ROLE OF EPIDERMAL CERAMIDES IN BARRIER FUNCTION

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Synopsis

Lipids are considered to play an important role in the structure, differentiation and function of the epidermis.
During the process of keratinisation and epidermal differentiation the lipid composition of the skin changes dramatically. These changes are consistent with the requirement for cutaneous waterproofing. In this view, many reports have shown the importance of sphingolipids in maintaining the optimal mammalian epidermal permeability barrier function.
Stratum corneum lipids major constituent, ceramide, has been shown to be associated to both water retaining capacity of the skin and permeability barrier; ceramide is thought to derive primarily from glucosylceramide which is pratically absent in the exterior layer of the stratum corneum.
Altogether these data lead to the hypothesis that the hydrolysis of glucosylceramide to ceramide, mediated by a lysosomal-like β-glucosylceramidase, plays an important role in the formation and maintenance of epidermal permeability barrier.
These evidences will be critically evaluated in the light of our experimental data on ceramide and glucosylceramide content in the skin of patients with Gaucher's disease. This sphingolipidosis, due to the deficiency of lysosomal β-glucosylceramidase, is often associated with cutaneous abnormalities.

Riassunto

È definitivamente accertato che i lipidi giochino un ruolo molto importante nella organizzazione strutturale e funzionale dell'epidermide e dei processi di differenziamento ad essa correlati.
Durante il processo di cheratizzazione e differenziamento la composizione in lipidi della cute si modofica sostanzialmente nell'intento di mantenere una ottimale omeostasi del contenuto d'acqua dell'epidernide. In questa ottica un ruolo molto importante è svolto dai glicosfingolipidi; infatti il principale costituente lipidico dello strato corneo, il ceramide, è coinvolto sia nella ritenzione d'acqua sia nella funzione barriera svolta dall'epidermide. Si ritiene comunemente che questo sfingolipide derivi per idrolisi catalizzata da un enzima specifico, β-glucosilceramidosi, dal glucosilceramide presente nei corpi lamellari. Questi dati verranno criticamente valutati sulla base dei risultati ottenuti nel laboratorio dei relatori in pazienti affetti da malattia di Gaucher, sfingolipidosi caratterizzata dall'assenza, geneticamente determinata, dell'enzima β-glucosilceramidasi.

1. Keratinization process and lipid synthesis

Keratinocytes are the predominant epidermal cell-type: they produce the keratins, a family of related proteins responsible for specific skin functions including resistence against environmental attacks and impermeability to substances which get in touch with the skin.

Epidermis is made of different cell layers referred as to basal, spinous, granular and horny layers. Keratinocytes move from the former to the latter, changing their morphology, shape and metabolic activity. In the basal layer cells show intense metabolic activity due to their rapid division; in the spinous layer cells develop organelles necessary for the synthesis of a keratin precursor protein, keratohyaline, which is converted to keratin by spcific enzymes; moreover they produce keratinosomes or Odland bodies containing lipid compounds in the form of bistratified discs stacked one on top to the other. Moving up the cell becomes flat, loses its regular shape, the nucleous is expelled. The cell is so transformed in a flattened envelope which internal walls are covered with lipids. In the granular layer the content of Odland bodies are poured into the intercellular spaces when they form the leaves of lipoid material found between the dead cells (corneocytes) of the horny layer. In the most external portion of this layer the corneocytes gradually lose their cohesion and are desquamated. They will be then replaced by keranocytes pushing up from the underlying layers. (1, 2)

This cyclic process is know as keratinization. The term refers either to the life of epidermal cells, which lasts about 28 days, or to the physiological processes, schematically described above, which occur during this period; as above indicated one of this process accounts for the production of epidermal lipids which are indispensable for the maintenance of epidermal integrity.

The hydrofobic material synthetised in the Odland bodies have been biochemically characterized and it is composed mainly of glucosylceramide and ceramide. Though other substances such as sphingosine, cholesterol esters and fatty acids are also present, it as been clearly demonstrated that only the ceramides play an essential role in barrier function.

2. Sebum: its composition and functions (3)

Production of sebum is not constant, and changes with different stages of life. In the foetus the sebaceous glands begin to secrete sebum during the fourth month. Later when the newborn baby is no longer receiving the maternal hormones which stimulate the glands, sebocytes enter a state of quiescence which only ends when the child reaches puberty; at this stage the sexual glands have developed and start to secrete sex hormones - predominantly androgens in the male and estrogens in the female. In females there is a reduction in sebum production after the menopause caused by the progressive inactivation of the ovaries. On synthesis, sebum has a different composition from that when it reaches the skin surface.

The composition of sebum on the skin surface is reported in the following table:

free fatty acids	20%
triglycerides	40%
squalene	15%
wax	20%
sterol, glyco and phospholipid	5%

Sebum is fundamental in all mammals as it represents the best protection for their fur and, in man, for the skin.

By emulsifying itself, that is, mixing with the water derived from sweating or traspiration, sebum forms a film over the skin which is able, within certain limits, to protect the epidermis from harmful chemical substances and from the action of pathogenic micro-organism.

3. The hydrolipid film (4)

The hydrolipid film is produced by the sebaceous glands, sweat and transpiration; its importance in the maintenance of the skin's emollience and defence against pathogenic bacteria is well established. Once produced the film can flow into the grooves from one region to neighbouring ones; differences in the distribution density of sweat and sebaceous glands mean that not all the areas of the skin are equally covered by the hydrolipid film.

Regions which are poorly supplied will have less natural emollience and, retaining less water, will have a higher tendency to desquamation. Furthermore, in some individuals the sebaceous glands produce hydrolipid film which is qualitatively altered and tends to irritate the skin itself.

The hydrolipid film is essentially composed of:

- sebum components
- cellular waste (keratinocyte residues)
- bacterial substances (derived from bacteria normally present on the skin)
- water (sweating and transpiration)
- exogenous substances (cosmetics, dirt)

An important point to remember regarding the hydrolipid film is that it constitutes part of the normal dirt that we remove daily from our skin by cleansing. The excessive removal of the hydrolipid film strips the epidermis of its protection and exposes the skin itself to environmental attack and dehydration.

4. Skin hydration (5)

Hydration represents the most important parameter for the health of the skin. Numerous factors determine the water content of the skin, although, overall it is directly related to the ambient humidity and the skin can only retain adeguate concentration of water at a relative humidity of 60%.

The skin's factors which govern the maintenance of its hydration are:

- epidermal lipid concentration and composition
- surface hydrolipid film distribution
- natural moisturizing factor (NMF) occurence
- horny layer physiologically normal architecture
- presence of organic substances (salts, amino acids, jaluronic acid)

Epidermal lipids have a fundamental role both in binding water and in occluding the intercellular spaces. Their action is correlated with the correct structure of the lamellae in the horny layer which otherswise would not be able to retain water.

The hydrolipid film covers the epidermis with a thin protective layer which softens and defends the epidermis itself against both chemical and bacterio-micotic agents. Moreover it slows down the desquamation rate. Removal of the film by excessive cleansing, for example, leads to damage of the horny layer which desquamates and loses intercellular lipids, so opening the barrier for the entry of germs and harmful substances.

The NMF are a series of substances produced by epidermal cells and by sweating which function to bind water both intracellularly and extracellularly toghether with the intercellular lipids. Of the NMFs the most important are PCA (pirrolidon carboxylic acid) and urea, but many other organic substances and mineral salts are part of the group.

EPIDERMAL LIPIDS (1, 6, 7)

The skin protects the body from excessive water loss and at the same time hinders the entrance of chemical substances, microorganisms and antigens. From a physiological and biochemical point of view the skin must ensur firmness, resistance and flexibility, production of keratinocytes, appropriate desquamation rate, rapid exchange of dead cells and formation of a semipermeable barrier which dispels water in a controlled way. As previously indicated the most external level of defence is the surface hydrolipid film, produced by the skin itself; in fact it is able to exert a selective antimicrobial action, thanks to the presence of medium-chain fatty acids and acidic pH and to increase the water retention capacity (WRC) of the horny layer.

On the basis of composition and origin, the surface lipids can be divided into two groups: those derived from the sebaceous glands and those derived from the epidermis. The latter are essential for the WRC of the horny layer, the hydratation state, the functioning of the semipermeable skin barrier (8) and in the light of recent knowledge, also in the control of the processes of maturation and desquamation of the horny layer. In this view the sphingosine derived from the hydrolysis of ceramides is thought to stimulate keratinocyte production and differentiation. (9,10)

Morphologically speaking the epidermal lipids originate from the Odland bodies, appear in the intracellular spaces at the upper limit of the granular layer and are found as laminar structures between the corneocytes.(11) The horny layer is a bi-compartmental system: cells rich in proteins and without lipids embedded in intracellular spaces packed with lipids.

Two different groups of epidermal lipids can be distinguished on the basis of their functions:

- lipids not containing sphingosine
- sphingolipids.

The former are: glycerophospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine), triglycerides, free fatty acids (oleic, palmitic, palmitoleic, linoleic), sterols (cholesterol, squalene, cholesterol esters) and n-alcans. The sphingolipids are: glycosphingolipids, sphingomyelin and six classes of ceramides. (12)

Epidermal cholesterol is a product of keratinocyte metabolism; it is synthesized in the lower regions of the epidermis and subsequently converted into cholesterol sulphate (which is found in highest concentrations in the granular layer) by a sulphotransferase. Steroid sulphatase finally reconstitutes cholesterol so that cholesterol sulphate is not present in the desquamated horny lamellae. (13,14,15)

Based ont the most recent findings, cholesterol is thought to be fundamental in the cohesion - desquamation rate of the corneocytes. (15)

The free or esterified fatty acids found in man have a length varying between C-14 and C-24, the majority of molecules being C-16 and C-18. In normal skin the ratio of saturated fatty acids to unsaturated is approximately one, while in dry skin a decrease in linoleic acid and an increase in palmitoleic acid was shown. The application of emulsions rich in linoleic acid restablishes a distribution of fatty acids which is similar to that of normal skin. (16)

Since the discovery of ceramides, the modifications induced by the lack of essential fatty acids in the skin have been explained as alterations in the ceramide backbone, of which one of the essential constituent is linoleic acid. (17)

Phospholipids are essential to maintain the lipid bi- layer of many cell membranes. They are present in large quantities in the germinative layer of the epidermis; on the contrary in the outer layers, the horny one for example, the phospholipid subclasses (phosphatidylcholine and phosphatidylethanolamine) which were present in the germinative layer have virtually disappeared and new classes of lipids, like the ceramides, appear. (18)

The barrier function of the skin depends mostly on the structural organisation of the lipids rather then the quantity of any single class of lipids present in the horny layer. (19)

SPECIFIC FUNCTIONS OF EPIDERMAL GLICOLIPIDS AND CERAMIDES.

As already mentioned lipids are considered to play an important role in the structure, differentiation and function of the epidermis.
During the process of keratinisation and epidermal differentiation the lipid composition of the skin changes dramatically. (20,21) These changes are consistent with the requirement for cutaneous waterproofing. (21, 22, 23, 24)
In this view, many reports have shown the importance of sphingolipids in maintaining the optimal mammalian epidermal permeability barrier function, (25, 26, 27, 28) and solvent extraction studies have shown that the progressive removal of sphingolipids, rather than non polar lipids, is associated with proportional abnormalities in barrier function. (28)
Stratum corneum lipids major constituent, ceramides, has been shown to be associated to both water retaining capacity of the skin and permeability barrier; ceramides are thought to derive primarily from glucosylceramides which are pratically absent in the exterior layer of the stratum corneum. (29) This suggests the presence in the horny layer of hydrolytic enzymes; in fact, data from the literature show that a number of catabolic enzyme, such as sphingomyelinase, triacylglycerol hydrolase (30), phospholipase A (31), steroid sulphatase (15), and β-glycosidase (32, 33) are localised in the lower part of the horny layer. It was suggested that the last enzyme (E.C.: 3.2.1.45 β-D-glucosyl-N-acylsphingosine glycohydrolase) should have similar characteristic with the lysosomal isoform, even though their topology is different. This suggestion is further supported by the ascertained presence in the lamellar bodies of a proton pump to secure the acidic conditions for the optimum of the activity of the enzyme. (34)
Due to these similarities we investigated the glycolipids and ceramide distribution in the stratum corneum of Gaucher's patients, whose dry skin might have been due to a "storage" of glucosylceramide, compaired with the same parameters in healthy donors. Gaucher's disease is a lysosomal storage disease, characterized by the absence, genetically determined, of β-glucosylceramide glycohydrolase.
Our results indicate that the glucosidase present in the lamellar bodies of Gaucher's patients stratum corneum failed to hydrolyze the glucosylceramide to ceramide, as shown by the high percentage in the pathological samples of the former (range: 48-69% of the total glycolipids), and the complete absence of the latter. (35)
We are evaluating the usefulness of topical therapy (in addition to the replacement therapy which became recently availabe for the treatment of Gaucher's patients) with a lotion containing commercially available 3 hydroxy ceramide, which fatty acids composition is closely related to that of the main ceramide present in human skin. (10) The results so far obtained seem to be very promising.
Altogether these data lead to the concept that the hydrolysis of glucosylceramide to ceramide plays an important role in the formation and maintenance of epidermal permeability barrier.
This prospect introduces new roles for functional cosmetics, which should be made not only by inert substances but also by molecules suitable to interact with the skin and to contribute to the restoration of its correct physiology, biochemystry and structure.

References

1. **Berra B., Rapelli S. (1994)** Biochemistry of the skin. Structural and metabolic aspects of carbohydrates, proteins and lipids. In: *The skin as a dynamic barrier and the function of cosmetics.* RTC Acta, vol. **3**, pp.49-67.
2. **Elias P.M. (1981)** Epidermal lipid membranes and keratinization. *Int. J. Derm.*, **20**, 1-12.
3. **Wheatley V.R. (1986)** The Sebaceous Glands. In: *The physiology and pathophysiology of the skin.* A. Jarret (Ed) vol. 9 Academic Press.
4. **Celleno L. (1994)** Structure and function of the skin. In: *The skin as a dynamic barrier and the function of cosmetics.* RTC Acta, vol. **3**, pp.41-42.
5. **Celleno L. (1994)** Structure and function of the skin. In: *The skin as a dynamic barrier and the function of cosmetics.* RTC Acta, vol. **3**, pp.42-43.
6. **Berra B., Rapelli S. (1991)** Role of lipids in cellular membrane and skin biology. Proc. Intern. Congress: "Progress in Cosmetic Dermatology: Science and Safety.", Rome October 30 - Nov. 2, 1991.
7. **Giannetti A., Di Nardo A. (1994)** The skin as a barrier in dynamic equilibrium. In: *The skin as a dynamic barrier and the function of cosmetics.* RTC Acta, vol. **3**, pp.77-81.
8. **Schurer N.J., Elias P.M. (1991)** The biochemistry and Function of Stratum Corneum Lipids. In: *Advances in Lipid Research,* vol. 24 Skin Lipids P.M. Elias (Ed) Academic Press Inc., pp. 27-56.
9. **Uchida Y., Iwamori M., Nagai Y. (1990)** Activation of keratinization of keratinocytes from fetal rat skin with N-(O- Linoleolyl) w-Hydroxy Fatty Acyl Sphingosyl Glucose (Lipokeratinogenoside) as a marker of epidermis. *Biochem. Biophys. Res. Commun.* **170**, pp. 162-168.
10. **Uchida Y., Ogawa T., Iwamori M., Nagai Y. (1991)** Enhancement of keratin synthesis induced by lipokeratinogenoside, N-(O- Linoleolyl) w-Hydroxy Fatty Acyl Sphingosyl Glucose, in association with alteration of the intracellular Ca2+ content and protein kinase in cultured keratonocytes (FRSK). *J. Biochem.* **109**, pp. 462-465.
11. **Wertz P.W., Downing D.T., Freinkel R.K. and Traczyk T.N. (1985)** Sphingolipids of the stratum corneum and lamellar granules of fetal rat epidermis. *J. Invest. Dermat.*, **83**, pp.193-195.
12. **Lampe M.A., Burlingame A.L., Whitney J., Williams M.I., Brown H.E., Roitman E., Elias P.M. (1983)**. Human stratum corneum lipids: characterization and regional variations. *J. Lipid Res.*, **24**, pp.120-130.
13. **Proksch E., Elias P.M., Feingold K.R. (1990)** Regulation of 3-Hydroxy-3-methylglutaryl-CoA reductase activity in murine epidermis: modulation of enzyme content and activation state by barrier requirements. *J. Clin. Invest.*, **85**, pp. 874-882.
14. **Feingold K.R., Man M.Q., Menon G.K., Cho S.S., Brown B.E., Elias P.M. (1990)** Cholesterol synthesis is required for cutaneous barrier function in mice. *J. Clin. Invest.*, **86**, pp. 1738-1745.
15. **P.M. Elias, M.L. William, M.E. Maloney, J.A. Bonifas, B.E. Brown, S. Grayson, E.H. Epstein Jr. (1984)** Stratum corneum lipids in disorders of cornification: steroid sulfatase and cholesterol sulfate in normal desquamation and the pathogenesis of recessive X-linked ichthyosis. *J. Clin. Invest.*, **74**, pp. 1414-1421.
16. **M. Press, P.J. Hartop, C. Prottey (1974)** Correction of essential fatty acid deficiency in man by the cutaneous application of sun-flower-seed oil. *Lancet,* **1**, pp. 597-599.
17. **S. Hamanaka, C. Asagami, M. Suzuki, F. Inagaki, A. Suzuki (1989)** Structure determination of glucosyl-β1-N- (w-o-linoleoyl)-acylsphingosines of human epidermis. *J. Biochem.* (Tokyo), **105,** pp. 684-690.

18. **L.A. Goldshmidt (1983)** *Biochemistry and Physiology of the Skin.* Oxford Univ. Press, Oxford.
19. **G. Grubauer, K.R. Feingold, R.M. Harris, P.M. Elias. (1989)** Lipid content and lipid type as determinant of the epidermis permeability barrier. *J. Lipid Res.*, **30**, pp. 89-96.
20. **G.M. Gray and H.J. Yardley (1975)** Different populations of pig epidermal cells: isolation and lipid composition. *J. Lipid Res.*, **16**, pp. 441-447.
21. **P.M. Elias, B.E. Brown, P. Fritch, J. Goerke, G.M. Gray and J. White (1979)** Localization and composition of lipids in neonatal mouse stratum granulosum and stratum corneum. *J. Invest. Dermatol.*, **73**, pp. 339-348.
22. **P. M. Elias. (1983)** Epidermal lipids, barrier function, and desquamation. *J. Invest. Dermatol.*, **80,** pp. 40-49.
23. **M.L. Williams and P.M. Elias. (1987)** The extracellular matrix of stratum corneum: role of lipids in normal and pathological function. *Crit. Rev. Ther. Drug Carrier Syst.*, **3**, pp. 95-122.
24. **H.J. Yardley and R. Summerly (1981)** Lipid composition and metabolism in normal and diseased epidermis. *Pharmacol. Ther.,* **13**, pp. 357-383.
25. **P.W. Wertz, D.T. Downing (1982)** Glycolipids in mammalian epidermis: structure and function in the water barrier. *Science* (Wash. DC), **217**, pp.1261-1262.
26. **W.M. Holleran, K.R. Feringold, M. Mao-Qiang, W.N. Gao, J.M. Lee and P.M. Elias. (1991)** Regulation of epidermal sphingolipid synthesis by permeability barrier function. *J. Lipid Res.*, **32**, pp. 1151-1158.
27. **W.M. Holleran, M.Mao Qiang, G.K. Menon, P.M. Elias and K.R. Feingold. (1991)** Sphingolipids are required for mammalian barrier function: inhibition of sphingolipid synthesis delays barrier recevery after acute perturbation. *J.Clin. Invest.*, **88**, pp. 1338-1345.
28. **G. Grubaner, K.R. Feingold and P.M. Elias (1989)** Lipid content and lipid type as determinants of the epidermal permeability barrier. *J. Lipid Res.*, **30**, pp. 89-96.
29. **W.M. Holleran, Y. Takagi, G.K. Menon, G. Legler, K.R. Feingold, P.M. Elias. (1993)** Processing of epidermal glycosylceramides is required for optimal mammalian cutaneous permeability barrier function. *J.Clin. Invest.*, **91**, pp. 1656-1664.
30. **G.K. Menon, S. Grayson and P.M. Elias. (1986)** Cytochemical and biochemical localization of lipase and sphingomyelinase activity in mammalian epidermis. *J. Invest. Dermatol.*, **86**, pp. 591-597.
31. **P.M. Elias, G.K. Menon, S. Grayson and B.E. Brown.(1988)** Membrane structural alterations in murine stratum corneum: relationship to the localization of polar lipids and phospholipases. *J. Invest. Dermatol.*, **91**, pp. 3-10.
32. **M.K. Nemanic, J.S. Whitehead and P.M. Elias (1983)** Alterations in membrane sugars during epidermal differentiation: visualization with lectins and role of glycosidase. *J. Histochem. Cytochem.*, **31**, pp. 887-897.
33. **W.M. Holleran, Y. Takagi, G. lmokawa, S. Jackson, K.R. Feingold and P.M. Elias. (1992)** β-Glucocerebrosidase activity in murine epidermis: characterization and localization in relationship to differentiation. *J. Lipid Res.*, **33**, pp. 1201-1209.
34. **S.J. Chapman, A. Walsh. (1989)** Membrane-coating granules are acidic organelles which possess proton pumps. *J. Invest. Dermatol.*, **93**, pp. 466-470.
35. **L. Adorni, S. Rapelli, R. Ciuffo, B. Berra. (1995)** Skin lipid abnormalities in Gaucher's disease. Submitted for pubblication.