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A mechanistic analysis to characterize oramucosal permeation properties

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Abstract

The hypotheses of this study are that the permeation of ionizable molecules follows the pH-partition theory, that the preferred transport pathway for penetrants depends on their charge status and that transport resistance is related to the membrane-coating granules (MCG). Transcellular resistance is believed to be proportional to the volume of MCG in the intracellular space while paracellular resistance is believed to result from the extrusion of the lipid contents of the MCG into the intercellular space. Nicotine, an ionizable model compound with two pK_a values (3.4 and 8.2), was chosen as a molecular probe to investigate the pH-partition theory on permeation through porcine oramucosae, to characterize the differences in permeability among various oramucosae, and to explore the preferred transport pathways of each nicotine species through oramucosae. The pH-partition theory was proved from the observations that permeability, partition coefficient and diffusivity of nicotine varied as a function of pH. The keratinized gingiva was found to have greater permeability than the non-keratinized buccal and sublingual mucosae. The neutral nicotine species had a higher permeability than the ionized species due to its higher partition coefficient and diffusivity. A mechanistic analysis (permeability ratio-pH profile) was conducted to determine the preferred transport pathway of each nicotine species. The permeability of neutral nicotine was found to be proportional to the occupied volume of MCG in the intracellular space. This indicates that the preferred transport pathway for neutral nicotine is transcellular. As the solution pH was decreased, and a greater fraction of nicotine became protonated, the transport of hydrophilic, charged nicotine species along the intercellular pathway was preferred.

Keywords: Nicotine; Oral mucosae; Permeation; pH-partition theory; Transport pathways; Membrane-coating granules (MCG)

1. Introduction

Drug delivery through mucosae that line the oral cavity offers the possibility of circumventing the hepatic 'first-pass' elimination that follows gastrointestinal absorption. In addition, gastric acid- or digestive enzyme-mediated degradation in the gastrointestinal tract is also avoided. Moreover, absorption following oramucosal administration is not influenced by the potential variation

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in the gastric-emptying rate or the presence of food. These advantages are of value in the systemic delivery of drugs that are subject to extensive hepatic clearance and are particularly useful for the systemic delivery of bioengineered therapeutic peptides.

The oramucosae, like the skin, are accessible for the precise localization of dosage forms for continuous and prolonged drug delivery. However, the mucosal lining of the oral cavity has been reported to be more permeable to drugs than the skin (Galey et al., 1976; Lecsh et al., 1989). Since the oramucosae are routinely exposed to various physical forces and a multitude of different foreign substances that are contained in foods and drinks, they have evolved as robust membranes that are less prone to irreversible damage by drugs, drug dosage forms or formulation excipients (Merkle and Wolany, 1992). Moreover, the robustness may permit the coadministration of permeation enhancers to improve oramucosal permeability without causing permanent damage.

The entire oral cavity is lined with a stratified squamous epithelium supported by a connective tissue lamina propria (Wertz and Squier, 1991). Regions such as the gingiva and hard palate are subject to the mechanical forces of mastication. The epithelia in these regions have a cornified surface that resembles that of the epidermis (Squier and Hill, 1989). This type of mucosa represents about 24% of the total surface area of the oral mucosae (Collins and Dawes, 1987). Regions of the oramucosae, such as the cheeks (buccal mucosa), the floor of mouth (sublingual), and the underside of tongue, are stretched or compressed during speech and mastication. These regions have a surface lining that consists of non-keratinized, stratified squamous epithelium (Squier and Hill, 1989) and are referred to as lining mucosae. They occupy about 60% of the area of the oral mucosa (Collins and Dawes, 1987). Patterns of epithelial differentiation in the oramucosae are determined by the functional demands placed on the tissue (Alvares and Meyer, 1971; Squier et al., 1976). In keratinized epithelia, these demands are met by a flattened, dehydrated, mechanically tough and chemically resistant surface layer. In non-keratinized regions, the surface is less able to resist mechanical abrasion, but is flexible and extensible to permit mobility of the lining regions (Wertz and Squier, 1991).

For both keratinized and non-keratinized oral epithelia, it is believed that the volume of membrane-coating granules (MCG) in the intracellular space and the presence of extruded lipids (from MCG) into the intercellular region constitute the major resistance through transcellular and paracellular pathways (Squier, 1973, 1977; Squier and Rooney, 1976). MCG are spherical or oval organelles, 100–300 nm in diameter, and are found in the intermediate cell layers of many stratified epithelia (Matoltsy, 1976). A unique characteristic of the MCG is their progressive migration towards the distal plasma membrane. The distribution corresponds well with the proposed role of the granules as a precursor of the material that will form the superficial intercellular permeability barrier (Squier, 1973; Elias and Friend, 1975). The MCG first appear around the midpoint of the epithelium, concentrated close to the distal cell membrane; in the third quarter of the epithelium, they appear to fuse with the cell membrane and discharge their contents into the intercellular spaces of the epithelium. This event appears to be associated with the formation of a barrier to the free movement of substances through the intercellular spaces of the epithelium (Squier and Rooney, 1976).

There are several independent arguments that propose a relationship between MCG and the formation of an intercellular permeability barrier. There is good evidence that the major lipid components in the intercellular space are derived from these granules. A relationship between the relative volume occupied by MCG in various human stratified squamous epithelia and permeability to water was derived from the data of Schroeder (1981) by Lecsh et al. (1989). It was observed that, for each type of epithelium, a greater volume of MCG was associated with a lower water permeability (Lecsh et al., 1989).

In this paper, nicotine, which is a diacidic base (pK_as = 3.4 and 8.2) (Cordell, 1981), was used as a molecular probe to evaluate mucosal permeation (permeability, partition coefficient and diffusivity) as a function of pH. The transport resistance and

barrier function of each mucosa was also correlated with the volume of MCG in the cytoplasm, and the preferred transport pathway of different nicotine species was evaluated.

2. Materials and methods

2.1. Materials

Unless stated otherwise, all materials were ACS grade or better and were used as received. (−) Nicotine was obtained from Sigma (St. Louis, MO). Disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, sodium chloride, citric acid monohydrate, phosphoric acid, orthoboric acid, hydrochloric acid, sodium hydroxide, methanol and acetonitrile were all reagent grade and were purchased from Fisher (Fair Lawn, NJ). White domestic pigs (female, unscalded, 40 ~ 60 pounds) were purchased from Dealaman (Warren, NJ). Valia-Chien permeation cells were fabricated by Crown Glass (Somerville, NJ).

2.2. In vitro permeation studies

Various oramucosae (buccal, sublingual and gingival) were excised from freshly slaughtered domestic pigs. Buccal and sublingual membranes were excised and mounted onto the openings (the available surface area was 0.64 cm^2) of the Valia–Chien permeation cells. A specially designed membrane holder with an available surface of 0.05 cm^2 was used for the gingival mucosa to compensate for the small available surface area of gingiva. Each mucosa was sandwiched between two half cells that served as donor and receptor compartments. Nicotine solutions (50 mg/ml) at various pHs (2.0, 3.0, 5.4, 7.8 and 8.8) were prepared using citrate phosphate buffer and were used as the donor solutions. Isotonic phosphate buffer at pH 7.4 was used as the receptor medium. The donor and receptor cells were filled with 3.3 ml of nicotine solution and isotonic phosphate buffer, respectively. Sink conditions were maintained throughout the experiment. Samples (100 μl) were withdrawn from the receptor cell at predetermined time intervals (2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 16 h) and were replaced with the same volume of nicotine-free phosphate buffer. All samples were analyzed by high-performance liquid chromatography (HPLC). The nicotine concentration for each sample was corrected for the amount of drug that was lost due to sampling and dilution. Triplicate experiments were conducted for each treatment. The permeation of tritiated water across the mucosae was studied and the physical integrity of the membranes over the study period was confirmed (Nair and Chien, 1993; Nair et al., 1997). In addition, the linearity of transport data may be used as an indicator of the physical integrity of tissues (Harris and Robinson, 1992). The linearity of the permeation profiles over the period of study confirmed that mucosal integrity was maintained.

2.3. Partitioning studies

Samples of buccal mucosa, with an average thickness of 1.23 mm and a surface area of 0.64 cm^2, were soaked in nicotine solutions (2.0 ml of 50 μg/ml) of different pHs (2.0, 3.0, 5.4, 7.8 and 8.8) for 24 h in a shaking water bath (37°C) to reach the equilibrium state (Nair et al., 1997). Nicotine concentration was analyzed by HPLC at the end of 24 h to measure the amount lost from solution. Nicotine stability at each selected pH, in the absence of buccal mucosa, was conducted as a control study. These studies indicated that there was negligible loss of nicotine, through degradation or adsorption, for 24 h. Therefore according to mass balance, it was assumed that the amount lost from solution, partitioned into the buccal mucosa. The partition coefficient for nicotine in the buccal/buffer system was determined (concentration ratio between buccal and buffer) for each studied pH. Triplicate experiments were performed for each pH.

2.4. Analytical method

A Waters HPLC (Millipore, MA) with a WISP 712 autoinjection system and a Spectroflow 783 UV detector (Kratos, NJ) was used to analyze nicotine. A μBondapak reversed phase C_{18}

column (3.9 x 150 mm) was used to analyze nicotine with a mobile phase of 0.06 M phosphate buffer (pH 7.4) and acetonitrile (80:20) at a flow rate of 1 ml/min. UV detection of nicotine at 256 nm was used and a retention time of 3.0 min was obtained. The analytical sensitivity for nicotine was 10 μg/ml.

2.5. Data analysis

According to Fick's law, the steady-state flux (J_{ss}) of nicotine across an oramucosa is:

$$J_{ss} = P_T(C_d - C_r) = K_T D_T C_d / h \quad (1)$$

where P_T is the apparent (overall) permeability coefficient through the whole thickness of oramucosa; K_T is the apparent partition coefficient between mucosa and donor medium; D_T is the apparent diffusivity through the whole thickness (h) of the oramucosa; and C_d and C_r are concentrations in the donor and the receptor solutions, respectively.

For an ionizable penetrant with two pK_a values, such as nicotine, the overall permeability (P_T), partition coefficient (K_T) and diffusivity (D_T) are summations of the individual contributions from the neutral and charged species. The overall permeability, partition coefficient and diffusivity can be expressed by the following equations:

$$P_T = (P_n X_n + P_{(+)} X_{(+)} + P_{(++)} X_{(++)}) \quad (2)$$

$$K_T = (K_n X_n + K_{(+)} X_{(+)} + K_{(++)} X_{(++)}) \quad (3)$$

$$D_T = (D_n X_n + D_{(+)} X_{(+)} + D_{(++)} X_{(++)}) \quad (4)$$

in which, P_n, $P_{(+)}$ and $P_{(++)}$ are, respectively, the specific permeabilities for neutral, mono-protonated and di-protonated nicotine. K_n, $K_{(+)}$ and $K_{(++)}$ are, respectively, the specific partition coefficients of neutral, mono-protonated and di-protonated nicotine. D_n, $D_{(+)}$ and $D_{(++)}$ are, respectively, the specific diffusivities of neutral, mono-protonated and di-protonated nicotine. X_n, $X_{(+)}$ and $X_{(++)}$ are the fractions of neutral, mono- and di-protonated nicotine at a particular pH, respectively. The degree of nicotine protonation follows the Henderson–Hasselbach equation where the fraction of each species, as a function of pH, can be calculated from the following equations:

$$X_n = \frac{K_{a1}K_{a2}}{(K_{a1}K_{a2} + K_{a1}[H^+] + [H^+]^2)} \quad (5a)$$

$$X_{(+)} = \frac{K_{a1}[H^+]}{(K_{a1}K_{a2} + K_{a1}[H^+] + [H^+]^2)} \quad (5b)$$

$$X_{(++)} = \frac{[H^+]^2}{(K_{a1}K_{a2} + K_{a1}[H^+] + [H^+]^2)} \quad (5c)$$

2.6. Statistical analysis

Experimental results were statistically analyzed by a one-way analysis of variance (ANOVA) with pair-wise multiple comparisons of the Student–Newman–Keuls method.

3. Results and discussion

The mole fractions of each nicotine species [neutral (NN), mono-protonated (NNH^+) and di-protonated (NH^+NH^+)] as a function of pH was determined by the Henderson–Hasselbach equations (Eqs. (5a), (5b) and (5c)).

The permeation profiles of nicotine through the buccal mucosa at pHs 8.8, 5.4 and 2.0 are illustrated in Fig. 1 (Nair et al., 1997). Significantly lower nicotine permeation was observed at pH 5.4 and 2.0 than that at pH 8.8. The steady-state flux was determined for each studied pH (8.8, 7.8, 5.4, 3.0 and 2.0). The permeation of unionized nicotine was much higher than that of the monoprotonated species. The transoramucosal permeation of diprotonated nicotine was the lowest. The differences between each of the respective species were statistically significant ($P < 0.001$). The overall permeability (P_T) at each pH was calculated according to Eq. (1). The fraction (X_n, $X_{(+)}$ and $X_{(++)}$) of nicotine species (NN, NNH^+ and NH^+NH^+) at each pH was estimated from Eqs. (5a), (5b) and (5c). The specific permeability (P_n, $P_{(+)}$ and $P_{(++)}$) for each nicotine species was computed according to Eqs. (2), (5a), (5b) and (5c) and is shown in Table 1. One permeability value with no S.D. for each species was obtained since only the mean overall permeability (P_T) at pH 8.8, 5.4 and 2.0 was used for solving P_n, $P_{(+)}$ and $P_{(++)}$ in Eq. (2). As demonstrated in Table 1, the permeability of neutral nicotine

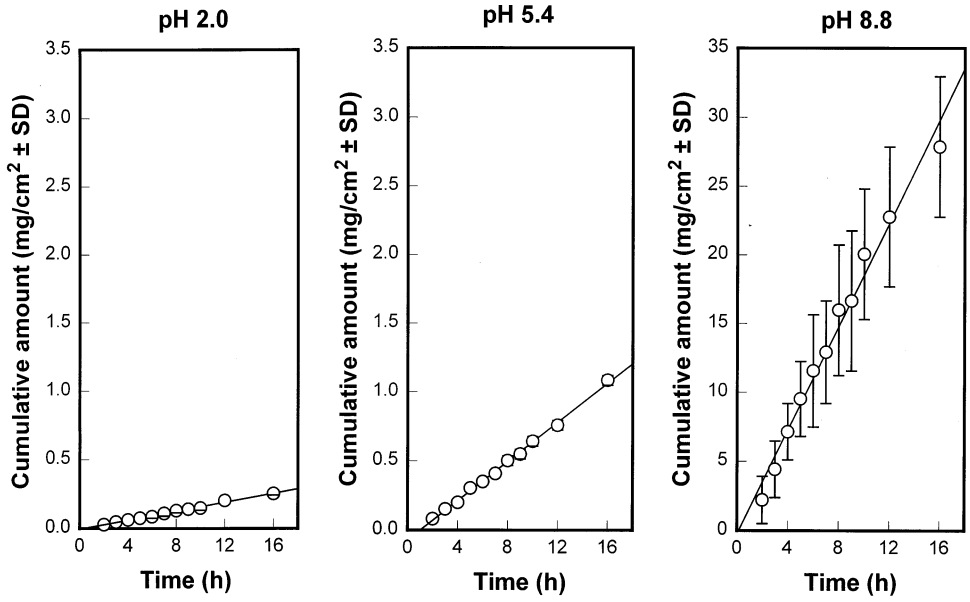

Fig. 1. Comparison of nicotine permeation through the non-keratinized buccal mucosa under different pHs. Significantly higher permeation of nicotine at pH 8.8 was obtained.

through the lipophilic buccal mucosa was much higher than that of mono- and di-protonated nicotine. The overall permeability (P_T) of nicotine through the buccal mucosa as a function of pH was computed using the three specific permeabilities shown in Table 1 and Eqs. (2), (5a), (5b) and (5c). A permeability-pH profile with two inflection points that reflected the dissociation constants (pH 3.4 and 8.2) of nicotine was obtained as shown in Fig. 2A.

In the same manner that was used to compute specific permeability, three overall partition coefficients (K_T) of nicotine in buccal/buffer systems (pH 8.8, 5.4, and 2.0) were used to estimate the specific partition coefficient (K_n, $K_{(+)}$ and $K_{(++)}$) for each nicotine species (Table 1). Neutral nicotine is shown to have a substantially greater partition coefficient than mono- and di-protonated nicotine. Again, the overall partition coefficient, as a function of pH, was computed according to Eq. (3) using K_n, $K_{(+)}$ and $K_{(++)}$ from Table 1. A pH-dependent partition coefficient profile with two inflection points, one at each pK_a, was obtained and is shown in Fig. 2B. The double sigmoidal curves indicate that nicotine molecules follow the pH-permeability pattern that is characteristic of passive diffusion.

As the transport mechanism of nicotine, through the buccal mucosa, was found to be passive diffusion, the overall diffusivity (D_T) for each studied pH was computed by dividing the overall permeability (P_T) by the overall partition coefficient (K_T) and multiplying by the thickness of the buccal mucosa. The calculated overall diffusivities for three studied pHs (8.8, 7.8 and 2.0) were used in Eq. (4) to obtain the specific diffusiv-

Table 1
Specific permeability, partition coefficient and diffusivity of various nicotine species through the nonkeratinized buccal mucosa

	Neutral	Mono-protonated	Di-protonated
Permeability $\times 10^4$ (cm/h)	479.0	12.97	2.603
Partition coefficient	1.499	0.410	0.233
Diffusivity $\times 10^4$ (cm²/h)	39.304	3.891	1.374

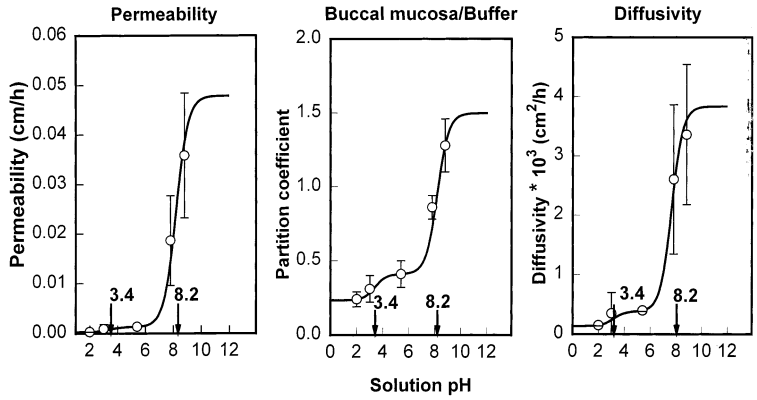

Fig. 2. Nicotine permeability (A), partition coefficient (B) and diffusivity (C) through the non-keratinized buccal mucosa as a function of pH. ○, experimental data of overall permeability (P_T), partition coefficient (K_T) and diffusivity (D_T); —, predicted overall permeability (P_T), partition coefficient (K_T) and diffusivity (D_T) as a function of pH calculated from Table 1, and Eqs. (2)–(4), (5a), (5b) and (5c).

ity for each species (listed in Table 1). The specific diffusivity (D_n) of neutral nicotine was found to be 10-fold greater than that of mono-protonated nicotine ($D_{(+)}$) and 29-fold greater than that of di-protonated nicotine ($D_{(++)}$). The hydrodynamic radius of neutral, mono- and di-protonated nicotine is expected to be the same. Therefore the difference in D is believed to result from different preferred transport pathways for neutral and protonated nicotine. The preferred transport pathway for neutral nicotine is expected to be the lipophilic intracellular space. It is therefore believed that the protonated species follow the more hydrophilic diffusion pathway, which is the intercellular space. The overall diffusivity of nicotine as a function of pH was plotted according to Eq. (4) and the specific diffusivity of each nicotine species is presented in Table 1. A pH-dependent profile for nicotine diffusivity is demonstrated in Fig. 2C.

The permeation of nicotine through different oramucosae (non-keratinized buccal and sublingual, and keratinized gingival) under the same conditions (pH 8.8) was compared and is depicted in Fig. 3 (Nair et al., 1997). The keratinized gingival mucosa shows higher nicotine permeation than the non-keratinized buccal and sublingual mucosae. The specific permeability for each species and mucosa was computed using Eq. (2) and the overall permeabilities at pH 8.8, 5.4 and 2.0 are summarized in Table 2. Irrespective of the charge status of nicotine, the permeability of nicotine through the keratinized gingiva was highest among the studied oramucosae. Three double-sigmoidal curves were obtained for the permeability-pH profiles (Fig. 4), each with two inflection points that corresponded to the two pK_as of nicotine. The results indicate that nicotine molecules follow pH-permeability relationships that are characteristic of passive diffusion in all three oramucosal tissues.

The permeability differences among the various oramucosae and the permeability-pH dependencies are distinctly demonstrated in Fig. 4. In order to investigate the relationship between permeabilities for the various oramucosae as a function of pH, the permeability ratios between each pair of oramucosae (gingival versus buccal, gingival versus sublingual and sublingual versus buccal) were determined from Fig. 4. The permeability ratio for each pair of oramucosae is shown in Fig. 5. The permeability ratio for the two non-keratinized buccal and sublingual mucosae was found to remain constant (2.20) and was independent of pH. This observation suggests that the transport resistance of passive diffusion through the buccal mucosa by either the transcellular and/or paracellular pathways was 2.20-fold greater than that through the sublingual mucosa.

As discussed in Section 1, the major barrier to the permeation of molecules across both kera-

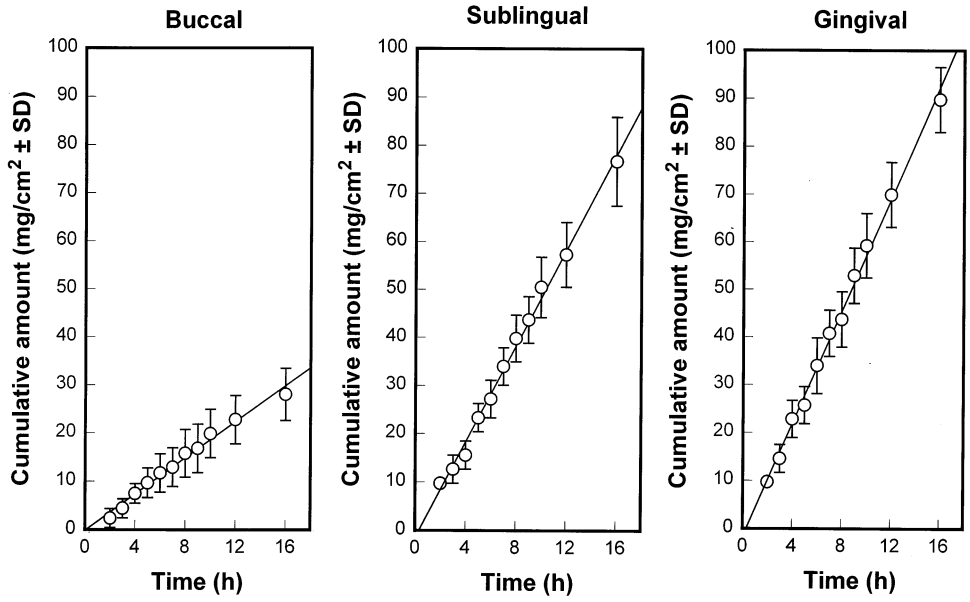

Fig. 3. The permeation profiles of nicotine through various oral mucosae at pH 8.8. Significantly higher permeation of nicotine through the keratinized gingival mucosa was observed.

tinized and non-keratinized oral epithelia by passive diffusion is believed to result from the presence of MCG in the intracellular space. A relationship between the relative volume of MCG in the cytoplasm and the permeability of water for various human stratified squamous epithelia was established by Lecsh et al. (1989) from the data of Schroeder (1981). It was concluded that lower water permeability was associated with the existence of a greater volume of MCG in each type of epithelium. Due to the similarity between human oramucosae and porcine oramucosae (Squier and Hall, 1985), the relative ratio of the occupied volumes of MCG in the intracellular space in human oramucosae (buccal/sublingual) was assumed to represent the ratios for porcine buccal and sublingual mucosae. Therefore, Schroeder's human data were utilized to calculate the relative cytoplasmic volume ratios occupied by MCG in the non-keratinized buccal and sublingual mucosae and the keratinized gingival mucosa (Table 3). The inter-mucosal permeability ratio-pH profile for porcine sublingual:buccal mucosae maintained a constant level (2.20) that was approximately equal to the ratio (2.14) of the occupied volume of MCG for buccal versus sublingual mucosae. This clearly illustrates the barrier function of MCG in cytoplasm.

The inter-mucosal permeability ratio-pH profiles for each pair of the non-keratinized and the keratinized oramucosae were significantly affected by pH. The permeability ratios between each pair of the keratinized and the non-keratinized oramucosae remained constant (2.79 and 1.27 for gingival:buccal and gingival:sublingual, respectively) when pH was higher than 8.0. It is noteworthy

Table 2
Specific permeability of various nicotine species across selected oramucosae

Species	Specific permeability $\times 10^4$ (cm/h)		
	Non-keratinized		Keratinized
	Buccal	Sublingual	Gingival
Neutral	479.0	1056	1338
Mono-protonated	12.97	18.08	78.39
Di-protonated	2.603	6.559	26.47

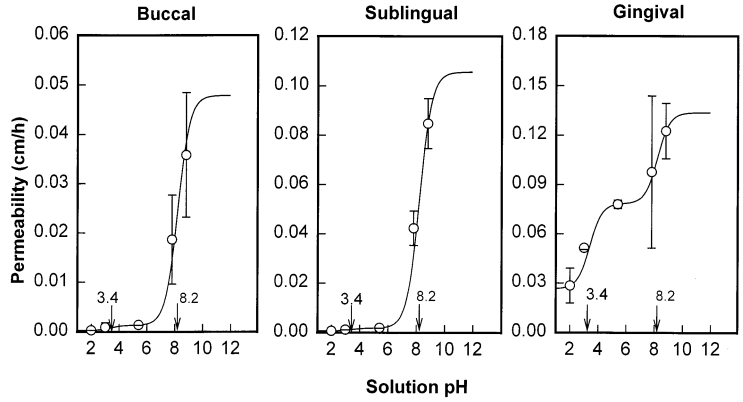

Fig. 4. Comparison of the permeability profiles of nicotine across various oramucosae as a function of pH. ○, experimental data of overall permeability (P_T); —, predicted permeability as a function of pH which was computed from Table 2 and Eqs. (2), (5a), (5b) and (5c).

that these constant permeability ratios were approximately equal to the ratio (2.65 and 1.23 for buccal:gingival and sublingual:gingival, respectively) of the occupied volume of MCG for each pair as indicated in Table 3. The volume of MCG, which is believed to be responsible for diffusional resistance through intracellular space, was found to be inversely proportional to the permeability of neutral molecules, as shown in Table 3 and Fig. 5. Therefore, it is believed that the major and preferred transport pathway for neutral nicotine is transcellular. As pH was decreased, the permeability ratio changed substantially. These observations imply that transport resistance changed due to the switch of the diffusion pathway, from transcellular to paracellular, as nicotine became protonated. The greater paracellular transport resistance through the non-keratinized oramucosae, such as the buccal and sublingual mucosae, than through the keratinized gingival tissue was observed from Fig. 5. Therefore, it is believed that biochemical differences between the non-keratinized and the keratinized oramucosae, such as lipid composition, could result in significantly different degrees of diffusional resistance for charged nicotine molecules via the paracellular pathway due to the lipids extruded from MCG into the intercellular space during the course of differentiation.

Barrier properties are known to vary from one tissue region to another due to the variation in lipid composition. When compared to the keratinized epithelia (Squier et al., 1986; Wertz et al., 1986), the non-keratinized epithelia have been reported to contain a higher percentage of glycosylceramides, but a lower percentage of ceramides, in the intercellular spaces. The differences are due to the absence of glycosidases (the enzymes required for the conversion of glycosylceramides to ceramides) in the non-keratinized epithelia (Chang et al., 1991; Wertz et al., 1984). The non-keratinized oral mucosae also contain much higher levels of sterol esters than the keratinized epithelia (Squier et al., 1986, 1991; Wertz et al., 1986). The high levels of glycosylceramides and sterol esters in the intercellular space of the non-keratinized epithelia may be responsible for the higher diffusional resistance to the permeation of hydrophilic/charged nicotine through the paracellular pathway for the non-keratinized mucosae as shown in Fig. 5.

4. Conclusion

The permeability of nicotine through the buccal mucosa was found to be a function of solution pH because the dissociation of nicotine significantly changed its partition coefficient and diffu-

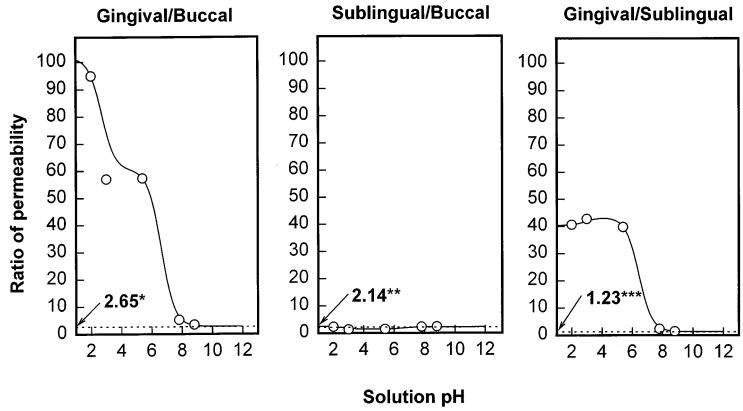

Fig. 5. Inter-mucosal permeability ratios as a function of pH based on data in Fig. 4. The values (2.65, 2.14 and 1.23) and dashed lines represent the ratio of the occupied volumes of membrane-coating granules (MCG) in the intracellular space for each pair of oramucosae as indicated in Table 3. ○, permeability ratios calculated from experimental results in Fig. 4; —, predicted permeability ratios from the predicted permeability in Fig. 4.

Table 3
Relative cytoplasmic volume of membrane-coating granules (MCG) in various human oral epithelia and the content ratio between oramucosal pairs

MCG	Gingival	Sublingual	Buccal
Relative volume[a] (mm^3/cm^3)	307.89	380.36	816.84
Ratio of MCG between oramucosae			
Buccal/gingival		2.65	
Buccal/sublingual		2.14	
Sublingual/gingival		1.23	

[a] Source: Lecsh et al. (1989).

sivity. The pH-partition coefficient and pH-diffusivity relationships of nicotine in the buccal mucosa were characteristic of relationships that demonstrate passive diffusion as the transport mechanism.

The permeability of nicotine through the keratinized gingival mucosa was higher than the permeability through both the non-keratinized buccal and sublingual mucosae. The transport pathway of neutral nicotine is expected to be transcellular. The transport resistance of nicotine through the transcellular pathway was observed to be inversely proportional to the occupied volume of MCG in the intracellular space. It is believed that the preferred transport pathway for nicotine switched from the transcellular pathway to the paracellular pathway due to its protonation at low pH. It is also believed that the transport resistance of charged nicotine through the paracellular pathway in the non-keratinized buccal and sublingual mucosae was higher than that in the keratinized gingival mucosae. This is assumed to be due to differences in lipids that are extruded into the intercellular space from MCG between the non-keratinized and keratinized mucosae during differentiation. The high levels of glycosylceramides and sterol esters in the intercellular spaces of the non-keratinized oral mucosae are possibly responsible for the higher diffusional resistance of paracellular transport.

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