Lipid Content and Water Permeability of Skin and Oral Mucosa

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It has been claimed that total lipid content may be the critical factor determining the water permeability of skin. The present study examined this relationship in various oral epithelia and epidermis. Epithelia was heat separated from specimens of porcine skin, gingiva, buccal mucosa, palate, and floor of mouth. Lipids were solvent extracted and separated by thin layer chromatography with appropriate standards. The plates were sprayed with sulfuric acid and charred, and the concentration of lipids was determined by densitometry as mg lipid/gm tissue dry weight. Permeability constants were determined for each tissue by using tritiated water in perfusion chambers. When these values were compared over all regions, total lipid did not appear to be related to the permeability of these tissues. However, in the keratinized regions (epidermis, gingiva, and palate) a lower water permeability was related to a greater content of total lipid, nonpolar lipid, ceramide, and glucosylceramide. In non-keratinized tissues, a lower permeability corresponded to increased amounts of an unidentified glycosylceramide. The role of lipid in the permeability barrier of these tissues was further demonstrated by extracting specimens of skin and oral mucosa with chloroform/methanol and then determining Kp values; in both tissue regions, there was a significant increase in water permeability. Thus, although lipid is a component of the water permeability barrier in both skin and oral mucosa, different lipid components subserve this function in keratinized and non-keratinized tissues. *J Invest Dermatol 96:123–126, 1991*

It has been demonstrated that for skin, the major determinant of barrier function is the lipid content of the epidermal stratum corneum, rather than the thickness or number of corneocyte layers present [1–3]. Although the epithelium lining the oral cavity has an important barrier function, it shows morphologic diversity, ranging from regions of orthokeratinized mucosa to nonkeratinized mucosa [4]. As in skin, the major permeability barrier in oral mucosa is located in the superficial strata of the epithelia [5,6]. In porcine keratinized oral epithelia, the lipid components present are similar to those in epidermis; some of them are believed to be associated with a superficial intercellular permeability barrier [7], having a composition resembling that described in epidermis [1,8,9]. In non-keratinized oral regions, there are differences in the proportions as well as in the representation of these lipid components [9]. The water permeability of oral epithelia also shows marked regional variation and differs from that of epidermis [10]. In the present study, we have examined water permeability and lipid content of porcine epidermis and oral epithelium to determine whether the relationship described for skin by Elias and co-workers [1–3] extends to other tissues.

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MATERIALS AND METHODS

Portions of skin were taken from the midline of the belly, keratinized oral mucosa was from the gingiva and hard palate, and nonkeratinized mucosa was from the cheek and the floor of mouth of pigs. Tissue from 21 pigs was used for the lipid analysis, and from ten pigs for the permeability measurements. All procedures were carried out within 1–2 h of tissue removal.

Lipid Analysis Sheets of epithelium (approximately 1 × 2 cm) from all five regions were separated from their underlying layers of connective tissue by placing the epithelial surface on a hot plate at 60°C for 90 sec, after which the epithelium could be easily stripped away. Specimens examined histologically showed that separation occurred cleanly at the epithelium-connective tissue interface.

The epithelia were dried overnight under vacuum and dry weights were determined. Lipids were extracted by 2-h immersions in each of three chloroform-methanol solutions, 2:1, 1:1, and 1:2, (HPLC grade, Fisher Scientific, Fair Lawn, NJ) by the procedures of Gray [11]. The solvents were evaporated under nitrogen and the total weights of extracted lipid determined. This is an efficient extraction procedure; subsequent re-extraction of the tissue did not yield any more lipid. After being re-dissolved in 1:1 chloroform-methanol at 25 mg/ml, 4 μl (100 μg) samples of the lipid solutions were spotted in scored lanes on 20- × 20-cm, thin-layer chromatography plates of 0.5-mm thick silica gel H (E.M. Reagents, Darmstadt, West Germany). The plates were then developed in one of three different solvent systems: (a) chloroform-methanol-water (40:10:1); (b) chloroform-methanol-acetic acid (190:9:1); or (c) hexane followed by toluene followed by hexane-diethylether-acetic acid (80:20:1). These solvent systems separated the total epithe-

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lial lipids into several identifiable classes. Known lipids were cochromatographed as standards for comparison. After drying, the plates were sprayed with 50% H_2SO_4 and charred at 220°C for 1 h. The migration of the lipid components of each of the epithelia was compared with that of the lipid standards.

Lipids were quantified by using a separate set of plates on which a density reference, consisting of 4 μg of cholesterol, was also included. These plates were scanned with a densitometer, and peak areas were determined by triangulation [12]. Duplicate assays were carried out on seven samples from each tissue region. Values were expressed as mg lipid/g dry weight of separated epithelium and are shown as a mean ± SE.

Permeability Measurements *Biopsies of full-thickness skin* and oral mucosa were cut into discs approximately 7 mm in diameter after removing excess fat and connective tissue. A soft rubber gasket with an internal diameter of 5 mm was sealed to the epithelial surface of each specimen with cyanoacrylate adhesive, and the tissue was clamped between the two halves of a glass perfusion chamber. For hard palate, portions of rugae were clamped between a chamber with an internal diameter of 2.5 mm so as to accommodate these smaller biopsies. Each chamber was filled with 6 ml of phosphate-buffered saline, and 2–10 μCi of tritiated water (Amersham Corp., Arlington Heights, IL; 5 mCi/ml) was added to the epithelial side of the chamber. The chambers were mounted in racks fabricated for this purpose and equipped with rotating magnetic stirrers, and small stirring fleas were placed in each half chamber. The measurements were carried out at room temperature (20°C) because previous studies have shown that penetration of compounds across oral mucosa is by diffusion [13] and is not affected by metabolic inhibitors [6]. Samples were taken at 2, 4, 6, 8, 10, 12, 24, and 26 h after the start of the experiment. The samples were mixed with scintillation fluid (Research Products International, Mount Prospect, IL) and counted in a Beckman liquid scintillation counter (Beckman Instruments, Irvine, CA) until a 2 sigma value of 1% was reached; quenching was corrected automatically in the counter.

A permeability constant, Kp, was calculated using the relationship $Kp = Q/A \cdot t \cdot (C_o - C_i)$ [13,14], where Q is the quantity of compound traversing the tissue in time t (min), C_o and C_i are the concentrations of the compound on the outer (epithelial) and inner (connective tissue) sides of the specimen, respectively, and A is the area of exposed tissue in cm^2. The units are cm/min.

Values for replicate chambers containing the same tissue region from an animal were measured and than compared between time periods by using an analysis of variance (ANOVA) and Duncan's multiple range test to identify means that were significantly different at a 95% confidence level. By this procedure, it was possible to identify the time sequence over which Kp values did not differ significantly, indicating that a steady state had been reached. This varied with different regions, but usually occurred after 2–4 h. Kp values at steady state were averaged to give the permeability constant for that tissue region.

To further examine the contribution of lipids to the water permeability barrier, we extracted specimens of skin from two animals and of buccal mucosa from four animals in chloroform : methanol (1 : 1) for 2 h, and a permeability coefficient for water was determined, as described above.

RESULTS

After lipid extraction, both skin and buccal mucosa showed a permeability coefficient that was significantly higher than unextracted specimens (Table I). Although the permeability of unextracted mucosa was significantly higher than that of skin, the values for extracted mucosa and skin did not differ significantly. The permeabilities of all tissue regions to water were significantly different from one another, except for palate and gingiva (Table II).

Given the effect that lipid extraction has on water permeability in keratinized and nonkeratinized regions, it is appropriate to examine permeability in terms of lipid content (Table II). If lipid content does determine water permeability, then tissues with lower Kp values might be expected to have the greater total lipid content. This relationship is evident in the keratinized regions, where the superior barrier properties of skin, as compared to gingiva and palate, seem to reflect a greater total lipid, non-polar lipid, ceramide, and glycosylceramide content. However, such relationships are not evident in the nonkeratinized tissues, where total lipid and non-polar lipid content approaches that in epidermis, although these tissues show the highest water permeability of all regions examined. Overall, the total ceramide content correlates best with water permeability, showing a decrease as permeability increases in keratinized regions, and being present in very small quantities (1 mg/g or less) in the permeable nonkeratinized regions. However, the relative differences in permeability between buccal mucosa and floor of mouth do not correspond exactly to the amount of ceramide, but seem to reflect the relative amounts of a glycosylceramide, which is not present in the keratinized epithelia. Thus, the less permeable buccal mucosa contains proportionately more of this glycosylceramide (18.4 mg/g) than the floor of mouth (7.5 mg/g), which is the most permeable of all the tissues examined.

Table I. Water Permeability of Skin and Oral Mucosa Before and After Lipid Extraction

	Kp × 10^{-7} cm/min ± SEM	
	Unextracted	Extracted
Skin	62 ± 5	2386 ± 234 [a]
Buccal mucosa	634 ± 19	2241 ± 145 [a]

[a] Values not significantly different (p = 0.05).

DISCUSSION

The significant increases in permeability seen in the keratinized tissue (skin) and a nonkeratinized tissue (buccal mucosa) after extraction with lipid solvents suggest that lipids represent a major constituent of the water permeability barrier in both tissues. Despite the significant differences in their normal (unextracted) permeability to water, both tissues after extraction reached similar values that were significantly higher than the unextracted values. This suggests that without an intact lipid barrier, widely different tissues show a similar diffusional resistance to water. Chloroform-methanol treatment completely and selectively removes lipids from the tissue [15], but it may damage epithelial structure. The extent to which this may contribute to increased permeability is unknown.

In order to relate extracted lipid components to the epithelial water permeability barrier, which is believed to be located in the intercellular regions of the superficial cell layers [1,16,17], it is necessary to determine their morphologic location. Histochemical localization of neutral lipids reveals a predominately intercellular localization in the stratum corneum of epidermis [18] and keratinized gingival and palatal epithelium [19]. In nonkeratinized oral epithelium, nonpolar lipids are found mainly intracellularly, whereas glycolipids that can be removed by lipid extraction are located between the cells of the superficial layers [19]. These are likely to represent the glycosylceramides described here.

As previously proposed [1–3], lipid content and type mirror water permeability not only in skin, but also in keratinized regions of oral mucosa. However, this relationship does not seem to hold for nonkeratinized oral epithelia, where there are larger quantities of phospholipids and nonpolar lipids than in the keratinized epithelia, despite a significantly higher water permeability. Clearly, these lipid components contribute little to barrier function in nonkeratinized regions, and permeability seems to be related to the amount of glycosylceramide present. Although the glycosylceramides of nonkeratinized epithelia show a similar mobility on thin-layer chroma-

Table II. Water Permeability and Lipid Content of Skin and Oral Mucosa

	Skin	Gingiva	Palate	Buccal Mucosa	Floor of Mouth
Water permeability Kp × 10^{-7} cm/min (±SEM)	62 ± 5[a]	364 ± 18[a]	412 ± 27[a]	634 ± 19[a]	808 ± 523[a]
Epidermal/epithelial lipid content mg/g tissue (±SEM)					
Sphingomyelin	9.5 ± 0.30	6.2 ± 0.48	5.7 ± 0.83	9.6 ± 0.38	10.7 ± 0.35
Phosphatidylcholine	16.7 ± 1.58	12.5 ± 1.05	16.2 ± 1.85	14.0 ± 0.80	24.1 ± 1.47
Phosphatidylserine	1.1 ± 0.83	1.1 ± 0.83	0.6 ± 0.18	2.6 ± 0.49	1.2 ± 0.20
Phosphatidylinositol	2.1 ± 0.26	0.8 ± 0.15	1.1 ± 0.81	1.6 ± 0.19	2.1 ± 0.26
Phosphatidylethanolamine	8.4 ± 0.38	6.4 ± 0.95	6.9 ± 0.98	14.9 ± 0.57	19.5 ± 0.45
Total phospholipids	37.8	27.0	30.5	42.7	57.6
Cholesterol sulphate	1.1 ± 0.15	3.0 ± 0.07	1.3 ± 0.15	8.8 ± 0.35	4.2 ± 0.61
Glycosylceramides	0	0	0	18.4 ± 0.88	7.5 ± 0.30
Glucosylceramides	3.6 ± 0.45	1.4 ± 0.15	1.4 ± 0.19	0	0
Acylglucosylceramide	4.9 ± 0.60	1.4 ± 0.17	2.2 ± 0.26	0	0
Total glycosylceramides	8.5	2.8	3.6	18.4	7.5
Ceramides[b]	25.3	4.8	2.7	0.9	1.0
Cholesterol	24.4 ± 1.47	14.1 ± 0.85	26.2 ± 1.32	15.1 ± 0.59	25.4 ± 1.32
Fatty acids	21.2 ± 1.07	3.3 ± 0.45	1.0 ± 0.70	1.8 ± 0.11	0.8 ± 0.15
Cholesterol esters	4.1 ± 0.45	0.7 ± 0.15	0.2 ± 0.04	6.6 ± 0.71	19.5 ± 0.41
Triglycerides	38.7 ± 1.39	11.4 ± 0.32	12.4 ± 1.02	17.6 ± 0.87	14.5 ± 0.57
Hydrocarbons	2.5 ± 0.87	1.3 ± 0.15	0	1.0 ± 0.18	0.8 ± 0.18
Total nonpolar lipids	49.7	18.1	27.4	23.5	45.7
Total lipids	122.4	55.7	65.5	94.3	116.0

[a] All values significantly different ($p < 0.05$) from one another except for gingiva and palate.
[b] Ceramides represent the sum of several chromatographically distinct ceramide types previously identified in porcine epidermis [35].

tographs to those of keratinized epithelium, they do not appear to be identical [9]. Furthermore, the very small quantities of ceramides present in nonkeratinized regions suggests that there is no mechanism for converting the glycosylceramide to ceramide, as occurs in keratinized epithelia [20–24]. Thus, the barrier material in nonkeratinized epithelia may be represented by a glycolipid that is, as yet, uncharacterized. In terms of other lipid components, it should be noted that recent studies [25,26] have shown that triglycerides are minor components of the endogenous epidermal lipids, but are found in relatively high concentrations in epidermis prepared from excised skin due to contamination by subcutaneous fat. Similarly, it has recently been demonstrated that the small and variable levels of hydrocarbons found in normal skin samples are environmental contaminants of petrogenic origin [26,27]. Given these considerations, it is likely that the values quoted here for these components are overestimates of actual epidermal content.

We know that the barrier region of nonkeratinized epithelium is located in the superficial one third to one quarter of the epithelium [5] and that different types of compounds penetrating the epithelium all appear to do so via an intercellular route [17]. It is, therefore, relevant to consider the available evidence for a barrier in the intercellular regions. Electronmicroscopic studies of a variety of nonkeratinized epithelia have revealed the presence of small intercellular granules that are located at the superficial boundary of cells in the middle third of the epithelium [28,29]. These granules are membrane bounded and contain an amorphous dense core; lamellae are rarely seen [28]. The contents of the granules are believed to be liberated into the intercellular space in the upper third of the epithelium, but the intercellular material remains amorphous and does not show evidence of organization into sheets of lamellae, as has been described in both epidermis and keratinized oral epithelium [30–33]. It is possible that the contents of the granules in nonkeratinized epithelia represent the unknown glycosylceramide described above. Such material would not form as effective a water permeability barrier as ceramides organized into intercellular lamellae, but could restrict the movement of larger molecules, such as proteins [6]. On the other hand, a glycosylceramide would be susceptible to the effect of lipid solvents and could account for the characterization of buccal mucosa as a "lipoid phase" [34] in terms of its drug permeability.

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