The Epidermal Permeability Barrier: From the Early Days at Harvard to Emerging Concepts

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Perhaps no tissue is so physically maligned by processing for light/electron microscopy as is the stratum corneum (SC). To further complicate matters, no tissue of such critical importance for survival has been so intellectually maligned as well. Because routine microscopic images of normal SC depict loosely attached corneocytes ("basket-weave pattern"), until the 1960s the barrier was thought to reside not in the SC but rather in the outer stratum granulosum (Table I). The key breakthroughs came from Albert Kligman's group, who found isolated SC to be not friable but instead extremely durable (Christophers and Kligman, 1964), and from the work of Irvin Blank and Robert Scheuplein in Dr Fitzpatrick's department at Harvard, who further demonstrated the highly impermeable nature of the SC (Blank, 1969; Scheuplein and Blank, 1971). Because Blank and Scheuplein found the water-transport characteristics of human SC to be similar to plastic wrap, the SC soon was analogized to a sheet of plastic or "Saran" wrap (Table I). According to this model, which still dominates the world view of skin biophysicists and physical chemists, hydrophilic and lipophilic molecules traverse a uniform SC "membrane" via a transcellular route without regard to tissue architecture or metabolic activity (Blank, 1969). Accordingly, percutaneous penetration is determined by the chemical characteristics of the penetrating molecule, as well as the diffusion path-length across the SC (= thickness of the membrane), as embodied in Fick's law (Scheuplein and Blank, 1971). Although commonsense alone (e.g., the hyperpermeability of the thickened SC of the palms and soles to water) immediately invalidates the "plastic wrap" model, the seminal work of Blank and Scheuplein nevertheless established the importance of the SC as the critical tissue determinant of the cutaneous permeability barrier. Perhaps of greater importance, it spawned an entirely new industry, devoted to transdermal drug delivery.

Developments after 1970 showed that the "plastic wrap" model did justice neither to the structural heterogeneity nor to the metabolic activity of the SC. Frozen sections of SC revealed the compression of corneocytes into exquisite geometric stacks of interlocking tetracaidodecahedra (24-sided cells) (Christophers and Kligman, 1964; Menton and Eisen, 1971). Frozen sections and freeze-fracture images revealed lipid stacks, localized to the intercellular spaces (Elias and Friend, 1975), which were shown to derive from the secreted contents of epidermal lamellar or Odland bodies (George Odland first realized the novelty and potential importance of this organelle, previously thought to be an effete mitochondrion; Odland and Holbrook, 1981). Lipid biochemistry, coupled with lipid histochemistry, revealed a unique extracellular membrane system, devoid of phospholipids, relying instead on an equimolar mixture of ceramides, cholesterol, and nonessential free fatty acids to form extracellular membranes (Gray and Yardley, 1975; Elias *et al*, 1979), which are riveted into parallel structures by linoleic-acid-bearing ω-hydroxy-esterified ceramides (acylceramides) (Wertz and Downing, 1987) – hence, the still-current, two-compartment "bricks and mortar" model of the SC (Table I).

Awareness that the lamellar body is enriched in hydrolytic enzymes initially led to speculation that this organelle could be a modified lysosome, whose primary function lay in desquamation (Wolff and Holubar, 1967; Wertz and Downing, 1987). Indeed, that suspicion has been borne out by recent studies, which have demonstrated a role for lamellar-body-derived enzymes (and structural proteins) in desquamation (see below). Yet, the lamellar body is clearly a secretory organelle, not a lysosome (Elias *et al*, 1998). Even more important than its role in desquamation is its role in the delivery to the SC interstices of a family of lipid hydrolases, which metabolize polar lipid precursors (cholesterol sulfate, phospholipids, sphingomyelin, and glucosylceramides) into their more nonpolar products, which together form the extracellular lamellar membrane system (Elias and Menon, 1991). This critical sequence, together called "lipid processing", also provides powerful evidence that the SC is not metabolically inert, i.e., the "living stratum corneum" (Table I). Finally, recent studies have shown that localized changes in acidity, i.e., within SC extracellular "microdomains", regulate lipid processing leading to barrier formation (Fluhr *et al*, 2001; Behne *et al*, 2002) (Fig 1). In fact, each SC subcellular compartment, i.e., corneocyte cytosol, cornified envelope, and extracellular domains, contains specific types of metabolic activity (Table II). Yet amazingly, the SC is still considered "dead" by regulatory agencies, such as the Food and Drug Administration.

Not only lipids, but also specialized junctional structures, corneodesmosomes, are segregated within SC intercellular domains. These simplified junctions lack many of the proteins of their counterparts in lower epidermal layers, but they are enriched in desmoglein 1, desmocollin 1, and a novel protein, corneodesmosin, which appears to coat their external surfaces (Lundstrom *et al*, 1994). By making corneodesmosomes initially resistant to proteolysis, this protein mediates the initial cohesiveness of corneocytes in the lower SC (Lundstrom *et al*, 1994). Corneodesmosomes eventually succumb to the relentless attack of secreted

Table I. Evolving concepts of SC

Outdated	1. Disorganized; no functional significance ("basket-weave")
	2. Homogeneous film ("plastic wrap")
Current	3. Two-compartment organization ("bricks and mortar")
	4. Persistent metabolic activity ("living stratum corneum")
	5. Homeostatic links to nucleated cell layers (barrier requirements regulate metabolic processes in underlying epidermis)
	6. Stratum corneum as a biosensor (external humidity alone regulates proteolysis of filaggrin; epidermal DNA/lipid synthesis; and initiation of inflammation)
	7. Pathophysiologic links to deeper skin layers (barrier abrogation initiates inflammation)

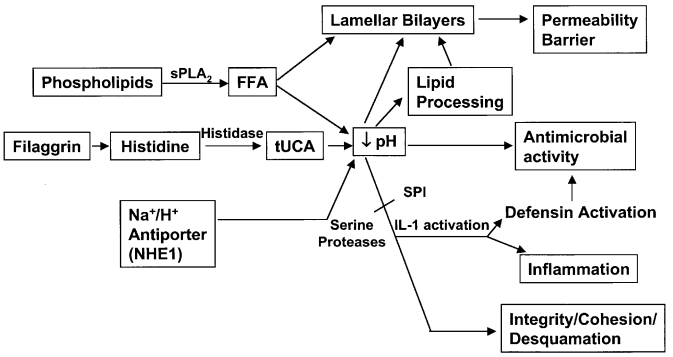


Figure 1
Endogenous pathways of SC acidification: regulated functions.

proteases (primarily serine, but also aspartate and thiol proteases), which degrade not only corneodesmosin but also desmoglein 1 and desmocollin 1 (Horikoshi *et al*, 1999; Eckholm *et al*, 2000). Many of the key participants in SC cohesion/desquamation, including corneodesmosin, as well as the serine protease SCCE and other proteases and glycosidases, whose specific roles are less well understood, are also lamellar body products. Like every known structure in the SC, however, even the lacunae that result from corneodesmosome degradation mediate a key function, i.e., they form an aqueous, expansile "pore" penetration pathway that bypasses both corneocytes and adjacent lamellar bilayers (Menon and Elias, 1997).

Recent studies suggest that both the initial cohesion and the ultimate desquamation of corneocytes from the SC surface may be orchestrated by localized changes in pH, which selectively activate different classes of extracellular proteases in a pH-dependent fashion (Fig 2). The most rigorously studied participants are the epidermis-specific serine proteases, the SC chymotryptic (SCCE) and SC trypic (SCTE) enzymes (Eckholm *et al*, 2000), which both exhibit neutral-to-alkaline pH optima. Because an acidic pH dominates in normal SC, we suspect that two other protease family members, thiol (cysteine) proteases (cathepspin L2) and an aspartate protease, cathepsin D (Cath D)

Table II. Examples of metabolic activity in SC

Corneocyte cytosol	1. Proteolysis of filaggrin to amino acids
	2. Deimination of amino acids into humectants and other bioactive molecules
	3. Primary cytokine activation
Corneocyte envelope	1. Progressive transglutaminase-mediated cross-linking (increased rigidity)
	2. Formation of corneocyte-bound lipid envelope (ceramidation, deglucosylation of ω-hydroxyceramides)
Extracellular matrix	1. Proteolysis of corneodesmosomes
	2. Conversion of lamellar-body-derived, polar lipid precursors into non-polar products

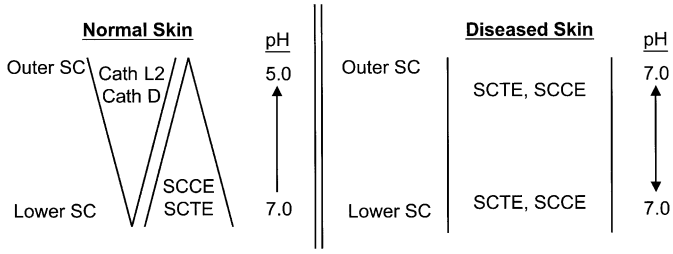


Figure 2
Proposed pH-dependent role of different proteases in desquamation.

(Horikoshi *et al*, 1999; Bernard *et al*, 2003), mediate desquamation in the outer layers of normal SC, whereas SCCE/SCTE could initiate corneodesmosome degradation in the lower layers of normal SC, and in diseased SC where a neutral pH predominates at all levels (Fig 2). Thus, permeability barrier homeostasis and cohesion/desquamation are both exquisitely self-regulated and pH-dependent processes that localize to the SC interstices.

Meanwhile, the SC cytosol is also far from inert. A cascade of hydrolytic and deiminating enzymes that localize to the corneocyte cytosol have been linked to several key SC functions, including SC hydration, UV filtration, and UV-induced immunosuppression, favoring skin cancer development, as well as possibly both antimicrobial activity and cytokine activation (Fig 3). The filaggrin–histidine–urocanic acid (UCA) pathway generates not only critical humectants but also the H^+ donor, UCA, which could mediate one or more functions shown in Figs 1 and 3 (Scott and Harding, 1986; Krien and Kermici, 2000). Importantly, the putative aspartate protease (cathepsin) that initiates this cascade is inversely regulated by changes in external humidity (Scott and Harding, 1986). Thus, the capacity of the corneocyte to hydrate above the stratum compactum is largely dependent upon activation of this pathway in response to a reduced external humidity (Fig 3). Yet, several other mechanisms, e.g., glycine deimination to pyrrolidone carboxylic acid, arginine deimination to citrulline by arginase, and glycerol generation from sebaceous-gland-derived glycerol (Fluhr *et al*, 2003), also contribute to hydration of the corneocyte cytosol (Fig 3).

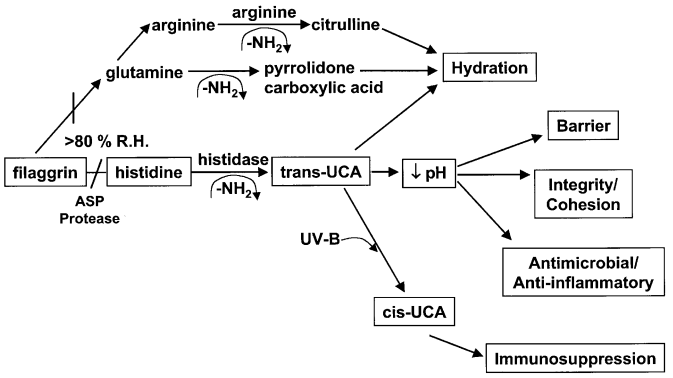
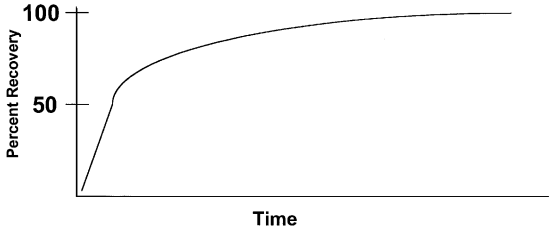


Figure 3
Functions potentially impacted by filaggrin metabolism in SC.



Assess:
- Metabolic responses to barrier abrogation (22)
- Underlying pathology (23)
- Topical therapeutics (24)
- Metabolically-based penetration enhancers (25)

Figure 4
Cutaneous stress test: applications.

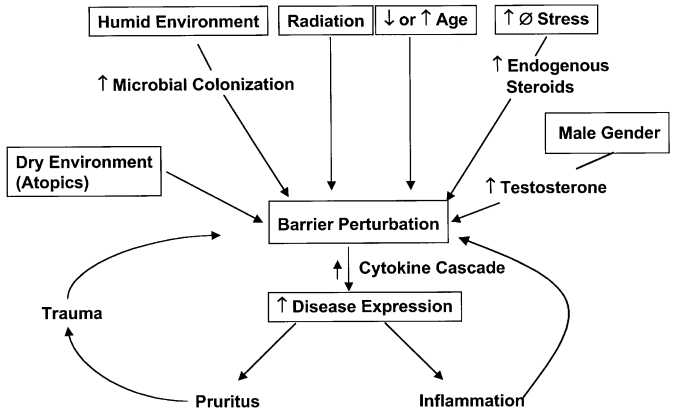


Figure 5
Cutaneous stress test reveals additional risk factors.

Table III. Signals of the repair response

Signal	Regulated response	Pathogenic signal
EXTRACELLULAR		
Ions: Ca^{2+}, $K+$	Lamellar body secretion; keratinocyte differentiation	No
Cytokines: TNFα, IL-1α, β, IL-1ra, GM-CSF, IL-6, IL-8	DNA synthesis; lipid synthesis (IL-1α)	Yes
Growth factors: NGF, TGFβ1, amphiregulin	DNA synthesis	Not known
INTRACELLULAR		
Sterol regulatory element binding proteins	Cholesterol/fatty acid synthesis; LDLr expression	No
Nuclear hormone receptors	Epidermal differentiation; epidermal proliferation; cutaneous inflammation	No

The most current view of the SC depicts this tissue as an exquisite biosensor (Table I). In response to barrier abrogation, external injury, altered pH alone, and even extremes of humidity, the SC elaborates a set of homeostatic responses that rapidly normalize permeability barrier homeostasis in normal skin (Elias *et al*, 1999). The rate of barrier recovery after acute abrogations constitutes a type of stress test (the Cutaneous "Treadmill" Exam), which was deployed first to discern a sequence of metabolic processes, such as increased lipid synthesis, lamellar body production/secretion, DNA synthesis, and lipid processing, linked specifically to maintenance of barrier function (Elias *et al*, 1999) (Fig 4). Subsequently, the cutaneous stress test was also used to identify underlying pathology in situations such as aged skin (Ghadially *et al*, 1995), where basal function is normal (Fig 5). Finally, this dynamic approach has also proved useful in the development and comparison of various "barrier repair" preparations (Mao-Qiang *et al*, 1995), and to identify metabolic approaches that enhance transdermal drug delivery (Elias *et al*, 2002) (Fig 4).

The "biosensor" concept implies the existence of signaling mechanisms between the SC and the nucleated cell layers, and recent studies have identified both extracellular and intracellular processes that are stimulated by barrier abrogation (Table III). One of the best-characterized classes of extracellular signaling molecules are primary cytokines, principally IL-1α and IL-1β, released in a non-energy-dependent fashion from their preformed pools in corneocytes subsequent to barrier abrogation (Wood *et al*, 1996), which then appear to regulate downstream processes, such as keratinocyte proliferation and lipid synthesis (Elias *et al*, 1999). A second, unrelated class of extracellular signals comprises alterations in calcium concentration in the outer epidermis, which regulate both lamellar body secretion (Menon *et al*, 1994) and epidermal differentiation (Elias *et al*, 2002). Two key intracellular signaling mechanisms are (1) a family of transcription factors, the class 1 and 2 families of nuclear hormone receptors; and (2) the sterol element binding proteins. These mechanisms regulate several specific steps in keratinocyte protein and lipid synthesis (Table III), which together lead to epidermal differentiation (Elias and Feingold, 2001).

The primary purpose of all these signaling events is to stimulate metabolic events in the underlying epidermis that normalize permeability barrier function, the principal function of the skin, without which life would not be possible in a terrestrial environment. Although much effort has been expended in elucidating the specific metabolic events that

restore barrier homeostasis, the signals that stimulate homeostatic responses can, if sustained, initiate a "cytokine cascade" that leads to inflammation and epidermal hyperplasia (Elias *et al*, 1999). Yet, although these cytokines are readily released from the SC, e.g., in response to an elevated pH, curiously their release alone does not lead to inflammation (Hachem *et al*, in press). Thus, the downstream regulation of SC-initiated inflammation comprises yet another insufficiently studied aspect of this tissue.

In summary, while the enzymatic processes that generate the mechanical and permeability barriers, as well as SC cohesion/desquamation, have been the subject of intense study, the regulation and localization of several other key defensive functions of the SC, such as the antimicrobial, antioxidant, and ultraviolet barriers, pathways of antigen access, the links between primary cytokine activation and inflammation, as well as the relative roles and compartmentalization of SC hydration, remain largely unexplored.

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