Transfer of Alcohols and Ureas across the Oral Mucosa Measured Using Streaming Potentials and Radioisotopes

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Abstract □ The permeability of the oral mucosa to an alcohol and a urea series was studied using radioisotope transfer and the measurement of streaming potentials. Both methods yielded similar quantitative estimates of permeability. The rate of transfer of the smallest member of both series (methanol and urea) was greater than the second member (ethanol and methylurea). In the alcohol series, permeability increased as the chain length increased from ethanol to butanol. In contrast, the permeability of the oral mucosa to ethylurea and propylurea was less than to methylurea. However, butylurea had a greater rate of transfer than either propylurea or ethylurea.

Keyphrases □ Permeability—oral mucosa to alcohol and urea series, radioisotope transfer, streaming potential, dogs □ Alcohol series—absorption through oral mucosa, radioisotope transfer, streaming potential, dogs □ Urea series—absorption through oral mucosa, radioisotope transfer, streaming potential, dogs □ Absorption—alcohol and urea series through dog oral mucosa, radioisotope transfer, streaming potential

A major function of the oral mucosa is to act as a protective barrier between substances present in the oral cavity and the underlying tissues. However, this barrier is not perfectly impermeable and many substances can cross it. For instance, the work of Brunton (1) established that nitroglycerin can be absorbed through the oral mucosa in sufficient quantities to cause pharmacological responses. Later, Walton (2) studied the sublingual absorption of several alkaloidal drugs. He pointed out that the oil-to-water distribution coefficient was the primary factor determining the extent of sublingual absorption of these drugs. More recently, buccal absorption was studied as an example of an *in vivo* model of passive transfer through a lipid membrane (3).

Despite the abundance of evidence suggesting that the oral mucosa acts as a passive semipermeable barrier, a recent review (4) pointed out that there is a paucity of quantitative data on the permeability of this barrier to specific compounds.

In this report, two methods were utilized to obtain quantitative estimates of the permeability of the lingual frenulum to selected compounds. The first method relies on the measurement of the streaming potential developed when water moves through a charged membrane in response to an osmotic gradient. This method has been extensively used in determining the permeability of the rabbit gallbladder to nonelectrolytes (5). The second method is the measurement of the rate of transfer of radioactively labeled compounds across the lingual frenulum of the dog. This method was used in earlier studies on transmucosal absorption of local anesthetics (6).

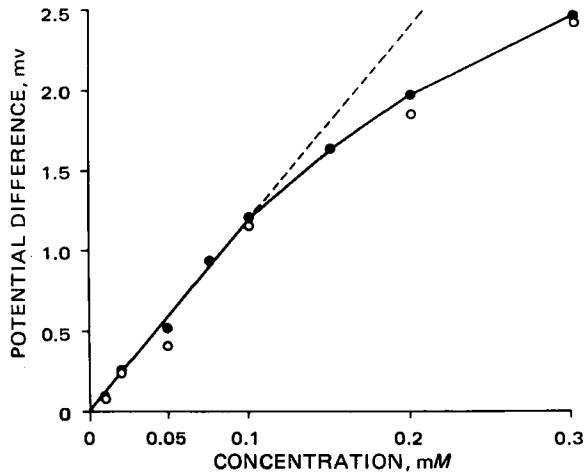

Figure 1—*Potential difference across the frenulum of the dog in response to osmotic gradients of sucrose (●) and mannitol (○). The dashed line is a continuation of the linear relationship that exists up to a concentration of 0.1 mM. Each point represents the mean value of at least three determinations on each of four separate pieces of tissue.*

EXPERIMENTAL

Streaming Potential Measurements—The lingual frenulum was removed from adult male mongrel dogs anesthetized with 30 mg/kg of pentobarbital sodium. The tissue was placed in oxygenated Krebs–Ringer phosphate solution and split along the cut edges, exposing an inner blood side surface as well as an outer oral side surface. The composition of the Krebs–Ringer phosphate solution was potassium (5 m*M*), sodium (148 m*M*), magnesium (1.33 m*M*), calcium (2.0 m*M*), chloride (154 m*M*), sulfate (1.33 m*M*), glucose (0.1%), and pH 7.4 phosphate buffer (8.6 m*M*).

The inner surface was carefully cleaned free of extraneous tissue, and the resulting thin membrane was placed in a modified Ussing (7) chamber. The methods described for determining streaming potentials in rat intestine (8) were adapted to this *in vitro* lingual frenulum preparation. Electric potential differences across the membrane were measured using polyethylene bridges filled with 4% agar in 150 m*M* sodium chloride. One bridge was placed in the solution facing the oral side of the membrane, and another bridge was placed in the solution facing the inner side. Each bridge led to a beaker of potassium chloride solution, which was connected through a potassium chloride–agar bridge to a second beaker of saturated potassium chloride; a calomel electrode was placed in the second beaker.

The potential difference between the electrodes was measured and recorded[1]. The asymmetry potential of the circuit was measured as the potential difference with both agar–sodium chloride bridges dipping into the solution bathing the same side of the tissue.

The solution bathing both sides of the membrane was identical (Krebs–Ringer phosphate), except that a known concentration (0.1

[1] Keithley model 602 electrometer and Keithley recorder.

Table I—Reflection Coefficients and Permeability Constants of Alcohols and Ureas on the Canine Oral Frenulum *In Vitro*

Compound	Molecular Weight	Partition Coefficient[a]	Permeability Constant, ($K_p \times 10^{-5}$ cm/sec)[b]	Reflection Coefficient[c]
Urea series				
Urea	60	0.00047	2.7 ± 0.83	0.17 ± 0.08 (39)
Methylurea	74	0.0012	1.4 ± 0.75	0.29 ± 0.12 (15)
Ethylurea	88	0.0041	—	0.76 ± 0.10 (15)
Propylurea	102	—	—	0.91 ± 0.03 (15)
Butylurea	116	—	—	0.43 ± 0.16 (18)
Alcohol series				
Methanol	32	0.14	9.7 ± 4.6	0.02 ± 0.02 (15)
Ethanol	46	0.26	3.7 ± 2.5	0.09 ± 0.01 (27)
Propanol	60	1.9	5.9 ± 3.6	0.02 ± 0.00 (19)
Butanol	74	7.7	10.1 ± 4.3	0.01 ± 0.01 (15)

[a] Ether to water; values are from Collander (13). [b] Mean permeability constant ± *SD* calculated from 12 pieces of tissue. [c] Mean reflection coefficient ± *SD*. The total number of determinations is given in parentheses.

M, unless otherwise mentioned) of substance to be tested was placed on one side. Sucrose was used as a nonpermeant substance. The potential developed by the test substance was compared to that developed in the presence of the same concentration of sucrose, as described by Wright and Diamond (5). Measurements of the potential difference developed by test substances were always bracketed by measurement with sucrose.

Radioactive Transfer Measurements—The lingual frenulum was removed from the animals, prepared, and placed in chambers as already described. Then 3 μCi of ^{14}C-labeled compound[2] plus sufficient nonradioactive compound to bring the total concentration to 1–10 m*M* (in Krebs–Ringer phosphate solution) was added to the half chamber facing one side of the membrane, and the other half chamber was filled with Krebs–Ringer phosphate solution.

Ten-microliter aliquots were removed from both half chambers at 30-min intervals. These samples were counted for a total of 5000 counts[3] using conventional techniques. All counts were corrected for quenching. Permeability constants were calculated from a knowledge of the area of exposed tissue, the volumes and concentrations present on both sides of the frenulum at each time interval, and the area of tissue exposed to the solutions using Fick's general law of diffusion:

$$\frac{Q}{At} = J_s = K_p \Delta C_s \qquad \text{(Eq. 1)}$$

where Q is the amount of solute that penetrates the membrane, A is the area of membrane exposed to solute, t is the time, J_s is the amount of solute that penetrates per unit area in unit time, K_p is the permeability constant, and ΔC_s is the difference in concentration on the two sides of the exposed membrane.

RESULTS

Potential Difference across Frenulum in Absence of Osmotic Gradients—In the absence of an osmotic gradient across the frenulum *in vitro*, there is essentially no potential difference between the outer (oral) and inner (blood) sides. The average value (±*SD*) for 42 preparations in which Krebs–Ringer phosphate solution was on both sides of the membrane was −0.0076 ± 0.031 mv (oral side negative). The range of recorded potential difference was from −0.06 to + 0.05 mv.

Potential Difference across Frenulum in Presence of Osmotic Gradient—Figure 1 shows the effect of adding either sucrose or mannitol to the bathing solution on the outside of the frenulum. The outside of the membrane went positive with respect to the inside. The amount by which the outside went positive depended on the concentration of sucrose or mannitol present in the bathing solution. The relationship between the concentration of sucrose present and the electrical response recorded was essentially linear when concentrations of 0.1 *M* or lower were used. At higher concentrations, the relationship was nonlinear.

If, after a steady potential had been reached, the sucrose-containing solution on the oral side of the frenulum was replaced with Krebs–Ringer phosphate solution, then the potential quickly returned to close to the original value. The potential difference that developed when sucrose was added to the outer side of the membrane could also be brought back to approximately zero by adding the identical concentration to the inside of the membrane (three experiments). Thus, at least two ways of eliminating the osmotic gradient resulted in abolition of the potential across the frenulum.

When a more permeant substance than sucrose was placed in the outside bathing solution, the potential difference across the preparation was less than when sucrose was used. Figure 2 shows the results of one experiment in which 0.1 *M* solutions of sucrose, urea, and ethylurea were compared. The potential differences developed to 0.1 *M* gradients of these three substances were 0.58, 0.11, and 0.45 mv, respectively. These data correspond to reflection coefficients of 0.19 for urea and 0.78 for ethylurea.

Table I gives the average reflection coefficients for each substance tested. The reflection coefficients for the second member of both the alcohol and urea series were higher (indicating a lower rate of penetration) than the first member of the series. In the alcohol series, the reflection coefficient then decreased with further increases in chain length. In contrast, the reflection coefficient continued to increase in the urea series until propylurea and then decreased when butylurea was tested.

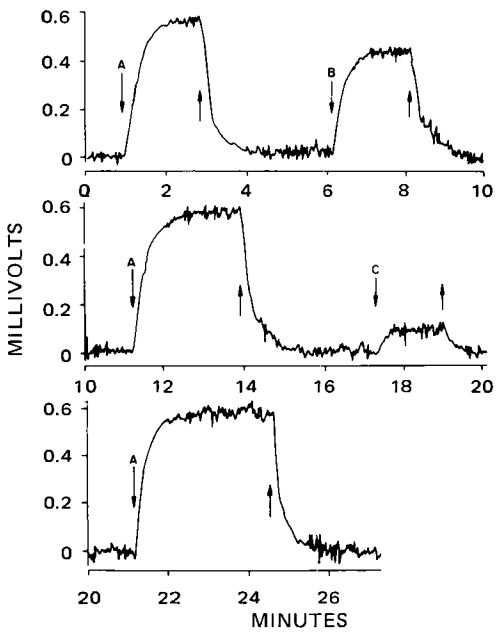

Figure 2—*Continuous record of the potential difference across the frenulum in response to 0.1* M *gradients of sucrose (A), ethylurea (B), and urea (C). Downward arrows indicate addition of the compound to the oral side of the preparation. Upward arrows indicate replacement of the compound-containing solution with Krebs–Ringer phosphate solution.*

[2] New England Nuclear Corp.
[3] Packard liquid scintillation counter.

Permeability Constant Measurements—The permeability of the frenulum to many compounds on which reflection coefficients were determined was measured by the rate of isotope penetration from the outside to the inside of the tissue. The permeability constants ranged from about 10^{-4} cm/sec for butanol to as low as 4×10^{-7} cm/sec for sucrose. The permeability for three of the compounds listed in Table I (ethylurea, propylurea, and butylurea) could not be determined because the labeled compounds were not commercially available.

Within both the alcohol and urea series, the permeability of the first member was higher than that of the second member of the series. This was true even though the ether-to-water distribution ratio is higher for the second member than the first. In the alcohol series, permeability then increased as chain length increased.

The electrical measurement of reflection coefficients is based on the principle that a flow of water across a charged membrane results in the development of an electrical potential difference across the membrane. The magnitude of these streaming potentials is directly proportional to the flow rate across the membrane (9). Since streaming potentials are set up rapidly, they offer a convenient means for determining osmotic flow. A fully impermeant molecule produces the greatest osmotic flow across a given membrane. The same concentration of a less permeant molecule results in a lower water flow and, consequently, a reduced potential across the membrane. Thus, as pointed out by Wright and Diamond (9), general agreement between direct chemical determination of permeability and results from the determination of reflection coefficients should be expected.

Collander (10, 11) made a detailed comparison of osmotic and chemical methods utilizing the alga *Nitella mucronata*. He measured the permeation of 17 nonionized compounds and demonstrated that similar permeability sequences were obtained by both methods. Our results using the dog frenulum also indicate that similar trends can be demonstrated by both methods.

Both methods have advantages and shortcomings. The primary advantage of the streaming potential technique is the rapidity of the measurement. Because each measurement takes only a few minutes, determinations of the streaming potential developed to a number of compounds can be performed on a single sample of tissue. An entire series of compounds can be run on the same tissue sample, thereby minimizing variations between tissue. Also, because of the short time of exposure to the test substances, there is less opportunity for damage to the tissue by a toxic compound.

However, higher concentrations of solute are required for the streaming potential measurement than for determinations using radioisotopes. Furthermore, the electrical signal generated under the conditions in this study was often less than 1 mv, particularly when very permeable compounds were tested. Thus, there are appreciable signal-to-noise ratio problems. These are particularly troublesome when either relatively highly permeable or impermeable compounds are compared.

The main advantage of the radioisotope method is that low concentrations of solute are used, which minimizes the opportunity for the test compound to injure the tissue. Also, when the isotope method is used, permeability constants rather than reflection coefficients can be calculated from the experimental results. These advantages are offset somewhat by the obvious disadvantages of the longer experimental time period and the necessity of making comparisons between tissues rather than on the same piece of frenulum.

Collander and Bärlund (12) concluded that lipid solubility is the most important factor determining the permeation of nonelectrolytes. However, they also pointed out that small molecules penetrate more rapidly than would be predicted from their lipid solubility. The current study of an alcohol series and a urea series suggests that a similar phenomenon exists for oral tissue. Both the reflection coefficient and the permeability constant data suggest that the smallest member of the series (one carbon) penetrates the frenulum more rapidly than some higher members of each series. This is true even though the ether-to-water partition coefficients are lower for the smaller compounds.

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