The Stratum Corneum Barrier: The Final Frontier

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ABSTRACT The stratum corneum (SC) is the differentiated end product of the mammalian epidermis. It is vital to constancy of the milieu interieur (the environment within) because it prevents water loss and the penetration by potentially toxic xenobiotics, damaging radiation, and pathogenic microbes. The intercorneocyte space contains complex nonpolar lipids that constitute the water barrier. The formation of the SC in the process of keratinization is complex providing multiple opportunities for disorders to arise. The final act of keratinization is desquamation and for this to occur the controlled release of single corneocytes is required in which proteases play an important role. Tests and techniques are described that measure the structure and function of the SC. J. Nutr. 134: 2017S–2021S, 2004.

KEY WORDS: • skin • stratum corneum • epidermis • keratinization

Introduction

The mammalian stratum corneum (SC) is a remarkable structure that appears lifeless and trivial to the histologist but in reality has almost unbelievable complexities, subtleties, and importance. It is the definitive boundary or frontier structure that sharply separates the body’s vulnerable organs and tissues from the variable and sometimes hazardous world outside.

Stratum corneum function

The SC is highly efficient at restricting the movement of water both in and out of the body although clearly it is the latter that is of most importance. In health ~0.5 L of water vapor is lost per day in what has come to be known as “insensible perspiration” or transepidermal water loss (TEWL). The SC becomes “leaky” allowing more water through the skin in a wide variety of disorders that will be described later. The barrier is also disturbed after comparatively minor injury such as solvent damage and adhesive tape stripping. After damage experimentally by stripping or chemical trauma the barrier reconstitutes itself in some 7–10 d but this can be accelerated by the application of emollients (1). Interestingly, the integrity of the SC barrier appears to be influenced by emotional stimuli because in one study subjects who had marital problems repaired damaged SC barrier more slowly than did matched controls (2). When the SC barrier is severely damaged all over the body as in generalized psoriasis up to 6 L of water may be lost per day—more than enough to make one seriously thirsty!

It is the intercorneocyte lipid that appears to be of major importance as far as the water barrier is concerned. This is formed by expulsion of ceramides from the lamellar layer to form broad sheets in the intercorneocyte space.

The SC also has the important task of preventing xenobiotics from penetrating the skin if contacted. Pharmacologists and those involved in the development of drugs for either topical or “transdermal” drug use are much concerned with the way that the SC prevents permeation of the skin by chemical agents with which it comes into contact because they need to try to make drugs cross into the skin from the creams and ointments in which they are formulated. The route(s) of penetration of drugs into the skin is still hotly debated. Some believe that hair follicles provide a route for drug penetration (3) but evidence appears to favor the view that the follicular route is insignificant and that interfollicular sites are of much greater importance (4). It is not certain whether all drugs take the same route through the SC itself. It is clear that molecular weight, solubility, and molecular configuration greatly influence the rate of penetration and it seems likely that different agents may adopt different pathways.

The SC provides a barrier against marauding pathogens. As we are all aware it isn’t always successful at protection but when one considers the range of potentially harmful bacteria, fungi, and viruses looking for a “chink in the armour” the SC performs well. The full range of the skin’s protective mechanisms against microbes has not as yet been characterized. The constant outward movement of corneocytes and their sloughing off at the surface in the process of desquamation must be a built-in mechanism inhibiting pathogens from gaining a foothold. In

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4 Abbreviations used: PAS, periodic acid Schiff; SC, stratum corneum; SCCE, stratum corneum chymotryptic enzyme; SEM, scanning electron microscopy; TEWL, transepidermal water loss.
addition it appears that the epidermis possesses chemical defenses against microbes including peptides known as “defensins” (5) and toll receptors (6).

Solar ultraviolet radiation is very damaging to complex biological macromolecules and protective strategies have evolved to avoid or at least minimize problems. The SC itself absorbs ultraviolet energy but it is the corneocytes complement of melanin particles that provide the most protection. The more melanin synthesized by epidermal melanocytes, and the larger the amounts of melanin donated to keratinocytes and ultimately to the corneocytes, the greater the protection against ultraviolet radiation.

Desquamation is not often thought of as a function but without the proper operation of this process of cell loss from the skin surface normal barrier function inevitably fails. The mechanisms underlying the orderly release of single corneocytes at the skin surface has been intensively investigated in recent years and some aspects will be addressed later.

Before leaving a description of the functions of the SC mention must be made of its physical abilities to extend and bend allowing movement. We rapidly appreciate the importance of this permissive function for movement when it is lost in abnormal keratinization, for example in dermatitis. The brittle SC produced in this disorder fissures during attempted movement (Fig. 1) compromising the healing process.

### Formation of the stratum corneum

The process of terminal differentiation of the epidermis or, as it is more usually known, keratinization is impressively complex. My aim in this section is to describe the process overall in general terms and then concentrate on some aspects in which there have been major developments in our understanding in recent years. Although the natural tendency is for me to concentrate on things human, we must recognize that most of the experimental work underlying our understanding of the process of keratinization was conducted in small mammals.

#### Overview of keratinization

The histologist’s view of the SC is distorted by the destructive actions of the dehydrating agents, fixatives, and stains used in tissue processing as well as the embedding and sectioning processes. A cryostat section of skin treated briefly with alkaline buffer and methylene blue demonstrates that the SC is a delicate membrane of some 15-μm thick composed of thin overlapping horn cells or corneocytes each <1 μm in thickness and some 900 μm² in area (Fig. 2). The epidermis contains 70% water as do most tissues, yet the SC contains only 15% water. Alongside this change in water content the keratinocyte nuclei and virtually all the subcellular organelles suddenly disappear in the granular cell layer leaving only melanin particles that persist throughout the SC and the minute bodies that expel their complex lipids into the first intercorneocyte space (see later). Interestingly some enzymes also persist, an important issue as far as desquamation is concerned (see later sections). The granular cell layers also become histochemically positive for the activities of a number of lysosomal enzymes, including lipases (demonstrated by the nonspecific esterase reaction) and proteases. Presumably these are at least in part responsible for the dissolution for the epidermal organelles. The water loss and markedly altered cellular content is accompanied by dramatic changes in cell shape. From an oval or polyhedral shape in the Malpighian layers the keratinocyte starts to flatten off in the granular cell layers and then assumes a spindle shape and finally becomes shield-like or disciform as a corneocyte. The cellular volumes of the keratinocyte and corneocyte are roughly similar but the density of the structure appears to increase with increasing maturation.

The corneocyte itself develops a tough chemically resistant proteinaceous band at the periphery of the cell. This so-called corneous band contains heavily cross-linked proteins formed from precursors present in the granular cell layer particularly involucrin, loricrin, and cornifin. The cross-linking is promoted by the enzyme transglutaminase that is detectable histochemically in the granular cell layer and lower segments of the stratum corneum. The γ-glutamyl link that results from transglutaminase activity is extremely chemically resistant.

#### The intercorneocyte space

The lipid content of the stratum corneum is markedly different from that of the epidermis that produces it. The major alteration is in the presence of nonpolar lipids rather than the polar lipids containing mainly phospholipids that predominate in the keratinocytes of the epidermis. These nonpolar lipids are

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**FIGURE 1** Hand affected by dermatitis showing severe fissuring due to the production of abnormal inflexible stratum corneum.

**FIGURE 2** Cryostat section of normal human skin treated with Sorensen’s alkaline buffer and methylene blue to show the stratum corneum (90×).
mainly sphingosine base containing ceramides (ceramides 1–9) for whose formation the rate-limiting step is the enzyme serine palmitoyl transferase (7). These ceramides are synthesized in the epidermal granular cell layer and in particular in the lamellar bodies (or membrane-coating granules). These minute organelles expel their content into the first intercorneocyte space where they form coherent sheets or lamellae. It is these latter that constitute the bulk of the water barrier property of the SC.

Desquamation

Until recently we did not have a clear idea as to how desquamation occurs. Single corneocytes are released in an orderly and imperceptible manner at the skin surface so that the forces that bind corneocytes together are in some way reduced to allow desquamation to occur. A popular belief was that the intercellular lipids are primarily involved although it was difficult to conceive of the control of corneocyte cohesion being under the control of lipids. The realization that desmosomes survived almost intact within the SC as “corneodesmosomes” suggested that proteolysis was likely to play an important role. The search by Lundstrom and Eagrud (8,9) lead to the characterization of the stratum corneum chymotryptic enzyme (SCCE). This 27-kDa protein is thought to be packaged in the lamellar bodies of the stratum granulosum. It is synthesized as an inactive pro-enzyme. Interestingly in one study the pro-enzyme was found, by its immunoreactivity to be present in the whole SC whereas the SCCE itself was associated with the corneodesmosomes where presumably it can hydrolyze the structure to initiate desquamation (10).

Methods for studying the stratum corneum structure

All the techniques used to study other issues are available for the stratum corneum although routine histological preparations of fixed tissue are not satisfactory for detailed studies (see previous sections). The SC swells when treated with an alkaline buffer and if skin treated in this way is sectioned with a freezing microtome a more elegant and instructive view of the ordered structure is obtained (Fig. 2). Ultrastructural details of SC can be inspected using standard preparative techniques and transmission electron microscopy as outlined by Haftek (11) using the careful methods outlined in Haftek’s publication the existence of corneodesmosomes in the SC, and their importance in desquamation are evident. Scanning electron microscopy (SEM) of the SC is of particular value as it is an ideal way of examining membranes. One of the best techniques for obtaining SEM images is by making and examining highly resolved skin surface replicas made by taking a silicone rubber (dental impression material) negative and then using this to make a hard positive with an epon adhesive or some other similar material. Another efficient way of obtaining SC samples for SEM is to use the skin surface biopsy technique (12,13). In this simple but useful technique rapidly bonding cyanocrylate adhesive is employed to detach a thin layer of SC from the skin. A drop of the adhesive is placed on a glass microscope slide, which is then pressed onto the skin. After ~20 s the slide is detached from the skin with a rolling motion taking with it an intact sample of SC some 2–3-cells thick. As the adhesive is transparent the SC can be inspected by straightforward light microscopy (Fig. 3) but for purposes of SEM a small piece is detached and stuck to an SEM stub, which is then sputter coated with gold (Fig. 4).

The skin surface biopsy method of obtaining samples of SC has been used to answer many questions concerning the SC. For example, the ability to observe the microbiology of the SC in situ (14) by staining with periodic acid Schiff reagent has been extremely useful in detecting the presence of dermatophytes (Fig. 5); scabies mites may also be seen. There are two major advantages of the method: the ability to examine the SC in the horizontal dimension and the ability to view the SC undisturbed as it is in vivo. These abilities provide a unique insight into the biology of the SC.

The constituent cells of the SC, the corneocytes, are easily obtained by scraping the skin surface aided by Triton X-100 detergent solution. The suspension so obtained is spread onto a microscope slide, as is done to inspect a sample of blood, and is best viewed by phase contrast microscopy (Fig. 6).

Functional

Numerous tests of function have been developed but only three tests of function will be briefly described here. Two are concerned with barrier function of the SC and the third is concerned with the function of desquamation. Arguably the most important aspect of barrier function is the control of the movement of water through the skin. The flux of water vapor
through the skin can be determined using an evaporimeter (15). This contains two water sensors mounted vertically in a chamber one above the other. When placed on the skin in a stable ambient environment the difference in water vapor values between the two sensors is a measure of the flow of water coming from the skin. There are several commercially available evaporimeters [e.g., Tewameter® Courage & Khazaka (köln)], which are widely used in clinical practice as well as in investigative skin biology. In particular, the determination of water loss has become important in the care of the newborn premature infant and the management of patients with burns. The technique is reliable and sensitive enough for most purposes, and as with all forms of instrumentation, care must be taken to ensure that the evaporimeter is adequately calibrated and that artifacts do not confuse the situation (16).

Testing barrier function in vivo with regard to the penetration of chemical agents is less straightforward. Applications to the skin surface of an agent that causes vasodilatation (a rubefacient), making the skin go red, such as methyl nicotinate, are sometimes used, taking the time for reddening to occur as the measure of penetration. To assess the penetration of drugs after application of a cream or ointment containing them to the skin surface, we have used the skin surface biopsy sampling technique described above. Samples are taken at different depths (by taking successive samples at the same site) and at different times after application (at adjoining sites) (17,18). The drug is estimated in each sample taken by the high-performance liquid chromatography (HPLC) method or an enzyme-linked immunosorbent assay (ELISA) test so that a profile of concentrations at different times and depths is built up.

The third test of function is an assessment of desquamation. For desquamation to occur there must be a loss of the binding forces between corneocytes. If there is some impediment to this loss of binding force the corneocytes do not separate singly but as “clumps” and are seen clinically as “scales”. Hence, if this is correct, all scaling disorders should be found to have an increased intracorneal binding force. We devised a technique for measurement of intracorneal cohesion using a device (the cohesograph) containing a piston that is stuck to the skin with cyanoacrylate glue. The force required to detach the piston (with a small segment of SC) is a measure of the binding forces within the SC (19). As surmised, the binding forces are increased in all the scaling disorders investigated (e.g., Table 1) and can be reduced by the use of emollients—agents that improve the scaling.

### Scaling in skin disease

Scaling is a cardinal sign of skin disease. It occurs in inflammatory disorders such as eczema and psoriasis, in skin infections such as ringworm, and in congenital disorders of keratinization—notably the ichthyoses. It also occurs in response to environmental trauma from low relative humidity and the use of soaps and detergents. The elderly are particularly vulnerable to these stimuli, presumably because there is a drop in the moisture content of the SC with age.

The presence of scaling signifies faulty keratinization and desquamation, and because of the complex multistep nature of terminal differentiation of the epidermis, there are many opportunities for faults to develop. The most common of the scaling dermatoses include psoriasis, eczema, and ichthyosis, and to help in the appreciation of the link between our understanding of SC biology and clinical dermatology, I will briefly describe their nature.

Psoriasis is a chronic remittent disorder characterized by the development of red scaling patches. It develops in 1–2% of the population and is of unknown cause. It appears to involve susceptibility genes particularly one on chromosome 6, which is why the disorder seems to be inherited irregularly as a dominant characteristic with incomplete penetrance. Although the ultimate cause is unknown the disorder is clearly driven by T-lymphocytes. Liberation of cytokines from activated lymphocytes cause the characteristic epidermal hyperproliferation and attract the infiltrate of polymorphonuclear leukocytes that result in incomplete keratinization and parakeratotic scaling.

### TABLE 1

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<th>Mean cohesive force ± SD (g)</th>
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<tr>
<td>Normals (n = 10)</td>
<td>95.2 ± 17.3</td>
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<tr>
<td>Ichthyosis (n = 5)</td>
<td>153.1 ± 43.8&lt;sup&gt;1&lt;/sup&gt;</td>
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<sup>1</sup> Significantly different from normal (P < 0.01).
Ecema (also known as dermatitis) is not a single disorder but is an epidermal reaction pattern to a number of pathogenic influences (immunological and infective) in which the epidermis in particular is affected. The commonest type of ecema is atopic ecema (infantile ecema), which now affects some 15% of the population aged under 12 y. Fluid collects between the keratinocytes in affected epidermis (spongiosis), disturbs keratinization, and causes hyperproliferation, both of which result in scaling.

Psoriasis and ecema affect the formation of the stratum corneum because there is an acquired underlying epidermal abnormality, but in the ichthyoses there is an intrinsic abnormality in the keratinization process itself that is the fault; i.e., there is a primary disorder of keratinization. Because terminal differentiation has so many steps, all governed by separate genes, all of which may develop mutations, there are many different inherited ichthyoses. The commonest form of ichthyosis is autosomal dominant ichthyosis characterized by generalized dryness and scaling of the skin surface. It has been said to occur in ~0.2% of the population but I suspect that it is actually more common than that. There appears to be a deficiency in filaggrin in this condition but the exact molecular fault eludes us still.

All scaling disorders cause disability because of the scaling itself as well as because of other clinical components of the disorders such as redness and pruritus. Scaling evokes a primitive distaste and even revulsion on the part of the observer. Scaling also causes a degree of physical disability due to a reduction in manual dexterity if the hands are involved; it also causes limitation of movement because of decreased extensibility and the presence of painful fissures in the skin.

Before leaving scaling it is worth remarking that there is no convenient technique for measuring the severity of scaling (20). The availability of such a technique would take a lot of the guesswork out of the evaluation of patients, both in the course of routine clinical care and in the context of clinical trials. Image-analysis techniques used to evaluate skin surface contour have been used but are currently cumbersome, expensive, and impractical. The best compromise currently is the use of sticky transparent discs known as Desquames (21). When pressed onto the skin surface, loosened scale is picked up by the disc. The amount of scale on the disc can be evaluated by reference to a scale or by a spectrophotometric technique and is related to the severity of the scaling.

**LITERATURE CITED**