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Review article

Contribution of lipid components to the permeability barrier of oral mucosa

A. Ganem-Quintanar [a], F. Falson-Rieg [a,b], P. Buri [a,c,*]

[a] *Pharmapeptides, Interuniversity Centre Geneva-Lyon, Archamps, France*
[b] *University Claude Bernard, Lyon, France*
[c] *University of Geneva, Geneva, Switzerland*

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Abstract

The aim of this paper is to review the lipid composition of different regions of the oral mucosa, bearing in mind that, in general, molecular diffusion occurs through the intercellular lipids. Lipid content and organization is described to explain the permeability differences between keratinized and nonkeratinized tissues. Some of the thermotropic and lyotropic transitions observed in simple lipid model systems, such as liposomes, are also taken into consideration, in an attempt to explain the contribution of each lipid class to the permeability barrier. © 1997 Elsevier Science B.V.

Keywords: Lipids; Phase transition; Polymorphism; Oral mucosa; Permeation enhancers

1. Introduction

The first report on the ability of substances to cross the oral mucosa was that of Brunton published in 1880, who described the effects of sublingually administered glyceryl trinitrate in alleviating the symptoms of angina pectoris [1]. The use of the oral mucosa as a drug delivery site has led to the assumption that it is a highly permeable tissue, but in reality, the permeability of the oral mucosa is a complex issue that depends not only on the structure, but also on the nature of the penetrants [2].

It is generally accepted that the diffusional resistance of oral mucosa is primarily associated with the intercellular lipids of the outer layers of the tissue [3–10]. Differences in the composition and organization of these lipids give rise to regional variations in permeability. Thus, the arrangement of lipids within the tissue, as well as their biophysical behaviour, are fundamental aspects not only to study absorption, but also to explain the action of substances applied on the epithelium.

Most enhancers have been reported to act up on the intercellular lipids, in many cases by fluidizing, extracting or reorganizing the lipid domains, and in this way, perturbing membrane integrity. These phenomena are often related to the thermotropic and lyotropic transitions undergone by lipids, when they are subjected either to temperature or hydration changes, or exposed to certain chemicals.

Therefore, the purpose of this article is to review the composition and organization of the lipid domains in the different regions of the oral mucosa, taking into

Abbreviations: CH, cholesterol; CS, cholesterol sulphate; DSC, differential scanning calorimetry; ΔH, enthalpy change; IR, infrared spectroscopy; MCG, membrane-coating granules; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; T_M, transition temperature.

* Corresponding author. School of Pharmacy, University of Geneva, Quai Ernest Ansermet 30, 1211 Geneva 4, Switzerland. Tel.: +41 22 7026568; fax: +41 22 7026567; e-mail: adriana.quintanar@pharm.unige.ch

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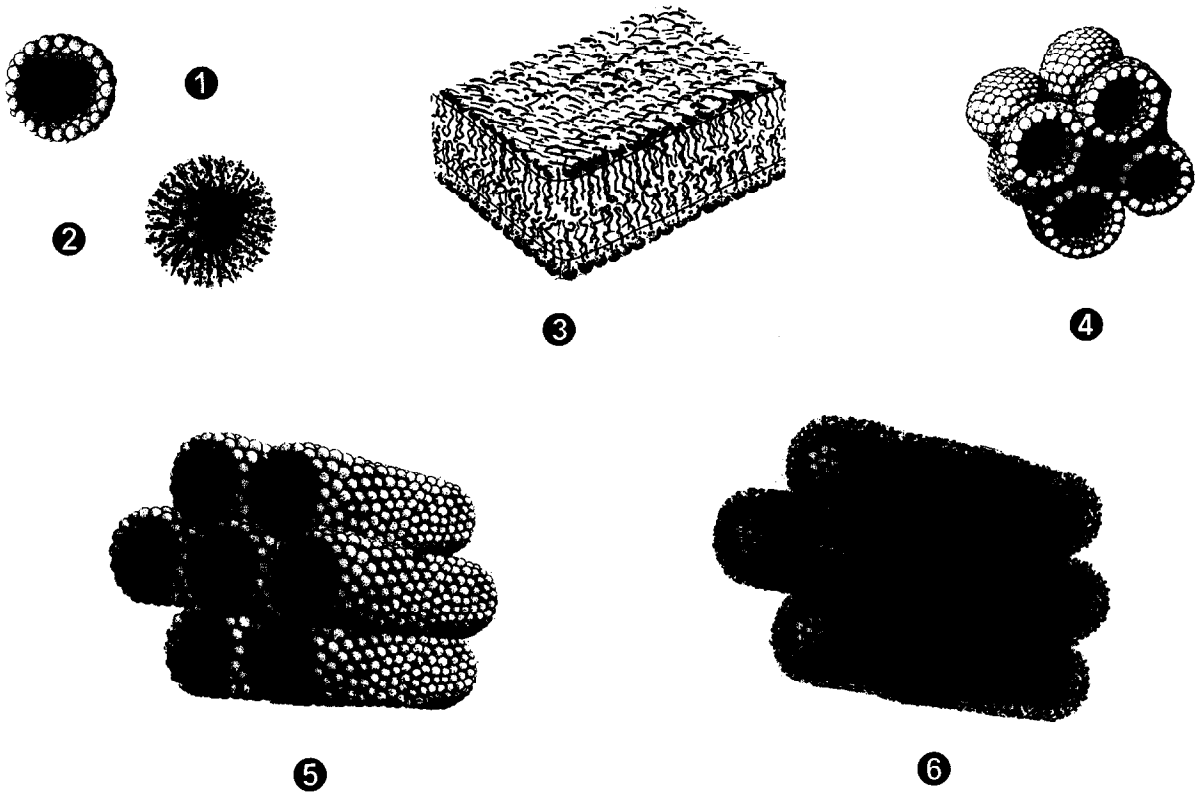

Fig. 1. Polymorphic phases available to hydrated liquid crystalline lipids. (1) Micellar; (2) inverted micellar; (3) bilayer; (4) cubic; (5) hexagonal (H_I); (6) hexagonal (H_{II}). (Modified from Brown et al., 1971 [25]).

consideration some biophysical aspects of lipid behaviour, namely, polymorphism and phase transitions, as phenomena capable of explaining the function of biological membranes, as well as the differences in permeability between the different oral regions. However, as excellent reviews are already available in the literature on the subject of the biophysical behaviour of lipids [11–15], this aspect is only described briefly. Interesting reviews about the use of permeation enhancers for oral mucosal drug delivery and their mechanisms of action are also available [4,16].

2. Biophysical behaviour of lipids

2.1. Structural aspects

Although a general molecular-level explanation of the role of lipid properties in membrane function has not been completely elucidated, it is clear that the integrity of natural membranes relies upon the interaction of a wide variety of polar and nonpolar lipids and proteins. However, there are certain conceptual problems involved in assuming a constant bilayer structure because it is difficult to reconcile a variety of functional capacities such as cell fusion, exo- and endocytosis, transbilayer movements of lipids ('flip-flop'), facilitated transport as well as protein insertion and orientation, with an inviolate bilayer structure [14,17–20]. The great variety of lipids found in biological membranes suggests that lipids play functional roles other than the simple formation of a bilayer structure; furthermore, when dispersed in water, membrane lipids can adopt a variety of aggregated structures (Fig. 1).

The ability to exist in several different phases, is an important property of pure lipids and lipid mixtures, which depends on temperature, hydration, lipid class, and the length and degree of saturation of their alkyl chains [13,15,21,22]. This ability lends itself to a view of biological membranes somewhat different from that suggested by previous models such as the fluid mosaic model [14,15,23,24]. It is possible that these non-bilayer aggregates may be related to biomembrane structure and function, thus allowing new possibilities for the dynamic participation of lipids in functional processes.

2.2. Phase transitions

A common feature of all membrane lipids, is the existence of a temperature-dependent reversible transition (Fig. 2). Below the transition temperature (T_M), lipids exist in a 'gel' state. An increase in temperature or hydration results in the transition to a more disordered fluid-like state (liquid–crystalline state), similar

to the fusion of a crystalline solid. However, when a given lipid molecule is heated, instead of melting directly into an isotropic liquid it may pass through intermediate states called mesophases or liquid crystals, characterized by residual order in some directions but lack of order in others [25]. Such molecules undergo thermotropic mesomorphism. Furthermore, certain molecules may be induced to form liquid crystals by the addition of a second chemical component, i.e. a solvent such as water (lyotropic mesomorphism) [15]. The disorder in the lipid structure, brought about by the thermotropic or lyotropic transitions, has been related to a decreased diffusional resistance of the tissue [26–37].

A great deal of information about membranes, has been obtained through the study of the thermotropic and lyotropic properties of pure synthetic lipids and simple lipid mixtures, synthetic lipids having the advantage of being homogeneous in their fatty acid composition. However, it is important to consider that different conformational and polymorphic states may exist in the isolated components, resulting in differences in thermal transitions when compared to the intact tissue samples. For example, thermal transitions of lipids extracted from the stratum corneum are sharper and occur at slightly lower temperatures than those observed using intact stratum corneum [26]. Much of the information on membrane lipid composition and fluidity has also been obtained from the study of easily manipulated bacterial membranes [38].

Artificial membranes with a unique lipid type undergo a phase transition at a well-defined temperature (the melting point of the membrane-component lipid). However, as biological membranes have a heterogeneous lipid composition, their phase transitions occur over a broad temperature range [11,13]. Calorimetric studies of many mammalian membranes suggest that most of the lipid is in the liquid–crystalline state at ambient temperature, although a small fraction of gel phase lipid may also be present [11].

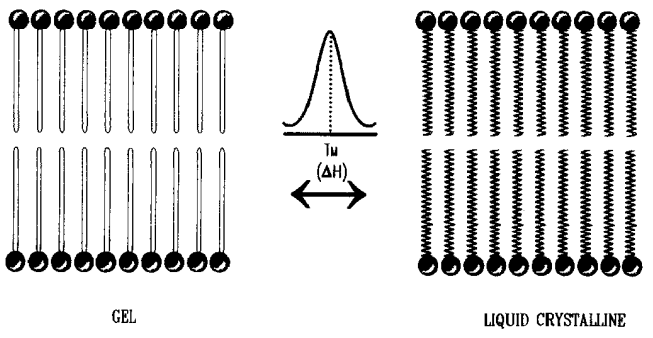

Fig. 2. Order–disorder transition in lamellar aqueous lipid dispersions. T_M, transition temperature; ΔH, enthalpy change. (Modified from Curatolo, 1987 [22]).

The thermal behaviour of lipid–water systems [39], as well as of stratum corneum [26,40–50] and rabbit buccal lipids [51,52], has been well characterized. These studies have shown a thermally reversible transition at ~ 60°C, and a transition at ~ 70°C, this latter transition was associated with a dramatic increase in lipid fluidity. X-ray diffraction studies [53], showed that at 70°C all of the alkyl chains were in the liquid crystalline state and, if an excess of water was present, lipids very likely formed an inversed hexagonal (H_{II}) phase. A marked decrease in the fluorescence anisotropy of liposomes composed of lipids commonly found in oral tissues (ceramide, cholesterol, cholesterol sulphate and fatty acid) was observed when temperature reached 70°C [54].

The C–H and C–D stretching vibrations measured by infrared (IR) spectroscopy and associated with the thermal motion of the lipid hydrocarbon chains, indicated an increased flexing of the hydrocarbon chain and an increased rotational isomerization about the C–C bonds along the chain as the temperature was raised [38,44,55–57]. Above 70°C, stratum corneum behaved like a lipid-deficient barrier, allowing an almost free diffusion of water. Furthermore, an irreversible thermal alteration of the stratum corneum barrier properties was observed at temperatures above 80°C [53].

Hydration has a great influence on lipid fluidity, altering the differential scanning calorimetry (DSC) profiles and IR spectra. Increased hydration results in a decreased T_M [26] and thus, an increased membrane fluidity [37]. Hydration-induced changes in skin permeability have been demonstrated by Behl et al. [27–29]. Thus, since oral mucosa, unlike the skin, has a moist surface, it may contribute to the higher permeability of the keratinized oral tissues when compared to the epidermis [58]. A decrease in T_M has also been observed with some substances frequently used as epidermal and oral mucosal permeation enhancers, i.e. fatty acids [59–62], surfactants [63–66], Azone® [67–69] and ethanol [23,24,35,36].

3. Lipid composition and permeability of the oral epithelium

3.1. Absorption pathways

From a histological point of view, the oral mucosa is covered by a stratified epithelium, very similar in its pattern of maturation to that of the skin, which provides a barrier against insult by endogenous or exogenous substances present in the oral cavity and also prevents the loss of material from the underlying tissues [58]. A major difference between the oral mucosa and the gastrointestinal tract, is the organization of the epithelium, which, for the latter is composed of a single

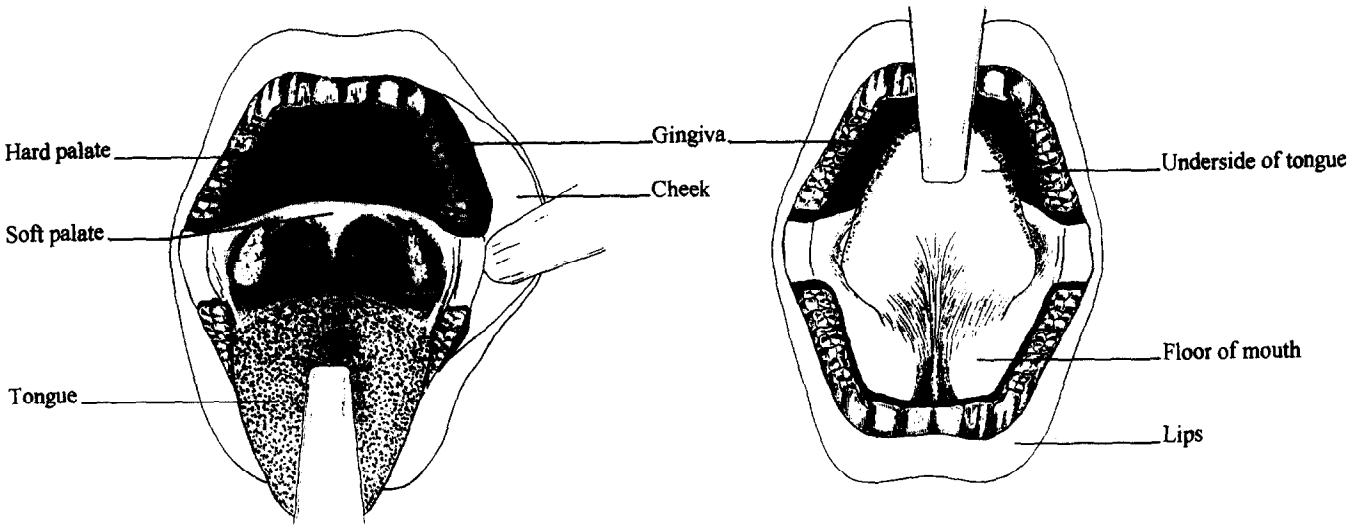

Fig. 3. Regional variations in oral mucosa: (shaded) keratinized; (open) nonkeratinized; (dotted) specialized. (Adapted from Legent et al., 1986 [156]).

layer of cells that facilitates absorption across the tissue.

The movement of substances through the stratified squamous epithelia seems to occur through the intercellular lipid matrix. Houk and Guy [6] have summarized the evidence in favour of intercellular pathway as the major route for percutaneous absorption:

1. Treatment with organic solvents reduced the resistance to solute penetration.
2. Autoradiographic techniques as well as a novel in situ precipitation method, have revealed some penetrant compounds in the intercellular spaces.
3. Mathematical analysis of the in vivo penetration of methyl nicotinate supports the intercellular rather than the transcellular route.
4. Conditions or agents which increase lipid fluidity, also increase permeability.

Several authors have used different techniques to define the position of the permeability barrier, for example Schreiner and Wolf [70], used electron microscopy to determine that intradermally injected horseradish peroxidase (a water soluble tracer) crosses the basement lamina and diffuses outwards through the intercellular spaces, to the boundary between the stratum granulosum and the stratum corneum where the contents of the membrane-coating granules or lamellar granules are extruded. Squier [71] demonstrated that, when applied topically, horseradish peroxidase penetrated only the most superficial cell layers. Similar observations were made by the same author for keratinized oral epithelium. These results were confirmed later [7] using lanthanum salts, smaller tracers of lower molecular weight than horseradish peroxidase. Squier and Lesch [8] showed by autoradiography that radiolabeled water, ethanol and cholesterol, follow an intercellular route through the epidermis, keratinized and nonkeratinized oral epithelium. And finally, recent work with laser confocal microscopy [9,10], revealed that the distribution of fluorescein isothiocyanate labeled dextran 4000 in buccal epithelium was intercellular. Furthermore, it has been proposed that within the intercellular space there exist two pathways: a hydrophobic route through the lipid bilayers and a hydrophilic route along the aqueous channels associated with the polar head groups of the lipids [3].

3.2. Differences in the oral lining tissue

The type and differentiation of the multiple cell layers reflects the functional demands placed on the tissue, such as a requirement of flexibility or rigidity and resistance to mechanical damage [72]. As shown in Fig. 3, three types of oral mucosa are recognized [2,3,5]:

(A) *Masticatory mucosa.* This covers the gingiva and hard palate, regions which are particularly susceptible to the stress and strains resulting from masticatory activity. These regions are covered by a keratinized epithelium that closely resembles the skin in its pattern of maturation.

(B) *Specialized mucosa.* This is found on the dorsum of the tongue and has a surface consisting of areas of both keratinized and nonkeratinized epithelium.

(C) *Lining mucosa.* This covers the remaining regions, such as the lips and the cheeks. Its elastic surface is capable of stretching during mastication and speech. The epithelium is nonkeratinized, can vary in thickness in different oral regions and is more permeable than the keratinized regions. While the permeability differences between keratinized and nonkeratinized epithelia seem

to be related to the type of lipids present in the tissue, the differences in permeability among the different regions of keratinized epithelia are attributed to lipid composition [73]. The water permeability among epidermis, gingiva and palate is inversely correlated with the lipid content, particularly the amount of ceramide [3,74].

3.3. The membrane-coating granules

As cells of epithelia mature, small organelles, known as membrane-coating granules (MCG), probably derived from the Golgi complex appear in the prickle cell layer. In the late stages of differentiation, they migrate towards the superficial part of the cell at the junction of the granular and cornified layers in the keratinized tissues and in the deeper part of the superficial cell layer in nonkeratinized tissues. The bounding membrane fuses with the cell membrane, and the contents of the granules are discharged into the intercellular space. During fusion, the bounding membrane of the granules is introduced into the plasma membrane of the epithelial cell. The extruded material, composed primarily of lipids is then organized into multiple stacked lipid sheets [3,75–78]. This is believed to provide an intercellular permeability barrier.

These granules, were first observed in epidermis by Selby [79] and Odland [80]. There appears to be no difference between the MCG of epidermis and keratinized oral epithelium, they are ovoid, 0.1–0.3 μm in length and electron microscopy studies have shown that the contents of the lamellar granules consist of alternating dense and light lines which define the lamellae present within the granules [75]. Apparently, these lamellae form a series of disks and from their appearance, it is possible that each one of these disks represents a flattened liposome [81,82].

The analysis of MCG from keratinized epithelia indicated a high ratio of lipid to protein, about 2.0, according to Freinkel and Traczyk [83]. The lipids include phospholipids, cholesterol, cholesterol esters, fatty acids, ceramides and several other neutral lipids [82–84]. An elevated amount of an unusual acylglucosylceramide [85] led to the suggestion that the ω-hydroxyacyl chain would completely span one bilayer, while the linoleate inserts into an adjacent bilayer, thus assisting in the assembly of the stacks of disks that are seen in the lamellar granules [81,86]. This hypothesis was supported by the observation that incorporation of acylglucosylceramide into phosphatidylcholine/cholesterol liposomes, resulted in liposome flattening and stacking, while other glycolipids did not cause this phenomenon [87].

In the epidermis of essential fatty acid deficient rats, the linoleate of the normal acylglucosylceramide was replaced by oleate, and it was shown that this substitute molecule was unable to mediate the assembly of the lamellar granules [88]. Furthermore, the lamellar granules appeared empty [81]. A number of hydrolytic enzymes, like glucosidase and acid phospholipase, associated with the MCG may participate in the biochemical transformation of lipids to form the lamellar sheets [4,83].

In nonkeratinized epithelium, MCG have a similar distribution within the epithelium and a similar chemical composition to those of keratinized tissue [89–91]. They are spherical, approximately 0.2 μm in diameter, but contrary to MCG from keratinized epithelia, they contain an electron-dense amorphous core from which delicate strands radiate, without an internal lamellar structure [3,91]. Moreover, the differentiation process in nonkeratinized tissues involves less dramatic changes in cell shape, contents, and lipid composition than in keratinized tissues.

The beginning of the permeability barrier has been located at the level where MCG extrude their content. In the keratinized oral epithelium, this barrier is placed at the junction of the granular and cornified layers. In nonkeratinized epithelium, it is located at one fourth to one third of the surface of the tissue [5]. A relationship between MCG and permeability has been established, thus, a greater volume of MCG is associated with a lower permeability [3].

3.4. Lipid composition of the oral epithelium

The intercellular lamellar sheets resulting from the arrangement of the extruded disks from MCG, as revealed by transmission electron microscopy [76,92], consist of alternating dark bands of varying intensity (corresponding to regions of high electron density) with intervening lucent bands; the differences in appearance between the dense bands support the concept of lipid asymmetry (a well known phenomenon in biological membranes [93]).

In keratinized tissues, during fusion of the bounding membrane of MCG with the plasma membrane, a lipid monolayer emerges. It is a lipid envelope apparently covalently linked through ester or thioester bonds to the outer surface of the cornified envelope that plays an apparent role in the cohesion of corneocytes [94]. The covalently bound lipids consist mainly of ω-hydroxyceramide with smaller amounts of ω-hydroxyacid and normal fatty acid [95]. As shown in Table 1, the total amount of covalently bound lipids represents 0.24% of the dry weight of palatal stratum corneum and 0.20% of gingival stratum. As the covalently bound lipids cover only about 10% of the corneocyte surface, oral keratinocytes do not have a lipid envelope comparable to that of epidermal corneocytes [78,96].

In addition to the lipids, that in the palatal stratum corneum make up 47 ± 6 μg lipid/mg tissue (cf. $115 \pm$

Table 1
Composition (weight percent) of lipids from skin and oral epithelia

Lipids	Skin	Gingiva	Palate	Floor of mouth	Buccal mucosa	Model[b]	Author (Ref)	Year
Total phospho-lipids	24.1	42.3	39.1	44.2	38.2	Pig epith.	Wertz [89]	1986
	37.8[a]	27.0[a]	30.5[a]	57.6[a]	42.7[a]	Pig epith.	Squier [90,101]	1991, 1986
	0.0		12.1			Pig barrier	Wertz [4,5,97]	1996, 1993, 1992
					34.6	Pig epith.	Wertz [4,5]	1996, 1993
Sphingomyelin	9.5[a]	6.2[a]	5.7[a]	10.7[a]	9.6[a]	Pig epith.	Squier [90]	1991
	0.0		5.2		11.9	Pig barrier	Wertz [5]	1993
Phosphatidyl-choline	16.7	12.5[a]	16.23[a]	24.1[a]	14.0[a]	Pig epith.	Squier [90]	1991
	0.0		2.7		9.4	Pig barrier	Wertz [5]	1993
Phosphatidylserine	1.1[a]	1.1[a]	0.6[a]	1.2[a]	2.6[a]	Pig epith.	Squier [90]	1991
	0.0		2.1		5.1	Pig barrier	Wertz [5]	1993
Phosphatidylinosi-tol	2.1[a]	0.8[a]	1.1[a]	2.1[a]	1.6[a]	Pig epith.	Squier [90]	1991
	0.0		1.2		2.2	Pig barrier	Wertz [5]	1993
Phosphatidyl-ethanolamine	8.4[a]	6.4[a]	6.9[a]	19.5[a]	14.9[a]	Pig epith.	Squier [90]	1991
	0.0		0.9		6.0	Pig barrier	Wertz [5]	1993
Cholesterol	15.4	21.0	33.6	19.5	13.6	Pig epith.	Wertz [89]	1986
	24.4[a]	14.1[a]	26.2[a]	25.4[a]	15.1[a]	Pig epith.	Squier [90]	1991
		4.0				Pig epith.	Rabinowitz [98]	1972
			25.0			Human epith.	Lekholm [99]	1977
	28.4		22.5		16.6	Pig barrier	Wertz [4,5]	1996, 1993
Cholesterol sul-phate	0.2	2.0	1.7	3.2	7.8	Pig epith.	Wertz [89]	1986
	1.1[a]	3.0[a]	1.3[a]	4.2[a]	8.8[a]	Pig epith.	Squier [90,101]	1991, 1986
	2.0		9.8		0.9	Pig barrier	Wertz [4,5]	1996, 1993
Cholesterol esters	2.6	1.1	0.2	15.0	5.9	Pig epith.	Wertz [89]	1986
	4.1[a]	0.7[a]	0.2[a]	19.5[a]	6.6[a]	Pig epith.	Squier [90,101]	1991, 1986
		5.0				Pig epith.	Rabinowitz [98]	1972
			1.0			Human epith.	Lekholm [99]	1977
	15.5		1.3		3.6	Pig barrier	Wertz [4,5]	1996, 1993
Acylceramides	1.7	0.4	0.2	0.0	0.0	Pig epith.	Wertz [89]	1986
	6.0		0.9		0.0	Pig barrier	Wertz [4,5]	1996, 1993
Acylglycosylce-ramides	3.2	2.1	2.8	0.0	0.0	Pig epith.	Wertz [89]	1986
	4.9[a]	1.4[a]	2.2[a]	0.0[a]	0.0[a]	Pig epith.	Squier [90]	1991
	0.0		0.0		0.0	Pig barrier	Wertz [4]	1996
Glycosylceramides	2.3	2.1	1.8	5.8	16.5	Pig epith.	Wertz [89]	1986
	0.0[a]	0.0[a]	0.0[a]	7.5[a]	18.4[a]	Pig epith.	Squier [90,101]	1991, 1986
	0.7		2.6		19.0	Pig barrier	Wertz [4,5]	1996, 1993
Total ceramides	12.2	6.6	3.3	0.7	0.8	Pig epith.	Wertz [89]	1986
	25.3[a]	4.8[a]	2.7[a]	1.0[a]	0.9[a]	Pig epith.	Squier [90,101]	1991, 198
	39.7		21.7		8.4	Pig barrier	Wertz [5]	1993
Ceramide 1	6.0		0.9		0.0	Pig barrier	Wertz [5,97]	1993, 1992
Ceramide 2	13.0		8.9		8.4	Pig barrier	Wertz [5,97]	1993, 1992
Ceramide 3	5.2		2.1		0.0	Pig barrier	Wertz [5,97]	1993, 1992
Ceramide 4	4.1		2.0		0.0	Pig barrier	Wertz [5,97]	1993, 1992
Ceramide 5	3.5		2.1		0.0	Pig barrier	Wertz [5,97]	1993, 1992
Ceramide 6	7.2		5.7		0.0	Pig barrier	Wertz [5,97]	1993, 1992
Fatty acids	13.6	5.0	1.3	0.6	1.6	Pig epith.	Wertz [89]	1986
	21.2[a]	3.3[a]	1.0[a]	0.8[a]	1.8[a]	Pig epith.	Squier [90,101]	1991, 1986
		12.0				Pig epith.	Rabinowitz [98]	1972
			15.0			Human epith.	Lekholm [99]	1977
	11.2		7.6		11.8	Pig barrier	Wertz [4,5]	1996, 1993
Triglycerides	24.7	16.9	15.9	11.1	15.7	Pig epith.	Wertz [89]	1986
	38.7[a]	11.4[a]	12.4	14.5[a]	17.6[a]	Pig epith	Squier [90]	1991
		8.0				Pig epith.	Rabinowitz [98]	1972
			14.0			Human epith.	Lekholm [99]	1977
	1.3		21.2		5.2	Pig barrier	Wertz [5]	1993

Table 1 (Continued)

Lipids	Skin	Gingiva	Palate	Floor of mouth	Buccal mucosa	Model[b]	Author (Ref)	Year
Total bound lipids	2.4	0.2	0.24			Pig barrier	Chang [96]	1993
Total lipids	122.4[a]	55.7[a]	65.5[a]	116.0[a]	94.3[a]	Pig epith.	Squier [90]	1991
			47.0[a]			Pig barrier	Wertz [97]	1992
					3.8[c]	Rabbit epith.	Chien [100]	1995

[a] Expressed as mg/g dry tissue.
[b] epith., total epithelium; barrier, s.c. for keratinized and mucosal barrier for nonkeratinized tissues.
[c] Percentage related to protein and unbound water.

16 μg lipid/cm^2 of stratum corneum surface) [97], one of the major differences between epidermal and oral stratum corneum is that desmosomes make up $\sim$15% of the intercellular space of epidermal stratum corneum, while in the palatal stratum corneum they reach 47% [78]. In addition, Swartzendruber et al. [78] found that about 10% of the intercellular space of palatal stratum corneum is occupied by saccules which probably contain nonlamellar phase lipids, for example, liquid phase cholesterol esters.

Lipids including small amounts of ceramides, monohexosylceramides, cholesterol esters, cholesterol sulphate (CS), fatty acids and a high proportion of phospholipids, triglycerides and cholesterol (CH), fill in the intercellular space of oral keratinized tissue.

In the nonkeratinized regions, the chemical nature of the intercellular material is less well defined than that in the keratinized epithelia. Since the intercellular spaces of nonkeratinized epithelia appear to contain amorphous material, it is possible that the lipids within them are in a nonlamellar liquid phase, with only occasional short stacks of lipid lamellae. This may result in a barrier that is less efficient than that from keratinized regions [4]. As shown in Table 1, nonkeratinized epithelia contain little free ceramide (only ceramide 2 was reported to be present in buccal mucosa), fatty acids and CS; moderate levels of cholesterol esters, but phospholipids, glycosylceramides, triglycerides and CH are abundant.

3.4.1. Phospholipids

The presence of phospholipids in the epithelial oral surface, particularly in the cornified regions, could be related to lower levels of phospholipases in the oral epithelium compared to epidermis [102]. It has been suggested that phospholipid catabolism is required in order to permit membrane fusion, necessary for the conversion of short lamellar disks into broad lamellar sheets, as found in epidermis. This could explain the presence of short stacks of lamellae that persist in the surface of the oral epithelium, and thus, the differences in permeability between epidermis and keratinized oral tissue [96,97]. With respect to the nonkeratinized epithelium, transmission electron microscopy showed that the intercellular space of the outer layers appears empty, with only some membranous structures and also short stacks of lipid lamellae present [4].

Variations in the size, shape, polarity and charge of the polar head groups of phospholipids were reported to play a significant role in the structure of micelles, monolayers and bilayers of lipids [103–105]. Among phospholipids present in the oral cavity, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are the most abundant. At pH 7.0 these two phosphoglycerides are dipolar zwitterions and have no net electrical charge. Phosphatidylserine, present in a lower quantity, has a negative charge at physiological pH. With regard to the degree of unsaturation, it was observed that the different transition temperatures reflect the fatty acid compositions; i.e. the highly unsaturated soya PE has a lower bilayer–hexagonal transition temperature than the 18:1$_c$/18:1$_c$ PE. An increased unsaturation may encourage the formation of inverted phases such as the hexagonal (H_{II}) phase, with the polar headgroup at the inner side of the structure [106].

Sphingomyelin (SM), is structurally different from the glycerol-based phospholipids present in the oral cavity, and exhibits bilayer structures and a thermotropic behaviour which is similar but not identical, to those of the other phospholipids. The existence of SM in one or more metastable conformations at physiological temperatures, has been observed to depend on the degree of hydration and the degree of hydrogen bonding between adjacent SM molecules [107].

The gel $\rightarrow$ liquid crystalline transitions of phospholipids as a function of temperature and water content, have been widely studied [108–115]. Increasing temperature, lecithins undergo a chain melting transition $L_{\beta'}$–L_α. This transition may involve different intermediate phases [13,108,113–116]. However, of the several bilayer phases of hydrated phospholipids, the most biologically relevant is probably the liquid-crystalline phase (L_α), in which, the hydrocarbon chains are conformationally disordered and the lipid molecules can diffuse in the plane of the bilayer [15]. An unusual phase, called the interdigitated phase, in which asymmetric lipid hydrocarbon chains from apposing monolayers interpenetrate, has been observed naturally in

biological membranes. This phase has been reported to be responsible for the coupling together of the two opposing leaflets of a bilayer [117]. Interdigitation of phospholipid bilayers has been reported to be induced by several molecules, drugs or solvents when they are included in the membrane, such as chlorpromazine, tetracaine, benzyl alcohol, phenylethanol, phenylbutanol, methanol, ethanol, ethylene glycol and glycerol, among others [23,24,36,118].

3.4.2. Ceramides

Ceramides represent ~3–6% of the total lipid content in keratinized epithelium and only ~0.7% in nonkeratinized tissue. They are derived from glycosylceramides, which are deglycosylated at the moment of extrusion from MCG. However, the presence of glycosylceramides in the oral epithelial surface (~2.6% in keratinized and ~19% in nonkeratinized tissue) reflects the lack of appropriate glycosidases for the conversion of glycosylceramides into ceramides, and this may result in the higher water permeability of these regions [119]. On the contrary, in nonkeratinized regions, permeability has been proposed to be related to the total glycosylceramide content, i.e. the less permeable buccal mucosa contains proportionately more of this glycosylceramide (16.5%) than the floor of the mouth (5.8%) which is the most permeable of the oral tissues [90,101]. As shown in Table 1, only the palate and the gingiva contain acylglycosylceramides and acylceramides.

Ceramides, can be separated into six fractions. The least polar is ceramide 1 or acylceramide, this has an unusual chemical structure. Like the acylglucosylceramide from which it is derived, the acylceramide contains very long ω-hydroxyacids amide-linked to sphingosine bases and linoleic acid ester-linked to the ω-hydroxyl group [3]. Ceramide 1, which is intimately related to barrier function, is present in a much lower concentration in the palatal stratum corneum than in the epidermal stratum corneum (Table 1) [97]. By analogy with the proposed function of acylglucosylceramide in the MCG, in the tissue, ceramide 1 may serve as a molecular rivet stabilizing the multilamellar lipid structure. The linoleate chain, present in this ceramide, being essentially the only polyunsaturated chain in stratum corneum, may, on the other hand, confer a certain fluidity to the membrane [120]. However, more important is the crucial role of this fatty acid for the formation and maintenance of the epidermal water barrier [81,121]. As for MCG, in fatty acid-deficient states, although the overall degree of lipid unsaturation is maintained nearly unchanged, the unavailability of linoleic acid results in an important loss of barrier function. This suggests that the geometry and position of the double bonds of the fatty acid are critical for the formation of the barrier to water transport [88].

Ceramide 2 is the most abundant ceramide in keratinized tissue, and is apparently the only ceramide present in nonkeratinized regions. Ceramides 2–6 are highly saturated and the absence of methyl branches and cis double bonds in the acyl chains, should confer resistance to oxidative damage on exposure to the atmosphere and should permit a near crystalline packing of the chains within the bilayer [3].

Structural studies with glycolipids have shown the presence of complex thermotropic and lyotropic transitions and the formation of different metastable states [122–128]. In general, the transition associated with the melting of the hydrocarbon chains of glycolipids occurs at a higher temperature and involves a higher enthalpy change than that of phospholipids. For example, it has been found that cerebroside dispersions undergo a melting chain transition at 82°C ($\Delta H = 17.5$ kcal/mol), while phospholipids, particularly dipalmitoyllecithins, melt at 41.6°C ($\Delta H = 8.7$ kcal/mol) [107,108,124,129]. The high enthalpy associated with glycolipid phase transitions, correlates well with the important role of ceramides in the mechanical and permeability properties of a tissue. Comparison of the crystal structures of ceramides and other sphingolipids, suggested that the hydrogen-bonding capacity of the sphingosine moiety could facilitate intermolecular interactions different from those found with phospholipids [130]. Ceramides confer stability to the bilayer due to the formation of hydrogen bonds between their polar heads and other lipids or water molecules. Furthermore, the great diversity of chain lengths found in ceramides, may permit a high degree of chain interdigitation and additionally, ceramides, like cholesterol and fatty acids, lack bulky polar head groups, which, in conjunction with their straight saturated chains, permits the formation of tightly packed, highly ordered crystalline arrays. Such bilayers can be expected to be particularly effective in resisting the permeation of water [3,131].

3.4.3. Sterols

The predominant sterol found in oral tissues both, keratinized and nonkeratinized, is CH (Table 1). Contrary to the aliphatic lipids which are synthesized via phospholipids, CH is directly synthesized from acetate and its synthesis is regulated by barrier requirements [132,133]. It has been suggested that CH can regulate the permeability of biological membranes by affecting the internal viscosity of the lipids [134]. Apparently, CH can exhibit a condensing effect on lipids in the liquid–crystalline state and a liquefying effect of lipids in the crystalline state, leading to an intermediate gel state (Fig. 4). It has been proposed that CH is the main component responsible for the solidity of the stratum corneum and that its omission results in an increased bilayer fluidity [54]. When mixed with unsaturated phospholipids, CH lowered the T_M between the gel and

the liquid–crystalline phases and the temperature at which non-bilayer phases are formed, favouring the formation of hexagonal (H_{II}) phase. On the contrary, with saturated phospholipids, CH stabilized the bilayer structure [106,135,136]. In general, it was observed that the incorporation of cholesterol into ceramide-fatty acid mixtures decreased the melting entropy and enhanced the mobility of lipids [128].

Other sterols such as cholesterol sulphate (CS) are also present. CS reaches its highest concentration in the granular layer, but persists in the cornified layer of keratinized tissues, representing ~9.8% of the lipids present in palatal stratum corneum. It has been suggested that CS is involved in cohesiveness of the stratum corneum and its hydrolysis is associated with desquamation [137–139]. Nonkeratinized regions contain much higher levels of sterol esters, which are not bilayer-forming lipids, than their keratinized correlates [4].

3.4.4. Triglycerides and fatty acids

Triglycerides represent about 15 wt% of the lipids in the oral epithelium. As cholesterol esters, they are not-bilayer forming lipids and may be organized as a separate non-lamellar lipid phase [97], which may give a certain fluidity, particularly to the cornified layer, that contains (~ 1%, versus only 5% in the buccal mucosa.

As already noted, fatty acids are components of several lipid classes, whose structural behaviour is strongly influenced by the characteristics of their fatty acid components. However, in the oral cavity fatty acids are also present in the free form, where they represent ~8% of the total lipids of the cornified layer and ~12% of the lipids in the buccal mucosa barrier. The length and degree of unsaturation of the alkyl chain are factors that influence the melting point of a fatty acid. For saturated fatty acids, the longer the hydrocarbon chain, the higher the melting point. Saturated hydrocarbon chains are flexible around the C–C bonds, therefore, the presence of one or more cis double bonds hinders this rotation and introduces an increased flexion in the hydrocarbon chain, which prevents the formation of well-ordered, compact crystals [103]. The fatty acids present in the oral cavity are mainly saturated, straight-chained species of 14–28 carbons, the most abundant being the 22:0 and 24:0 fatty acids. This should result in the presence of regions of close-packed crystalline state [81].

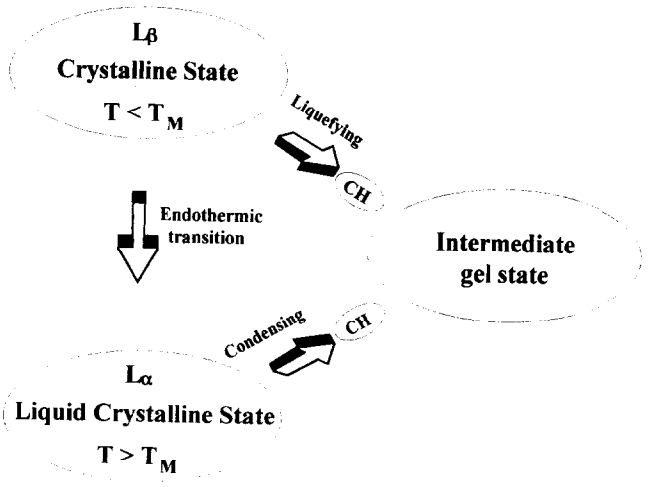

Fig. 4. Effects of cholesterol (CH) on lipids in the crystalline and liquid crystalline states. (Adapted from Demel and Kruyff, 1976 [134]).

4. The action of enhancers correlates well with lipid fluidity

As mentioned above, diffusion of substances through oral epithelia occurs principally through the intercellular lipids [8–10]. Therefore, one mechanism to enhance oral mucosal absorption is by disrupting the structure of these lipids.

When studying the action of permeation enhancers, two factors might be considered. First, a structure–effect relationship, to understand the interactions between the enhancer and the membrane constituents. Therefore, the physicochemical properties of an enhancer, such as the nature, conformation, degree of unsaturation, ionization state and chain length, will influence the magnitude of enhancement. Second, the lipid composition within the membrane, because differences in the type, content and organization of these lipids, will result in regional variation in the permeability of the oral mucosa.

In general, the extent of enhancement, observed with some permeation enhancers has been shown to be proportional to the increase of disorder or fluidity of the lipid domains. An increased fluidity has been observed when fatty acids are used to promote the absorption of insulin [140] and propranolol [141] through buccal mucosa. Differential scanning calorimetry (DSC) and infrared spectroscopy (IR) have shown significant increases in lipid fluidity when *cis*-unsaturated fatty acids are used [59,142]. The analysis showed a reduction of the T_M associated with the intercellular lipids, as well as a shift to a higher frequency and a broadening of the IR stretching vibrations peaks [59,143,144]. It has been proposed that the presence of *cis*-double bonds prevents the formation of well-ordered compact crystals [103,145]. The inclusion of fatty acids in phospholipid membranes may affect the lamellar phase structure, by forming hydrogen bonds between the fatty acid carboxyl group and the phosphate group of the phospholipid [63] and by favouring the direct $L_{\beta'}$–H_{II} phase transition, without an intervening fluid lamellar phase (L_α) [146]. In human buccal epithe-

lial cells it was shown that oleic acid disrupts strongly the polar head and the hydrophobic region of the membrane lipids [147].

Another possible enhancing mechanism is the extraction of intercellular lipids. Bile salts, have been shown to be able to solubilize buccal lipids [148]. Different studies with liposomes, showed that surfactants decrease T_M and broaden the interval over which the transition takes place, as a consequence of the perturbation of the packing state of the bilayer [65]. Lipid extraction is the most generally accepted mode of action of ethanol [147].

Substances like Azone®, developed specifically as a skin permeation enhancer, has also been used to promote the absorption of different solutes through the oral mucosa [67,149–152]. It was proposed that Azone® disrupts electrostatic interactions, by inserting into the alkyl chain regions of the lipids. This may be expected to reduce T_M and increase lipid fluidity [153].

However, alteration of the lipid structure is not the only way to increase permeability, other mechanisms such as the alteration of cellular proteins [51], and the protection of drugs, i.e. peptides, from oral proteolytic enzymes [154,155] have also been proposed.

5. Concluding remarks

The diffusional resistance of the tissue is determined by the intercellular lipids. The variation in permeability observed for oral mucosa relies to a great extent, on the presence or absence of a keratinized epithelium, and in the composition and organization of membrane lipids. The great variety of lipids that constitute biological membranes suggests that lipids are organized in an heterogeneous fashion, creating mixed gel state lipids that coexist with lipids in a liquid–crystalline phase. Thus, information about the behaviour and molecular arrangement of these lipids is important because it contributes to understanding membrane structure and function and it is, therefore, also essential for the study of drug absorption and permeation enhancement.

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