Chapter 1
Epidermal function

As we age, the skin changes not only in appearance but also in functionality. Many people who had no special problems with the skin begin to complain about its increased irritability in older age, including inadequate reaction to cold and hot temperatures, rashes after washing, unpleasant subjective sensations (feeling of tightness, itching), and so on. Those who have experienced dermatologic problems (atopic dermatitis, seborrheic dermatitis, infectious lesions, etc.) may notice their exacerbation.

Behind the external and functional alterations, there are changes in the structure of skin tissues. However, the problem of high sensitivity is primarily related to changes at the epidermis level because this is where the main defense mechanisms of the skin are localized.

Figure V-1-1 summarizes all the major epidermal changes observed with aging (Wang Z. et al., 2020) described in detail in the sections that follow.

1.1. Barrier function alterations

The barrier function of the *stratum corneum* is assessed by measuring TEWL, which increases dramatically when the barrier is compromised.

In intact skin, mean normal TEWL values vary depending on gender, part of the body, and skin pigmentation. Although the correlation between TEWL and age is insufficiently explored, available data show that average TEWL values on some body parts in older people may be lower than in younger individuals (Boireau-Adamezyk E. et al., 2014), indicating the more reliable barrier in older skin. However, there is an age-related increase in TEWL in the décolletage area, suggesting the opposite. On the neck, forearms, and hands, young and older women have comparable TEWL levels (Luebberding S. et al., 2013a). TEWL is also higher on average in older women than in older men (Luebberding S. et al., 2013b).

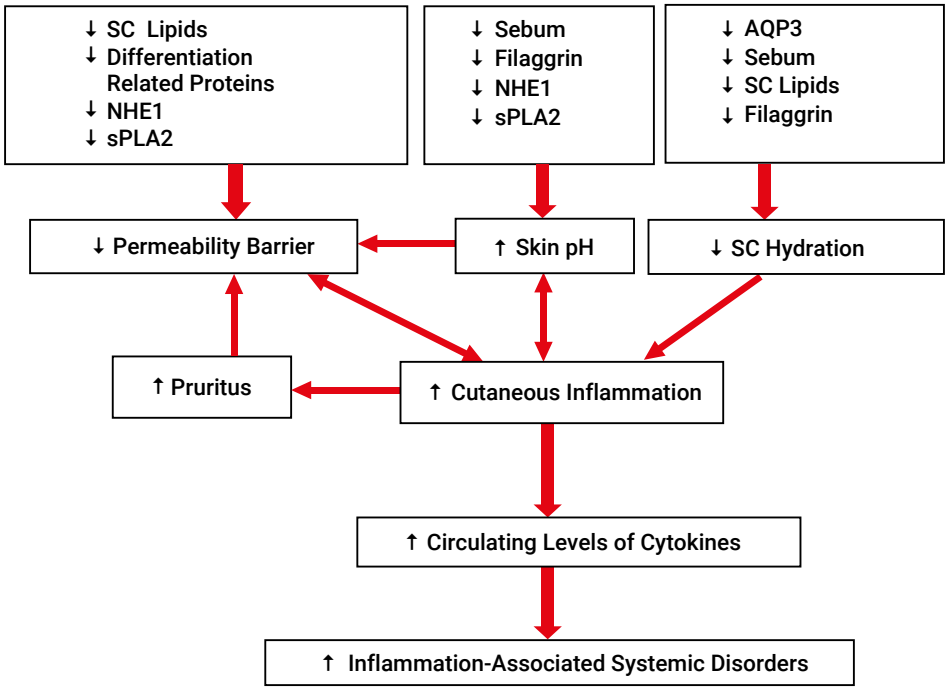

Figure V-1-1. Age-related epidermal changes and their clinical significance (Wang Z., et al., 2020)

Despite the scattered results, one thing is clear: the TEWL levels in healthy, intact skin of people of different ages, although slightly different, are not so critical to speak about the pathological disturbance of the barrier function during aging. However, there is still an age-related problem: after damage to the *stratum corneum*, the restoration of barrier function in elderly people is much slower than in younger individuals due to the biochemical and structural changes in the epidermis that occur with age.

Recall that the skin permeability barrier is localized in the *stratum corneum*. It consists of keratin-filled corneocytes surrounded by a cornified envelope made of proteins and alternating lipid and water layers between the corneocytes regulating the diffusion of low-molecular-weight substances through the *stratum corneum*. The functioning of the barrier is largely determined by the quantity and quality of protein and lipid substances formed during the maturation of keratinocytes and their final transformation into corneocytes.

1.1.1. Epidermal growth factor

In the epidermis of aging skin, the level of epidermal growth factor decreases along with a decline in the basal keratinocyte division rate. At the same time, the number of keratinocyte apoptoses increases. All these processes lead to the thinning of both living layers of the epidermis and the *stratum corneum* (Gilhar A. et al., 2004; Kinn P.M. et al., 2015).

1.1.2. Calcium ion concentration gradient

With age, there is a change in the gradient of Ca^{2+} in the epidermis, another important factor controlling the division and maturation of keratinocytes and the formation of the *stratum corneum*. Thus, in the basal and spiny layers of the epidermis of elderly people, Ca^{2+} concentration is higher, which inhibits keratinocyte proliferation (Denda M. et al., 2003; Micallef L. at al., 2009).

In contrast, in the granular layer, Ca^{2+} levels fall, and this impairs the maturation of *stratum corneum* proteins (filaggrin, loricrin, etc.) (Takahashi M. & Tezuka T., 2004; Rinnerthaler M. et al., 2013), which can lead to the formation of defective corneocytes and alterations in the permeability barrier (Scharschmidt T.C. et al., 2009).

1.1.3. Extracellular lipid sheets of the *stratum corneum* (lipid barrier)

Extracellular lipid layers also exhibit age-related changes. The formation of lipid barrier requires cholesterol, free fatty acids, and ceramides in approximately equal molar ratios (Man M.Q. et al., 1996). These lipids are synthesized by keratinocytes, and deficiency in any of them can lead to defects in barrier structures (Feingold K.R. et al., 2014). Studies have shown that the «old» *stratum corneum* has >30% lower total lipid content compared to the «young» *stratum corneum* (Ghadially R. et al., 1995), which is associated with a decrease in the synthetic activity of keratinocytes both in the intact and injured skin. Thus, applying a mixture of barrier lipids can improve barrier function in the elderly, which confirms the presence of age-related barrier dysfunction (Zettersten E.M. et al., 1997).

1.1.4. pH gradient across the *stratum corneum*

One of the stages of lipid barrier formation is the enzymatic transformation of lipid precursors into barrier lipids. This transformation is already carried out outside keratinocytes in the extracellular spaces of the *stratum corneum* (Man M.Q. et al., 1995). Besides enzymes responsible for the lipid barrier, there are enzymes in the *stratum corneum* that ensure the timely desquamation of horny scales by breaking down corneodesmosomes (proteolytic enzymes). All these enzymes, like any other enzymes, are very sensitive to the pH level. There is a pH gradient in the *stratum corneum*, from an acidic value of about 5.5 (the hydrolipid mantle on the skin surface) to a slightly alkaline value of about 7.2 (at the border with the granular layer). Thus, different layers of the *stratum corneum* will have distinct pH levels, controlling the enzyme activity at a certain depth (**Fig. V-1-2**).

With age, surface pH tends to increase (Choi E.H. et al., 2007; Man M.Q. et al., 2009; Schreml S. et al., 2012), which changes the pH gradient across the *stratum corneum*, and this affects enzyme activity. Specifically, proteolytic enzymes in the middle and on the top of the *stratum corneum* are activated at higher pH, which accelerates exfoliation. In contrast, enzymes responsible for the lipid barrier are depressed at higher pH, resulting in an altered lipid barrier. All these processes combine to weaken the permeability barrier of the *stratum corneum* (**Figures V-1-3** and **V-1-4**).

Application of preparations with neutral pH delays barrier repair; in contrast, acidification of the *stratum corneum* accelerates barrier repair in both young and aging skin (Hachem J.P., 2003; Choi E.H. et al., 2007; Hachem J.P. et al., 2010).

1.1.5. Glucocorticoids and cortisol

Biological aging is accompanied by increased glucocorticoid and cortisol levels in the skin (Yiallouris A. et al., 2019). Studies have shown that systemic or topical application of glucocorticoids inhibits keratinocyte proliferation and weakens the barrier (Kao J.S. et al., 2003). In the skin, cortisone is converted to its active form, cortisol, by 11β-hydroxysteroid dehydrogenase 1 (Tomlinson J.W. et al., 2004). In aging skin, the activity of this enzyme increases (Tiganescu A. et al., 2011), and this negatively affects the epidermis' ability to repair and form a barrier (Choe S.J. et al., 2018). Inhibition

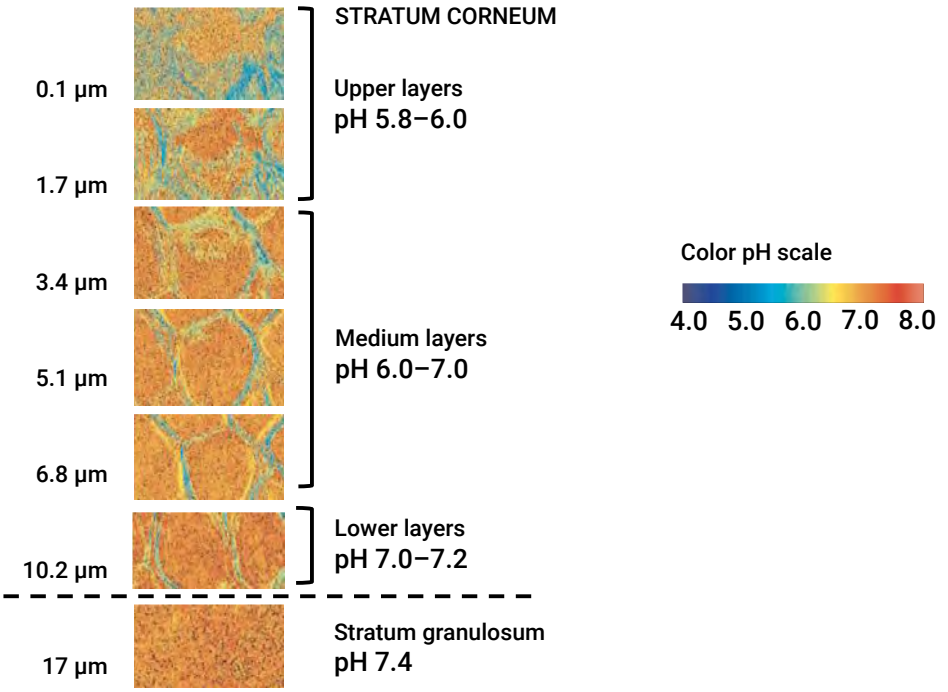

Figure V-1-2. pH change in the *stratum corneum*: assessment by two-photon spectroscopy (Hanson K.M., et al., 2002)

Special molecules, so-called fluorescent probes, are applied to the skin. They penetrate the *stratum corneum* and, upon further irradiation with light of a certain wavelength, become excited and release excess energy in photons. This secondary emission is called fluorescence and can be recorded. To determine the pH of the *stratum corneum*, a probe was chosen that can emit in both acidic and alkaline environments, but this emission will be at different wavelengths. In the resulting image, the luminescence in an acidic environment is indicated in blue and in a neutral-alkaline environment in orange. The resulting color images can be used to calculate the average pH at different depths of the *stratum corneum* based on the ratio of blue to orange areas.

The closer to the surface, the bluer the color. The calculated average pH in the upper layers of the *stratum corneum* is slightly higher than in the hydrolipid mantle but still acidic — less than 7.0. In the middle of the *stratum corneum*, the pH approaches neutral values, and it becomes progressively more alkaline at further depths.

Color distribution in the *stratum corneum* is uneven, as indicated by clearly demarcated blue (acidic) and orange (neutral) areas. The *stratum corneum* consists of dense, almost water-free horny scales, within which the pH is neutral. Free water in the *stratum corneum* is in the extracellular space, and this, too, as clearly shown here, will be acidified.

That is, even in the lowest layers of the *stratum corneum* we still see areas with acidic pH, although there are fewer of them. But under the *stratum corneum*, water is found both in the cells and in the extracellular space. As the pH here is slightly alkaline, we do not see individual cells but a uniform orange coloring.

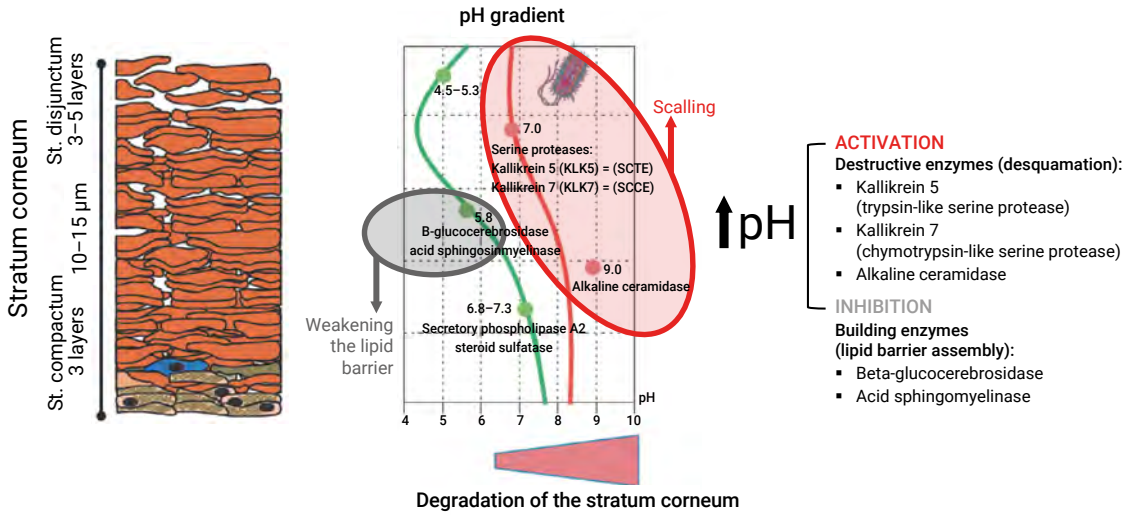

Figure V-1-3. An increase in surface pH modulates the pH gradient across the *stratum corneum* and results in enzymatic activity alterations

As the pH value increases, enzymes responsible for lipid barrier assembly are inhibited, leading to the formation of desordered lipid layers and the weakening of the permeability barrier. Conversely, proteolytic enzymes that degrade corneodesmosomes are activated, resulting in increased desquamation and visible scaling.

Increased serine protease activity in atopic skin: orange staining intensity correlates with serine protease activity (fluorescence micrograph, photo: Peter Elias)

pH

- Atopic dermatitis
- Ichthyosis vulgaris
- Seborrhea, acne, seborrheic dermatitis
- Diaper dermatitis
- Irritant contact dermatitis
- Patients on dialysis
- Candida intertrigo (*Candida albicans*)
- Onychomycosis (*tinea pedis*)

Figure V-1-4. Skin pathologies associated with elevated surface pH