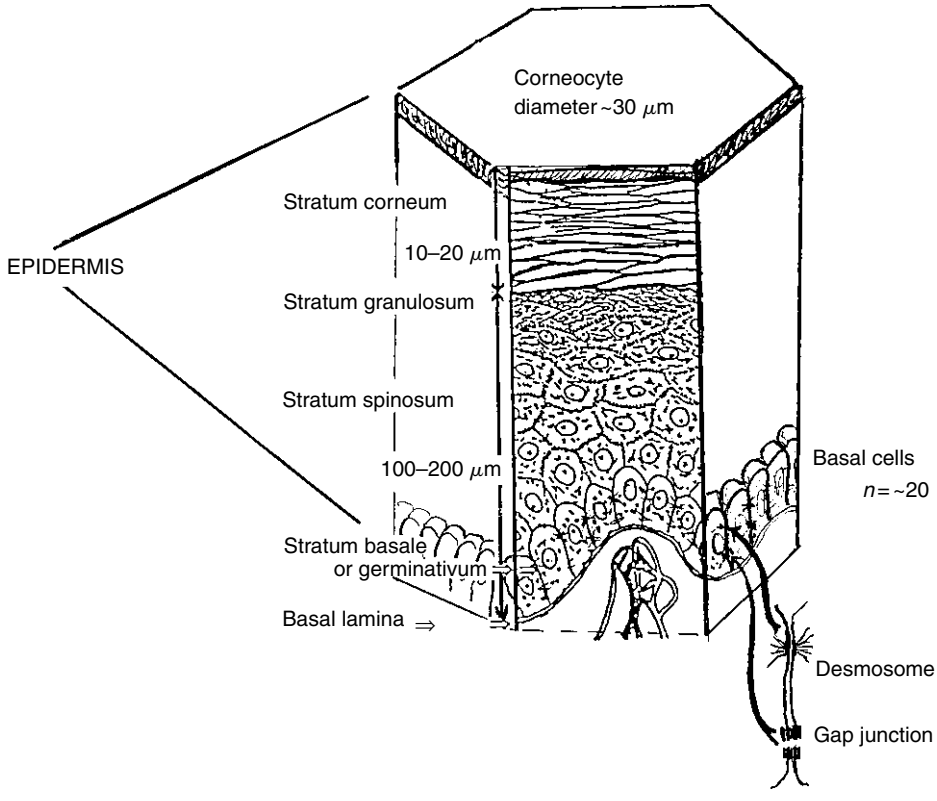

FIGURE 2.1 The proliferative unit as deduced from Potten.[5,6]

travel from the stratum basale to the stratum corneum at a pace that ensures a smooth surface.[7] An additional controlling mechanism may be the shift in the Na/K ratio that occurs as the cells move into the stratum spinosum.[8] Thus, higher than normal Na and lower than normal K concentrations within the cell of the upper stratum will effectively hinder the cell to enter the cell division cycle.

2.3 CORNEOCYTE STRUCTURE

A corneocyte can be described as a very flat cell, about 30 μm in diameter and ~0.3 μm thick, filled with keratin inside a protein envelope. Keratin is a highly hydrophilic material that can bind substantial amounts of water, and we discern a fibrous component as well as an amorphous one. The fibrils, 8 nm in diameter, span the inside of the corneocyte and thus constitute an internal reinforcement ensuring that the cell form in the plane of the skin remains virtually unchanged even at long exposures to water. This is achieved by an orientation of the fibrils in the plane of the cell (Figure 2.2[a]). In the vertical dimension there are virtually no reinforcement fibrils, and thus the cells have more freedom to swell in this direction. Norlén et al.[9] have actually shown that the swelling is less than 5% in the horizontal dimension, but can be more than 25% in the vertical dimension. This ensures a minimal roughness of the skin surface even at maximal swelling, thus minimizing the risk of surface breaks at mechanical stress on wet skin. The conspicuously thicker stratum corneum

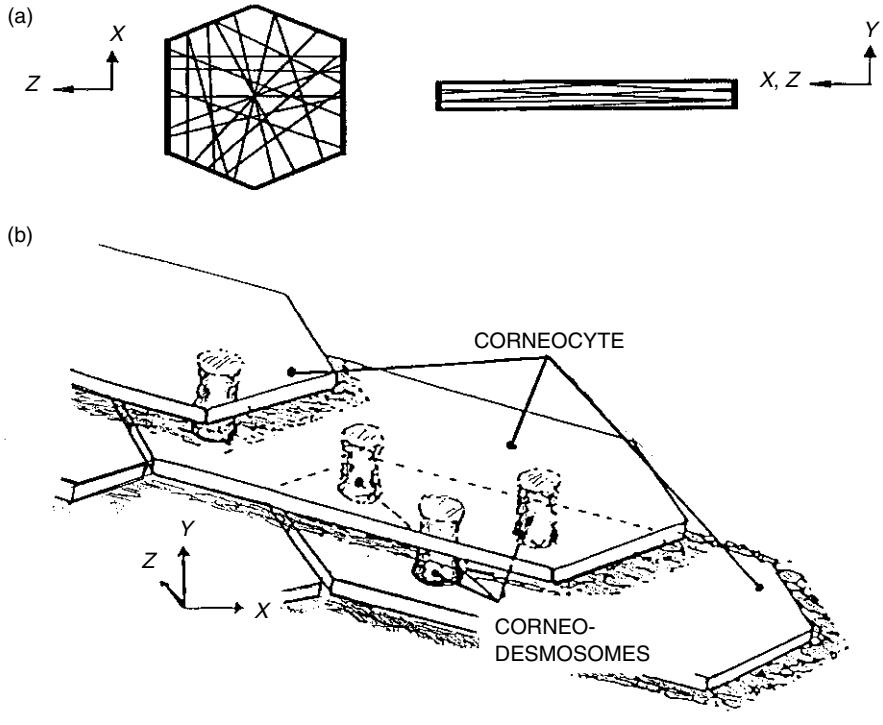

FIGURE 2.2 (a) The corneocyte is a flat, hexagonal-like structure with a surface area of about 1000 μm^2 and a thickness of 0.3 μm. A protein envelope encloses a cell compartment containing only fibrous and amorphous keratin. The keratin fibrils inside the cell are randomly oriented in the plane of the cell and constitute an internal reinforcement, which ensures that the cell form in the plane of the skin is preserved within very narrow limits. (b) The classic view of corneocytes coupled to each other through protein "rivets," corneodesmosomes. This arrangement makes a mechanically rigid scaffold. The lipid bilayers, which are separated by thin water sheaths and are mechanically very soft, are protected from sliding relative to each other and being directly exposed to mechanical shear that would break up the structure.

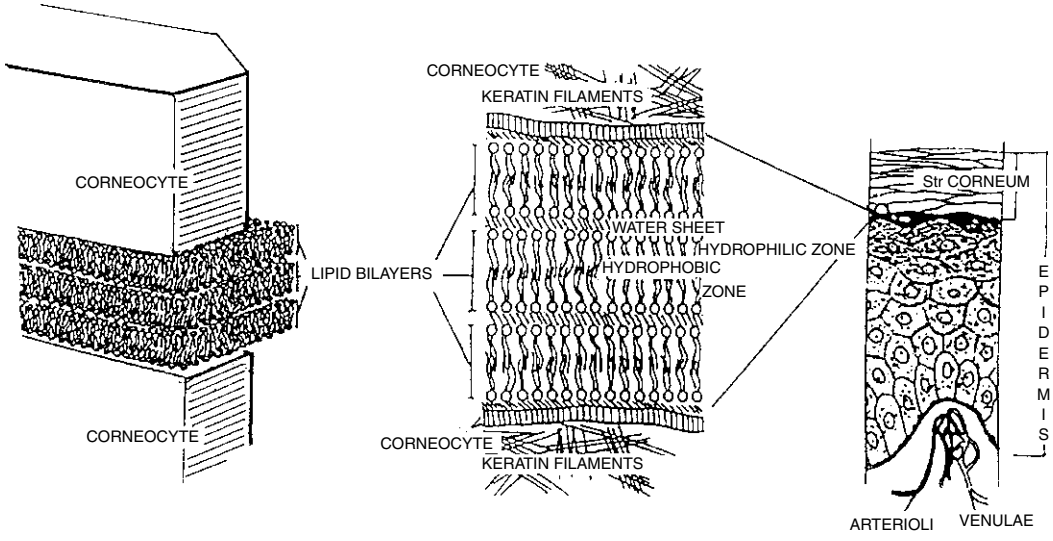

FIGURE 2.3 Stacked bilayers of lipids are inserted into the extracellular space of the corneocyte scaffold to form the lipid barrier of the skin.

of the palms and foot soles do indeed become wrinkled at maximal swelling, but here a conspicuous thickness of the stratum corneum compensates for this roughness.

The classical view of stratum corneum includes the presence of desmosome rivets ("corneosomes"). The corneocytes are mutually joined by these desmosome rivets that effectively hinder the cells to move in relation to each other in the plane of the skin (Figure 2.2[b]). This prevents shearing forces from disrupting the stacked bilamellar lipid structures in the extracellular space (Figure 2.3). The desmosome "rivets" also prevent this space from being increased due to mechanical forces imposed on the skin. Today it is known that there is an ongoing, partly pH-dependent, enzyme activity in stratum corneum including both lipases and proteases, which are involved in the process of corneocyte desquamation.[10] The activity of these proteolytic enzymes, such as the stratum corneum tryptic enzyme,[11] is necessary for degrading protein structures allowing for the desquamation of corneocytes. A new view of the ultrastructure of the skin and especially stratum corneum structures has presently been published.[12,13] By using a new method with instant freezing of a tissue sample allowing for a complete vitrification, it has become possible to produce skin sections for low temperature cryotransmission electron microscopy. The newly obtained structural information on the protein–lipid interaction in stratum corneum suggests that the classical view of the desmosome rivets has to be reevaluated (Lars Norlén, pers. comm.).

2.4 THE HYDROPHILIC AND THE HYDROPHOBIC PATHWAYS THROUGH THE SKIN BARRIER

Looking at the barrier in more detail, we find that it can be described as composed of two main components. Interspersed between the corneocytes we find the "hydrophobic" (water-repellent) substance, the barrier lipids. The keratinized corneocytes containing fibrous and amorphous proteins represent a "hydrophilic" (water-attracting) component. Neutral lipids (fatty acids, cholesterol) and ceramides dominate the lipid phase, and it is mainly these lipids that are responsible for the control and limitation of water transport through the skin.[14] Visualization of the penetration pathway through the skin by tracer methods has demonstrated that the extracellular pathway is likely to be the only route through the barrier for substances other than water.[15] Water diffusion through the keratinocytes

is not expected to occur freely due to the fact that keratin will adsorb water. The bound water is likely to take on a certain degree of structured organization; hence the amount of freely diffusible water will be comparatively small. Consequently, the water transport through the keratinocytes will be impeded. Norlén et al.[16] have shown that water permeation through lipid-extracted stratum corneum membranes is only about three times higher than through a nonextracted stratum corneum membrane.

2.5 THE PHYSICAL STATE OF THE LIPIDS DETERMINES THE PROPERTIES OF A LIPID MEMBRANE OR BARRIER

Lipids that can form biological membranes are characterized by a hydrophilic head group and a hydrophobic part, usually a carbon chain (cf. fatty acids versus cholesterol). From physical, thermodynamic considerations it can be shown that it takes a lot of energy to keep the hydrophobic part of a lipid dissolved in a water solution.[17] For this reason lipids tend to aggregate in micelles or bilayers. This means that they form a hydrophobic compartment [or phase], which encloses the carbon chains that separate them from water. The hydrophilic head groups face the water and thus constitute a border between a hydrophobic phase and water (Figure 2.4). A number of factors determine how stable such aggregates are.[18] These include temperature, the length of the hydrophobic carbon chain, their degree of unsaturation [double bonds], the temperature, the water content, the presence of divalent ions, etc.

Temperature is in general an important factor for lipid membrane configuration. It has been demonstrated that lipid membranes exist in two main physical states: one extremely close-packed, the crystalline state (Figure 2.4[a]), and the other, the liquid crystalline state (see Figure 2.4[b]). In the latter state the structure is more open, and the lipid units are free to diffuse in the plane of the membrane. This actually allows water molecules to pass right through the membrane. The transition between these two main states is determined by the so-called transition temperature, and this is in turn dependent on the particular properties of the lipids forming the membrane[17] (Figure 2.4[c]). Lipids with short chains and lipids that are unsaturated have their transition temperature at lower temperatures than long-chain and saturated lipids. Biological membranes (bilayers) are generally complex mixtures of different lipid species, and the transition temperature for such a structure is expected to vary with the actual proportions of the lipid components. This also means that the transition occurs within a comparatively broad temperature interval compared to the corresponding sharply defined interval of a single lipid species.[8]

As a generalization, we may be allowed to state that the transition temperature for cell membranes in biological living systems is found between 0 and 40°C and the chain lengths are between 16 and 18 carbons. This is in conspicuous contrast to the lipids of the stratum corneum barrier where chain lengths up to and over 30 carbons have been demonstrated.[14,19] From such facts we expect the transition temperature of the skin barrier lipids to be around 40°C, and this has also been substantiated in a number of investigations.[20–22] This means that under normal conditions with a skin temperature about 30°C, the barrier will essentially be impermeable to water.

Straight carbon chains can be housed in comparatively small volumes and allow van der Waal's forces to act and cause a close packing (Figure 2.4[a]). The van der Waal's forces are not effective if the distance between the atoms is several atoms in diameter.[18] Double bonds tend to create kinks on the carbon chains, preventing them from close apposition with neighbor chains, which is a prerequisite for allowing the weak van der Waal's forces to contribute to a close packing of the chains. Thus, kinked carbon chains hinder close packing of the lipid chains and promote a liquid crystalline state of the bilayer where the lipid units are allowed to diffuse in the plane of the bilayer[23] (see Figure 2.4[b]). A cell membrane is actually this kind of structure with a very rapid diffusion of lipids within the membrane and therefore allows almost free passage of water in both directions over the membrane. The important message here is that the cell membrane is not a water barrier.

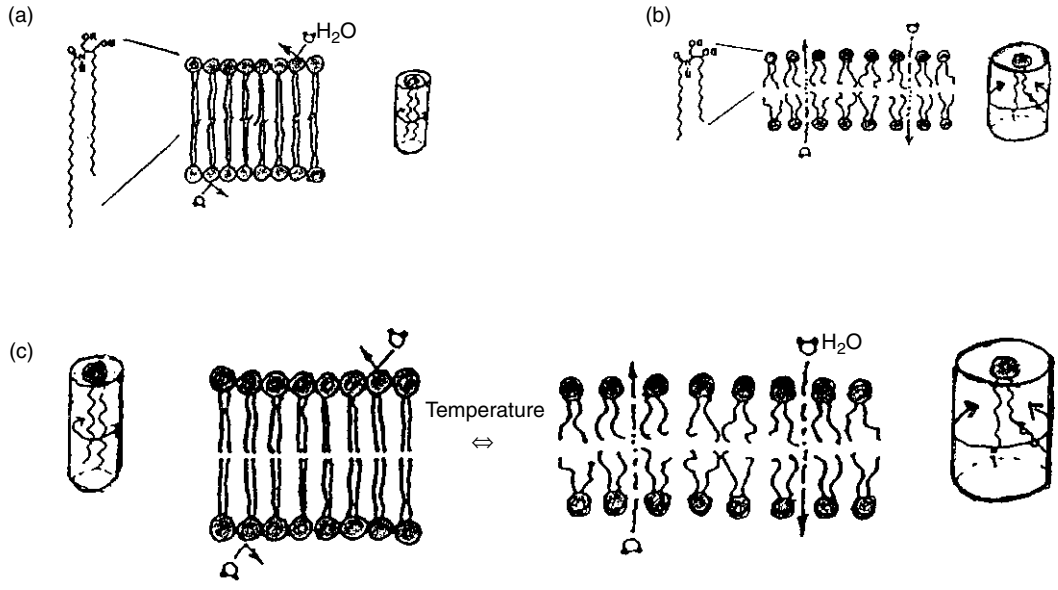

FIGURE 2.4 (a) Long, saturated carbon chains can attract each other through van der Waal's forces, and this causes a tight, close-packed crystalline structure that is impermeable to water. Straight (saturated) carbon chains demand less space than kinked (unsaturated) chains. Saturated long aliphatic chains ($C > 20$ carbons) tend to pack close at skin temperatures (26 to 32°C). When associated with water, there may still be a freedom of rotation along the carbon chain axis and the structure is sometimes denoted gel phase. (b) Short carbon chains and carbon chains with a double bond form liquid crystalline structures, where the chains of the bilayer show high degrees of freedom to diffuse in the plane of the bilayer. The liquid crystalline state thus becomes favored if one of the carbon chains is unsaturated. (c) The transition temperature of bilamellar lipid structures. Long, saturated carbon chains (left), tightly close packed form a crystalline structure that is impermeable to water. Short carbon chains (right) form liquid crystalline structures where the chains of the bilayer show high degrees of freedom to diffuse in the plane of the bilayer. The transition between these two states is dependent on temperature, chain length, and degree of unsaturation of the chain. If the temperature is lowered, the thermal movements of the chains decrease and van der Waal's attraction forces become operative; the structure becomes crystalline and impermeable to water. Thus, the transition between these two states depends on the parameters of temperature, chain length, and degree of unsaturation of the chain. Saturated, long aliphatic chains ($C > 20$ carbons) tend to pack close at skin temperatures (26 to 32°C).

This is in sharp contrast to the conditions in stratum corneum where the lipid membranes are almost impermeable to water. As a consequence of these facts, we expect the bulk of lipids that form the skin barrier to be in a crystalline (gel) state, that is, to have long carbon chains ($C > 20$:0) to comply with the physical requirement that the transition temperature should be higher than normal skin temperature (>35°C). A physiological mixture of ceramides, free fatty acids (FFA), and cholesterol is indeed needed for a normal barrier function.

2.6 THE CERAMIDES OF THE HUMAN SKIN BARRIER

At physiological pH the long-chain ceramides of the horny layer barrier in the presence of cholesterol and fatty acids have been shown to have equal capacity to form lamellar lipid structures as have phospholipids.[24,25] The chain length of the ceramides is to a great extent longer than 18 carbons, even up to 34 carbons in one of the chains, and this suggests close packing of the crystalline type at normal skin temperatures.

Several classes of ceramides have been described in human skin.[14] Today it is considered that the ceramides are essential for the barrier properties. It has been suggested that the lower amount of ceramides found in stratum corneum in atopic dermatitis[26,27] explains the increased TEWL seen in dry atopic skin. In this context it is of special interest to note that part of the long-chain ceramides of the horny layer are covalently bound to the proteins forming the corneocyte envelope.[25] This suggests that such lipids constitute anchors of the hydrophobic phase to the corneocytes and thereby add to the cohesion of the cells of the horny layer.

2.7 FREE FATTY ACIDS AND CHOLESTEROL

The recent data of Norlén et al.[16,28] demonstrate that the FFA retrieved from stripped lower arm skin (and therefore essentially uncontaminated by sebum lipids) are all saturated and long-chain species ($C > 20$). This harmonizes with lipid data from epidermal cysts, which are virtually free from triglycerides of sebum origin.[29] Furthermore, the ceramides of the barrier lipids are all long-chain species and therefore also comply with the requirement set up for a water-impermeable barrier.

The third class of lipids found in stratum corneum extracts is represented by cholesterol and cholesteryl esters. The actual role of cholesterol remains enigmatic, and no clear reason for its role in the barrier function has been proposed so far. However, it is possible that contrary to what is the role in cell membranes where cholesterol increases close packing of phospholipids, it can act as kind of a detergent in lipid bilayers of long-chain, saturated lipids.[30,31] This would allow some fraction of the barrier to be in a liquid crystalline state, hence water permeable in spite of the fact that not only ceramides, but also fatty acids found in the barrier are saturated, long-chain species.[28,32]

2.8 LIPID GRADIENTS WITHIN STRATUM CORNEUM

Some data indicates that there is a change in composition and arrangement of the lipids during the transition through stratum corneum.[30,31] This can in part depend on the presence of lipids from sebum secretion. Together with the decrease in water across stratum corneum it is possible that there is a rearrangement of the lipid structure, which in turn can be of importance for the desquamation process.

2.9 STRUCTURE OF STRATUM CORNEUM — BARRIER MODELS

2.9.1 The Brick and Mortar Model

In 1975, Michaels et al.[33] presented a conceptual model of the arrangement of corneocytes and lipids in stratum corneum. They envisaged stratum corneum as a brick and mortar structure with the keratin filled corneocytes as bricks and the intercellular lipids as mortar. This model was further explored by Elias and co-worker.[34–37] This model does not per se include a structure–function perspective on the barrier but has had a tremendous impact on the research on stratum corneum and its composition, function, and the regulation of homeostasis.

2.9.2 The Domain Mosaic Model

In 1994 Forslind presented a more structure–function orientated model, the domain mosaic model.[38] With the background given previously, the requirements on the stratum corneum barrier can be summarized as follows: the barrier should be watertight but still allow a small, controlled amount of water to leak from the system in order to keep the corneocyte keratin hydrated.

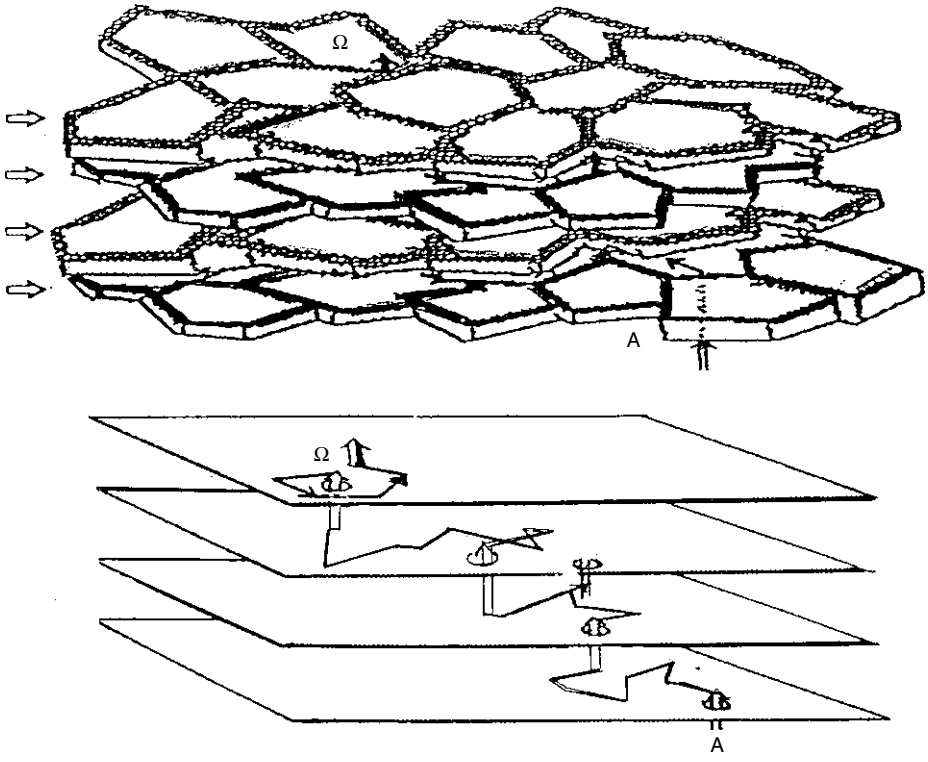

FIGURE 2.5 The stacked bilayers of the skin barrier are envisioned as composed of crystalline domains separated by fringes of lipids in the liquid crystalline state.[38] The fringe zones may actually oscillate in a very small time scale between a liquid crystalline state and a crystalline (gel) state. Such a tentative idea would mean that the barrier is open just temporarily at a certain location since penetration must occur in the liquid crystalline areas. Thus, the action of a penetration enhancer would be to "stabilize" a liquid crystalline state or transform it into another type of structure, for example, a cubic phase.

From these requirements we may infer a structure where the bulk of the intercellular lipids exist in the crystalline, close-packed state in stacked bilayer structures (Figure 2.5) due to the large amounts of long-chain saturated species. However, circumstantial evidence, for example, TEWL, indicates that a fraction of the lipid compartment should be in the liquid crystalline state, but as yet we do not know the composition of this fraction. Again the role of cholesterol may be crucial, as mentioned earlier.

Accepting that the bulk of barrier lipids are in the watertight crystalline state we may depict the bilayers as composed of crystalline domains separated by lipids in the liquid crystalline state.[38,39] The cross section of a domain can tentatively be assumed to be of the same size as the cross section of a lamellar granule, the structure from which the lipids are extruded into the extracellular space of the stratum corneum, that is, ~200 nm. Several bilayers are stacked on top of each other and separated by a thin film of water adherent to the hydrophilic head groups (Figure 2.3). Since it is unlikely that the crystalline domains are exactly uniform in size and form, we do not expect the fluid crystalline interdomain areas to overlap precisely. A water molecule leaving the body via the stratum corneum on a downhill diffusion gradient will therefore have to suffer a tortuous, meandering way through the lipid barrier.[40,41] (Figure 2.5). In the water sheath separating the bilayers, the water molecule will diffuse randomly until it finds a "hole-in-the-roof," that is, a liquid crystalline phase through which it can tunnel into the next, overlaying water sheath. Considering the fact that it, in addition to a number of water molecules, will have to circumvent water-saturated corneocytes, shows us that the path out to the environment will be extremely long, hence the actual low value of the TEWL.

2.9.3 The Single Gel Model and the Sandwich Model

Models of stratum corneum have been further developed. During the past few years two major and substantially different models have been proposed, the Single gel phase model by Norlén (for reviews, cf.[30,42]) and the Sandwich model by Pilgram and Bouwstra (for reviews, cf.[31,43]). The basic concept of the Single gel model is that the lipids forming the lipid phase in stratum corneum are present in one, continues gel phase without phase separation.[44] The model also includes a new view on the formation of the lipid phase of stratum corneum.[45] Based on ultrastructural analysis of serial sections and freeze sections of vitrified skin biopsies[12] it is postulated that the lipid phase is formed as a continuous tubular system within the upper part of the epidermal keratinocytes, also continuous with the cell membrane. At the interface between stratum granulosum and stratum corneum the tubular structures are unfolded into the intercellular space. This model of formation contradicts the classical view of lamellar bodies with preformed lipid membranes in the keratinocytes, the Landmann model.[46] It is postulated that this model would be compatible with lowest energy cost for producing the lipid phase of stratum corneum. In the Sandwich model the lipids of stratum corneum are proposed to be arranged in membranes with alternating crystalline and liquid crystalline phases. The importance of cholesterol sulphate, pH, and calcium ions has been highlighted in this model.[47,48] Pros and cons for these models have been discussed extensively.[30,31,49,50]

2.10 PROPERTIES OF THE LAMELLAR BARRIER — EFFECTS OF PENETRATION ENHANCERS

Based on the concept of the domain mosaic model and the Fick model for downhill gradients over a barrier, Engström[51,52] has presented arguments to show that only a fraction of the total lipid mass of the barrier has to be involved in structural changes that will open up or prevent barrier passage. These ideas were more extensively presented in a sequel publication which demonstrated that enhancement factors for barrier penetration of the order of 100 could easily be obtained for substances with partition coefficients far from one.[40] This is true even if the fraction of the extracellular bilayer that has undergone structural transformation, for example, to a hexagonal or cubic phase, is small, that is, 1 to 10% (Figure 2.6). It is to be noted that the structural transformations, for example, conversion of a lamellar phase into a hexagonal phase, a bicontinuous cubic phase, or a sponge phase, are expected to occur only in the liquid crystalline phase regions between the crystalline domains, hence only a very small part of the total barrier is involved in the process.

It must be realized that structural changes of these kinds are local phenomena. This reasoning implies that a penetration enhancer introduced into the lipid barrier is expected to diffuse in the liquid crystalline phase and exert its structure transformation effects more or less exclusively there. Within a relatively short time it will also be diluted through this diffusion process and then the bilayer structure will be restored and the normal barrier function will be regained.

A problem that is rarely taken into account is related to the fact that the water concentration shows a conspicuous gradient within the stratum corneum thickness. These factors are likely to influence the physical state of lipids in bilayer formations, and therefore we expect lipid barrier structure to vary within the thickness of the stratum corneum.[53]

2.11 CONCLUSIONS — BARRIER PENETRATION IN A FUNCTIONAL PERSPECTIVE

In the past, barrier research for the most part had the character of “black box” descriptions of the dynamics of substance penetration through the skin. Today, skin barrier research is oriented toward an understanding of the molecular structures of penetrants and the lipid bilayers including

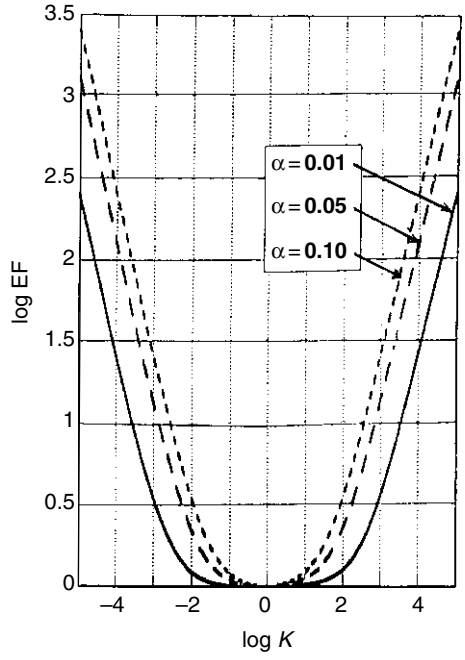

FIGURE 2.6 The enhancement factor EF (log EF) plotted versus the partition coefficient (log K) demonstrates that small changes in the fraction a of the liquid crystalline phase of the barrier that undergoes a structural transformation from lamellar to cubic, hexagonal, etc. phase causes vast changes in the EF.[51]

processes and structural events occurring at penetration. Our knowledge of the actual lipid barrier structure(s) and its detailed function is starting to emerge. Biophysical techniques such as x-ray diffraction, NMR, and FTIR have confirmed that a large part of the barrier lipids are in a crystalline state.[31,43,54–56] This is supported by lipid analyses of stripped human skin extracted *in vivo*, which has demonstrated that the FFA and the ceramides are long-chain species ($C > 22$) and hence should pack in crystalline (gel) structures at skin temperature. The role of cholesterol remains enigmatic, but is likely to influence the structural organization of the FFA and ceramides. New structural evidences contributing to a broader understanding of the organization and function of stratum corneum[12,44–45] is now published. These findings are to some extent contra dictionary to the structural data obtained by other techniques.

The lipid bilayers of the stratum corneum not only constitute a barrier, but may also function as a pool from which substances can slowly penetrate into the system on a downhill gradient. The actual effect of solvents and detergents on barrier lipid structure is not known in any satisfactory detail. Likewise, we are only starting to understand how different moisturizers might influence the structure and function of the barrier. We still lack an understanding of how the composition of the ceramide, FFA, and cholesterol influences the defect barrier in some pathological disorders, for example, dry atopic skin.

The unique character and the particular composition of the human skin barrier lipids call for investigations on human skin, possibly pig skin, and to a great extent preclude rodents as models for barrier function in penetration studies.

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3 Epidermal Lipids and Formation of the Barrier of the Skin

Philip W. Wertz

CONTENTS

3.1 LIPIDS IN THE EPIDERMIS

3.1.1 Introduction

The evolution of life in the relatively dry terrestrial environment required the development of a waterproof integument.[1] In the terrestrial vertebrates, the stratum corneum provides the primary barrier to water loss. The barrier function of the stratum corneum depends upon a unique mixture of lipids that form lamellar structures in the intercellular spaces.[2–5] This generally consists of ceramides, cholesterol, and long chain fatty acids.

In human epidermis, the lipid end products of differentiation consist of cholesterol, 22- through 28-carbon straight chain saturated fatty acids, and nine different series of ceramides.[5,6] The building blocks from which the ceramides are composed include sphingosine, phytosphingosine, and 6-hydroxysphingosine as the base components and normal saturated fatty acids, α-hydroxyacids, and ω-hydroxyacids as the amide-linked fatty acids. In addition, the ω-hydroxyacid-containing ceramides bear ester-linked linoleate on the ω-hydroxyl group. All nine possible combinations of base–acid pairings are formed.[6] Representative structures are shown in Figure 3.1.

In addition to the free lipids found in the intercellular spaces of the stratum corneum ω-hydroxyceramides, ω-hydroxyacids, and fatty acids are covalently attached to the outer surface of the cornified envelope.[7–9] The hydroxyceramides and hydroxyacids are thought to be attached through ester-linkages involving glutamic or aspartic acid side chains,[10] while the fatty acids are thought to be attached through formation of ester linkages with serine or threonine hydroxyl groups. Evidence has been presented indicating that transglutaminase 1 may be responsible for attachment of the hydroxyceramides to the envelope.[11] Representative structures of the covalently bound lipids are presented in Figure 3.2.

The literature is replete with the use of chromatographic fraction numbers to indicate ceramide structural types. This can be very confusing because different laboratories have achieved different degrees of separation and because, even with the best resolution achieved, there is at least one fraction

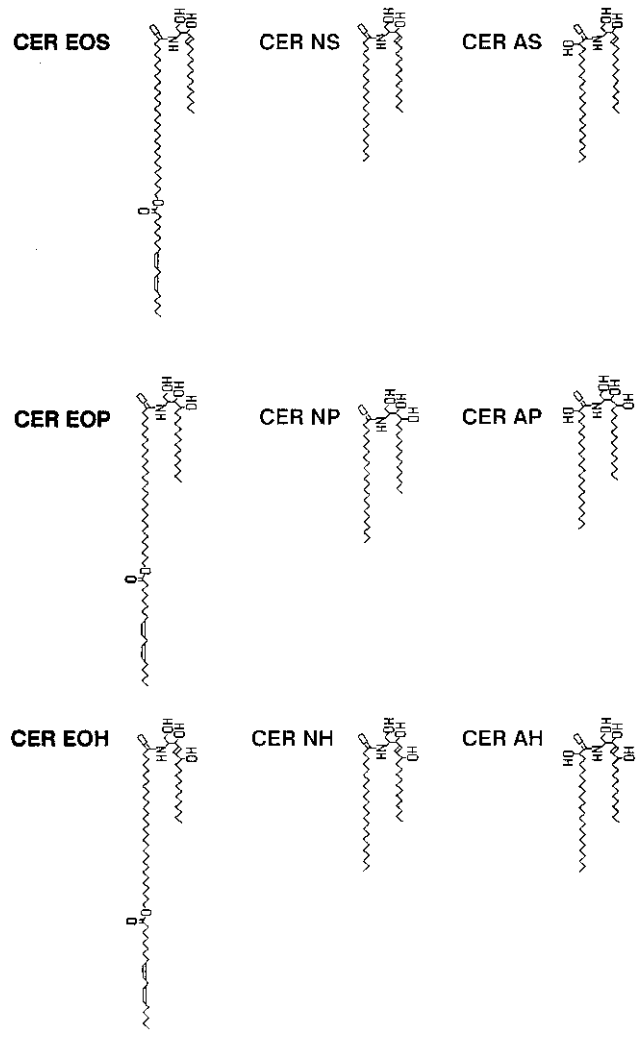

FIGURE 3.1 Representative structures of human stratum corneum ceramides.

that contains two structural types of ceramides. A solution to this problem is a nomenclature system in which the long chain base and amide-linked fatty acids are designated by single letters[12]: S for sphingosine, P for phytosphingosine, H for 6-hydroxysphingosine, N for normal fatty acid, A for α-hydroxyacid, and O for ω-hydroxyacid. The presence of an ester-linked fatty acid is indicated by a prefix E. Thus the acylceramide in which ω-hydroxyacid is amide-linked to sphingosine and linoleate is ester-linked to the ω-hydroxyl group would be designated as ceramide EOS. Similarly, the ceramide consisting of normal fatty acids amide-linked to phytosphingosine would be ceramide NP. This nomenclature is used in Figure 3.1 and Figure 3.2.

Both x-ray diffraction studies and investigations using transmission electron microscopy have indicated that the intercellular lipids are organized into 13 nm trilaminar structures.[13–15] The formation of these trilaminar units seems to require ceramide EOS,[16,17] although to a lesser extent supplementation of lipid mixtures with synthetic EOP can promote self assembly.[18] The possible role of ceramide EOH has not been studied directly; however, it is clear that the natural proportion of ceramide EOH[6] is probably insufficient to promote self assembly of 13 nm units. A transmission electron micrograph of the intercellular lipid lamellae is shown in Figure 3.3. Controversy exists regarding details of the organizational state of the intercellular lipids.[19–21]

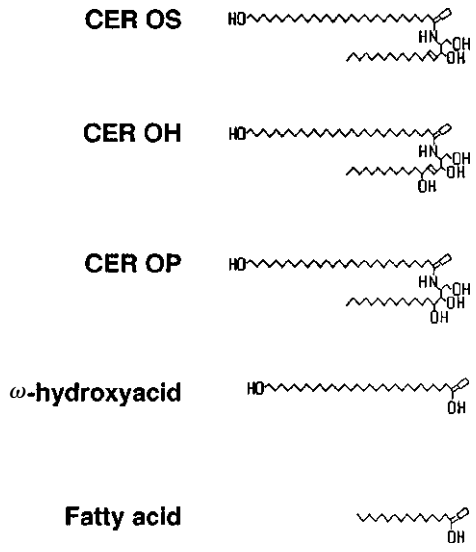

FIGURE 3.2 Representative structures of covalently bound lipids from human stratum corneum.

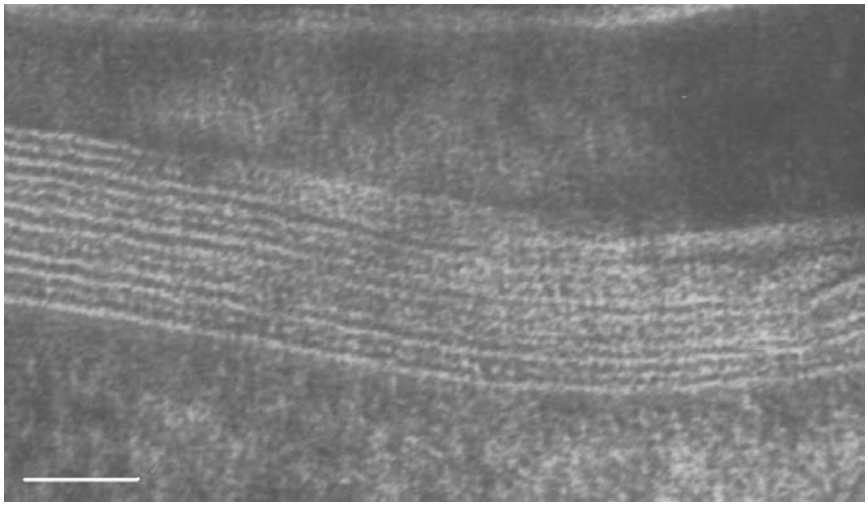

FIGURE 3.3 Intercellular lipid lamellae in the stratum corneum. Bar equals 30 nm.

Interactions between water and the polar head groups of lipid molecules are necessary for the formation of lamellar phases; however, it appears that there is no free water associated with the 13 nm trilaminar units. This is supported by the observation that this periodicity does not increase with increasing stratum corneum water content.[22] There is likely water hydrogen bonded to the polar regions of the lamellae. In contrast, the minor short periodicity swells from 5.8 to 6.6 nm as the water content of stratum corneum increases from 12 to 50%.[23] This suggests that the lipid lamellae are simple individual bilayers and free water molecules can exist between adjacent bilayers, thus causing the increase in the lamellar spacing.

3.1.2 Carbon Sources

Linoleic acid is the parent essential fatty acid of the ω-6 series, and as such, must be obtained in the diet.[24] It is primarily derived from vegetables and nontropical vegetable oils. When linoleate was

injected intradermally into porcine skin, it was initially taken up into a small, rapidly turning over pool of triglycerides.[25] It was rapidly transferred to phosphoglycerides, then to an acylglucosylceramide (glycosylated version of ceramide EOS), and finally to ceramide EOS. Basal keratinocytes have low density lipoprotein (LDL) receptors and can thereby derive cholesterol from the circulation; however, once keratinocytes move upward and out of the basal layer, the LDL receptors are internalized and degraded.[26] Except for linoleate and lipid internalized via basal cell LDL receptors, it is thought that most of the remaining carbon for epidermal lipid synthesis is derived from circulating acetate.[27] Although cultured keratinocytes have been shown to incorporate carbon from glucose into lipids, when radiolabelled glucose was injected intradermally, only the glycerol moiety of the phosphoglycerides became labeled.[27]

3.1.3 Energy Production

Adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) are required to support lipid biosynthesis. Basal keratinocytes have functional mitochondria and are thought to produce energy by β-oxidation of fatty acids.[28,29] The enzymes necessary for glycolysis are also present, but this is much less efficient than β-oxidation and the mitochondrial system. However, as cells move upward metabolism becomes increasingly more anaerobic until in the granular layer energy is produced entirely by anaerobic glycolysis with reduction of pyruvate to lactate. In fact, the mitochondria are degraded. The degradation of mitochondria and other internal membranous organelles would result in the release of calcium previously sequestered by these structures. The generation of the calcium gradient is one of the factors driving differentiation.[30]

3.1.4 Major Biosynthetic Pathways

All the major biosynthetic pathways use acetyl-CoA as the basic building block, and in each pathway the rate limiting enzyme is regulated by phosphorylation with the phosphorylated enzyme being active. In the biosynthesis of cholesterol, the rate limiting step is catalyzed by hydroxymethylglutaryl-CoA (HMG-CoA) reductase. Initially, three molecules of acetyl-CoA are condensed to produce β-HMG-CoA. HMG-CoA reductase then uses two NADPH molecules to reduce HMG-CoA to mevalonate-CoA. The remaining steps in cholesterol biosynthesis are numerous and well-documented.

The rate limiting step in fatty acid synthesis is catalyzed by acetyl-CoA carboxylase to produce malonyl-CoA at the expense of one ATP.[31] Malonate and acetate are transferred from CoA to acyl carrier protein in the cytosolic fatty acid synthetase complex, where chain extension leads to the production of palmitate. Palmitate can then be transferred back to CoA, and the chain can be extended two carbons at a time through the action of a fatty acid elongase system located in the endoplasmic reticulum. The ω-hydroxylation that produces the ω-hydroxyacids of the acylceramides is thought to be mediated by a cytochrome p450 just when the fatty acid is long enough to span the endoplasmic reticular membrane.

The rate limiting step for all sphingolipid biosynthesis is serine palomitoyl transferase, which condenses palmitoyl-CoA with serine to produce 3-ketodihydrosphingosine.[32] The keto group is rapidly reduced, and the resulting dihydrosphingosine group is N-acylated to produce a simple ceramide. The 4,5-*trans* double bond can then be introduced in the base component, and various positions can be hydroxylated to produce α-hydroxyacids, phytosphingosines, and 6-hydroxysphingosines. These hydroxylation reactions require vitamin C.[33]

3.2 LAMELLAR GRANULES

Much of the lipid that accumulates with keratinization is packaged in small organelles called lamellar granules.[4,34] These small organelles have also been called Odland bodies, keratinosomes, membrane

coating granules, lamellar bodies, and cementsomes. They are derived from the Golgi apparatus and are generally round to ovoid in shape and about 0.2 μm in diameter. They consist of a unit bounding membrane surrounding one or several internal stacks of lipidic disks. They are lipid rich, and therefore, have a low buoyant density. This property has been exploited to isolate lamellar granules from rodent and porcine epidermis.[35–37] They are particularly rich in glycolipids, especially the glucosylated analogue of ceramide EOS, and phospholipids, and contain a relatively high proportion of cholesterol. They contain little ceramide or free fatty acids. It has been suggested that glucosylceramide EOS may be involved in assembly of the internal lamellae of the lamellar granules. More recently, it has been suggested that a large portion of the lamellar granule-associated glucosylceramide EOS is actually in the bounding membrane.[5] This pool of glucosylceramide EOS would be introduced to the cell periphery when the bounding membrane of the organelle fuses with the cell plasma membrane, and could be the precursor of the covalently bound hydroxyceramide on the cornified envelope.

3.3 CATABOLISM

In addition to delivering lipids to the intercellular space between the granular layer and the stratum corneum, lamellar granules also deliver a battery of hydrolytic enzymes that convert the initially extruded phospholipid- and glycolipid-rich lipid mixture into the fatty acids and ceramides of the stratum corneum intercellular spaces.[38,39] In rodent epidermis, some of this lipid processing continues in the intercellular spaces of the stratum corneum; however, with porcine and human epidermis conversion to the mature barrier lipids is completed at the stratum granulosum–stratum corneum interface. The enzymes that mediate this transformation are mainly acid hydrolases and include a glucocerebrosidase to convert glucosylceramides to ceramides, and acid sphingomyelinase to convert sphingomyelin into ceramides and a battery of phospholipases to release fatty acids from phosphoglycerides.

3.4 COMPOSITION

The literature regarding the composition of human stratum corneum lipids has recently been reviewed.[40] In general, there is a great deal of variation among the published compositions. Some of this probably reflects differences in the analytical methods that were used; however, much of the variation reflects failures to recognize contaminants including sebaceous lipids, subcutaneous fat, and environmental hydrocarbons. When the known contaminants are factored out, it is apparent that the main stratum corneum lipids are ceramides, cholesterol, and free fatty acids in the ratio of 50:27:12 by weight. When the average molecular weights are taken into consideration, these major components are present in roughly 1:1:1 molar proportions. Mixtures of ceramides:cholesterol and fatty acids in a 1:1:1 molar ratio have been used by a number of investigators to approximate stratum corneum lipids for studies of physical properties.[41–43]

The remaining 11% of the stratum corneum lipid mass consists mainly of cholesterol sulfate and cholesterol esters.[44] The cholesterol sulfate has been implicated in regulation of the desquamation process. It has been shown that cholesterol sulfate inhibits serine proteases of the types that degrade desmosomal proteins leading ultimately to cell shedding. A sterol sulfatase must act on cholesterol sulfate to make the proteolytic degradation of the desmosomes possible. The degradation of cholesterol sulfate in association with desquamation has been demonstrated both with an organ culture model and with human skin *in vivo*. Cholesterol esters have long been cited as a hallmark of keratinization; however, these liquid phase lipids are probably not found within the intercellular lamellae. Late in the keratinization process, oleate is transferred to cholesterol to produce cholesterol oleate. This cholesterol ester is not accommodated well by membranes, and it has been suggested that it phase separates into isolated pockets within the intercellular space. The cholesterol ester deposits are

thought to be reflected in amorphous pockets within the intercellular spaces in transmission electron micrographs. The transfer of oleate to cholesterol and subsequent phase separation of cholesterol oleate may provide a mechanism for keeping oleic acid, a well-known permeability enhancer, out of the lamellar domains that provide the barrier function.

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4 Lipid Structures in the Permeability Barrier

Lars Norlén

CONTENTS

4.1 INTRODUCTION

The physical state and molecular organization of the stratum corneum intercellular lipid matrix largely determines the hydration-level of the stratum corneum and thus, indirectly, the mechanical properties and appearance of the skin. A better understanding of stratum corneum lipid organization may thus aid the development of more efficient cosmetic formulations.

Notwithstanding the spectacular progress made during the last 25 years in the field of stratum corneum research, the structure and dynamics of the lipid matrix of stratum corneum intercellular space is still largely undetermined. This may partly be due to the compositional as well as functional complexity of the stratum corneum intercellular lipid matrix and partly due to the technical difficulties involved in this research field. For example, uncontaminated compositional skin lipid data are difficult to obtain (Wertz and Norlén, 2002). Furthermore, the skin separates two very different compartments (i.e., inside and outside the organism) and consequently several pronounced gradients are present over the stratum corneum. The endogenous stratum corneum thus represents an open (i.e., out of equilibrium) system, and is therefore difficult to model *in vitro*. Also, *ex vivo* techniques, such as conventional electron microscopy and x-ray diffraction, usually requires extensive skin sample preparation which may alter the endogenous lipid organization of the stratum corneum intercellular space. Although everyone knows that sample preparation for conventional electron microscopy may yield but a poor representation of reality, few may be aware of how profoundly different the molecular organization as well as higher order structure of endogenous skin may be with respect to its representations in conventional electron micrographs (Figure 4.1). Using

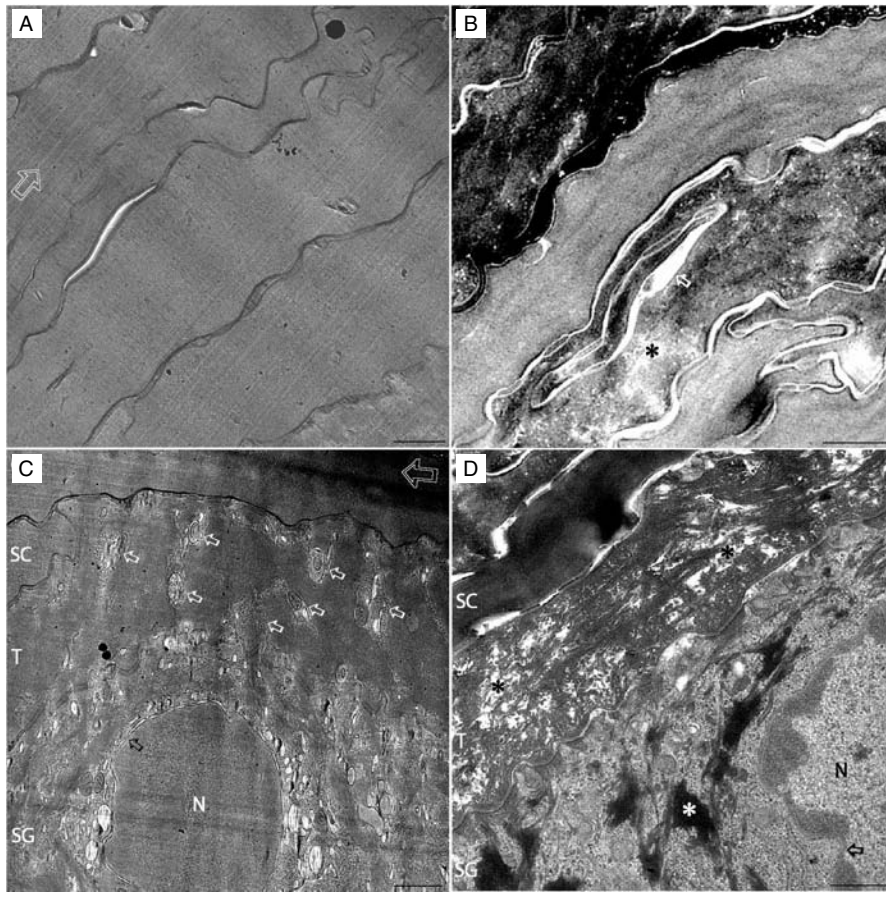

FIGURE 4.1 Conventional sample preparation for electron microscopy results in important losses of epidermal biomaterial. Low magnification transmission electron micrographs of human epidermis at the interzone between viable and cornified cell layers (A, B: lowermost stratum corneum; C, D: uppermost stratum granulosum). (A, C): cryo-electron micrographs of vitreous sections of native epidermis. (B, D): conventional electron micrographs of resin embedded sections. In the vitreous cryo-fixed epidermis (A, C) cellular as well as intercellular space appears densely packed with organic material, while in the conventionally fixed epidermis (B, D) the distribution of biomaterial is characteristically inhomogeneous. Loss of biomaterial appears to have taken place in (B, D), both in the cytoplasmic (black asterix) and intercellular (white arrow) space. Large portions of the biomass of the viable cells appear as aggregated, heavily stained clusters, so-called keratohyalin granules (D, white asterix). Furthermore, the rich variety of cytoplasmic organelles and multigranular structures present in the stratum corneum/stratum granulosum transition (T) cells of native epidermis (C) (white arrows) are replaced by empty space in resin-embedded samples (D) (black asterix). Inner and outer nuclear envelopes and nuclear pores are clearly distinguished in the native cryo-fixed nonstained specimen (C) (black arrow) while they are difficult to distinguish in the conventionally-fixed stained specimen (D) (black arrow). Electron dense single-spot in (A) and double-spot in (C) correspond to surface ice contamination. SG: uppermost stratum granulosum cell; T: transition cell; SC: lowermost stratum corneum cell; N: nucleus; open white double-arrow (A, C): section cutting direction. Section thicknesses ~100 nm (A, C), ~50 nm (B, D). Scale bars 500 nm (A–D). A–D adapted from Norlén and Al-Amoudi (2004). With permission from the Blackwell Science Publications.

conventional tissue embedding methods for electron microscopic observation, the skin is chemically fixed during minutes and subsequently dehydrated by organic solvent, with important loss and precipitation of biomaterial. Methods such as freeze-substitution or low-temperature embedding are less destructive, but the skin is still dry and the use of staining means that it is the local ability to bind stain, rather than the biomaterial per se, which is observed. More precisely, the contrast in conventional epoxy-sections is ascribed to the difference between the inherent electron scattering properties of individual biological elements plus the additional electron scattering of osmium, uranium, and lead, and the epoxy embedding medium itself (Kondo, 1995).

To better understand the structure, function, and dynamics of the endogenous lipid matrix of the stratum corneum intercellular space some general principles of lipid phase behavior, dynamics, and structural organization may represent a useful starting point. Further follows a short overview of some basic physico-chemical principles that may be of relevance for stratum corneum lipid research, followed by a presentation of the new technique cryo-transmission electron microscopy of fully hydrated vitreous skin sections and how this technique recently has been applied to the study of the structural organization and formation of the lipid matrix of the stratum corneum intercellular space.

4.2 LIPIDS AND LIPID ORGANIZATION

4.2.1 Lipid Classification

The term lipid can be defined as fatty acids, their derivatives, and substances related biosynthetically or functionally to these compounds. This definition encompasses cholesterol and bile acids, but does not include other steroids, fat-soluble vitamins, carotenoids, or terpenes (Christie, 1987, p. 42).

From a physical point of view lipids can further be subdivided into nonpolar and polar lipids. Fatty acids, triacylglycerols, diacylglycerols, sterols, and their esters are relatively nonpolar, while monoacylglycerols, phospholipids, galactosylglycerolipids, and sphingolipids belong to the group of polar lipids (Larsson, 1994, pp. 1–3).

4.2.2 Lipid Self-Assembly

Lipids with a hydrophilic (headgroup) and a hydrophobic end (hydrocarbon chain) are termed amphiphilic. The degree of hydrophilicity of molecular groups is important for the understanding of interactions and associations of amphiphilic molecules. These molecules can self-associate or self-assemble into larger three-dimensional (3D) aggregates such as micelles, bilayers, or biological membranes when certain conditions are fulfilled. Such aggregates are fluid-like since the forces that hold these aggregates together are weak screened electrostatic-, van der Waals-, hydrophobic-, and hydrogen-bonding interactions (Israelachvili, 1992, p. 341; Larsson, 1994, p. 47; Hyde et al., 1997). A change in solvent conditions, such as pH or electrolyte concentration, will not only affect the interactions between the aggregates but also the intermolecular forces within each aggregate with resulting changes of structure, shape, and size.

A difference in the cohesive energies between the molecules in the aggregated and the dispersed states is a prerequisite for aggregates to be formed. The monomer concentration at which aggregation formation starts is termed the critical aggregation concentration (CAC) or critical micelle concentration (CMC) (cf. Israelachvili, 1992, pp. 348–352). The major forces involved in self-assembly of lipids are the hydrophobic attraction at the hydrocarbon/water interface and the hydrophilic, steric, or ionic repulsion of the headgroups. The former tending to decrease and the latter to increase the interfacial headgroup area per molecule. To understand the phase-behavior of a system, the complex separate force contributions do not need to be known in detail since one can expect the first term in any energy expansion to be inversely proportional to the surface area occupied per headgroup. The headgroup surface area at which the total free energy per molecule in a system is at a minimum

is termed the optimal surface area, defined at the hydrocarbon/water interface. This implies that, to a first approximation, the interaction energy between lipids has a minimum at a certain headgroup area, a_0. In fact, self-assembly of lipid aggregates can largely be quantitatively understood from geometrical considerations. The packing properties of amphiphilic molecules depend on their optimal headgroup area, a_0, hydrocarbon chain volume, v, and the critical chain length, l_c (maximum effective hydrocarbon chain length). Once these three parameters are determined one can to a good approximation estimate into which structures the molecules can self-assembly. Entropy will then favor the structure with the smallest aggregation number. The dimensionless critical packing parameter, cpp $= v/(a_0 l_c)$, can be used to determine whether the lipids will form bilayers ($\frac{1}{2} < v/(a_0 l_c) < 1$), spherical micelles ($\frac{1}{3} < v/(a_0 l_c) < \frac{1}{2}$), or reversed structures ($v/(a_0 l_c) > 1$). Each of these structures will exist as the smallest aggregate in which all the lipids have minimum free energy (cf. Israelachvili, 1992, pp. 368–371).

As indicated above, the lipid structures formed can to a large extent be modified by the ion-concentration, pH, and temperature of their environment as well as by the degree of unsaturation of the hydrocarbon chains. For anionic headgroups the headgroup area can be decreased by increasing the salt concentration, particularly of divalent ions like Ca^{2+}, or by lowering the pH. This also has the effect of condensing the hydrocarbon chains. Introduction of branched chains, particularly of *cis* double bonds, reduces the critical chain length and consequently increases the critical packing parameter. This will ultimately favor reversed structures. An increase in temperature reduces the critical chain length due to increased hydrocarbon chain motion. However, it can also alter the optimal headgroup area in both directions depending on the specific headgroup characteristics (cf. Israelachvili, 1992, p. 380).

4.2.3 Lipid Phase Behavior

4.2.3.1 Solid State

In the solid state the translational motion of the molecules is slow and the molecules are arranged with long-range orientational and positional order. However, for compounds with long hydrocarbon chains the molecules may rotate in their lattice sites at the same time as they maintain full positional order, forming so-called "plastic crystals" (Evans and Wennerström, 1994, p. 412). The stability of these "plastic" crystalline phases (α-forms) increases with chain length and with the presence of impurities (e.g., broad chain-length distributions) (Larsson, 1994, p. 27).

Lipid molecules have a unique property in that they often can be packed in different ways in the solid state, that is, they exhibit polymorphism, although there is only one best packing mode. The main mechanisms behind polymorphism are variations in the tilt of molecules of the bilayer and variations in hydrocarbon chain packing (Larsson, 1994, p. 11). The best way to describe the chain packing is to use the subcell corresponding to the smallest repetition unit within the unit cell (Hernquist, 1984, p. 14). The main polymorphic forms are the α-, β'-, and β-form. A melt crystallizes into an α-form upon cooling. Using x-ray diffraction, α-forms show one strong diffraction line at 4.15 Å. This corresponds to the hexagonal chain packing, which expresses rotational disorder along the hydrocarbon chain axes. When an α-form is stored there is usually an irreversible phase transition into a crystal form with fixed hydrocarbon chain planes (i.e., this crystal form has a higher melting point than corresponding α-forms). An orthorhombic chain packing may then be formed, where every second hydrocarbon chain plane is perpendicular to the others. This crystal form is called a β'-form. It is characterized by two diffraction lines at 3.8 and 4.2 Å. (Hernquist, 1984, pp. 16–18, 24–31; Small, 1986, pp. 98–101; Larsson, 1994, pp. 13–14, 27).

Crystals of most polar lipids can swell in the presence of water. The corresponding phases, gel-phases, with lamellarly packed lipid, and water layers, are sometimes thermodynamically stable (Larsson, 1994, p. 41). Also, the hydrocarbon chain packing of gel-phases usually show some axial rotational disorder. The alkyl chain cross-sectional area is close to 20 $Å^2$ in a plane perpendicular

to the direction of the chain (Evans and Wennerström, 1994, p. 247). If the headgroup area and the cross-sectional area of the alkyl chain is similar, a lamellar L_β-phase with chains perpendicular to the bilayer surface is formed. If the headgroup area is dissimilar to the cross-sectional alkyl-chain area a lamellar $L_{\beta'}$-phase with tilted chains or a rippled $P_{\beta'}$-phase results (Evans and Wennerström, 1994, p. 247).

4.2.3.2 Liquid State

The transition from the crystalline to the liquid state is accompanied by absorption of heat, a loss of long-range order, and an increase in molecular volume. However, many long chain lipids show only small volume changes (10–20%) during the transition from solid to liquid, which indicates that some short-range order should remain in the liquid state (Small, 1986, pp. 56–57).

4.2.3.3 Liquid Crystalline State

When a lipid molecule in the crystal state is heated it may pass through intermediate states called mesophases or liquid crystals (Friedel, 1922) instead of melting directly into an isotropic liquid. These liquid crystals are characterized by residual long-range order, but lack of short-range order (Small, 1986, p. 49). Since they have both some degree of order and of fluidity they possess the characteristics of both liquids and of crystalline solids. In the liquid crystal the hydrocarbon chains are "melted" and consequently there is solely a crystalline periodicity in the direction corresponding to the bilayer thickness. This is because the forces between the polar headgroups are stronger than the van der Waals interaction between the hydrocarbon chains. Consequently, when energy is added to the system, the thermal motions will overcome the forces between the hydrocarbon chains but not the forces in the polar headgroup sheets, resulting in hydrocarbon chain disorder but unchanged bilayer structure (Larsson, 1994, p. 47).

The change from a crystalline into a liquid crystalline state can be brought about by changes in, for example, temperature or pressure. Furthermore, some molecules may be induced to form liquid crystals by the addition of a solvent such as water. This behavior is in reality a liquid crystalline formation in a two component system and is called solvent-induced liquid crystal formation or lyotropic mesomorphism (Small, 1986, p. 49).

Liquid crystals can be in the smectic, nematic, or isotropic states. In the smectic liquid crystalline state there is a long-range order in the direction of the long axis of the molecules. These molecules may be in single- or bilayer conformation, have molecular axis normal or tilted to the plane of the layer, and frozen or melted chains. In the nematic liquid crystalline state the molecules are aligned side by side but not in specific layers. The isotropic liquid crystalline state is more or less a liquid state, but where clusters with short-range order persist (Small, 1986, pp. 49–51).

As described above, different phases and geometries can be formed by self-assembly of liquid crystalline amphiphilic molecules depending on their optimal headgroup area, a_0, hydrocarbon chain volume, v, and the critical chain length, l_c (maximum effective hydrocarbon chain length) (Israelachvili, 1992, p. 370). To form a lamellar phase (L_α) a cross-sectional area of 28 to 32 Å^2 is required (Larsson, 1994, p. 48). By increasing the temperature and/or decreasing the relative amount of water and/or decreasing the optimal headgroup area a reversed hexagonal phase (H_{II}) and eventually a reversed micellar phase (L_2) may be induced. In contrast, by decreasing the temperature and/or increasing the relative amount of polar solvent and/or increasing the optimal headgroup area a normal hexagonal phase (H_I) and eventually a normal micellar phase (L_1) is formed. Other phase geometries may appear between the L_1, H_I, L_α, H_{II}, and L_2-phases. These are most commonly the descrete (I_1, I_2) and bicontinuous (V_1, V_2) cubic phases (Figure 4.2) (Larsson, 1989). The reversed bicontinuous cubic phases (V_2) represent infinite periodical minimal surfaces (IPMS) (Larsson, 1994, pp. 50–55) (cf. Section 4.2.4).

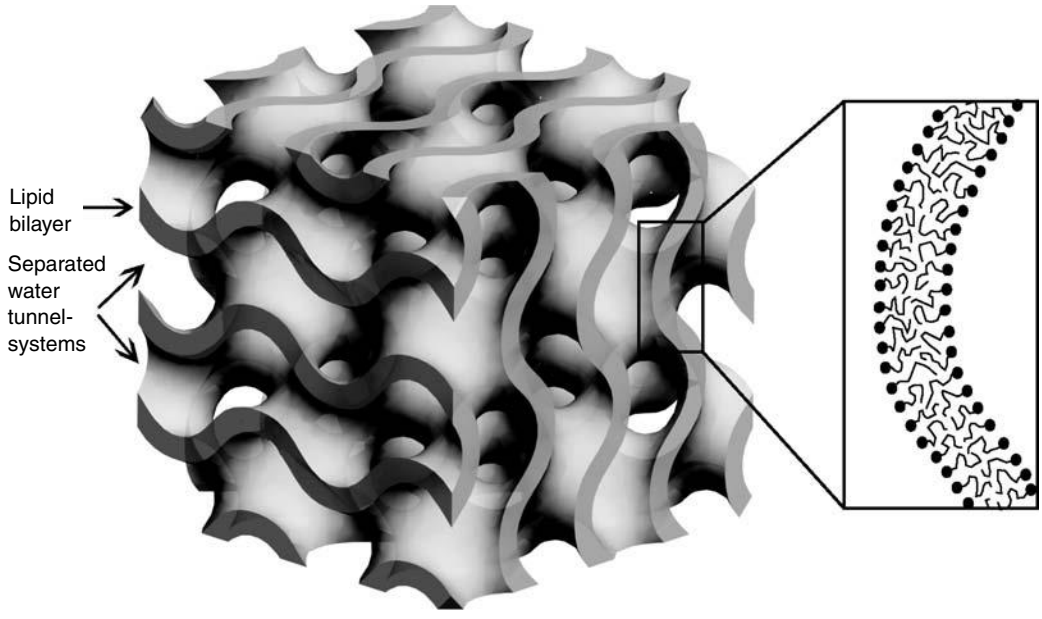

FIGURE 4.2 Schematic illustration of $2 \times 2 \times 2$ unit cells of a lipid/water phase with gyroid cubic symmetry. In reversed bicontinuous cubic phases the lipid bilayer membrane separates two intertwined water-filled subvolumes resembling 3D arrays of interconnected tunnels. Black box (right) represents an enlargement of a part of the folded liquid crystalline lipid bilayer membrane structure.

4.2.4 Liquid Crystalline Lipid/Water Phases with Cubic Symmetry

A central issue in the field of lipid self-assembly is the structure of liquid crystalline mesophases denoted bicontinuous cubic phases (Figure 4.2). Cubic lipid/water phases were detected by Luzatti et al. and Fontell in the 1960s although they were believed to be rare in comparison with the classical lamellar, hexagonal, and micellar mesophases. It is now clear that these phases are ubiquitous in lipid systems (Hyde et al., 1997). Further a number of cubic phases can occur in the same system as the temperature or solvent concentration/composition is varied. It is surely no coincidence that the symmetries of these lipid/water phases are precisely those of low genus three-periodic minimal mathematical surfaces with cubic symmetry. The simplest three-periodic hyperbolic (i.e., saddle shaped, or, more specifically, with negative average Gaussian curvature) surfaces are IPMS of the primitive (P), gyroid (G), and diamond (D) types. For these surfaces the mean curvature is constant and everywhere identically zero, just like for a flat surface. In fact, geometrical analysis indicates that reversed (bilayer) bicontinuous cubic phases are only to be found in lipid/water systems that also form lamellar phases readily (i.e., where the average molecular shape is close to cylindrical ($v/a\mathrm{l} \sim 1$)). This close geometrical resemblance between the lamellar and the bicontinuous cubic phases is further emphasized by the low enthalpy difference between these two phases (~0.5 kJ/mol lipid) (Engström et al., 1992) as compared to the enthalpy difference between the lamellar and reversed hexagonal phases (~5 to 10 kJ/mol lipid) (Seddon et al., 1983). These facts strongly suggest that hyperbolic mesophases with cubic symmetry may be presented in biological cells, which typically express lamellar membrane bilayer morphologies. In fact, most biological membranes contain at least one lipid species that can form a cubic and/or hexagonal phase (Lindblom and Rilfors, 1989).

Recently it was shown that biological membranes with cubic symmetry are indeed present in many living systems, and, as suspected on theoretical grounds (cf. earlier), often closely associated

with lamellar membrane morphologies (Landh, 1996; Hyde et al., 1997). There are, however, several differences between the hitherto identified cellular membranes with cubic symmetry and cubic lipid/water equilibrium phases (Bouligand, 1990; Landh, 1996, pp. 171–173). Perhaps the most striking difference is that the observed periodicities in biological membrane systems with cubic symmetry studied so far by conventional electron microscopy are much larger (unit cell size ~50 to 2000 nm) than for corresponding cubic lipid/water equilibrium phases (unit cell size ~10 to 30 nm). Other differences are that reversed bicontinuous cubic lipid/water phases usually are balanced (i.e., the two subvolumes separated by the lipid bilayer are of equal size; Figure 4.2) and constituted by a single bilayer leaflet, which often is not the case for hitherto identified biological membranes with cubic symmetry (Landh, 1996). In other words, in the nonequilibrium situation *in vivo* it is not clear whether lipid composition (i.e., average molecular shape) can be directly related to membrane geometry. However, using cryo-electron microscopy on vitreous sections of native human skin the existence of cubic-like cellular membrane morphologies of dimensions corresponding to those of cubic lipid/water equilibrium phases space was recently indicated (Al-Amoudi et al., 2004). Such membrane structures may be of central importance for the formation of the lipid matrix of the stratum corneum extracellular space as well as for the formation of the stratum corneum keratin network (Norlén and Al-Amoudi, 2004).

4.3 CRYO-TRANSMISSION ELECTRON MICROSCOPY OF VITREOUS SKIN SECTIONS

Water is a major constituent of skin. However, most of our knowledge of skin ultrastructure has been gained from observation on dehydrated epidermis (e.g., using conventional electron microscopy on dehydrated skin samples or x-ray diffraction on isolated stratum corneum). It is possible, therefore, that the deleterious effects of conventional specimen preparation may not have been fully acknowledged in the present perception of epidermal cellular/molecular organization. This may be particularly true for biological liquid crystalline structures such as lipid membranes as water activity represents a major factor determining lipid phase behavior and structural organization (Guldbrand et al., 1982; Small, 1986; Israelachvili, 1992; Evans and Wennerström, 1994; Larsson, 1994).

Cryo-electron microscopy of vitreous sections of freshly taken, fully hydrated, noncryoprotected, nonstained (i.e., native) skin samples has several major advantages over conventional electron microscopy of chemically fixed specimens. These are: (i) no loss of biomaterial (including water); (ii) infinite preparational reproducibility (in the case of successful complete tissue vitrification); (iii) biostructures may be preserved down to atomic resolution; (iv) the optical density of the recorded image is directly related to the local density of the biological material of the sample; (v) immobilization of skin can be achieved within seconds after sample acquisition; (vi) total tissue fixation time is in the millisecond range. These advantages should, however, be weighed against possible pressure-induced artifacts, cutting-induced deformations, and electron beam damage. Furthermore, even a millisecond cryo-fixation time is long compared with the characteristic time of many dynamical biological processes. Another drawback is that section handling and microscopy is demanding, rendering serial sectioning and 3D-reconstruction more difficult. Low electron dose computerized cryo-electron tomography on vitreous skin sections may, however, in future largely overcome this limitation.

Recently, it was reported that a freshly-taken, fully hydrated full thickness human epidermis can be completely vitrified, directly, without the use of cryoprotectants or any other pretreatment (Norlén et al., 2003). As mentioned above, successful tissue vitrification has the potential to preserve biostructures down to atomic resolution. Consequently, the native ultrastructure of epidermal biomolecular complexes could now therefore, theoretically, be observed at subnanometer resolution *in situ*.

Due to optimization of the cryo-sectioning method (i.e., minimized section thickness) and microscopy technique (i.e., minimized electron dose), native epidermal ultrastructural *in situ* data, with a resolution of a few nanometer, has recently been presented of the skin barrier lipid organization and its formation process (including desmosomal differentiation) (Al-Amoudi et al., 2005) as well as on the structural organization and formation of the corneocyte cell matrix (i.e., the keratin intermediate filament network) (Norlén and Al-Amoudi, 2004). These high-resolution cryo-electron microscopic data are closer to the biological reality, more detailed and differ from those obtained by conventional electron microscopy of resin embedded epidermis. Notably they indicate (a) that the skin barrier formation process may evolve via a lamellar "unfolding" of a small lattice parameter lipid "phase" with cubic-like symmetry with subsequent "crystallization," or "condensation," (including lamellar reorganization) of the epidermal intercellular lipid matrix and b) that the multilamellar lipid organization of the stratum corneum intercellular space may be more complex than earlier thought (Al-Amoudi et al., 2005) (Figure 4.3). Notably, "lamellar body discs" (cf. Landmann, 1986) at the interface between stratum granulosum and stratum corneum, as well as "13 nm lamellar repeats" (cf. Bouwstra et al., 2000; McIntosh, 2003; Hill and Wertz, 2003) of the stratum corneum intercellular space are conspicuous by their apparent absence in cryo-electron micrographs of vitreous skin sections (Norlén et al., 2003; Al-Amoudi et al., 2005).

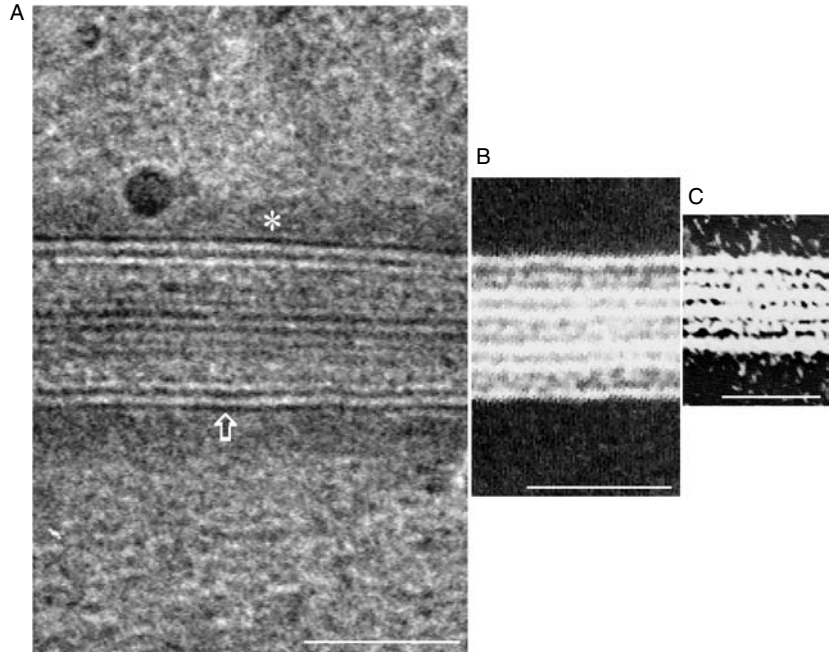

FIGURE 4.3 High magnification transmission electron micrographs of multilamellar membrane structures in the intercellular space of the cornified part of human epidermis. (A): cryo-electron micrograph of vitreous section. (B, C): conventional electron micrographs of resin embedded sections. The cell plasma membranes appear as 3.8 nm wide bilayers in (A) (open white arrow). A 16 nm broad zone of electron dense material, the cornified cell envelope (white asterix), is directly apposed to the cytoplasmic side of the bilayer plasma membranes in the native sample (A) (open white arrow). Scale bar 50 nm (A). Scale bars 25 nm (B, C) adapted from measures given in Swartzendruber et al. (1989). (A) reprinted from Norlén (2003). With permission from Blackwell Science Publications. (B, C) reprinted from Swartzendruber et al. (1989). With permission from Blackwell Science Publications.

4.4 LIPID ORGANIZATION OF THE STRATUM CORNEUM INTERCELLULAR SPACE — A SINGLE AND COHERENT MULTILAMELLAR "DRM"?

Water homeostasis is a strict requirement for normal physiological function. The most important task of the human skin is thus to create a watertight enclosure of the body to prevent water loss. It is the intercellular lipid matrix of the outermost keratinized horny layer of the skin (possibly together with recently reported claudin-based tight-junctions; Furuse et al., 2002) that represents the skin barrier proper as once this lipid matrix (composed foremostly of saturated long chain ceramides (∼50% wt/wt) and cholesterol (∼30% wt/wt) (Wertz and Norlén, 2002)) has been removed, substances diffuse freely into or out of the body system (Blank, 1952; Breathnach et al., 1973; Elias and Friend, 1975). At the same time the intercellular lipid matrix ensures that the stratum corneum remains hydrated and thus the skin surface appears healthy and smooth.

The strong gradients in, for example, water concentration, present over the stratum corneum *in vivo* suggests that the structure and function of the intercellular lipid matrix cannot be fully understood unless the processes involved in its formation are considered. The conventional view of the formation of the stratum corneum intercellular lipid matrix is essentially that "lamellar bodies" (i.e., discrete spherical lipid bilayer vesicles), containing in their turn "lamellar disks" (i.e., discrete flattened lipid bilayer vesicles), bud off from the *trans*-Golgi network and diffuse toward the plasma membrane of the differentiating stratum granulosum cells (i.e., topmost viable epidermal cells facing stratum corneum). After fusion of the limiting membrane of the "lamellar bodies" with the plasma membrane of the stratum granulosum transition cell, the lamellar body lipid content is thought to be discharged into the intercellular space where the "lamellar discs" merge into intercellular lamellar sheets via a second fusion process (Landmann, 1986). However, cryo-electron microscopic indications of the existence of "lamellar bodies" and "lamellar body discs" in native skin is lacking (Norlén et al., 2003; Al-Amoudi et al., 2005). Quite differently, the skin barrier formation process may instead take place as a lamellar "unfolding" (or "phase transition") of a small lattice parameter liquid crystalline lipid "phase" with cubic-like symmetry with subsequent "crystallization" or "condensation" (including possible lamellar reorganization) of the intercellular lipid matrix (Norlén, 2001a). Furthermore, the idea of a direct close-packing (crystallization or condensation), without marked sorting of lipid species during the close-packing process, of such a liquid crystalline structure into a single multilamellar gel-like structure (Norlén, 2001b, 2002), reduces to a minimum introduction of unknown features when trying to explain the formation, structure, and function of the lipid matrix of the stratum corneum intercellular space.

Cholesterol may represent a key entity for the proper formation, molecular packing, and function of the lipid matrix of the stratum corneum intercellular space. Generally, in the presence of cholesterol the movements of *liquid* crystalline lipid chains are strongly reduced with resulting diminished distances between the hydrocarbon chains and thus increased van der Waals interaction. Consequently, liquid hydrocarbon chains seem to be "condensed" toward the cholesterol skeleton, however, without crystallizing and without loosing all mobility. For saturated, *crystallized* lipids, a competition arises for the hydrocarbon chains (e.g., those of skin ceramides and free fatty acids) between cholesterol and the crystalline aggregate. Cholesterol may consequently "steal" hydrocarbon chains from the crystalline aggregate by offering these a more favorable van der Waals interaction. In such an aggregate with cholesterol, the saturated hydrocarbon chains cannot be in all-*trans* conformation and are thus, by definition, liquid crystalline (i.e., they cannot give rise to crystalline wide-angle reflections in x-ray experiments). From a crystallographic point of view, the single gel-phase predicted in the single gel-phase model (Norlén, 2001b) may thus *in cholesterol-rich regions* be an unusually close-packed *liquid crystalline* structure. Consequently, the endogenous lipid organization of the stratum corneum intercellular space may resemble that of nonionic detergent resistant membrane fragments (DRMs) isolated from a variety of eukaryotic cells. These, like the skin barrier lipid matrix, are composed of a mixture of saturated long acyl-chain sphingolipids and cholesterol and, likewise, may

exist as a *liquid ordered* structure (i.e., a "gel phase"; Ahmed et al., 1997; Brown and London, 1997; Brown, 1998; Ge et al., 1999; Xu and London, 2000).

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5 Particle Probes and Skin Physiology

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CONTENTS

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5.1 INTRODUCTION

The condition of the skin, whether it is normal, dry, eczematous, etc., reflects its physiology/pathophysiology. Only during the past three decades has it been possible to probe the physiology of human skin, and this has been achieved through the means of particle probes. The electron and the proton probe both rely on the production of secondary x-ray quanta emission, which allows identification as well as quantification of elements. Since there exists no well-established model to substitute for human skin in experimental approaches to clinically normal and pathological skin conditions, this chapter is devoted to the study of element and particularly trace element distributions in normal and pathological human skin. It is interesting to note that the development of modern medicine from the moment of the discovery of x-rays has been closely linked to the development of physics. Almost immediately after his discovery of x-rays in December 1895, Konrad Röntgen made an image of his left hand carrying a finger ring. From a historical point of view, this can truly be regarded as the first clinical x-ray image. It is a fact that this image had a tremendous impact on the contemporary medical body, and the clinical applications of the method were greeted with great enthusiasm among medical doctors. It became obvious that the density of the material was related to the degree of x-ray absorption, that is, the bones were seen easily against the background of soft tissue. In clinical practice this resulted in the invention of contrast media which allowed, for example, the intestinal system to be imaged with a fair amount of detail.

Early on, x-rays were used for structure determination, and Bragg, father and son, are justly regarded as portal figures in this basic research application of the "mysterious rays." The development of this and other analytical methods based on x-ray techniques has had a pronounced impact on modern biology. This chapter will briefly outline the history of x-ray absorption in biological research and then concentrate on the application of micro probes with special reference to the proton probe in experimental dermatology. References to results from other techniques will, however, be included.

5.2 THE BEGINNING — QUANTITATIVE MICRORADIOGRAPHY

The UV-absorption method, with which quantitative determination of DNA in tissue sections can be done, provided the inspiration for the development of x-ray spectrographic methods at the end of the 1940s. The quantitative x-ray analysis methods were developed to provide quantitative elementary analysis on a histochemical and cytochemical scale, that is, quantitative elemental analysis of tissues *in situ* and at a subcellular level. Engström[1] formulated how the problem could be attacked in the following way:

1. *Alternative 1*: Quantitative analysis of the element in question in a very small piece of tissue, microdrop, or something similar; the localization of the element in question in the tissue being obtained in the preparation of the analysis object
2. *Alternative 2*: Quantitative determinations of the element in question, which has a relatively low atomic number, within a cell or a very small area in a microscopic section of a tissue, but retaining the structure; resulting in the analysis being directly correlated to the cytological structure

The second alternative was developed in Engström's thesis, "Quantitative micro- and histochemical elementary analysis by roentgen absorption spectrography," which he published in 1946, and his method became known as quantitative microradiography.[1] A satisfactory resolution was granted by fine-grain Lippmann emulsions, available in the 1930s, and this film material allowed a resolution in the 10-μm range.

This spectrographic method that allowed chemical elementary analysis of single mammalian cells was based on the selective absorption of monochromatic x-rays measured directly in the spectrometer

or by photometry of the x-ray photographic image of the cell/tissue. By exposing the same object for x-rays on each side of the absorption edge for the element to be determined, quantitative data were obtained, for example, concentrations when the mass of the exposed area/volume was determined by a "white" radiation exposure. In early studies, ^{15}P and ^{20}Ca were determined in 10-μm bone sections within an area of $10 * 10$ μm, and the amounts determined were of the order of 10^{-9} to 10^{-12} g. The error of the analysis was estimated to be 5 to 10%. The photographic recording provided a precise localization of the area measured, and grain density can be determined by photometry, out of which quantitative data can be calculated. Engström's method was further developed and refined by the work of Lindström[2] (Figure 5.1). He expanded the theoretical basis for x-ray absorption spectrophotometry and constructed an x-ray spectrophotometer with a bent crystal that produced high intensity monochromatic x-rays of varying wavelengths. The basis provided by the work of Engström and Lindström is presently put to good use in fully-automatic microradiographic systems and standard tools in, for example, dental research.

5.3 INERT PREPARATION — CRYO-METHODS FOR ELEMENTAL ANALYSIS OF TISSUE SAMPLES

The content of a cell can be regarded as a gel in which ions are free to move at appreciable speed with minor restrictions. The study of the physiology of a cell in a particular phase of its activity must be done on a sample where all ionic movements have been instantaneously arrested. Chemical fixation relies on the diffusion of the fixing agent into the cell and its contents, and will obviously perturb the particular conditions sought. If the tissue temperature can be instantaneously lowered to produce vitreous ice, this would be an ideal preparative choice.[3–6] However, the heat conductive properties of organic material are far from excellent, and, therefore, we expect a gradient of temperature to move down into a tissue block exposed to a freezing medium. It has been shown that the depth to which a complete momentary freezing will reach is only about 50 to 100 μm. Further down in the tissue a temperature gradient will cause ice crystals to form, and these ice crystals not only disrupt the morphology of the cell, but also create redistribution of movable ions in a freezing-out process. Therefore, only a surface portion of a cryo-fixed tissue block is suitable.

After the subsequent sectioning of only the outer part of the frozen tissue block, we should ideally have a tissue section with a vitrified cellular gel containing all ions in their "natural" morphological positions. However, cryo-sectioning is actually a process of shearing. The shearing process may actually cause a rise in the temperature of the section surface unless precautions against this are taken. Samples aimed for high resolution analysis require that sectioning be performed preferably in a temperature-controlled chamber at an ambient temperature of lower than -100°C. Also, the knife temperature must be controlled and kept very close to this temperature if very thin sections ($<$200 nm) are desired.[4] This is especially the case for x-ray microanalysis (EMP) in the scanning transmission electron microscope (STEM).

5.4 ENERGY DISPERSIVE X-RAY MICROANALYSIS IN THE ELECTRON MICROSCOPE

The original electron probes were wavelength dispersive, utilizing a crystal spectrometer for analyzing the particular characteristic x-ray emission from an element sought for. It was realized in the 1960s that the scanning electron microscope (SEM) actually represented an analysis system, that is, had a potential of being a versatile analysis instrument. In addition to the secondary electrons used for imaging, the electron beam of an SEM produces a number of signals, for example, back-scattered electrons, x-rays, cadluminiscent light, Auger electrons, electric current, etc.

In the early 1970s when the energy dispersive detectors of semiconductor origin were commercially introduced, they actually revolutionized elemental analysis in the electron microscope. X-ray

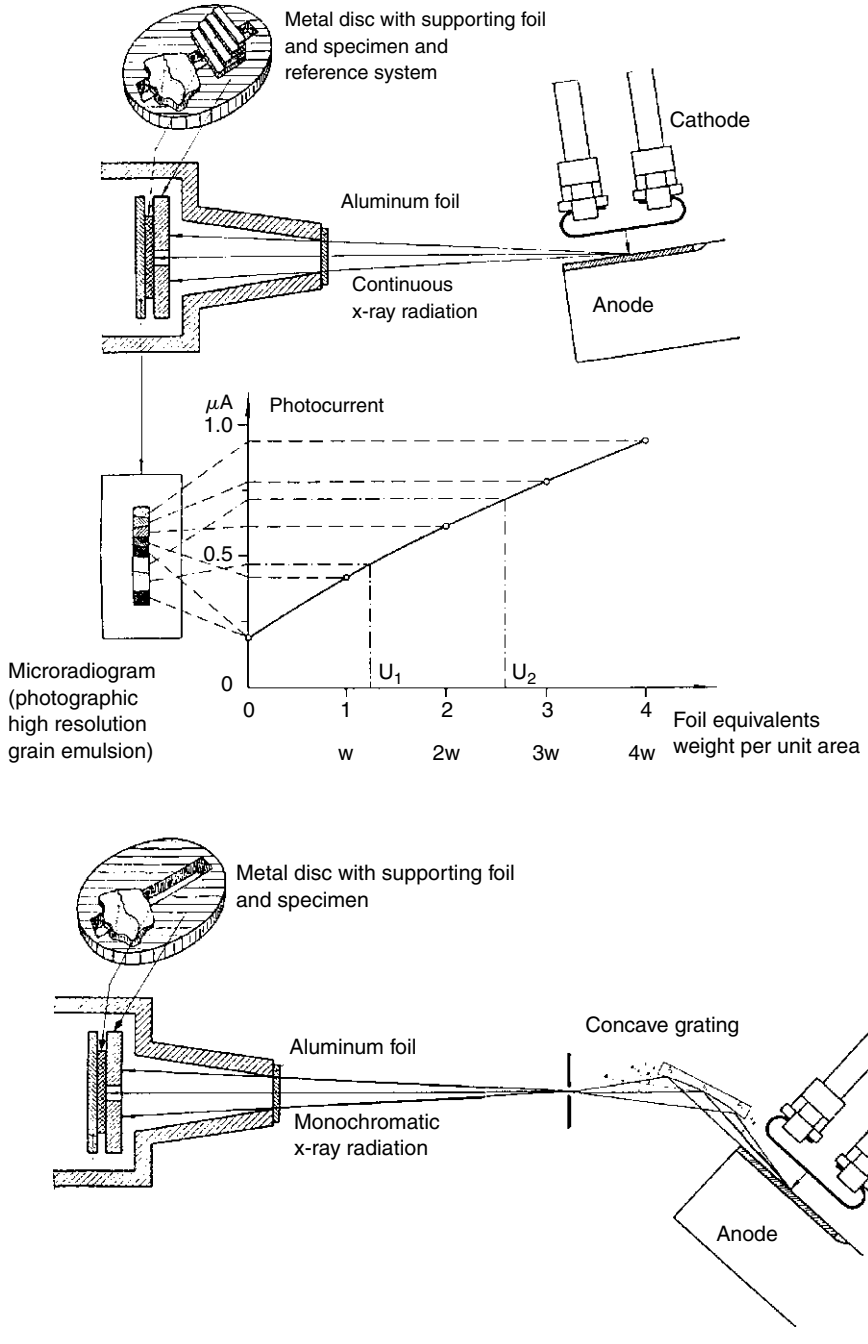

FIGURE 5.1 Top: The principle of microradiography — an x-ray absorption technique for quantitative assessment of dry weight (mass). Bottom: In addition to the mass information, a specified element can be quantitatively assessed by using two monochromatic radiation wavelengths on each side of an absorption edge for the element. (Adapted from Lindström, B., *Acta Radiologica Suppl.* 125, 206, 1955.)

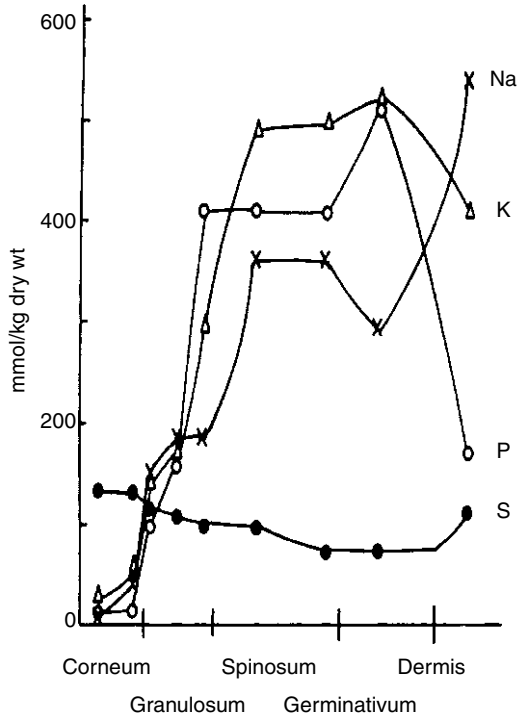

FIGURE 5.2 STEM x-ray spectrum from a skin sample.[48] Note that there is a conspicuous shift in the Na/K ratio moving from the dermis into the basal cell layer. Again, moving into the stratum spinosum, Na increases and K is lowered, a ratio shift, which suggests that the spinosum cells are incapable of entering the mitosis cycle.

microanalysis (XRMA) almost immediately found numerous applications in medical and biological sciences. The energy-dispersive detectors allowed a simultaneous recording of "all" elements in the irradiated volume. Cytochemical methods are hampered by the obvious drawback of not allowing multielement analysis in the same section. The particle probes present a great advantage because virtually all elements of physiological interest can be measured simultaneously within one and the same volume. Consequently, comparisons of the relative contents and formation of elemental ratios, for example, Na/K, that provide sensitive markers for cellular function[7] (Figure 5.2), are often more sensitive indicators of a physiological change than the absolute amounts of an ion (i.e., an element). The additional fact that the electron beam could scan a surface area of the object meant that elemental mapping now in principle was possible. However, it is clear for what was hinted previously that the XRMA technique requires inert preparation in order to minimize ion flux during the preparation and analysis. In the past three decades, cryo-fixation and cryo-sectioning methods, as well as freeze-drying techniques, have consequently been the focus for preparation technique development.[3,5,8]

5.5 PROTON PROBE ANALYSIS

The use of particles heavier than electrons, and especially proton-induced x-ray emission analysis, was developed under the supervison of Professor Sven Johansson at Lund University, Sweden, during the 1970s.[9] Generally referred to as PIXE (particle- or proton-induced x-ray emission) analysis, it has proven to be a sensitive trace element technique. The initial response among medical researchers was a cautious one, most likely due to the fact that the problems of specimen preparation initially were

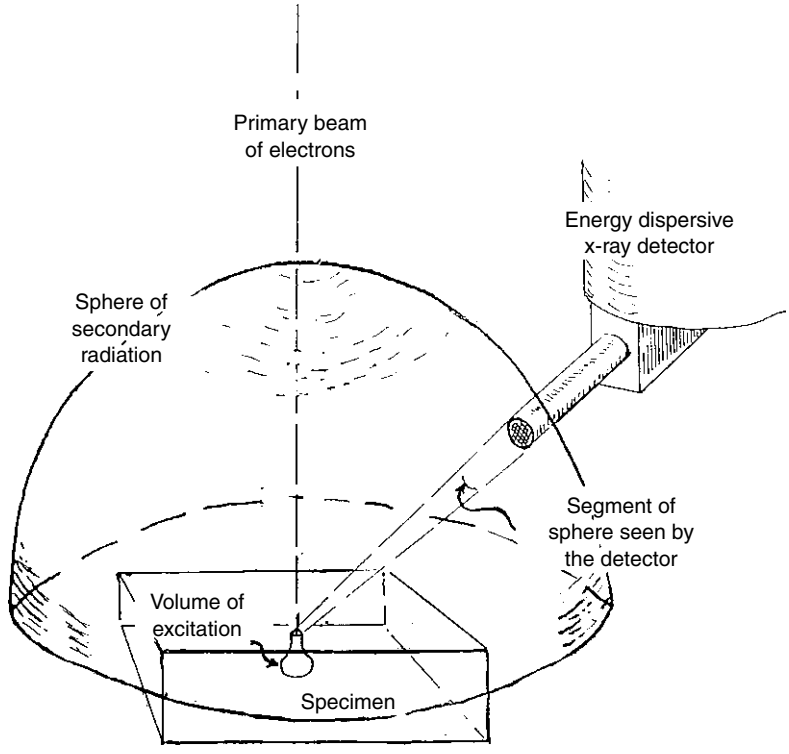

FIGURE 5.3 Sphere of secondary radiation. The detector "sees" only a fraction of the total number of emitted x-ray quanta. (Adapted from Lindström, B., *Acta Radiologica Suppl.* 125, 206, 1955.)

not fully appreciated by the physicists or even among biologists. Hence, the interpretation of results obtained from ill-prepared specimens were difficult, if not impossible, to make. Today technical and preparative problems are well-acknowledged and proton probe analysis is used in medical or biological applications by several research groups around the world.

5.6 DETECTION OF THE X-RAY SIGNAL

In particle probe analysis systems, x-rays are generated from the elements due to an excitation caused by the impinging particles, whether they are electrons or protons, and these secondary x-rays are emitted in all directions. However, the detector can only cover a small part of the sphere of secondary radiation (Figure 5.3), even if the geometry of the experimental setup allows the detector to come very close to the object, which will increase the spatial angle from which the detector "sees" the volume of analysis. Here we see a factor which influences markedly the sensitivity of the analysis method.

The x-rays generated represent quanta of energy. Since characteristic x-ray quanta represent "fingerprints" of the atom they are originating from, a detector that can sort quanta according to its energy will allow identification of elements present in the excited specimen volume. The number of quanta recorded will be proportional to the amount of that particular element present (Figure 5.4). The energy-dispersive system is a fast detector system that uses a signal processor, which transforms the incoming x-ray quanta into electric pulses, subsequently fed into a multichannel analyzer that recognizes the different energies. The energy resolution of the system depends on the channel width that is usually set to 10 to 20 eV (Figure 5.4). The information collected in such a multichannel analyzer can be presented as an energy spectrum, which presents the relative intensities of the x-ray

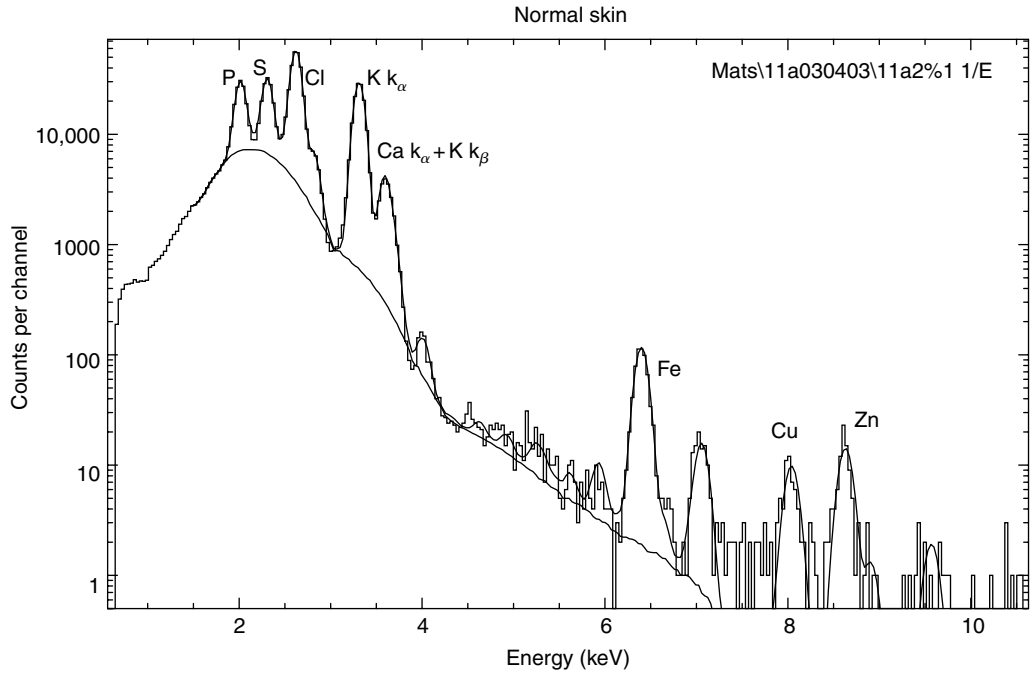

FIGURE 5.4 An x-ray spectrum from a normal skin sample obtained by PIXE-analysis. Detectable peaks are seen above the continuous background (Bremsstrahlung) radiation. Note that the Na peak is not detectable (due to absorption in the sample and the detector window).

signals from the object. As is generally the rule for spectrographic techniques, calibration of the system is done for absolute quantification.

5.7 COMPARISONS BETWEEN ELECTRON AND PROTON PROBES

From what was given earlier, it is clear that PIXE analysis of tissue physiology requires cryo-methods for tissue preparation. But are the data obtained with PIXE compatible with those given by the EMP (XRMA)?

Using standards for biological quantitation as specimens, we have compared XRMA and PIXE[10] and obtained identical results — a correlation coefficient of 0.996 between the methods was obtained for elements such as ^{16}S and ^{28}Ni. Hence, as analysis techniques these methods are fully complementary.

But how well do the XRMA and PIXE compare in practice? The advantages and disadvantages of the two techniques are summarized in Table 5.1. It can be recognized that even if EMP and PIXE are fully compatible for a number of physiologically important elements, there are some notable differences in two important aspects: their sensitivity (e.g., to trace elements) and their spatial resolution.

5.7.1 Sensitivity

The PIXE analysis has a sensitivity that allows analysis of most of the physiologically important elements down to 1 ppm level with the notable exception of Na. The characteristic x-ray quanta from Na have a low energy and are, therefore, to a great extent, suffering self-absorption within the

TABLE 5.1
The Advantages and Disadvantages of XRMA and PIXE

XRMA		PIXE	
Advantages	**Disadvantages**	**Advantages**	**Disadvantages**
High spatial resolution, 0.2 μm (200 nm)	Need for very thin *cryo-sections (<200 nm), cumbersome preparation*	*High sensitivity, <1 ppm* Allows trace element analysis, for example, Ca, Fe, Zn	Comparatively *low spatial resolution* $\leq$5 μm, which can be improved with loss of sensitivity
Sensitivity ~200 ppm	Absolute quantitation is not a straightforward procedure, appropriate *standards* and some approximations of *correction factors* are always involved in the practical application	Sensitivity 1 ppm	Need for rather cumbersome *cryo-preparation*
		Low thermic load on specimen due to scanning data acquisition	Thick samples require *correction factors*
			Quantitation of thin samples is straightforward
Simultaneous recording of elements within a specified volume that allows formation of elemental ratios, which can be used as sensitive monitors of physiological balance and unbalance in cells and tissues, for example, Na/K	Rapid *burnout of the organic scaffold* causes registration of higher than normal contents of elements	*Simultaneous recording* of elements within a specified volume that allows formation of elemental ratios, which can be used as sensitive monitors of physiological balance and unbalance in cells and tissues, for example, Ca/K, Ca/Zn, Fe/Zn	*Very long acquisition* times may result in *burnout of the organic scaffold*, causing a virtual higher than normal contents of elements
	Mapping, even of small areas, requires very long analysis time		
Mass determination by background absorption		*Mass determination* by back scattered protons or STIM, light element detection by nuclear complementary techniques, e.g., back scattering (C,N,O), photon tagged nuclear reaction analysis, pNRA for detection of B, Li, Na	
Elemental mapping to depict distributions of elements over the mass distribution image or a secondary electron image		*Elemental mapping* to depict distributions of elements over tissue section is a routine	

specimen, and also attenuation by the detector window. However, Na can be quantified concomitantly with energy detection via g-detectors by utilizing the nuclear reaction occurring as a result of proton capture by the Na nucleus.[11]

The heavy particles in the probe used in PIXE analysis are not as easily retarded as electrons by biological materials, and this results in a negligible background production allowing even weak secondary x-rays to be detected, hence the high sensitivity of 1 ppm.

The XRMA can generally be said to analyze elements down to contents of 200 ppm and is therefore essentially insensitive to elements such as Ca, Fe, and Zn occurring at low concentrations in the tissue and thus are denoted trace elements in biological tissues. The reason for this insensitivity is the fact that the light electrons impinging on the section are subject to multiple scattering and retardation, effects that produce a significant background of continuous radiation in which the weak trace element signals are buried.

5.7.2 Spatial Resolution

In EMP the cross section of the electron probe is often of the order of 2 nm, and with a section thickness approximately 100 nm a resolution at the subcellular level can be obtained. Considering the fact that physiologically interesting elements generally are freely dispersed in the cytosol, it is clear that local variations in concentration in biological tissues are to be expected. Therefore, analysis data are retrieved from sets of spots in regions of the tissue deemed to be representative of the structure under investigation.

In order to get reasonable acquisition times for data in PIXE-analysis, the sections used are generally 15 μm or thicker. This thickness causes an overlap of the secondary x-ray emission information from cellular structures in the depth of the section precluding a subcellular resolution at analysis (Figure 5.5). Furthermore, as the width of the PIXE probe is $\leq$5 μm, this represents another factor that diminishes the spatial resolution of PIXE. Thus, PIXE analysis superposes data from the intra- and extracellular compartments in the tissue during analysis due to the comparatively low spatial resolution of the measuring system and on the thickness of the sections ($\sim$15 μm or more). These facts relate to the considerable smoothing of curves describing the elemental and mass distribution over the cellular layers of a differentiated epidermis (Figure 5.6). The effect is perhaps most conspicuous in the very narrow stratum corneum region (a total width in a section of approximately 10 μm) where the mass curve is rather wide in these experiments. In general terms, this means that generally analysis within a defined cell compartment is not possible, and data are generally referred to as originating from a defined morphological entity, for example, a stratum of an epithelium, a special structure in the brain, etc.

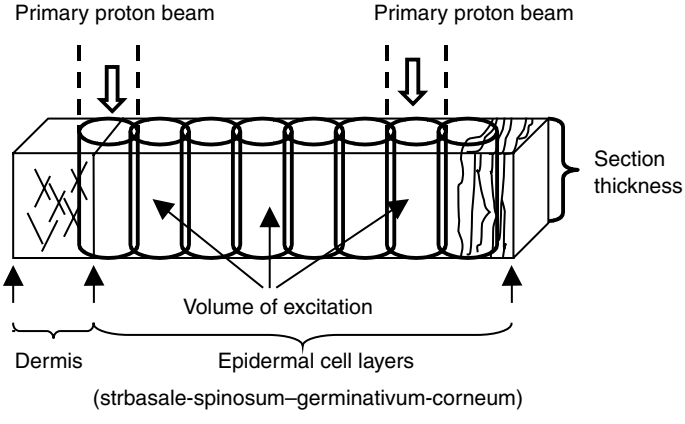

FIGURE 5.5 The secondary x-ray information will emerge from cellular structures in the depth of the section within volume of the proton beam volume of excitation. In addition, a probe diameter of 5 μm will result in lateral overlap of cellular compartments. Hence, the spatial resolution of the proton probe is restricted to strata rather than single cells. The resolution can be improved by diminishing the probe diameter ($<$2 μm) and the section thickness ($<$6 μm) at the cost of a substantial increase in acquisition time.

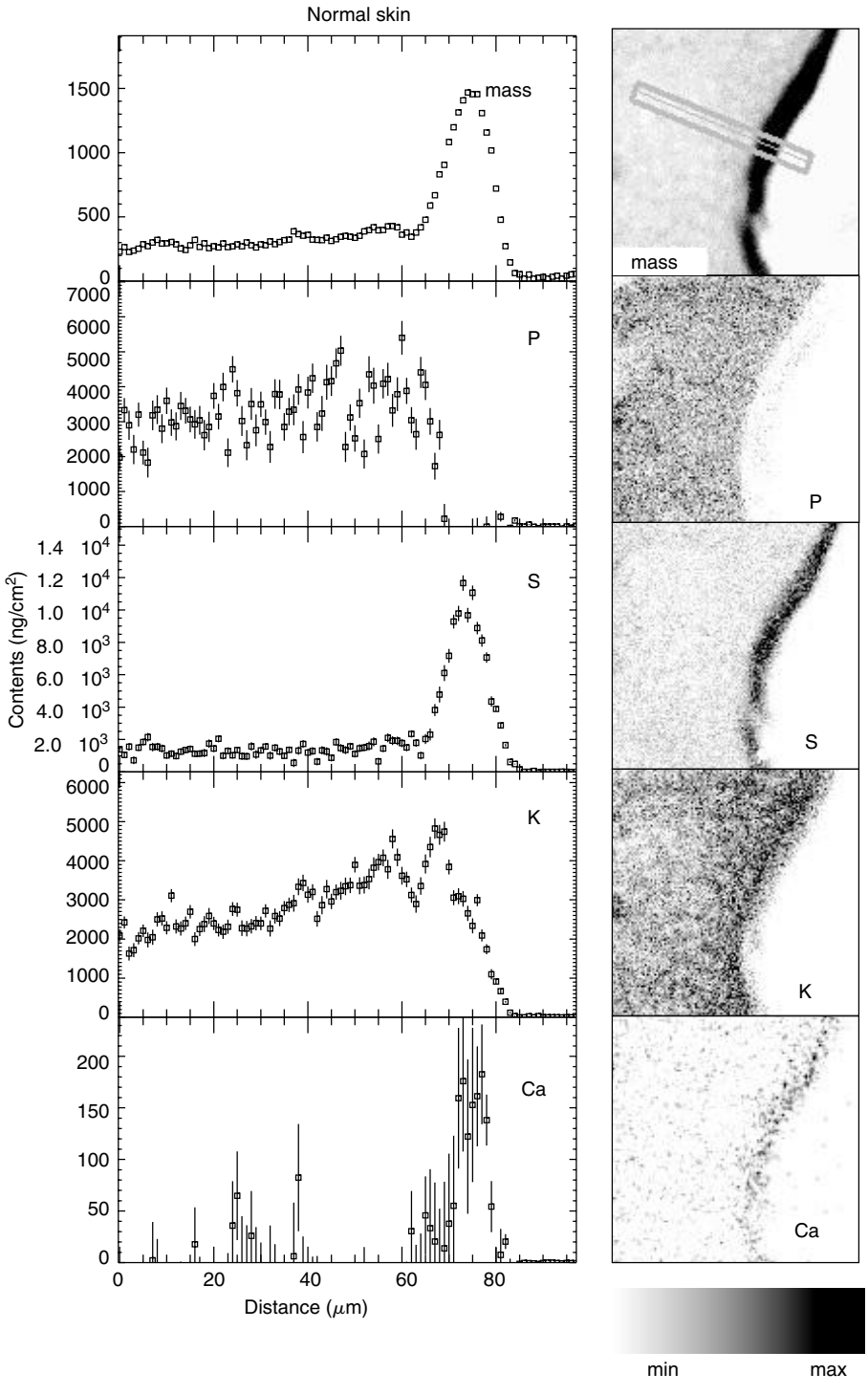

FIGURE 5.6 Proton probe analysis of a normal skin sample. The left panel demonstrates mass, P, S, K, and Ca distributions. To the right, the top picture represents a "vertical" scan across epidermis perpendicular to the skin surface (stratum corneum to the right; the basal cell layer to the left). The pixel mapping for P, S, K, and Ca are given in the right panel with the corresponding profiles in the left panel. Distance given in the left panel indicating the dermal–epidermal border at 0 and the outer part of stratum corneum at 80.

5.7.3 Limitations of PIXE Analysis

The number of specimens investigated in a PIXE study may appear small in comparison with the corresponding numbers used in different types of light microscopic investigations or biochemical studies. The typical acquisition time for a pixel map from a single section is generally about 30 to 45 min when trace elements are analyzed. Cost/benefit aspects of running an experiment will obviously require optimization of data acquisition for evaluation. To assess a number of data that would allow statistical analysis by the algorithm used, the time allowed has not been sufficient so far to quantify such elements as Mg, Cu, Ni, and Se.

5.8 ELEMENTAL MAPPING

In PIXE applications spot analysis was initially the dominating analysis method, but during the 1980s scanning procedures were developed, which allowed pixel mapping of the specimen. With a probe size of 5 μm, a tissue surface of $200 * 200$ μm can be covered by the pixel map in an acquisition time of approximately 30 to 45 min, that is, within this time period a representative area of the tissue section can be analyzed for all elements and mass (Figure 5.6). A further important advantage in PIXE mapping is the markedly diminished thermal load on the volumes of analysis that results in more reliable data when the influence of burnout of the organic matrix material was minimized.

From pixel maps obtained by the scanning proton probe, cross-section profiles of elemental distributions can be extracted (Figure 5.6). For elements present in trace amounts, long acquisition times are needed as mentioned. A corresponding map obtained with the EMP would require at least a five times longer acquisition time, which is the reason why elemental mapping for elements present in low concentrations has not been favored in XRMA.

5.8.1 Pixel Maps Provide Information on the Dynamics of Tissue Activity

Analysis of PIXE pixel maps reveals as one conspicuous feature the variation in the distribution of elements and trace elements seen between different strata of the skin as well as between different sections. This is found both in the normal and pathological skin. Such variations in the distribution of elements, and especially trace elements, indicate that there are obvious differences in the detailed cellular physiology of the differentiating keratinocytes. Such findings also harmonize with our previous experience from electron microscopic studies of irritant contact reactions.[12–14] These studies show that the correlation between the morphological image and the quantitative elemental data is not a direct one. Although morphologically similar, certain cells reveal their different stage of differentiation in the patterns of elemental distributions as suggested by the elemental maps. Combining other techniques (e.g., immunological, molecular, genetic) with elemental analysis should provide an additional detailed insight into the keratinization process programed to develop a complete stratum corneum with a functional barrier.

5.8.2 Multivariate Analysis Identifies Co-Variations of Elements and Strata

A recent development of proton probe analysis involves multidimensional statistical analysis of all data extracted from mass and elemental maps. The SIMCA™ program, which essentially analyzes

covariations of factors, allows not only comparisons of elemental levels within strata of an epidermal cross section, but it also allows comparisons between strata, elements, individuals, etc.[15]

5.9 TRACE ELEMENT ANALYSIS IS POSSIBLE WITH THE PROTON PROBES

In the initial studies of trace elements of human skin cross sections, a Ca profile evolved which was increasing from the basal, germinative level of the epidermis toward the horny layer. The drastic concentration drop of Ca concentration down to threshold values at the border of the stratum germinativum and the stratum corneum was a particularly interesting feature.[15–17] Only a few years later this finding could be correlated to the fact that a Ca concentration >0.1 mmol was essential if a fully cornified stratum corneum was to be obtained in cell culture. This relationship between Ca and terminal differentiation of the epidermal cells was verified in a PIXE study of epidermal cell cultures.[18]

5.9.1 The Ca^{2+} Signal

The influence of trace elements on the normal and abnormal physiology of the skin has not yet reached a full understanding. The role of calcium for epidermal differentiation has been demonstrated in a series of elegant studies by Elias and coworkers[19–21] using different techniques such as ion capture cytochemistry at transmission electron microscopic resolution. In the normal murine skin the most prominent localization of Ca^{2+} is noted in the upper stratum granulosum and the dermis, whereas the basal region is virtually free from Ca^{2+} precipitates. When the barrier was broken by acetone treatment, a redistribution of Ca^{2+} took place with a conspicuous accumulation of precipitates in the extracellular space of the stratum corneum and loss of the stratum granulosum localization. Treating the barrier-disrupted skin with iso-osmolar sucrose containing Ca^{2+} replenished the epidermal Ca^{2+} reservoir. However, the secretion of lamellar bodies was impeded and hence barrier recovery. Conversely, treatment with iso-osmolar sucrose only lead to barrier repair through lamellar body secretion in the absence of the normal Ca^{2+} gradient. The conclusion of the authors was that loss of the Ca^{2+} reservoir is an important signal for restoration of barrier function after damage. It has also been postulated that the elemental gradient across epidermis is crucial for the maintenance of a normal barrier function (stratum corneum).[22,23] In spite of these investigations there is a need for fully quantitative data to support these findings, and such quantitative data can be obtained using PIXE analysis.

5.9.2 Ca^{2+} and Programmed Cell Death or Apoptosis

The importance of Ca^{2+} as a signal for various cell functions is now established. A further aspect of the Ca^{2+} function is its property to promote "programmed cell death" (or apoptosis). Zn^{2+} has been shown to inhibit this effect.[24] As of new, it is not clear what role such a phenomenon plays in the sequel of cellular differentiation of the epidermis. This process can be regarded as a kind of programed cell death, which involves the complete dissolution of nucleic acid material in the stratum granulosum.[25] One indication on the importance of increased Ca levels in this cellular stratum is related to the finding that a full differentiation of the epidermis does not occur in tissue culture unless the Ca content equals at least 0.1 mM.[26] One may speculate that these findings suggest an explanation to the sporadic occurrence of parakeratotic cells in the stratum corneum in the paralesional psoriatic epidermis where unusually high zinc levels are recorded in the stratum granulosum zone. Whether this effect is directly coupled to an increased cellular activity in the germinative pool may be a matter of speculation, but the actual high levels of iron (Fe) compared to normal skin suggest such

an increased activity. Further detailed analyses, including particle probe studies, are required before this question can be settled.

The skin dependence on appropriate availability of Zn^{2+} for normal function is a subject that has not been completely resolved.[27,28] However, it is conceivable that these problems can at least partially be solved using particle probe analysis.

5.9.3 Iron and Zinc

Iron has a high peak value in the basal cell region, drops to values less than half the peak value in the uppermost epidermal layers, and is not detectable in the stratum corneum region.[16] Zinc (Zn), which is represented in the dermis by concentrations below or just at the detection level of the system, shows a comparatively stable level over the Malpighian epidermis and disappears coincidentally with Fe in the stratum corneum region.[15,16] So far, the PIXE data obtained from normal skin suggest an approximate ratio of Zn content in epidermis/dermis of 3:1 to be compared with a 6:1 ratio given by neutron activation analysis.[29] Copper (Cu) is just barely detectable in the Malpighian layers, and generally no quantitation is possible within the acquisition times used in most of our experiments.

5.9.4 Mass and Elemental Distributions of the Epidermis — PIXE Data

The mass distribution curves reach a peak in the stratum corneum region, flatten out in the basal cell region, and then rise again in the dermis in full agreement with the previous XRMA data. The S distribution curves follow the mass curves in concert. Around 30 to 50 μm below the maximum mass peak, at a region corresponding to the stratum spinosum, we find the P distribution peak. Chlorine (Cl) has a weak minimum approximately where the P distribution has its peak. In the vicinity of stratum corneum, which is virtually free from Cl, there is a conspicuous drop in the Cl content. K reaches its highest levels in stratum granulosum and drops to nil in the stratum corneum.

5.9.5 Recycling of Diffusible Ions

Summing up the data on the distribution of physiologically important diffuable elements, it is interesting to note that at the border between the viable epidermis and the stratum corneum their contents are close to or below the detection limit of the particle probes with the particular exception of calcium (see Chapter 8). The disappearance of the diffusible ions can be understood if we consider the fact that the water content is roughly constant over the epidermal cross section, finally dropping to low values within the stratum corneum.[21,30,31] The mass content of the cells increase continuously on the passage from the cells of the basal layer to the final fully cornified corneocyte. Since keratin binds water, we can see that the amount of free water available for the freely diffusible ions decreases with increasing mass. This creates a downhill gradient directed toward the dermis. Therefore, the recycling of freely diffusible ions requires no special energy-consuming mechanism.

5.9.6 Local Variations of Element and Trace Element Distributions

There are slight, but obvious variations in the elemental distribution patterns from one section to another, although a general trend can clearly be discerned. The Fe and Zn distributions have their centers of gravity in the stratum spinosum/stratum granulosum area, but Zn is more clearly confined to the basal layer.

5.9.7 Horizontal Elemental Distributions

The element distribution curves have generally been extracted from pixel "channels," which cover the cross section from stratum corneum down into the papillary dermis. When data are retrieved so as to represent pixel channels that cover a specified stratum horizontally, it can be seen that the mass distribution along the basal lamina of normal skin varies somewhat along the horizontal scan. However, it remains approximately constant at the upper level (the level of the stratum spinosum/granulosum). This obviously relates to the fact that the basal cells may be in different phases of the cell division cycle, whereas the stratum spinosum cells are more synchronized in their development. K and Cl covary with mass in the basal region, but the variation is more independent in the stratum spinosum region as expected from cross-section distribution.[13] As expected S covaries to a great extent with the mass distribution. In stratum spinosum and stratum basal more conspicuous variations in relation to mass are seen in the P distribution.

There is an extensive variation in the Fe and Zn distributions in the basal region. The Fe content is close to or below the detection limit in the upper region; the Zn content shows some peaks above the detection limit. Single off-limit values in these trace elements were seen.

Ca appears to stay rather constant within each horizontally scanned band, which is consistent with the fact that the increase in Ca toward the stratum corneum is likely to be related to the physiological and regulatory effects of this ion.

5.10 ELECTRON AND PROTON PROBE DATA FROM PATHOLOGICAL SKIN

5.10.1 Psoriasis

An early XRMA study in which compared skin from healthy, normal persons with uninvolved and involved (a stable plaque) psoriatic skin revealed that Mg, P, and K were increased in the involved skin corresponding to what is recorded in highly proliferative, nonneoplastic cells.[32,33] Previously Burkhart and Burnham had recorded a significant increase in P and Ca content in involved psoriatic skin compared to uninvolved skin from the same patients.[34] Later, Kurtz et al. reported corresponding findings from a PIXE study of psoriatic skin, but found no difference between the Zn content of control skin and uninvolved skin from psoriatic patients.[35] However, in pinpoint lesions they recorded a significant increase of Zn corresponding to neutron activation analysis data given by Molin and Wester (Table IV).[36,37] Some interesting aspects on the elemental distribution were revealed in a study of uninvolved psoriatic skin.[38] Our PIXE data demonstrated that uninvolved psoriatic skin has a mass distribution with the same general features as that of normal skin, although generally at a lower level (absolute mass content). The P and S distributions are not conspicuously different from those of normal skin.

Ca shows a twofold or even higher increase in the stratum granulosum region compared to normal skin, but in contrast to the Ca distribution in normal skin, that of psoriasis follows the mass distribution more closely. In many sections there is an additional Ca peak in the vicinity of the basal cell layer, but the full significance of this is not clear.

The trace element distributions of uninvolved psoriatic skin merit special comments. The main Fe peak appears closer to the mass distribution peak than in normal skin. Also, there are obvious variations in the Fe content in different strata (cell layers), and the lowermost values are consistently at least twice as high as those in normal skin. Our PIXE investigation substantiates the previously reported finding that psoriatic patients lose Fe through the shedding of stratum corneum cells in lesional areas by demonstrating that clinically normal skin of psoriatic patients contains higher than normal amounts of Fe.[36,37]

The Zn content of the uninvolved psoriatic skin is increased in the stratum spinosum especially, except in one single section where the Zn follows suit with Fe distribution. Such variations are likely to occur as a function of the cell cycle position of a particular cell.

5.10.2 Elemental Distributions in Different Strata of Psoriatic Normal-Looking Skin — Horizontal Scans

In comparison to the control skin there are high mean values and prominent variations in the trace elements, notably Fe and Zn, in the upper level of the epidermis. However, the mass distribution pattern essentially follows that of normal control skin with some variations in the upper layer.

In spite of the fact that the spatial resolution does not allow discrimination between the intra- and extracellular compartments, PIXE data nevertheless reveal some crucial points concerning the physiology of normal-appearing psoriatic skin as opposed to the normal skin. The Ca distribution profile, which in a normal skin remains at an almost constant level over the skin cross section, shows a slight increase in the stratum granulosum region in certain specimens. This differs from data of a previous preliminary study based on selected point measurements in different strata of skin sections.[15] However, with the new information obtained from the elemental maps such a variation is likely to occur as an expression of the continuous changes occurring *in vivo*. Continuous changes like these are represented in similar studies using quench frozen specimens by a "snap-shot" depicting momentarily what are actually transient processes.

In order to elucidate the background to these abnormal elemental distributions, further studies including psoriatic plaques will be needed. Recent data from dry skin of atopics also present elemental distributions, which vary conspicuously from those found in normal.[15,39] These facts challenge our experimental imagination to produce answers to what faults in the cellular mechanisms are at hand in these skin disorders.

5.10.3 Metal Allergy

In the Western Hemisphere Ni allergy has very rapidly grown to be a major dermatological problem. The penetration profile of this metal ion through the skin remained largely unknown, in spite of previous studies on Ni penetration through human skin using XRMA, due to the insensitivity of the method.[13,40,41] In a PIXE study of skin samples from individuals tested for Ni allergy, it was demonstrated that the Ni accumulated in the stratum corneum and that only trace levels passed through the skin barrier.[42] These findings suggest that extremely minute amounts of Ni are needed to elicit an allergic reaction in an Ni-sensitized individual. It corresponds to the observation that just a single, very brief contact with a dry nickel-plated object may elicit an allergic reaction.

5.10.4 Irritant Contact Dermatitis and Effects on the Keratinocytes — EMP Data

Irritant contact dermatitis is a public health problem. Skin exposure to irritants (e.g., water, detergents, and solvents) causes damage to the barrier and induces an inflammation, ultimately contact dermatitis.[43] By combining microprobe analysis with other techniques it is possible to correlate physiological changes (e.g., barrier repair processes) with effects on the keratinocytes. In a series

of *in vivo* and *in vitro* experiments[12–14,44–46] we have been able to relate changes in the Na/K ratio and P content of the keratinocytes to changes in proliferation and up-regulation of inflammatory mediators.

5.10.5 Multidimensional Statistical Analysis Using SIMCA

Primary data are not always easy to interpret to give a functional picture of the tissue physiology. Ratios of elements such as Ca/Zn can provide interesting information when one realizes that Zn may be antagonistic to effects elicited by Ca. But comparisons and correlations of data from different strata, individuals, and disorders are still problematic. From a physiological/biological point of view correlations may provide more pertinent information than straightforward statistics which just provide statistical significances. The recent introduction of multidimensional statistical analysis (SIMCA) conspicuously broadens the possibility of meaningful interpretations of primary data. Using SIMCA to study the dry skin of atopic individuals, it turns out that when we look for correlations between strata of the epidermis and elements, the dry atopic skin proves to be very immature compared to the unafflicted skin of normal (control) individuals. A SIMCA scatter plot shows that the stratum basale and spinosum covariate in the atopic skin, but are well separated in the control skin.[47] Such information suggests that the stratum spinosum of the atopic skin is immature. Multidimensional statistical analysis allows us to understand data in physiological terms and will undoubtedly have an impact on the analysis of skin disorder obtained by biochemical and immunological means.

In a recent study of clinically normal skin from patients with psoriasis, a high Fe content of the horny layer was demonstrated, whereas there was no detectable Fe in the horny layer of normal healthy control individuals.[38] Obviously, this finding in psoriasis demonstrates that the entire differentiating epidermis of these patients is involved in the disorder, whether clinically expressed or not.

5.11 SUMMARY AND CONCLUSIONS

In this chapter, as well as in Chapter 8, the feasibility of skin physiology studies using particle probe analysis has been demonstrated. The EMP or XRMA analysis of biological tissues has allowed the study of physiological processes, which cannot be attacked using common physiological techniques, e.g., microelectrode registrations. An example of "impossible physiology" that was subsequently allowed by the XRMA is the study of the physiology of the differentiating epidermis (Figure 5.2).[48] Thus, we were able to show that the cells on the basal lamina separating the fibrous tissue of the dermis from the cellular tissue of the epidermis are the only cells upholding a normal Na/K ratio. The next cellular level has already suffered an increase in the Na and a decrease in K, meaning that these cells either leak ion or that their membrane pumps are deficient. A consequence of this is that only the basal cells with a normal Na/K ratio can go through the mitosis cycle producing a progeny. The biological meaning of this is obviously one of cell division control, resulting in a smooth skin surface.

Further, the EMP has allowed studies of the water profile over the skin cross section[30,31] the physiological changes at irritant reactions[12–14,44–46] and psoriasis.[32] A comprehensive overview of the EMP application is given in two overview papers.[49,50]

The application of x-ray analysis methods to biological problems has proven to be of great, sometimes unsurpassed, value. Recent developments of computer software, statistical programs, etc. have tremendously broadened the possibility of data retrieval and handling. The reason why we do not find more biologically oriented work in the literature is obviously due to an information gap, that is, an educational problem — biologists/medical researchers know too little about x-ray physics, and physicists know too little about biological systems and the effects of biological tissue preparation.

Particle probe analysis and, in particular, proton probe analysis, which is sensitive to trace element levels in tissue sections have been demonstrated to reveal important details about cellular physiology in the differentiating epidermis of normal and pathological skin. Such a physiological approach will serve to complement data from other techniques. A future collective approach of this kind will make it possible to understand how a dry and eczematous skin develops and also what the mechanisms of subsequent healing are.

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6 Role of Calcium Ions in the Regulation of Skin Barrier Homeostasis

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CONTENTS

6.1 INTRODUCTION

Dry skin symptoms are frequently linked to an impaired skin barrier function, as observed in psoriasis, ichtyosis, atopic skin, and contact eczemas.[1] More precisely, this skin barrier function is connected to the chemical and physical condition of the stratum corneum (SC), the uppermost layer of the epidermis. SC gives protection against desiccation and environmental challenge by regulating water flux and retention.[2] The optimal level of hydration maintained in SC is largely dependent on three components, which are constantly regenerated in this particular skin layer, namely (1) intercellular lamellar lipids, as an effective barrier to the passage of water; (2) corneocytes (SC cells), which provide the tortuous diffusion path, created by the SC layers and corneocyte envelopes, that retard water loss, and (3) natural moisturizing factor (NMF), a complex mixture of low-molecular-weight, water-soluble compounds first formed within the corneocytes by degradation of the histidine-rich protein known as filaggrin. Disturbance to the regeneration processes of these components results in dry, flaky skin conditions.[3]

The importance of calcium in the regulation of skin barrier homeostasis is apparent as calcium is involved in the regeneration process of skin barrier components.[4] Hence, the balance of calcium level in skin is closely related to hydration of the skin. Apart from the skin, this ion plays a crucial role in various processes in the body, including the growth, death, differentiation, and function of immune cells. The role of calcium in skin is found to be more complex than previously assumed. The elucidation of calcium regulation mechanism in skin could be useful to understand and solve skin problems.

6.2 MECHANISM OF CALCIUM CELL SIGNALING

In the body, calcium, in the form of the ion Ca^{2+}, is the most abundant metal ion and fifth (after H, O, C, and N) most abundant element in the body, on both an atom and weight basis. Over 90% of body

calcium resides in bones and tooth enamel. The rest, described as mobile Ca^{2+}, is found throughout body fluids and takes part in various processes, including muscle contraction, blood clotting, nerve excitability, intercellular communication, membrane transport of molecules, hormonal responses, exocytosis, cell fusion, adhesion, and growth.[5]

Mobile calcium is a universal messenger for living things, even in simple organisms and plants. The unique combination of its ionic radius and double charge allows Ca^{2+} to be specifically recognized and to yield tighter binding to receptors to the exclusion of other ions, leading to strong, specific binding.[6] The specificity enables cells to form special receptors to assess signals from calcium. For many parts of the body, Ca^{2+} often acts as a second messenger in a manner similar to cAMP. Transient increases in cytosolic Ca^{2+} concentration trigger numerous cellular responses including muscle contraction, release of neurotransmitters, and glycogen breakdown (glycogenolysis), and also act as an important activator of oxidative metabolism (7, pp. 496–498). Ca^{2+} does not need to be synthesized and degraded with each message transmission, so it is an energy-efficient signal for the cell.[8]

In skin, calcium can provide signals for the cells, either extracellular or intracellular (in the cytosol). The extra- and intracellular signaling may be connected to each other, but may also act separately. In cultured keratinocytes, extracellular calcium levels influence growth and differentiation.[9,10] Low extracellular calcium levels (<0.1 m*M*) induce the growth of keratinocytes as a monolayer with a high proliferation rate, rapidly becoming confluent. In this condition keratinocytes never stratify, but possess many of the characteristics of basal cells; the cells synthesize keratin proteins and are connected by occasional gap junctions but not by desmosomes. High extracellular calcium levels (>1 m*M*) induce differentiation of keratinocytes. Keratinocytes rapidly flatten, form desmosomes, and differentiate with stratification. Moreover, cornified envelopes form in cells of the uppermost layers.[9,10]

The response to signaling is also shown in a progressive way. Keratinocytes grown in a low-calcium media proliferate. Increased extracellular Ca^{2+} inhibits proliferation, while it induces differentiation.[11] On the other hand, differentiation of keratinocytes causes a decrease in responsiveness to extracellular calcium, which may facilitate the maintenance of the high level of intracellular calcium required for differentiation.[12]

Intracellular Ca^{2+} increases with raised extracellular Ca^{2+}.[13–15] This implies that increased intracellular Ca^{2+} is the actual signal to trigger keratinocyte differentiation. Intracellular Ca^{2+} signals are assessed through calcium-binding proteins to induce responses. The major calcium-binding protein in skin is calmodulin. Calmodulin regulates target protein by modulating protein–protein interactions in a calcium-dependent way. Calmodulin regulates many enzymes, for example, adenyl and guanyl cyclase, phosphodiesterase, ornithine decarboxylase, calcium-calmodulin-dependent protein kinase, transglutaminase, and phospholipase, which are also found in skin.[8]

Both intracellular release and transmembrane flux contribute to the rise in intracellular Ca^{2+}.[14,15] The rise in keratinocyte intracellular Ca^{2+} in response to raised extracellular Ca^{2+} has two phases: (a) an initial peak, not dependent on extracellular Ca^{2+} and (b) a later phase that requires extracellular Ca^{2+}.[14] An early response of human keratinocytes to increases in extracellular Ca^{2+} is an acute increase in intracellular Ca^{2+}. Stepwise addition of extracellular Ca^{2+} to neonatal human keratinocytes is followed by a progressive increase in intracellular Ca^{2+}, where the initial spike of increased intracellular Ca^{2+} is followed by a prolonged plateau of higher intracellular Ca^{2+}.[16] The response of intracellular Ca^{2+} to increased extracellular Ca^{2+} in keratinocytes is saturated at 2.0 m*M* extracellular Ca^{2+}.[16,17] The response of intracellular Ca^{2+} to increased extracellular Ca^{2+} in keratinocytes resembles the response in parathyroid cells, in that a rapid and transient increase in intracellular Ca^{2+} is followed by a sustained increase in intracellular Ca^{2+} above basal level. This multiphasic response is attributed to an initial release of Ca^{2+} from intracellular stores followed by an increased influx of Ca^{2+} through voltage-independent cation channels. The keratinocyte and parathyroid cell contains a similar cell membrane calcium receptor thought to mediate this response to extracellular Ca^{2+}. This receptor can activate the phospholipase-C pathway, leading to an increase

in the levels of inositol 1,4,5-triphosphate (IP_3) and *sn*-l,2-diacylglycerol (DAG) — both of which are important messengers — as well as stimulating Ca^{2+} influx and chloride currents,[18,19] IP_3 causes release of Ca^{2+} from internal stores, such as endoplasmic reticulum, further increasing intracellular level to precede a number of calcium-stimulated cellular events.[20] DAG forms a quarternary complex with phosphatidylserine, calcium, and protein kinase C to activate the kinase. This will accelerate terminal differentiation (Hennings et al., 1983). The signal transduction mediated through calmodulin induces other proteins, for example, desmocalmin, which is associated with the formation of desmosomes.[21]

Keratinocytes grown in low-calcium medium (0.02 m*M*) maintained intracellular calcium levels adequate for arachidonic acid metabolism and actually showed increased prostaglandin (mainly PGE_2 and PGF_2) production up to 4.5 times compared to cells grown at normal calcium level (1.2 m*M*).[22] If this is true for the *in vivo* condition, a low level of extracellular calcium — for instance, due to a defective skin barrier — may cause an increase in prostaglandin synthesis, leading to hyperproliferative epidermal disorders, such as psoriasis, which are often associated with abnormalities in prostaglandin production.[23]

6.3 REGULATION OF CALCIUM

The regulation of calcium in skin shows an ingenious adaptation of living organisms to the presence of ions. As Ca^{2+} cannot be metabolized like other second-messenger molecules, cells tightly regulate intracellular levels through numerous binding and specialized extrusion proteins.[24] The concentration of calcium in extracellular spaces (generally $\sim$1.5 m*M*) is four orders of magnitude higher than in the cytosol ($\sim$0.1 μM). In excitable cells, for example, muscle cells, the extracellular concentration of calcium must be closely regulated to keep it at its normal level of $\sim$1.5 m*M*, so that it cannot accidentally trigger the muscle contraction, the transmission of nerve impulses, and blood clotting (7, p. 1144). In other cells, including keratinocytes, the extracellular level is maintained in a specific equilibrium with the intracellular concentration.

What is the importance to keep the intracellular calcium level low? A low calcium concentration makes the use of the ion as an intracellular messenger energetically inexpensive. The movement of calcium ions across membranes requires energy, usually supplied by ATP. If the resting level of calcium in the cell were high, a large number of ions would need to be transported into the cytoplasm to raise the concentration by the factor of ten that is ordinarily needed to activate an enzyme; afterward the excess calcium would have to be expelled from the cell. Normally low calcium level means that relatively few ions need to be moved, with a relatively small expenditure of energy, to regulate an enzyme. In contrast, energetic cost of regulation by the other important intracellular messenger, cyclic adenosine monophosphate (cyclic AMP), is high; it must be synthesized and broken down each time it carries a message, and both steps requires a significant investment of energy.[6] Furthermore, low intracellular calcium is a necessary condition for the phosphate-driven metabolism characteristic of higher organisms. The energy-rich fuel for most cellular processes is adenosine triphosphate (ATP). Its breakdown releases inorganic phosphate. If the intracellular concentration of calcium were high, the phosphate and the calcium would combine to form a precipitate of hydroxyapatite crystals — the same stony substance found in bone. Ultimately calcification would doom the cell.[6] This is likely the case with long-term occupational exposure to high levels of dissolved calcium, for example, in miners,[25] agricultural laborers,[26] and oil field workers,[27] which can result in calcinosis cutis, a benign and reversible hardening of the exposed skin.

The large concentration gradient between extracellular spaces and cytosol is maintained by the active transport of Ca^{2+} across the plasma membrane, the endoplasmic reticulum (or the sarcoplasmic reticulum in muscle), and the mitochondrial inner membrane. Generally, plasma membrane and endoplasmic reticulum each contain a Ca^{2+}-ATPase that actively pumps Ca^{2+} out of the cytosol at the expense of ATP hydrolysis (7, pp. 496–498). Mitochondria act as a "buffer" for cytosolic Ca^{2+}:

if cytosolic concentration of calcium rises, the rate of mitochondrial Ca^{2+} influx increases while that of Ca^{2+} efflux remains constant, causing the mitochondrial concentration of calcium to increase, while the cytosolic concentration of calcium decreases to its original level (its set-point). Conversely, a decrease in cytosolic concentration of calcium reduces the mitochondrial influx rate, causing net efflux of calcium from mitochondria and an increase of cytosolic concentration of calcium back to the set-point (7, p. 531).

Besides the already mentioned Ca^{2+}-ATPase, the transport of Ca^{2+} is regulated by a series of calcium pumps, transport systems, and ion channels. The availability of certain regulatory systems is dependent on the activity of the cells. In excitable cells such as cardiac muscle, the influx of Ca^{2+} to cytosol is regulated by voltage- (or potential-) dependent channels, while the efflux (out of cytosol) is regulated by cation exchanger, such as Na^{+}–Ca^{2+} exchanger.[8] Undifferentiated keratinocytes in the basal layer have different sets of Ca^{2+} transport system than differentiated cells in the upper layers. In basal layer, the system consists of 14-pS nonspecific cation channels (NSCC)[28] and does not possess functional voltage-sensitive Ca^{2+} channels.[15] Differentiated keratinocytes are likely to possess at least two and possibly three pathways of Ca^{2+} influx: (a) nicotinic channel (nAChR); (b) voltage-sensitive Ca^{2+} channels (VSCC, which can be blocked by nifedipine or verapamil); and (c) NSCC, which is not activated by nicotine.[29]

The permeability of skin to Ca^{2+} ions has been known from some dermatoses, such as calcinosis cutis[25–27] and perforating verruciform collagenoma.[30] In a shorter term, calcinosis cutis developed after a 24 h (at least) topical application of an electrode paste containing saturated calcium chloride solution, bentonite, and glycerin, used for examination by electroencephalography or electromyography.[31,32] The permeability of human skin to Ca^{2+} ions *in vitro* shows a marked dependence upon anatomic site. In agreement with the data observed for nonelectrolytes, permeation decreased in the following order: foreskin > mammary > scalp > thigh. Mouse and guinea pig skin show comparable permeability to that of human scalp. Ca^{2+} transport from dermis across epidermis is higher than that from epidermis to dermis.[33,34]

A technique was developed to continuously monitor the low level of Ca^{2+} flux across human SC *in vitro*. The study showed that the flux through untreated human SC was sigmoidal. The steady-state flux had an average of 7×10^{-12} mol/cm^2/s. After the SC was pretreated with acetone or sodium lauryl sulfate, the shape of the curve was similar but the Ca^{2+} flux was significantly higher.[35]

6.4 CALCIUM GRADIENT

As mentioned earlier, there is a high calcium gradient between extra- and intracellular domains of keratinocytes, which requires tight regulation. Moreover, a calcium gradient is present within the epidermis, with higher quantities of Ca^{2+} in the upper than in the lower epidermis (as the cell moves from the basal layer to the stratum granulosum).[36] Ca^{2+} concentration increases steadily from the basal region to SC, while this is not the case with other ions.[37] Figure 6.1 illustrates the calcium gradient in human skin in comparison with an actual literature data.[38] Such a gradient is not observed in skin abnormalities related to the formation of abnormal barrier function, such as psoriasis.[39] Studies in mice and rats showed that this gradient exists at the same time as the formation of a maturing skin barrier at the end of gestation. The gradient is then maintained from the newborn throughout the adult life.[40]

It is not yet clear whether the calcium gradient leads to the formation of a mature barrier or the barrier caused the gradient. It may even be both, if the regulation uses a feedback mechanism, as the differentiation will eventually form a barrier leading to the accumulation of calcium ions in the upper epidermis. This high level of calcium will, in turn, guarantee the ongoing process of differentiation toward the formation of corneocytes (horny cells in the SC). The mechanism is thus almost completely autonomous, perpetual, and, if it runs smoothly, requires little correction from the body.

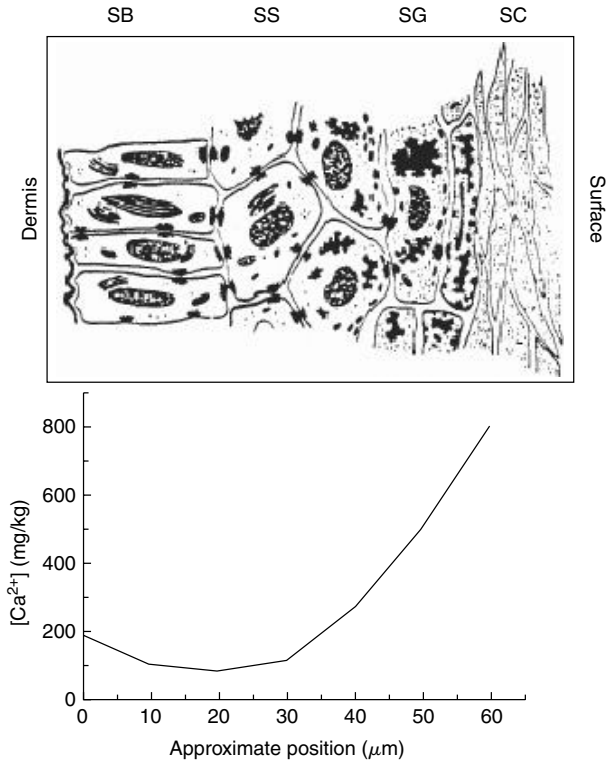

FIGURE 6.1 Illustration of calcium gradient in epidermis based on literature data (proton induced x-ray emission analysis of calcium in sectioned human skin) (Malmqvist et al., 1987). SB, stratum basale/basallayer; SS, stratum spinosum; SG, stratum granulosum; SC, stratum corneum.

6.5 CALCIUM AND BARRIER REPAIR MECHANISM

Disruption of the barrier with acetone treatment or tape stripping depletes Ca^{2+} from the upper epidermis, resulting in the loss of the Ca^{2+} gradient.[41–43] This is due to accelerated water transit that leads to the increased passive loss of Ca^{2+} into and through the SC.[41,43] One *in vitro* study showed that the permeability of human SC to Ca^{2+} dramatically increased after the SC was pretreated with acetone or sodium lauryl sulfate solution.[35] The decrease in Ca^{2+} levels in the outer epidermis is associated with enhanced lamellar body secretion and lipid synthesis (important components in repair responses).[41,44] However, if Ca^{2+} gradient is preserved by the addition of Ca^{2+} into the media, lamellar body secretion, lipid synthesis, and barrier recovery are inhibited.[44] The inhibition raised by high extracellular concentration of calcium is potentiated by high extracellular K^+.[45] Another study confirmed that barrier recovery is accelerated by the low concentrations of calcium and also potassium during an increased water loss, since water loss may induce a decrease in the concentration of Ca^{2+} in the upper epidermis, which, in turn, may stimulate lamellar body secretion and barrier repair.[46] Furthermore, the inhibition raised by high extracellular concentration of calcium is reversed by nifedipine or verapamil, specific calcium channel blockers.[45] In another study, administration of Ca^{2+} free solutions by sonophoresis resulted in a marked decrease in Ca^{2+} content in the upper epidermis, and subsequently the loss of the Ca^{2+} gradient was accompanied by accelerated lamellar body secretion (a sign of skin barrier repair).[47]

The process of barrier repair in connection with transepidermal water loss and calcium gradient is illustrated in Figure 6.2. Experiment in mice shows that the calcium gradient disappears after acute permeability barrier disruption, and returns after 6 h in parallel with barrier recovery, barrier

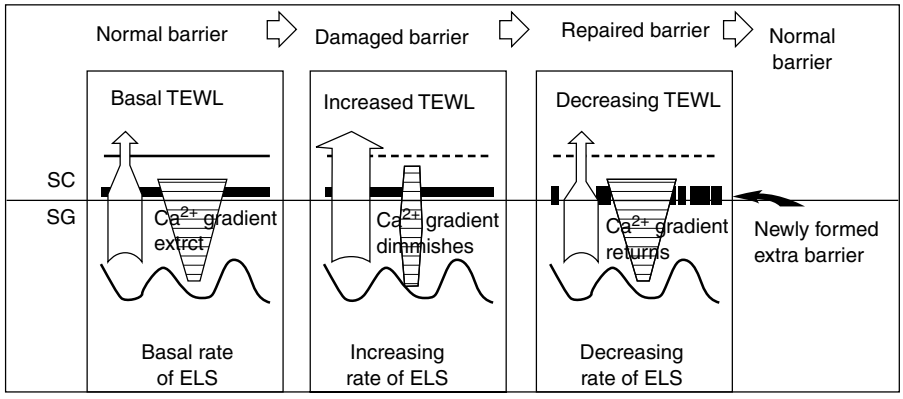

FIGURE 6.2 Illustration of skin barrier repair in epidermis. SC, stratum corneum; SG, stratum granulosum; TEWL, transepidermal water loss; ELS, epidermal lipid synthesis.

function (through restriction of transcutaneous water movement) could regulate the formation of the epidermal calcium gradient.[48]

"It should be noted that the barrier repair in response to the skin barrier disruption is not the same as the normal barrier regeneration process. The response is an emergency step to quickly reduce the transepidermal water loss to its set-point and thereby returning the calcium gradient to its natural condition (41). Once the calcium gradient is normalized, the normal skin barrier regeneration takes place. It is confirmed that addition of high calcium concentration during the barrier disruption process will induce higher influx of calcium into epidermal keratinocytes which delays the emergency skin barrier repair process (new ref. 1). However, during this delay and if the applied calcium concentration is within the right physiological range, the normal skin regeneration process can take place and the normal barrier function is restored without the formation of intermediate emergency barrier. This is indicated in a study on the cultured keratinocytes that extracellular calcium in physiological range of concentration is not a sufficient signal for growth arrest when other growth conditions are optimized (new ref. 2). The restoration of normal barrier function during the application of high concentration of calcium is evident from the effect of bathing in the calcium-rich Dead Sea water to improve skin diseases related to skin barrier impairment (new ref. 3) as well as to enhance skin hydration and reduce inflammation in atopic dry skin (new ref. 4) ."

6.6 CONCLUSION

Calcium ions play an important role in the homeostasis of skin barrier. A change in the barrier will change the calcium ion gradient in skin and lead to disturbance in the skin barrier regeneration process. A severe change might lead to a high degree of calcium signaling, which may induce the activation of various processes, from increased synthesis of skin components or messengers to the inflammatory reactions. All of these are important factors leading to dry skin conditions. The regulation of calcium in skin is therefore necessary to maintain a good skin barrier function and to avoid dry skin symptoms.

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7 Desquamation

Torbjörn Egelrud

CONTENTS

7.1 INTRODUCTION

The stratum corneum is a cellular tissue. Its building blocks, the corneocytes, are highly resistant to physical and chemical trauma. The mechanical strength of an individual corneocyte, emanating from its tightly packed keratin bundles and the cross-linked proteins of the cornified envelope, is outstanding. The mechanical resistance of individual corneocytes is mirrored by the pronounced mechanical strength of the entire stratum corneum, implying a strong cell cohesion within the tissue. The corneocytes and their intercellular cohesive structures are prerequisites for the function of the stratum corneum as the physical–chemical barrier between body interior and exterior, serving as an important part of the barrier as well as a backbone for the intercellular barrier lipids.

The stratum corneum is continuously being formed in the process of terminal keratinocyte differentiation. The rate of stratum corneum renewal is determined by the rate of cell proliferation in the basal layer of the epidermis. The fact that the thickness of the stratum corneum is fairly constant at a given body site implies that a fraction of the most superficial parts of the stratum corneum must be continuously shed at a rate that balances *de novo* production of corneocytes. This process, desquamation, normally occurs invisibly with shedding of individual cells or small aggregates of cells, resulting in the smooth appearance of the skin surface associated with a "normal" skin condition. Disturbances in this process, due to either increased production of corneocytes or a decreased rate of cell shedding, results in the accumulation on the skin surface of only partially detached cells with or without a concomitant thickening of the stratum corneum. The severity of the disturbance may vary from modest to very pronounced, from a barely visible scaling combined with a feeling of roughness and dryness of the skin surface to the accumulation of thick brittle scales such as in psoriasis or in the various forms of ichthyosis.

Thus, it can be concluded that there must be mechanisms within the stratum corneum, which are responsible for a well-regulated desquamation. A closer look at the criteria that must be fulfilled by these mechanisms suggests that they are likely to be of significant complexity. As stated previously, the barrier function of the stratum corneum depends on a strong cohesion between individual

corneocytes. The elimination of cell cohesion, a prerequisite for desquamation, would be deleterious if it took place in the barrier-forming parts of the stratum corneum. Under normal conditions the turnover time of the stratum corneum is two to four weeks. Moreover, corneocytes are "dead" in the sense that they have no protein synthesis, they have no active turnover of cell surface structures, and they are unresponsive to cellular signaling. Thus, chemical reactions leading to structural and functional changes within the stratum corneum may be considered as the final steps of a series of events initiated in viable parts of the epidermis. The process, which occur spontaneously without further input of regulatory signals, but yet in a well-regulated manner, depends on enzymes and other components produced by still living keratinocytes. In other words, at the time when a viable keratinocyte of the stratum granulosum is transformed to a corneocyte of the stratum corneum, the cell and the tissue it becomes part of must be "programed" in a way that allows the cell to be strongly linked to contiguous cells for a certain period of time, after which its cohesion to its neighbors should decrease to an extent, which will eventually allow it to be shed from the skin surface.

It seems reasonable to believe that a better understanding of desquamation and the mechanisms involved would give us possibilities to design better treatments for skin disorders associated with disturbances in stratum corneum turnover, be they common "dry skin problems" or results of more or less handicapping skin diseases. One strategy to understand desquamation would be to first identify mechanisms of cell cohesion in the stratum corneum, the structures involved, and the changes these structures undergo as cell cohesion decreases. The next step would be the identification of chemical reactions taking place, which would immediately give clues as to the nature of enzymes likely to be involved. Another fruitful strategy would be to elucidate the molecular basis and pathophysiology of diseases such as ichthyoses (see Chapter 8). The elucidation of ichthyosis-like conditions induced by certain drugs may also be expected to be productive in this context.[1,2]

The most likely site at that the events that eventually lead to desquamation take place is the stratum corneum intercellular space. As described in other chapters of this book, the chemical composition, organization, and interactions of this part of the stratum corneum are extremely complex. The stratum corneum intercellular space may be considered as a multiphase system consisting of a complex mixture of lipids in which structural proteins, enzymes, and other nonstructural proteins; a range of low molecular weight substances with different degrees of hydrophilicity; and water in low but significant concentrations are dispersed and interact with each other. A full understanding of stratum corneum cell cohesion and desquamation will rely on our understanding of the complex interactions of the many constituents of the intercorneocyte space. Although important steps forward have been taken in recent years, much has still to be learned. It should therefore be stated that our present knowledge about desquamation is quite rudimentary. Some clues have emerged, however, and will be summarized below.

7.2 SKIN DISEASES WITH DESQUAMATION DISTURBANCES

An accumulation of scales on the skin surface may be due to either an increased production of corneocytes, such as in psoriasis, or to a delayed desquamation. It may be predicted that conditions with delayed desquamation, once their pathophysiology on the molecular level is understood, will be highly informative with regard to the understanding of desquamation. Two such conditions are recessive X-linked ichthyosis (RXI) and lamellar ichthyosis.

The elucidation of the molecular genetics RXI has had a major impact on our understanding of stratum corneum turnover. Individuals with RXI lack an enzyme, cholesterol sulfatase,[3,4] which catalyzes the transformation of cholesterol sulfate (CS) to cholesterol and free sulfate. As a result there is an accumulation of CS in the stratum corneum intercellular space. Possible mechanisms by which this change in intercellular lipid composition of the stratum corneum can cause disturbances in desquamation, leading to ichthyosis, will be discussed later.

A group of individuals with severe ichthyosis (recessive autosomal lamellar ichthyosis) has been found to have mutations in the gene for epidermal transglutaminase.[5–7] By means of catalyzing cross-linking of constituent proteins, this enzyme plays a crucial role in the formation of the cornified envelope of the corneocyte. How this type of molecular defect can cause ichthyosis is unknown. It may be expected that further studies on this condition will give important contributions to our understanding of desquamation. Similarly, we can expect that the soon-to-come elucidation of the molecular genetics of inherited lamellar ichthyoses with similar phenotypes, but without transglutaminase mutations,[8] will be informative.

7.3 STRATUM CORNEUM CELL DISSOCIATION INVOLVES PROTEOLYSIS

Experimental evidence that protein structures are involved in stratum corneum cell cohesion was presented by Bisset et al.[9] They induced cell dissociation in pig and human nonpalmo-plantar stratum corneum by means of incubation of the tissue in the presence of the zwitterionic surfactant 6-octadecyldimethyl ammoniohexanoate. Cell dissociation could not be induced when the tissue had been pretreated with the serine protease inhibitor phenylmethylsulfonyl fluoride (PMSF). The fact that cell dissociation was found only in the presence of EDTA suggested a role also for calcium in stratum corneum cell cohesion.

Lundström and Egelrud[10] found a unipolar spontaneous cell dissociation in pieces of hypertrophic human plantar stratum corneum incubated in a simple buffer. The cell dissociation occurred only at the surface that had faced outward *in vivo*. The rate of cell dissociation was increased in the presence of EDTA. It was inhibited by inhibitors of serine proteases, but not by inhibitors of other groups of proteases. Since the tissue had not been treated with exogenous proteases before the experiments, it was concluded that the observed cell dissociation was mediated by an endogenous serine protease. This experimental system has been used as an *in vitro* model of desquamation. In addition to information about the enzyme(s) involved in the cell dissociation, it has provided information about the nature of the cohesive structures in the stratum corneum.

There is evidence that protein structures are also responsible for cell cohesion in nonpalmo-plantar stratum corneum. When punch biopsies of normal human gluteal skin were incubated in a buffer containing a mixture of the zwitterionic surfactant *N*,*N*,-dimethyldodecylamine and the anionic surfactant sodium dodecyl sulfate,[11] there was dissociation of cells in the stratum corneum but not in the rest of the epidermis. The cell dissociation took place only in the presence of EDTA and was inhibited by the serine protease inhibitor aprotinin.[12] Suzuki et al.[13,14] presented evidence that spontaneous cell dissociation in nonpalmo-plantar stratum corneum could be inhibited by a combination of inhibitors of trypsin-like and chymotrypsin-like enzymes. Thus, nonpalmo-plantar stratum corneum contains endogenous proteases that mediate cell dissociation.

7.4 DESMOSOMES AND CORNEODESMOSOMES

Desmosomes mediate mechanical contacts between viable epithelial cells such as keratinocytes.[15–18] A desmosome is a round or oval, button-like structure with a diameter of 0.2 to 1 μm. It consists of two symmetrical halves, each one belonging to one of two contiguous cells and consisting of an intracellular, a transmembranal, and an extracellular part. Inside the cell, just below the plasma membrane, is the desmosomal plaque. To this structure are linked intracellular keratin filaments as well as glycoproteins belonging to the cadherin family named desmogleins and desmocollins (for a review of desmosomal cadherins, see Reference 19). These glycoproteins cross the plasma membrane, and their glycosylated parts occupy the extracellular space where they interact with their counterparts from the contiguous cell, thus forming a cohesive structure between the cells. In the electron microscope the desmosomal plaque is visible as an electron dense structure, approximately

15 nm in width, on the inner aspect of the plasma membrane. The extracellular parts of desmosomes between uncornified keratinocytes has a moderately electron dense, plate-like appearance, approximately 30 nm in width, and has a zigzag formed electron dense central line. Desmosomes and keratin filaments form functional units, the desmosome-intermediate filament complexes.[17] These complexes link the keratin filament cytoskeleton of individual cells into a network comprising the whole epithelium.

The corneodesmosomes, that is, desmosomes in the stratum corneum, have a somewhat different appearance in the electron microscope.[20–22] Due to the densely packed and electron dense intracellular keratin filaments, it is not possible to identify the intracellular desmosomal plaque. The extracellular plate-like parts of corneodesmosomes have a homogenous and high electron density with no visible central line. Analyses of total number of desmosomes, measured as percentage of the cell periphery occupied by extracellular parts of desmosomes, showed a difference between the stratum corneum in palms and soles and stratum corneum at other body sites. In nonpalmo-plantar stratum corneum the number of desmosomes in deeper layers was comparable to the number of desmosomes in the stratum granulosum, whereas it was only around 20% of this number in the superficial layers close to the skin surface. This was true, however, only if the whole corneocyte periphery was considered. Whereas there were few desmosomes in the central parts of superficial corneocytes, the number of desmosomes per unit length of cell periphery at the overlapping edges of corneocytes was essentially the same as in deeper layers of the tissue. Thus, extracellular parts of desmosomes in the central parts of corneocytes disappear as the cells move upward in the stratum corneum, whereas desmosomes at the edges remain as long as the cells have not been shed. In palmo-plantar stratum corneum the number of desmosomes per unit length of corneocyte periphery is constant and high throughout the tissue until the cells are shed.[23]

The ultrastructural appearance of corneodesmosomes suggest that they are modified during the transition between viable and cornified epidermal layers. Part of this modification may be due to the incorporation of a recently discovered protein, corneodesmosin.[24–26] This is a 52-kDa protein, which is specifically expressed in keratinizing epithelia. In the stratum granulosum it is found intracellularly in association with lamellar bodies. In the transition zone between the stratum granulosum and the stratum corneum, coinciding with the change in the ultrastructural appearance of the desmosomes, corneodesmosin is translocated to the extracellular parts of desmosomes.[27] Immunoblot analyses have suggested that corneodesmosin is continuously degraded to smaller components in the stratum corneum.[26] It is not yet known to what extent this protein contributes to the cohesive capacity of corneodesmosomes. It has been speculated that corneodesmosin degradation may be part of the regulatory events involved in desquamation.[26]

7.5 DESQUAMATION INVOLVES DEGRADATION OF CORNEODESMOSOMES

Evidence that degradation of corneodesmosomes is a prerequisite for desquamation comes from ultrastructural and immunochemical studies. In the so-called retention ichthyoses, in which it is believed that a delayed desquamation causes the thickening of the stratum corneum and the accumulation of squames, there is an increased number of corneodesmosomes in the superficial layers of the stratum corneum.[28,29] In plantar stratum corneum undergoing spontaneous cell dissociation, electron microscopy of dissociating cells suggested that degradation of the intercellular parts of desmosomes preceded the widening of the intercellular space.[30] Chapman and Walsh[31] showed by means of electron microscopy that desquamation in pig skin was associated with morphological signs of desmosomal degradation.

Immunoblot analyses with antibodies specific for the transmembranal desmosomal glycoprotein desmoglein I (DG I) of plantar stratum corneum undergoing spontaneous cell dissociation[30] showed that although the still cohesive tissue contained only intact DG I, dissociated cells contained no

intact DG I, instead they contained degradation products of this protein. Analyses of surface cells that had been shed from plantar skin *in vivo* gave similar results.[32] In xerotic skin superficial stratum corneum contained more extractable intact DG I than in normal skin,[33] suggesting that delayed desmosomal degradation may contribute to the accumulation of squames. Increased amounts of intact DG I in superficial stratum corneum was found also in a mouse model with experimentally induced scaling.[34] Taken together, these ultrastructural and immunochemical results strongly suggest that corneodesmosomes are responsible for cell cohesion in the stratum corneum and that proteolytic degradation of their extracellular parts is a prerequisite for desquamation.

7.6 ENZYMES INVOLVED IN DESQUAMATION

The best-characterized enzyme so far with a proposed function in desquamation is stratum corneum chymotryptic enzyme (SCCE, also named human kallikrein 7; hK 7).[35] The discovery of SCCE was a result of the search for the enzyme responsible for the degradation of cohesive structures in the *in vitro* model of desquamation in hypertrophic plantar stratum corneum. SCCE has several properties compatible with a role in desquamation also *in vivo*.[36,37] SCCE has been purified from plantar stratum corneum.[38] It has been cloned and expressed in mammalian cells.[39] In reduced form SCCE has a molecular mass of around 28 kDa, it is partially glycosylated, and it has a basic isoelectric point. Although having a neutral to alkaline pH-optimum, it is active also at pH 5.5, that is, it is active at the pH of the stratum corneum.[40] SCCE is produced as an inactive precursor with a propeptide seven amino acid residues long. Removal of the propeptide by means of trypsin treatment of recombinant pro-SCCE yields a proteolytically active enzyme.[39] The mechanisms of SCCE activation *in vivo* remain to be elucidated. The deduced amino acid sequence contains the conserved regions typical of serine proteases, but is otherwise, at most, only around 40% homologous with other known human enzymes. SCCE shows similarities, but also significant differences regarding the activity on peptide substrates and the sensitivity to various protease inhibitors when compared to other chymotryptic enzymes such as bovine chymotrypsin and human cathepsin G.[38] This may be explained, at least partially, by the fact that in SCCE there is an asparagine residue in the bottom of the deduced primary substrate binding pouch, whereas this site is occupied by serine and alanine residues in chymotrypsin and cathepsin G, respectively.[39]

Analyses of mRNA from a large number of various human tissues has shown high expression of SCCE only in the skin.[39] Immunohistochemical studies have shown that SCCE is expressed in high suprabasal keratinocytes in the epidermis. In hair follicles and sebaceous glands it is expressed at a site where there is formation of cornified keratinocytes and hence a need for desquamation-like processes. In the oral cavity SCCE staining is found in the cornified epithelium of the hard palate, but not in the buccal mucosa or at other sites with noncornified epithelium. Thus, these findings suggest that SCCE expression is related to a differentiation process, leading to the formation of a cornified squamous epithelium.[41–44]

Results from enzymologic studies have suggested that SCCE has an extracellular localization in the stratum corneum.[45] This has been corroborated by means of immunoelectron microscopy. With this method SCCE was found intracellularly in association with lamellar bodies in the stratum granulosum. In the transition between the stratum granulosum and the stratum corneum, SCCE is extruded to the extracellular space together with the lamellar bodies. In the stratum corneum specific labeling is found only in the extracellular space, often in association with corneodesmosomes.[46]

Results from *in vitro* experiments, catalytic properties, and tissue localization are all compatible with the role of SCCE in the degradation of intercellular cohesive structures in the stratum corneum as part of the events leading to remodeling of the tissue and eventually to desquamation. Increased expression of SCCE in the epidermis of transgenic mice leads to impaired barrier function with increased transepidermal water loss. The transgenic animals have a thickened epidermis and a marked hyperkeratosis, possibly reflecting compensatory reactions.[47–48] There are also other proteases

present in the stratum corneum, some of which may be involved in desquamation.[13,14,37,38,49–51] Of these proteases, a 33 kDa serine protease named stratum corneum tryptic enzyme (SCTE; human kallikrein 5; hK 5)[35,52] with trypsin-like primary substrate specificity may be of special interest. The tissue distribution of SCTE is similar as for SCCE and it has been postulated that SCTE has a complementary role to that of SCCE in degradation of structures involved in stratum corneum cell cohesion during desquamation.[13,53] In addition SCTE is a candidate for being responsible for the activation of the SCCE precursor. Additional information in this respect will be crucial for the understanding of the role of SCCE and related enzymes in the formation and turnover of the stratum corneum.

7.7 REGULATION OF DESQUAMATION

We are very far from an understanding of how and by which mechanisms desquamation is regulated. If we assume, however, that proteolytic degradation of corneodesmosomes plays a major role in desquamation, a number of possible mechanisms can be postulated on the basis of the present knowledge. These are summarized in Table 7.1.

The activation of enzyme precursors is likely to be of central importance. A significant fraction of the total SCCE present in the stratum corneum is in the form of inactive proenzyme.[53,54] A change in the ratio of precursor to active enzyme may be expected to cause marked changes in the rate of corneodesmosomal degradation. *In vitro* pro-SCCE can be activated by pancreatic trypsin.[39] As mentioned earlier SCTE has been suggested to act as an SCCE activator, but this remains to be elucidated. It is possible that SCCE is just one of a number of enzymes constituting a "proteolytic cascade" in the stratum corneum, in which one enzyme serves as activator of another enzyme.

The stratum corneum is likely to contain a number of inhibitors of the various proteases present. CS may be of special interest. Accumulation of CS in the stratum corneum in RXI may be causative of this disease, in which there is evidence of a delayed degradation of desmosomes.[28] CS has been shown to inhibit pancreatic serine proteases *in vitro*, and application of CS on mouse skin *in vivo* causes a scaling condition.[34] In addition to direct effects on enzymes, CS could cause delayed desquamation by acting as a substrate modifier or by changing the physical–chemical conditions in the stratum corneum extracellular space.

Also, in autosomal recessive ichthyosis there are findings indicative of an impaired desmosome degradation in the stratum corneum.[29] The mechanisms involved have not been elucidated.

As mentioned previously for CS, substrate modifications could be of significant importance as regulating factors in proteolytic degradation of cohesive structures. Walsh and Chapman showed that pretreatment with glycosidases made preparations of stratum corneum more susceptible to cell

TABLE 7.1
Mechanisms which may be involved in regulation of desquamation

Enzyme activation
Activation of SCCE
Enzyme inhibition
Cholesterol sulfate
Antileukoprotease
Other protease inhibitors in the stratum corneum
Substrate modification
Glycosylation
pH? Water? Ions? Lipids?

Note: See text for references.

dissociation induced by exogenous proteases, suggesting that proteins involved in cell cohesion may be protected by carbohydrates against proteolytic degradation.[55]

A number of protein protease inhibitors are present in the stratum corneum. Antileukoprotease has been shown to be an efficient inhibitor of SCCE at physiological concentrations.[56] Extracts of plantar stratum corneum contains covalent complexes between SCCE and α1-antitrypsin (Egelrud, T., unpublished observation). Recent findings in the human genetic disease Netherton's syndrome (NS) have given new insights on the potential role of serine proteases and their inhibitors for epidermal homeostasis. In NS there is severe impairment of skin barrier function. The causing mutations have been found in a gene, Serine Protease Inhibitor Kazal type 5 (SPINK5),[57] encoding a complex protein, which after post-translational modifications gives rise to a number of serine protease inhibitors called LympoEpithelial Kazal-Type related Inhibitor (LEKTI).[58] It has been suggested that the lack of LEKTI, which is highly expressed in the stratum granulosum of normal epidermis,[59] may lead to increased and uncontrolled activity of epidermal serine proteases, which in turn would result in a deteriorated barrier.

There are a vast number of other factors which may be expected to influence the rate of desquamation, for instance, by affecting the rate of proteolytic reactions. pH, water, and ion concentrations, and lipid composition may all be expected to be of importance. Experimental data in this area are very scarce, but some speculations can be made. For instance, the pH dependency of SCCE activity could be of importance. SCCE has optimal activity at pH 7 to 8, but close to half its maximal activity at pH 5.5.[36,37] This implies that rather small variations in either direction of the pH of the extracellular space should have effects on the rate of SCCE-mediated protein degradation. In support of this, the rate of spontaneous cell dissociation observed in plantar stratum corneum *in vitro* showed a marked pH dependency, being highest at neutral to weakly alkaline pH and decreasing at lower pH values.[10]

The effects of chelating agents in *in vitro* models for desquamation suggest that divalent ions such as calcium may play a role in the regulation of desquamation.[12,26,60]

The composition of the stratum corneum intercellular lipids may have profound effects on desquamation. In addition to modifying effects on, for example, proteolytic enzymes and their substrates,[34] lipids may also be directly involved in corneocyte cohesion. The effects of cholesterol sulfate have already been mentioned. In addition to RXI, there are a number of other hereditary diseases with disorders of desquamation associated with disturbances in lipid metabolism. Furthermore, scaling as a result of treatment with lipid-lowering drugs has been observed (for review, see References 1 and 2).

7.8 CONCLUSION

A normal desquamation is of crucial importance for the maintenance of the function of the stratum corneum and for a normal skin appearance. In recent years some basic knowledge about stratum corneum cell cohesion and the role of proteolysis in desquamation has evolved. Much still has to be learned, however. In the near future we may expect to obtain information about further enzymes involved in desquamation, and the ongoing elucidation of hereditary skin diseases will give new clues with regards to regulation of mechanisms involved in desquamation. Similarly, further studies on the physical chemistry and the chemical composition, including identification of hitherto unknown proteins, of the stratum corneum intercellular space may be expected to give important contributions to this central area of skin biology.

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Dry Skin and Hyperkeratotic Conditions

8 Ichthyosis — An Inborn Dryness and Scaliness of the Skin

Anders Vahlquist

CONTENTS

8.1 INTRODUCTION

The term *ichthyosis* (latin for "scaly fish dermatosis") encompasses a wide range of various keratinizing disorders with different etiologies (Figure 8.1), but with the common feature of widespread epidermal hyperkeratosis and a dry and scaly skin (for a recent review see reference 1). Although acquired forms of ichthyosis exist (e.g., in association with malignancy, severe malnutrition, lepra, and treatment with lipid-lowering drugs), only the inherited forms will be discussed here.

Depending on the type of ichthyosis and the variable influence of individual and environmental factors, the severity of the skin symptoms may range from mild xerosis and scaling on the extremities mainly appearing in winter time (as in ichthyosis vulgaris) to massive hyperkeratosis and scaling all over the body (as in lamellar ichthyosis). The more severe forms are frequently associated with keratoderma (thickening of the palms and soles), ectropion (constricted eyelids), anhidrosis (inability to sweat), and patchy alopecia (hairloss). Some patients also experience skin erosions and blisters as in bullous ichthyosis, or epidermolytic hyperkeratosis. Pathogenetically, the latter form of ichtyosis is reminiscent of epidermolysis bullosa simplex and pachonychia congenita, that is, genodermatoses, which are also due to reduced cell-cohesion in epidermis but usually not defined as ichthyosis.

Some patients with ichthyosis also have noncutaneous symptoms, which are due to the same genetic defect as the skin condition albeit expressed in another tissue (CNS, immune system, skeleton, etc.); these diseases are collectively called "ichtyosiforme syndromes."

Over the last decade, many new etiologies for ichthyosis have been elucidated, making it easier to correctly identify various subtypes of the disease and to give proper genetic councelling to the patients and parents. Hopefully, this knowledge will also lead to novel therapies for different forms of ichthyosis, including perhaps somatic gene therapy for the most severely affected patients (for review, see reference 2). Today however the therapy is mainly symptomatic and based on topical emollients, keratolytic agents and, in more severe cases, oral retinoids (vitamin A analogs). Because the patients

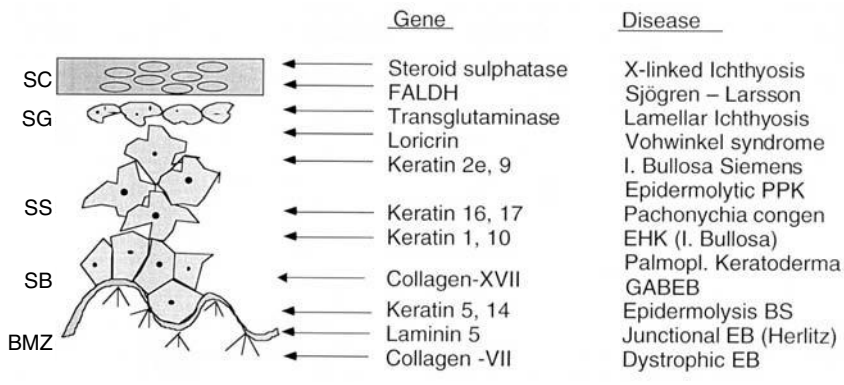

FIGURE 8.1 Examples of gene products which are incriminated in keratinizing disorders in human skin. The top eight of these disorders can be classified as ichthyosis. The lower half of the figure indicates mechano-bullous disorders which are pathogenetically related to some forms of ichthyosis although expressed around the basal membrane thus causing skin fragility and blistering rather than dry scaling. Data compiled from the literature. Abbreviations: SC — stratum corneum, SG — stratum granulosum, SS — stratum spinosum, SB — stratum basale, BMZ — basal membrane zone, FALDH — fatty aldehyde dehydrogenase, PPK — palmo-plantar keratoderma, EHK — epidermoltyic hyperkeratosis, EB — epidermolysis bullosa.

often require life-long treatment with daily applications of emollients all over the body, they are potentially big consumers of such products and usually become experts in their own right on how to treat their skin.

8.2 THE COMMON TYPES OF ICHTHYOSIS

The most common forms of ichthyosis — autosomal dominant ichthyosis vulgaris (IV) and X-linked recessive ichthyosis (XRI) — in many countries occur at frequencies a high as 1/300 and 1/2500, respectively. In fact, the genetic traits for IV and XRI are so frequent that the two diseases sometimes coexist in one and the same family, which may cause confusion as to the inheritance pattern. Although the incidence of IV and XRI is probably similar around the world, climate differences in particular will affect the severity of the disease, and hence its notification by the health care system.

In both types of common ichthyosis, scaling is usually most apparent on the extensor surface of the extremities, but it may also appear on the trunk, especially in XRI (Figure 8.2). Xerosis of the skin is a prominent feature in most patients, but there is no skin inflammation unless ichthyosis is complicated by, for example, atopic eczema (common in IV) or microbial infections.

Clinically, IV and XRI may appear indistinguishable at first sight, explaining why they were not recognized as separate entities until the 1960s when Wells and Kerr did their pioneering work [3]. By and large, XRI starts earlier in life — it may event present at birth[4] — and is usually more severe than IV. When laboratory diagnosing of XRI became possible in the late 1970s, based on the discovery of steroid sulfatase deficiency in these patients,[5] several new features distinguishing XRI from IV emerged[6] (Table 8.1). While histologically the affected skin will show orthohyperkeratosis in both diseases, due to retention of mature corneocytes, the stratum granulosum (SG) is normal in XRI but thin or even missing altogether in IV.[7] On electron microscopy (EM) this defect in IV epidermis will appear as tiny and crumbled keratohyalin granules due to defect processing of profilaggrin.[8,9] The exact genetic mechanism causing IV has not been determined. In fact a recent report claims that there are two forms of IV, one with missing SG and one with normal appearing SG, obviously with different pathoetiologies.[10] This illustrates the difficulty in distinguishing IV and XRI based on histopathology alone. Diagnosing XRI is however facilitated by the fact that on serum electrophoresis the β-lipoprotein fraction of an affected person will show abnormal mobility due to the accumulation of negatively charged lipids.[11]

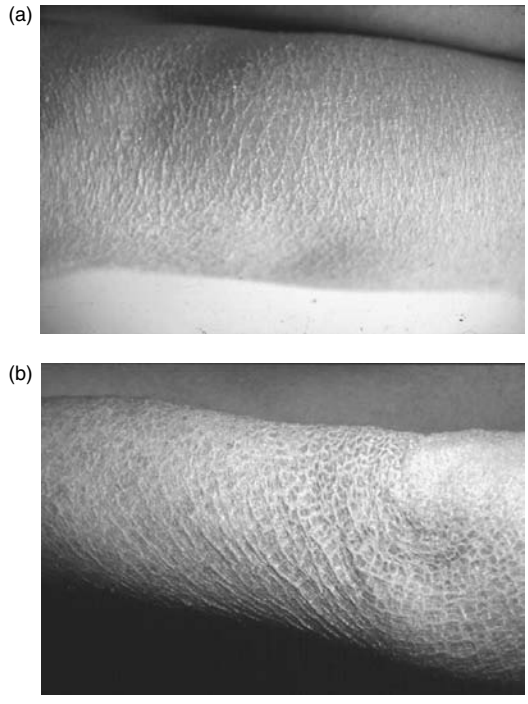

FIGURE 8.2 Extremities showing (a) ichthyosis vulgaris in a 40-year-old woman and (b) X-linked ichthyosis in a 20-year-old man (from the author's files).

TABLE 8.1
Clinical and Biological Features Distinguishing *Ichthyosis vulgaris* (IV) from X-linked Recessive Ichthyosis (XRI)

Features	IV	XRI
Symptoms appearing <6 months	Rare	Frequent
Brownish scales on the trunk	Rare	Frequent
Flexural involvement	Rare	Frequent
Testicular nondescendence	Rare	Frequent
Corneal opacity	Rare	Frequent
Accentuation of palmar creases	Frequent	Rare
Associated atopic eczema	Frequent	Rare
Associated keratosis follicularis	Frequent	Rare
Scanty or absent stratum granulosum	+	−
High CS/low free cholesterol in stratum corneum	−	+
Abnormal mobility of β-lipoprotein	−	+
Deletions or mutations in the STS gene	−	+

8.3 BIOCHEMICAL DIFFERENCES OF THE HORNY LAYER IN IV AND XRI

The lack of filaggrin and keratohyaline in IV horny layer results in a deficiency of the natural moisturizing factor (NMF) — composed of urocanic acid and pyrrolidone carboxylic acid (PCA) — that is, breakdown products of amino acids in profilaggrin.[12] In XRI, on the other hand, there is

TABLE 8.2
Cholesterol Sulfate (CS) and the Skin

Is normally present in stratum granulosum and stratum corneum
Is probably important for the pH gradient in stratum corneum17
Accumulates in stratum corneum of patients with X-linked ichthyosis13
Accumulates in epidermis during chemical carcinogenesis47
Is growth inhibitory to human keratinocytes48
Activates PKC (which phosphorylates TGM1)49
Induces transcription of the TGM gene50
Inhibits certain proteases (SCCE, etc.) in stratum corneum16
Is reduced in epidermis during retinoid therapy51

an accumulation of cholesterol sulfate (CS) and a concurrent decrease in cholesterol in the stratum corneum (SC) due to steroid sulfatase deficiency.[13] Clearly, intercellular lipids are very important for corneocyte cohesion and barrier function. Thus a careful monitoring of transepidermal water loss (TEWL) in XRI patients has shown repeatedly that, despite hyperkeratosis, there is a slight impairment of the epidermal barrier function.[14,15] Interestingly, this impairment can be reproduced in experimental animals via topical application of CS.[15] Recent observations about the multiple effects of CS in epidermis (Table 8.2), makes it possible to speculate about the delayed shedding of corneocytes in XRI. Presumably, hyperkeratosis is not a compensatory mechanism, but reflects a retardation of the desquamation process by CS,[16] possibly via enzyme inhibition.

It is noteworthy that CS, which is a weak organic acid, has been hypothesized to be involved in the formation of a pH gradient over the horny layer. Thus, in an intervention study of patients with ichthyosis[17] we found that the pH gradient, which normally spans from 7 in stratum granulosum to pH 4.5 to 5 on the skin surface, is shifted to more acidic values in XRI patients and to more basic values in IV patients (Figure 8.3). These findings are consistent with the accumulation of CS in XRI, and the lack of urocanic acid and PCA in IV skin. Speculatively, changes in the pH gradient may not just reflect the altered chemical compostion of the horny layer, but might also influence the activity of pH-dependent enzymes operating in the intercorneocyte space.[18] Whether pH variations also influence the transcorneal diffusion of topically applied acids and bases (e.g., salicylic acid, alpha-hydroxy acids, and certain other drugs) remains to be determined.

8.4 TREATMENT OF COMMON ICHTHYOSIS

A primary objective in treating ichthyosis is to remove scales and reduce the dryness of the skin. To accomplish this, several items have to be taken into consideration when prescribing a topical treatment: (1) the age and sex of the patient (children have a thinner skin and a higher skin surface area/body weight ratio than adults thus increasing the risk for systemic toxicity; females of childbearing age should not uncritically be exposed to potentially teratogenic compound), (2) the severity of the disease (thick scales require keratolytic agents, xerosis requires emollients), (3) the extent and location of the skin lesions (whole body application increases the risk for systemic toxicity; face and flexural sites usually need less potent therapy), (4) presence of fissures, erosions, and bacteria in the skin lesions (precludes the use of irritating creams). It is also essential that the health provider evaluates the patient's willingness and ability to apply creams all over the body 1 to 2 times daily for longer periods of time, and has an open attitude to individual preferences regarding cream formulations. It should be kept in mind that cosmetic acceptability of a cream is a *sine qua non* for good compliance and that there are probably as many opinions about "the best cream formulation" as there are patients.

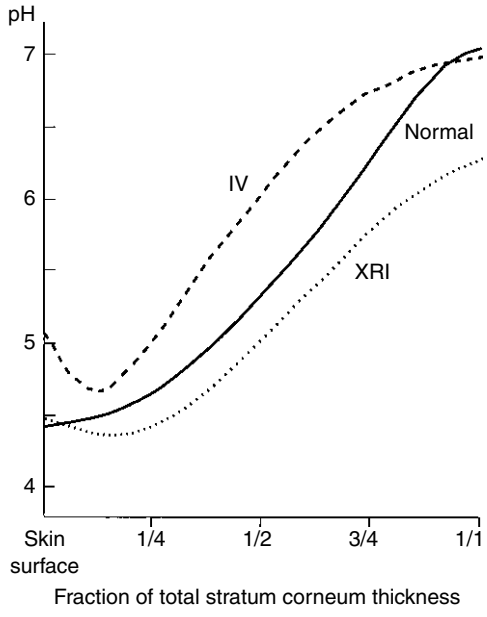

FIGURE 8.3 Schematic representation of the pH gradients over stratum corneum in normal skin, *Ichthyosis vulgaris* (IV), and X-linked Recessive Ichthyosis (XRI) skin, respectively. (Reproduced from Öhman, H. and Vahlquist, A., *J. Invest. Dermatol.*, 111, 674, 1998. With permission.)

In theory, one way of treating IV and XRI would be to substitute the missing components in SC, viz. NMF and cholesterol, respectively. Indeed some success has been reported using cholesterol-containing creams in XRI patients,[19] but on the whole the substitution therapy concept is no viable today. Instead, standard treatment of mild to moderate IV and XRI relies on daily application of emollients containing 2–10% urea,[20] 5–15% lactic acid,[21] or 10–25% propylene glycol or glycerol. Considering that each ichthyosis patient represents a potential mega-user of topical therapy, with a calculated life-time consumption of creams or ointments in the order of 1 t, surprisingly few clinical trials have addressed this problem; in fact, ichthyosis is not even mentioned in the text book on *Evidence Based Dermatology*.[22]

In a recent German study, urea in a new lotion base was found to be highly effective and well tolerated also by children.[23] Using a semiocclusive cream formulation containing lactic acid and propylene glycol (see Section 8.6) we have observed good results in severe cases of IV and XRI (Vahlquist et al., unpubl. observ.). Other treatment options include regular baths (salt and oil), UV-irradiation, and climate therapy in winter. Oral retinoid therapy is rarely indicated for common ichthyosis, except perhaps in the most severe cases of XRI. Topical corticosteroids and vitamin D derivatives (calcipotriol) are usually contraindicated in common ichthyosis because (1) they do not alleviate the disease processes and (2) they are associated with a significant risk for systemic absorption when used extensively on large areas of the body.

8.5 RARER FORMS OF ICHTHYOSIS

The two most severe types of ichthyosis, lamellar ichthyosis (LI) and epidermolytic hyperkeratosis (EHK), are distinct families of diseases with completely different etiologies (see Figure 8.1 and Table 8.3). Nevertheless, LI and EHK have several things in common: they are rare, congenital diseases (prevalence $<1/100{,}000$) with more or less generalized hyperkeratosis and a defective skin barrier, and they usually demands vigorous therapy.

TABLE 8.3
Common and Rare Forms of Ichthyosis

	Ichthyosis vulgaris	X-Linked ichthyosis	Lamellar ichthyosis (nonbullous ichthyosiform erythroderma)	Epidermolytic hyperkeratosis (bullous ichthyosis)
Incidence	1/300	1/3,000 (boys)	1/100,000	1/300,000
Etiology	Defective keratohyaline	Steroid sulfatase deficiency	Transglutaminase 1-plus several deficiency (other causes)	Keratin mutations
Inheritance	Autosomal dominant	Recessive X-linked	Autosomal recessive	Autosomal dominant
Appearance	Early in childhood	Early in childhood	Congenital	Congenital
Symptoms	Retention hyperkeratosis on extremities; better in summer	Brown scales all over the body; associated features; maternal pregnancy abn.	"Collodion baby"; generalized scaling; large, thick scales; ektropion; hypohidrosis	Intense blistering at birth; later verrucous hyperkeratosis, esp. in body folds; keratoderma ±

LI and its closely related variants, nonbullous ichthyosiform congenital erythroderma (CIE) or erythrodermic lamellar ichthyosis (ELI) 1, and congenital ichthyosis with fine/focal scaling (CIFS) or non-LI/non-CIE 4 (Figure 8.4), seem mostly to be caused by abnormalitishing the cornified cell envelope or defective deposition of intercorneocyte lipids. Thus mutations in the gene encoding for keratinocyte transglutaminase type 1 (*TGM1*) cause a recessive disorder characterized by deficient TG-1 activity in the upper epidermis and defective cross-linking of envelope proteins, such as involucrin and loricrin.[24,25] This can be visualized by EM as absent or faint marginals of the corneocytes.[8] We and others have found that about 50% of all cases of LI/ELI are due to *TGM1* mutations usually of the compound heterozygous type.[26] Less readily explained EM findings in LI and ELI include numerous lipid droplets and cholesterol clefts in SC and bizarre accumulations of membrane-like structures in cells from both the granular and the horny layers.[8] These findings point to a multifactorial pathogenesis of LI, CIE, and CIFS, also supported by recent disclosures of several new chromosomal loci and candidate genes for this group of diseases.[27,28] Finding the remaining causes of nonbullous congenital ichthyosis will probably be instrumental in elucidating some of the still unknown mechanisms during normal cornification and also raises the hope for future gene therapy of the most severely affected patients.

8.6 TREATMENT OF LAMELLAR ICHTHYOSIS

The introduction of oral retinoid therapy in the late 1970s was a break-through for many patients with LI, and the pros and cons of this therapy have been discussed at length in the literature (for review, see reference 29). This chapter will focus on external remedies that remain a mainstay of therapy in LI and CIE.

Both emollients and keratolytic agents are commonly prescribed but the treatment traditions differ from one country to another and even from one doctor to another. For example, although urea-containing lipophilic creams are popular in many European countries, mixtures containing propylene glycol or alpha-hydroxy acids (AHA) seem to be the first choice for treating severe ichthyosis in United States. Importantly, the commonly used keratolytic agent, salicylic acid, should be avoided when treating large, eroded skin areas or in small children owing to the risk of systemic toxicity. This

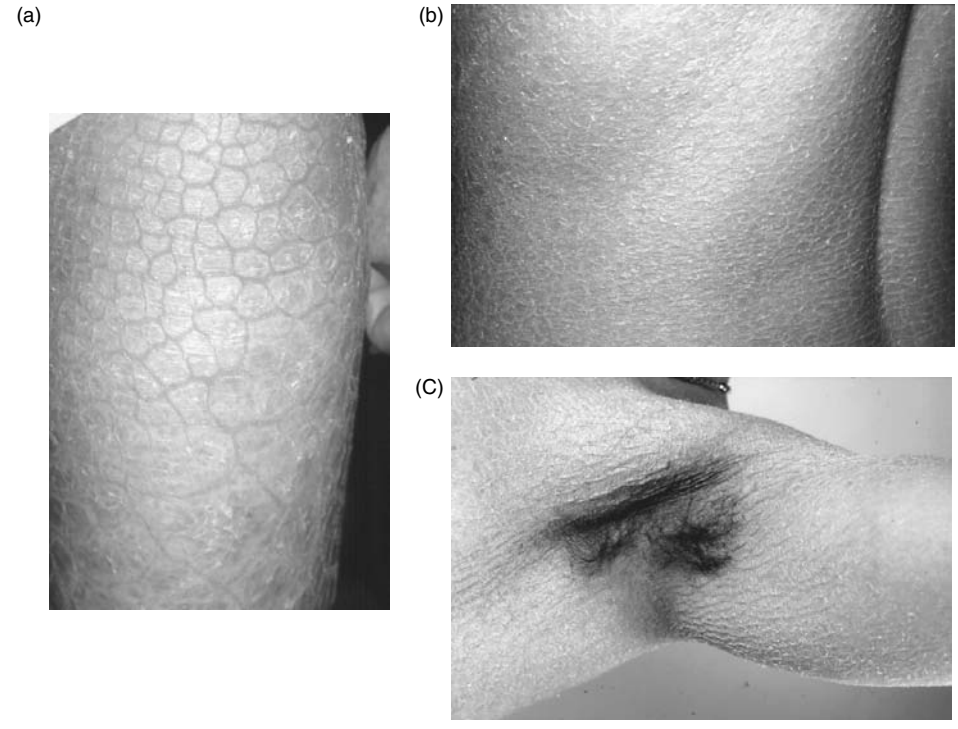

FIGURE 8.4 Examples of clinical variants of lamellar ichthyosis (LI) and nonbullous congenital ichthyosiform erythroderma (NBCIE). (a) Large scales on the thigh of a man with LI due to transglutaminase −1(*TGM1*) mutations, (b) generalized scaling on the trunk of a woman without *TGM1* mutations, and (c) scaling and mild erythroderma in the axillae of a woman also without *TGM1* mutations. (Photos from the author's files.)

is especially important to remember in collodion babies who may otherwise suffer fatal salicylism. By and large, these babies should only be treated with bland, semiocclusive emollients, possibly containing glycerol; even usage of occlusive ointments, such as petrolatum, may increase the risk of bacterial skin infections and septicemia.[30]

In adults, the side effects associated with topical treatment are minimal but the reasons for treatment failures are numerous. Needless-to-say the selection of a cream base (hydrophilic or lipophilic, nonocclusive or semiocclusive) is important not only for the antimicrobial and pharmacologic effects, but also for compliance reasons.

By combining two or more keratolytic agents and moisturizers in the same cream base it is often possible to achieve additative or even synergistic effects without the need of using irritating concentrations of either agent alone. Thus, in a double-blind trial of four different cream mixtures in 20 patients with LI, a mixture of 5% lactic acid and 20% propylene glycol in a semi-occlusive cream for four weeks twice daily was significantly more effective than 20% propylene glycol or 5% urea alone in the same vehicle.[31] However, while hyperkeratosis was virtually abolished in some patients (Figure 8.5) and most patients tolerated continued therapy for many months, the TEWL data actually showed that the skin barrier deteriorated. This pin points an inherent drawback when trying to reduce scaling and hyperkeratosis associated with LI: the excessive accumulation of defective corneocytes in LI probably represents a homeostatically controlled repair mechanism by which epidermis partially compensates for an intrinsic failure of the skin barrier (due to the deficiency of TG-1 or intercellular lipids); therefore an efficient therapeutic removal of corneocytes is likely to precipitate the underlying barrier defect. Admittedly, TEWL is a very sensitive gauge of the barrier function and a modest increase in TEWL may not represent a real problem to the patient. However, also a minor deterioration of the barrier function may increase the transcutaneous penetration of, for example, topically applied drugs and chemicals, a matter of great concern especially in children.

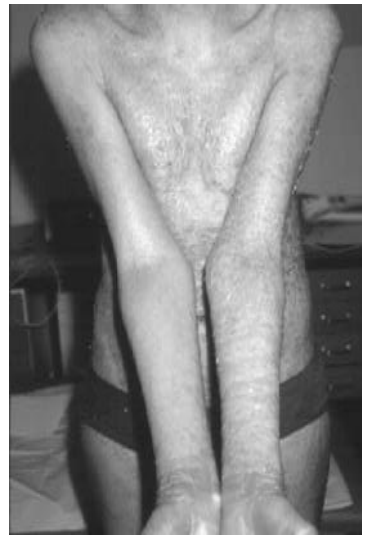

FIGURE 8.5 Patient with lamellar Ichthyosis (due to *TGM1* mutations) who twice daily for 2 mo. received a cream formulation containing lactic acid (5%) and propylene glycol (20%) on the right arm as compared to on the left arm (Reproduced from . . . 31)

Apart from emollients and keratolytic agents, topical applications of more specific drugs, such as retinoids,[32] N-acetylcysteine,[33] liarozole,[34] and calcipotriol[35] have also been tried in LI. Some of these drugs probably act through reducing epidermal hyperproliferation associated with certain forms of LI. Others affect keratinocyte differentiations and hence corneocyte function. Because of the obvious risk of systemic side effects when using these drugs extensively on the skin, and the notorious differences in therapeutic effects seen in different skin regions (strong effects in skin folds, poor effects on palms and soles, etc.), they have not become very popular in the treatment of LI and are usually not available for this indication.

Although treatment of xerosis and scaling is a *sine qua non* in LI therapy, other skin symptoms also need to be taken care of. Thus, ectropion, alopecia, and finger contractions are usually unresponsive to medical treatment, and only occasional patients with anhidrosis will improve the sweating capacity when using potent oral drugs for LI.[36]

8.7 BULLOUS ICHTHYOSIS, A KERATIN DISORDER

EHK and the closely related diseases, ichthyosis bulluosa of Siemens, epidermolytic palmo-plantar hyperkeratosis, and pachonychia congenita, are all due to dominant negative keratin mutations expressed in the suprabasal layers of epidermis.[37,38] Depending on which pair of keratin molecules is affected (K1/10, K2e/9, or K6/16,17), keratinocytes in different parts of suprabasal epidermis will collapse when exposed to mechanical stress. This results in superficial blisters and erosions that easily become infected. Concurrently, other parts of the epidermis may be hyperkeratotic, leading to a mixture of oozing and dry skin lesions with a characteristic malodor.[1] The flexural areas are usually most severely affected, but some patients have a widespread verrucous type of ichthyosis occasionally coupled to palmo-plantar keratoderma (Figure 8.6).

The treatment of EHK is complicated. On the one hand, hyperkeratosis must be reduced to minimize the disfiguring and foul-smelling scales. On the other hand, skin blisters and erosions must be protected from irritation in order to heal. A too potent keratolytic treatment will often aggravate the condition by disrupting the epidermal barrier and increasing the risk for painful and

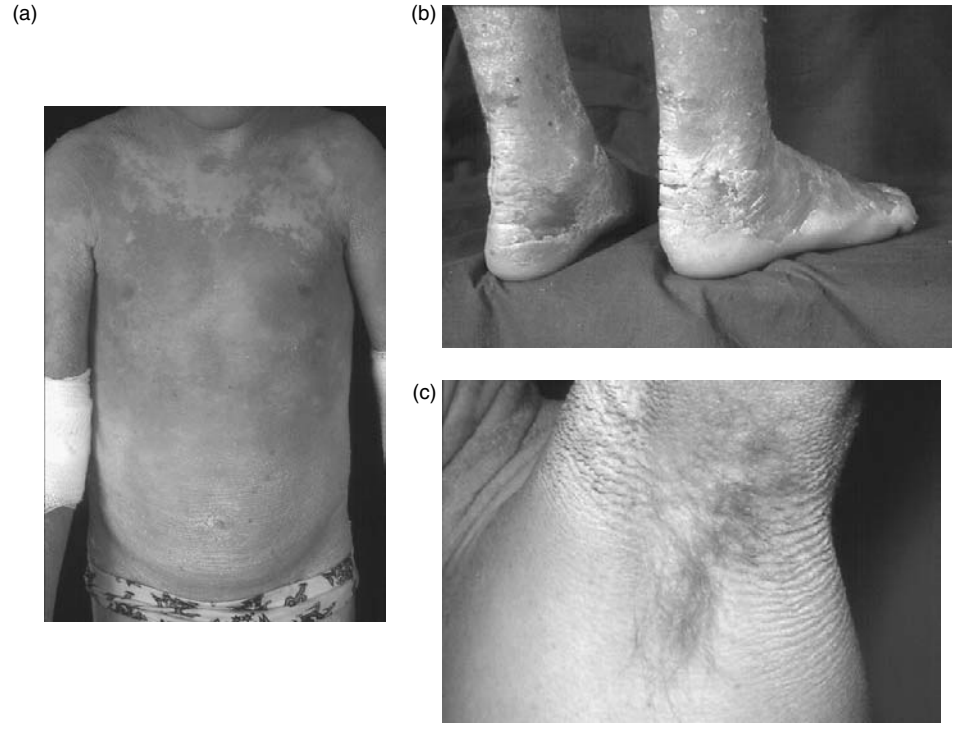

FIGURE 8.6 Epidermolytic hyperkeratosis in a 10-year-old boy showing (a) extensive hyperkeratosis on the trunk, (b) erosions and scaling around the ankle, and (c) verrucous hyperkeratosis in the axilla of a 25-year-old man carrying a spontaneous deletion in one of his K1 alleles (Vahlquist et al., unpubl. observ.).

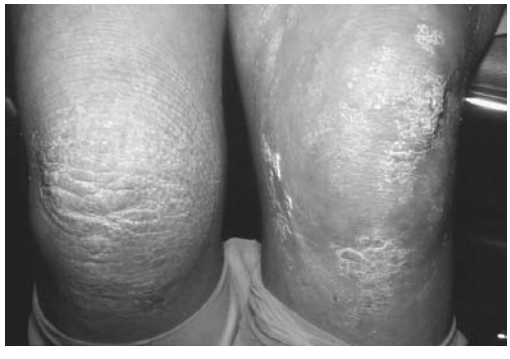

FIGURE 8.7 Successful topical treatment of EHK with a tretinoin-containing cream on the left knee of a woman with inherited K10 mutation (From Virtanen et al, *Acta Derm Venerol* 81, 163–70, 2001. With permission)[40].

easily infected erosions. It is more important than ever in EHK that the treatment of different body areas is individualized.

Retinoids, topical as well as systemic, have been tried in EHK but were often found to be irritating. Nonetheless some patients are improved by oral acitretin,[39] but the dose must be kept low in order to avoid the epidermolytic side effect of the drug. If correctly used, topical tretinoin and tazarotene may also be effective in some patients with EHK (Figure 8.7). Interestingly, the response to retinoid therapy seems to be partially determined by which keratin gene (K1, K2e, or K10) is mutated; patients with K2e and K10 mutations have the best response probably because they tolerate a retinoid-induced down-regulation of K2e expression better than other patients.[40] However the

topical treatment of EHK also relies on the use of bland emollients and a liberal prescription of antiseptics and antibiotics to prevent bacterial infection of the skin.

8.8 ICHTHYOSIFORME SYNDROMES AND OTHER RARE ICHTHYOSIS-LIKE CONDITIONS

Netherton's syndrome (ichthyosis circumflexa and atopy) is due to a deficiency of LEKT1 — a protease inhibitor normally expressed in both epidermis and the thymus.[41] This leads in the skin to exaggerated degradation of corneodesmosomes and a deficient epidermal barrier, which neonatally is reflected in generalized erythroderma and life-threatening hypernatrinemia and septicemia. When the child grows older, ichthyosis and atopy usually become more prominent features.[42] However, the skin barrier remains defective, which must be taken into consideration when prescribing topical treatments.

Darier disease (keratosis follicularis) is an autosomal dominant disorder of keratinization, which usually starts at puberty. Mutations in an endoplasmatic ATPase, which pumps calcium ions across membranes in keratinocytes, have been found to cause the disease.[43]

The patient's skin problems somewhat resemble those in EHK, that is, hyperkeratosis coexists with acantholysis and the epidermis is fragile and easily infected but can be effectively treated with low doses of retinoids.[29]

Among the many types of neuroectodermal syndromes having ichthyosis as presenting symptom, two well-known examples are *Sjögren–Larsson syndrome* and *Refsum disease*, both of which represent inborn errors of the lipid metabolism.[44,45] Although treatment aiming at rectifying the abnormal accumulation of lipid metabolites in skin and nervous tissue improves ichthyosis in some cases of Refsum disease, most patients require additional therapy of the same type as in common ichthyosis. The skin symptoms in Sjögren-Larsson syndrome respond quite well to oral retinoids,[46] but topical treatment with lactic acid/propylene glycol in a semiocclusive cream base (see under lamellar ichthyosis) is also very effective (Gånemo, Jagell, Vahlquist, unpubl. observ.).

8.9 CONCLUSIONS AND PROSPECTS FOR THE FUTURE

Ichthyosis can be a disabling condition requiring laborious treatments several times a day, but may also be a relatively mild disorder, which only occasionally needs application of emollients. From a diagnostic as well as therapeutic point of view, the many different subtypes of ichthyosis represent a problem for the caring physician. For example, a paradoxic combination of barrier failure and massive hyperkeratosis in some types of ichthyosis demands special attention. Choosing the right treatment will be even more important in future when new therapeutic regimes based on more-detailed knowledge about the pathogenesis of ichthyosis will emerge.

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9 Dry Skin in Atopic Dermatitis and Patients on Hemodialysis

Motoji Takahashi and Zenro Ikezawa

CONTENTS

SUMMARY

We investigated the characteristics of the dry skin in patients with atopic dermatitis (AD) and those on hemodialysis (HD) using noninvasive methods. Transepidermal water loss (TEWL), water content, parakeratotic cells, free amino acid and ceramide in stratum corneum (SC), and skin surface pH were examined on lesional and nonlesional skin in the dorsolumbar part of AD patients, HD patients, and healthy normal controls. The water content in SC on lesional and nonlesional skin was markedly lower in the AD patients than in the normal controls. The water content in SC was also lower in HD patients. The level of free amino acids, which represents the natural moisturizing factor (NMF) in SC, was decreased in both patient groups, which corresponded with the decrease of their water content in SC. TEWL was high in AD patients, but that in HD patients was almost similar to that in the controls. The level of the ceramides, which are closely related to the barrier function of SC, was lower in AD patients than in HD patients or in the controls, which was in agreement with the results of TEWL. In the composition of ceramides, the HD patients showed a higher percentage of ceramides 2

and 3 and a lower ratio of 1, 4/5, and 6 in comparison with the controls. No parakeratotic corneocytes were found in the controls or HD patients, while they were found in not only the lesional but also nonlesional skin of AD patients, indicating the presence of mild inflammation even in nonlesional skin. The conversion ratio of ornithine, which is a free amino acid component in SC, to citrulline was lower in AD patients than in HD patients or the controls. This suggested increased epidermal proliferation in AD patients. The skin surface pH value was high in both AD and HD patients, and the latter showed a higher value than the former. Except for pH, the results of all of the measurements in the nonlesional skin of AD patients were found to be intermediate between those of the lesional skin and the normal controls, showing that the lesional skin is physiologically different from the nonlesional skin.

These findings suggested that the decrease in free amino acids (= NMF) and inferior barrier function of SC caused the dry skin of AD patients, but the decrease of NMF mainly caused the dry skin in HD patients.

9.1 INTRODUCTION

Many important allergic aspects of AD have been reported. For example, most patients show high serum IgE levels due to high response of IgE antibody to various allergens such as foods, dust, mites, and fungi. Also, they show an increase of eosinophils and eosinophil-derived mediators such as eosinophilic cationic protein and major basic proteins in blood during exacerbation of AD. Among the nonallergic aspects of AD, the skin is also tends to overreact to irritation and become dry, particularly in winter. Many researchers have analyzed the characteristics of the dryness in skin lesions of AD from the viewpoint of the functions of the SC. Decreased water content in SC,[1,2] enhanced TEWL,[3,4] shortening of the turnover time of SC,[3] reduction of ceramide levels,[5–7] decrease of free amino acid level,[3] and increase of skin surface pH[8] have been reported. However, few investigators have analyzed, compared, and discussed these properties in lesional and nonlesional skin.

On the other hand, although dry skin or skin itchiness has frequently been recognized together with pigmentation in the patients with chronic kidney failure and receiving HD,[9] only a few reports have been made on the characteristics of dry skin (e.g., high pH,[10] decrease of water content in SC, and low TEWL.[11])

Here, we will report the characteristics of dry skin observed in lesional and nonlesional skin of AD patients and patients with chronic kidney failure and undergoing HD from the viewpoints of various functions of SC.

9.2 SUBJECTS AND METHODS

9.2.1 Subjects

The study subjects included 48 patients with AD (27 male and 21 female), 22 patients undergoing HD (7 male and 15 female), and 30 healthy volunteers (15 male and 15 female) who served as controls (Table 9.1).

9.2.2 Skin Regions and Timing for Measurements

Skin measurements were always made on the dorsolumbar region in HD patients and healthy subjects and whenever possible for lesional and nonlesional skin in AD patients. In some cases, meaurements on the nonlesional skin could not be made because a sufficient area could not be obtained.

The targeted skin regions were cleaned with ethanol and distilled water 30 min before the start of measurement. Measurements were made at an ambient temperature of 21 to 23°C and relative humidity of 35 to 50% from December to February when the skin was apt to become dry.

TABLE 9.1
Age Distribution of Subjects

Age distribution (Years)	Atopic dermatitis		Hemodialysis		Normal controls	
	Male	Female	Male	Female	Male	Female
3–9	1	5				
10–19	11	8				1
20–29	7	5			8	11
30–39	6	1			6	3
40–49	1			2	1	
50–59		1		6		
60–69		1	4	4		
70–79	1		3	3		
Total	27	21	7	15	15	15
Mean ± SD	23.3 ± 14.4		64.0 ± 10.1		27.8 ± 4.9	

9.3 MEASUREMENTS

9.3.1 Water Content in Stratum Corneum

Skin surface conductance (μS) was determined with Skicon-100 (IBS Company, Hamamatsu, Japan) by measurement of water content in SC. Nine measurements were repeated at a single point, and the mean was taken after excluding the highest and lowest values.

9.3.2 Transepidermal Water Loss

Transepidermal water loss (g/m^2/h) was determined with Evaporimeter EP-1 (Servo Med Company, Sweden). Measurements were repeated twice, and the mean was calculated.

9.3.3 Skin Surface pH

Distilled water was dropped on the surface of the skin, and pH was determined with a pH meter (Model D-12, Horiba Manufacturing Co., Ltd., Tokyo, Japan).

9.3.4 Parakeratotic Index of Stratum Corneum

The method of Koyama et al.[12] was used to determine the parakeratotic index of SC. A glass plate was attached to the skin with Scotch tape (Sumitomo 3M, Tokyo, Japan) measuring 25 × 19 mm to remove corneocytes. The adherent horny material was stained with hematoxylin–eosin solution for microscopic inspection of nuclei. The results were scored depending on the number of the nucleated cells in the visual field (0 = none, 1 = small, 2 = relatively large, 3 = very large).

9.3.5 Free Amino Acid in Stratum Corneum

The method proposed by Horii et al.[13] was used for the measurement of free amino acid. The SC was stripped with adhesive cellophane tape (Cello-tape, Nichiban Co. Ltd., Tokyo, Japan). The tape was immersed in toluene to remove the SC, which was then washed with toluene several times and dried in a vacuum desiccator. One milligram of dried sample of SC was precisely weighed and homogenized with 0.1% of sulfosalicylic acid. After centrifugation the supernatant was analyzed with a high-speed amino acid analyzer (Model 835, Hitachi, Tokyo, Japan) to determine the level of total

amino acid, ornithine, and citrulline. The conversion ratio of ornithine to citrulline {Cit/(Orn + Cit)} was calculated as an index of amino acid metabolism in the epidermis.

9.3.6 Ceramides in Stratum Corneum

The method of Denda et al.[14] was used to measure ceramides. After SC was stripped with adhesive cellophane tape, it was removed from the tape and washed several times with hexane, followed by drying in a vacuum desiccator. Lipids were extracted from the SC sample in a mixture of chloroform and methanol (2:1). Ceramides were separated with a silica gel column (Bond Elut Sl, Analytichem International, United States) and purified for measurement by gas chromatography (GC-14A, Shimazu Manufacturing Co., Ltd., Japan). The composition of ceramides was obtained by high-performance thin layer chromatography and scanned on a recording photodensitometer (TLC Scanner CS930, Shimazu, Japan).

9.4 RESULTS

9.4.1 Water Content in Stratum Corneum

The water content at both lesional and nonlesional sites was markedly lower in the AD patients than in the normal controls (Figure 9.1). Furthermore, the water content in the lesional skin was lower than that in nonlesional skin, but no significant difference could be recognized between them. The skin surface conductance was also lower in HD patients than in the controls (under one tenth), and the skin of HD patients was dry to the same extent as in AD patients. As the mean age of patients undergoing HD was higher than that in the controls, it is difficult to make a precise comparison without matching the age of the two groups. However, considering the finding that aging did not affect the skin surface conductance on the face,[15] and Kumasaka's[16] report that skin conductance on

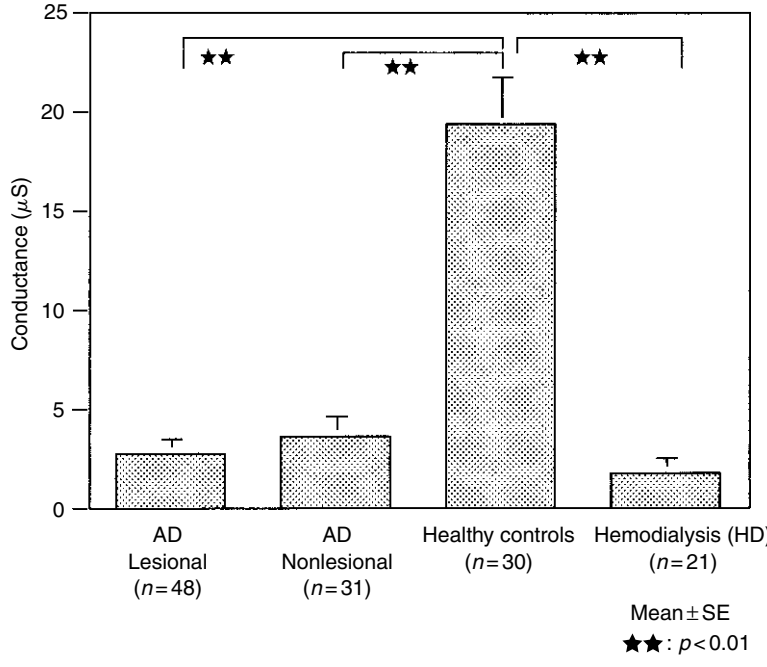

FIGURE 9.1 Water content in the SC of patients with AD and those on HD.

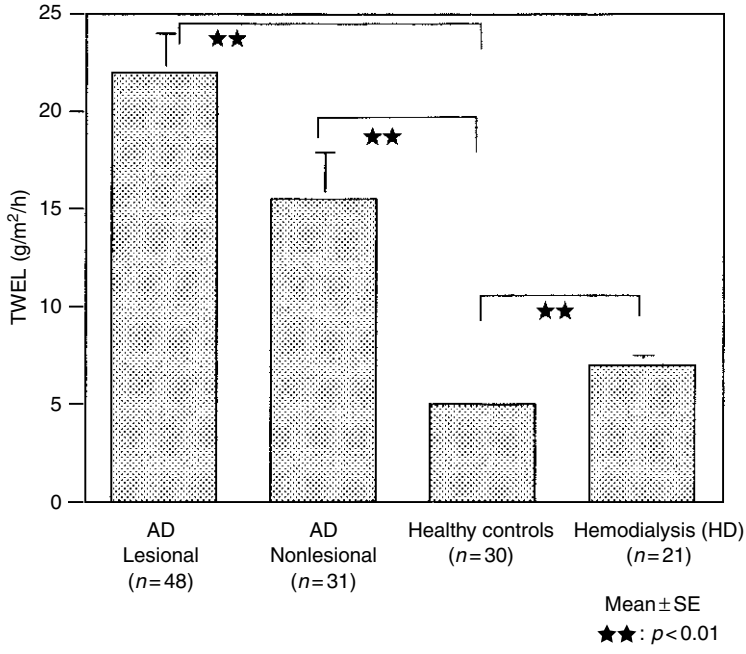

FIGURE 9.2 The TEWL in patients with AD and those on HD.

the crus in the young was about three times as large as that in the elderly, the extent of the dryness in HD patients was larger than the aging effect.

9.4.2 Transepidermal Water Loss

The TEWL in both regions was three to four times higher in AD patients than in the control (Figure 9.2), and the lesional skin gave higher values than the nonlesional skin ($p < 0.05$). On the other hand, the TEWL was only slightly higher in HD patients than in the control (mean value: HD patients, 7.0 g/m^2/h, the control, 5.0 g/m^2/h). The barrier function of SC in HD patients was similar to that in the control.

9.4.3 Skin Surface pH

The AD patients showed a higher pH at lesional and nonlesional sites than the controls ($p < 0.01$). However, the difference between the two sites was not significant. The pH was significantly higher ($p < 0.01$) in HD patients than in the controls (Figure 9.3).

9.4.4 Parakeratotic Cells in Stratum Corneum

Figure 9.4 shows the appearance of typical parakeratotic cells in SC obtained by skin surface biopsy with tape stripping. As Figure 9.5 shows, no nucleated cells were found in either the controls or HD patients, but they were observed frequently on the lesional skin of AD patients. In some AD patients (16 of 31), they were also found on the nonlesional skin.

9.4.5 Free Amino Acid Content in Stratum Corneum

As Figure 9.6 shows, the levels of free amino acids contained in 1 mg of dry SC were in decreasing order of the controls, HD patients, nonlesional skin of AD patients, and lesional skin of AD patients.

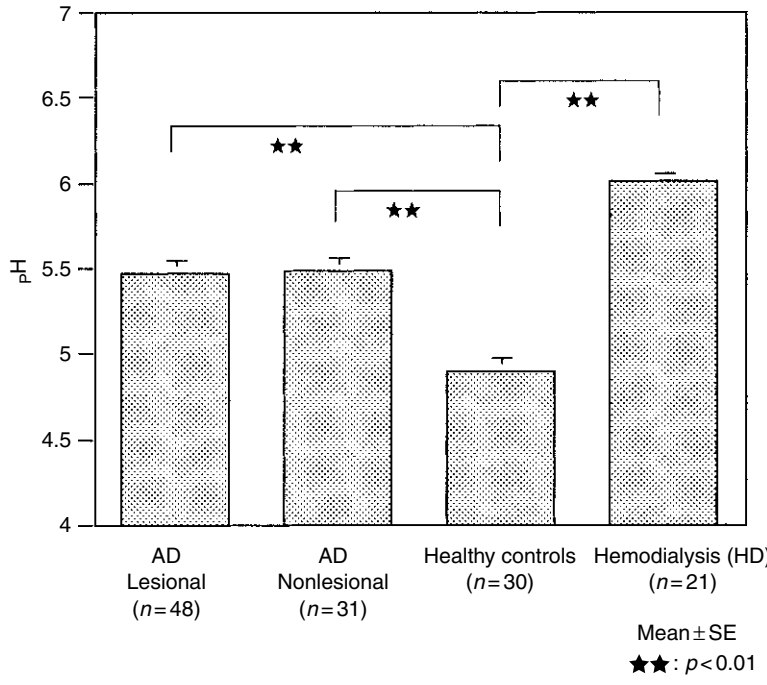

FIGURE 9.3 Skin surface pH in patients with AD and those on HD.

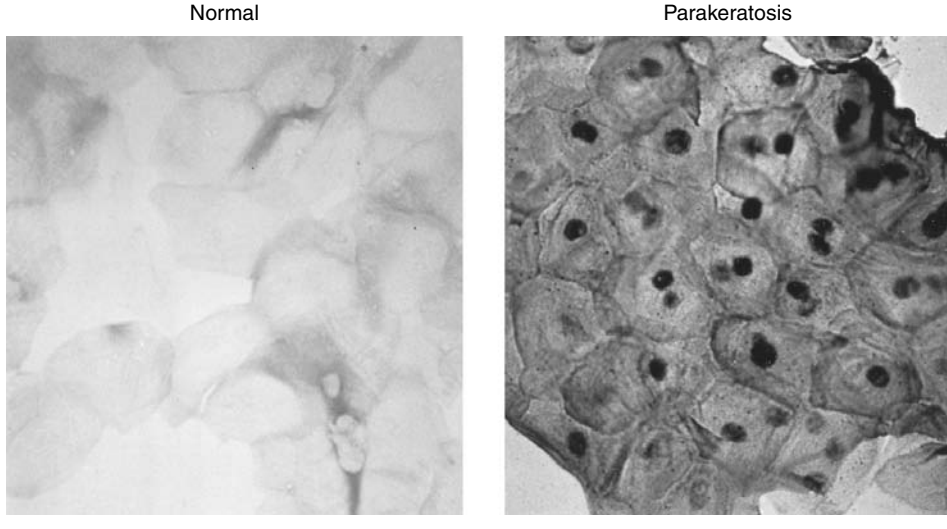

FIGURE 9.4 Parakeratotic cells detected in the SC obtained by tape stripping. Cells were stained with a hematoxylin–eosin solution.

Free amino acids act as a moisture holding factor in SC,[17] and their contents are correlated with skin surface conductance.[13] The amino acid levels in both AD and HD patients who had low skin surface conductance were less than half of the control levels. The conversion ratio of ornithine to citrulline {Cit/(Orn+Cit)} is related to the degree of cornification disorder and is negatively correlated with the epidermal proliferation rate.[12] As Table 9.2 shows, conversion ratios were similar in HD patients and the controls, but showed a significant difference ($p < 0.05$). On the other hand, the conversion

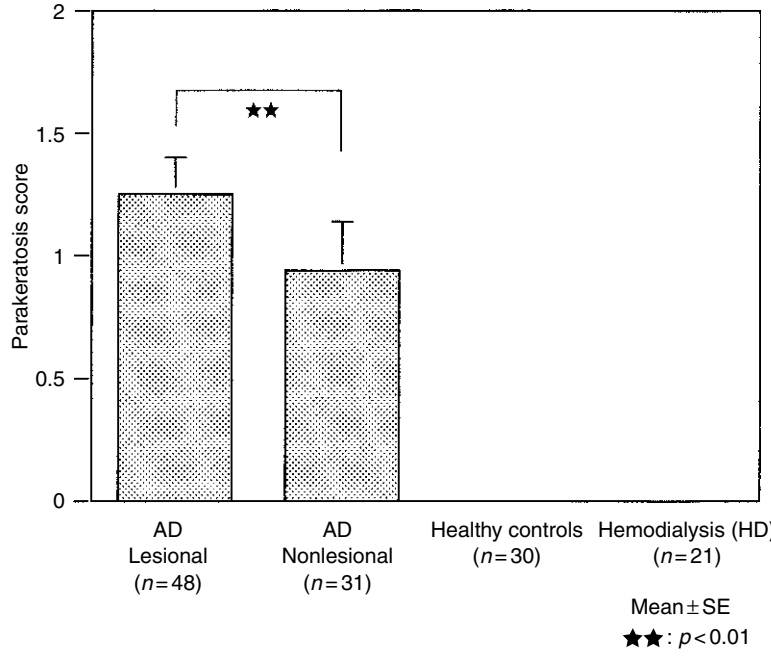

FIGURE 9.5 Parakeratotic cells in the superficial SC of patients with AD and those on HD.

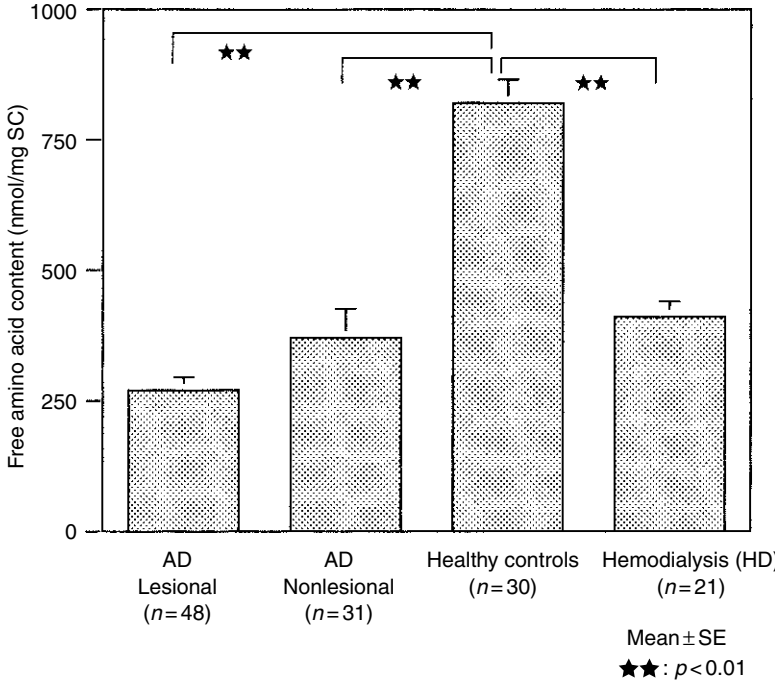

FIGURE 9.6 Free amino acids in the SC of patients with AD and those on HD.

TABLE 9.2
Conversion Ratio of Ornithine to Citrulline

	n	Cit/(Orn+Cit) %
AD lesional	48	69.8 ± 2.8[a]
AD nonlesional	31	75.0 ± 2.6[a]
Hemodialysis	21	88.3 ± 1.3[b]
Healthy controls	30	91.8 ± 1.0
		mean ± S.E.

[a] $p < 0.01$ compared with healthy controls.
[b] $p < 0.05$ compared with healthy controls.

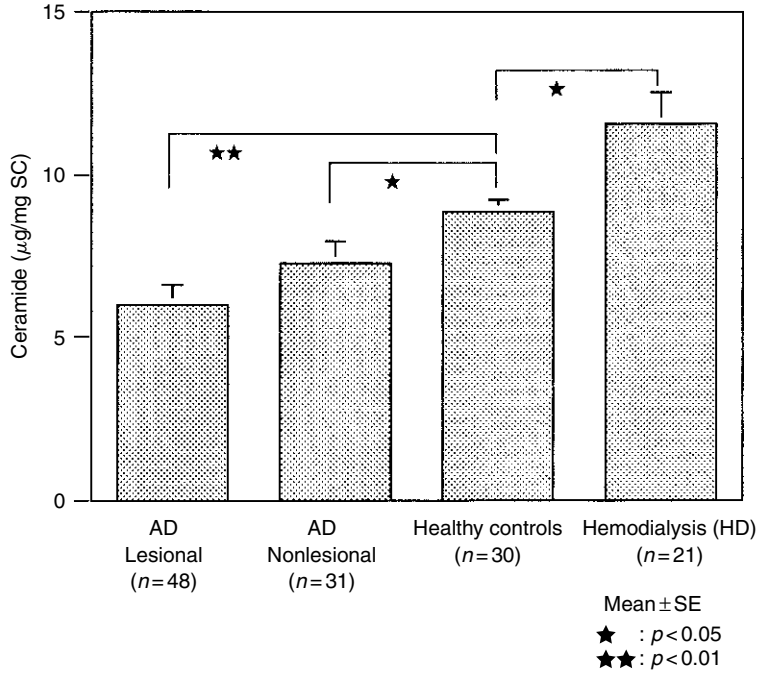

FIGURE 9.7 Ceramide in the SC of patients with AD and those on HD.

ratio of ornithine to citrulline was markedly lower in AD patients, which indicated that the epidermal proliferation was increased in AD patients.

9.4.6 Ceramide Levels in Stratum Corneum

In both lesional and nonlesional sites the ceramide levels were lower in the AD patients than in the controls (Figure 9.7). This result was in agreement with previous reports.[6,7] The lesional skin gave lower levels than the nonlesional skin on average, but no significant difference could be found. However, the levels were higher in HD patients than in the controls ($p < 0.05$). The quantity of intercellular lipids (or ceramide) was closely related to TEWL.[18,19] This study revealed that ceramide levels were high in the control and HD patients whose TEWLs were low, while AD patients with high TEWL showed low ceramide levels.

TABLE 9.3
Characteristics of the stratum corneum in various types of dry skin

Dry skin	Water content in stratum corneum	TEWL	Free amino acids in stratum corneum	Ceramides in stratum corneum	References
Atopic xerosis	Lower	Higher	Lower	Lower	(3),(35)
Senile xerosis	Lower	Normal	Lower	Slightly lower	(20)
Hemodialysis	Lower	Normal – Slightly higher	Lower	Higher	
Seasonal allergic rhinitis	Lower	Normal	Lower	N.D.	(36)
Ichthyosis valgaris	Lower	N.D.	Lower	N.D.	(13)
Induced by tape stripping	Lower	Higher	Lower	Normal	(14)
Induced by surfactant	Lower	Higher	Lower	Normal	(23)

N.D.: not determined

Ceramides are classfied into five species (ceramide 1, 2, 3, 4/5, and 6) according to their polarity. Yamamoto et al.[6] and Imokawa et al.[7] have reported that AD shows a significant decrease in the proportion of ceramide 1, which is a carrier of linoleate and responsible for water-barrier function.

In HD patients, the proportions of ceramides 2 and 3 were high and those of ceramides 1, 4/5, and 6 were low in comparison with the normal controls.

9.4.7 Characteristics of Stratum Corneum in Various Types of Dry Skin

The characteristics of hydration level and barrier function of SC in various types of dry skin were reviewed and summarized in Table 9.3. They are senile xerosis, seasonal allergic rhinitis, ichthyosis valgaris, and experimentally induced dry skin including atopic xerosis and dry skin by hemodialysis. The water content decreased in every type of dry skin and the free amino acids content also decreased corresponding to the decrease of the water content. However, the TEWL or the ceramide levels showed no clear tendency throughout every type of dry skin, especially ceramides showed higher or lower value even though the water content in SC was consistently lower in every type of dry skin.

9.5 DISCUSSION

The characteristics of the dry skin of AD patients have been widely studied. However, those of HD patients have not been studied in detail, although they also show dry skin with itchiness as in AD patients. In this study, we investigated the functions of SC to characterize the dry skin of AD and HD patients by noninvasive methods.

The water content in SC was low both in AD and HD patients, and their skin was obviously dry. However, there was a great difference between them in TEWL. The TEWL was high in AD patients accompanied with extremely inferior barrier function of SC, while HD patients showed a slightly higher TEWL than the controls and their barrier function proved to be in the mostly normal range. The findings obtained in HD patients resembled the symptoms of senile xerosis[20,21] and coincided with those reported by Kamiya et al.[11] The difference in TEWL between AD and HD patients and the

low water content in SC in both groups were explained by the difference in ceramide levels and low free amino acid levels, respectively.

The extent of parakeratosis was determined by the microscopic inspection of nucleated cells in SC. Parakeratotic cells were detected at a high frequency on the lesional skin of AD patients and were also found on the nonlesional skin in some cases, but at a much lower frequency. This indicates the presence of slight inflammation even at the nonlesional site. On the other hand, the absence of nucleated cells in SC indicated that the skin of HD patients as well as normal controls had no inflammation.

Free amino acids in SC are the metabolites of filaggrin originating from keratohyalin granules.[22] Furthermore, histidine is converted into urocanic acid, ornithine to citrulline, and glutamic acid to pyrrolidone carbonic acid.[12] The conversion ratio of ornithine to citrulline [Cit/(Orn+Cit)] and free amino acid content in SC decreased on the scaly inflammatory skin induced by surface active agents or tape stripping.[12,14] In the dry skin, caused by ichthyosis vulgaris[13] or radiation of UV rays,[24] which shows a short turnover time of SC (namely, high proliferation rate in basal cells), the reduced free amino acid levels were recognized.[14,23] The significantly low conversion ratio of amino acids and the observation of some parakeratotic cells even in nonlesional skin suggested that hyperproliferation of keratinocytes induced by slight inflammation caused the decrease in free amino acid levels and the amino acid conversion ratio in AD.

On the other hand, the decrease of free amino acid levels in HD patients could be attributed to the decrease of keratohyalin granules or filaggrin, as found on the crus of the patient with senile xerosis[20,25] or of the elderly,[26] and not to the inflammation nor to the increased epidermal proliferation, because the conversion ratio of amino acid was similar to those in the control and no parakeratotic cells could be observed.

A decrease in ceramide levels and abnormalities in the formation of lamellar bodies and in the extrude process of their components into the corneocytes spaces have been reported in AD patients.[28,29] According to Holleran et al.[30] and Menon et al.,[31] in normal skin ceramides are produced in SC by degradation of glucosylceramides by beta-glucocerebrosidase and by hydrolysis of sphingomyelin by sphingomyelinase. In the epidermis of AD patients an altered metabolic pathway of sphingomyelin was suggested by Murata et al.[32] The activity of sphingomyelin acylase is enhanced, and then large amounts of sphingosylphosphorylcholine and free fatty acids are formed, but the amount of ceramides is decreased. However, no reports on the intercellular lipids in HD patients are available. The ceramide levels were higher than those in the controls. Nevertheless, they had slightly higher TEWL. This might be due to the difference in the composition of ceramides. Though TEWL is correlated with the amount of intercellular lipids, barrier function of the SC also depends on the composition or structure of the intercellular lipids.[33,34] We reported previously[14,23] that on dry skin caused by surface active agents or tape stripping, TEWL was increased with the lack of change in ceramide levels, but the proportion of ceramide 2 was increased while that of ceramide 4/5 decreased. HD patients showed a similar change, and the rise of TEWL was supposed to be caused by the disturbance of the orientation or structure of intercellular lipids. Both AD and HD patients showed a higher skin surface pH than the controls. Ishida et al.[10] have reported that HD patients showed a higher pH on the forehead, forearm, palm, and crus. Anderson[8] also reported that the dry skin in AD or ichthyosis vulgaris showed higher pH. Factors related to skin surface pH are (1) environmental factors, including atmospheric temperature and humidity, and bathing; (2) intracorporeal factors such as menstruation; and (3) factors based on the composition of the skin itself such as SC, sweat, and sebum. However, no established explanations are available yet.

It is quite interesting that the rise in skin surface pH might be related to the drying or itching of the skin in AD or HD patients. The higher pH in HD patients than in AD patients might be partially due to the higher frequency of parhidrosis in the former.

A significant difference was found only in TEWL between the lesional and nonlesional skin of AD patients. However, all the measurements on the nonlesional skin, except for skin surface pH, gave values intermediate between those on the lesional skin and those in the controls.

Therefore, lesional and nonlesional skin might have some differences physiologically as well as clinically.

AD patients showed mild inflammation with induction of the parakeratosis, decreased moisture holding ability, and inferior barrier function, while HD patients showed only reduced moisture holding ability with almost normal skin barrier function but without inflammation. Then, it was concluded that the dry skin of AD patients resulted from the lack of moisture holding factor (free amino acids, NMF) and inferior barrier function of SC, while that in HD patients was mainly attributed to the decrease of the moisture holding factors.

Finally, it is concluded that the hydration state of the skin surface is strongly depend on the content of free amino acids of SC in dry skin, but not on ceramides in lamellar lipids.

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10 Experimentally Induced Dry Skin

Mitsuhiro Denda

CONTENTS

10.1 INTRODUCTION

Dry, scaly skin is characterized by a decrease in the water retention capacity of the stratum corneum (SC),[1] with water content diminished to less than 10%. Barrier function of the SC is usually declined, and transepidermal water loss (TEWL) is increased because of an abnormality on barrier homeostasis.[2] People feel tightness of their skin, and the skin surface becomes rough, scaly, and sensitive. Hyperkeratosis, abnormal scaling, and epidermal hyperplasia are usually observed in the dry skin.[2] Keratinization also shows abnormal features.[2] These phenomena are commonly observed in atopic dermatitis and psoriasis.[3] Dermatitis induced by environmental factors such as exposure to chemicals, low humidity, and UV radiation also shows these features. Thus, many researchers have been investigating the cause and treatment of dry skin, and there is currently great interest in adequate model systems for dry skin studies. In this chapter, I will describe several model systems of dry skin for clinical research of dermatitis associated with skin surface dryness and also mention recent studies to improve the dry skin.

10.2 EXPERIMENTALLY INDUCED DRY SKIN

10.2.1 Dry, Scaly Skin Induced by Barrier Disruption

Barrier disruption is observed in variously induced scaly skin[4] and is known to cause changes in epidermal biochemical processes, including lipid biosynthesis,[5] DNA synthesis,[6] calcium localization,[7] and cytokine production.[8] Up-regulation of specific keratin molecules and adhesion

molecules associated with the inflammatory response is also observed.[9] Because a decline of SC barrier function might be related to many types of skin abnormalities, the role of the SC barrier function has recently become the focus of intense research.

In our daily life, the SC barrier is potentially perturbated by chemicals such as surfactants, detergents, and organic solvents. As a good model of this, Gruneward et al.[10] demonstrated damage of the skin by repetitive washing with surfactant solutions. They treated skin following the repeated use of SLS and N-cocoyl protein condensate sodium as a mild wash substance for one week. In their report, they suggested that repeated washing with even a mild surfactant damaged skin.

Recent studies suggested that intrinsic factors also affect cutaneous barrier homeostasis. Psychological stress delays barrier recovery after artificial barrier disruption.[11] Also, the SC barrier becomes fragile and the recovery rate is delayed with aging.[12] Thus, a dry skin model induced by barrier disruption might be a good model for clinical research.

Previously[13] investigators usually used back or forearm skin for the experiment. It was easier to induce scaly skin on back skin than on forearm skin. In the case of back skin, we stripped SC nine times with adhesive cellophane tape. At that time, the transepidermal water loss (TEWL) value was over 10 mg/cm^2/h and most of the SC was removed. In the case of forearm, to induce dry, scaly skin, stripping for 30 to 50 times was needed. One week after treatment, TEWL was higher than the normal level, skin surface conductance decreased, and SC cell area also decreased (Table 10.1). The skin surface became scaly and flaky. Figure 10.1 shows skin surface pictures of the forearm skin with and without barrier disruption. Abnormal scaling is observed on the surface of skin, which was treated with tape stripping. These phenomena are commonly observed in natural dry skin, such as atopic dermatitis and psoriasis.

Acetone treatment is also used for barrier disruption.[14] Compared to tape stripping, this treatment breaks the SC barrier homogeneously. On the other hand, it takes a longer period of time to break the barrier than by tape stripping.

Treatment with the surfactant is another way to break the barrier, as described earlier.[10] The efficacy depends on each surfactant. Yang et al.[15] suggested that some kinds of anionic surfactant, such as sodium dodecyl sulfate (SDS), affect not only the SC barrier, but also the nucleous layer of the epidermis. Fartasch demonstrated[16] that the topical application of SDS caused cell damage to the nucleated cells of the epidermis and acetone treatment disrupted the lipid structure only in the SC. Thus, if one wants to investigate the effect of the disruption of the SC barrier function, tape stripping or acetone treatment would be better for the study.

The UV radiation also causes decline of barrier function, but this method potentially induces various kinds of responses not only in the epidermis, but also in the dermis.[17]

TABLE 10.1
Change of Skin Surface Condition One Week after Tape Stripping, Measured at 22°C and 55% RH

	TEWL (g/m^{2}h)	Conductance (Mean value/control)	SC cell area (mm^2)
Before treatment	6.3 ± 1.9	1.1 ± 0.3	1047 ± 81
After treatment	8.1 ± 2.5*	0.6 ± 0.3*	956 ± 69***

*$p < 0.05$ and ***$p < 0.001$, significance of difference between normal and scaly skin.

Note: Each value is the mean ± standard deviation from nine subjects. Each value is the mean ± standard deviation from nine subjects.

Source: From Denda M. et al. (1992) *Arch. Dermatol. Res.* 363–367 (with permission).

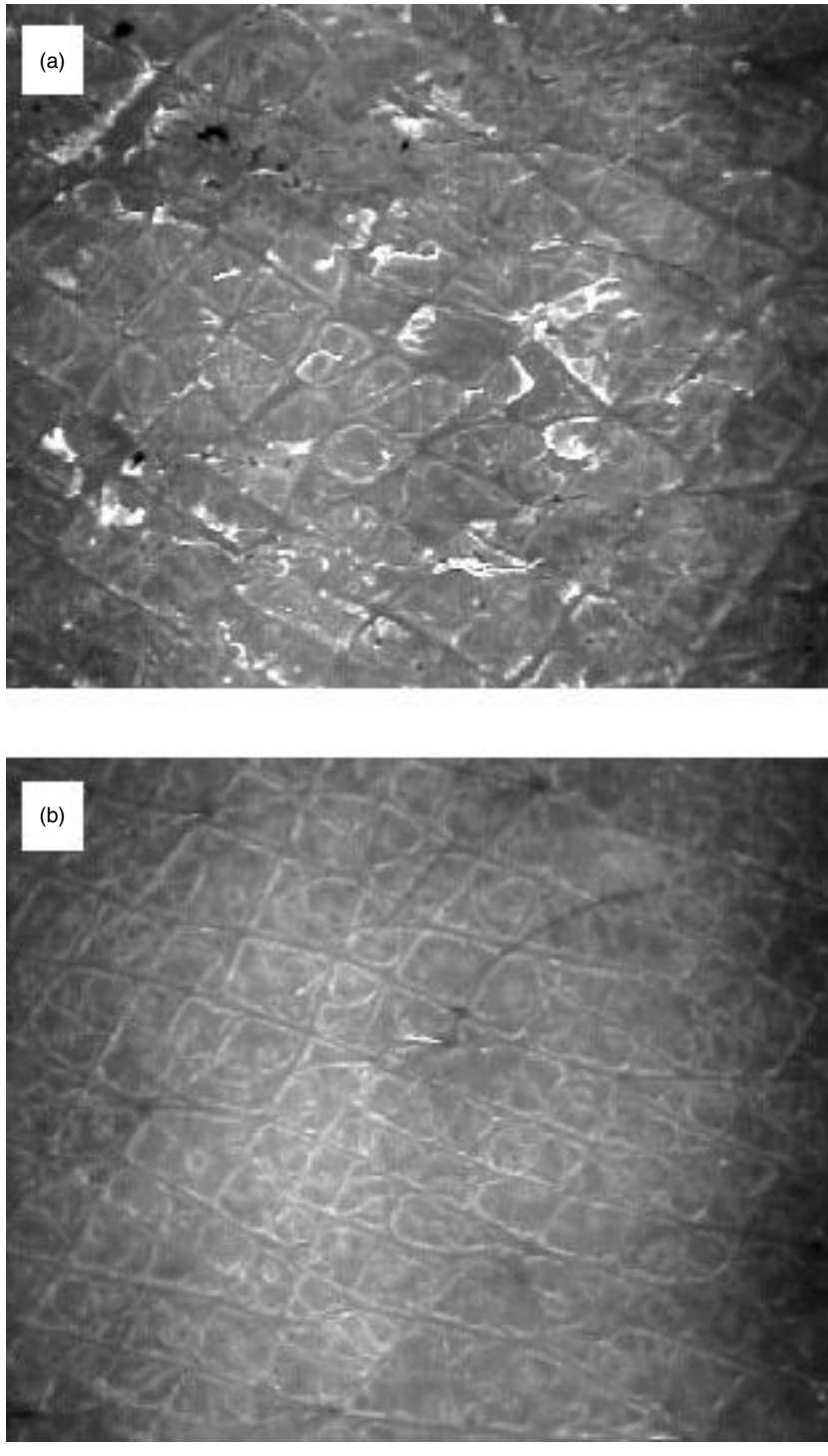

FIGURE 10.1 Dry, scaly skin induced by tape stripping (a) and untreated control (b). Forearm skin was stripped with adhesive cellophane tape 50 times, and 1 week later, skin surface was observed with a microvision system (Hi-Scope, NH-2000, Panasonic, Japan).

The degree of epidermal hyperplasia correlated with the level and duration of barrier disruption.[18] The effects of repeated barrier disruption have been examined using hairless mice. Not only epidermal hyperplasia, but also cutaneous inflammation, was observed in the case of a longer and higher level of repeated barrier disruption by tape stripping and acetone treatment. Since neither the increase in epidermal cytokine production nor the described changes in cutaneous pathology were prevented by occlusion, the hyperplasia in this model should not be attributed to increased water loss, but rather to epidermal injury resulting in the production and release of epidermal cytokines.

Although repeated barrier disruption induces inflammation, epidermal hyperplasia, and abnormal keratinization, there are several histological differences between this model and psoriasis. Gerritsen et al.[19] reported the absence of some characteristic features of psoriasis in the dry skin induced by repeated tape stripping. They also demonstrated that filaggrin expression in the model system was different from that in psoriasis. The mechanism underlying the clonical skin diseases such as psoriasis remains to be investigated.

10.2.2 Dry, Scaly Skin Induced by an Occlusive Surfactant Dressing

Surfactants have also been used to cause artificial dry skin. Many researchers have reported surfactant-induced dry skin.[20] In our daily life, surfactants, that is, detergents, are a potential cause of dermatitis. Thus, the dry skin induced by surfactants has been studied not only as a model system of dry skin, but also for clinical study of skin trouble in our daily life.

The effect of a surfactant on skin depends on the type of surfactant as described earlier. Wilhelm et al. demonstrated the irritation potential of anionic surfactants.[21] They evaluated the effects of sodium salts of n-alkyl sulfates with variable carbon chain length on TEWL and found that a C12 analog gave a maximum response. They suggested that the mechanisms responsible for the hydration of SC are related to the irritation properties of the surfactants. Leveque et al. also suggested[22] that the hyperhydration of SC is consecutive to the inflammation process. They demonstrated that the increase of TEWL was induced by SDS without removal of SC lipids. SDS might influence not only SC barrier function, but also the nucleated layer of epidermis and dermal system associated with inflammation.[23] Recently, no correlation was found between the level of epidermal hyperplasia and TEWL increase on the SDS-irritated skin.[23] Further work would be needed to determine the effects of surfactants on skin.

In our previous study[4] we used human forearm skin or back skin for the study. The forearm skin was treated with a 5% aqueous solution of SDS and an occlusive dressing was applied. After treatment, we washed off the surfactant solution with water and then continuously measured TEWL, skin surface conductance, and SC lipid morphology by ATR-IR for 14 days. The lipid morphology in the SC was altered by the treatment, but recovered to normal within two days. On the other hand, both TEWL and skin surface conductance were abnormal even two weeks after the SDS treatment. Single application of the barrier disruption by tape stripping or acetone treatment did not cause such obvious changes. Thus, the occlusive surfactant dressing affects skin not only on the SC, but also on the nucleated layer of the epidermis and dermis as described earlier. Potentially, this method damaged the skin too much. One should pay careful attention to the concentration of the surfactant solution and period of the occlusive dressing. The damage of skin is different in each person. Application of an occlusive dressing substantially increases the irritant response of the skin to repeated short-term treatments with the surfactant.

During the past decade, several reports have demonstrated the decline of sphingolipid metabolism in atopic dermatitis[24,25] and the cause of dry skin has been shown to be the abnormality of sphingolipid metabolism.[26] However, in experimentally induced dry skin, the total amount of SC ceramide did not change[13,26] but the amino acid content decreased.[13] Recently, Tanaka et al. reported[27] that amino acid content was reduced in the SC in atopic respiratory disease. They suggested that the free amino acid content is the crucial factor of the dry, scaly features of not only experimentally induced

dry skin but also atopic respiratory disease. This is a good example showing that the experimentally induced dry skin model is quite a useful method to investigate dermatitis associated with skin surface dryness.

10.2.3 Dry, Scaly Skin Induced by Dry Environment

Seasonal changes affect the condition of normal skin and may trigger various cutaneous disorders.[28,29] In common dermatitis, a decline in barrier function often parallels the increased severity of clinical symptomatology. All these conditions tend to worsen during the winter season when humidity is low.[30,31] Abundant indirect evidence indicates that decreased humidity precipitates these disorders, whereas, in contrast, increased skin hydration appears to ameliorate these conditions. The mechanisms by which alterations in relative humidity might influence cutaneous function and induce cutaneous pathology are poorly understood.

Recently, low humidity has been shown to stimulate epidermal DNA synthesis and to amplify the hyperproliferative response to barrier disruption.[30,31] SC morphology was also influenced by a dry environment,[32] and abnormal desquamation was observed under low humidity.[33] These results suggest that this model system, that is, dry skin induced by dry environment, is also an important model for clinical research of skin diseases associated with skin surface dryness.

In our study, we used hairless mice.[32,33] Before each experiment, animals were caged separately for at least four days. These cages were maintained in a room kept at a temperature of 22–26°C and at a relative humidity of 40–70%. Animals were kept separately in 7.2-l cages in which the relative humidity (RH) was maintained at either 10% with dry air or 80% with humid air. The temperature was same in all cases (22 to 26°C), and fresh air was circulated 100 times per hour. Animals were kept out of the direct stream of air. The level of NH_3 was always below 1 ppm.

Under a dry condition, epidermal DNA synthesis increased within 12 h.[31] Abnormal scaling and an increase of SC thickness were also observed within 2 to 3 days.[33] When we treated flank skin of the animals, which were kept in a dry condition for 48 h with acetone, obvious epidermal hyperplasia and mast cell degranulation were observed 48 h after the acetone treatment.[30] When the animals were kept in dry condition for more than 1 week, the barrier function was enhanced.[34] However, drastic decrease of environmental humidity induced barrier abonormality,[35] and decrease of water retention capacity of SC.[36] Ashida et al. demonstrated the increase of IL-1α in the epidermis[37] and histamine and mast cell in the dermis[38] under dry environment. Hosoi et al. demonstrated the allergic response enhanced by low environmental humidity.[39] These studies provide evidence that changes in environmental humidity contribute to the seasonal exacerbations and ameliorations of cutaneous disorders such as atopic dermatitis and psoriasis, diseases, which are characterized by a defective barrier, epidermal hyperplasia, and inflammation. Because these responses were prevented by occlusion with plastic membrane, petrolatum, and humectant[30] this dry skin model is a good system to evaluate clinical methods to solve skin problems.

10.3 NEW STRATEGIES TO IMPROVE DRY SKIN

As described earlier, dry, scaly skin looks like skin diseases can be induced by several artificial methods. These are useful model to find out new strategy to improve dry, scaly skin condition. Following are the examples of recent studies about new methods to cure dry, scaly skin.

10.3.1 Protease Inhibitor

Denda et al. previously demonstrated[40] that *trans*-4-aminomethyl cyclohexane carboxylic acid (*t*-AMCHA), an anti-fibrinolytic agent that activates plasminogen, improved the barrier homeostasis and whole skin condition. After barrier disruption, proteolytic activity in the epidermis increased within 1 to 2 h. This increase was inhibited by *t*-AMCHA. Topical application of *t*-AMCHA or

trypsin-like serine protease inhibitors accelerated the barrier recovery. Moreover, topical application of *t*-AMCHA mitigated epidermal hyperplasia induced by repeated barrier disruption. These findings suggested that manipulations that injure the stratum corneum activate the plasminogen/plasmin system and the increase of the extracellular protease activity is detrimental to barrier repair and may induce pathologic changes in the skin. Kitamura et al. also reported[41] the efficacy of this agent to dry skin. The protease balance might be important for the barrier homeostasis and skin pathology.

10.3.2 Nuclear Hormone Receptor Activator

Feingold and his coworkers demonstrated an important role of nuclear hormone receptor on epidermal differentiation and stratum corneum barrier formation. Activation of PPARα Peroxisome proliferator-activated receptor α by farnesol also stimulated the differentiation of epidermal keratinocytes.[42] Cornified envelope formation, involcrin, and transglutaminase protein, and mRNA levels were also increased by the activation of PPARα. Interestingly, the inflammatory response was also inhibited by the treatment.[43] They also showed that topical application of PPARα activators accelerated the barrier recovery after tape stripping or acetone treatment and prevented the epidermal hyperplasia induced by repeated barrier disruption.[42] Regulation of the nuclear hormone receptor would open a new possibility for improvement of the cutaneous barrier.

10.3.3 Histamine Receptor Antagonist

Histamine receptors are related to skin barrier function.[44] Three different types of histamine receptors, H1, H2, and H3 have been reported. First, topical application of histamine H1 and H2 receptor antagonists accelerated the barrier repair. Histamine itself, H2 receptor agonist, and histamine releaser delayed the barrier repair. Histamine H3 receptor antagonist and agonist did not affect the barrier recovery rate. Topical application of the H1 and H2 receptor antagonists prevented the epidermal hyperplasia induced by barrier disruption under low humidity. The mechanism of the relationship between the histamine receptors and the barrier repair process has not been elucidated yet.

10.3.4 Regulation of Receptors of Neurotransmitters in the Keratinocytes

Recently we demonstrated that a variety of receptors of neurotransmitters exist in epidermal keratinocytes and moreover, they are strongly associated with skin permeability barrier condition and epidermal hyperproliferation.[45–47]

Activation of calcium permeable receptors such as purinergic P2X receptor or NMDA receptor delayed the barrier recovery after barrier disruption and enhanced epidermal hyperplasia induced by barrier disruption.[45,47] Topical application of antagonists of these receptors prevented these pathological changes. On the other hand, topical application of agonists of chloride permeable receptors such as GABA(A) receptor or glycine receptor accelerated the barrier repair and prevented the epidermal hyperplasia.[46]

Not only ionotropic receptors but also metabotropic receptors are associated with cutaneous barrier homeostasis. β2-adrenergic receptor antagonist prevented epidermal hyperplasia induced by barrier disruption.[48] In the case of metabotropic receptors, the level of intracellular cAMP in the epidermal keratinocytes is associated with cutaneous barrier homeostasis and epidermal hyperplasia.[49]

Ectoderm-derived keratinocytes and neurons show a similar expression of those receptors. Both of them play a crucial role as the interface of information between body and environment. Both systems are regulated by nonlinear ion dynamics. A physicochemical study of the nonlinear dynamics is necessary for further understanding of both intelligent systems.

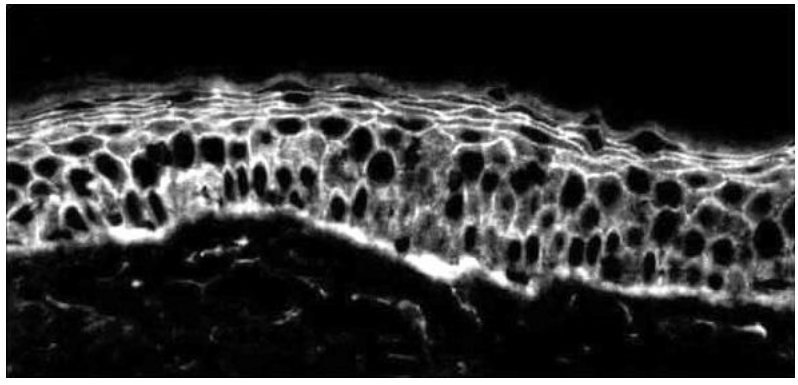

FIGURE 10.2 Pain receptor VR1(TRPV1) is localized in human epidermal keratinocytes.

10.4 THINKING EPIDERMIS: *SENSOR DEVICES IN THE EPIDERMIS*

Epidermis is an interface between body and environment. The basic mechanism of the smart system of the epidermis has not been clarified yet, but epidermal ion dynamics in the epidermis plays an important role as a signaling system.[47,49] As described earlier, a variety of receptors of neurotransmitters are found in the keratinocytes. Recently, the existence and function of vanilloid receptor subtype 1 (VR1, TRPV1) in epidermal keratinocytes has been demonstrated (Figure 10.2).[50,51]

VR1 is a cation channel and originally it was discovered as a polymodal pain receptor in the nerve system.[52] The role of VR1 in the epidermal keratinocytes has not been clarified. It might be associated with sensory perception of the skin. Temperature and osmotic pressure sensitive receptors are also found in the epidermal keratinocytes.[53,54] Epidermal keratinocytes might play a crucial role as a sensor against environmental changes.

Previous reports demonstrated that exposure to a dry environment made skin more sensitive pathologically.[34,37–39] The signaling system of the epidermis against environmental changes has not been clarified yet. The cultured keratinocyte, however, an oscillation of intercellular calcium is induced by the air exposure (see Figure 10.3).

The mechanism of the induction and role of the calcium oscillation have not been clarified. The nonlinear ion dynamics might play an important role in the sensor system of the epidermis.

10.5 CONCLUSION

As described previously, one can induce dry, scaly skin, which shows features very similar to dermatitis such as atopic dermatitis and psoriasis. Use of this experimentally induced dry skin should enable the discovery of a new clinical methodology to cure or care for skin problems. Recently, several excellent *in vitro* skin models have been reported. Although they are also very useful models for the study of cutaneous metabolism, their function and microstructure are still different from those of intact skin. On the other hand, the mechanisms underlying abnormal desquamation, that is, scaling in the dry skin such as atopic dermatitis, are not completely known. Sato et al. reported[55] the inhibition of protease in the SC induced scale without affecting epidermal mitosis. This result seems to be no direct relationship between skin surface appearance and epidermal proliferation. However, decline of SC barrier function induced epidermal hyperplasia, as described earlier.[30] The loss of water content from SC also induced epidermal DNA synthesis.[30] Further mechanistic studies on each of the dry skin features are required.

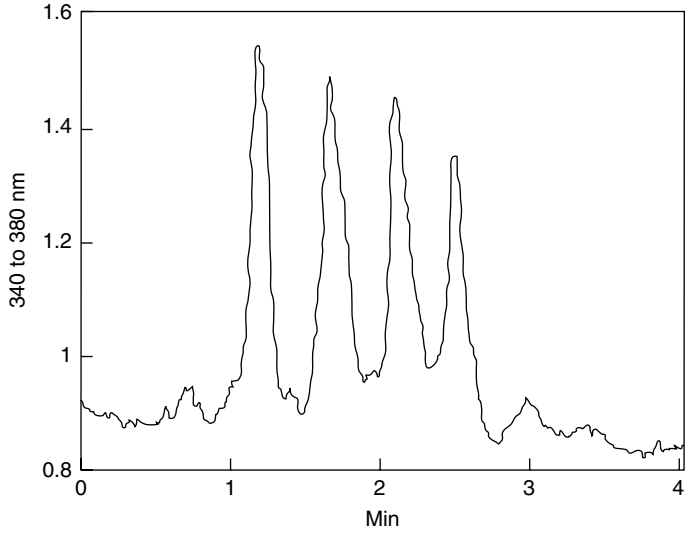

FIGURE 10.3 Oscillation of intercellular calcium in the cultured keratinocyte immediately after air exposure. Vertical axis shows the ratio of emission 340 to 380 nm.

The models described in this section should help clarify the biochemical mechanism of dry skin and lead to improvements in the clinical treatment of various skin problems associated to skin surface dryness.

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11 Dryness in Chronologically and Photo-Aged Skin

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CONTENTS

11.1 INTRODUCTION

Elderly people complain about their skin status in high proportion; this proportion may be as high as over 80% at the age of 80. They most often complain about the unsightliness of the skin, skin spots, pruritus, and stinging. Sometimes they complain about what is called "senile pruritus." In most cases "senile pruritus" may result from dry skin conditions. Dry skin is often localized on the lower legs and the outsides of the upper arms; dryness may also occur in the face. Dry skin may develop into eczéma craquelé, especially when stressed with repeated exposure to hot water and detergents. This condition may even develop into a generalized eczema and erythroderma. Erythroderma is much more common in the elderly. The cause of erythroderma is often difficult to identify. The most common cause for erythroderma in the elderly is generalized eczema. Eczema may be related to atopic constitution or to contact dermatitis. Dry skin in the elderly may also be related to psoriasis or irritated (eczemateous) psoriasis. The incidence of psoriasis increases with age. Dry skin may also be related to T-cell lymphoma, which causes localized or generalized mycosis fungoides. The initial stage, often called parapsoriasis, is especially difficult to distinguish from eczema. A special type of mycosis fungoides, Sézary syndrome, shows erythroderma and itching and is sometimes also difficult to distinguish from generalized eczema or psoriasis. Dry skin can be an important finding in renal and hepatic diseases and in hypothyreodism, diseases, which are much more frequent in aging. Very seldomly eczéma craquelé may be an indicator of an internal malignancy.[1]

11.2 SKIN AGING

In developed countries, interest in cutaneous aging is the result of a progressive, dramatic rise over the past century in the absolute number and the proportion of the population who are eldery.[2] Skin aging is due to the conjunction of intrinsic (chronological aging) and extrinsic factors (principally photo-aging, but several other environmental factors, including cigarette smoking, also contribute).

Chronological aging is a generalized process involving every organ and every cell of the body including the skin. The appearance of the skin may be an indicator of the conditions of internal organs. For example, people with senile dry skin commonly also exhibit dry eyes, which may be part of Sjögren's syndrome.[3] Werner's syndrome (WS), a representative progeroid syndrome with chromosomal instability caused by the mutation of RecQ type DNA/RNA helicase, manifests skin changes similar to those observed in systemic sclerosis (SSc). In addition, patients with WS show a variety of the signs and symptoms of normal aging in early stages of life: gray hair, alopecia, muscle atrophy, osteoporosis, cataracts, hypogonadism, diabetes mellitus, hyperlipidemia, atherosclerosis, malignancy, brain atrophy, and senile dementia.[4]

The mechanisms of aging are still only partly known. The process of intrinsic skin aging resembles that seen in most internal organs and is thought to involve decreased proliferative capacity leading to cellular senescense and altered biosynthetic activity of skin derived cells. Senescent cells undergo three phenotypic changes: they irreversibly arrest growth, they acquire resistance to apoptotic death, and they acquire altered differentiated functions. The growth arrest is very likely critical for the role of replicative senescence in tumor suppression, but may be less important for the aging of organs such as the skin. On the other hand, the altered differentiation may be critical for compromising the function and integrity of organs like the skin during aging. Senescent keratinocytes and fibroblasts appear to accumulate with age in human skin. Moreover, senescent cells express genes that have long-range, pleiotropic effects — degradative enzymes, growth factors, and inflammatory cytokines. Thus, relatively few senescent cells might compromise skin function and integrity. Moreover, by altering the tissue microenvironment, senescent cells may also contribute to the rise in cancer that occurs with age.[5] Cellular manifestation of intrinsic aging includes decreased life span of cells, decreased responsiveness of cells to growth signals, which may reflect loss of cellular receptors to growth factors, and increased responsiveness to growth inhibitors. Cells from patients with WS have a striking limitation in their *in vitro* replicative life spans and undergo extensive chromosomal rearrangements.[6] Many syndromes associated with premature aging involve dry skin.

Extrinsic aging, more commonly termed photo-aging, also involves changes in cellular biosynthetic activity, but leads to gross disorganization of the dermal matrix. Environmental factors are atmospheric pollution, wounds, infections, trauma, anoxia, cigarette smoke, and hormonal status. These factors have a role in increasing the rate of accumulation of molecular modifications and have thus been termed "factors of aging." All these factors share as a common feature the capability to directly or indirectly induce one of the steps of the micro-inflammatory cycle. This triggers a process leading to the accumulation of damage in the skin resulting in skin aging. Factors involved are intercellular adhesion molecule-1 (ICAM-1), extracellular matrix, collagenases, myeloperoxidases, and reactive oxygen species.[7] Dermal fibroblasts possess a finite replicative capacity of 50 to 100 doublings, and then cease replicating in response to growth factors. Senescent dermal fibroblasts overexpress metalloproteinase activities that may explain the age-related atrophy of extracellular matrix architecture. Also structural changes in the telomeric region of the genome have been discussed in skin aging.[8]

Skin aging is subject to both endogenous and exogenous factors. Some areas, such as on the trunk, age mainly indogenously. Other areas, especially the face or hands, are also affected by exogenous factors. This has a significant impact on the condition and appearance of the skin. Clinically, chronologically aged skin has a pale appearance and shows fine wrinkling. Solar irradiation causes skin to appear prematurely aged. This photo-aged skin shows deeper wrinkle formation, laxity, and leathery appearance. Both types of aged skin share fragility, impaired wound healing capacities, and

higher vulnerability.[9] Aged skin also shows mottled pigmentation. Histology revealed an overall thinning of the skin including the epidermis, dermis, and subcutaneous fat tissue in chronologically aged skin. In sun-damaged skin there is a thinning of the epidermis, but with a marked stratum corneum hyperplasia. The dermis exhibits thinning, curling, and frequent branching of elastic fibers evolving into an almost complete replacement of disorganized fibers with accompanying amorphous masses. The photo-aging correlates with increased solar elastosis and is inherently different from chronologic aging. Warren et al.[10] found an increase in elastine synthesis and increased degradation of collagen fibers subsequently degrading to what histopathology perceives as solar elastosis. This has also been described as an accumulation of elastotic material. The staining intensity of elastin in protected skin significantly decreased, whereas in sun-exposed skin the intensity gradually increased. The accumulated elastin in facial skin was morphologically abnormal and appeared to occupy the areas of lost collagen. In facial skin the collagen fiber architecture appeared disorganized after the fouth decade. In protected skin, type I and III collagen staining was altered only after the eighth decade, while in sun-exposed skin the relative staining intensity significantly decreased with age.[11] Also, the water content of the skin is greatly influenced by ground substances, which may be responsible for wrinkling and laxity of the skin accompanying cutaneous aging. Therefore, water content in the skin is presumed to be a critical determinant in cutaneous aging. Jung et al. aimed at clarifying the change in water content and the content of glycosaminoglycans (GAG) of rat skin in relation to aging. A significant decrease of water content in aged rat skin, which may be related to the change of GAG with intrinsic aging of the skin, was found.[12]

11.3 BIOPHYSICAL MEASUREMENTS IN AGED DRY SKIN

Obviously, measurements of stratum corneum hydration in aged and aged dry skin are important. In a comprehensive study comparing the skin of the lower leg in elderly versus young subjects with either normal or dry skin, we found a slight, but not significant, increase in stratum corneum hydration in normal aged compared to normal young skin using the well-known Corneometer®. The stratum corneum should have at least 10% water; some authors claim that 20 to 30% is ideal.[13] Hydration was significantly reduced in young and aged dry skin compared to the age-matched controls. The lowest values were obtained in young dry skin (but not significantly lower than aged dry skin).[14] An additional study on the skin of the lower leg found a striking decrease in skin surface hydration in elderly xerotic skin.[15]

Transepidermal water loss (TEWL) in aged and aged dry skin has been examined in several publications. We found decreased TEWL in aged compared to young normal skin. Also, dry skin showed decreased TEWL in young as well as in aged skin. Overall, aged dry skin had the lowest TEWL. We suggest that the described increase in the thickness of the stratum corneum in aged and especially in aged dry skin, together with reduced skin temperature and blood flow, may be the reason for the low TEWL under basal conditions.[14] Wilhelm et al. examined biophysical parameters in skin aging, comparing a group with a mean age of 27 to a group with a mean age of 71 years. They did not find significant differences in sebum, capacity (as a marker of hydration), and pH. However, TEWL was significantly lower in the aged population.[16] This shows an unperturbed barrier function in aged skin under basal conditions. However, after stressing the skin by tape stripping, barrier repair was delayed in aged skin.[17] Barrier repair in aged dry skin has not yet been examined.

11.4 LIGHT AND ELECTRON MICROSCOPY STUDIES IN AGED DRY SKIN

Histologically, elderly xerotic skin showed an atrophic nucleated epidermis and a threefold increase in corneocyte size. This was accompanied by an approximately 50% increase in the number of

stratum corneum cell layers as well as a corresponding decrease in stratum corneum turnover time. Electron microscopy revealed decreased size and number of keratohyalin granules and failure of the granules to aggregate.[15]

11.5 EPIDERMAL DIFFERENTIATION IN DRY AND AGED DRY SKIN

The hallmarks of dry skin (xerosis) are scaliness and loss of elasticity. Decreased hydration and disturbed lipid content of the stratum corneum are also well-known features. The frequency of dry skin increases with age. Previously, we examined whether the known features of dry skin are related to changes in epidermal proliferation and differentiation. In addition, age-related changes in normal and in dry skin were examined. Sixty-two volunteers were divided by clinical grading and biophysical measurements into groups with young/normal, young/dry, aged/normal, and aged/dry skin. Biopsy samples taken from the lower legs (exhibiting the most severe dryness) were examined by two-dimensional gel electrophoresis and by immunohistochemistry for epidermal proliferation, epidermal keratins, and cornified envelope proteins. We found a slight increase in proliferation in both groups with dry skin compared with normal skin of the corresponding age. In aged/normal compared with young/normal skin there was a significant decrease in proliferation. However, epidermal proliferation was the same in aged/dry skin as in young/normal skin. For epidermal differentiation, an age-independent decrease of keratins K1 and K10 and an associated increase in the basal keratins K5 and K14 was detected in dry skin. There was also an age-independent premature expression of the cornified envelope protein involucrin. In contrast, loricrin expression was not influenced by dry skin conditions. Therefore, we concluded that normal epidermal differentiation is necessary for skin homeostasis. Stimulation of aged skin by skincare products should not cause hyperproliferation with disturbed epidermal differentiation. Stimulated skin should be carefully examined by appropriate immumunohistochemical and biophysical methods to detect any alterations in epidermal proliferation, differentiation, and skin barrier function.[14]

11.6 AMINO ACIDS AND FILAGGRIN IN DRY AND AGED DRY SKIN

Dry skin (xerosis) is one of the characteristics of aged skin. Both intrinsically and extrinsically aged skin is prone to dry skin conditions. Dry skin mainly results from changes of the stratum corneum, which, in turn, result from changes in epidermal differentiation. Stratum corneum proteins and lipids are crucially involved in proper water binding, as the elasticity of the stratum corneum needs a certain amount of water. Water content and thickness of the stratum corneum contribute to skin surface morphology.[18] Dry skin may be caused by a decrease in stratum corneum amino acids, which are natural moisturizing factors and which may be derived from filaggrin breakdown.[19] Jacobson et al. examined the effects of aging in xerosis on the amino acid composition of human skin. They found reduced content of free amino acids in old/normal compared to young/normal subjects. Xerosis did not appear to effect the amino acid composition of samples from young or old subjects.[20]

Filaggrin is regarded as a cornified envelope protein[21]; the cornified envelope proteins are responsible for the mechanical resistance of the stratum corneum. Filaggrin breakdown products were supposed to be an important source of the water binding proteins in the stratum corneum.[19] In aged skin, filaggrin is immunohistochemically decreased compared with to levels found in young skin. However, a recent study by Takahashi and Tezuka (2004), showed that the total amount of amino acids in the stratum corneum was larger in aged senile dry skin than in young skin. The expression of filaggrin mRNA in aged skin was, however, similar to that in young skin. This finding suggests that the immunohistochemical decrease in filaggrin in aged skin may be caused by promotion of filaggrin proteolysis in the upper layers of the stratum spinosum.[22] Previously, Tezuka examined

terminal differentiation of aged facial epidermis immunohistochemically. In old age, the epidermis tends to become dry and flaky, especially on the lower legs. However, this is less common in the face, although long-term ultraviolet light irradiation has important effects on the differentiation of facial keratinocytes. The authors found a striking decrease in filaggrin content in the skin of the lower legs but not in the face in aged skin.[23] However, Scott experimentally found alterations in the metabolism of filaggrin in the skin after chemical- and ultraviolet-induced erythema. The time between formation and breakdown of the filaggrin was much reduced in the hyperplastic epidermis resulting from the UV irradiation.[19] This altered filaggrin metabolism may be the cause for dryness in the photo-aged facial skin.[25] We recently found a broadening of the staining zone for filaggrin in atopic dermatitis lesional skin (with reduced hydration). However, the staining intensity was reduced,[24] a quantification of filaggrin content in atopic dermatis (which has not yet been performed) may reveal a decreased amount of the protein in eczema. Overall, the role of amino acids and filaggrin in dry and aged dry skin needs further examination.

11.7 UREA AND GLYCEROL IN AGED DRY SKIN

Wellner et al.[26] described reduced stratum corneum content of water-binding urea in atopic dermatitis and in dry skin. In aged skin either a slightly reduced urea content after the age of 65 in callus skin[27] or a highly significant reduction[28] in the stratum corneum have been reported. These findings may explain the effectiveness of urea-containing emulsion in the treatment of aged and aged dry skin.

Glycerol is effective in enhancing hydration of the stratum corneum in dry skin.[29] Recently it has been shown that the epidermal water/glycerol transporter aquaporin-3 in deficient mice leads to severely impaired stratum corneum hydration.[9] In these mice, stratum corneum glycerol content was reduced threefold. And it was shown that glycerol replacement corrects the defects in these mice.[30] However, it has not yet been published whether changes in the aquaporin-3 transporter occur in human dry skin or in aged dry skin.

11.8 EPIDERMAL LIPIDS IN AGED DRY SKIN

Several works have focused on the role of stratum corneum lipids in aging. Rogers et al. found significantly decreased levels of all major lipid species, in particular ceramides, with increasing age. The relative levels of ceramide-1-linoleate were reduced in aged skin, whereas ceramide-1-oleate levels were increased.[31] To elucidate the mechanisms involved in the decrease of ceramide levels in aged skin, Jin et al. examined both the activities of beta-glucocerebrosidase, which is a major enzyme in ceramide production, and of ceramidase, which is an essential enzyme in ceramide degradation in the stratum corneum of aged skin. The authors found no changes in beta-glucocerebrosidase activity in aged skin; however, there was an age-related upregulation in ceramidase activity. The increase in ceramidase activity may be a cause for the reduced ceramide content in aging.[32] Ghadially et al. examined changes in lipids in aged mice. Although the total lipid content was decreased in the stratum corneum of aged mice, the distribution of ceramides (including ceramide 1), cholesterol, and free amino acid was unchanged. Moreover, a normal composition of esterified, very long-chain fatty acids were present. Finally, stratum corneum lamellar bodies displayed normal structure and dimensions, but were focally decreased in number, with decreased secretion of lamellar body contents.[17] De Paepe et al. described that the decrease in lipid concentration during aging depends on the anatomical site. Therefore, these variables should be controlled in a reproducible and standardized way in order to study the direct relationship between skin condition and barrier lipid composition.[33]

Akimoto quantified stratum corneum lipids in xerosis and asteatotic eczema in aging. On leg skin they found an age-related decline in total lipids. In healthy leg skin, there was age-related decline in the total ceramide. In contrast, in xerosis and in asteatotic eczema suffering significantly reduced water-holding properties, no definite decrease, but rather a slight increase in ceramide quantity, with

the same composition of each individual ceramide as compared to healthy age-matched controls, was found. The authors stated that the observed decrease in the stratum corneum total lipid content may well explain the high incidence of winter dry skin in older people. However, the progression toward asteatotic eczema cannot be accompanied solely by a decrease in ceramide quantity.[34]

Lipid composition and epidermal differentiation in photo-aged human dry skin has not been examined in detail. It has been shown that acute UV-irradiation increases lipid and ceramide content.[35,36] Also, it has been described that the levels of free fatty acids (FFA) and squalene in aged skin were significantly lower. The composition of linoleic acid decreased in the older group by 40%, suggesting age-dependent loss of oxidatively vulnerable polyunsaturated fatty acid. Sunlight exposure for 1.5 h did not change levels of FFA and squalene, or FFA composition. However, squalene hydroperoxide increased by 60-fold, suggesting that hydroperoxide is produced by singlet oxygen.[37]

The UV iradiation has been shown to be involved in membrane lipid peroxidation and this induces matrix metalloproteinase-1-expression.[38] Therefore, the use of antioxidants has been suggested in treating and preventing skin aging.[39] Whether this also works in preventing dry skin in aging is unknown.

11.9 SEBACEOUS LIPIDS IN DRY AND AGED DRY SKIN

The role of sebaceous lipids in dry skin remains controversial. Although some authors denied a function of sebaceous lipids in dry skin,[40] others described that the excretion of sebum onto the skin surface in xerosis is reduced, suggesting that either the activity or the number of functioning sebaceous glands is reduced and that selective changes in the synthesis of specific sebaceous lipids occur in dry skin.[41] Akimoto et al.[34] analyzed sebum-derived lipids present in the stratum corneum and found a significant decline in free fatty acids in xerosis and asteatotic eczema as compared to age-matched healthy controls, and a similar decline in triglycerides in the above three groups when compared to younger controls. While the number of sebaceous glands remains the same during life, sebum levels tend to decrease after menopause in females, whereas no major changes appear until the eighth decade of life in men.[42] This parallels the decline in androgen levels, but this cannot be the sole factor because there is so much overlap in sebaceous gland activity between men and women. Downing et al. further stated that the increased occurrence of dry skin in the elderly has been shown to be unrelated to the sebum secretion rate. This is not unexpected, as children, with even less sebum, rarely have dry skin.[40] Therefore, sebum secretion may be one factor of many in dry skin of the elderly.

11.10 BATHING AND CLEANSING IN DRY AND AGED DRY SKIN

Skin care in aged or photo-aged dry skin to ameliorate the signs and symptoms is important. The value of bathing and cleansing in dry skin has been discussed in several publications. It is certainly important to avoid contact with high concentrations of soaps or detergents and hot water for a prolonged period in dry skin, and especially in aged dry skin. In my opinion the composition of the detergent is more important than the type of detergent or its pH. The value of bath and shower oils also remains controversial. Several commercial products claim the importance of bath oils for the treatment of dry skin, however, in a recent publication the irritant potential of bath and shower oils has been described and it has been found that there are significant differences between several products. Instead of protecting the skin some formulations may induce subclinical injuries and delays in barrier function recovery.[43] Aged dry skin is prone to irritation.[44] Nevertheless, some people with dry skin report an improvement after using bath oil. In my opinion, after using bath oil, an additional treatment with creams or ointments should be performed in dry and aged dry skin.

11.11 THERAPY OF AGED DRY SKIN

The therapy of aged dry skin is not very different from the general treatment of dry skin. In general, lipid enriched ointments (water in oil emulsion) may be used. However, very greasy ointments based on petrolatum may induce perioral dermatitis in the face. Therefore, in the face it is better to use oil in water creams. Also the treatment depends on the environmental conditions. In winter it is generally advisable to use an ointment for dry skin, whereas in summer (where dry skin is not so severe) a cream may be used. In addition, substances with water binding ability may be used. Urea is widely used for the treatment of dry skin. The concentration of urea in creams and ointments is normally between 3 and 10%.[45] In eczema stinging and burning may occur after treatment with urea-containing ointments. This is less pronounced in dry skin only. In addition, several other compounds may be used for the treatment of dry skin. Glycerol shows positive effects in measurements of hydration[46] taken with the commonly used Corneometer® (however determination of skin hydration by biophysical devices serves as a marker for hydration only and may not necessarily reflect the water content important for the stratum corneum). In addition, lactate and potassium salts have been used to improve dry skin.[47]

Also, ceramide containing creams are in use. It has been shown that lipid supplementation in aging containing a mixture of natural ceramides improves the resistance of aged skin against sodium laurylsulfate-induced dermatitis.[48] Zettersten et al. examined barrier recovery in chronologically aged mouse skin after application of different lipid mixtures. They found that a cholesterol-dominant, optimal ratio of lipids containing cream accelerated barrier recovery.[49] Also, a report on the effects of "physiological mixtures" of lipids including ceramides in atopic dermatitis has been published.[50] However, attempts by several companies to introduce ceramide containing creams or ointments were not as successfully as expected. Because the levels of unsaturated fatty acids are lower in aged and photo-aged skin,[37] treatment with linoleic or gamma-linolenic acid may be of value in aged and photo-aged dry skin.[51] For severely dry skin with a tendency toward eczema, corticosteroids of mild to moderate potency may be used with good response.

The UV radiation causes premature skin aging. This photo-aging is characterized by wrinkles, mottled pigmentation, dry and rough skin, and loss of skin tone. Use of topical vitamin A derivatives like tretinoin can improve photo-aged skin mainly by changing epidermal differentiation.[52] However, skin dryness does not improve and even worsens with retinoid therapy, as well known from its systemic and topical use in several diseases in clinical dermatology.

The role of estrogens in preventing skin aging has been discussed in detail. Estrogen administration has been claimed to ameliorate nearly every sign of aging including skin signs. The skin is a target organ for various hormones and sex steroids, which have a profound influence on the aging process. A decrease in sex steroids thus induces a reduction of those skin functions under hormonal control. Keratinocytes, Langerhans' cells, melanocytes, sebaceous glands, collagen content, and the synthesis of hyaluronic acid, for example, are under hormonal influence. A study on the effect of non-contraceptive estrogen use on skin wrinkling, dryness, and atrophy involving 3875 postmenopausal women in the United States with a mean age of 62 years had been performed. Estrogen use was associated with a statistically significant decrease in the likelihood of senile dry skin and wrinkling. The authors stated that the results strongly suggest that estrogen use prevents dry skin and skin wrinkling, thus extending the potential benefits of postmenopausal estrogen therapy to include protection against selected age- and menopause-associated dermatologic conditions.[53] Gynecologists have seen their future in lifestyle medicine, preventing aging in general and also preventing skin aging by prescribing estrogens. However, a rumor of increased breast cancer rates related to hormone replacement therapy has been confirmed in the highly rated Million Women Study.[54] Therefore, the value of estrogen replacement therapy must be discussed. To minimize cancer risk derivatives of estrogen including phyto-estrogen may be used. Also, topically applied estrogen may show fewer side effects. Topical application of estrogens has a positive effect on skin aging parameters.[55] Phytohormones have structural similarity to 17 beta-estradiol, explaining their estrogen-like effects. However, isoflavonoids

exhibit an inferior biological potency to synthetic estrogens. Although a large number of publications have documented the effects of sex hormones on the aging process, it is obvious that hormone replacement should not be administered as an independent treatment for skin aging.[56]

11.12 SUMMARY

Dry skin is very common in elderly people and steadily increases with age. Dryness may be due to chronological aging superimposed with photo-aging. The pathophysiology involves impaired epidermal differentiation and lipid composition. Aged dry skin is prone to irritation, thus cleansing in aged dry skin should be very mild. Lipid-enriched creams or ointments should be used for the treatment of aged dry skin. Urea, glycerol, and other water binding compounds may be included in topical preparations.

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12 Itch Associated with Dryness of the Skin: the Pathophysiology and Influence of Moisturizers

Brett T. Summey Jr. and Gil Yosipovitch

CONTENTS

12.1 INTRODUCTION

Xeroderma (dry skin) is characterized by a scaly, rough, cracked, and fissured surface, and is intimately associated with the somatosensory sensation of itch. Nearly every dermatological condition that manifests with xerotic skin is accompanied by itch. Atopic dermatitis is the hallmark and most prevalent of the conditions manifesting with dry skin and itch. Other clinical conditions involving dry skin and pruritus include common diseases like psoriasis, seasonal xerosis, pruritus of the elderly, asteatotic eczema, certain ichythoses, as well as systemic diseases such as uremia and human immunodeficiency virus infection. Quite interestingly, patients with ichythosis vulgaris, which is associated with significant xerosis, do not complain of itch.[1]

In addition to primary skin processes, itchy xerotic skin can also be induced secondarily. For example, environmental factors such as rapid variations in relative humidity as well as the extremely windy, cold, and dry weather associated with the winter season induce skin dryness and itch. In addition, many commonly used topical products containing soaps, detergents, alcohol, and other irritants as well as hot water alone also lead to dry, itchy skin.

Despite the well-known clinical coupling of dry skin and itch, studies to objectively compare the degree of skin hydration or measurements of the transepidermal water losses with severity of pruritus have provided conflicting results on this close association. This review will discuss the existing data, breakdown the pathophysiology of xerotic itch, and describe the role of moisturizers in alleviating both entities.

12.2 SPECIFIC PRIMARY CONDITIONS WITH BARRIER MALFUNCTION AND ITCH

12.2.1 Atopic Dermatitis

Atopic dermatitis is the most common itchy dermatosis, with well-documented alteration in the stratum corneum function. Numerous studies have revealed an increase in the basal transepidermal water loss (TEWL) in the stratum corneum of patients with this condition. Of note, this increase in TEWL was also described in the clinically unaffected skin of atopics.[2–4] There have been direct correlations shown between the degree of inflammation and severity of barrier impairment in atopic dermatitis. Despite these findings, to date there have been no definitive reports correlating degree of barrier function with itch variability.

12.2.2 Senile Xerosis

Pruritus of the elderly is another common dermatosis associated with itch.[5] One study has shown that elderly patients with generalized pruritus had a higher degree of skin dryness than in age matched control subjects.[6] This study also demonstrated that skin surface conductance, a marker of stratum corneum water content, was decreased in elderly patients with generalized pruritus. The study was also successful in demonstrating an acquired abnormality in keratinization in these patients. This abnormality manifested with increased intracorneal cohesion compared with the controls. Another study showed an increase in histamine release and hypersensitivity in patients with senile pruritus.[7] Paradoxically, oral anti-histamines are not very efficacious in the treatment of senile pruritus.

12.2.3 Dry Skin and Uremic Itch

Dry skin is the most common dermatologic problem among patients requiring hemodialysis.[8] Among this cohort, itch is also present in 60 to 90%.[9] The association between the degree of itch and the degree of skin dryness in hemodialysis patients has been investigated in several studies; however most could not identify any connection. Young et al. were able to demonstrate this connection, however subsequent studies have not.[10] One study found no difference in the capacitance, a measure of skin hydration, of the skin between hemodialysis patients with and without itch.[11] We have studied stratum corneum hydration in hemodialysis patients versus healthy controls and found skin hydration to be significantly lower in the dialysis group. However, we did not show that this finding correlated with itch.[12] In a separate study we also tried to link skin surface pH changes in hemodialysis patients to itch severity, which was unsuccessful.[13] Of note, however, we were able to show skin surface pH levels to be significantly higher in hemodialysis patients at all body sites. A direct correlation has been demonstrated between impairment of barrier function with elevation in skin surface pH.[14,15] Therefore, this finding provided evidence of an abnormal stratum corneum barrier function in dialysis patients. In a recent study we performed on assessing skin barrier integrity in uremics with end stage renal failure on dialysis, we found a significant impairment in barrier integrity (unpublished results). Although no correlation was found between abnormal barrier integrity and itch, several patients with severe itch had significant impairment in their barrier integrity. Future studies will assess possible correlation between this barrier abnormality and severe itch. Kato et al. studied stratum corneum water content using high-frequency conductance measurements with a surface hygrometer.[16] In addition, the study also assessed the resorption (water uptake) and desorption (water loss) before and after dialysis treatments.[17] Despite these sophisticated approaches, Kato et al. were unable to demonstrate a correlation between skin xerosis and itch.[16] Recently, a clinical study did report subjective evidence of a significant connection between intensity of xerosis and intensity of itch. They reported more hemodialysis patients with moderate to severe dry skin had itch than the patients on hemodialysis with mildly dry skin.[18]

12.2.4 Human Immunodeficiency Virus (HIV) Infection and Xerosis

Xerosis with itch is present in more than 20% of patients with HIV infection, and most commonly is localized on the lower extremities.[19] One study revealed the cutaneous neural tissue density within the papillary dermis and epidermis to be significantly decreased in HIV infected patients versus healthy controls.[20] The study also reported significant differences in neuropeptide concentrations within HIV infected individuals. For example, calcitonin gene-related peptide (CGRP) was reduced in the epidermis in both the upper arms and legs, whereas substance P was found to be reduced only in the upper arms of HIV patients with itch. Dry skin and itch can also be induced iatrogenically via commonly prescribed medications for HIV such as indinavir etc. The standard in HIV treatment involves protease inhibitors, such as indinavir, which have been shown to induce dry skin and itch in over 40% of patients.[21]

12.3 PATHOPHYSIOLOGY OF ITCH IN XERODERMA

The pathophysiology of dry skin involves a complex cycle of interactions beginning ultimately with an alteration in the structure of the stratum corneum. This initial defect can lead to subsequent abnormalities in cell proliferation, keratin expression, surface lipid deposition, pH levels, cytokine concentrations, and water metabolism.[22] Any one of the aforementioned abnormalities could serve as the nidus of itch stimulation. A recent study in mice provided new insights into the mechanism of itch induction in dry skin. Miyamoto et al. artificially disrupted the stratum corneum barrier by the use of either tape stripping, 1% sodium lauryl sulfate, 1:1 mixture of acetone:ether, or plain water followed by 1:1 acetone:ether. The study involved applying one of these techniques to the rostral back of mice, and observed if the decrease in barrier function had some correlation with itch induction. Interestingly, only the mice pre-treated with water and followed by acetone:ether were noted to have increased scratching behavior. In addition, this was also the only group with a significant increase in TEWL. This report points to the properties of water and their effects on the stratum corneum as a possible culprit in itch elicitation. Water can remove the intrinsic surface lipids that serve to keep the stratum corneum hydrated and prevent water loss. In addition, water can also cause transient swelling of the stratum corneum followed by drying out of the surface layers. A possible mechanism for itch stimulation may rest in the epidermal C nerve fibers depolarizing in response to these structural changes (i.e., swelling and shrinking) within the stratum corneum.

The role of mast cells and histamine inducing itch remains unclear in dry skin. It has been shown that histamine concentrations increase 48 hours following acetone treatment in a dry environment.[23] A subsequent study demonstrated an increased number of mast cells and histamine levels in the dermis of hairless mice in response to low environmental humidity.[46] The authors did not examine a relationship between scratching behavior with the increase in mast cells and histamine. Miyamoto et al. used the mouse model treated with water followed by 1:1 acetone:ether to see if they could demonstrate an increase in mast cell number or degranulation; however, they found no difference.[24] Furthermore, they performed the same study on mast cell deficient mice and were able to induce a similar scratching behavior, which suggests that mast cells may not play a definite role in the mechanism of itch in dry skin.

12.4 CROSS-TALK BETWEEN STRATUM CORNEUM AND NERVOUS SYSTEM — A POSSIBLE MECHANISM

Nojima et al. recently demonstrated an increase of scratching behavior and scaly skin in rats treated with water followed by 1:1 acetone:ether. In addition, this report also revealed an increase in Fos-like immunoreactivity within the superficial dorsal horn of these rats. This immunoreactivity serves as a marker of the nerves that are directly stimulated by impairment in barrier function.[25] A correlation

between the increase of immunoreactivity in the lamina I spinothalamic tract and duration of scratching was clear. These results are consistent with former reports of neural fibers in lamina I that are histamine specific in cats and humans.[26,27] These neurons have also been associated with patients suffering from chronic itch that responded to iontophoresis of histamine.[28] This study clearly shows that a cross-talk between the stratum corneum and peripheral as well as central nervous systems exists. This cross-talk is further validated by studies demonstrating that *mu*-receptor opioid antagonists, which are well-known to inhibit itch in the central nervous system, are capable of suppressing itch in the mouse model treated with water + 1:1 acetone:ether.[29,47] Bigliardi et al. used confocal microscopy to demonstrate *mu*-receptor presence within the stratum granulosum.[30] This reveals that keratinocytes may use opioid receptors as another method of cross-talk with itch neural fibers.

With the recent exploration into nerve fiber increases in areas of barrier damage, offer another mechanism of itch transmission in dry skin. Takamori et al. showed that the skin of patients with xerosis had a high density of intraepidermal fibers.[31] Furthermore, studies in rats showed not only an increase in nerve fiber density, but also a significant increase in the expression of nerve growth factor (NGF) in the epidermis. This report offers a mediator, NGF, of neural proliferation; and more specifically offers a mechanism of increased C fiber elongation and penetration in the epidermis of barrier impaired skin. It should be noted that these findings contradict results shown in HIV xerosis, where patients have a global reduction in the nerve fiber density. An explanation for these discrepancies might be that different neuromediators are involved, since NGF has not been found elevated in HIV xerosis to date.

Miyamoto et al. have also demonstrated in the dry skin and itch mouse model (water + acetone:ether treated) that the scratching response can be inhibited by the use of atropine, a nonspecific muscarinic acetylcholine receptor (mAChR) antagonist, and 4-diphenyl-acetoxy-N-methyl-piperidine (4-DAMP), an M_3 mAChR antagonist.[32] They further showed that M_1 and M_2 mAChR antagonist were not able to inhibit the scratch response. This report suggests the role of acetylcholine, and the M3 specific receptor as a potential player in dry-skin-associated pruritus. In addition, skin biopsies in human subjects with atopic dermatitis were found to have increased levels of acetylcholine compared with normal controls, which suggests that abnormal concentrations of neurotransmitters may also be involved in itch secondary to xeroderma.[33]

Serine proteases like stratum corneum chymotryptic enzyme (SCCE) have been shown to induce pathologic changes in the barrier function causing hyperkeratosis and severe itch.[34] A mouse model has been created with hyperexpression of the SCCE gene; the phenotype reveals dry skin with chronic pruritus. Elevations in epidermal SCCE have also been demonstrated in patients with psoriasis and atopic dermatitis.[35] Other mediators, which may be involved in eliciting itch in dry skin, include tryptase (another serine protease), cytokines (i.e., tumor necrosis factor-alpha), and interleukins.[36] One study reported tryptase to be elevated four times the normal range in patients with atopic dermatitis; and further studies revealed a specific tryptase receptor, proteinase-activating receptor 2 (PAR2), to be present on afferent nerves and keratinocytes with increased expression within the epidermis.[37,48] It is possible, in light of these findings, that serine protease elevations secondary to barrier perturbation are responsible for itch fiber activation in dry skin.

Several neural receptors have been isolated recently within the epidermis of humans and mice. Many of these receptors, such as vanilloid receptor 1, are associated with ion dynamics in the peripheral nervous system.[38] Since skin barrier function is also predicated on ion gradient stability,[15,39] these receptors might be inducing transmission of itch related to barrier impairment.

12.5 THE ROLE OF MOISTURIZERS IN ITCH AND DRY SKIN

Moisturizers and emollients have been used for years in patients with dry skin with some relief in pruritus. Moisturizers are one of the gold standards of treatment in atopic dermatitis, the hallmark

of itchy dry skin.[40] Moisturizers' mechanism of action involves retention of water in the stratum corneum and providing an exogenous barrier to prevent transepidermal water loss. However, the antipruritic mechanism of this treatment remains unclear. Moisturizers containing salicylic acids are known to exert anti-pruritic effects with an uncertain mechanism.[41] Moisturizers such as urea, glycerol, and lactic acid promote desquamation and corneodesmolysis. In dry skin, it is likely that abnormal retention of these cohesive proteins coupled with reduced hydration will alter the mechanical properties of the stratum corneum. This alteration may lead to the stimulation of underlying nerve fibers in the epidermis.

Importantly, not all moisturizers provide the same effect in restoration of the barrier function. Certain lipid mixtures or an inadequate concentration of physiologic lipids actually have been demonstrated to inhibit barrier restoration.[42,43] Newer ceramide-dominant emollients have been developed in efforts to restore the intrinsic physiologic lipid concentration of the skin. One type of ceramide-dominant emollient was shown to significantly improve the overall severity of atopic dermatitis and demonstrated correction of transepidermal water losses in these patients.[44] Unfortunately, studies using ceramide-dominant emollients for patients with atopic dermatitis did not use itch improvement as an endpoint. However, these types of moisturizers likely have a role in the improvement of itch associated with dry skin.

In addition to using moisturizers to improve barrier function with hopes of itch reduction subsequent to this improvement, another approach is to formulate topicals containing both moisturizers and anti-pruritics. Some common topical pruritics currently being used with success are pramoxine and polidocanol.[1,45] This approach has been used extensively internationally, but remains underutilized in the United States. Studies using ceramide-dominant emollients compounded with anti-pruritics would be of interest.

12.6 CONCLUSION

The association between primary and secondary causes of dry skin with pruritus is well known. The mechanism of itch transmission as a result of dry skin and barrier impairment is not as clear. However, a large body of evidence suggests that a cross-talk exists between the stratum corneum and nerve fibers in the epidermis. This cross-talk through an array of possible mediators is the likely process of itch transmission to the central nervous system. Future studies focused on this interaction will help shed light on the mechanisms of itch stimulation in dry skin. Currently, the treatment of itch using moisturizers is limited. Newer ceramide-dominant emollients and concomitant moisturizer and anti-pruritic formulations offer promise. Future investigations may offer specific topical targets and greatly improve the efficacy of topical therapy.

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13 Effects of Moisturizer in Psoriasis

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CONTENTS

13.1 INTRODUCTION

Psoriasis is universal in occurrence. It is a disease of the skin characterized by variable clinical features. The cutaneous lesions are usually so distinct that a clinical diagnosis is easy to make. Psoriatic lesions are classified as erythrosquamous, which indicates that both the vasculature and the epidermis are involved.[1]

Psoriasis is a chronic disease with hyperproliferation of the epidermis and inflammatory reactions of the dermis and epidermis. Psoriasis is characterized by an elevated turnover rate of keratinocytes. The duration of the cell cycle is shortened. Inflammation is characterized by the release of cytokines and an expression of CD4+ cells in psoriatic lesions of affected patients. Scaling marks the clinical feature associated with hyperkeratosis, pruritus, inflammation, and stratum corneum dryness.

The onset of psoriasis constitutes a lifelong treat. The different treatment modalities of psoriasis do not offer cure to the patient. Only disease control or suppressive therapy is possible. The available treatments are intended to minimize the development of skin lesions and the associated symptoms.[2] The aim of any treatment should be the decrease or remission of scaling, pruritus, inflammation, burning, and dryness. The classical treatment is of topical nature including dithranol, coal tar, keratolytical agents, and emollients. Photo-chemotherapy with systemic PUVA, bath PUVA and cream PUVA, photo-therapy with classical UVB-light (wavelength: 300 to 320 nm) have shown to be effective. Methotrexate, etretinate, fumaric acid, and recently biologicals (Efalizumab, Alefacept, Etanercept, Infliximab) have shown their efficacy especially in severe cases.[3–9] Topical therapies are adequate in patients with limited plaque psoriasis or less than 20% body surface area involved. The agent of choice depends on the affected anatomical site, patient preference, cost of medication, likelihood of remission, and possible side effects. In some cases a combination therapy with more than one medication may be indicated.[2] The aim of the present chapter is to analyze the actual knowledge on moisturizing agents in the topical treatment of psoriasis. Psoriasis requires a lasting, stabilizing, stage-adjusted topical treatment. A main component of this treatment in a complete therapeutical concept consists in an adjuvant basic therapy with oil baths and emollients. The most important indications of emollients and moisturizing agents are an adjuvant therapy of classical

psoriasis treatment modalities and the supportive treatment in relapse free phases. Very mild forms of psoriasis should be treated with compounds showing low side-effect rats and good cosmetic acceptance.

For topical therapy Greaves et al.[10] scored emollients *lowest*, taking into account:

- Relapse rate
- Side effects
- Cosmetic acceptance
- Efficacy

followed by keratolytical agents, coal tar, dithranol, and corticosteroids. For the adjuvant therapy of mild cases of psoriasis a low risk rate (side effects, cosmetic problems) and no necessity for a strong and rapid efficacy is required. These requirements are met by emollients, moisturizing and keratolytical agents reducing scaling and subjective discomfort and inducing a better hydration of the stratum corneum. Altered structure and function of the skin measured by increased transepidermal water loss (TEWL), dysfunction of bilamellar barrier lipids, impaired skin permeability and skin roughness can be improved, releaving clinical symptoms and decreasing relapses.[11] Therapeutic moisturizers help to maintain hydration and overall integrity of the stratum corneum.[12]

A second indication for keratolytical and some moisturizing agents (e.g., urea) is the penetration enhancement of topically applied antipsoriatic drugs (e.g., salicylic acid in the dithranol and coal tar treatment or corticosteroids). This may result in an economical benefit.[11]

13.2 EFFECTS OF MOISTURIZER AND KERATOLYTICAL AGENTS IN PSORIASIS

13.2.1 Salicylic Acid

Since the beginning of the 20th century salicylic acid is known to exert a keratoplastic effect. Salicylic acid is widely used as a keratolytic agent in the treatment of hyperkeratotic dermatoses, for example, psoriasis.[13] It is mainly used in concentrations of 0.5 to 60% in almost any vehicle. As mechanism of action for topical salicylic acid desolution intercellular corneodesmosomes resulting in corneocyte desquamation, stratum corneum hydration, corneocyte swelling, and subsequently stratum corneum softening have been proposed.[13] Salicylic acid is most beneficial in extremely thick or scaly psoriatic plaques.[2] It is the most effective of the known keratolytics. Several over-the-counter medicated shampoos and scalp solutions aimed for treatment of the scaly scalp contain salicylic acid. Furthermore compounded ointments with salicylic acid are helpful for localized psoriasis.[13] Moncorps reported in 1929 about different penetration properties of salicylic acid from different vehicles.[14] The concentration does not only depend on the concentration within the same vehicle but also on the type of ointment.[14] The resorption rate of salicylic acid on psoriatic lesions is higher with a faster and longer resorption than on the skin of healthy subjects.[15] The resorption rate also depends on the severity of the inflammation.[15] But the liberation of salicylic acid from different formulations does not correlate with the penetration rate into the skin.[16] An additional study of the same group showed a dose-depending percutaneous absorption of salicylic acid *in vivo*.[17] In contrast to the antihyperplastic properties of salicylic acid on pathological hyperproliferation of the epidermis, a promotion of the epidermopoiesis in normal guinea pig skin has been shown with a 1% salicylic acid–acetone–ethanol solution.[18] The mitotic index rose by 17%, epidermis thickness was increased by 40%, and the thickness of the deep epidermis by 19%.[18] Pullmann et al. in contrast did not find a change in the proliferation rate of psoriatic epidermal cells in humans in an autoradiographic study.[19] Roberts et al. however could show a reduction of stratum corneum cell layers after three weeks.[20] The keratolytic effect was visualized by surfometry and scanning

electron microscopy.[21] Huber and Christophers for a 50% salicylic acid solution could show that corneocytes did not change their morphology while the intercellular structure was altered.[22] This treatment resulted in a desquamation of the corneocytes. *In vivo*, with the silver nitrate staining technique, Nook could prove a keratolytic effect for the combination of a water-soluble ointment containing 5% salicylic acid and 10% urea in comparison to 5 and 10% salicylic acid alone in petrolatum.[23] The combination therapy was as effective as 10% salicylic acid, and significantly more effective than a 5% salicylic acid formulation. The keratolytic effect of salicylic acid 6% in an isopropyl solution has been shown with the cantharidin blister method.[24] Going et al. reported the successful treatment of scalp psoriasis with a salicylic acid gel with a negligible systemic absorption of salicylic acid.[25] The negative interaction of dithranol and zinc oxide in pastes could be partly inhibited by the addition of salicylic acid.[26] The addition of salicylic acid to dithranol formulations improves the clinical efficacy of dithranol due to the antioxidant properties of salicylic acid.[27]

Salicylic acid can be helpful as a monotherapy. Witman reported that it is most commonly used in combination with corticosteroids, enhancing their penetration. Concentration of salicylic acid used for this purpose is 2 to 10%.[2] Such combinations require compounding by a pharmacist and carry the risk of imprecise formulations that are potentially unstable, unsafe, or ineffective.[2]

The major risk resulting from topical treatment of psoriasis with salicylic acid is the potential chronic or acute systemic intoxication with the symptoms of burning of oral mucosa, frontal headache, CNS symptoms, pH deviation (metabolic acidosis), tinnitus, nausea, vomiting, and gastric symptoms.[28–30] These symptoms may occur in topical treatment of large body surfaces, especially in children.[31–33] Even lethal cases have been reported.[34,35] Therefore, a concentration higher than 10%, and an application on larger surfaces especially in children are not suitable. Salicylic acid should not be applied to more than 20% of the body surface area.[13] It should be noted that some topical treatments of psoriasis such as calcipotriol are inactivated by salicylic acid.[36]

13.2.2 Urea

The moisturizing effect of urea in dry and scaly skin conditions is widely studied and accepted.[37–40] Urea is known to exert a proteolytical, keratolytical, hydrating, hygroscopical, penetration enhancing, epidermis thinning, and antipruritic effect.[41] An increased water-binding capacity could be shown under a treatment with a w/o-emulsion containing 10% urea.[42] An increased hydration comparing 10% urea to 5% was not detectable in both o/w- and w/o-emulsions.[43]

In vitro and *in vivo* data showed a decreased DNA-synthesis index with a thinning of the epidermis and a reduction of the epidermal cells.[44] The mechanisms of urea on the epidermis result in an epidermal thinning (approx. −20%), reduction of the cells in the DNA-synthesis in basal layers (approx. −45%) and a prolonged generation time of postmitotic epidermal cells.[45] Recent data suggest that lipid biosynthesis may also be increased by topical application of high concentration of urea.[37] An improved drug liberation of steroids from ointments containing urea has been reported.[46] Furthermore, penetration enhancement for glucocorticosteroids by urea is well-studied.[47–53] Such a penetration enhancement leads to a steroid sparing effect and an increased clinical effectivity of steroid ointments containing urea. The maximum of the steroid penetration is within psoriatic lesions. But it remains still unclear whether the penetration enhancement exert a clinical benefit.

Dithranol in combination with urea is widely used in psoriasis to improve the clinical efficacy, to minimize the dithranol concentration, to achieve the desired effect, to shorten the contact, to get a better hydration of the stratum corneum, and to decrease the proliferation rate of the keratinocytes. Gabard and Bieli showed an increased keratolytical effect of salicylic acid by adding 10% urea.[54] Hagemann and Proksch[55] showed in 10 patients with psoriasis under a 2-week treatment with a 10% urea ointment: increased stratum corneum hydration, a small decrease in TEWL, a reduction in epidermal thickness (−29%), and a decreased epidermal proliferation (−51%). The altered expression of involucrin and cytokeratins as marker for epidermal proliferation was partially reversed.[55]

With topically applied 10% urea ointment, Sasaki et al. showed an improvement of stratum corneum water content, hygroscopicity, and TEWL, in psoriasic patients.[56]

Shemer reported a treatment of scalp seborrheic dermatitis and psoriasis with a 40% urea–1% bifonazole ointment, showing a potential benefit of the combination over bifonazole alone.[57] These authors reported that urea reduces the plaque thickness concluding that this treatment regime is safe, well-tolerated, and effective.[58] Own studies showed that salicylic acid and high-dose urea in five different compounded formulations (German Formulatory NRF) have a keratolytic effect on stratum corneum. The used formulations were an ointment with 20% salicylic acid, an oil containing 10% salicylic acid, a gel with 6% salicylic acid, and ethanol and salicylic acid 6% in an isopropyl solution as well as a paste containing 40% urea. Twenty healthy test persons were investigated. Their stratum corneum was stained with silver nitrate and a photographic developer, and a chromameter was used to determine the amount of discoloration produced by the different study products and drug free vehicles. After 24 h semiocclusive application and 24 h later a significant keratolysis in all areas treated with the salicylic acid containing formulations versus drug free vehicles was found. All formulations were effective, with a significant keratolytic activity measured after occlusion. A special notice was made with oil with 10% urea: here the keratolytical effect was first observed 24 h after occlusion. In this formulation the liberation of urea is retarded.[59]

13.2.3 Alpha-Hydroxy Acids

Alpha-hydroxy acids, for example, glycolic acid or lactic acid, are organic acids present in natural sources such as fruits, wine, and milk. They exert specific and unique benefits on the structure and function of the skin.[60] Alpha-hydroxy acids have been proposed as therapeutic options against exfoliative skin conditions such as psoriasis. They penetrate the epidermal layers, provoking an increase in stratum corneum turnover. The precise mechanism by which alpha-hydroxy acids regulate desquamation is not fully understood.[60] Alpha-hydroxy acids appear to increase cohesion of the corneocytes.[61] Kostarelos revealed synergistic effects between alpha-hydroxy acids and betamethasone lotions in the topical treatment of scalp psoriasis.[62] A combination of a 10% (w/w) glycolic acid with 0.1% (w/w) betamethasone was applied twice daily for eight weeks on the scalp. A synergistic effect between alpha-hydroxy acid and betamethasone on scalp psoriasis was apparent. Furthermore, there was no systemic or topical side-effect experienced by the patients and no irritation was observed.[62]

In an own controlled study we treated 12 psoriatic patients with a glycolic acid lotion 15% versus a 0.05% betamethasone valerate cream.[63] TEWL (Evaporimeter EP1, ServoMed, Sweden), Laser Doppler (Perimed-Periflux, Sweden), and skin color (Chroma Meter CR-200, Minolta, Japan) were taken at baseline and on day 5–10–15 on psoriatic lesions. Erythema a^* value was used for monitoring. The results of the study showed that:

1. The TEWL values decreased significantly within 15 days on both sites, particularly for the corticosteroid treated site ($P < .01$ glycolic, $P < .005$ betamethasone). However, no significant differences in TEWL between glycolic acid and betamethasone could be detected.
2. a^* values decreased significantly during the treatment. Significant differences were found between basal and final readings (glycolic $P < .01$, betamethasone $P < .009$). No significant differences were found between glycolic acid and betamethasone.
3. Laser Doppler values decreased significantly during the study (glycolic $P < .001$, betamethasone $P < .0001$); significant differences appeared at days 5, 10, and 15 between the two products with lower values in the corticosteroid treated site ($P < .05$, .01, and .05, respectively). The results showed significant improvement of TEWL, a^* value, and Laser Doppler after treatment with both products. No significant differences appeared in TEWL and erythema between glycolic acid and betamethasone; on the other hand, a significantly decreased Laser Doppler was recorded in the sites treated with betamethasone confirming

the higher effect of corticosteroid compounds in terms of vasoconstriction and reduction of inflammation. The results were confirmed clinically with a reduction of hyperkeratosis and erythema induced by both treatments. The study shows that AHAs are useful not only in the control of hyperkeratosis, but also in the modulation of keratinocyte proliferation, which occurs in the disorders of keratinization such as psoriasis. AHAs can be regarded as an adjuvant therapy in psoriasis.

13.2.4 ω-Fatty Acids and Psoriasis

It has been shown that oral or topical supplements of eicosapentaenoic acid (EPA) and ω-3 derivatives can decrease, not only skin dryness and scaling, but also the severity of inflammatory skin diseases such as psoriasis.[57,64] ω-3 derivatives can be incorporated into cell membranes. They are utilized as a substrate for phospholipase activity. This may lead to an increase of free EPA, which can be used as a substrate for cyclooxygenase and lipooxygenase activities resulting in an increased production of anti-inflammatory leukotrienes LTB5 and PG3.[65] Abnormal serum fatty acid profiles in Darier's disease, ichthyosis vulgaris, psoriasis, and Sjögren–Larsson syndrome have been reported.[66] Hartop et al.[67] monitored TEWL on different psoriatic plaques treated topically with linoleic acid in comparison to clobetasole.

In an own study we tested topical corticosteroids in combination with 5% lanolic acid.[68] An improvement of barrier function could be detected. Formulations containing ω-3 and ω-6 fatty acids may help in the restoration of barrier properties. Higher efficacy of these products may be achieved by combining different classes of stratum corneum lipids.[68] Escobar et al.[64] showed a clinical improvement of scaling and plaque thickness for topical fish oil compared to the base-treated site in a four week treatment.[64]

In a double-blind, placebo-controlled multicenter study with highly purified ω-3-polyunsaturated fatty acids for topical treatment in psoriasis no statistical or clinical differences between the ω-3-polyunsaturated fatty acid and the placebo-treated lesions were found.[69]

13.3 EMOLLIENTS IN PSORIASIS

Emollients are agents designed to make the stratum corneum softer and more pliant by increasing its hydration. They are the most frequently used products in dermatology.[70] They induce a relative occlusive film that limits evaporation of water from the skin and allows the stratum corneum to rehydrate itself. Three mechanisms of emollients on the hydration of the stratum corneum have been proposed. They can exert a direct hydrating effect by liberating water from the formulation itself. Another mechanism is that the occlusive effect of the formulation can influence stratum corneum hydration and finally they are able to bind water evaporating from deeper part of the skin.[71] Regular use of an emollient or moisturizer is important. Several products are available today, for example, moisturizing creams and ointments as well as bath oils. Creams or ointments are preferable to lotions in psoriatic skin. They tend to be thicker, more occlusive, and therefore more effective.[2] Emollients do not work as a monotherapy and should be used in combination with other therapies. It has been reported, that in chronic plaque psoriasis water-in-oil emollients could be used as a steroid-sparing agent,[72] their capacity of hydrating the stratum corneum leads to enhanced delivery of corticosteroids. The replacement of one of the twice daily application of bethametasone dipropionate treatment by a water-in-oil emollient showed the same efficacy than twice per day application of the same glucocorticoids.[72] In an early study it has been shown, that white soft paraffin may inhibit the development of Koebner response in psoriasis.[73] Finlay reported an effective cream therapy adjunct to dithranol for the treatment of chronic plaque psoriasis.[74] Nola reported that the electrical properties of the stratum corneum change after application of an emollient and that there is also an anti-inflammatory activity of these substances.[70] Witman proposed that patients with psoriasis

should be encouraged to take a daily bath in warm water followed by generalized application of moisturizer containing cream or ointment.[2] A second or third application of a moisturizer during the day might be also beneficial.[2] Tanghetti made an observation study that showed that[75] the use of an emollient or a corticosteroid enhanced the efficacy of tazarotene treatment.

Emollients can cause a few side effects, such as irritant dermatitis, allergic contact dermatitis, fragrance allergy or allergy to other constituents, stinging, acne cosmetica, and pigmentary disorders.[70] The patient acceptance of emollients is generally excellent. An additional advantage of these therapies is the fact that they are inexpensive.

13.4 SUMMARY

In summary psoriasis is a chronic disease and requires lifelong treatment. Treatment options include topical agents, photo-chemotherapy, methotrexate, etretinate, fumaric acid, and biologicals. Topical agents beside dithranol are keratolytical agents and emollients. The most important indications of emollients and moisturizing agents are an adjuvant therapy of classical psoriasis treatment modalities next to supportive treatment in relapse free phases. A second indication for keratolytical and some moisturizing agents is the penetration enhancement of topically applied antipsoriatic drugs. Salicylic acid is widely used as a keratolytic agent in the treatment of psoriasis, mainly in concentrations of 0.5 to 60% in almost any vehicle. It is the most effective keratoltic agent. Different investigations showed the positive effects of salicylic acid and reported of benefits in the treatment of psoriasis. Another effect of salicylic acid is the enhanced penetration of corticosteroids in combinations of these treatments. The major problem in topical treatment with salicylic acid is the risk of an acute or systemic chronic intoxication, so some special features have to be attended when salicylic acid is chosen for topical therapy. Urea has a moisturizing effect that is used in the treatment of psoriasis. It exerts different positive effects on psoriatic skin. Also an improved drug liberation of steroids is known. Urea in combination with salicylic acid or dithranol improves the clinical efficacy and the effects of these therapies. Alpha-hydroxy acids are organic acids that exert specific and unique benefits on structure and function of the skin. Synergistic effects of alpha-hydroxy acids and betamethasone have been reported. AHAs can be regarded as an adjuvant therapy in psoriasis. Emollients are the most frequently used products in dermatology and were designed to make the stratum corneum softer. The regular use of an emollient is important in psoriatic therapy although they do not work as a monotherapy and should be used in combination with other therapies. A water-in-oil emollient can show a steroid-sparing effect and the efficacy of tazarotene was enhanced by use of an emollient. Few authors reported the positive effects of emollients in psoriasis. These agents can cause a few side effects, but the patient acceptance is generally excellent.

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