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

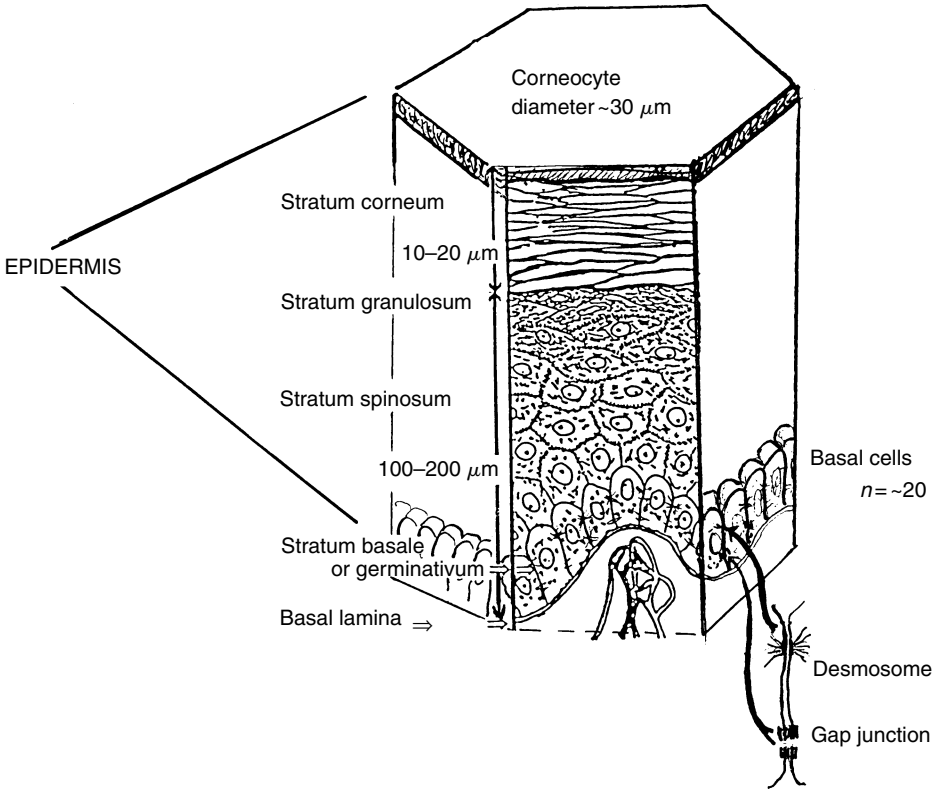

FIGURE 2.1 The proliferative unit as deduced from Potten.[5,6]

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

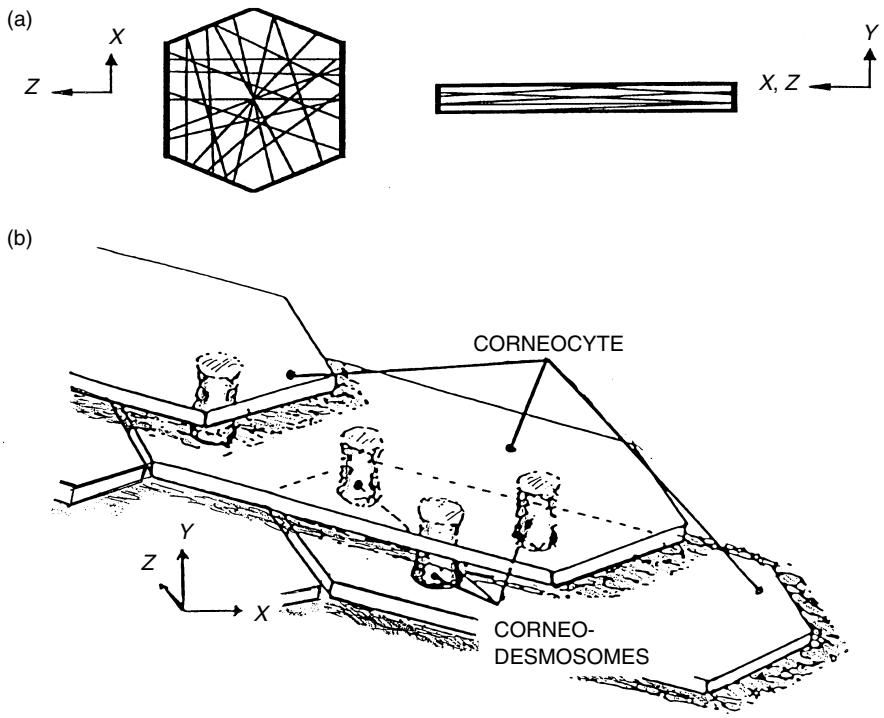

FIGURE 2.2 (a) The corneocyte is a flat, hexagonal-like structure with a surface area of about 1000 μm^2 and a thickness of 0.3 μm. A protein envelope encloses a cell compartment containing only fibrous and amorphous keratin. The keratin fibrils inside the cell are randomly oriented in the plane of the 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.

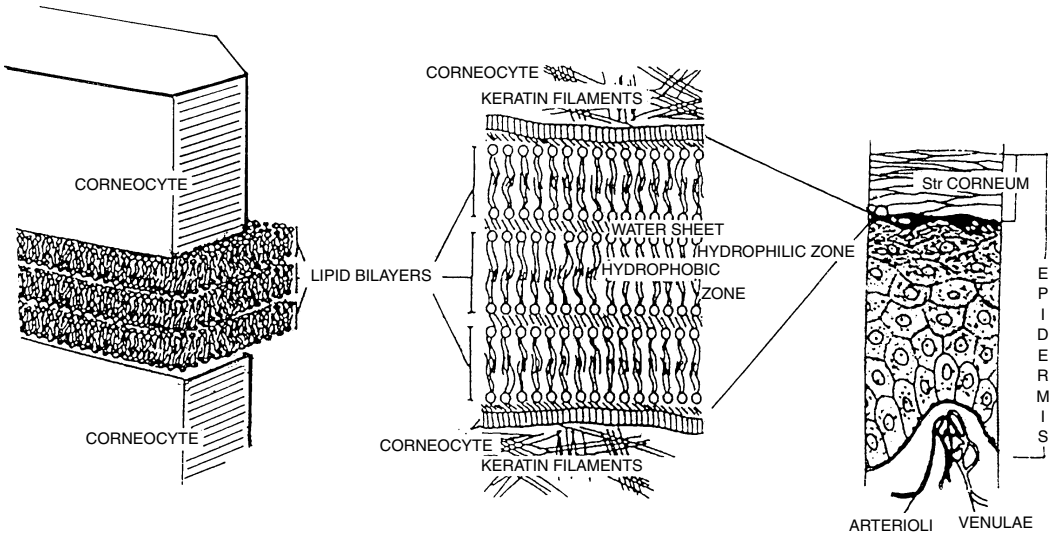

FIGURE 2.3 Stacked bilayers of lipids are inserted into the extracellular space of the corneocyte scaffold to form the lipid barrier of the skin.

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.

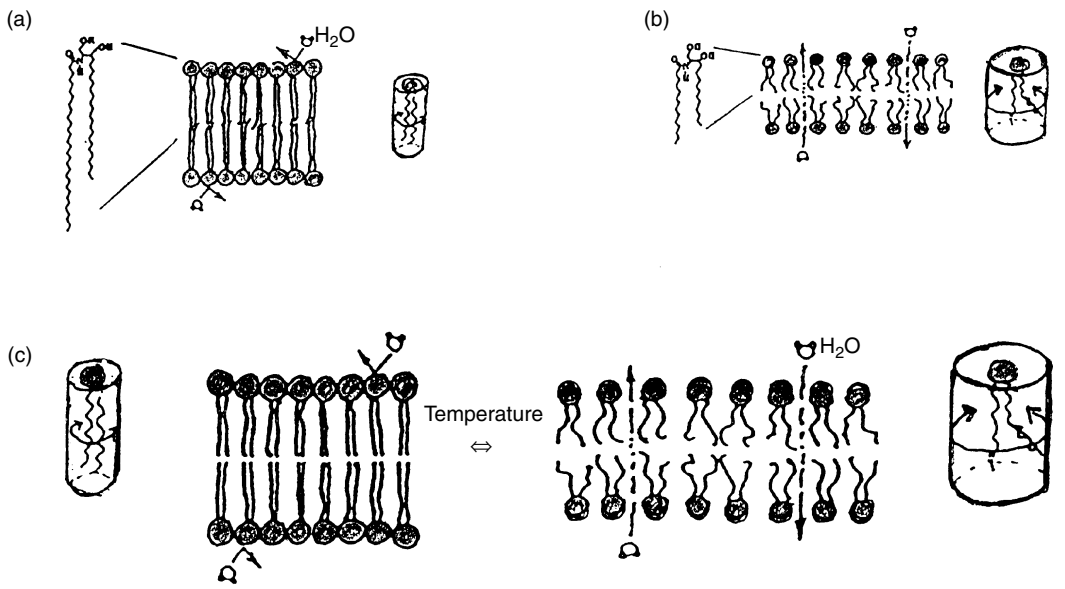

FIGURE 2.4 (a) Long, saturated carbon chains can attract each other through van der Waal's forces, and this causes a tight, close-packed crystalline structure that is impermeable to water. Straight (saturated) carbon chains demand less space than kinked (unsaturated) chains. Saturated long aliphatic 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.

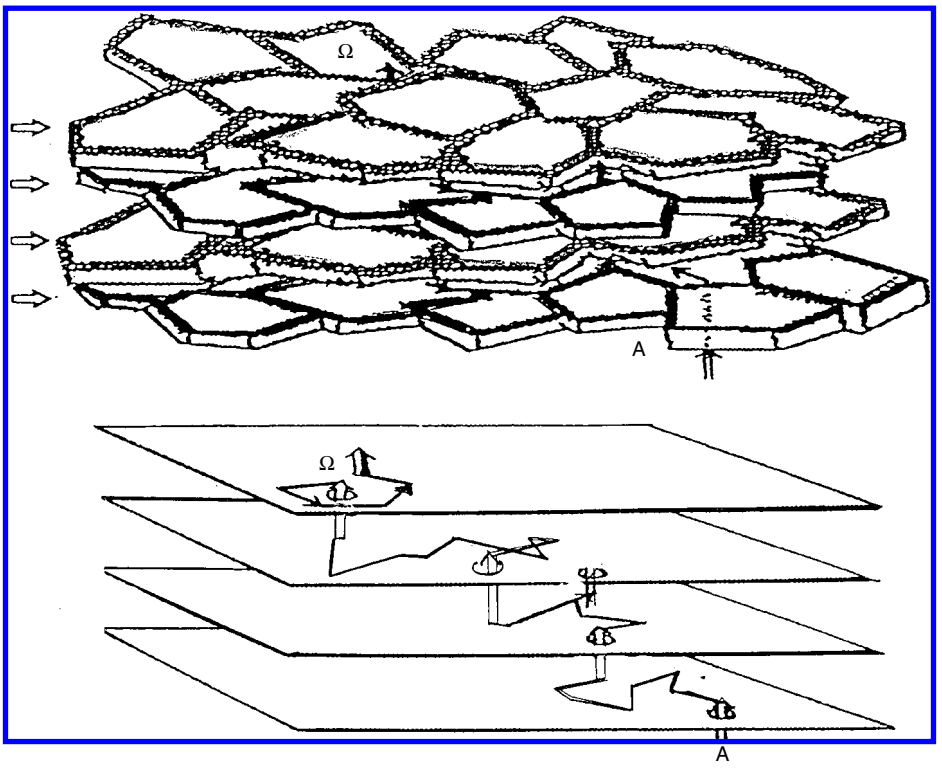

FIGURE 2.5 The stacked bilayers of the skin barrier are envisioned as composed of crystalline domains separated by fringes of lipids in the liquid crystalline state.[38] The fringe zones may actually oscillate in a very small time scale between a liquid crystalline state and a crystalline (gel) state. 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

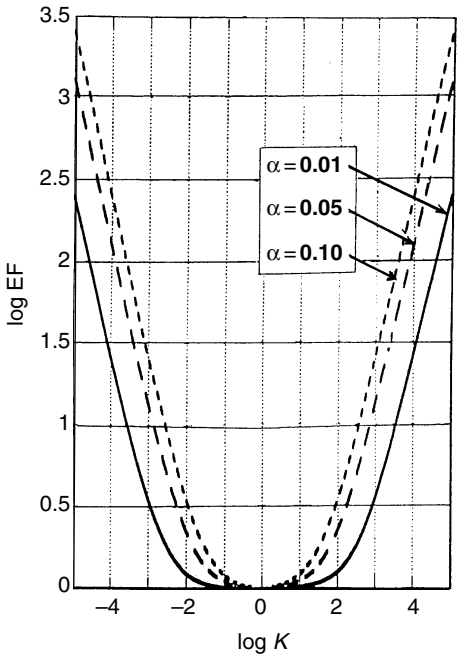

FIGURE 2.6 The enhancement factor EF (log EF) plotted versus the partition coefficient (log K) demonstrates that small changes in the fraction a of the liquid crystalline phase of the barrier that undergoes a structural transformation from lamellar to cubic, hexagonal, etc. phase causes vast changes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