SKIN BARRIER FUNCTION: MORPHOLOGICAL BASIS AND REGULATORY MECHANISMS

R. Darlenski[1], J. Kazandjieva[2], N. Tsankov[1]
[1]Department of Dermatology and Venereology, Tokuda Hospital-Sofia
[2]Department of Dermatology and Venereology, Faculty of Medicine- Sofia

Keywords: ***stratum corneum, transepidermal water loss, hydration, ceramides, keratin, filaggrin***
Contact details: ***R. Darlenski, 110 A Hipodruma res. distr., 1612 Sofia, Bulgaria, tel: +359 890 545599, e-mail: darlenski@abv.bg***

Abstract: Skin covers the body and fulfils different functions: defensive, thermoregulatory, excretory, resorptive, metabolite and sensory. It accomplishes the protection of the mammalian organism against exogenous physical, chemical, and biological agents, as well as it sustains the organism's homeostasis. Almost 90% of the skin barrier function resides in the stratum corneum. The major constituents of the barrier – lipid bilayers and corneocytes embedded in a cornified envelope interact to perform the multiple defensive functions. Herein the mechanisms for the regulation of the barrier homeostasis are discussed.

Review Article

Introduction

Skin is the largest organ in the mammalian organism and its direct defence from external factors.[1,2] The skin is not merely an inert body coverage but it fulfils several important functions: defensive, thermoregulatory, excretory, resorptive, metabolite and sensory. Healthy skin provides protection of the body from environmental factors: physical (mechanical trauma, thermal injury, radiation), chemical (destructive agents, surface active substances, xenobiotics, allergens) and biological (bacteria, viruses, etc.).[3] A major element of the defensive function of the skin is to maintain homeostasis by preventing the uncontrolled loss of water, ions and serum proteins from the organism into the environment. The epidermis is not absolutely impermeable to substances applied directly onto the skin surface - a phenomenon on which the topical dermatology treatment is based as well as the application of transcutaneous therapeutic systems for transdermal drug transport.[4]

For the first time, in the 20^{s} of the 20th century, Marchionini and Schade applied scientific evidence for the protective function of the water-lipid mantle of the skin and introduced the concept of ***the skin barrier***.[5] According to the definition, ***barrier*** refers to an object that separates two distinct spaces and/or prevents the free passage between two environments.[6] In this sense, the skin barrier ensures the integrity of the body and controls the exchange of substances with the environment.

The concept of skin barrier has been constantly evolving in parallel with the advance of the research methods. Currently, it is considered that over 90% of the skin barrier function resides in the epidermis and particularly in its outermost corneous layer (stratum corneum).[2,7] The morphological structures, constituting the skin barrier develop relatively late in the ontogenesis (about 34th gestational week).[8,9] The skin

J Clin Med. 2011; 4(1):36-45

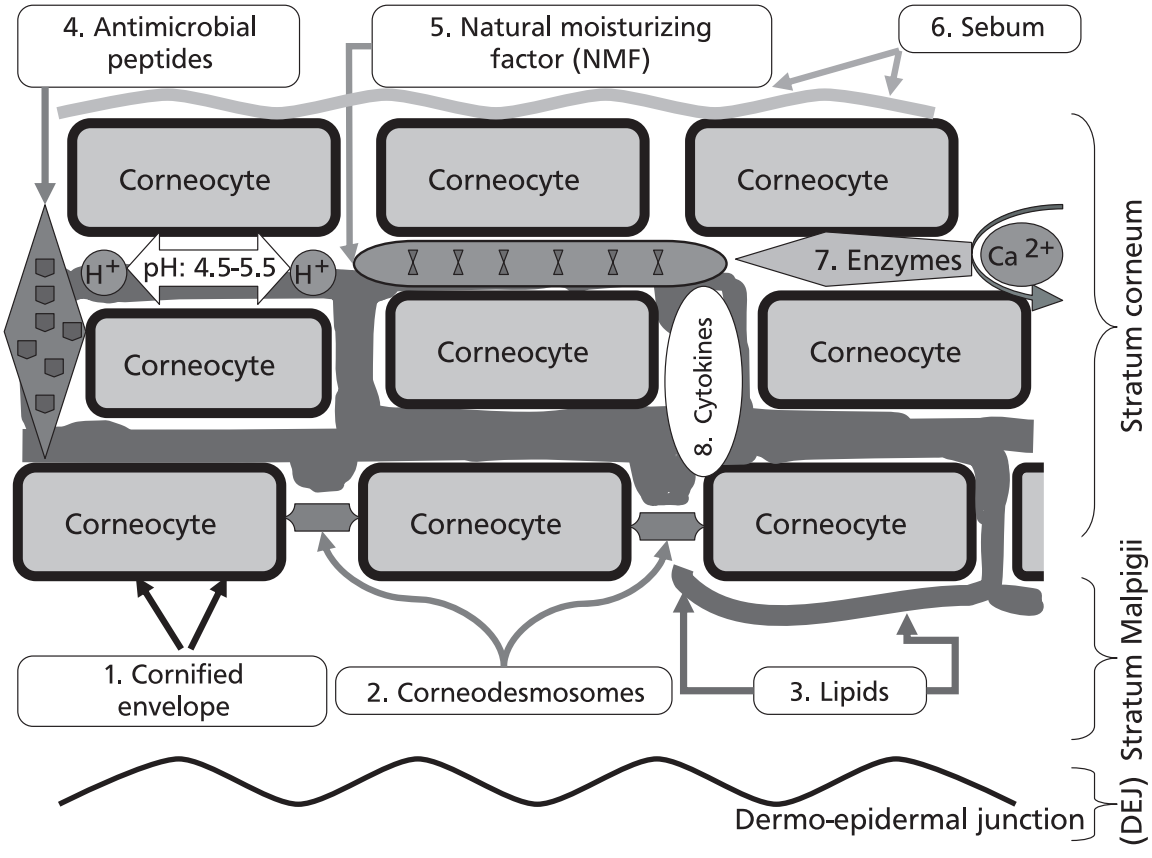
barrier is not fully competent immediately after birth and develops in the earliest stages of the neonatal period.[10,11] Exploring the morphological and biochemical bases of the epidermal barrier is essential for understanding its main functions. Initially, stratum corneum (SC) was considered an inert layer of dead cells formed in the keratopoesis, interconnected through intercellular lipid layer. In recent decades, the understanding of the structure of SC has been updated and the classical model (***cement*** and ***concrete***) has now developed into the concept that SC is a dynamic system with metabolic activity, which responds to external influences through the process of regulation in the synthesis of DNA and structural proteins, proteolysis and ion transport.[12] The schematic structure of the epidermal barrier is illustrated in figure 1.

SC with its main constituents the corneocytes and the intercellular lamellar lipid bilayers, are considered the main structures determining the speed of the transcutaneous exchange of substances.[3,13] The mechanical resistance of the epidermal barrier is mainly due to the corneocytes embedded in the so-called ***cornified envelope***. It is composed of proteins such as the dynamically linked loricrin, involucrin and filaggrin.[14] The adjacent lipid layers are responsible for the water permeability and the exchange of substances with the external environment. The main biochemical components of the skin barrier are lipids and proteins.

1. Lipids

Lipids involved in the composition of SC are 50% ceramides, 25% cholesterol and cholesterol esters, 15% free fatty acids and other lipids presented in small concentrations.[15] The lipids of the SC are substantially different from those constituting other biological membranes in the body.[16] Ceramides are structurally a heterogeneous family of lipids that contain long main hydrocarbon chain omega-hydroxy acids and 6-hydroxysphingosine as a binder. New members of the ceramide family are constantly being discovered, but ceramide-1 (the least polar ceramide in the structure of SC) plays a crucial role in the organization of the inter-

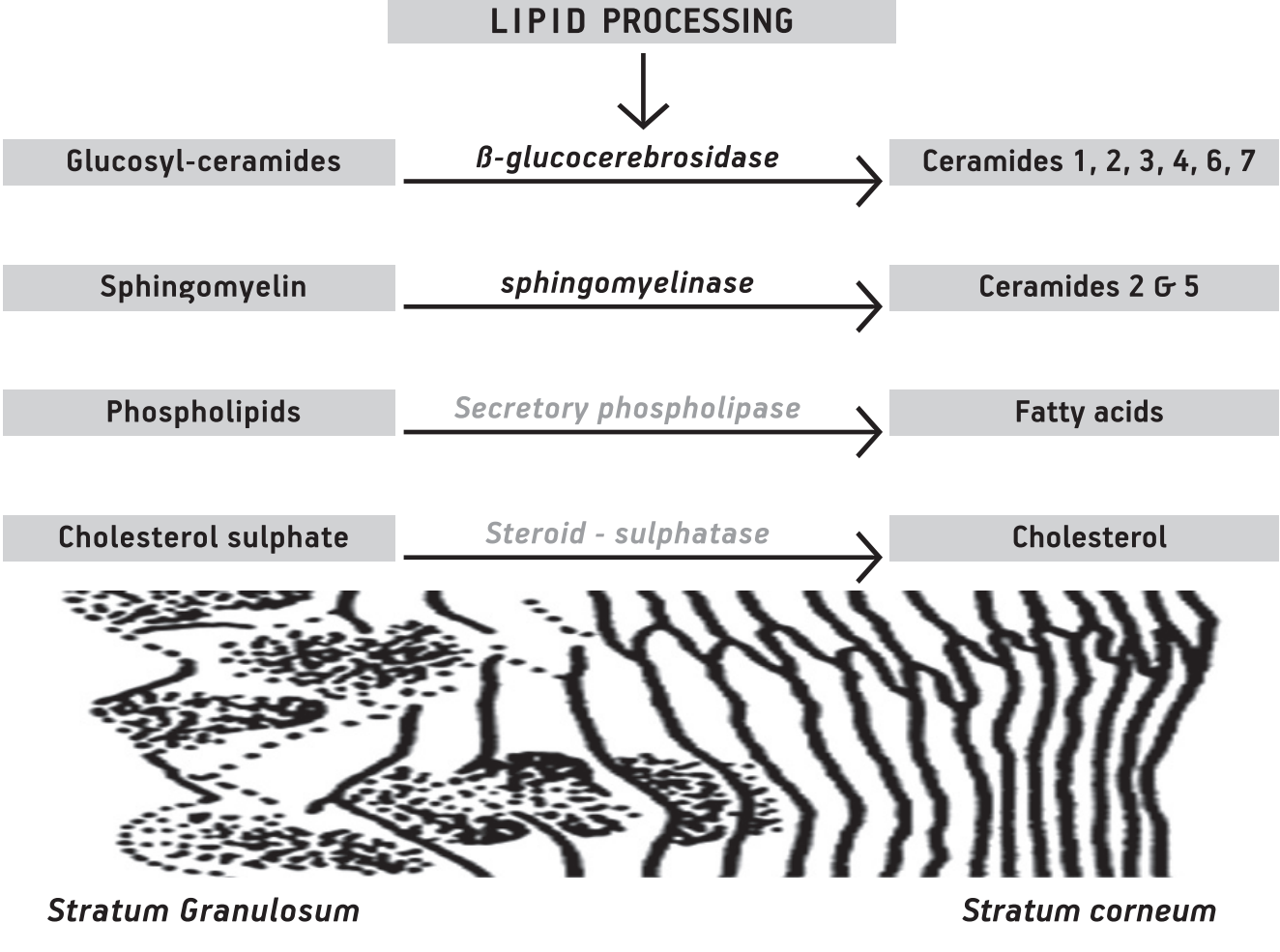
Figure 1: Shematic structure of the epidermal barrier with its morphological and functional elements



cellular lipid layers.[17] Double-stranded layers of omega-hydroxy ceramides are covalently bound to proteins of the cornified envelope surrounding the corneocytes, thus determining the homogeneity and unity of the stratum corneum.[18] Cholesterol increases the fluidity of the intercellular lipids, and thus contributes to the elastic properties of skin.[19,20]

The three main classes of SC lipids are derived from their precursors - phospholipids glycoceramides, sphingomyelin, and unbound sterols - delivered to the SC through ovoid structures, surrounded by membrane, called lamellar bodies or Odland bodies.[15] The Odland bodies contain enzymes such as hydrolases and proteases, responsible for the synthesis of lipids of the corneous layer and the physiological process of desquamation. Once secreted into the intercellular space of the SC, lipid precursors undergo enzymatic conversion to ceramides, cholesterol and free fatty acids by the enzymes of the lamellar bodies **(Figure 2)**. The process is called lipid processing and its goal is the biochemical conversion of polar precursors to nonpolar lipids and their organisation into lipid layers. Different models are proposed for the organization of intercellular lipids in SC: a "sandwich"[19], a "mosaic"[21] and a "gel-phase" model.[22] Electron microscopy studies using ruthenium tetroxide as postfixation reagent reveals the composition of the intercellular lipid layers:

FIGURE 2. SCHEMATIC REPRESENTATION OF THE LIPID PROCESSING CARRIED OUT BY ENZYMES ORIGINATING FROM THE ODLAND BODIES
The black coloured enzymes have acidic pH-optimum range while those in *gray* - alkaline pH-optimum.



the light lamellae have a bilayer organization and are partly interrupted by electron-dense areas.[23]

Various factors, such as the change in the acidity of the skin surface, the mechanical disruption of the epidermal barrier and others, influence the process of lipid processing. Inhibiting the activity of secretory phospholipase (converting phospholipids to free fatty acids - **Figure 2**) leads to defects in the structure of intercellular lipid layers.[24] Experimental destruction of the skin barrier leads to increased synthesis of cholesterol, associated with higher activity of the enzyme HMG-CoA-reductase (a key enzyme in cholesterol synthesis).[15] Lack of β-glucocerebrosidase and acidic sphingomyelinase, respectively in Gaucher and Niemann-Pick disease, leads to an impaired organization of intercellular lipids in the areas of SC and clinical manifestations of impaired skin barrier function.[15]

The sebum, produced by sebaceous glands, is involved in the formation of water-lipid mantle of the skin surface and thus participates in the formation of epidermal barrier. The composition of human sebum was analyzed by a thin layer chromatography: fatty acids (47%), wax esters (17%), ceramides (13%), squalene (11%), cholesterol (7%), triglycerides (3%) and cholesterol esters (2%).[25] Fatty acids serve as a substrate for the formation of skin surface acidity – an important defensive mechanism against pathogenic flora, as well as a maintenance of barrier homeostasis.[26,27] Reduced sebum secretion is a key element in the pathogenesis of senile xerosis and atopic skin.[28]

2. Proteins

Several proteins such as keratins, loricrin, involucrin, filaggrin and corneodesmosin participate in building the corneocyte cytosol, the cornified envelope and the links between the corneocytes.

The keratins form the intermediate filaments of the corneocyte cytoskeleton. The major keratins, expressed in the suprabasal layers are keratins 1, 2f and 10, constituting about 80% of the corneocyte weight.[14] Keratins 1 and 10 forming the corneocyte cytoskeleton are connected with desmoglein 1 and desmocollin 1 through desmosomal plaque proteins – plakoglobin and desmoplakins. The keratins are attached to the cornified envelope proteins surrounding the corneocyte, thus contributing to the stability of the epidermal barrier. Mutations in the gene responsible for the synthesis of keratin 1 in ichthyosis hystrix, leads to incomplete formation of intermediate filaments and impaired barrier function.[29]

Filaggrin belongs to the family of the S100 Ca^{2+}-binding proteins. The enzymatic transformation of profilaggrin located in keratohyalin granules in stratum granulosum, leads to the formation of two products filaggrin and N-terminal peptide.[14] Filaggrin provides the formation of keratin filaments into macrofibrils in the lowest layers of SC. The filaggrin is an important source for maintaining skin moisture.[30] By reaching the skin surface, filaggrin is degraded to free amino acids – the basic components of a highly absorbent complex called natural moisturizing factor (NMF).[31] A small part of filaggrin binds with loricrin for the formation of the corneocyte cornified envelope.[32] The N-terminal peptide is involved in regulation of the corneocyte programmed cell differentiation.[33]

Mutations in the filaggrin gene are associated with impaired barrier function in diseases such as ichthyosis vulgaris and atopic dermatitis.[34,36]

Structurally different proteins build up the hydrophobic cornified envelope which wraps the corneocytes, for example - involucrin, loricrin, envoplakin and proteins containing proline.[32,37] The loricrin, supplied to the SC by keratohyalin granules, is a major component of the cornified envelope and represents about 70% of its mass.[37] Patients with mutations in the loricrin gene, clinically develop the Vohwinkel syndrome[38], characterized by digital constrictures (pseudo-ainhum), keratodermia, mild to moderate ichthyosis and impaired skin barrier function.

The involucrin is located mainly at the outer layers of the cornified envelope and is considered the primary precursor for its formation.[1] The process of forming bonds between proteins in the composition of the cornified envelope is accomplished by an enzyme – transglutaminase -1.[39,40] Although there are at least two other isoforms of this enzyme, their activity is not sufficient to compensate for the deficiency of transglutaminase-1 in ichthyosis lamellaris.[39,40]

Corneodesmosomes (the desmosoms in SC) are made of different proteins - desmosomal cadherins, desmogleins, desmocollins, desmosomal plaque proteins and extracellular corneodesmosin.[41] The corneodesmosin secreted by the lamellar bodies binds to proteins of the cornified envelope, corresponding with the location of the corneodesmosomes.[41] This protein plays an important role in the physiological desquamation process demonstrated by its increased degradation and the increased corneocyte detachment from the skin surface.[42]

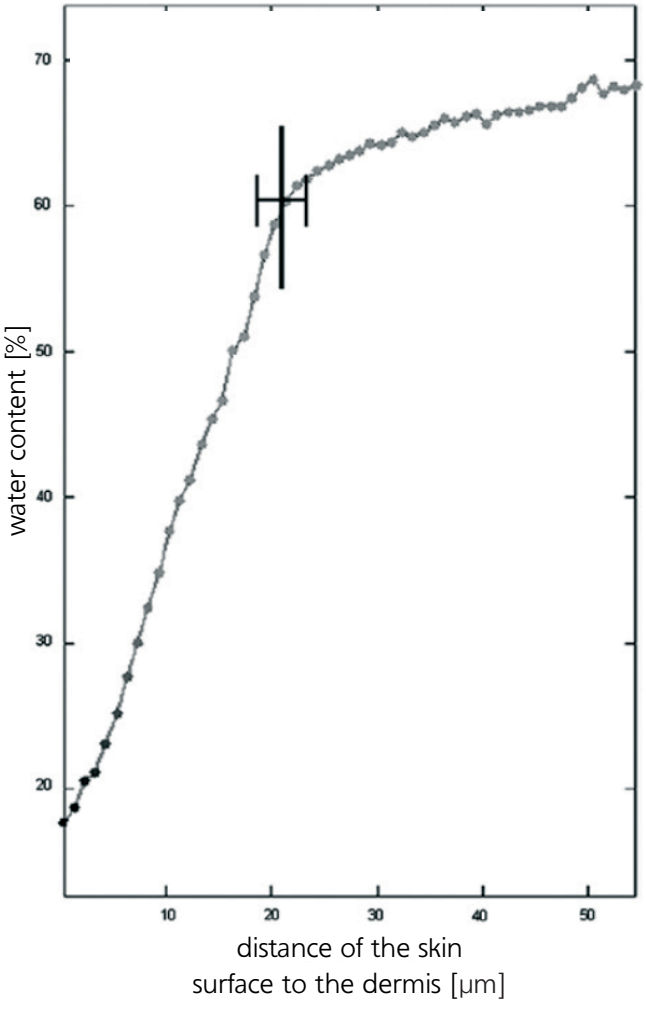
3. Regulation mechanisms of the skin barrier homeostasis

The skin barrier fulfils various defensive functions: a) protection from environmental factors (physical, chemical, biological); b) antimicrobial protection c) regulates the transport of water and the exchange of substances with the environment (excretion, secretion, resorption) and d) protection against oxidative stress.[1] A precise regulation is needed for the proper implementation of these function. There are several interrelated mechanisms and signaling systems for the formation and maintenance of the epidermal barrier:

3.1. Hydration of the stratum corneum

Water is essential for the functioning of the physiological processes in the mammalian organism. The uncontrolled passage of water to the external environment mainly depends on the integrity of the stratum corneum of the epidermis.[14] Water retention contributes to the skin elasticity. Water distribution is not homogeneous in the epidermis and its water gradient is determined through a new method - ***in vivo*** confocal Raman microspectroscopy (CRM).[43,44] The authors demonstrate a gradual increase in water content in SC from 15% to 25%, which reaches 40% at the border of SC and stratum granulosum.[43] With the transition to the deeper layers of the epidermis there is a rapid increase in the water content up to 70% which remains constant throughout the epidermis. A typical epidermal water distribution curve obtained by CRM in healthy volunteers is presented in **Figure 3** (modified by Darlenski, R. et al. Non-invasive ***in vivo*** methods for investigation of the skin barrier physical properties. Eur J Pharm Biopharm 2009; 72 (2):295-303).

Figure 3: A curve demonstrating the actual water distribution in the epidermis, obtained by in vivo confocal Raman microspectroscopy of the forearm volar surface in healthy volunteers. A marker placed at a depth of about 20 μm corresponds to the border between the stratum corneum and stratum granulosum.



Many physiological processes depend directly on the hydration of SC. The degradation of corneodesmosomes is carried out by enzymes (glycosidase and serine-proteases) which require water for functioning. In this way the process of desquamation depends on the water content in SC.[45] Impaired desquamation occurs in diseases and conditions with reduced hydration of the SC, such as ichthyosis vulgaris and xerosis senilis.[46]

The enzymatic degradation of filaggrin is dependent also on the water content in SC.[44] The reduced hydra-

J Clin Med. 2011; 4(1):36-45

tion of SC activates the filaggrin degradation to hygroscopic amino acids (the composition of the natural moisturizing factor) which retain water in the stratum corneum.[47] Impairment of this regulatory mechanism in ichthyosis vulgaris is associated with reduced levels of natural moisturizing factor and impaired skin barrier function.[45]

Stratum corneum contains pre-formed pro-inflammatory cytokines.[48] The low humidity environment causes a release of proinflammatory mediators such as interleukin-1.[49] This may explain the progression of some inflammatory skin diseases in low-humidity environment (e.g. in winter).

3.2. Calcium ions gradient in the epidermis

The quantitative distribution of calcium ions (Ca^{2+}) in the epidermis is inhomogeneous. Ca^{2+} levels are low in basale and spinous layers of the epidermis, and peak in stratum granulosum.[50] Experimental damage to the skin barrier stimulates recovery processes that occur at low Ca^{2+} concentrations in the epidermal surface layers. It has been demonstrated that reducing Ca^{2+} in the violation of the barrier integrity stimulates the secretion of lamellar bodies at the border between stratum granulosum and stratum corneum.[51,52] The barrier recovery is hindered in a Ca^{2+} rich environment. On the other hand, calcium is essential for cell differentiation and development of intercellular contacts in the epidermis. The formation of intercellular contacts through cadherins is difficult in the absence of Ca^{2+}.[53]

The disturbance in Ca^{2+} gradient has a clinical significance. In diseases such as psoriasis vulgaris and X-linked ichthyosis, such a disturbance in the Ca^{2+} gradient is observed in parallel with the impaired skin barrier function.[54,55]

3.3. Skin surface acidity as a regulatory mechanism

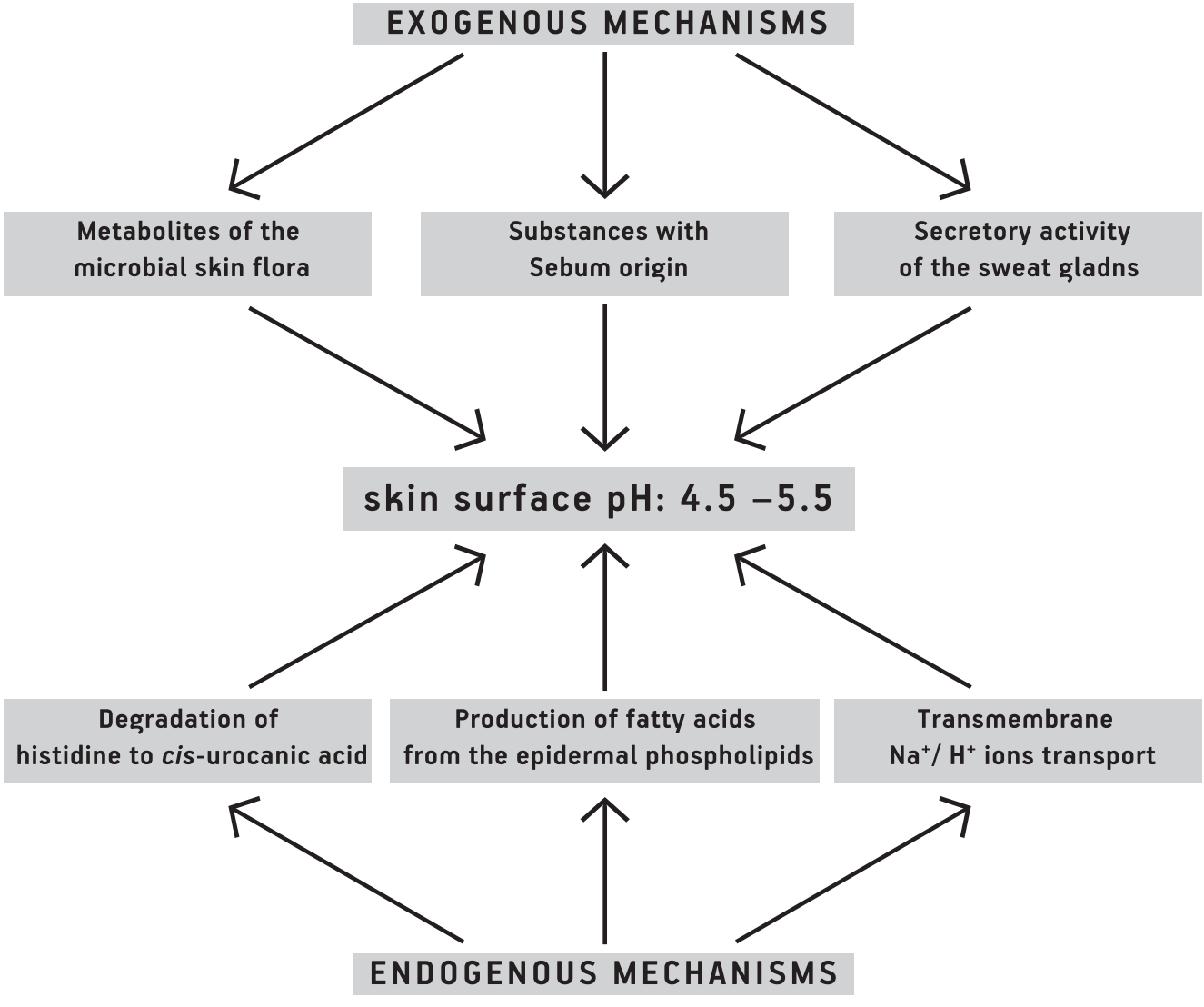
The acidic pH of the skin surface layers is important for the defensive function of the epidermal barrier and for the formation and maintenance of its integrity.[42,56] The mechanisms for the formation of the skin pH are exogenous and endogenous and are presented in figure 4.

The skin barrier function is competent at birth, while the skin surface pH is neutral.[11,57] Neutral pH is the cause of delayed recovery of experimentally damaged epidermal barrier in neonatal rats.[11] The authors explain this phenomenon with reduced activity of β-glucocerebrosidase and acidic sphingomyelinase (involved in the lipid processing of SC and with acidic pH optima) and increased activity of serine-protease (degrading the corneodesmosomes and with alkaline pH optima).[58,59] The role of pH is confirmed by the normalization of the recovery process through acidification of the environment.[11] The clinical significance of these results is demonstrated by the pathogenetic vicious circle in diaper dermatitis. The insufficient acidity of SC in infants and the alkaline environment (from ammonium salts in the urine) activate enzymes (originating from faeces) - trypsin, and lipase, causing further damage and irritation of the skin barrier.[60,61]

The increased epidermal pH at birth predisposes to more frequent bacterial and fungal infections in neonates.[56,57,61] High pH values favor the development of the skin surface pathogens such as ***Staphylococcus aureus*** and ***Candida albicans***.[60,61]

In conclusion, the violation of a single regulatory mechanism leads to the impairment of various skin barrier functions. The integrity of the skin barrier is essential for carrying out the defensive function of skin.

FIGURE 4. SCHEMATIC REPRESENTATION OF THE CONTEMPORARY UNDERSTANDING FOR THE MECHANISMS WHICH GENERATE AND MAINTAIN THE ACIDIC SKIN SURFACE PH



Bibliography:

1 Elias PM: Skin barrier function. Curr Allergy Asthma Rep. 2008;8:299-305.

2 Elias PM, Schmuth M: Abnormal skin barrier in the etiopathogenesis of atopic dermatitis. Curr Opin Allergy Clin Immunol. 2009;9:437-446.

3 Elias PM, Choi EH: Interactions among stratum corneum defensive functions. Exp Dermatol. 2005;14:719-726.

4 Wagner H, Kostka KH, Lehr CM, Schaefer UF: Interrelation of permeation and penetration parameters obtained from in vitro experiments with human skin and skin equivalents. J Control Release. 2001;75:283-295.

5 Schade T, Marchionini A: Zur physikalischen chemie der hautoberfläche. Arch Dermatol Syph. 1928;1

6 Hornby AS: Oxford advanced learner's dictionary of current english; in Wehmeier S (ed). Oxford, Oxford University Press, 2004, pp 88.

7 Clark RA, Ghosh K, Tonnesen MG: Tissue engineering for cutaneous wounds. J Invest Dermatol. 2007;127:1018-1029.

8 Kalia YN, Nonato LB, Lund CH, Guy RH: Development of skin barrier function in premature infants. J Invest Dermatol. 1998;111:320-326.

9 Williams ML, Hanley K, Elias PM, Feingold KR: Ontogeny of the epidermal permeability barrier. J Investig Dermatol Symp Proc. 1998;3:75-79.

10 Fluhr JW, Crumrine D, Mao-Qiang M, Moskowitz DG, Elias PM, Feingold KR: Topical liver x receptor activators accelerate postnatal acidification of stratum corneum and improve function in the neonate. J Invest Dermatol. 2005;125:1206-1214.

11 Fluhr JW, Mao-Qiang M, Brown BE, Hachem JP, Moskowitz DG, Demerjian M, Haftek M, Serre G, Crumrine D, Mauro TM, Elias PM, Feingold KR: Functional consequences of a neutral ph in neonatal rat stratum corneum. J Invest Dermatol. 2004;123:140-151.

12 Harding CR: The stratum corneum: Structure and function in health and disease. Dermatol Ther. 2004;17 Suppl 1:6-15.

13 Herkenne C, Alberti I, Naik A, Kalia YN, Mathy FX, Preat V, Guy RH: In vivo methods for the assessment of topical drug bioavailability. Pharm Res. 2008;25:87-103.

14 Elias P, Feingold K: Permeability barrier homeostasis; in Elias P, Feingold K (eds): Skin barrier New York, Taylor & Francis Group, LCC, 2006, pp 337-361.

15 Feingold KR: The role of epidermal lipids in cutaneous permeability barrier homeostasis. J Lipid Res. 2007

16 Feingold KR, Schmuth M, Elias PM: The regulation of permeability barrier homeostasis. J Invest Dermatol. 2007;127:1574-1576.

17 Bouwstra JA, Gooris GS, Dubbelaar FE, Weerheim AM, Ijzerman AP, Ponec M: Role of ceramide 1 in the molecular organization of the stratum corneum lipids. J Lipid Res. 1998;39:186-196.

18 Wertz PW: Biochemistry of human stratum corneum lipids; in Elias PM, Feingold KR (eds): Skin barrier. New York, Taylor & Francis, 2006, pp 33-42.

19 Bouwstra JA, Dubbelaar FE, Gooris GS, Ponec M: The lipid organisation in the skin barrier. Acta Derm Venereol Suppl (Stockh). 2000;208:23-30.

20 Tanaka M, Okada M, Zhen YX, Inamura N, Kitano T, Shirai S, Sakamoto K, Inamura T, Tagami H: Decreased hydration state of the stratum corneum and reduced amino acid content of the skin surface in patients with seasonal allergic rhinitis. Br J Dermatol. 1998;139:618-621.

21 Forslind B: A domain mosaic model of the skin barrier. Acta Derm Venereol. 1994;74:1-6.

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

23 Hill JR, Wertz PW: Molecular models of the intercellular lipid lamellae from epidermal stratum corneum. Biochim Biophys Acta. 2003;1616:121-126.

24 Mao-Qiang M, Jain M, Feingold KR, Elias PM: Secretory phospholipase a2 activity is required for permeability barrier homeostasis. J Invest Dermatol. 1996;106:57-63.

25 Willea JJ, Kydonieus A: Palmitoleic acid isomer (c16:1delta6) in human skin sebum is effective against gram-positive bacteria. Skin Pharmacology and Applied Skin Physiology. 2003;16:176-187.

26 Puhvel SM, Reisner RM, Sakamoto M: Analysis of lipid composition of isolated human sebaceous gland homogenates after incubation with cutaneous bacteria. Thin-layer chromatography. J Invest Dermatol. 1975;64:406-411.

27 Fluhr JW, Kao J, Jain M, Ahn SK, Feingold KR, Elias PM: Generation of free fatty acids from phospholipids regulates stratum corneum acidification and integrity. J Invest Dermatol. 2001;117:44-51.

28 Tagami H, Kobayashi H, O'Goshi K, Kikuchi K: Atopic xerosis: Employment of noninvasive biophysical instrumentation for the functional analyses of the mildly abnormal stratum corneum and for the efficacy assessment of skin care products. J Cosmet Dermatol. 2006;5:140-149.

29 Sprecher E, Ishida-Yamamoto A, Becker OM, Marekov L, Miller CJ, Steinert PM, Neldner K, Richard G: Evidence for novel functions of the keratin tail emerging from a mutation causing ichthyosis hystrix. J Invest Dermatol. 2001;116:511-519.

30 Rawlings AV, Matts PJ: Stratum corneum moisturization at the molecular level: An update in relation to the dry skin cycle. J Invest Dermatol. 2005;124:1099-1110.

31 Rawlings AV: Sources and role of stratum corneum hydration; in Elias PM, Feingold KR (eds): Skin barrier. New Yourk, Taylor & Francis Group, LCC, 2006, pp 399-425.

32 Candi E, Schmidt R, Melino G: The cornified envelope: A model of cell death in the skin. Nat Rev Mol Cell Biol. 2005;6:328-340.

33 Pearton DJ, Dale BA, Presland RB: Functional analysis of the profilaggrin n-terminal peptide: Identification of domains that regulate nuclear and cytoplasmic distribution. J Invest Dermatol. 2002;119:661-669.

34 Oji V, Seller N, Sandilands A, Gruber R, Gerss J, Huffmeier U, Hamm H, Emmert S, Aufenvenne K, Metze D, Luger T, Loser K, Hausser I, Traupe H, McLean WH: Ichthyosis vulgaris: Novel flg mutations in the german population and high presence of cd1a+ cells in the epidermis of the atopic subgroup. Br J Dermatol. 2009;160:771-781.

35 O'Regan GM, Sandilands A, McLean WH, Irvine AD: Filaggrin in atopic dermatitis. J Allergy Clin Immunol. 2009;124:R2-R6.

36 Sandilands A, Terron-Kwiatkowski A, Hull PR, O'Regan GM, Clayton TH, Watson RM, Carrick T, Evans AT, Liao H, Zhao Y, Campbell LE, Schmuth M, Gruber R, Janecke AR, Elias PM, van Steensel MA, Nagtzaam I, van Geel M, Steijlen PM, Munro CS, Bradley DG, Palmer CN, Smith FJ, McLean WH, Irvine AD: Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. Nat Genet. 2007;39:650-654.

37 Steven AC, Steinert PM: Protein composition of cornified cell envelopes of epidermal keratinocytes. J Cell Sci 1994;107 (Pt 2):693-700.

38 Schmuth M, Fluhr JW, Crumrine DC, Uchida Y, Hachem JP, Behne M, Moskowitz DG, Christiano AM, Feingold KR, Elias PM: Structural and functional consequences of loricrin mutations in human loricrin keratoderma (vohwinkel syndrome with ichthyosis). J Invest Dermatol. 2004;122:909-922.

39 Jeon S, Djian P, Green H: Inability of keratinocytes lacking their specific transglutaminase to form cross-linked envelopes: Absence of envelopes as a simple diagnostic test for lamellar ichthyosis. Proc Natl Acad Sci U S A. 1998;95:687-690.

40 Nemes Z, Marekov LN, Steinert PM: Involucrin cross-linking by transglutaminase 1. Binding to membranes directs residue specificity. J Biol Chem. 1999;274:11013-11021.

41 Simon M, Jonca N, Guerrin M, Haftek M, Bernard D, Caubet C, Egelrud T, Schmidt R, Serre G: Refined characterization of corneodesmosin proteolysis during terminal differentiation of human epidermis and its relationship to desquamation. J Biol Chem. 2001;276:20292-20299.

42 Hachem JP, Crumrine D, Fluhr J, Brown BE, Feingold KR, Elias PM: Ph directly regulates epidermal permeability barrier homeostasis, and stratum corneum integrity/cohesion. J Invest Dermatol. 2003;121:345-353.

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

44 Caspers PJ, Lucassen GW, Puppels GJ: Combined in vivo confocal raman spectroscopy and confocal microscopy of human skin. Biophys J. 2003;85:572-580.

45 Madison KC: Barrier function of the skin: „La raison d'etre" Of the epidermis. J Invest Dermatol. 2003;121:231-241.

46 Rawlings AV: Trends in stratum corneum research and the management of dry skin conditions. International Journal of Cosmetic Science. 2003;25:63-95.

47 Scott IR, Harding CR: Filaggrin breakdown to water binding compounds during development of the rat stratum corneum is controlled by the water activity of the environment. Dev Biol. 1986;115:84-92.

48 Wood LC, Elias PM, Calhoun C, Tsai JC, Grunfeld C, Feingold KR: Barrier disruption stimulates interleukin-1 alpha expression and release from a pre-formed pool in murine epidermis. J Invest Dermatol. 1996;106:397-403.

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

50 Cornelissen LH, Oomens CW, Huyghe JM, Baaijens FP: Mechanisms that play a role in the maintenance of the calcium gradient in the epidermis. Skin Res Technol. 2007;13:369-376.

51 Mao-Qiang M, Mauro T, Bench G, Warren R, Elias PM, Feingold KR: Calcium and potassium inhibit barrier recovery after disruption, independent of the type of insult in hairless mice. Exp Dermatol. 1997;6:36-40.

52 Choi EH, Kim MJ, Yeh BI, Ahn SK, Lee SH: Iontophoresis and sonophoresis stimulate epidermal cytokine expression at energies that do not provoke a barrier abnormality: Lamellar body secretion and cytokine expression are linked to altered epidermal calcium levels. J Invest Dermatol. 2003;121:1138-1144.

53 Furukawa F, Fujii K, Horiguchi Y, Matsuyoshi N, Fujita M, Toda K-I, Imamura S, Wakita H, Shirahama S, Takigawa M: Roles of e- and p-cadherin in the human skin. Microscopy Research and Technique. 1997;38:343-352.

J Clin Med. 2011; 4(1):36-45

54 Elias PM, Crumrine D, Rassner U, Hachem JP, Menon GK, Man W, Choy MH, Leypoldt L, Feingold KR, Williams ML: Basis for abnormal desquamation and permeability barrier dysfunction in rxli. J Invest Dermatol. 2004;122:314-319.

55 Menon GK, Elias PM: Ultrastructural localization of calcium in psoriatic and normal human epidermis. Arch Dermatol. 1991;127:57-63.

56 Leyden JJ, Kligman AM: The role of microorganisms in diaper dermatitis. Arch Dermatol. 1978;114:56-59.

57 Behne MJ, Meyer JW, Hanson KM, Barry NP, Murata S, Crumrine D, Clegg RW, Gratton E, Holleran WM, Elias PM, Mauro TM: Nhe1 regulates the stratum corneum permeability barrier homeostasis. Microenvironment acidification assessed with fluorescence lifetime imaging. J Biol Chem. 2002;277:47399-47406.

58 Fluhr JW, Behne MJ, Brown BE, Moskowitz DG, Selden C, Mao-Qiang M, Mauro TM, Elias PM, Feingold KR: Stratum corneum acidification in neonatal skin: Secretory phospholipase a2 and the sodium/hydrogen antiporter-1 acidify neonatal rat stratum corneum. J Invest Dermatol. 2004;122:320-329.

59 Hachem JP, Man MQ, Crumrine D, Uchida Y, Brown BE, Rogiers V, Roseeuw D, Feingold KR, Elias PM: Sustained serine proteases activity by prolonged increase in ph leads to degradation of lipid processing enzymes and profound alterations of barrier function and stratum corneum integrity. J Invest Dermatol. 2005;125:510-520.

60 Korting HC, Hubner K, Greiner K, Hamm G, Braun-Falco O: Differences in the skin surface ph and bacterial microflora due to the long-term application of synthetic detergent preparations of ph 5.5 and ph 7.0. Results of a crossover trial in healthy volunteers. Acta Derm Venereol. 1990;70:429-431.

61 Schmid-Wendtner MH, Korting HC: The ph of the skin surface and its impact on the barrier function. Skin Pharmacol Physiol. 2006;19:296-302.