STRATUM CORNEUM MOISTURIZATION AT THE MOLECULAR LEVEL: AN UPDATE IN RELATION TO THE DRY SKIN CYCLE

Anthony V. Rawlings, Ph.D. and Paul J. Matts, Ph.D.*
AVR Consulting Ltd, Northwich, Cheshire, UK
*Procter & Gamble, Rusham Park, Egham Surrey, UK

Introduction

'Stratum Corneum Moisturization At The Molecular Level' [1] was published over a decade ago to review what was known about the biology of a common cosmetic problem called 'dry skin' **(Figure 1)**. At the same time Warner and Lilly [2] in 1994 published 'The correlation of water content with ultrastructure in the stratum corneum' and demonstrated for the first time the precise location of the reduced water content of the skin in dry skin conditions, namely the outermost layers of the stratum corneum (SC). These key publications described the current state of the art of dry skin knowledge. Since then significant advances have been made in our understanding of the pathophysiology of dry skin.
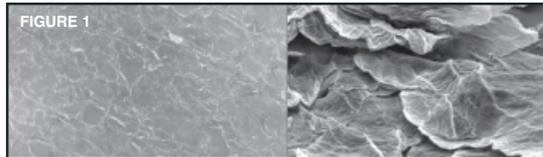

FIGURE 1. – *(Left)* Visible light macrograph of dry skin on the outer lower leg (approx. 50x), showing lifting squame. *(Right)* SEM micrograph of carbon tape applied to dry outer lower leg skin (500x); note compacted corneocytes in disarray.

Under normal circumstances, the SC must be as impermeable as possible except for a small amount of water loss to (a) hydrate the outer layers of the stratum corneum to maintain its flexibility and (b) to provide enough water to allow enzyme reactions that facilitate stratum corneum maturation events, together with corneodesmolysis and ultimately desquamation **(Figure 2)** [3-5].

Key in precipitating the condition we call 'dry skin' or cosmetic xerosis is a perturbation of water gradients within the SC. The only study to demonstrate changes in SC water gradients in dry skin is that of Warner et al [2] where about one third of the outer layers of the stratum corneum are reported to contain less than 10% water content **(Figure 3)**. As was originally reported by Blank [6] at this water content the SC will be dysfunctional and brittle.
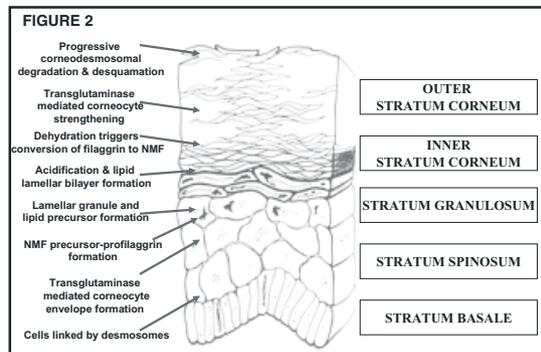

FIGURE 2. Typical structure of the epidermis and critical steps in formation of the stratum corneum. Modified from Rawlings, AV; Scott, IR; Harding, CR & Bowser, PA. Stratum Corneum Moisturization at the Molecular Level. J. Invest. Dermatol. 103 (5) 731-740 (1994) and Rawlings, AV & Harding CR. Moisturization & skin barrier function. Dermatologic Therapy. 17: 43-48 (2004).
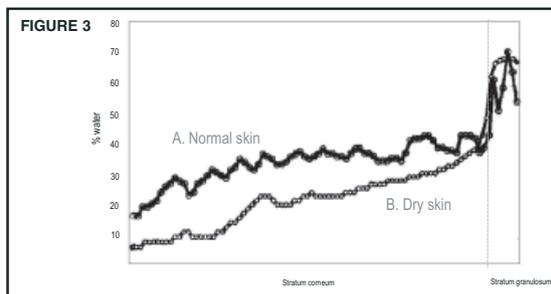

FIGURE 3. Water profile averaged over a single rectangular region of a cryosection obtained from an individual with normal skin, grade 0.5 (A). The horizontal axis is distance across the SC with the SC/granulosum junction indicated by a vertical line. Water profile averaged over a single rectangular region of a cryosection obtained from an individual with dry skin, grade 4 (B). From Warner, RR & Lilly, NA. Correlation of water content with ultrastructure in the stratum corneum. In: Bioengineering of the skin: Water & the stratum corneum. Pp 3-12. Eds: Elsner, P, Berardesca, E & Maibach HI. CRC Press Inc. (1994)

The SC uses three main mechanisms to hold onto water:

- the intercellular lamellar lipids whose physical conformation, predominantly an orthorhombic laterally-packed gel and 13nm long periodicity lamellar phase, provide a tight and semi-permeable barrier to the passage of water through the tissue,

- the presence of fully matured corneodesmosome-bound and ceramide hydrophobed corneocytes which influence the tortuosity of the SC and thereby the diffusion path length of water and

- the presence of both intracellular and extracellular hygroscopic materials called "natural moisturizing factors" (NMF).

This paper will review the latest understanding of these mechanisms and how they are disturbed in dry skin but equally review the implications of the micro-inflammatory state in such conditions and introduce the concept of a dry skin cycle.

Stratum Corneum & Epidermal Structure

Our original picture of the stratum corneum as a 'basket weave' appearance at the histological level and a stratum compactum-stratum disjunctum at the electron microscope level has come under question over the last decade. Pfeiffer et al [7] developed new high pressure freezing followed by freeze substitution techniques and visualized lipid stacks within lamellar bodies and organized microdomain-like areas in the cytosol of keratinocytes but, more importantly, the stratum corneum appeared more compact with smaller intercellular spaces and hence tighter cell-cell interactions. In the bottom layers of the SC the corneocytes were less transparent and this transparency increased towards the skin surface. No keratohyalin granules were observed in the epidermis. Norlen [8] has also developed cryo-transmission electron microscopy techniques to image vitreous sections of skin without the use of cryo-protectants. More densely-packed images are apparent compared with conventional images and new organelles or tubular structures have been observed in the epidermis. Cubic-like membrane structures with a lattice parameter corresponding to that of cubic/water phases in vitro were observed. Using the same methodology Norlen [9] has further proposed a cubic rod packing model for stratum corneum keratin structures. However, even with a more compacted stratum corneum, several hydration zones have been established by Bouwstra et al [10] and Richter et al [11] upon hydration which will be important in the development of new moisturizing technologies.

Stratum Corneum Lipid Chemistry and Biophysics

Ceramides constitute on a weight basis approximately 47%, cholesterol 24%, fatty acids 11% and cholesterol esters 18% [12]. Cholesterol has an important role to play in the lipid mixtures as it can increase the chain mobility of lipids present 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 glucosyl-ceramides to ceramides in the lower and upper regions of the stratum corneum. However, ceramides are the most complex of SC lipids and, given this diversity together with the identification of new ceramides, a new nomenclature based on structure, rather than the original chromatographic migration characteristics, was proposed by Motta et al [13]. In this system, ceramides are 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, O respectively whereas sphingosines, phytosphingosines and 6-hydroxysphingosine are indicated by S, P and H. Sphinganine (not previously classified) is proposed to be SP in this nomenclature system. A novel long-chain ceramide containing branched chain fatty acids is also found in vernix caseosa [14]. Typical structures of human ceramides are given in (Figure 4). Newly identified ceramides have also been found attached to the corneocyte envelope. In addition to ceramide A (sphingosine) and ceramide B (6-hydroxysphingosine), Chopart et al [15] recently identified covalently-bound omega hydroxyl fatty acid containing sphinganine and phytosphingosine ceramides. These covalently-bound ceramides should now be named CER OS, CER OH, CER OSP and CER OP.
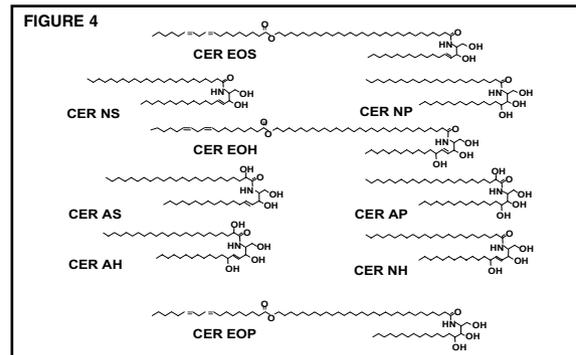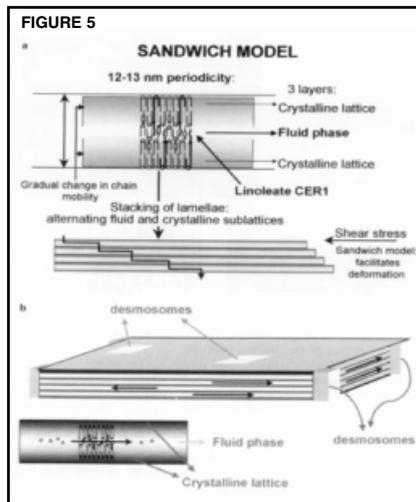

FIGURE 4. Structures of human stratum corneum ceramides.

All of the SC ceramides are synthesised from glucosyl-ceramides, epidermosides and sphingomyelin. Epidermosides are glycated precursors of omega hydroxyl-containing ceramides. The studies of Hamanaka et al [16] have demonstrated that sphingomyelin 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, CER EOH & CER EOP.

Lipids in vivo appear to exist as a balance between a solid crystalline state (orthorhombic packing) and gel (hexagonal packing) or liquid crystalline states. The orthorhombically-packed lipids are the most tightly packed conformation and have the better barrier properties and, 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 [17]. This is consistent with a weakening of the barrier towards the outer layers of the stratum corneum. It is believed that short chain fatty acids from sebum contribute to the crystalline to gel transition in the upper stratum corneum layers [18]. Bouwstra et al [19] 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 (Figure 5). 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. CER EOS, EOH and EOP play an essential role in formation of the additional lamellar arrangements. The repeated distances were found to be 13nm in dimension, composed of two units measuring approximately 5nm each and one unit measuring approximately 3nm in thickness. These repeat lamellar patterns were also observed by X ray diffraction studies and were named the "long periodicity" (LPP) and "short periodicity" (SPP) phases respectively.

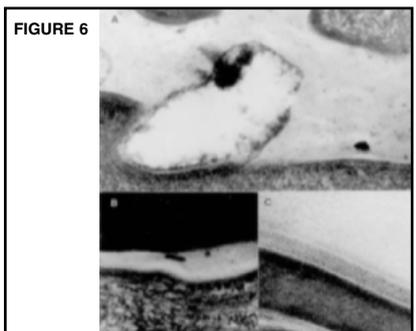In the absence of CER EOS, mostly hexagonal phases are also observed for total lipid mixtures. Nevertheless, the inclusion of CER EOS induces the formation of the LPP. Moreover, the importance of ceramide 1 or CER EOS in facilitating the formation of the long 13.4nm LPP has been further elaborated by understanding the influence of the type of fatty acid esterified to the omega hydroxyl fatty acid [20].

FIGURE 5

SANDWICH MODEL

12-13 nm periodicity:

FIGURE 5a. 'Sandwich model', the characteristics of which are: (1) the liquid sublattice is located in the central lipid layer of this phase, in this layer mainly unsaturated linoleic acid and cholesterol are present; (2) in the sublattice adjacent to the central layer a gradual change in lipid mobility occurs due to the presence of less mobile long saturated hydrocarbon chains; (3) 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. 5b. The liquid phase parallel to the basal layers of the lamellae facilitates transport and therefore communication between the desmosomes. Modified from Bouwstra J, Pilgram G, Gooris G, Koerten H and Ponec M. New aspects of the skin barrier organization. Skin Pharm Appl Skin Physiol 14: 52-62 (2001).

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. 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. It is important to remember in vivo that the fatty acid composition of CER EOS is highly complex but contains a large proportion of linoleic acid.

Changes to the composition of the SC lipids could, therefore, dramatically influence the condition of the skin. In the surface layers of the stratum corneum, cholesterol sulphate & CER EOS are hydrolysed, concentrations of short chain length fatty acids are increased while pH and water levels are decreased, increased crystallisation of cholesterol occurs and decreased ceramide levels occur and, as a result, perturbations to the lamellar ordering can be observed. In this respect, using electron microscopy of tape strippings from the outer layers of normal healthy skin, Rawlings et al [21] reported complete loss of lamellar ordering in the outer layers of the stratum corneum **(Figure 6)**. These results have been confirmed by Warner et al [22] and Berry et al. [23].

FIGURE 6

FIGURE 6. Organisation of stratum corneum lipids in tape-strippings of individuals with clinically normal skin. Transmission electron micrographs of tape-strippings. Ultrastructural changes in lipid organisation 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. (x200,000). From: Rawlings, AV, Watkinson, A, Rogers, J, Mayo, AM, Hope J and IR Scott, Abnormalities in stratum corneum structure, lipid composition and desmosomal degradation in soap-induced winter xerosis, J Soc Cosmet Chem 45 203-220 (1994)

Stratum Corneum Corneodesmosomes and Corneodesmolysis

A fuller description of the original 'brick and mortar' model introduced "corneodesmosomes", modified and specialized desmosomes. The corneodesmosomes [24] 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 & Dsc 1 are linked to keratin filaments via corneodesmosomal plaque proteins such as plakoglobin, desmoplakins and plakophilins. The corneodesmosomal protein, corneodesmosin (Cdsn), after secretion by the lamellar bodies with the intercellular lipids, and certain proteases, becomes associated with the desmosomal proteins just before transformation of desmosomes into corneodesmosomes. As these proteins are cross-linked into the complex by transglutaminase, their controlled disruption must occur by proteolysis. Indeed, Rawlings et al [21 and **Figure 7**] demonstrated degradation of the corneodesmosomes towards the surface of the SC in humans.
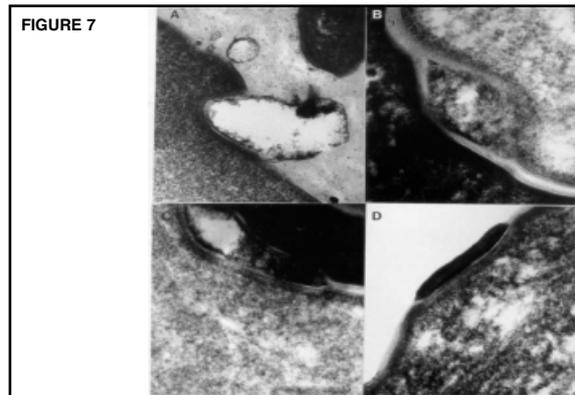
FIGURE 7

FIGURE 7. Electron micrographs of tape strippings of normal skin (grade 1). Degradation of corneodesmosomes (CD) toward the surface of the stratum corneum: A. First strip; CD fully degraded. B. Second strip; CD partially degraded and encapsulated by lipid lamellae. C. Third strip; CD partially degraded, vaculation of structure. D. Third strip, normal CD in contact with lamellar lipids. From: Rawlings, AV, Watkinson, A, Rogers, J, Mayo, AM, Hope J and IR Scott, Abnormalities in stratum corneum structure, lipid composition and desmosomal degradation in soap-induced winter xerosis, J Soc Cosmet Chem 45 203-220 (1994)

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 corneodesmosomal linkages. Currently, 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 now known as Cathepsin L-2), cathepsin E and the aspartic protease cathepsin D. SCCE & SCTE are alkaline-optimal enzymes whereas the latter ones are acidic-optimum enzymes [25-29]. Cathepsin L has also recently been implicated in corneodesmosin hydrolysis [30]. Only SCTE and not SCCE, however, was capable of degrading Dsg1 [31]. This enzyme was also reported to be involved in the processing of pro-SCCE. Bernard et al [32] has also identified an endoglycosidase, heparanase 1, within the stratum corneum, thought to play a role in the pre-proteolytic processing of the protecting sugar moieties on corneodesmosomal proteins. Cdsn undergoes several proteolytic steps. Cleavage of the N terminal glycine loop domain occurs first at the compactum disjunctum interface (48-46KDa to 36-30 KDa transition), followed by cleavage of the C terminal glycine loop domain in exfoliated corneocytes

(36-30KDa to 15KDa transition) [33]. The last step appears to be inhibited by calcium resulting in residual intercorneocyte cohesion. Nevertheless, the presence of oligosaccharides did not protect Cdsn against proteolysis by SCCE [31]. A list of the putative desquamatory enzymes is given in **Table 1**.

TABLE 1 - ENZYMES INVOLVED IN THE DESQUAMATORY PROCESS

Sphingoid Hydrolases Ceramidase		
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/L2)
	Stratum corneum cathepsin L-like enzyme	(SCCL)
Aspartic Proteases	Stratum corneum cathepsin D-like enzyme	(SCCDE)
	Stratum corneum cathepsin E-like enzyme	(SCCEE)

Many of these enzymes are thought to exist as proforms and have been immunolocalised to the intercorneocyte lipid lamellae. Sondell et al [34] used antibodies that immuno-react precisely with pro-SCCE to confirm 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 [35] demonstrated that the processed enzyme was more associated with the corneodesmosomal plaque. More recently Igarashi et al [36] have immunolocalised cathepsin D to the intercellular space, whereas cathepsin E was localised within the corneocytes. Finally, KLK8 has also been reported to be localised to the intercellular spaces of the SC [37].

As some of the desquamatory enzymes have been immunolocalised to the intercellular space, the physical properties of the stratum corneum lipids, together with the water activity in this microenvironment, will influence the activity of these enzymes. 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 [38]. 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, alpha-1-antichymotrypsin and the SPINK5 derived peptides [39]. Nevertheless, anti-leukoprotease is believed to be the major physiological inhibitor of SCCE; the serpins are too low in concentration to be physiologically relevant [40]. Caubet et al [31] recently speculated in a new model of desquamation that SPINK5 may also inhibit SCTE.

Currently, little is understood of the molecular activation mechanisms of SCCE or other enzymes within the stratum corneum. Clearly, stratum corneum pH and water content will influence enzymic activity. As the stratum corneum pH declines towards the surface of the skin, the activity of SCTE and SCCE may be reduced and perhaps the acid optimal cathepsin enzymes mediate the final desquamatory steps.

Corneocyte Envelope Maturation & The Role Of Transglutaminases

The corneocyte envelope is an extremely stable and insol-uble 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 [41]. 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, 2, 3 & 5. However, only TGase 1, 3 and 5 are thought to be involved in keratinocyte differentiation.

At early time points in the keratinocyte differentiation process, envoplakin and periplakin are expressed and become associated with desmosomes in the viable epider-mis. Subsequently, involucrin (the glutamyl rich protein that covalently-bound lipids become attached to) is expressed at the same time as TGase 1 [42-45]. 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 reinforce-ment 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 suggests that TGase 3 and possibly TGase 5 initiate the heterodimerisation and homodimerisation cross-links between these proteins before TGase 1 finally cross-links them into the pre-existing scaffold. The toughness, strength and flexibility of the corneo-cyte envelopes are thought to be dictated by the SPRR proteins. There are two general groups of proteins: Group 1 (SPRR 1A, 1B, 3 and 4) and Group 2 (SPRR 2A, 2B, 2C, 2D, 2E, 2F and 2G). Group 2 proteins are the most flexible [46].

Using Normarski microscopy, the corneocyte envelopes (CE's) 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). Mils et al [47] reported that about 80% of corneocytes from volar forearm skin were smooth and rigid, whereas 90% from foot sole were rough or fragile cells. They can also be further differentiated by their binding of tetra methyl rhodamine isothiocyanate (TRITC), with the rigid envelopes staining to a greater extent **(Figure 8)** [48]. However, Hirao et al [49] have used a more elegant method
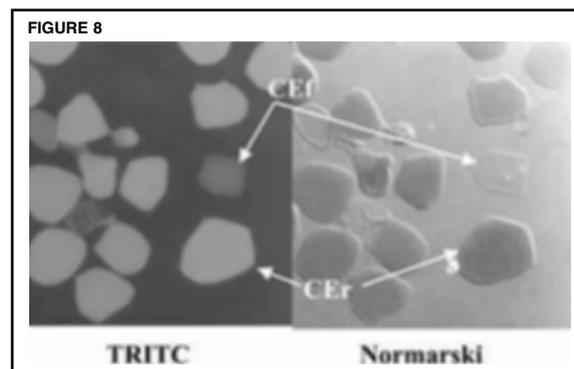

FIGURE 8

FIGURE 8. Fluorescence & Normaski phase contrast microscopy of TRIC stained cornified envelopes demonstrating increased fluorescence labelling of Cer compared with CEf. Harding CR, Long S, Richardson J, Rogers J, Zhang Z, Bush A & Rawlings AV. The cornified cell envelope: an important marker of stratum corneum maturation in healthy and dry skin. Int. J. Cosmet Sci. 25:1-11 (2003).

to identify corneocyte envelopes based upon their hydropho-bicity (staining with Nile red) and antigenicity (to anti-involu-crin; **Figure 9)**. 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
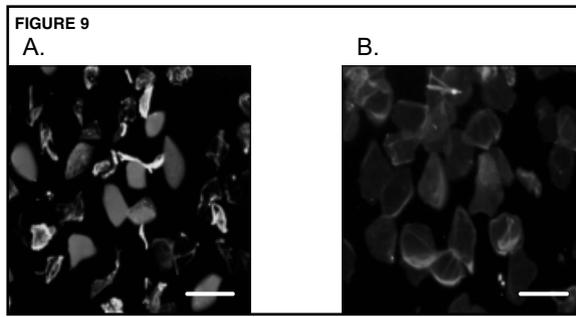
FIGURE 9. Double Staining of CE's with Nile red and anti-involucrin. A. Face. B. Upper Arm. Modified from Hirao T, Denda M and Takahashi M. Identification of immature cornfield envelopes in the barrier-impaired epidermis by characterization of their hydrophobicity and antigenicities of the components. Exp. Dermatol. 10: 35-44, (2001).

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). More recent work from Kashibuchi et al [50] using atomic force microscopy confirmed these structural changes in corneocytes from the deeper layers of the stratum corneum.

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 **(Figure 10)** [48]. Supporting this concept of increasing CE strength, gamma glutamyl-lysine cross-links also increase in the
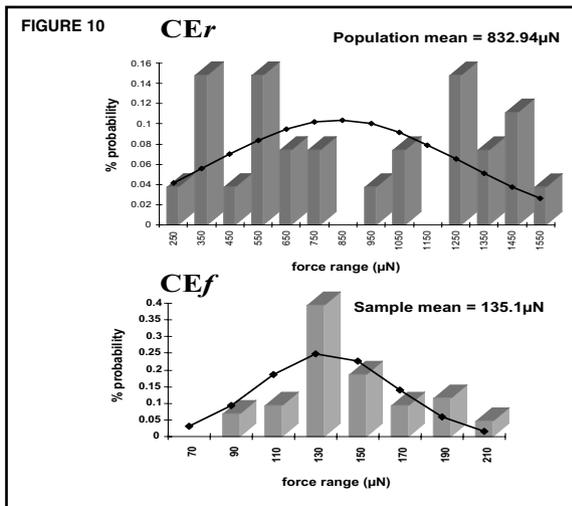

FIGURE 10. Distribution profile of the maximal compressional forces (uN) of individual CE's. Top panel shows the force range for Cer and the bottom for CEf. The maximal compression force was significantly different between the corneocytes. Harding CR, Long S, Richardson J, Rogers J, Zhang Z, Bush A & Rawlings AV. The cornified cell envelope: an important marker of stratum corneum maturation in healthy and dry skin. Int. J. Cosmet. Sci. 25:1-11 (2003).

subsequent layers of the stratum corneum, due to enhanced TGase activity. Three pools of TGase activity have been identified in the stratum corneum which 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.

Stratum Corneum Natural Moisturising Factors (NMF)

A historical perspective on filaggrin biology and generation was given by Rawlings et al [1] but, for completeness, a

summary diagram is provided in **(Figure 11)**. Biologically, NMF allows the outermost layers of the SC to retain moisture against the desiccating action of the environment. Traditionally, it was believed that this water plasticized the SC, keeping it resilient by preventing cracking and flaking which might occur due to mechanical stresses. The general mechanisms by which these NMF components influence SC functionality have been studied extensively. From a physical chemistry perspective, the specific ionic interaction between keratin and NMF, accompanied by a decreased mobility of water, leads to a reduction of intermolecular forces between the keratin fibers and increased elastic behaviour. Recent studies have emphasized that it is the neutral and basic free amino acids [51], in particular, that are important for the plasticization properties of the SC.
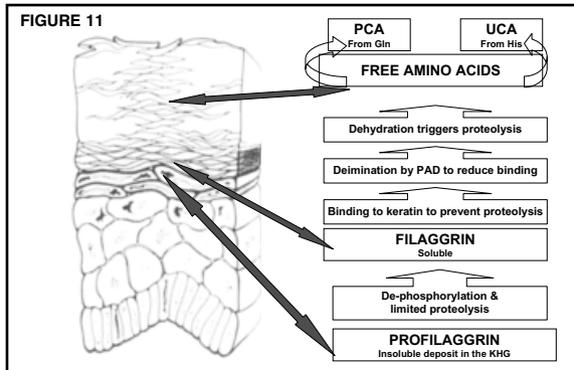

FIGURE 11. Schematic representation of profilaggrin catabolism and filaggrin hydrolysis to NMF within the SC.

Recently, hyaluronic acid has been shown to be present naturally in the SC [52] as has glycerol. Glycerol will also be derived from sebaceous triglyceride breakdown and again, to emphasize the importance of this molecule, studies by Fluhr et al [53] have indicated that topically-applied glycerol can completely restore the poor quality of SC observed in asebic mice (that have no sebaceous secretions) to normal. However, typically, these two molecules have been largely ignored in descriptions of NMF composition [1]. Recent data also indicates that lactate plays a critical role in influencing the physical properties of the SC. Lactate and potassium were found to be the only components of the NMF analyzed that correlated significantly with the state of hydration, stiffness and pH, in the SC [54].

The generation and maintenance of an acid pH within the SC, the so-called "acid mantle", is critical to the correct functioning of this tissue. Studies point to an essential role of free fatty acids generated through phospholipase activity as being vital for SC acidification [55], whilst Krein and Kermici [56] have recently proposed that urocanic acid (UCA) plays a vital role in the regulation of SC pH. However, studies on the histidase-deficient mouse (which cannot generate UCA through catabolism of free histidine), indicate SC pH is within the normal range, and this observation argues against the importance of UCA. Nevertheless, it is likely that NMF components contribute significantly to the overall maintenance of pH.

Other components of NMF are also not derived from filaggrin and urea, like lactate, may also be derived in part from sweat. However, the presence of sugars in the SC represents primarily the activity of the enzyme beta-D-glucocerebrosidase, as it catalyses the removal of glucose from glucosylceramides to initiate lipid lamellae organization in the deep stratum corneum [1].

Nevertheless, the most important development in this area of SC research has not been a greater understanding of the biology but has been the development of new measurement tools. Caspers et al [57] have pioneered the use of confocal Raman microspectroscopy to determine the

concentration of defined NMF components, non-invasively, in vivo within the SC [59]. Typical depth-concentration profiles can be seen in **(Figure 12)**.
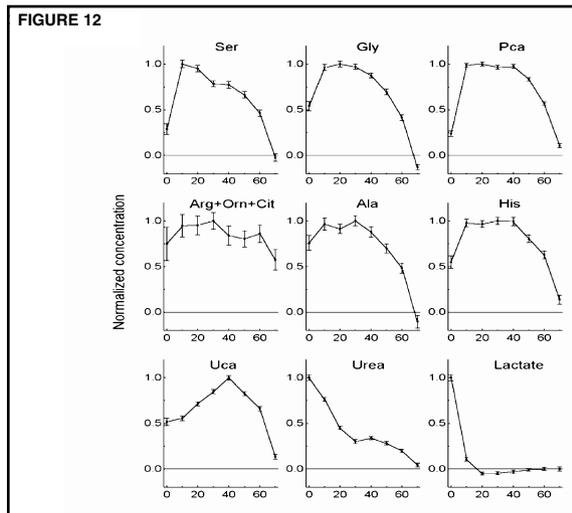

FIGURE 12. Semiquantitative in vivo concentration profiles of NMF and sweat constituents in the stratum corneum of the thenar as determined by Raman spectroscopy. From Caspers PJ, Lucassen GW, Carter EA, Bruining HA and Puppels GJ. In vivo confocal raman microspectroscopy of the skin: non-invasive determination of molecular concentration profiles. J. Invest. Dermatol. 116: 434-442 (2001).

The effect of humidity on epidermal differentiation and stratum corneum quality

Before considering the biology of dry skin, it is important to review the effect of environmental conditions on the SC, as these are the primary initiating events for the precipitation of the condition. In studies conducted in the summer and winter months of the year in the UK, Rogers et al [58] demonstrated that there was a significant reduction in the levels of SC ceramides, fatty acids, together with linoleate-containing CER EOS in subjects in winter. Similar differences in scalp lipid levels have been observed between the wet and dry seasons in Thailand [59]. However, more recently and more importantly, Declercq et al [60] have reported an adaptive response in human barrier function, where subjects living in a dry climate like Arizona (compared with a humid climate in New York) had much stronger barrier function and less dry skin due to increased ceramide levels and increased desquamatory enzyme levels (SCCE & SCTE).

Several animal studies have been conducted that support these findings. TEWL was reduced by approximately 30% in animals exposed to a dry (<10%RH) environment due to increased lipid biosynthesis, increased lamellar body extrusion and a slightly thicker SC layer whereas, in animals exposed to a high humidity environment (80%RH), this induction of lipid biosynthesis was reduced [61]. However, abrupt changes in environmental humidity can also influence stratum corneum moisturization [62]. After transferring animals from a humid (80%RH) to dry (<10%RH) environment, a six fold increase in TEWL occurred. Barrier function returned to normal within 7 days due to normal lipid repair processes. These changes did not occur in animals transferred from a normal to dry humidity environment. Similarly, findings were reported for the water-holding capacity and free amino acid content of the stratum corneum. Katagiri et al [63] demonstrated that exposure of mice to a humid environment, and subsequent transfer to a dry one, reduced skin conductance and amino acid levels even 7 days after the transfer to the new environment whereas, after transfer from a normal environment, decreased amino acid levels recovered within three days.

Exposure to low humidity conditions also increases epidermal DNA synthesis and amplifies the DNA synthetic response to barrier disruption [64]. Equally, when in a dry environment epidermal IL-1 levels increased and increased levels of this cytokine were greater when the barrier was experimentally-challenged [65]. More recently, the same group also reported increased numbers of mast cells and increased dermal histamine levels (but unchanged epidermal histamine levels) [66]. Others have reported increased SC nerve growth factor levels [67] and increased c-fibers in dry skin, elicited by dry environments [68]. These events would lead to increased skin itching in these conditions.

These changes in barrier properties of the SC are attributable to changes in SC moisture content and provide evidence that changes in environmental humidities contribute to the seasonal exacerbation or amelioration of xerotic skin conditions which are characterized by a defective barrier, epidermal hyperplasia and inflammation.

The pathophysiology of winter & soap induced dry skin

The differences in SC water concentration profiles between normal and dry skin influence the enzymic reactions in the SC. 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 [21] but have been confirmed more recently by Simon et al [69]. 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 [70] and Rawlings et al [21]. Dsc 1 levels were also reported to be increased by the latter group [71]. More recently Cdsn and plakoglobin were found elevated in dry skin [69]. Interestingly, however, in winter xerosis, the accumulation of the corneodesmosomal proteins, Dsg 1 and plakoglobin, correlate with each. Cdsn protein levels, which were also increased, do not, however, have such an association suggesting that different proteolytic mechanisms occur for the different corneodesmosomal components during desquamation. As suggested by Simon et al [69], 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 Dsg1 was observed. Perhaps the intracellular portions of Dsg 1 are also degraded within the corneocyte (for example, plakoglobin by the trypsin-like activity or cathepsin E activity reported within the corneocyte matrix. 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 [21] showing that corneodesmosomes become internally vacuolated, followed by complete detachment of the protein structures from the corneocyte envelope **(Figure 7)**.

The lamellar lipid matrix is also perturbed dramatically in dry skin **(Figure 13)** [21]. As the main desquamatory enzymes are found within this lipid matrix, the physical properties of the lamellar lipids will, therefore, influence enzyme activity.

Rawlings et al [4] originally reported that stratum corneum SCCE levels were reduced in the outer layers of xerotic stratum corneum compared with normal skin. This has been confirmed recently in more extensive studies by Van Overloop et al [72] who also found that the equally important stratum corneum SCTE activities were also reduced. Conversely, in SLS-induced dry skin, increased activities of these enzymes are reported [26]. More recently, the over-activation of the plasminogen cascade has been associated with dry skin. Normally only observed in the epidermal basal layers, skin plasmin is widely distributed
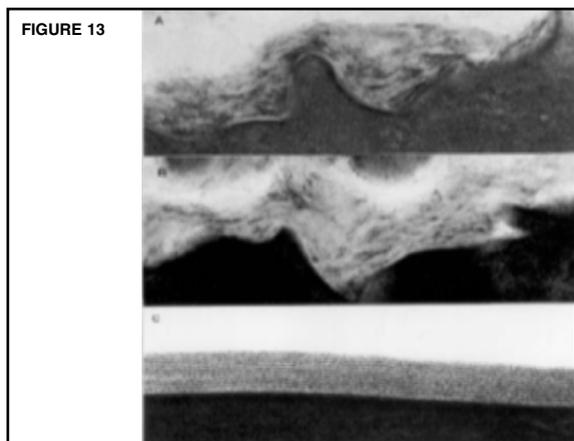
FIGURE 13. Organisation of stratum corneum lipids in tape-stripping of subjects with winter xerosis. Transmission electron micrographs of tape strippings of individuals with severe xerosis. Perturbation in lipid organisation towards the surface of the stratum corneum. D. First strip; disorganised lipid lamellae. E. Second strip; disorganisd lipid lamellae. F. Third strip; normal lipid lamellae (x200,000). From: Rawlings, AV, Watkinson, A, Rogers, J, Mayo, AM, Hope J and IR Scott, Abnormalities in stratum corneum structure, lipid composition and desmosomal degradation in soap-induced winter xerosis, J Soc Cosmet Chem 45 203-220 (1994)

through the epidermis in dry skin. Interestingly, a urokinase-type plasminogen activator also exists in the stratum corneum [73]. Clearly these and other enzymes are potentially involved in the inflammatory and hyperproliferative aspects of dry skin.

It has been well established that, in hyperproliferative disorders such as dry skin, there is a change in stratum corneum lipid composition. In particular, 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 the SC of subjects with dry skin. Fulmer & Kramer [74] 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 [75] could not find any changes in ceramide levels in dry skin, but found increased fatty acid levels. Rawlings et al demonstrated the reduced levels of ceramides at the surface of the SC in winter xerosis [21]. At this time, the full complexity of the different ceramide structure was not known but, more recently, Chopart et al [76] observed dramatic reductions in the levels of phytosphingosine-containing ceramides in dry skin (approximately 50%), together with a shortening and lengthening of the acyl sphingoid bases sphingosine and 6-hydroxysphingosine, respectively. Van Overloop et al [72] also clearly demonstrated that the phytosphingosine-containing ceramides were reduced to a greater extent than other ceramides, with increasing dryness levels. Fulmer and Kramer also observed dramatic reductions in the levels of long chain fatty acids in dry skin [74]. Imokawa et al [77] did not find reduced ceramide levels in xerotic skin (but only average levels, rather than superficial levels, were measured).

These changes in lipid composition will, of course, influence the lamellar packing of the lipids. In fact, Schreiner et al [78] established a reduction of CER EOS & EOH with increased concentrations of sphingosine-containing ceramides (CER NS & CER AS) and crystalline cholesterol in association with a loss of the LPP. However, although the lipid ultrastructure is clearly aberrant in the outer layers of dry skin [21], more work is needed to ascribe a particular lipid phase.

The proportions the different corneocyte envelope

phenotypes also change in subjects with dry skin [41, 48]. Soap washing leads to a dramatic increase in the levels of the fragile envelope phenotype at the expense of the rigid phenotype **(Figure 14)**. 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.
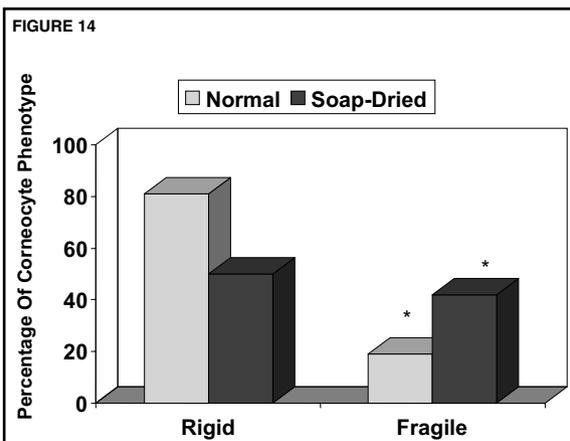

FIGURE 14. Percentage distribution of Cer and CEf in normal and soap dried dry skin. *p<0.05. Harding CR, Long S, Richardson J, Rogers J, Zhang Z, Bush A & Rawlings AV. The cornified cell envelope: an important marker of stratum corneum maturation in healthy and dry skin. Int. J. Cosmet. Sci. 25:1-11 (2003).

Reduced NMF levels are also implicated in dry skin conditions. The loss of NMF generally reported with increased ageing, however, is not consistent with the recent observations of Takahashi and Tezuka [79] of increased NMF in subjects with senile xerosis suggests that our understanding of this process is far from complete..

The 'Dry Skin Cycle' model: a new way to describe induction & propagation of xerosis

Classically, dry skin has been described in two ways – (a) as a condition that is simply either present or not or (b) as a linear progression of sequelae, resulting in the concomitant development of clinical tools such as linear visual grading scales, etc. Whilst not refuting the validity of these, it is proposed that the induction and propagation of dry skin conditions may be best and most intuitively expressed as a cyclical model, dependent on stratum corneum integrity and particularly upon barrier function and homeostasis.

A cyclical model implies a spiralling deterioration in outcome that, without intervention, would lead to a progressive worsening in model endpoints. Additionally, it is implicit that intervention at one, or preferably multiple, points within this cycle is necessary to arrest the progression of this continuing downward spiral. This is indeed the case with most dry skin conditions and, moreover, reflects extremely well consumer perception of dry skin – the seeming repetitive cycle of product usage, re-usage, disappointment with treatment outcome and, often, a corresponding loss of compliance. The model described below describes several phases within this cycle and, therefore, possible targets against which treatments could be directed. Reference to the graphical depiction of the model below **(Figure 15)** may facilitate complete understanding of the relationship of these phases, one with another.
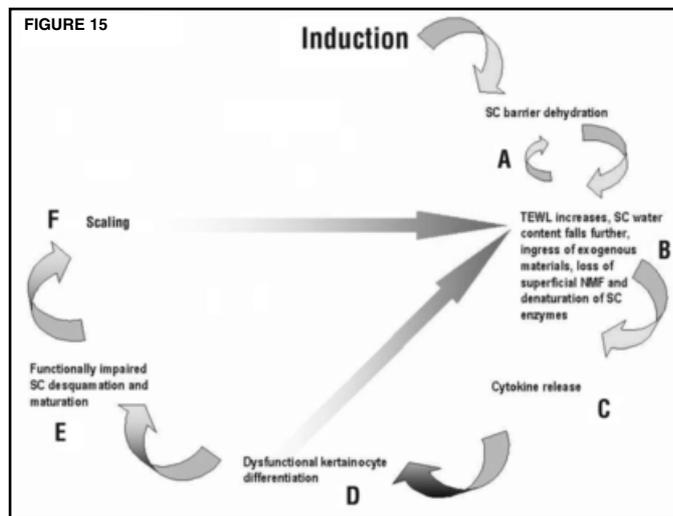
FIGURE 15. Schematic diagram showng pivotal events within the "dry skin cycle".

As discussed the induction phase can be mediated by a variety of different factors:

– low environmental temperature and humidity

– abrupt changes in environmental conditions which includes the effect of modern indoor climate-controlled environments

– surfactant dissolution of stratum corneum lipid & NMF

– chronological ageing & genetics

Once the skin has been provoked by one or more of these mechanisms, there is an inevitable sequence of events that may be described conveniently as a cycle.

Initially a mini-cycle of barrier deterioration is initiated and perpetuated. Blank estimated that the SC loses its flexibility once its water content falls below approximately 10% [6], the provocation for which may constitute one or a combination of the factors noted above. Without intervention, this quickly leads to a steeper SC hydration gradient, a decrease in net recondensation on the SC surface, a corresponding increase in evaporative water loss from the SC surface, a consequent further drop in SC water concentration and so on. The inevitable rapid consequence of this series of events is a decrease in the plastic or viscous properties of the SC (commonly interpreted as skin "softness" or "suppleness"), an increase in SC fragility / brittleness and an impairment of SC barrier function [80-83]. This surface dehydration is the first step in the development of the dry skin cycle and is further exacerbated by destruction of the normal barrier lipid lamellae in the outer layers of the stratum corneum during bathing [21]. The impaired barrier in the superficial layers of the stratum corneum allows leaching of NMF from the outermost skin cells, thereby reducing stratum corneum water activity. Whiteness between the dermatoglyphics (caused by backscatter from multiple tissue-air interfaces) and minor scaling due to the dehydration of individual corneocytes are the first visible steps in the cycle. Perturbation to the barrier then leads to further development of dry skin.

Due to the cyclical nature of these processes, therefore, it becomes virtually impossible to distinguish between dry skin conditions that are provoked initially by barrier disruption or by dehydration of the stratum corneum. However, once the barrier has been disrupted, even superficially, a new cascade of events is started primarily through the induction of a hyperproliferative state.

Acute and chronic insults to the SC barrier will lead to enhanced keratinocyte proliferation, consequent hyperker-

atosis and mild inflammatory changes, one of the hall-marks of dry skin conditions, as the skin attempts to repair itself. This response is mediated via production and secretion of cytokines and growth factors, many researchers citing the ratio between interleukin one receptor antagonist protein (IRAP) and interleukin one-alpha (IL-1α) as a key marker of this process [84-87]. The degree of hyperprofileration has been shown to be dependent upon the corresponding degree of barrier perturbation [88], probably reflecting both the ingress of exogenous irritants through the impaired barrier and the growing realisation that the SC barrier is itself a biosensor and that corneocytes and keratinocytes themselves participating in the release of these messengers. The hyperproliferation of the epidermis probably occurs as a result of the double paracrine signalling events between the epidermis and dermis. IL-1 acts on fibroblasts which in turn secrete KGF and GMCSF inducing hyperproliferation and dysfunctional differentiation of keratinocytes [89].

The induction of this inflammatory hyperproliferative state is absolutely key in the cycle of dry skin as it fundamentally leads to aberrant differentiation and the over-hasty production of a variety of poor quality materials and structures vital to the proper functioning of the SC barrier and normal healthy skin. These include:

(a) the production of smaller and immature corneocyte envelopes ,

(b) changes in epidermal lipid and particular ceramide biology,

(c) reduced transglutaminase activity

(d) reduced filaggrin synthesis and NMF levels

Finally a loss in efficiency of desquamation, due to reduced activity of desquamatory enzymes at the surface of the SC, and ensuing scaling, thickening and loss of hygroscopicity of the SC occurs. Marked scaling is, of course, one of the obvious consumer-noticeable expressions of "dry skin". The formation of a thicker SC with impaired desquamation has, again, immense biophysical importance. The water gradient across the thicker SC becomes steeper, leading to further increases in evaporative water loss, reducing further water concentration in the outer SC and propagating directly another round of the dry skin cycle.

Corneocytes that should be in a mature fully hydrophobed format are now replaced by fragile corneocytes. The resulting barrier protecting these corneocytes and their contents is now weaker due to changes in barrier lipid profiles and surface hydrophobicity. Equally, the hygroscopic (though highly water-labile) NMF present within corneocytes of normal SC, are depleted gradually through normal everyday activities such as cleansing and / or occupational duties [1, 59]. The corneocytes of dry SC are, therefore, subject to exaggerated insult such due to their changed biochemical and biophysical properties. The dry skin cycle, thus, is propagated further by an increased loss of NMF relative to normal skin and a corresponding loss in SC hygroscopicity.

Finally and most importantly, the development of an increasingly thick, dry SC results in a layer characterised, from a biomechanical viewpoint, by a dramatic increase in hardness and brittleness. The consumer perceives this as tightness. These properties create a SC barrier inherently susceptible to mechanical stress and fracture, another factor driving the impairment in barrier function cyclical nature of the dry skin cycle.

The clinical endpoint of "dry skin" cannot be regarded as static but rather is most fully described as a cycle that, without intervention, tends to perpetuate itself. Pivotal to

every stage of this cycle and its propagation is a compromised SC barrier. Interventions that truly break the dry skin cycle, therefore, by definition need not only to treat symptomatic manifestations, but repair and augment SC barrier function. This will yield a skin that is inherently better able to cope with the constantly changing external environment of the modern world.

The Management Of Dry Skin

Although a major analysis of dry skin treatments are outside of the scope of this review it is worth mentioning just briefly the biology that needs to be corrected in cosmetic dry skin condition and providing a few key examples. Traditionally, humectants, occlusives and emollients have been, and will continue to be, the mainstay of cosmetic treatments [90]. Glycerol, once thought to be "merely" a humectant, has been shown to be a lipid fluidizer [91] and corneodesmolytic moiety [92]. Bilayer-forming lipids such as ceramides have been introduced to supplement the SC barrier [93] and in this respect, on an equal-weight basis, bilayer lipids (when combined with glycerol), have been demonstrated to be clinically superior to petroleum jelly in relieving dry skin [94]. Hydroxyacids are being used to facilitate desquamation and improve lipid biosynthesis together with barrier function [95]. However, not all hydroxyacids perform equally. Glucanolactone and tartaric acid have been shown to be superior to glycolic acid and lactic acid in improving barrier function [96]. Ligands for nuclear receptors such as the peroxisomal proliferator activated receptor have been shown to improve epidermal differentiation, increasing ceramide and filaggrin levels [97]. Moreover, niacinamide has been introduced into lotions that up regulates endogenous epidermal lipid biosynthesis and, consequently, provides significant improvement in SC barrier function [98]. Moreover, when combined with glycerol, niacinamide-containing lotions have been shown to be more effective than traditional emollient and lactic acid-containing moisturisers in relieving dry skin in the treatment phase of a typical Kligman-type regression study **(Figure 16)**, as well as improving resistance to

SLS & tape-stripping induced induced barrier perturbation [99].

For more a complete review on dry skin -relieving technologies see the publication of Rawlings [100]

FIGURE 17. Summary of stratum corneum maturation & corneodesmolysis in normal (A) and dry skin (B).
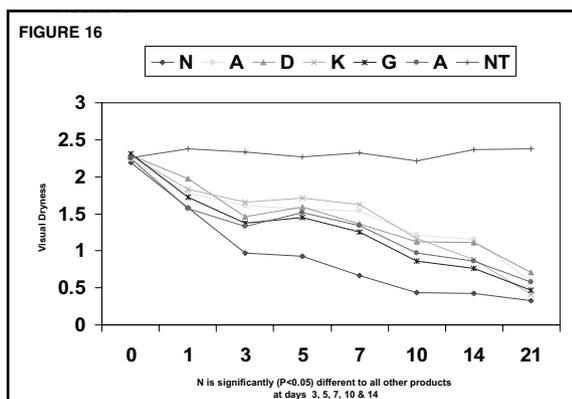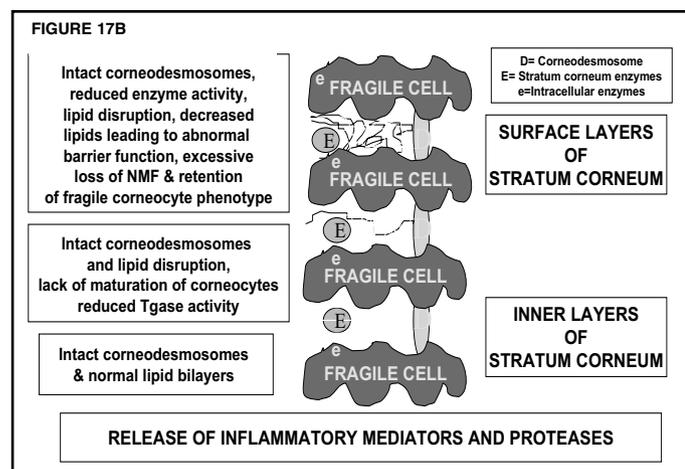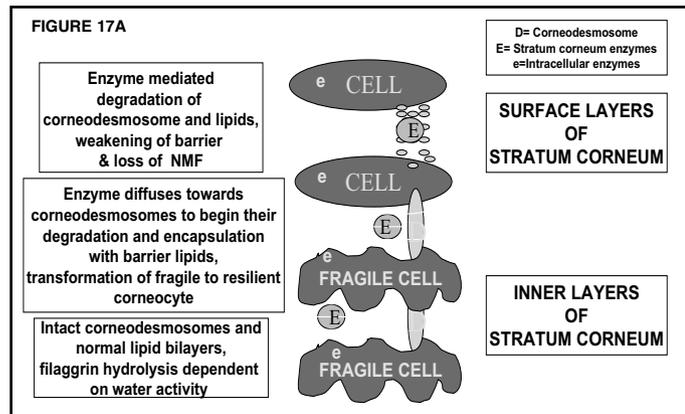

FIGURE 16. Results from the treatment phase of a Kligman-type regression study (products applied twice-daily at 2mg/cm2 to randomised sites on the outer, lower leg of female subjects [n=36] with inclusion of a no-treament control). Products represented high-efficacy commercial moisturisers with ingredients of differing dry skin relief mechanism. Key: NT = no treatment control; N = niacinamide-containing lotion; A = lactic acid-containing moisturiser; other product codes represent commercial products with high loadings of traditional humectants and emollients (including glycerin and petrolatum).

Summary & Conclusions

The maintenance of water balance in the stratum corneum is dependent on three major mechanisms:

- the intercellular lamellar lipids whose physical conformation, predominantly an orthorhombic laterally packed gel phase and 13nm long periodicity lamellar phase, provide a tight and effective barrier to the passage of water through the tissue,

- the presence of fully matured corneodesmosome-bound corneocytes which influence the tortuosity of the SC and thereby the diffusion path length of water and

- the presence of natural moisturizing factors (NMF).

The normal functioning of the SC, however, can be disturbed in dry, flaky skin conditions. On perturbation of SC barrier function, a futile cycle of events begins first with the superficial dehydration of the SC, and subsequent release of inflammatory mediators, induction of hyperproliferation of epidermal keratinocytes and disruption of epidermal differentiation, leading to an inferior SC. As has become apparent, reductions in SC water and NMF levels, changes in lipid ultrastructure and reductions in enzyme activities contribute to the reduced corneodesmolysis known to occur in these conditions. See **(Figure 17)** for a schematic summary of the differences in SC biology in normal & dry skin.

This understanding has led to new treatment modalities and new measurement techniques. These new measurement techniques will lead the way forward in further understanding SC water gradients in vivo in dry skin **(Figure 18)** building on the early work of Warner and Lilly [2].
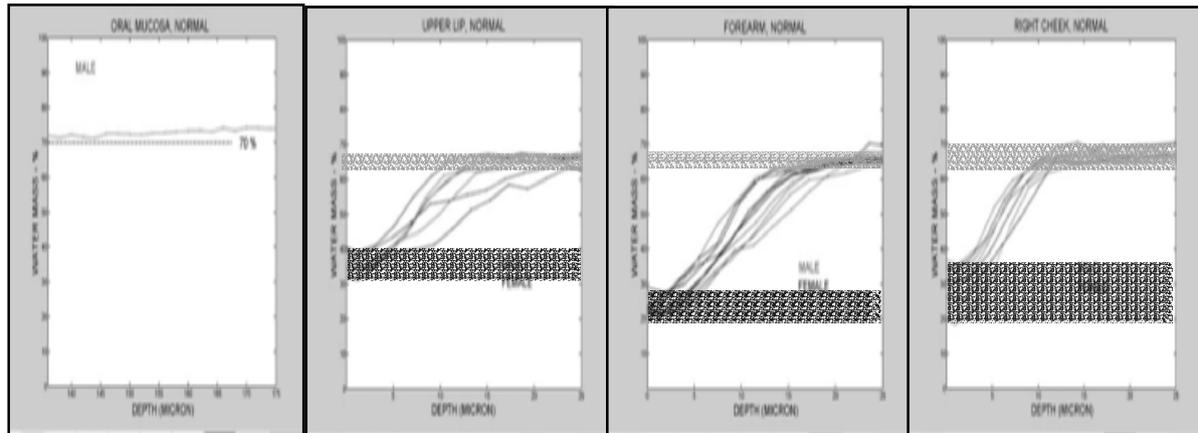
FIGURE 18

FIGURE 18. Semiquantitative in vivo concentration profiles of water on different body sites. Courtesy of Gerwin Puppels & Peter Caspers at River Diagnostics.

REFERENCES

1. Rawlings AV, Scott IR, Harding CR and Bowser PA. Stratum corneum moisturization at the molecular level. J Invest Dermatol 103: 731-740, 1994.

2. Warner RR & Lilly NA. Correlation of water content with ultrastructure in the stratum corneum. In: Elsner P, Berardesca E & Maibach HI eds. Bioengineering of the skin: Water & the stratum corneum. CRC Press Inc. 3-12, 1994.

3. Rawlings AV. Skin waxes: their composition, properties, structures and biological significance. Chapter 6, 221-256 (1994). In : Waxes: Chemistry, Molecular Biology & Functions. Eds Hamilton, RJ. The Oily Press, 1994.

4. Harding CR, Watkinson A and Rawlings AV. Dry skin, moisturization and corneodesmolysis. Int J Cosmet Sci 22: 21-52 2000.

5. Rawlings AV, Harding CR, Watkinson . and Scott IR. Dry & xerotic skin conditions. In: Skin moisturization. Eds: Leyden, J.J. and Rawlings, A.V. pp119-143, 2002.

6. Blank IH. Factors which influence the water content of the stratum corneum. J Invest Dermatol 18: 433-440, 1952.

7. Pfeiffer S, Vielhaber G, Vietzke JP, Wittern KP, Hintze U, Wepf R. High-pressure freezing provides new information on human epidermis: simultaneous protein antigen and lamellar lipid structure preservation. Study on human epidermis by cryoimmobilization. J Invest Dermatol. May;114(5):1030-8, 2000.

8. Norlen, L. Skin barrier structure and function: The single gel phase model. J. Invest. Dermatol. 117: 830-836, 2001.

9. Norlen L, Al-Amoudi A. Stratum corneum keratin structure, function, and formation: the cubic rod-packing and membrane templating model. J Invest Dermatol 123(4):715-32, 2004.

10. Bouwstra JA, de Graaff A, Gooris GS, Nijsse J, Wiechers JW, van Aelst AC. Water distribution and related morphology in human stratum corneum at different hydration levels. J Invest Dermatol 120(5):750-8, 2003.

11. Richter T, Peuckert C, Sattler M, Koenig K, Riemann I, Hintze U, Wittern KP, Wiesendanger R, Wepf R. Dead but highly dynamic--the stratum corneum is divided into three hydration zones. Skin Pharmacol Physiol 17(5):246-57, 2004.

12. Downing DT and Stewart ME. Epidermal composition. In: Dry skin and moisturizers chemistry and function. Eds:Loden, M. and Maibach, H.I. pp13-26, 2000.

13. Motta SM, Monti M, Sesana, S. et. al. Ceramide composition of psoriatic scale. Biochim Biophys Acta 1182: 147-151, 1993.

14. Oku H, Mimura K, Tokitsu Y, Onaga K, Iwasaki H, Chinen I. Biased distribution of the branched-chain fatty acids in ceramides of vernix caseosa. Lipids 35(4):373-81, 2000.

15. Chopart M, Castiel-Higounenc, I, Arbey E and Schmidt R. A new type of covalently bound ceramide in human epithelium. Stratum corneum III, 2001.

16. Hamanaka S, Hara M, Nishio H, Otsuk, F, Suzuki A And Uchida Y. Human Epidermal Glucosylceramides are Major Precursors of Stratum Corneum Ceramides. J Invest Dermatol 119: 416-423, 2002.

17. Pilgram GSK, Engelsma-van Pelt AM, Bouwstra JA and Koerten HK. Electron Diffraction Provides New Information on Human Stratum Corneum Lipid Organisation Studied in Relation to Depth and Temperature. J Invest Dermatol 113: 403-409, 1999.

18. Brancaleon L, Bamberg MP, Sakamaki T and Kollias N. Attenuated total reflection-fourier transform infrared spectroscopy as a possible method to investigate biophysical parameters of stratum corneum in vivo. J Invest Dermatol 116: 380-386, 2001.

19. Bouwstra J, Pilgram G, Gooris G, Koerten H and Ponec M. New aspects of the skin barrier organization. Skin Pharm Appl Skin Physiol 14: 52-62, 2001.

20. Bouwstra J, Gooris GS, Dubbelaar FER and Ponec M. Phase behaviour of stratum corneum lipid mixtures based on human ceramides: The role of natural and synthetic ceramide 1. J Invest Dermatol 118: 606-617, 2002.

21. Rawlings AV, Watkinson A, Rogers J. et al. Abnormalities in stratum corneum structure lipid composition and desmosome degradation in soap-induced winter xerosis. J Soc Cosmet Chem 45: 203-220, 1994.

22. Warner RR & Boissy YL. Effect of moisturizing products on the structure of lipids in the outer stratum corneum of humans. In: Loden M & Maibach HI eds. Dry skin & moisturisers. CRC Press Inc.:349-372, 2000.

23. Berry N, Charmeil C, Gouion C, Silvy A, Girard P, Corcuff C & Montastier C. A clinical, biometrological & ultrastructural study of xerotic skin. Int J Cosmet Sci 21: 241-249. 1999.

24. Serre, G., Mils, V., Haftek, M., Vincent, C., Croute, F., Reano, A., Ouhayoun, J.P., Bettinger, S. and Soleilhavoup, J.P. Identification of late differentiation antigens of human cornified epithelia, expressed in re-organized desmosomes and bound to cross-linked envelope. J Invest Dermatol 97: 1061-1072, 1991.

25. Lundstorm A and Egelud T. Cell shedding from human plantar skin in vitro: evidence that two different types of protein structures are degraded by a chymotrypsin-like enzyme. Arch Dermatol Res 282: 243-237, 1990.

26. Suzuki Y, Nomura J, Koyama J and Horii T. The role of proteases in stratum corneum: involvement in stratum corneum desquamation. Arch Dermatol Res 286: 249-253, 1994.

27. Horikoshi T, Igarashi S, Uchiwa H, Brysk H and Brysk MM. Role of endogenous cathepsin D-like and chymotrypsin-like proteolysis in human epidermal desquamation. Br J Dermatol 141: 453-9, 1999.

28. Horikoshi T, Arany I, Rajaraman S, Chen SH, Brysk H, Lei G, Tyring S. and Brysk MM. Isoforms of cathepsin D human epidermal differentiation. Biochimie 80: 605-12, 1998.

29. Watkinson A. Stratum corneum thiol protease (SCTP): a novel cysteine protease of late epidermal differentiation Arch Dermatol Res 291: 260-268, 1999.

30. Bernard D. et al. Analysis of proteins with caseinolytic activity in a human SC extract revealed a yet unidentified cysteine protease and identified the so called 'SC thiol protease' as Cathepsin L2. J Invest Dermatol 120: 592-600, 2003.

31. Caubet C et al. Degradation of corneodesmosome proteins by two serine proteases of the kallikrein family. J Invest Dermatol 122: 1235-1244, 2004.

32. Bernard D, Mehul B, Delattre C, Simonetti L, Thomas-Collignon A and Schmidt R. Purification and characterization of the endoglycosidase heparanase 1 from human plantar stratum corneum: a key enzyme in epidermal physiology. J Invest Dermatol 117: 1266-73, 2001.

33. Simon M, Jonca N, Guerrin M, Haftek M, Bernard D, Caubet C, Egelrud T, Schmidt R and Serre G. Refined Characterization of Corneodesmosin Proteolysis during Terminal Differentiation of Human Epidermis and its Relationship to Desquamation. J Bio. Chem 276: 20292-20299, 2001.

34. Sondell B, Thornell LE, Stigbrand T and Egelrud,T. Immunolocalization of SCCE in human skin. . Histo Cyto 42: 459-465, 1994.

35. Watkinson A, Smith C, Coan P. and Wiedow O. The role of Pro-SCCE and SCCE in desquamation. 21st IFSCC Congress pp16-25, 2000.

36. Igarashi S, Takizawa T, Yasuda Y. et al. Cathepsin D, and not cathepsin E, degrades desmosomes during epidermal desquamation. In press, 2005.

37. Ishida-Yamamoto A et al. Epidermal lamellar granules transport different cargoes as distinct aggregates. J Invest Dermatol. 122: 1145-1153, 2004.

38. Watkinson A, Harding C, Moore A and Coan P. Water modulation of stratum corneum chymotryptic enzyme activity and desquamation. Arch Dermatol Res 293: 470-476, 2001.

39. Komatsu N, Takata M, Otsuki N, Ohka R, Amano O, Takehara K and Saijoh K. Elevated Stratum Corneum Hydrolytic Activity in Netherton Syndrome Suggests an Inhibitory Regulation of Desquamation by SPINK5-Derived Peptides. J Invest Dermatol 118: 436-443, 2002.

40. Franzke CW, Baici A, Bartels J, Christophers E and Wiedow O. Antileukoprotease inhibits stratum corneum chymotryptic enzyme. J Biol Chem 271: 21886-21890, 1996.

41. Watkinson A, Harding CR and Rawlings AV. The cornified envelope: its role on stratum corneum structure and maturation. In: Skin Moisturization. Eds: Leyden, J.J. and Rawlings, A.V. pp95-117, 2002.

42. Candi E et al. Transglutaminase cross linking properties of the small proline rich 1 family of cornified envelope proteins. J Biol Chem 274: 7226-7237, 1999.

43. Kim IG, Gorman JJ, Park SC, Chung SI and Steinert PM. The deduced sequence of the novel protransglutaminase-E (TGase 3) of human & Mouse. J Biol Chem 268: 12682-12690, 1993.

44. Nemes Z, Marekov LN and Steinert PM. Involucrin cross linking by transglutaminase 1. J Biol. Chem 274: 11013-11021, 1999.

45. Steinert PM. The complexity and redundancy of epithelial barrier function. J Cell Biol 151: F5-F7, 2000.

46. Cabral A, Voskamp P, Cleton-Jansen .M. et al. Structural organisation and regulation of the small proline rich family of cornified envelope precursors suggest a role in adaptive barrier function. J Biol Chem 26: 19231-19237, 2001.

47. Mils V, Vincent C, Croute F and Serre G. The expression of desmosomal and corneodesmosomal antigens shows specific variations during the terminal differentiation of epidermis and hair follicle epithelia. J Histochem Cytochem 40: 1329-1337, 1992.

48. Harding CR, Long S, Richardson J, Rogers J, Zhang Z, Bush A & Rawlings AV. The cornified cell envelope: an important marker of stratum corneum maturation in healthy and dry skin. Int J Cosmet Sci 25:1-11, 2003.

49. Hirao T, Denda M and Takahashi M. Identification of immature cornfield envelopes in the barrier-impaired epidermis by characterization of their hydrophobicity and antigenicities of the components. Exp Dermatol 10: 35-44, 2001.

50. Kashibuchi N, Hirai Y, O'Goshi K and Tagami H. Three-dimensional analyses of individual corneocytes with atomic force microscope: morphological changes related to age, location and to the pathologic skin conditions. Skin Research and Technology 8: 203-211, 2002.

51. Jokura Y. et al. Molecular analysis of elastic properties of the stratum corneum by solid-state C-13-nuclear magnetic resonance spectroscopy, J Invest Dermatol 104: 806, 1995.

52. Sakai S. et al. Hyaluronan exists in the normal stratum corneum. J Invest Dermatol 114 :1184, 2000.

53. Fluhr JW. et al. Glycerol regulates stratum corneum hydration in sebaceous gland deficient (Asebia) mice J Invest Dermatol 120: 728, 2003.

54. Nakagawa, N et al. Relationship between NMF (potassium and lactate) content and the physical properties of the stratum corneum in healthy subjects, J Invest Dermatol 122: 755, 2004.

55. Fluhr JW. et al. Generation of free fatty acids from phospholipids regulates stratum corneum acidification and integrity. J Invest Dermatol 117: 44, 2001.

56. Krein PM and Kermici M. Evidence for the existence of a self-regulated enzymatic process within the human stratum corneum- an unexpected role for urocanic acid, J Invest Dermatol 115: 414, 2000.

57. Caspers PJ, Lucassen GW, Carter EA, Bruining HA and Puppels GJ. In vivo confocal raman microspectroscopy of the skin: non-invasive determination of molecular concentration profiles. J Invest Dermatol 116: 434-442, 2001.

58. Rogers J, Harding CR, Mayo A., Banks J & Rawlings AV. Stratum corneums lipids: the effect of ageing & the seasons. Arch Dermatol Res 288:765-770, 1996.

59. Meldrum H et al. The characteristic decrease in scalp stratum corneum lipids in dandruff is reversed by use of a ZnPTO containing shampoo. IFSCC Mag 6 (1), 3-6, 2003.

60. Declercq L, Muizzuddin N, Hellemans L, van Overloop L, Sparacio R, Marenus K and Maes D. Adaptation response in human skin barrier to a hot & dry environment. J Invest Dermatol 119:716, 2002.

61. Denda M, Sato J, MasudaY et. al. Exposure to a dry environment enhances epidermal permeability barrier function. J Invest Dermatol 111:858-863, 1998.

62. Sato J, Denda M, Chang S. et al. Abrupt decreases in environmental humidity induce abnormalities in permeability barrier homeostasis. J Invest Dermatol 119:900-904, 2002.

63. Katagiri C, Sato J, Nomura J et al. Changes in environmental humidity affect the water-holding capacity of the stratum corneum and its free amino acid content, and the expression of filaggrin in the epidermis of hairless mice. J Dermatol Sci 31: 29-35, 2003.

64. Denda M, Sato J, Tsuchiya T, Elias PM & Feingold KR. Low humidity stimulates epidermal DNA synthesis & amplifies the hyperproliferative response to barrier disruption: Implication for seasonal exacerbation of inflammatory dermatoses. J. Invest Dermatol 111:873-878, 1988.

65. Ashida Y, Ogo M & Denda M. Epidermal interleukin-1 generation is amplified at low humidity: implications for the pathogenesis of inflammatory dermatoses. Br J Dermatol 144:238-243, 2001.

66. Ashida Y & Denda M. Dry environment increases mast cell number & histamine content in dermis in hairless mice. Br J Dermatol 149:240-247, 2003.

67. Yokota T, Matsumoto M, Sakamaki T, Hikima R, Hayashi S, Yanagisawa M, Kuwahara H, Yamazaki S, Ogawa T & Hayase M. Classification of sensitive skin & development of a treatment system appropriate for each group. Proceedings 22nd IFSCC Congress. Volume I: Podium 17, 2002.

68. Urashima R & Mihara M. Cutaneous nerves in atopic dermatitis. A histological, immunochemical & electron microscopic study. Virchows Arch 432:363-370, 1998.

69. Simon M, Bernard D, Minondo AM, Camus C, Fiat F, Corcuff P, Schmidt R and Serre G. Persistence of both peripheral and non-peripheral corneodesmosomes in the upper stratum corneum of winter xerosis skin versus only peripheral in normal skin. J Invest Dermatol 116: 23-30, 2001.

70. Bartolone J, Doughty D and Egelrud T A non-invasive approach for assessing corneocyte cohesion: immunochemical detection of desmoglein 1. J Invest Dermatol 96: 596, 1991.

71. Long S, Banks J, Watkinson A, Harding C and Rawlings AV. Desmocollins: a key marker for desmosome processing in stratum corneum. J Invest Dermatol 106: 872, 1996.

72. Van Overloop L, Declercq L and Maes D. Visual scaling of human skin correlates to decreased ceramide levels and decreased stratum corneum protease activity. J Invest Dermatol 117: 811, 2001.

73. Kawai E, Kohno Y, Ogawa K et al. Can inorganic powders provide any biological benefit in stratum corneum while residing on the skin surface. 22nd IFSCC Congress Edinburgh P10, 2002.

74. Fulmer AW and Kramer GJ. Stratum corneum lipid abnormalities in surfactant-induced scaly skin. J Invest Dermatol 86 :598-602, 1986.

75. Saint-Leger D, Francois AM, Leveque JL, Stoudemayer TJ, Kligman AM and Grove G. Stratum corneum lipids in skin xerosis. Dermatologica 178: 151-155, 1989.

76. Chopart M, Castiel-Higounenc C, Arbey E. et al. Quantitative analysis of ceramides in stratum corneum of normal & dry skin. Stratum Corneum III, 2001.

77. Imokawa G. Ceramides as natural moisturizing factors and their efficacy in dry skin. In: Skin Moisturization. Eds: Leyden, J.J. and Rawlings, A.V. pp 267-302, 2002.

78. Schreiner V, Gooris GS, Pfeiffer S, Lanzenddorfer G, Wenck H, Diembeck W, Proksch E and Bouwstra J. Barrier characteristics of different human skin types investigated with x-ray diffraction, lipid analysis and electron microscopy imaging. J Invest Dermatol 114: 654-600 , 2000.

79. Tezuka T, Electron microscopical changes in xerotic senilis epidermis. Its abnormal membrane coating granule formation, Dermatologica 166: 57, 1983.

80. Matts, PJ & Goodyer, E, A new instrument to measure the mechanical properties of human skin in vivo, J Cosmet Sci 49: 321-333, 1998

81. Matts, PJ, Hardware and measurement principles: the Gas-Bearing Electrodynamometer and Linear Skin Rheometer, in Bioengineering of the Skin: Skin Biomechanics (Elsner, P, Beradesca, E, Wilhem, KP & Maibach HI, eds), CRC Press, Boca Raton, 2002.

82. Cooper, ER, Missel, PJ, Hannon, DP & Albright, GB, Mechanical properties of dry, normal and glycerol-treated skin as measured by the gas-bearing electrodynamometer, J Cosmet Sci 36: 335-348, 1985

83. Christensen MS, Hargens CW 3rd, Nacht S, Gans EH., Viscoelastic properties of intact human skin: instrumentation, hydration effects, and the contribution of the stratum corneum, J Invest Dermatol 69(3):282-6, 1977

84. Denda M, Wood LC, Emami S, Calhoun C, Brown BE, Elias PM, Feingold KR, The epidermal hyperplasia associated with repeated barrier disruption by acetone treatment or tape stripping cannot be attributed to increased water loss, Arch Dermatol Res 288(5-6):230-8, 1996

85. Kikuchi K, Kobayashi H, Hirao T, Ito A, Takahashi H, Tagami H, Improvement of mild inflammatory changes of the facial skin induced by winter environment with daily applications of a moisturizing cream. A half-side test of biophysical skin parameters, cytokine expression pattern and the formation of cornified envelope, Dermatology 207(3):269-75, 2003

86. Terui T, Hirao T, Sato Y et al. A increased ration of interleukin-1 receptor antagonist to interleukin-1 in inflammatory skin diseases, Exp Dermatol 7: 327-334, 1998

87. Proksch E, Jensen, J & Elias PM. Skin lipids and epidermal differentiation in atopic dermatitis. Clinics in Dermatology 21: 134-144, 2003

88. Proksch E, Feingold KR, Man MQ, Elias PM, Barrier function regulates epidermal DNA synthesis, J Clin Invest 87(5): 1668-73, 1991

89. Angel P, Szabowski A. Function of AP-1 target genes in mesenchymal-epithelial cross-talk in skin. Biochem Pharmacol. 64(5-6):949-56, 2002.

90. Rawlings AV, Canestrari DA & Dobkowski B. Moisturizer technology versus clinical performance. Dermatologic Therapy. 17: 49-56 2004.

91. Mattai J, Froebe C.L, Rhein LD. et al. Prevention of model stratum corneum lipid phase transitions in vitro bu cosmetic additives. J Soc Cosmet Chem 44: 89-100, 1993.

92. Rawlings AV, Watkinson A, Hope J , Harding C and Sabin R. The effect of glycerol and humidity on desmosome degradation in stratum corneum. Arch Dermatol Res 287: 457-464, 1995

93. Wollenweber U, Korevaar K, Rawlings AV & Schick. Application of a skin-identical lipid concentrate for enhanced skin moisturization and protection. SOFW-Journal. 130, 9-2004.

94. Summers RS, Summers B, Chandar P, Feinberg C, Gurskey R and Rawlings AV. The effect of lipids with and without humectant on skin xerosis. J Soc Cosmet Chem 47: 27-39, 1996.

95. Rawlings AV, Davies A, Carlomusto M, Pillai S, Zhang K, Kossturko R, Verdejo P, Feinberg C, Nguyen L and Chandar P. Effect of lactic acid isomers on keratinocyte ceramide synthesis, stratum corneum lipid levels and stratum corneum barrier function. Arch Derm Res 288: 383-390, 1996.

96. Berardesca E. et al. Alpha hydroxy acids modulate stratum corneum barrier function, Br J Dermatol 137: 934, 1997.

97. Watkinson A, Lee RS, Paterson SE, Marti VJ, Rawlings AV, Ginger RS, Donovan M, Green MR and Mayes AE. Peroxisome proliferator activated receptor alpha (PPAR·) activators: Petroselinic acid as a novel skin benefit agent for anti-perspirants. Proceedings of the 22nd IFSCC Congress, Edinburgh, Scotland, 1, podium presentation 11, 2002.

98. Matts PJ, Oblong JE and Bissett DL. A review of the range of effects of niacinamide in human skin. IFSCC Mag 5: 285-290, 2002.

99. Matts PJ Gray J & Rawlings AV. The "Dry Skin Cycle" – a new model of dry skin and mechanisms for intervention. The Royal Society of Medicine Press Ltd, International Congress and Symposium Series (In press), 2005.

100. Rawlings AV. Trends in stratum corneum research & the management of dry skin conditions. Int J Cosmet Sci 25: 63-95, 2003.