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Trends in stratum corneum research and the management of dry skin conditions

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Synopsis

The structure, composition, formation and function of the stratum corneum have been the subject of intense research over the last few decades. As has become apparent, stratum corneum barrier function is not only dependent on one single component but also on its total architecture. Recent developments in understanding lipid composition have led to a new ceramide nomenclature system, a new proposal for a molecular model of the interactions between ceramides, cholesterol and fatty acids, and the demonstration of the presence of crystalline orthorhombic and gel hexagonal lipid phases in the stratum corneum. Linoleate-containing ceramide one, now known as CER EOS, have been shown to be essential for the formation of the 13 nm long periodicity phase (LPP) observed by electron microscopy and X-ray diffraction studies, whereas long-chain fatty acids are important for the formation of the crystalline lipid phases essential for barrier function. The role of the corneocyte envelope, its constituent proteins and its transglutaminase-mediated maturation processes have been shown to be essential for good skin condition. Several proteases may have a role in corneodesmolysis, particularly serine and cathepsin-like enzymes. Novel filaggrin polymorphisms have been identified that may be involved in the expression of a dry skin phenotype. Disturbances in lipid packing states, reduction in ceramide levels (particularly the phytosphingosine-containing ceramides), reductions in the levels of long-chain fatty acids and loss of the LPP largely account for the perturbations in lipid structure that occur in dry skin. The reduced corneodesmolysis that occurs in this xerotic skin disorder is now well accepted and is caused by reductions in the levels and activities of stratum corneum proteases together with elevated levels of corneodesmosomal glycoproteins in the superficial layers of the stratum corneum. Additionally, increased levels of fragile corneocytes are associated with reduced transglutaminase activity and corneocyte envelope cross-linking events. However, in comparison with the advances in our understanding of the textural changes that occur in dry skin, the somatosensory changes are poorly understood and the itching associated with dry skin is still an under-researched area. The unique biosensor role of the stratum corneum essential for a competent natural moisturizing barrier may also have a role to play in the action of anti-ageing technologies by controlling the expression and secretion of epidermal cytokines and growth factors. Technologies to treat the surface textural skin problems, enhance the differentiation process, particularly lipid biosynthesis, and to control the somatosensory problems in dry skin have received much attention in the last decade. This paper will review the state of the art of stratum corneum biology and the trends in the management of dry skin.

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Résumé

Les structure, composition, formation et fonction du stratum corneum ont été, ces dernières décennies, l'objet d'intenses recherches. Il est ainsi devenu évident que la fonction barrière du stratum corneum ne dépend pas d'un composant unique mais de son

architecture entière. Des travaux récents portant sur la compréhension de la composition des lipides ont conduit à une nouvelle nomenclature de la classe des céramides, une nouvelle proposition d'un modèle moléculaire des interactions entre ces derniers, le cholestérol et les acides gras, ainsi que la démonstration de la présence de phases lipidiques cristallines orthorhombiques et de gel hexagonal au sein du stratum corneum. Un céramide à chaîne linoléique, référencé maintenant comme le CER EOS, a été montré responsable de la périodicité de phase de 13 nm (LPP) observée en Microscopie électronique et en diffraction par rayons X, tandis que les acides gras à longues chaînes sont montrés importants dans la formation des phases lipidiques cristallines, essentielles dans l'établissement de la fonction barrière.

Le rôle de l'enveloppe cornéocytaire, ses constituants protéiques et ses processus de maturation assurés par la transglutaminase, ont été montrés éléments essentiels d'un bon état cutané. Plusieurs protéases peuvent jouer un rôle dans la lyse des cornéodesmosomes, en particulier des enzymes à sérine et d'activité type cathepsine. De nouveaux polymorphismes de filaggrine ont été identifiés, pouvant être impliqués dans l'expression des phénotypes de peau sèche. Des perturbations dans les états d'agencements lipidiques, une réduction des taux de céramides (en particulier ceux à base de phytosphingosine), des chutes du taux d'acides gras à longues chaînes et une perte de la LPP, rendent grandement compte des perturbations de la structure lipidique rencontrées dans l'état de peau sèche. La lyse réduite des cornéodesmosomes observée dans l'état xérotique est aujourd'hui un fait accepté et est dû à des chutes des taux et activités des protéases du stratum corneum, et à des niveaux élevés des glycoprotéines des cornéodesmosomes des couches superficielles du stratum corneum.

De plus, des niveaux élevés de cornéocytes fragiles sont associés à une chute de l'activité de la transglutaminase et des mécanismes de réticulations de l'enveloppe cornéocytaire.

Cependant, comparées au progrès effectué dans la connaissance des modifications organizationnelles associées à la peau sèche, les modifications somatosensorielles restent peu appréhendées et la démangeaison associée à celle ci est un thème de recherche quasiment ignoré. Le rôle privilégié de bio-capteur du stratum corneum, essentiel à une barrière compétente et naturellement hydratée, peut aussi jouer un rôle dans l'action des technologies antivieillissement en contrôlant l'expression et la sécrétion des cytokines et des facteurs de croissance. Les technologies utilisées à des fins de traitement des problèmes de texture cutanée, l'amélioration du processus de différentiation, particulièrement dans la biosynthèse lipidique, et le contrôle des problèmes somato-sensoriels dans la peau sèche, ont été particulièrement étudiés durant cette dernière décennie.

Cet article vise donc à décrire l'état de l'art actuel de la biologie du stratum corneum ainsi que les tendances dans le traitement de la peau sèche.

Introduction

In their authoritative paper, on the 'Biophysical characterization of dry facial skin' in 1987, Leveque *et al.* [1] pointed out that 'while dry skin is a common disorder, which can make people feel miserable, the fact is we know very little about it'. In their subsequent studies, they demonstrated that dry skin was related to a slight increase in epidermopoiesis, leading in turn to a less stretchable stratum corneum, a physical property linked to both stratum corneum water content and thickness, but it was less well correlated with impaired barrier function. Using that holistic approach to functionally test dry skin increased our knowledge of the problem significantly. However, even at that stage, the basic structural defects that occur in dry skin had not been described and as the authors commented, 'electron microscopy has so far not illuminated the basic structural defect that underlies dry skin'. Since then, significant progress has been made.

Through individual efforts and tenacity of several scientists in the cosmetic industry and academia during the last few decades, there have been exciting advances in our understanding of not only the structure, composition and function of the stratum corneum but also its formation via a variety of transcription factors that are responsible for epidermal differentiation. This research has led to new paradigms in stratum corneum moisturization technologies. The initial research on developing a better surface barrier or improving skin surface moisturization in the outer layers of the stratum corneum leading to improved surface textural properties is now focused on identifying agents that are specifically delivered into the epidermis that assist the cellular differentiation process or act as precursors to vital stratum corneum components. As has become apparent, the stratum corneum and the epidermis, however, are unique biological sensors adapting to environmental conditions to ensure the proper

integrity of the natural moisturizing barrier. This review will discuss the recent advances in our knowledge of the biology of the stratum corneum, the defects in epidermal biology occurring in dry skin and the recent development in dry skin treatments. This will largely be an update on previous reviews except with reference to previously quoted work in the light of new research findings [2–6].

Stratum corneum lipid chemistry and biophysics

The raison d'etre for the stratum corneum barrier is to be as impermeable as possible except for a small amount of water loss to hydrate the outer layers of the stratum corneum to maintain its flexibility and to provide enough water to allow enzymes to facilitate stratum corneum maturation and desquamation. As a result, sharp phase separations between lipids should be minimized and from a functional point of view the skin barrier should be as 'homogeneous' as possible in order to maintain effective barrier function. As Norlen [7] has pointed out, this can only be achieved by heterogeneity in stratum corneum lipid composition, which broadens phase transition zones, and the lipids being in a plastic-crystalline state stabilized by cholesterol together with and heterogeneities in hydrocarbon chain length distributions in both ceramides and fatty acids [8].

The most characteristic features of stratum corneum lipid composition are its heterogeneity, dominant presence of saturated chemical species, extensive amounts of cholesterol and the complexity in ceramide chemistry. On a weight basis, ceramides constitute 47%, cholesterol 24%, fatty acids 11% and cholesterol esters 18%. Cholesterol has an important role to play in the lipid mixtures, as it can increase the chain mobility of lipids in the gel state and decrease their mobility in the liquid crystalline state, a property likely to be important for barrier function during processing of glucosylceramides to ceramides in the lower and upper regions of the stratum corneum. However, as will become evident, later the long-chain fatty acids are equally important in the formation of the correct lipid phases for barrier function.

Several investigators identified and described the complex mixture of stratum corneum lipids. However, Downing's group [9] clarified the structures of human ceramides, both those freely extractable by solvents and those covalently attached to the corneocyte envelopes. During this time, ceramides were classified by a numbering system (ceramide 1–6II) according to their polarity by chromatography. In humans, however some of the original ceramide bands separated by high-performance thin layer chromatography were later found to be composed of more than one type of ceramide chemical species. For instance, new ceramide structures based upon 6-hydroxysphingosines were identified. Given the diversity of the ceramide structures, a new nomenclature based on structure rather than chromatographic migration characteristics was proposed by Motta *et al.* [10]. In this system, ceramides were classified in general as CER FB, where F is the type of fatty acid and B indicates the type of base. When an ester-linked fatty acid is present, a prefix of E is used. Normal fatty acids (saturated or unsaturated), alpha-hydroxy fatty acids and omega-hydroxy fatty acids are N, A and O, respectively, whereas sphingosines, phytosphingosines and 6-hydroxysphingosine are indicated by S, P and H. Sphinganine not classified previously is proposed to be SP in this nomenclature system. Typical structures of human ceramides are given in Fig. 1.

Lipids covalently bound to involucrin on the corneocyte envelopes were originally identified as ceramide A (sphingosine) and ceramide B (6-hydroxysphingosine) together with omega hydroxy fatty acids [9]. Chopart *et al.* [11] at L'Oreal recently identified covalently bound omega-hydroxyl fatty acid-containing sphinganine and phytosphingosine ceramides. The relative functions of these different lipids are still largely unknown, but in general they are believed to act as a template for lamellar lipid organization of the freely extractable lipids. These covalently bound ceramides should now be named CER OS, CER OH, CER OSP and CER OP. SP is proposed as a classification for sphinganine as above.

All of these lipids are synthesized from glucosylceramides, epidermosides and sphingomeylin. Epidermosides are glycated precursors of omega hydroxyl-containing ceramides [12]. The studies of Hamanaka *et al.* in Kanebo [13] have demonstrated that the last lipid class provides some of CER NS and CER AS, whereas the glucosylceramides are precursors to ceramides and epidermosides are precursors to the covalently bound ceramides together with CER EOS and CER EOH. Still Vietzke *et al.* [14] at Beiersdorf reported that small amounts of other ceramides are present and that one of them might represent CER EOP.

Examination of stratum corneum by transmission electron microscopy following treatment with

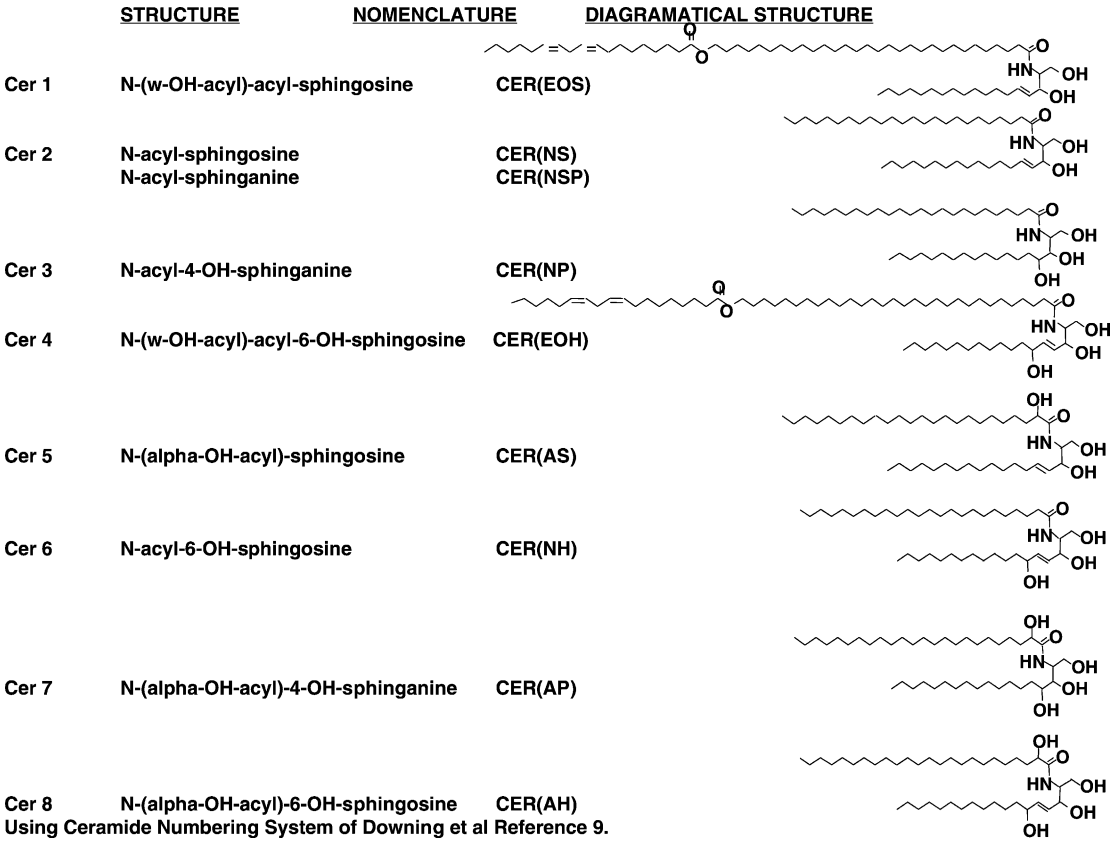

Figure 1 Ceramide nomenclature and structures.

ruthenium tetroxide has revealed that the number of lipid lamellae between corneocytes varies but in most regions there are multiples of three lamellae with a broad–narrow–broad spacing. When more lamellae are present, it is believed that CER EOS plays an essential role in the formation of the lamellar arrangements with six or more bands in multiples of three, where, in each six bands, the central pair of bilayers is linked by the omega-hydroxy acyl portion of CER EOS spanning from one bilayer and its linoleate tail inserted into the second bilayer. These repeated distances were found to be of 13 nm in dimension, composed of two units measuring approximately 5 nm each and one unit measuring approximately 3 nm in thickness. These repeat lamellar patterns were also observed by X-ray diffraction studies and were named as the long periodicity (LPP) and short periodicity (SPP) phases, respectively. Also, Garson *et al.* [15] at L'Oreal established that the lateral packing states for the bulk of human stratum corneum lipids was a crystalline orthorhombic phase. These findings led to the original molecular model of Swartzendruber *et al.* [16] where ceramides have a splayed chain conformation and the alkyl chains interdigitate between bilayers. However, Bouwstra *et al.* [8] recently proposed a new sandwich model consisting of two broad lipid layers with a crystalline structure separated by a narrow central lipid layer with fluid domains (Fig. 2). In this lipid arrangement, strong interaction within rather than between the tri-layer units would be expected. In fact, imperfections of lamellar stacking appear to be located between the tri-layers in stratum corneum samples from aged skin [8]. Cholesterol and ceramides are important for the formation of the lamellar phase whereas fatty acids play a greater role in the lateral packing of the lipids. Cholesterol is proposed to be located with the fatty acid tail of CER EOS in the fluid phase. The fluid phase could facilitate deformation of lipid lamellae during shear stress. Also, as several stratum corneum maturation events are dependent upon extracellular enzymes, it is possible that the desquamatory enzymes are located in this domain. Enzymes are unlikely to be active in crystalline phases.

Lipids *in vivo* appear to exist as a balance between a solid crystalline state (orthorhombic packing) and

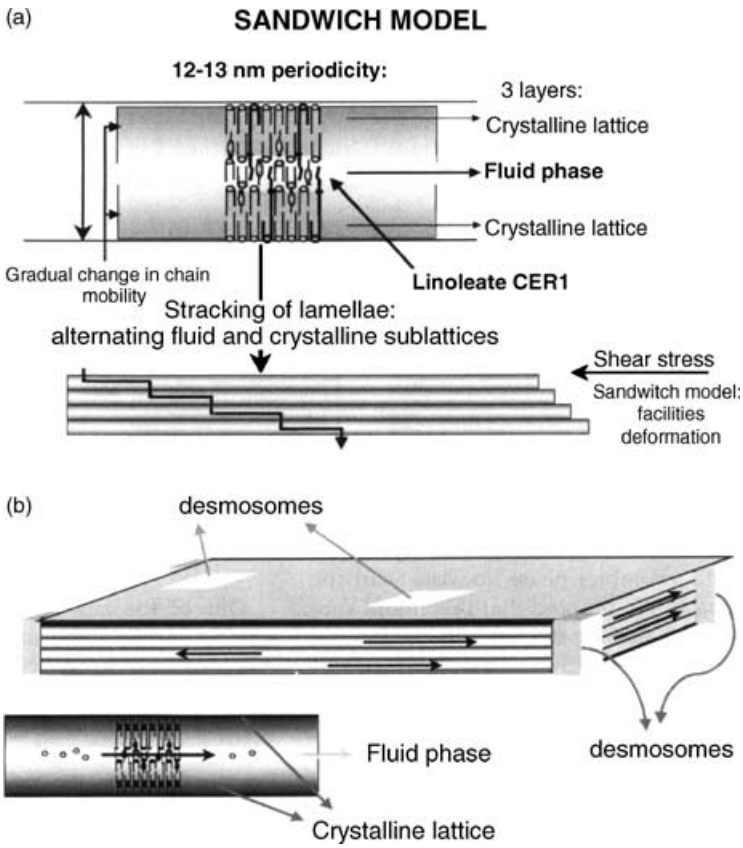

Figure 2 (a) 'Sandwich model', the characteristics of which are: (i) the liquid sublattice is located in the central lipid layer of this phase, in this layer mainly unsaturated linoleic acid and cholesterol are present; (ii) in the sublattice adjacent to the central layer a gradual change in lipid mobility occurs because the presence of less mobile long saturated hydrocarbon chains; (iii) only a small fraction of lipids forms a fluid phase in the SC, therefore one can assume that this central lipid layer is not a continuous phase. (b) The liquid phase parallel to the basal layers of the lamellae facilitates transport and therefore communication between the desmosomes. Modified from Bouwstra *et al. Skin Pharmacol. Appl. Skin Physiol.* **14**, 52–62 (2001) [8].

gel (hexagonal packing) or liquid crystalline states. The orthorhombically packed lipids are the most tightly packed conformation and have the better barrier properties. As shown by electron diffraction studies, these physical states change during migration of the corneocytes from the lower layers of the stratum corneum to the outer layers, where a greater proportion of hexagonally packed lipid conformations are observed. This is consistent with a weakening of the barrier towards the outer layers of the stratum corneum as has been determined by corneosurfametry methodologies. Electron diffraction studies have confirmed that the bulk of the stratum corneum lipids are present in an orthorhombic state. However, increased amounts of hexagonal gel phase are present in the outer layers of the stratum corneum particularly at skin surface temperatures. In fact, as much as 50% of the lipids in the surface layers of the skin can be in a hexagonal phase [17]. It is believed that short-chain fatty acids from sebum contribute to the crystalline to gel transition in the upper stratum corneum layers [18].

Mixtures of isolated human cholesterol and ceramides fractions pack together in a hexagonal state. Bearing in mind that most human ceramides have either a phytosphingosine or a 6-hydroxysphingosine base, these findings are consistent with the work of Rerek *et al.* [19], who demonstrated that phytosphingosine-containing ceramides compared with sphingosine-containing ceramides pack in a hexagonal phase. Examining the lateral packing states by wide-angle X-ray diffraction, the addition of long-chain fatty acids (C22–C26 but not C16–C18) induces a hexagonal to orthorhombic transition of equimolar ceramide and cholesterol mixtures. However, fatty acids also induce the formation of the SPP. pH has no effect on the lamellar ordering or lateral packing states of cholesterol and ceramide mixtures. However, when fatty acids are present the LPP is observed at pH 7.4, whereas both the LPP and SPP are observed at pH 5, probably because of ionization of the fatty acids. This indicates that in addition to the lateral packing states, changes in the lamellar ordering might also occur towards the surface of the stratum corneum. In fact, the complexity of ordering is even further highlighted by the loss of the LPP at full hydration of the corneum (60% water) [20–22]. Weichers *et al.* [23] have reported that

hydration of the stratum corneum does not affect the orthorhombic to hexagonal phase crystallization temperature.

Cholesterol sulphate is another intercellular lipid that is believed to be hydrolysed in the surface layers of the stratum corneum. *In vitro* addition of low levels of cholesterol sulphate (2%) to lipid mixtures has little effect on phase behaviour but at 10% it promotes the formation of a 12.8 nm lamellar phase, induces the formation of a fluid phase and increases the solubility of cholesterol in the lamellar phase [24].

In the absence of CER EOS, mostly hexagonal phases are also observed for total lipid mixtures. Nevertheless, the inclusion of CER EOS induces the formation of the LPP. Moreover, the importance of ceramide one or CER EOS in facilitating the formation of the long 13.4 nm LPP has been further elaborated by understanding the influence of the type of fatty acid esterified to the omega hydroxyl fatty acid [25]. As a consequence, the LPP is seen mainly with linoleate-containing CER EOS, less with oleate-containing CER EOS and is absent if only stearate-containing CER EOS is present in the lipid mixtures (Fig. 3). These studies indicate that for formation of the LPP, a certain fraction of the lipids has to form a liquid phase. If the liquid phase is too high as with the oleate-containing CER EOS or too low as with stearate-containing CER EOS, the levels of the SPP increase at the expense of the LPP. Similar changes are expected with increased concentrations of short- to medium-chain fatty acids from surface sebum levels.

These types of compositional changes could therefore dramatically influence the condition of the skin. The lipid packing states might not only influence the barrier properties of the skin but also its desquamation. On the surface of the stratum corneum cholesterol sulphate is hydrolysed together with CER EOS, concentrations of short chain length fatty acids are increased, pH and water levels decrease, increased crystallization of cholesterol and decreased ceramide levels occurs because of their extraction by cleansing. As a result, perturbations to the lamellar ordering can be expected. Using electron microscopy of tape strippings from the outer layers of normal healthy skin, Rawlings *et al.* [26] reported complete loss of lamellar ordering in the outer layers of the stratum corneum (Fig. 4).

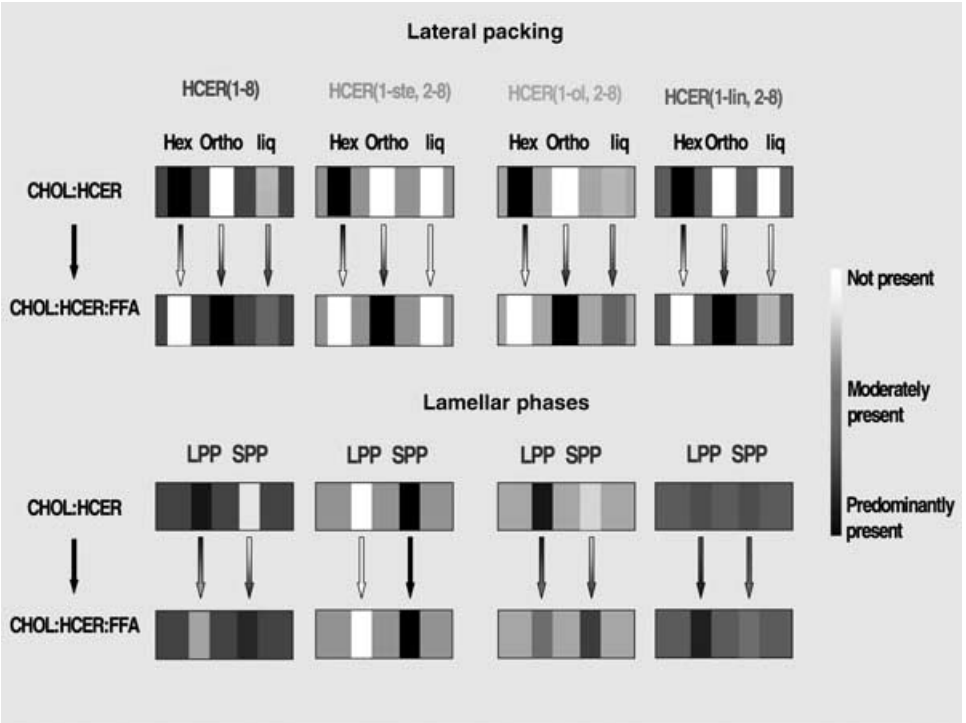

Figure 3 A summary of the lamellar phases and lateral packing in various lipid mixtures. HCER (1–8) mixtures in which HCER (EOS) is replaced with either synthetic stearate-containing CER (EOS), oleate-containing CER (EOS) or linoleate-containing CER (EOS). Modified from Bouwstra *et al. J. Invest. Dermatol.* **118**, 606–617 (2002) [25].

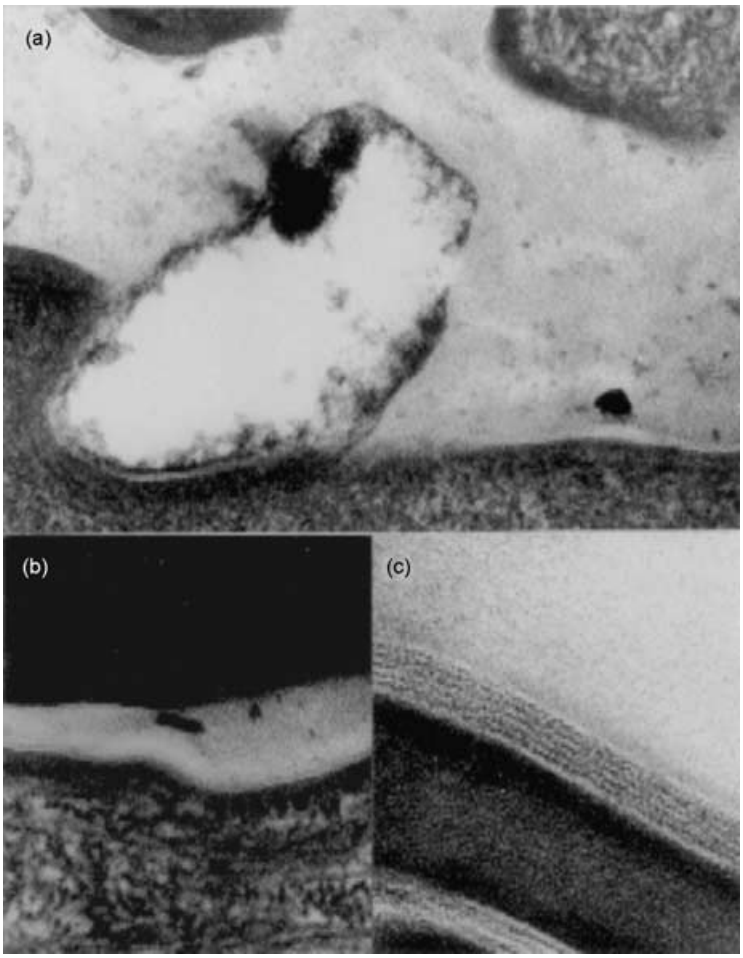

Figure 4 Organization of stratum corneum lipids in tape-strippings of individuals with clinically normal skin. Transmission electron micrographs of tape-strippings. Ultrastructural changes in lipid organization towards the surface of the stratum corneum: (a) First strip; absence of bilayers and presence of amorphous lipidic material. (b) Second strip; disruption of lipid lamellae. (c) Third strip; normal lipid lamellae. (200 000×). Modified from Rawlings *et al. J. Soc. Cosmet. Chem.* **45**, 203–220 (1994) [26].

Stratum corneum corneodesmosomes and corneodesmolysis

A further development of the original 'brick and mortar' model was the inclusion of the corneodesmosomes, modified and specialized desmosomes first named by Serre [27], which physically hold the cells together in the stratum corneum. The corneodesmosomes are macromolecular glycoprotein complexes incorporated into the corneocyte envelope (CE) and consist of the cadherin family of transmembrane glycoproteins, desmoglein 1 (Dsg 1) and desmocollin 1 (Dsc 1). These glycoproteins span the cornified envelope into the lipid-enriched intercellular space between the corneocytes and provide cohesion by binding homeophilically with proteins on adjacent cells. Within the corneocytes Dsg 1 and Dsc 1, these are functionally linked to keratin filaments via corneodesmosomal plaque proteins including plakoglobin, desmoplakins and plakophilins. Recently, the novel corneodesmosomal protein corneodesmosin (Cdsn) and its involvement in corneodesmosome formation was identified [28]. After secretion by the lamellar bodies with the intercellular lipids and some proteases, Cdsn becomes associated with the desmosomal proteins just before transformation of desmosomes into corneodesmosomes. However, in a fashion similar to the other corneodesmosomal proteins, it is progressively hydrolysed towards the surface of the stratum corneum.

The exfoliation of corneocytes from the surface of the skin is facilitated by the action of specific hydrolytic enzymes in the stratum corneum that degrade the corneodesmosomes and then allow cell loss by frictional forces. In the lower layers of the stratum corneum, corneodesmosomes are present all over the surfaces of the corneocytes. However, on maturation of the stratum corneum from the stratum compactum to the stratum disjunctum, there is a dramatic loss of non-peripheral corneodesmosomes

Table I Enzymes involved in the desquamatory process

Sulphatases	Steroid sulphatase	
Glycosidases	Heparanase 1	
Serine proteases	Stratum corneum chymotryptic-like enzyme	(SCCE/KLK7)
	Stratum corneum tryptic-like enzyme	(SCTE/KLK5)
Cysteine proteases	Stratum corneum thiol protease	(SCTP)
	Stratum corneum cathepsin E-like enzyme	(SCCEE)
Aspartic proteases	Stratum corneum cathepsin D-like enzyme	(SCCDE)

resulting in the major intercorneocyte forces only being at the overlapping edges of the corneocytes rather than on the total surface of the corneocytes. Several serine, cysteine and aspartic enzymes are believed to be involved in this process, namely, stratum corneum chymotryptic enzyme (SCCE), stratum corneum tryptic enzymes (SCTE), stratum corneum thiol protease (SCTP), cathepsin E and the aspartic protease cathepsin D. SCCE and SCTE are alkaline optimal enzymes, whereas the latter ones are acidic optimum enzymes. Lundstrom and Egelrud [29] demonstrated the importance of SCCE in the desquamatory process, while Suzuki *et al.* [30] identified the role of trypsin-like enzymes. The importance of cathepsin D in desquamation was established by Horikoshi *et al.* [31, 32] at Kanebo, demonstrating that only 30% inhibition of corneocyte release by PMSF, 40% by Pepstatin A, whereas Chymostatin inhibited it by 90% at pH 5.0. Using more specific protease inhibitors, Dr Horikoshi and his team has shown that at least 80% of the aspartic protease activity can be attributed to cathepsin D and 20% to cathepsin E. Cathepsin L has also recently been implicated in corneodesmosin hydrolysis [33].

Rawlings and Watkinson [34–36] demonstrated that exogenously applied SCCE, SCTE and a stratum corneum cathepsin D-like enzyme could facilitate desquamation by degrading Dsg 1 and Dsc 1. Watkinson [37] later discovered the stratum corneum thiol protease (SCTP) which also degraded Dsc 1. However, glycosidases are also involved in this process and synergistic activities of proteases and glycosidases has been demonstrated. It has been proposed that the selective loss of the non-peripheral corneodesmosomes may reflect differences in differential glycosylation patterns. In fact, Walsh and Chapman [38] described in 1991 that desquamation was the result of the sequential action of glycosidases and proteases. Examining corneodesmosomes by electron microscopy pretreatment of stratum corneum with a mixture of glycosidases enhanced proteolytic attack on the corneodesmosomes suggesting that sugar moieties are protecting corneodesmosomal proteins from hydrolysis. Rawlings *et al.* [39] further demonstrated the synergistic effect of combining glycosidases with a variety of proteases using *in vitro* desquamation models evaluating Dsg 1 hydrolysis and in clinical studies. However, the precise SC deglycosylase was not known at that time. More recently, Bernard *et al.* [40] have identified an endoglycosidase, heparanase 1, within the stratum corneum and that is thought to play a role in the preproteolytic processing of the protecting sugar moieties on corneodesmosomal proteins. A list of stratum corneum enzymes involved in the desquamatory process is given in Table I.

Incubating surfactant extracted epidermal proteins, Simon *et al.* [41] demonstrated that recombinant SCCE or an enriched fraction of SCTE can degrade Cdsn and to a lesser extent the desmocollins. Cdsn was hydrolysed by both enzymes at pH 7.4 and 5.6 but only SCCE degrades Dsc 1 at the lower pH. These enzymes, however, cannot degrade isolated Dsg 1 *in vitro*. This is not inconsistent with SCCE or SCTE inducing Dsg 1 degradation in the *ex vivo* corneocyte model reported by Rawlings *et al.* [34] or the effects of recombinant SCCE on Dsg 1 degradation [42] as these enzymes could be activating another endogenous protease. However, it is also possible that different epitopes of Dsg 1 are expressed in a lipid matrix compared with aqueous extracts. At the moment, it is unclear whether this enzyme or one of the other cysteine (cathepsin B, H or L or SCTP) or aspartic proteases (cathepsin D-like enzyme) cleaves desmoglein 1 *in vivo*. However, Horikoshi *et al.* demonstrated that a cathepsin D enzyme is involved in desquamation at low pH (Fig. 5, courtesy of Dr Horikoshi).

Many of these enzymes are thought to exist as pro-forms and have been immunolocalized to the intercorneocyte lipid lamellae. Using antibodies to chymotrypsin and trypsin Watkinson, Smith and

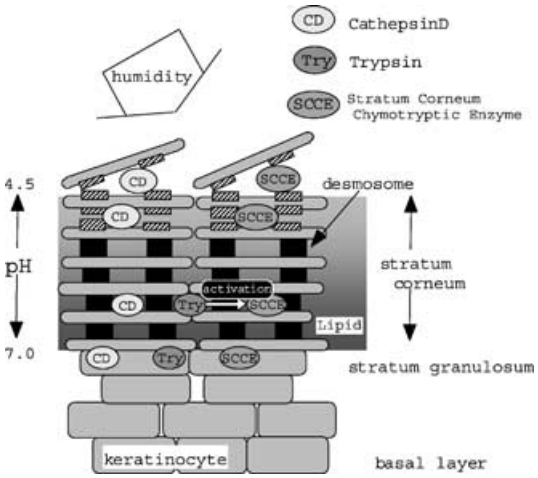

Figure 5 Diagrammatic representation of the mechanism of desquamation of human stratum corneum. Courtesy of Dr Horikoshi, Kanebo Ltd.

Rawlings [43] first demonstrated that immuno-reactivity for chymotrypsin was localized to the lipid lamellae and the corneocyte envelope whereas some trypsin immuno-reactivity was localized to the lipid lamellae but most was immunolocalized within the corneocytes. Sondell *et al.* [44], using antibodies that immuno-react precisely with pro-SCCE, confirmed that this enzyme is transported to the stratum corneum extracellular space via lamellar bodies. In later studies, using antibodies to both pro-SCCE and SCCE, Watkinson *et al.* [45] demonstrated that the processed enzyme was more associated with the corneodesmosomal plaque. More recently, Igarashi *et al.* [46] at Kanebo have immunolocalized cathepsin D to the intercellular space whereas cathepsin E was localized within the corneocytes. (Personal communication from Dr Horikoshi, Kanebo Ltd.).

Calcium appears to impair Cdsn hydrolysis but not Dsg 1 degradation. Incomplete desquamation *in vitro* at alkaline pH occurs without the presence of EDTA. Cells can, however, be 'forced' to desquamate with additional mechanical brushing together with agitation. This appears to be related to the degradation of corneodesmosin [47]. As indicated previously, Cdsn undergoes several proteolytic steps. Cleavage of the N-terminal glycine loop domain occurs first at the compactum disjunctum interface (48–46 to 36–30 kDa transition) followed by cleavage of the C-terminal glycine loop domain in exfoliated corneocytes (36–30 to 15 kDa transition). The last step appears to be inhibited by calcium resulting in residual intercorneocyte cohesion. Chelation of calcium allows this step to proceed [48]. The precise calcium chelator *in vivo* is currently unknown. Fatty acids are possible chelating agents.

As some of the desquamatory enzymes have been immunolocalized to the intercellular space, the physical properties of the stratum corneum lipids together with the water activity in that microenvironment of the stratum corneum will influence the activity of these enzymes. However, a variety of inhibitors are also present to attenuate their activities, cholesterol sulphate being one of them. Other protein and peptide inhibitors are present, such as elfin covalently bound to the corneocyte envelope, antileukoproteinase, alpha-1-antitrypsin and alpha-1-antichymotrypsin and the SPINK5-derived peptides [49]. Nevertheless, antileukoprotease is believed to be the major physiological inhibitor of SCCE; the serpins are too low in concentration to be physiologically relevant [50].

Currently, little is understood of the molecular activation mechanisms of SCCE or other enzymes within the stratum corneum. The role of desquamin or zinc 2 glycoprotein as it is now known remains elusive [51]. Clearly, stratum corneum pH and water content will influence enzymic activity. Interestingly, however, SCCE appears to have a greater tolerance to water deprivation than other proteolytic enzymes and this may be an adaptation to maintain enzyme activity even within the water depleted stratum corneum intercellular space [52]. As the stratum corneum pH declines towards the surface of the skin because of increasing NMF concentrations and free fatty acids from sebum or ceramide hydrolysis, the activity of SCTE and SCCE might be minimal and perhaps the acid optimal cathepsin enzymes mediate the final desquamatory steps especially the stratum corneum cathepsin D-like enzymes [31–33, 36]. Indeed, if the compactum pH is artificially elevated by inhibition of sPLA2, an enzyme which normally releases free fatty acids from phospholipids to acidify the lamellar matrix to allow the activity of the acid optimum beta-glucocerebrosidase and sphingomyelinase to cleave their substrates to form a ceramide-rich gel phase from a glucosyl ceramide-rich liquid crystalline phase, or by extensive prolonged (3 h) hydration of skin to neutral pH impaired barrier function and changes in stratum corneum cohesiveness occurs [53]. Premature corneodesmolysis occurred in the compactum layers, probably because of the maintenance of a liquid crystalline phase allowing complete diffusion of enzymes to the corneodesmosomes enhancing corneodesmolysis. There is no effect on the corneodesmosomes in the stratum

disjunctum as these lipids have already formed a gel phase.

Nevertheless, massive corneodesmolysis occurs at the stratum compactum–stratum disjunctum interface precisely where filaggrin in hydrolysed. The pH within the corneocytes will decrease dramatically and either activates acidic optimum enzymes present here or because of the swelling and smoothening out of the corneocytes allows enzymes to access their substrates or dissociates them from bound sites on the cells. The peripheral corneodesmosomes may still be physically separated from the proteases and protected.

Corneocyte envelope maturation and the role of transglutaminases

The primary purpose of epidermal differentiation is to form a fully functional stratum corneum that can act as a barrier, maintain tissue flexibility and can also desquamate. The corneocytes become stronger towards the surface layers of the skin presumably to help resist the mechanical effects of desiccation at the skin surface. As described above, the corneodesmosomes and covalently bound lipids are, however, covalently cross-linked into the corneocyte envelope. The functionality of the corneocytes, and thereby, the stratum corneum is therefore influenced by the action of the transglutaminase family of enzymes.

The corneocyte envelope is an extremely stable insoluble 10 nm thick proteinaceous layered structure. The stability of the envelope is attributed to the degree of cross-linking of envelope proteins by either disulphide, glutamyl–lysine isodipeptide bonds or glutamyl polyamine cross-linking of glutamine residues of several corneocyte envelope proteins [54]. The enzymes responsible for catalysing the gamma-glutamyl-epsilon-lysine isodipeptide bond formation are the calcium-dependent transglutaminases (TGase; glutamyl-amine aminotransferases EC 2.3.2.13) of which four are expressed in the epidermis 1–3 and 5. However, only TGase 1, 3 and 5 are thought to be involved in keratinocyte differentiation [55]. The catalytic mechanism involves the release of ammonia from the reactive glutamine residues and the residual glutamyl moieties form an acyl-enzyme thioester, which is a labile intermediate susceptible to nucleophilic attack by primary amines or alcohols. TGase 1 is expressed in the spinous cell layers as a 106 kDa protein, which, because of its *N*- and *S*-linked fatty acyl groups, is membrane bound. It is proteolytically cleaved by cathepsin D to give soluble components of 10, 33 and 67 kDa, the complex of which is membrane bound and has enhanced activity. TGase 3 is produced in the granular layer as a 77 kDa zymogen, which is activated by proteolysis to a 50 kDa protein [56].

At early time points in the keratinocyte differentiation process, envoplakin and periplakin are expressed and become associated with desmosomes in the viable epidermis. Subsequently, involucrin (the glutamyl-rich protein that covalently bound lipids become attached to) is expressed at the same time as TGase 1 [57]. TGase 1 then cross-links involucrin to the other early-expressed proteins, such as members of the small proline-rich (SPRR) family of proteins. Subsequently, other plasma membrane proteins become cross-linked and these form a scaffold for further reinforcement and maturation events. In the epidermis, the major CE reinforcement proteins are loricrin with smaller amounts of the SPRR proteins whereas in lips, palmoplantar or oral epithelia the opposite occurs. *In vitro* data suggest that TGase 3 and possibly TGase 5 initiate the hetero- and homodimerization cross-links between these proteins before TGase 1 finally cross-links them into the pre-existing scaffold [58].

The toughness, strength and flexibility of the corneocyte envelopes are thought to be dictated by the SPRR proteins. There are two general groups of proteins: Group 1 (SPRR 1 A, 1B, 3 and 4) and Group 2 (SPRR 2 A, 2B, 2C, 2D, 2E, 2F and 2G). Group 2 proteins are the most flexible [59]. Their expression profiles differ in dry versus wet epithelia. For instance, SPRR2G and SPRR4 are preferentially expressed in skin driven by the transcription factor Kruppel-like factor 4 (Klf4) [60]. SPRR4, 2C and 2G are consistently induced after UV irradiation and specifically incorporated into specific fragile envelopes. Interestingly, SPRR4 is expressed following UVC and UVB but not UVA [61].

In several reports, Barton *et al.* [62] using scanning electron microscopy originally identified corneocytes with a ruffled appearance in the deeper layers of the stratum corneum (Fig. 6). Later, using the Normarski microscopy, the corneocyte envelops (CEs) were shown to have a crumpled surface in the lower layers of the stratum corneum and a smoother, more flattened surface in the upper stratum corneum. These two populations of corneocyte envelopes were named fragile (CEf) and rigid (CEr). The fragile envelopes were initially observed in hyperproliferative disorders such as psoriasis and plantar corneum. Serre *et al.* [27] reported that about 80% of corneocytes from volar forearm skin were smooth and rigid

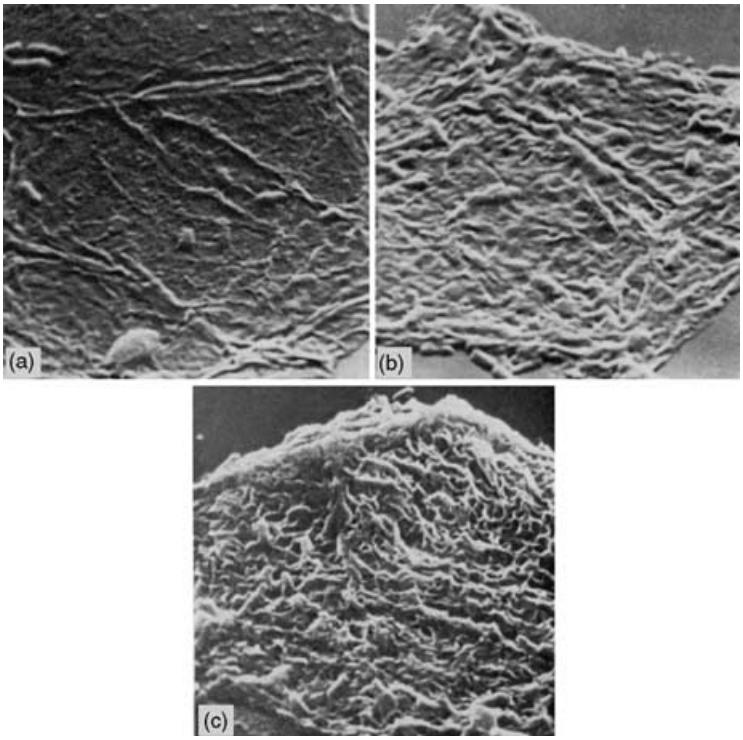

Figure 6 SEM of a typical surface corneocyte at (a) approximately 5000×; (b) approximately 5000× after 10 strippings; (c) after 20 strippings. The cell surface appears to have more microvillous projections deeper in the stratum corneum. Modified from *Br. J. Dermatol.* 100: 165–172, 1979.

whereas 90% from foot sole were rough or fragile cells. Antibodies directed against the fragile envelopes did not bind to the rigid ones. In particular, Cdsn [63] and Dsc 1 [64] have been associated with the fragile envelopes. They can also be further differentiated by their binding of tetramethyl rhodamine isothiocyanate (TRITC) with the rigid envelopes staining more greatly (Fig. 7). However, Hirao *et al.* [65] at Shiseido have used a more elegant method to identify corneocyte envelopes based upon their hydrophobicity (staining with Nile red) and antigenicity (to anti-involucrin; bright staining Fig. 8). It is clear from these studies that immature envelopes (CEf) occur in the deeper layers of the stratum corneum (involucrin positive and weak staining to Nile red or TRITC) and that mature envelopes occur in the surface layers of healthy skin (apparent involucrin staining lessened and increased staining with Nile red or TRITC). This conversion usually appears to occur at the stratum compactum–disjunctum interface together with filaggrin hydrolysis. Fragile envelopes also reacted with antibodies to loricrin, envoplakin, filaggrin and isopeptides. Thus, these cells are immature and less hydrophobic and their presence is closely related to impairment of barrier function. The maturation process appears to be different on different body sites. Usually those areas with a faster hyperproliferative rate contain greater quantities of CEf and the size of the corneocytes is also smaller. More recent work from Pola [66] using atomic force microscopy confirmed these structural changes in corneocytes from the deeper layers of the stratum corneum or in hyperproliferative disorders. As measured, the flatness index increased towards the surface of the stratum corneum and it was highly correlated with age. Interestingly, the volume of the corneocytes increased from the 1st to the 15th tape stripping but then slightly decreased deeper down. This may reflect the different locations of

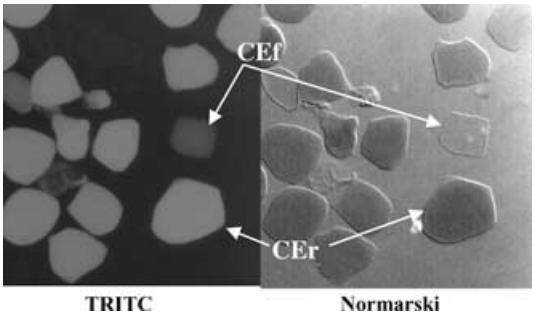

Figure 7 Fluorescence and Normaski phase contrast microscopy of TRITC-stained cornified envelopes demonstrating increased fluorescence labelling of CEr compared with CEf. Modified from Watkinson *et al.* In: *Skin Moisturization* (J.J. Leyden and A.V. Rawlings eds). pp 95–117. Marcel Dekker, New York (2002) [54].

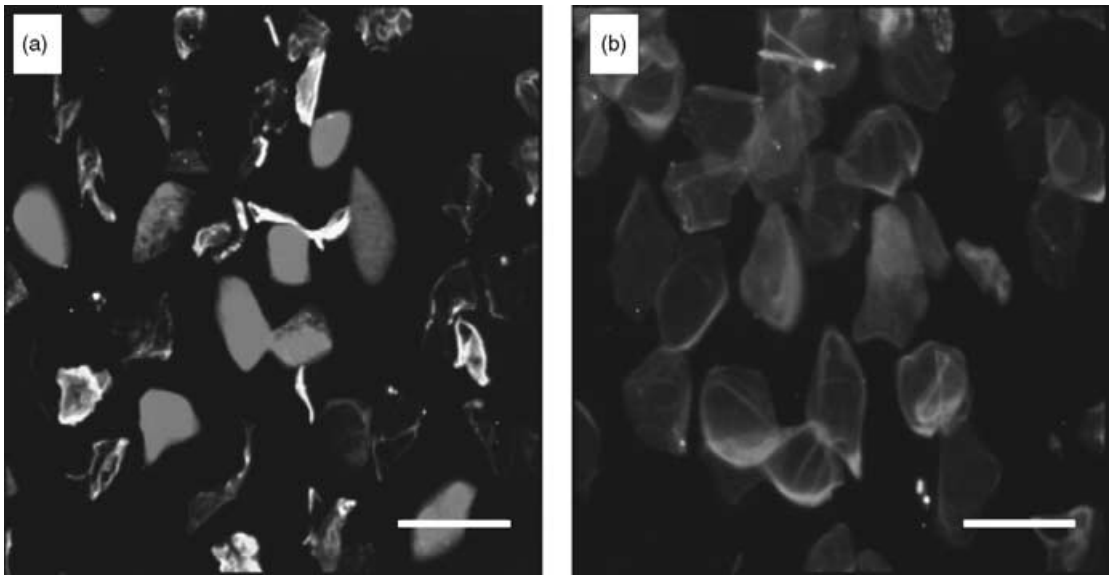

Figure 8 Double Staining of CEs with Nile red and anti-involucrin. (a) Face. (b) Upper Arm. Modified from Hirao *et al. Exp. Dermatol.* **10**, 35–44 (2001) [65]. See brighter staining of fragile envelopes on face.

filaggrin being hydrolysed to NMF and its loss towards the surface layers.

The morphological classification of fragile and rigid envelopes has subsequently been found to be a pertinent classification system as mechanically they have fragile and rigid characteristics under compressional force (Fig. 9) [54]. It is unlikely that the increases in corneocyte hydrophobicity lead to

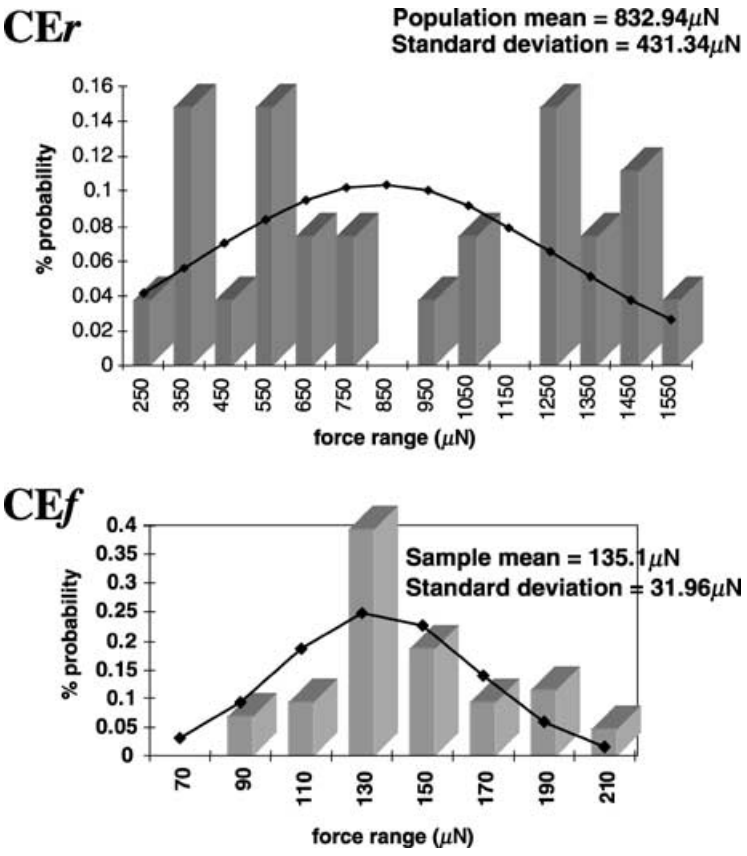

Figure 9 Distribution profile of the maximal compressional forces (uN) of individual CEs. Top panel shows the force range for CEr and the bottom for CEf. The maximal compression force was significantly different between the corneocytes. Modified from Watkinson *et al.* In: *Skin Moisturization* (J.J. Leyden and A.V. Rawlings eds). pp 95–117. Marcel Dekker, New York (2002) [54].

increased corneocyte resilience but it is more likely that further TGase-mediated cross-linking gives the CEr extra-mechanical strength. Supporting this concept of increasing CE rigidity, gamma glutamyl-lysine cross-links increase in the subsequent layers of the stratum corneum. Three pools of TGase activity in the stratum corneum have been classified based upon their solubility characteristics: a water soluble TGase (mainly TGase 1 and 3), a detergent-soluble TGase (TGase 1) and a particulate form that cannot be liberated from the corneocyte. Whether all enzyme fractions are active in this maturation process of CEf to CEr is currently not known [54].

A new marker of keratinocyte differentiation that might influence TGase activity is the calmodulin-like skin protein (CLSP). In the epidermis, together with the lower layers of the stratum corneum it binds TGase 3. Its precise role in differentiation, however, remains to be elucidated [67].

Stratum corneum and filaggrin

The production of NMF primarily through the hydrolysis of profilaggrin is critical to skin condition and for water binding in the outer layers of the stratum corneum. Recently, water uptake as a function of depth in the stratum corneum has been visualized using cryo-scanning electron microscopy [68]. These studies demonstrated that water is not homogeneously distributed over the whole of the stratum corneum. In these studies, at 70% w/w water content, the hydration of the central part of the corneum is higher than in the superficial and deeper layers of the corneum consistent with the location of filaggrin, NMF and finally leaching of NMF at the skin surface as reported by Rawlings *et al.* [2]. However, it has been shown that as corneocytes swell the swelling is almost perpendicular to the skin surface. The total length of the cell envelope/cross-sectional surface area decreases with stratum corneum hydration. At higher water content, the corneocyte envelope surrounds the cell content more efficiently compared to that at low water content. This indicates that the fragile cell villi on the surface of the cells observed by Barton *et al.* [62] are 'blown' out to give a smoother cell surface as filaggrin is hydrolysed and the osmotic forces within the corneocytes increases. However, the corneocytes still remain in contact at the peripheral edges of the cells.

As seen in Fig. 10, peptidyl–arginine–deiminase (PAD) is essential for the further processing of filaggrin by exposing filaggrin to filaggrinases after dissociation from filaggrin. Scott and Harding [69] have reported that in an occlusion-induced model of dry skin, filaggrin is not proteolysed to free amino acid but that it is deiminated, suggesting that the filaggrinases are the rate-limiting factor in filaggrin processing. How dehydration controls filaggrin proteolysis is not understood. Nevertheless, this occlusion model is not true dry skin and PAD should be further investigated in xerotic skin conditions.

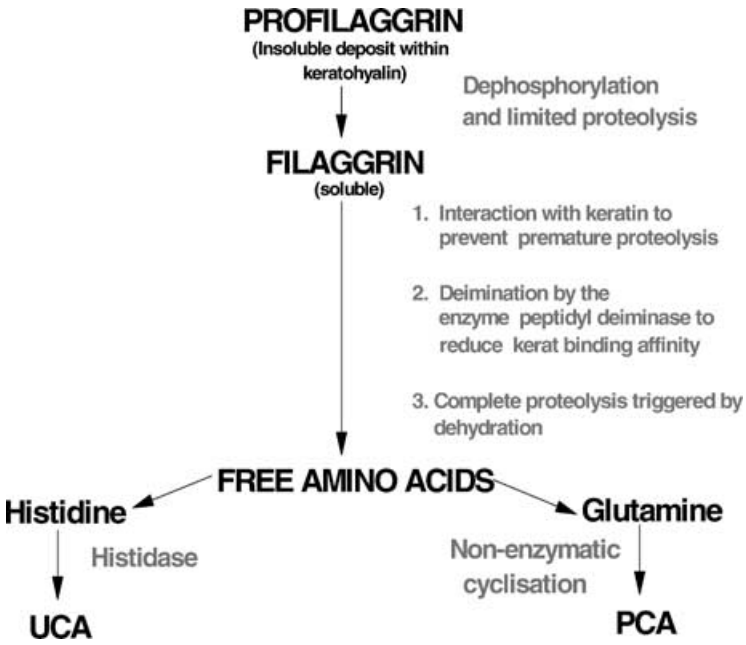

Figure 10 Schematic representation of profilaggrin catabolism and filaggrin hydrolysis to NMF within the SC.

The subtilisin-like proprotein convertases family of calcium-dependent serine proteases are involved in pro-filaggrin processing. Pearton *et al.* [70] have reported that four of these enzymes are expressed in the epidermis (furin, PACE4, PC5/6 and PC7/8). Furin is present in two forms with or without a transmembrane domain. Profilaggrin found in the granular layer is specifically cleaved by furin and PACE4 *in vitro*. These enzymes as well as the intracorneocyte proteases need further investigation to decipher filaggrinolysis.

Filaggrin is highly polymorphic and three allelic variants encoding 10, 11 or 12 filaggrin repeats have been identified. More recently, Ginger *et al.* [71] have established that the 12 repeat allele is associated with reduced dry skin frequency whereas the 11 repeat allele was associated with increased dry skin. Individuals with no 12 repeat allele are four times more likely to report frequent dry skin than those with at least one 12 repeat allele despite protein expression levels being equal. These polymorphisms may be markers of another linked polymorphism that has a role in epidermal barrier function or desquamation.

Changes in stratum corneum composition, structure and function in dry skin

Desquamation results in the degradation of non-peripheral corneodesmosomes at the stratum compactum–disjunctum interface and the peripheral corneodesmosomes are then finally degraded in the upper layers of the stratum corneum leading to loss of corneocytes from the surface of the skin by frictional forces. However, in dry flaky skin conditions corneodesmosomes are not degraded efficiently and corneocytes accumulate on the skin's surface layer. Increased levels of corneodesmosomes in soap-induced dry skin were first reported by Rawlings *et al.* [26] using electron microscopy but more recently scientists at the University of Toulouse and L'Oreal [72] have produced some beautiful freeze fracture visualization of the corneodesmosomes in dry skin (Fig. 11). Increased corneodesmosomes are clearly apparent in these studies. Equally impressive cryo-electron micrographs have been reported by Pilgram *et al.* [73]. The corneodesmosomes also had an irregular appearance at all depths in the skin in atopic stratum corneum (Fig. 12).

Many corneodesmosomal proteins are now also reported to be increased in the surface layers of xerotic skin. Stratum corneum Dsg 1 levels were reported to be increased by Bartolone *et al.* [74] and Rawlings *et al.* [26]. Dsc 1 levels were also reported to be increased by the latter group [64]. More recently, Cdsn and plakoglobin were found elevated in dry skin [72]. Interestingly, however, in winter xerosis the accumulation of the corneodemosomal proteins, Dsg 1 and plakoglobin correlate with each other, whereas Cdsn protein levels, which were also increased, do not have such an association suggesting that different proteolytic mechanisms occur for the different corneodesmosomal components during desquamation. As suggested by Simon *et al.* [72], as plakoglobin is a cytoplasmic protein this would indicate that at least the cytoplasmic domain of Dsg 1 may be cleaved. In fact, immunoreactivity to the carboxy terminal tail of the cytoplasmic portion of Dsg 1 was observed. Perhaps the intracellular portions of Dsg 1 are also degraded within the corneocyte-like plakoglobin by the trypsin-like activity [43, 45] or cathepsin E activity [46] reported within the corneocyte matrix or some other intracellular proteases, e.g. the filaggrinases. Conversely, Cdsn might be degraded by SCCE, SCTE or cathepsin D in the lamellar matrix. This is consistent with the early electron microscope images of Rawlings *et al.* [26] showing that corneodesmosomes become internally vacuolated but then the protein structures completely detach from the corneocyte envelope.

The lamellar lipid matrix is also perturbed dramatically in dry skin (Fig. 13) and although controversial at the time, Rawlings *et al.* [26] reported that stratum corneum ceramide levels were dramatically lower in the outer layers of xerotic stratum corneum. As the main desquamatory enzymes, but probably not the only desquamatory enzymes, are found within this lipid matrix, the physical properties of the lamellar lipids will influence enzyme activity. Using cryo-EM Pilgram [73] also observed that although the fracture across a lipid layer was not different in atopic skin compared with healthy skin, the fracture plane often contained rough structures.

Rawlings *et al.* [2] originally reported that stratum corneum SCCE levels are reduced in the outer layers of xerotic stratum corneum compared with normal skin. This has been confirmed more recently in more extensive studies by Van Overloop *et al.* [75] but equally important stratum corneum SCTE activities were also reduced. Conversely, in SLS-induced dry skin, increased activities of these enzymes are reported [30]. It is important to remember that dry skin is more alkaline than normal skin and if the aspartic and cysteine proteases are involved in

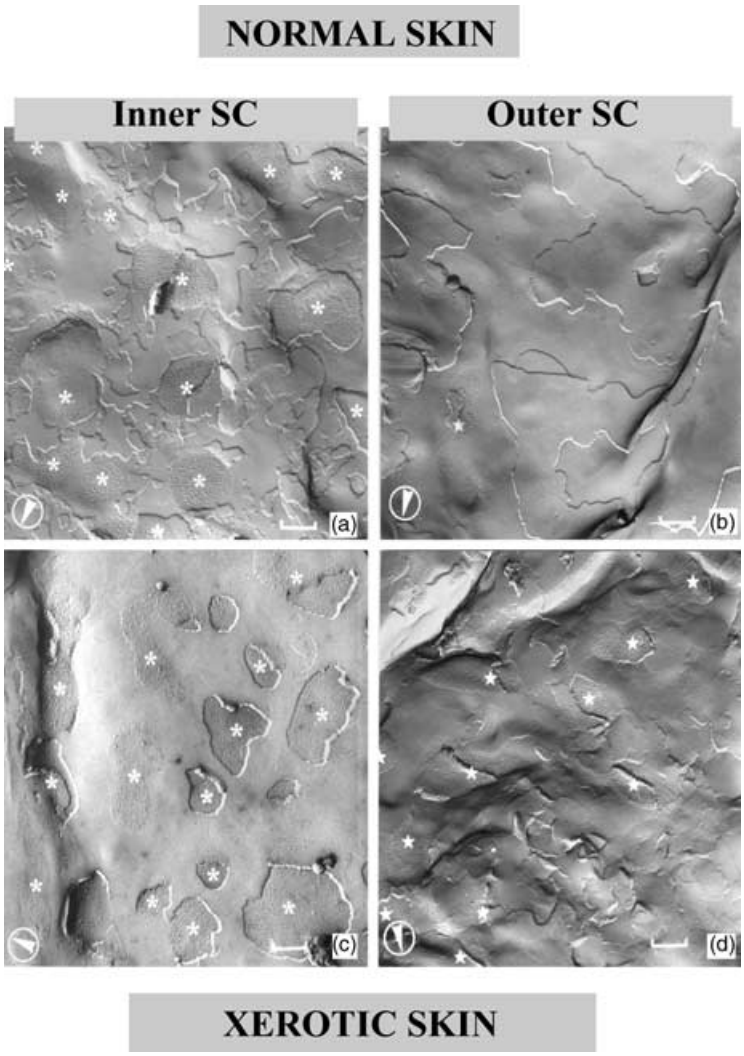

Figure 11 Freeze fracture replicas show an increased number of corneodesmosomes on the surface of corneocytes from the outer SC of winter xerosis compared to those of normal skin. The morphology of intercellular spaces within the inner SC (a,c) and outer SC (b,d) from normal (a,b) and xerotic (c,d) skin strippings was analysed on freeze fracture electron micrographs. Corneodesmosomes appear as particle aggregates (asterisks and stars). Arrowheads indicate the direction of shadowing. Scale bar 250 nm. From Simon *et al. J. Invest. Dermatol.* **116**, 23–30 (2001) [72].

desquamation, their activities will be minimal at this pH. More recently, the overactivation of the plasminogen cascade has been associated with dry skin. Normally only observed in the epidermal basal layers, skin plasmin is widely distributed through the epidermis in dry skin. Interestingly, a urokinase-type plasminogen activator also exists in the stratum corneum [76]. Clearly, these and other enzymes are potentially involved in the inflammatory and hyperproliferative aspects of dry skin.

It has been well established in hyperproliferative disorders such as dry skin that there is a change in the stratum corneum lipid composition. Particularly, the composition of the ceramide subtypes change and a predominance of sphingosine-containing ceramides at the expense of the phytosphingosine-containing ceramides has been observed. In 1986, the pioneering work of Fulmer and Kramer at P&G [77] first identified these changes in SDS-induced dry skin (increased levels of ceramide 2 and 4 and reduced levels of ceramide 3). However, Saint-Leger *et al.* [78] at L'Oreal observed no changes in ceramide levels in dry skin but found increased fatty acid levels. At this time, the full complexity of the different ceramide structures were not known but more recently Chopart *et al.* from L'Oreal [79] observed that dramatic reductions in the levels of phytosphingosine-containing ceramides in particular (approximately 50%) occur in dry skin together with a shortening and lengthening of the acyl sphingoid bases sphingosine and 6-hydroxysphingosine, respectively. Van Overloop *et al.* [75] at Estee Lauder also clearly demonstrated that the phytosphingosine-containing ceramides were reduced to a greater extent than

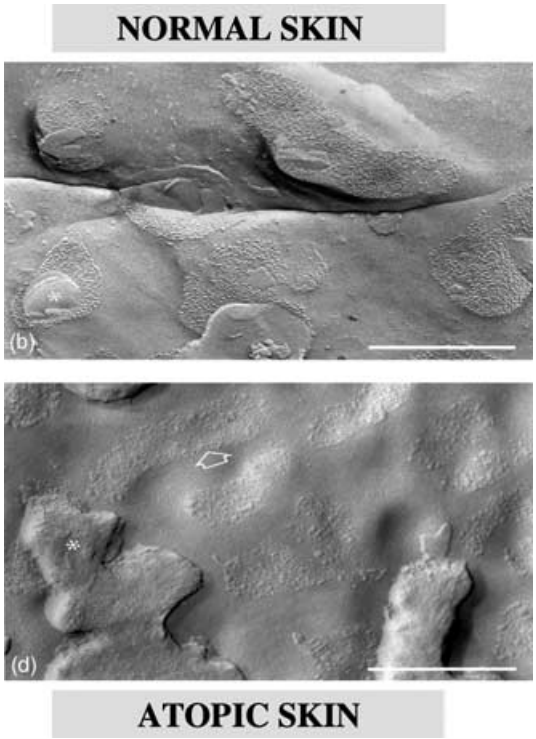

Figure 12 Representative electron micrographs of replicas from healthy and atopic stratum corneum. (a) Regular distribution of protein particles in corneodesmosomes from healthy stratum corneum. (b) Irregular distribution of protein particles which are not clearly defined in corneodesmosomes from a patient with atopic dermatitis. (Scale bar 500 nm). Modified from Pilgram *et al. J. Invest. Dermatol.* **117**, 710–717 (2001) [73].

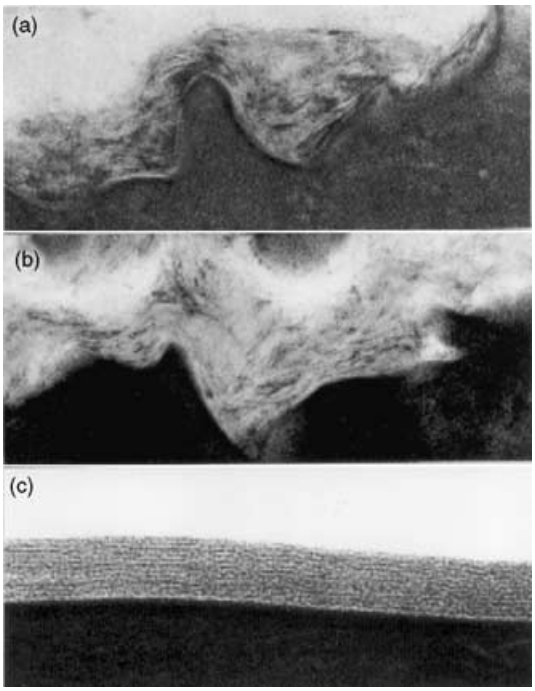

Figure 13 Organization of stratum corneum lipids in tape-stripping of subjects with winter xerosis. Transmission electron micrographs of tape strippings of individuals with severe xerosis. Perturbation in lipid organization towards the surface of the stratum corneum. (a) First strip; disorganized lipid lamellae. (b) Second strip; disorganisd lipid lamellae. (c) Third strip; normal lipid lamellae (200 000×). Modified from Rawlings *et al. J. Soc. Cosmet. Chem.* **45**, 203–220 (1994) [26].

other ceramides with increasing dryness levels. In the studies of P&G, dramatic reductions in the levels of long-chain fatty acids also occurred in dry skin [77].

Imokawa *et al.* at Kao [80] did not find reduced ceramide levels in xerotic skin; however, decreased ceramide levels and especially CER EOS were observed in stratum corneum from atopic skin. This was thought to be a result of the overexpression of sphingomeylin deacylase in atopics. Rather than producing ceramide from sphingomeylin by the action of sphingomyelinase, this enzyme yields sphingosylphosphorylcholine. However, there also appears to be a hydrophobic chain length deficiency in the ceramides and fatty acids in subjects with hyperproliferative skin disorders such as atopic dermatitis [81]. Covalently bound hydroxyceramides also decreased from approximately 50% in healthy skin to 25% in atopic skin, much like that reported for the freely extractable lipids. The fatty acid fractions increased at the expense of the ceramides. The biosynthesis of glucosylceramides *ex vivo* was also reduced in subjects with atopy, especially ceramide EOH and NP.

These changes in lipid composition will obviously influence the lamellar packing of the lipids. In fact, Schreiner *et al.* [82] at Beiersdorf established a reduction of CER EOS and EOH with increased concentrations of sphingosine-containing ceramides (CER NS and CER AS) and crystalline cholesterol in association with a loss of the LPP. Surprisingly, however, there were no changes in lipid ultrastructure in the subjects with dry skin in contrast to previous studies. This study demonstrates the importance of CER EOS for formation of the LPP *in vivo*. However, although the lipid ultrastructure is clearly aberrant in the outer layers of dry skin [26], more work is needed to ascribe a particular lipid phase.

The proportions of the different corneocyte envelope phenotypes also change in different body sites, especially areas with dry skin [54]. Soap-washing

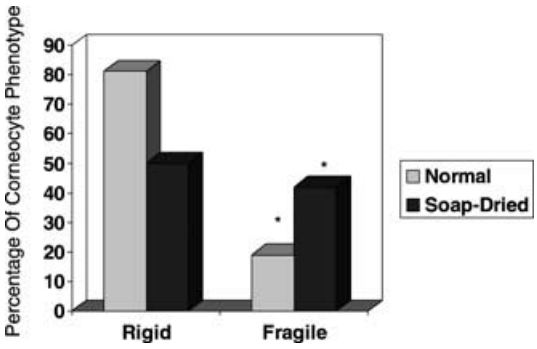

Figure 14 Percentage distribution of CEr and CEf in normal and soap dried dry skin. $^{*}P < 0.05$.

leads to a dramatic increase in the levels of the fragile phenotype at the expense of the rigid types (Fig. 14). These immature envelopes are also found in inflammatory dermatosis such as atopic dermatitis and also on the faces of subjects with apparently healthy skin but with some impairment of barrier function. This latter relationship was not dependent on race or age but was dramatically influenced by season with increasing amounts of CEf occurring in the winter months of the year. It is known that stratum corneum transglutaminase activities increase towards the surface of the stratum corneum, particularly the detergent-soluble and particulate fractions. Although the same trend of the relative increase in TGase between the inner and outer corneum is true of dry skin; TGase activities are dramatically lowered in dry skin compared with healthy skin, particularly the detergent-soluble fraction which contains mainly TGase 1. All of these changes will have a dramatic effect on the textural properties of skin.

Other physiological changes in stratum corneum composition

As discussed earlier, the precise fatty acid esterified to CER EOS also has a dramatic effect on lipid mesophases. As a result of the complexity of the lipid profiles *in vivo*, the differences in structure will likely be apparent. It is interesting that increased levels of oleate-containing CER EOS occurs at the expense of linoleate-containing CER EOS in samples of stratum corneum in winter versus summer (ratio of oleate to linoleate 1.74 : 0.51, respectively) and that even further increases in this ratio occur with ageing (0.3) [83]. These changes together with the general reduction in ceramide levels indicate that the LPP will potentially decrease in these conditions and might partly explain the susceptibility to dry skin and reduced barrier function in winter and during ageing. It is important to point out that others have found similar seasonal changes in epidermal glucosylceramides [84] but seasonal variations in stratum corneum ceramides were not observed in a Japanese population. It has been suggested that the skin does not produce vitamin D in winter in northern latitudes. Perhaps this accounts for the differences in skin functionality and epidermal differentiation. Barger-Lux *et al.* [85] have shown that median levels of circulating vitamin D decrease from 122 nmol L^{-1} in summer to 74 nmol L^{-1} in winter. Similar changes in other essential nutrients and hormones are anticipated to influence skin condition. As vitamin D cannot be used in cosmetic products, this could represent an opportunity for oral supplementation to improve skin condition.

Interestingly, adaptive responses in skin barrier function in subjects living in a hot or a humid environment [154]. Subjects in Arizona possessed a stronger skin barrier than those living in New York, and they also had a greater amount of ceramides. In this respect, further insights into differences in stratum corneum composition have also become evident on other body sites, especially the more humid axillary region of the body. Changes in the ceramide to cholesterol ratio has been described in the axillary region and using FTIR a more ordered lipid lamellae behaviour was found, suggesting that the elevated cholesterol levels might phase separate causing a reduced barrier function [86].

Recently, Yakota *et al.* [87] at Kanebo classified subjects with sensitive skin using a variety of techniques including TEWL. Type I was classified as a low barrier function group with high TEWL and abnormal desquamation. Type II was defined as having normal barrier function but with a thickened epidermis and high density of dermal papillae. Type III was specified as a pseudo healthy group with normal barrier function. All groups were sensitive in a lactic acid stinging test. More interestingly, all groups had higher levels of stratum corneum nerve growth factor (NGF) compared with a normal control group. In sensitive skin, hypersensitivity can be expected to be accompanied with changes in the nerve system. For instance, it has been reported that increased nerve fibres are found in dry skin and atopics [88]. Although treatments for the most affected group were reported (see below), further investigation of the NGF abnormalities are required. Other useful markers of sensitive skin include the IRAP/Il-1 ratio as explained by Takahashi at Kanebo [89].

Little is known of the physiological changes in stratum corneum enzymes except in dry skin. However, differences in activity levels are observed on different body sites. The stratum corneum of the axilla appears to be unique in that the levels of SCCE are dramatically lowered indicating a potential for aberrant desquamation which may be because of the increased hydration of this body site. A similar adaptation response is seen in hot versus humid climate, where a greater amount of SCCE is found in subjects living in Arizona [75]. Surprisingly, an age-related decline in SCTE but not SCCE exists [90]. Cathepsin D also decreases in ageing and in psorasis [91]. Differences in activity of cathepsin D have also been reported with lower levels in forearm skin compared with forehead stratum corneum. Conversely, SCCE activities were similar on these two body sites.

Corneotherapy and the management of dry skin

From the current understanding of the compositional changes in dry skin, five aspects of stratum corneum lipid biochemistry need to be corrected:

1 The lowered levels of ceramides generally
2 The phytosphingosine-containing ceramide insufficiency
3 The ceramide one linoleate (CEOS) insufficiency
4 The lowered covalently bound ceramides
5 The precise chain length of the ceramide sphingoid bases and free fatty acids.

Overall, however, the lipid lamellar architecture in the outer layers of the stratum corneum needs to be normalized in dry flaky skin conditions. Evidence also indicates that a reduction in long-chain fatty acids also occurs in SLS-induced dry skin. As these lipids are important for inducing an orthorhombic lateral packing state, these will also need to be supplied to the skin to more effectively correct barrier function. As will be discussed, routes 1–3 have been investigated to differing degrees. Moreover, correction of the reduction of stratum corneum NMF levels and correction of the aberration of corneocyte envelope maturation and the impaired corneodesmolysis are needed for dry skin treatments.

Several clinical studies evaluating the effects of ceramides have been conducted recently. However, it is important to remember that to derive the full benefits of ceramide technology formulation into heavy emulsions, where other emollients dominate the formulation, will be difficult to discern unless the ceramides are at a high enough concentration. Nevertheless, two studies investigating the properties of Locobase repair cream have found opposite effects on barrier recovery. Barany *et al.* [92] could not find any improvements to placebo, whereas Kucharekova *et al.* [93] found that the CER NP-containing cream significantly reduced TEWL, erythema and epidermal proliferation compared to placebo cream. Nevertheless, further improvements in function are observed with complete lipid mixtures. De Paepe *et al.* [94] have demonstrated improvements in barrier functionality and stratum corneum hydration from a lipid mixture of CER NP (0.2%), CER AS (0.1%) and CER UP (0.2%) together with cholesterol (0.25%), linoleic acid (0.25%) and phytosphingosine (0.5%) compared with placebo lotions and a lotion containing only CER NP (0.6%) and CER UP (0.4%). The percentages of increases in TEWL and stratum corneum hydration are shown in Fig. 15. To be consistant with the ceramide nomenclature, 'U' refers to unsaturated fatty acid. Berardesca *et al.* [95] have also established that balanced lipid mixtures containing CER NP are effective in improving the barrier properties and clinical condition of skin in subjects with contact dermatitis. Equally convincing are the studies of Chamlin *et al.* [96] showing that a ceramide dominant barrier repair lipid cream alleviates childhood atopic dermatitis. Over the 6-week treatment period TEWL values decreased by 50% and the number of desquamatory tape-strippings required to break the barrier increased from approximately 12 to 22 strippings, indicating a stronger stratum corneum barrier function.

The water content in the peripheral layers of the skin can be a rate-limiting factor in the desquamatory process but the role of glycerol in ameliorating dry skin is more complex than simple provision of water. Mattai *et al.* [97] at Colgate demonstrated that glycerol modifies the phase behaviour of the lipid matrix at low humidity. Presumably, the maintenance of a lamellar phase versus a solid crystalline phase allows the desquamatory enzymes to their corneodesmosome substrates. Although gel phases have been observed in the outer layers of the stratum corneum in dry skin conditions, the lamellar matrix is totally disrupted in the outer layers of the stratum corneum. Presumably, glycerol is fluidizing this amorphous lipid mixture rather than preventing a liquid crystal to crystal transition as previously suggested. More CEr are found on exposed body sites such as the face and further increases are observed in the winter months of the year. However, the

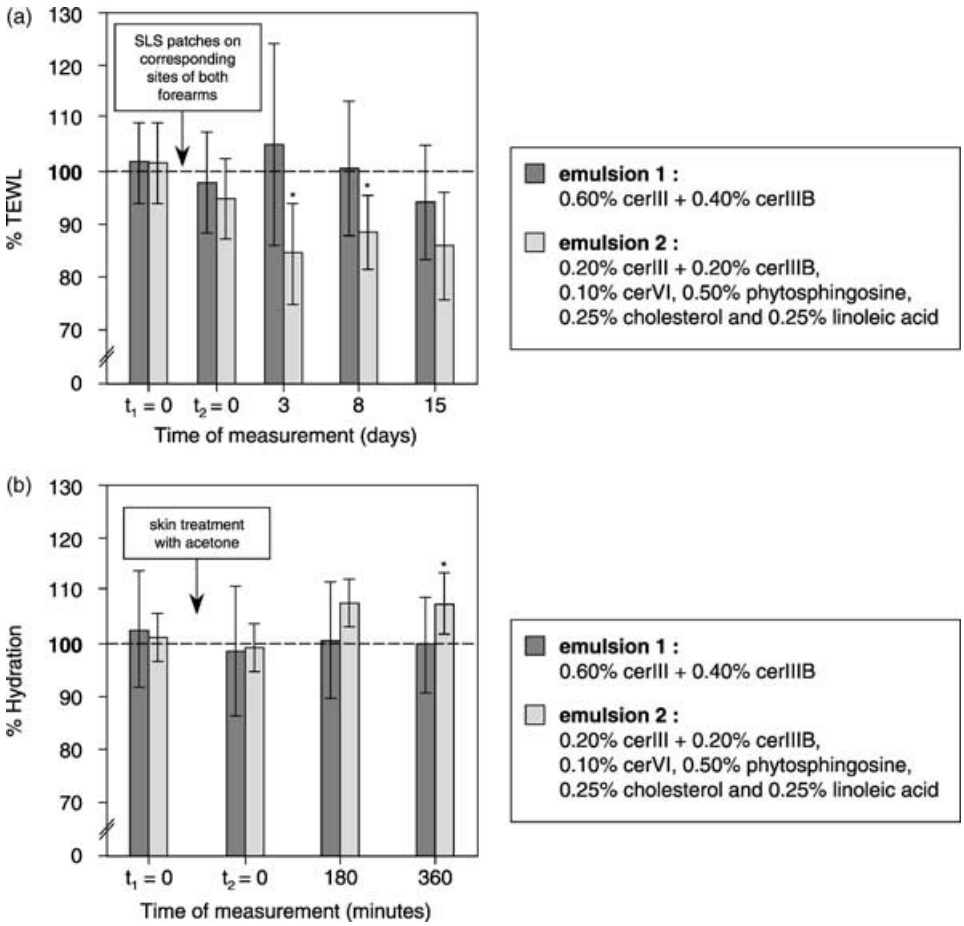

Figure 15 (a) TEWL values of the skin before ($t_1 = 0$) and after ($t_2 = 0$) SLS damage. At each data point, values and SD are given, expressed as a percentage of the controls arbitrarily set at 100%. On days 3 and 8, significant differences are shown between the TEWL values after topical application of emulsion 2 which decreased the TEWL values of the damaged forearm skin more effectively than emulsion 1. (b) SC hydration before and after acetone exposure. Measurements were also performed at 180 and 360 min after application of the emulsions. Emulsion 2 improved the hydration greater than emulsion 1. ($P < 0.05$). Modified from De Paepe *et al. JEADV.* **16**, 587–594 (2002) [94].

corneocytes retain the capability of maturing as can be seen by the application of moisturisers [98]. *In vivo* studies have demonstrated that simple moisturisers can reduce the quantity of fragile envelopes and improve barrier function. Increased TGase activities have been shown *ex vivo* to increase the maturation status of the envelopes at high humidity, by the action of a moisturiser (glycerol) or by TGase-activating substances such as calcium chloride and DTT. More recently, De Paepe [99, 102] has shown that maximal hydration at 10% glycerol levels and glycerol is superior to other general humectants. However, mixtures of sugars, saccharide isomerates (Pentavitin, Pentapharm, Ltd) that mimic those found within the stratum corneum, have been shown to possess a greater moisturizing capacity than glycerol alone [100]. As can been observed in Fig. 16, these saccharide isomerates have been proven to reduce the visual appearance of dry skin, increase skin hydration and reduce stinging to lactic acid.

The importance of glycerol and bilayer forming lipids in alleviating dry skin has been exemplified by the studies comparing bilayer-forming lipid lotion with a corresponding petroleum jelly lotion or just an emollient lotion [101]. The former delivered a faster clinical benefit (Fig. 17) as a result of a combination of the resistance of the product to soap washing and its facilitating desquamation. In a new *in vitro* soap-damaged model of corneocyte release glycerol, lotions have been shown to improve desquamation almost to a level comparable to undamaged stratum corneum [102]. Knubel *et al.* [103] at Henkel have also

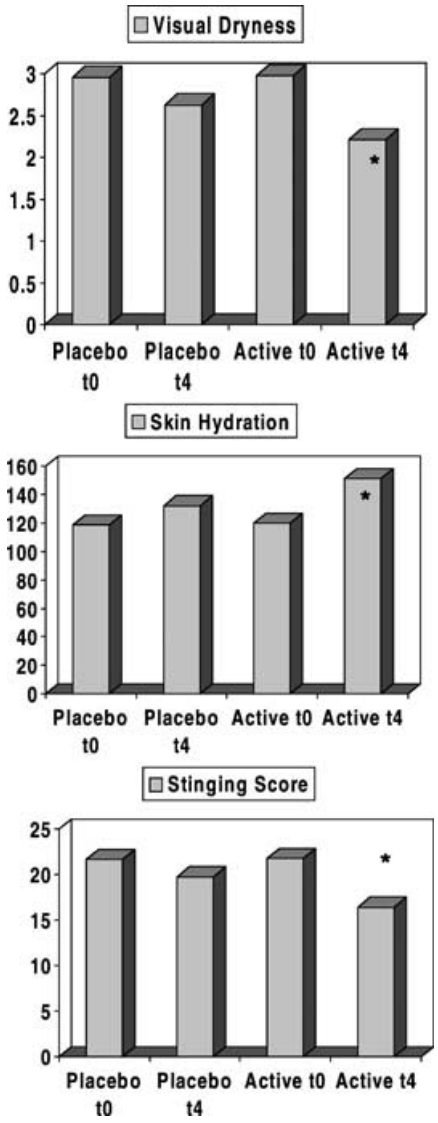

Figure 16 Effect of saccharide isomerte on visual dryness, skin hydration and facial stinging, $P < 0.05$.

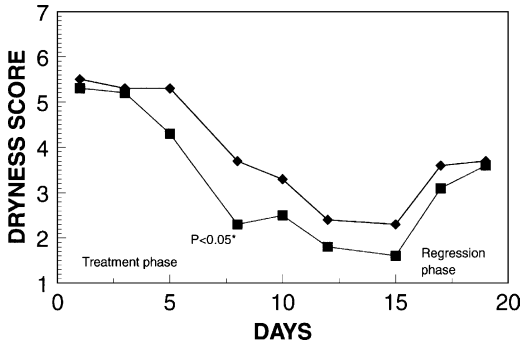

Figure 17 Moisturization efficacy test comparing the effect of a lotion containing 1% phospholipid, 2% cholesterol, 1% stearic acid plus 5% glycerol (treatment a) to a lotion containing 1% petrolatum, 2% cholesterol, 1% stearic acid plus 5% glycerol (treatment b). Modified from Summers *et al. J. Sol. Cosmet. Chem.* **47**, 27–29 (1996) [101].

developed lamellar creams similar but not identical to SC lipids that increase the degree of order of the alkyl chains of the skin lipid film, while conventional w/o cream reduces this degree of order. This resulted in greater skin moisture content in the first 6 h after treatment, but both creams were identical at 12 h as their components penetrated into the corneum.

The influence of alpha and beta hydroxy acids [104] on desquamation is now well established but new lipophilic variants of salicylic acid appear to influence corneodesmolysis differently. Whereas lactic and salicylic acid act on all corneodesmosomes, LSA act only on the stratum disjunctum corneodesmosomes. These lipophilic variants appear to act on the whole structure of the corneodesmosomes whereas the 'ordinary' acids fractionate the corneodesmosomes. Working with scientists at Avon Fartasch *et al.* [105] also demonstrated that the action of glycolic acid on corneodesmolysis was restricted to the stratum disjunctum suggesting a targeted action without compromising barrier function. Medium-chain fatty acids have also been reported by Rawlings and co-workers [106] not only to improve stratum corneum flexibility but also to assist in the relief of dry skin in combination with barrier lipids. Further enhanced dry skin relief was observed in the presence of barrier lipids [107] and the L-isomer of alpha hydroxy acids, in particular, increased stratum corneum extensibility and keratinocyte proliferation. Rawlings *et al.* [108] also reported that longer chain hydroxyacids were more effective short-chain fatty acids at facilitating corneocyte cell release in the presence of several calcium chelators. This may be the result of a fluidizing effect of these longer-chain fatty acids on the lamellar lipids as in stratum corneum extensibility studies using extensions where only lipids are believed to be extended longer-chain alphahydroxyacids plasticize the corneum [109].

Stratum corneum turnover time measured by dansyl chloride. (a measure of epidermal proliferation matched by desquamation) increased by 15% by applying a moisturizing cream at pH 3.8. However, further increases were observed with increasing concentration of the free acid of glycolic acid or by decreasing the pH of the base. At 8% glycolic acid concentration (4% free acid), Johnson [110] reported

approximately 30% increase in stratum corneum turnover time. The increased turnover time needs to be matched by increased desquamation, otherwise retention hyperkeratosis would occur, which clearly does not, in fact the opposite occurs. So, desquamation must also be enhanced by further activating acidic optimum enzymes or by also chelating calcium, which is known to reduce the final processing steps involved in Cdsn degradation.

As all corneodesmosomal proteins persist in the superficial layers of the stratum corneum in dry skin, either the full spectrum of skin desquamatory enzymes will be required to maximally induce exfoliation or broad specificities will be needed in a single enzyme. Bissett *et al.* at P&G [111] using stratum corneum cell disaggregation assays in the presence of the zwitterionic surfactant 6-octadecylammoniohexanoate demonstrated that both trypsin and subtilisin can accelerate cell dissociation. Use of broad-spectrum proteases in clinical studies has been reported by groups at Unilever, P&G and Kose [112]. It has been established that topical enzymes give a dramatic relief of dry skin faster than any ordinary moisturiser [113]. The extent of relief of dry skin can be seen in Fig. 18(a). Topical application of chymotrypsin alleviated skin scaling by at least a 2-grade change compared with 100% occlusion within 3 h. Broad-specificity bacterial proteases from *Bacillus licheniformis* were shown to be more effective than topical pancreatic chymotrypsin and papain in clinically alleviating the flaking and scaling. Morphological and immunological analysis of bacterial enzyme-treated skin revealed that topically applied protease specifically induced degradation of the corneodesmosomes thereby promoting desquamation (Fig. 18). The most recent is the work of P&G investigating the effects of proteases in hand dish-washing liquids. Immersing the hands of Korean or Japanese

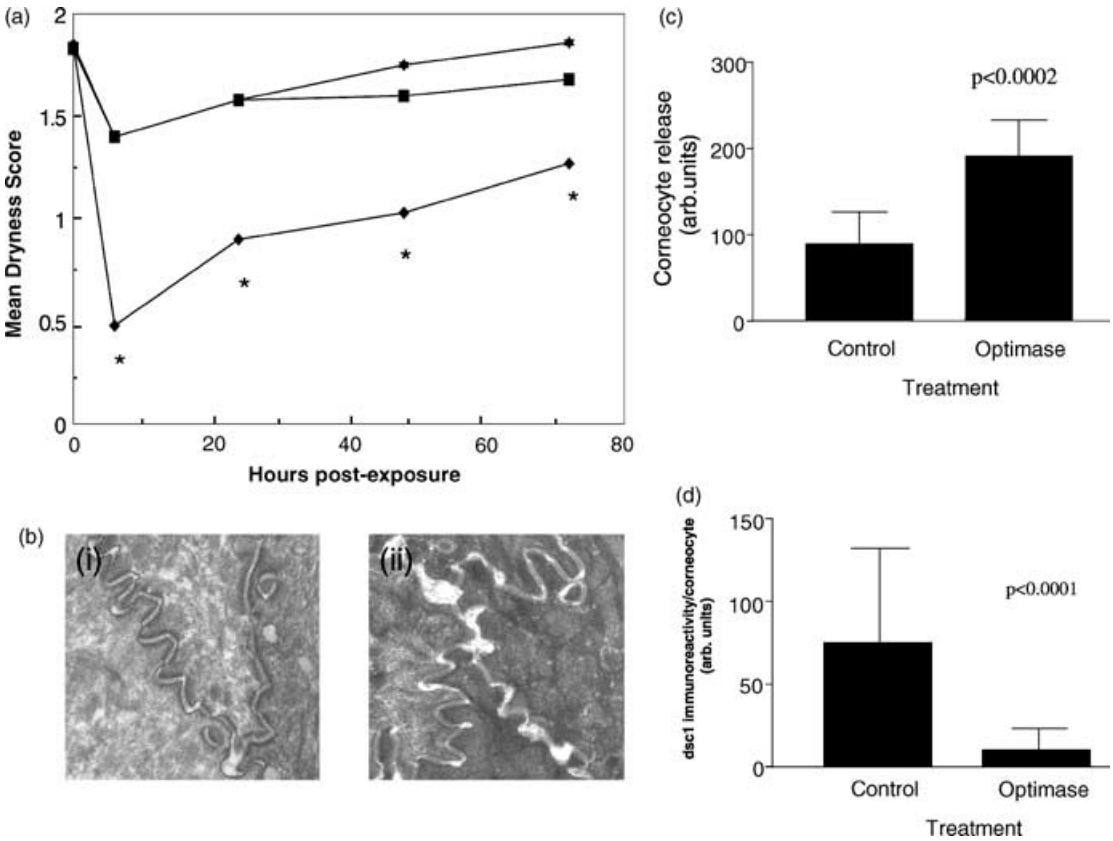

Figure 18 (a) Effect of chymotrypsin on visual scaling after 3 h occluded application. Vehicle buffer (square), heat inactivated enzyme (star) and active enzyme (diamond). $^*P < 0.05$. (b) Typical electron micrograph images of control (i) and plantar-treated (ii) stratum corneum. Optimase treated SC shows more degraded corneodesmosomes (arrow). (c) Effect of optimase on desquamation *in vitro*. (d) Effect of Optimase on desmocollin 1 degradation. Modified from Pocalyko *et al.* In: *Skin Moisturization* (J.J. Leyden and A.V. Rawlings, eds). pp 365–384 (2002) [113].

subjects for 15 min in products containing 0.005–0.02% protease for 4–9 days, the protease-containing dish-washing liquid was less irritating to skin as judged by less dry skin [114]. Topical application of cathepsin D enzyme from mushroom extract has also been shown to be beneficial for the treatment of xerosis [115].

More recently, the overactivation of the plasminogen cascade has been associated with dry skin [116]. Normally only observed in the epidermal basal layers, skin plasmin is widely distributed through the epidermis in dry skin. A new technology based upon *trans*-aminomethylcyclohexanecarboxylic acid has been shown to inhibit plasmin activity, accelerate recovery of stratum corneum barrier function in tape stripped, acetone and SLS perturbed skin, prevent epidermal hyperplasia and correct the dry skin condition. Several other trypsin-like serine protease inhibitors (leupeptin, TLCK and PMSF) accelerated barrier repair, whereas other types of serine protease metalloprotease, cysteine and aspartic protease inhibitors did not (EDTA, pepstatin, *N*-ethylmaleimide, chymostatin and TPCK [117]). Declercq *et al.* [118] at Estee Lauder, observed similar improvements in skin barrier function using egg white protease inhibitors.

Epidermotherapy: lipid synthesis enhancers

Changes in lipid levels and types can be corrected by topically applying agents to manipulate the lipid synthesis process within the viable epidermis (for a review, see Elias and Ghadially [119]). However, as described above in dry skin conditions the epidermis makes less phytosphingosine-containing ceramides, changes the carbon chain lengths of other sphingoid bases and syntheses less long-chain fatty acids. These results suggest that changes in the levels or activities of the different fatty acid synthetases as well as the enzymes involved in phytosphingosine synthesis occur in dry skin. The biology of these enzymes is yet to be described in these conditions.

Elias and co-workers [120], however, has used lipid mixtures to aid barrier recovery in acetone damaged barrier studies. Cholesterol itself was shown to aid barrier recovery in a tape stripping model in aged skin but not young skin. In fact, any incomplete mixture of one or two of the three major lipid species slows barrier recovery in this model. The equimolar mixture of the three dominant SC lipids allows normal rates of barrier recovery in normal skin whereas its further adjustment to a 3 : 1 : 1 molar ratio accelerates barrier recovery. As expected, the requirements for optimal barrier recovery in aged skin is different and it has been shown that a cholesterol dominant lipid mixture accelerates barrier recovery in aged skin whereas a fatty acid dominant mixture delays barrier recovery. In young skin, any of the lipid species can be the dominant lipid and the barrier will recover more quickly with one exception and that is in atopic dermatitis where a ceramide dominant mixture is required [121]. Further studies on the use of long-chain fatty acids are recommended. Exploiting these facts, workers from Kanebo [122] have used mevalonic acid the product of the rate-limiting enzyme HMGCoA reductase to increase cholesterol biosynthesis.

Several other routes have been shown to increase ceramide synthesis *in vivo* and improve barrier function. Alpha-hydroxy acids well known for their desquamatory properties also stimulate lipid biosynthesis. Lactic acid and especially the L-isomer increases ceramide biosynthesis *in vitro* and *in vivo*. Presumably, lactic acid achieves this by acting as a general lipid precursor by providing acetate and by providing more reducing power in the form of NADH or NADPH [123]. Corresponding improvements in barrier function were reported (Fig. 19). Interestingly,

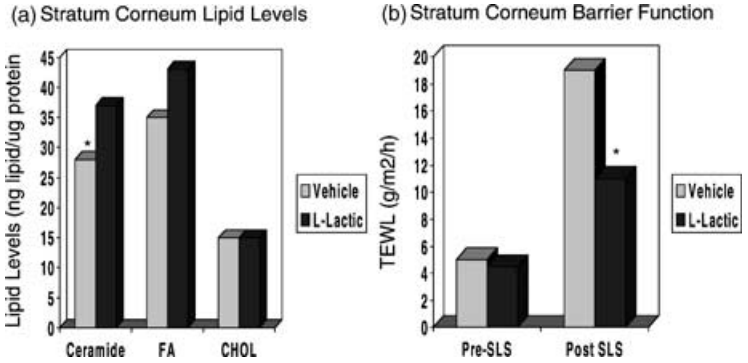

Figure 19 Effect of lactic acid on: (a) SC lipid levels and (b) SC barrier function following a 1-month topical application of 4% lactic acid in an aqueous vehicle. TEWL evaluated before application of SLS patch and 24 h after removal. $^{*}P = 0.05$. Modified from Rawlings *et al. Arch. Dermatol. Res.* **288**, 383–390 (1996) [123].

lactic acid also increased the levels of linoleate-containing CER EOS which may be contributing to these improvements in skin functionality.

The pleotropic skin benefits of niacinamide have been the subject of intense study by Procter & Gamble and have been excellently reviewed by Matts *et al.* [124]. Niacinamide has been reported to stimulate the synthesis of glucosylceramides, sphingomeylin, cholesterol and fatty acids by keratinocytes *in vitro* [125]. The increases in ceramide synthesis were achieved by enhancing the activity of SPT together with the expression of LCB 1 and 2. *In vivo*, however, increased levels of stratum corneum fatty acid (67%) and ceramide (34%) levels were observed. Similar to studies with lactic acid, increases in the levels of stratum corneum cholesterol seems to be refractory to change. In their further studies Tanno *et al.* [89] at Kanebo have also been investigating the changes in skin functionality with the presence of sensitive skin. In their most recent studies, topical application of niacinamide improved the barrier of the most severely affected subject with sensitive skin with a concomitant improvement in stinging score. Ertel *et al.* [126] observed similar improvements in barrier functionality together with an increased stratum corneum turnover rate using a 2% niacinamide cream. Draelos *et al.* [127] similarly observed a significant improvement in stratum corneum barrier function and improvement in global skin condition in subjects with stage 1/11 Rosacea.

Topical application of phytosphingosine and its derivatives has also been shown to increase stratum corneum ceramide levels and barrier function [128]. This is especially important as the phytosphingosine-containing ceramides are deficient in dry skin. Although increases in the total levels of ceramides were observed, greater increases in CER EOS and CERAS were found when combined with juniperic acid and linoleic acid. Linoleic acid on its own has also been proven to be incorporated into CER EOS *in vivo* [129], which is obviously important for the lipid phase behaviour and skin properties. Lipid fractions from unsaponifiable fractions of avocado (furanyl-8–11-*cis*-heptadecadiene) and sunflower oleodistillates (mainly linoleic and oleic acids) also increased ceramide and cholesterol biosynthesis *ex vivo* [130].

The effects of increasing stratum corneum lipid levels by stimulating ceramide biosynthesis have been investigated extensively by Denda *et al.* [131] at Shiseido. Histamine antagonists and certain fragrances stimulate lipid biosynthesis. Mixtures of magnesium and calcium salts have also been shown to accelerate skin barrier recovery and improve surfactant- or tape-stripping-induced dry skin. Although these studies indicate the importance of these ions for epidermal homeostasis more work is needed with cosmetic formulations. More recently, it has been demonstrated that gamma-aminobutyric (GABA) type A receptor agonists, musimol and isoguvacine accelerate barrier recovery following barrier disruption. Conversely, ATP (purinergic) receptor (P2X) agonists delay barrier recovery, whereas P2Y antagonists accelerate it. These also reduced the epidermal hyperproliferative response induced by acetone treatment under low environmental humidity.

Other agents have been shown to stimulate ceramide synthesis *in vitro*. Lipoic acid and *N*-acetylcysteine were also reported to increase ceramide synthesis *in vitro* [132]. Recently vitamin C has been shown to activate PKC, increase ceramide synthesis and improve the ceramide subspecies profile in epidermal skin equivalents [133]. Yarosh and Brown [134] also demonstrated that ursolic acid increased ceramides in human skin. For a complete analysis of agents that stimulate lipid biosynthesis see Table II.

Epidermotherapy: differentiation enhancers

Ellas and his team have recently identified the importance of the ligands of nuclear hormone receptors for epidermal differentiation [135–137]. This superfamily of nuclear transcription receptors includes the retinoic acid receptors, the steroid receptors, the thyroid receptors, and the vitamin D receptors and also the peroxisome proliferator activated receptor (PPAR), together with farnesol activated receptor (FXR) and the liver activated receptor (LXR). These transcription factors bind their respective ligands and regulate many of the aspects of cellular proliferation and differentiation. Fatty acids are important ligands for the PPAR receptor, farnesol for the FXR and hydroxylated cholesterol derivatives or cholestenoic acid for the LXR. All of these pathways stimulated epidermal differentiation and increased the synthesis of involucrin, filaggrin and enzymes of the ceramide synthesis pathway.

The transcription factor most intensively investigated is the PPAR. There are three main PPAR isoforms: alpha; beta/delta and gamma. Nevertheless, PPAR delta was recently observed to be the predominant PPAR subtype in human keratinocytes, whereas PPAR alpha and gamma were only induced

Table II Agents that increase ceramide biosynthesis

Lipids	Optimized mixtures of ceramides, cholesterol and fatty acids
Lipid precursors	Phytosphingosine, tetraacetylphytosphingosine, omega-hydroxyfatty acids, linoleic acid
Alphahydroxyacids	L-Lactic acid
Humectants	Glycerol, urea
Vitamins	Niacinamide, lipoic acid, ascorbic acid
Protease inhibitors	Aminocyclohexanecarboxylic acid, egg white lysozyme
Minerals	Magnesium, calcium
Histamine receptor	H1 receptor antagonist
Antagonists	H2 receptor antagonist
PPAR	PPAR alpha agonists
Electrical potential	Negative potential
Triterpenoids	Ursolic acid
GABA agonists	GABA type A agonists (musimol, isoguvacine)
Purinergic receptor	P2Y antagonists
Fragrances	Fragrances
GC receptor	Glucocorticoid receptor antagonists

during epidermal differentiation suggesting non-redundant functions during differentiation [138]. Respective ligands for all of these isoforms increased epidermal differentiation. Scientists at L'Oreal and Galderma have demonstrated that the pharmaceutical ligands for the PPAR receptors increase ceramide synthesis *in vitro* by increasing the expression of SPT, glucosyl ceramide synthase and glucocerebrosidase but not sphingomyelinase [139] (Fig. 20). More recently, PPAR delta ligands were found to be the most potent in inducing epidermal differentiation (tetrathioacetic acid) by increasing involucrin and transglutaminase while decreasing proliferation.

Petroselinic acid [140] and conjugated linoleic acid [141] have been identified as potent PPAR alpha activators that improve epidermal differentiation, reduce inflammation, increase extracellular matrix components and elicit skin lightening. *In vitro* increased levels of transglutaminase, involucrin, filaggrin and corneocyte envelope formation were observed in keratinocytes after treatment with petroselinic acid. These effects were confirmed *in vivo* by short-term patch-testing studies over 3 weeks, and increases in involucrin and filaggrin were also observed. Using this technology, improvements in the signs of photodamage and skin tone in a 12-week clinical study on forearm skin [142] were observed.

Niacinamide or its free acid is also a potential PPAR ligand and Oblong *et al.* [143] reported that niacinamide up-regulated the synthesis of involucrin and filaggrin by keratinocytes. The combination of these effects with those of the improvements of lipid biosynthesis probably account for the improvement in skin surface texture observed by multiple angle reflectance spectroscopy by Matts and Solechnick [144] at P&G.

Somatosensory problems in dry skin

Itch is a very common somatosensory problem associated with 'dry and flaky skin'. The itch sensation is mediated by the peripheral cutaneous nerve fibres (c-fibres) which respond to chemical mediators, of which the best characterized is histamine. On binding to its receptors, histamine provokes the somatosensory and vascular response. However, a variety of other mediators have been suggested to be involved with itch, such as neuropeptides, biogenic amines, cytokines and proteolytic enzymes. Classical dermatological treatments for itch, e.g. steroids and antihistamines are often either ineffective or have to be used at dosages which give unwanted side-effects The identification of molecules only expressed in skin and with a role in pruritus should be an attractive strategy for the development of new anti-itch treatments. Hansson *et al.* [145] at Arexis have developed an SCCE transgenic mouse model which overexpresses human SCCE. This phenotype spontaneously itches and has a weakened barrier function. The histochemical changes seen in the mouse model is similar to those observed in the inflammatory skin diseases. Inhibitors are being sought to control skin itch.

Other research has identified that cannabinoid agonists reduce histamine-induced itch and inflammation [146]. Both of the cannabinoid receptors are

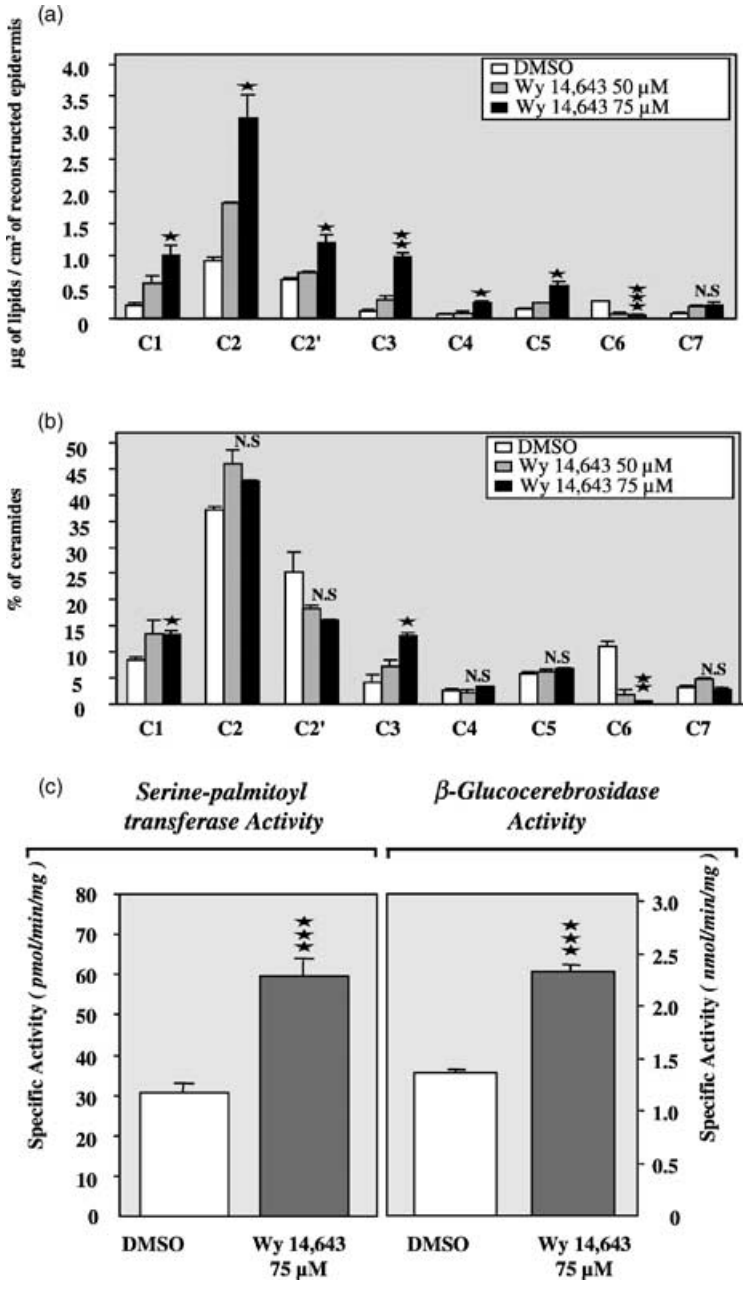

Figure 20 Activation of PPAR alpha with selective agonist Wy 14, 643 increases the ceramide content of reconstructed epidermis. (a) Mass levels of lipids (ug cm^{-2}). (b) Relative percentage of ceramides. (c) SPT and beta-glucocerebrosidase activities increased after PPAR activation. Modified from Rivier *et al. J. Invest. Dermatol.* **114**, 681–687 (2000) [139].

found in skin (CB1R and CB2R) [147]. Arachidonylethanolamide (anandamide) binds to both receptors whereas palmitolyethanolamide (PEA) and 2 arachidonylglycerol are CB2R agonists. Ligands that activate these receptors are known to be analgesic. In the recent studies, these ligands have been shown to reduce histamine-induced itch (Fig. 21). PEA in particular reduces itch whereas other *N*-acylethanolamides can increase the itching response [146] (US2002182159). Vanilloid receptors especially VR1 have also been identified in keratinocytes. This is a polymodal detector of pain producing chemicals such as capsaicin and protons in primary afferent neurones [148].

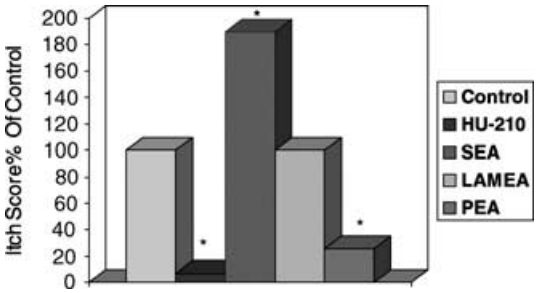

Figure 21 Agents were topically applied to forearm skin allowed to dry and then histamine was applied by iontophoresis to induce itching. The itching response was recorded on a visual analogue scale. Results are represented as percentage of control. $^{*}P < 0.05$.

Role of the stratum corneum in epidermal–dermal signalling

The role of the barrier in epidermal homeostasis has been clearly documented. However, many of the released cytokines and growth factors also have dermal effects such as vasodilatation and inflammatory cell infiltration. Nevertheless, some of these factors could also deliver a positive skin effect. In many ways, controlled transient barrier disruption could be beneficial providing that a strong barrier returns after the treatment. Smith [149] has demonstrated that chronic barrier disruption by daily tape stripping can mediate changes in skin physiology consistent with anti-ageing effects such as increases in epidermal and dermal firmness, thickness and smoothness, effects similar to that of the alphahydroxy acids. This disruption of normal barrier function would result in the generation of signals responsible for these effects such as VEGF, etc. Retinoic acid also stimulates the epidermal synthesis of a variety of growth factors and delivers similar effects to skin via this route. Equally, the thinning of the corneum and slight impairment of the barrier might contribute to the known activities of retinol by further stimulating the release of growth factors and cytokines. However, retinoic acid needs to be converted from retinol for its effects. The bioavailability of retinoic acid is primarily regulated by retinol esterification enzymes and retinoic acid degrading enzymes within the epidermis. Inhibition or activation of these enzyme systems has been the subject of intense research over the last decade, particularly lecithin retinol acyl transferase and retinoic acid 4-hydroxylase (several patents are published, a key one is EP0932387, [150]). Enhanced improvements in skin texture have recently been demonstrated when modulating these and other enzymes involved in retinoid metabolism [151]. Synergy with other nuclear receptors (PPAR, etc.) [152] has also been established and the combination of all of these routes will likely deliver the ultimate in clinical performance.

Summary and conclusions

Tremendous advances in the understanding of epidermal and stratum corneum biology have been made over the last decade. The changes in lipid biochemistry, particularly decreases in the levels of phytosphingosine-containing ceramides, reductions in the levels of long-chain fatty acids and CER EOS, together with the reductions in the levels and activity of proteases and transglutaminases, reduced corneocyte envelope maturation, and the consequent aberrant corneodesmolysis is now well established. Significant progress has been made in understanding of lipid phase behaviour and lamellar packing, particularly the presence of an orthorhombic lamellar packing together with the LPP and SPP periodicities. These lipids will either directly or indirectly influence enzyme activities in the corneum that are involved in corneocyte maturation events. Still more research is needed to understand the changes in fatty acid and ceramide biosynthetic enzymes together with the precise enzymes involved in corneodesmolysis but especially filaggrinolysis. The synthesis or processing of filaggrin may be a key factor in controlling the expression of dry skin. In its absence or when filaggrin proteolysis is reduced, corneocyte envelope maturation does not occur. Use of novel *in vivo* confocal Raman spectroscopy methodologies (Fig. 22) will help to decipher the precise location of filaggrin degradation and NMF profiles [153].

Significant progress has also been made in the management of dry skin. We now have a better understanding of the plethora of effects of a simple humectant-like glycerol. Because of the complexity of the changes in ceramide composition in different skin conditions and resulting changes in lipid packing, there is still more work to be done to develop even more effective products. However, it is clear that moisturisers containing bilayer-forming lipids are clinically more effective in alleviating dry skin compared with conventional technologies. Corneodesmolysis is the key to reducing the scaling associated with dry skin. Broad-spectrum proteases have been shown to rapidly alleviate the signs of dry skin. Optimal therapy with lipid enhancement is anticipated.

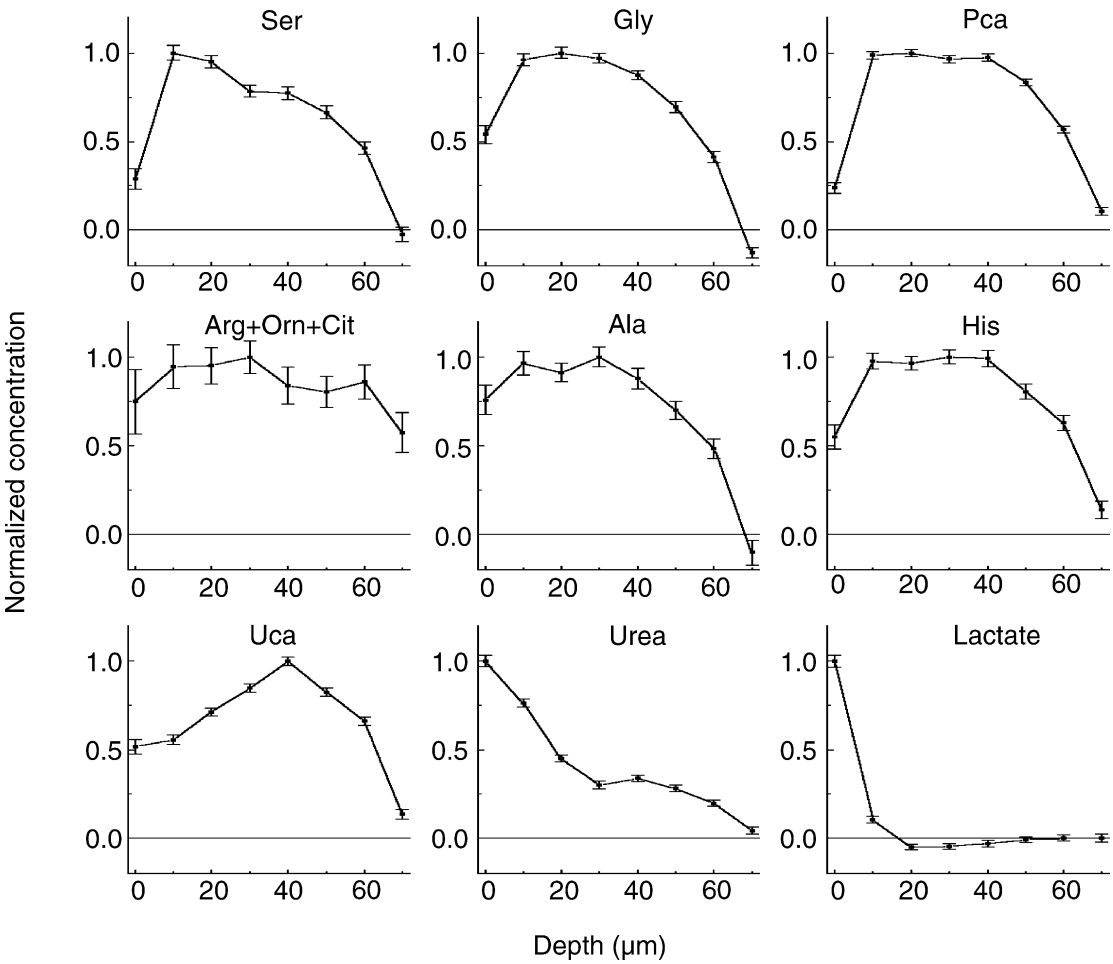

Figure 22 Semiquantitative *in vivo* concentration profiles of NMF and sweat constituents in the stratum corneum of the thenar as determined by Raman spectroscopy. From Caspers *et al. J. Invest. Dermatol.* **116**, 434–442 (2001) [153].

Lipid nutrition has become a reality with products containing ceramide precursors, such as phytosphingosine, lactic acid, linoleic acid or niacinamide. However, optimal combinations of lipid precursors targeting all the routes to stimulate lipid synthesis have not yet been exploited. The current state of our understanding of the lipid changes in dry skin indicates that we need to increase ceramide levels, particularly the levels of phytosphingosine-containing ceramides and linoleate-containing CER EOS.

Itch is still an aspect of the dry skin condition that is poorly treated. Antihistamines repair barrier function but do not always ameliorate itching. Cannabanoid agonists are an interesting technology option for itch. Controlling the inflammatory aspects of dry skin is important and protease inhibitors have been shown to improve barrier functionality.

The complexity of interactions between transcription factors during differentiation has become apparent over the last 5 years. The interplay between these transcription factors and how much redundancy there is in the system is yet to be understood. Ligands for the PPAR clearly improve epidermal differentiation and the performance of the natural moisture barrier. These types of agents can be used topically but, as many other ligands are food-derived, improvements in skin condition may be possible from a dietary perspective. This may be especially important as epidermal differentiation is sub normal in winter versus summer and may predispose the skin to xerosis. It has been argued that reduced vitamin D levels may account for this difference as a result of a reduction in UV-induced biosynthesis of vitamin D by the skin. As vitamin D cannot be used topically,

oral supplementation could be a possible route to improve skin condition.

The last decade of skin condition research has been really exciting and significant progress has been made. It is expected that research over the next decade will be even more stimulating and will lead to the development of technologies that would further control the textural and somatosensory problems associated with dry skin from a topical and/or oral perspective.

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