Permeability of tongue epithelium and its relation to taste

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MISTRETTA, CHARLOTTE M. Permeability of tongue epithelium and its relation to taste. Am. J. Physiol. 220(5): 1162–1167. 1971.—The permeability of rat tongue epithelium was investigated to provide information on the accessibility of free lingual nerve endings to chemical stimuli. Permeability of rat belly skin was also studied. Dorsal tongue epithelium was removed after subepithelial injection of collagenase. Abdominal skin was removed by gross dissection. The epithelium was placed in a diffusion cell and radioactive penetrants were applied to the dorsal surface. Permeability coefficients of 13 nonelectrolytes were determined according to Fick's law. Methanol, ethanol, butanol, ethylene glycol, and propionamide penetrate the tongue and belly relatively rapidly; thiourea, acetamide, glycerol, sodium butyrate, fructose, glucose, and mannitol penetrate slowly. For the tongue and belly, an increase in penetration rate is related to an increase in the ether/water partition coefficient of the penetrant. Ethanol, a known stimulant of the trigeminal part of the lingual nerve, penetrates tongue epithelium rapidly. In general, though, tongue epithelium is as effective a barrier to chemical penetration as belly skin, the free endings of the lingual nerve are not readily accessible to chemicals.

No matter how near to the surface one estimates the depth of the lingual nerve receptors, chemicals would have to penetrate the keratinized layer and at least some part of the underlying dorsal tongue epithelium to reach the sensory fibers of the lingual nerve. And so the question arises, How effective a barrier does dorsal tongue epithelium present to chemical penetrants?

METHODS

Dissertation of tissues and histology. Rat dorsal tongue epithelium and whole belly skin were used in the permeability experiments. Male rats weighing 200–250 g were anesthetized with an intraperitoneal injection of chloral hydrate (36 mg/100 g body wt). To obtain tongue epithelium, 0.5 ml of a 1.0% solution of collagenase (Worthington collagenase, code: CLS), Worthington Biochemical Corporation, N.J. (pH 7.4, 37 C) was injected under the dorsal surface of the rat's tongue, according to the method of Beidler (4). One-half hour after injection, the dorsal tongue epithelium with taste buds intact was easily stripped off. Only epithelium from the anterior two-thirds of the tongue was studied (Fig. 1).

The area of tongue epithelium which was eventually clamped off for penetration measurements contained an average of four fungiform papillae or four taste buds. In Fig. 1 one fungiform papilla is seen to the left of the field. The taste bud is situated in the middle of the papilla. The layer of keratinized tissue over the fungiform papilla is about 5 μ thick. This layer is broken by a taste pore at the center of the taste bud. The pore diameter is 5 μ. The keratin layer over the filiform papillae (seen to the right of the fungiform) is 30–50 μ thick.

Attempts to remove intact abdominal epidermis were not successful and so whole belly skin was used. To obtain rat abdominal skin, an area of the rat's belly was first clipped with an electric shaver until only very short hairs remained. A piece of skin about 1 cm² was then removed with scissors. The underlying fat and connective tissue containing the blood vessels were dissected away under a binocular dissecting microscope (Fig. 2).

The integrity of the tissue was routinely checked by histological techniques. Sections of tongue epithelium were found to contain taste buds and an intact layer of basal cells (Fig. 1).

Permeability apparatus. A modification of techniques described by Treherne (18) and Ainsworth (1) for studying the permeability of skin was used to investigate penetration.
PERMEABILITY OF TONGUE EPITHELIUM

FIG. 1. Photomicrograph of a cross section of stripped rat tongue epithelium. Magnification: × 320. A single fungiform papilla with intact taste bud is seen at left. Filiform papillae are to right of this section.

FIG. 2. Photomicrograph of a cross section of rat belly skin prepared as for a permeability experiment. Magnification: × 126.

The methods used are standard in the study of skin permeability (17) and permit a direct comparison of results with values in the literature. The tissue to be studied was placed over an opening (0.049 cm²) of a stainless steel diffusion cell which was filled with mammalian Ringer solution. The dermal side of the tissue faced the Ringer solution. The fragile tongue epithelium was supported on both sides by electron microscope grids. Such a support was not necessary for the abdominal skin.

Once the tissue was positioned over the aperture in the cell, a Lucite column (1.5 cm high) was clamped into place over the tissue. The column provided a reservoir for holding the penetrant and effectively clamped off 0.049 cm² area of tissue so that no leaks occurred.

A constant volume of 120 µl of radioactive penetrant was maintained on top of the epithelium. The penetrant was removed from the dermal side of the tissue by mammalian Ringer flowing beneath the epithelium at a rate of 1 ml/perform minutes. One-ml samples were then collected every 10 min by means of a fraction collector. The temperature of the diffusion cell was maintained at 33°C by circulating water from a constant temperature bath through a separate chamber in the bottom of the cell.

The radioactivity, in counts per minute of the collected samples, was counted in a Packard liquid scintillation counter. All experiments were run for at least 3.5 hr; the average length of experiments was 6 hr. At least four experiments were run (two for belly skin, two for tongue epithelium) for every penetrant with the exception of mannitol (one experiment only, on tongue epithelium).

The penetrants investigated and their concentrations are listed in Table 1. All penetrants were diluted in distilled water with the exception of mannitol which was solubilized in ethanol. These penetrants were chosen on the basis of the following considerations: 1) many of the same chemicals have been used by other workers to investigate the permeability of skin; 2) the chemicals represent a fairly wide range of molecular weights; 3) the ether/water partition coefficients cover a wide range.

Treatment of data. Counts per minute for collected samples were first corrected according to the percent efficiency of
TABLE 1. Permeability coefficients for rat tongue epithelium and belly skin for all substances studied

<table>
<thead>
<tr>
<th>Penetrant</th>
<th>Concentration</th>
<th>Permeability Coefficient for Tongue, cm/min X 10⁻⁶</th>
<th>No. of Exps</th>
<th>Permeability Coefficient for Belly, cm/min X 10⁻⁶</th>
<th>No. of Exps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol-¹⁴C</td>
<td>30.0</td>
<td>263 ± 9.8</td>
<td>2</td>
<td>119 ± 40.9</td>
<td>2</td>
</tr>
<tr>
<td>Ethyl carbamate-carboxyl-¹⁴C</td>
<td>46.0</td>
<td>249 ± 9.2</td>
<td>2</td>
<td>12 ± 2.6</td>
<td>3</td>
</tr>
<tr>
<td>Butanol-¹⁴C</td>
<td>50.0</td>
<td>212 ± 38.0</td>
<td>2</td>
<td>71 ± 45.1</td>
<td>3</td>
</tr>
<tr>
<td>Ethanol-¹⁴C</td>
<td>11.0</td>
<td>182 ± 45.6</td>
<td>2</td>
<td>28 ± 3.2</td>
<td>2</td>
</tr>
<tr>
<td>Ethylene glycol-¹⁴C</td>
<td>50.0</td>
<td>164 ± 3.7</td>
<td>2</td>
<td>59 ± 15.9</td>
<td>2</td>
</tr>
<tr>
<td>Propionamide-¹⁴C</td>
<td>48.0</td>
<td>156 ± 0.95</td>
<td>2</td>
<td>51 ± 30.5</td>
<td>2</td>
</tr>
<tr>
<td>Thiourea-¹⁴C</td>
<td>30.0</td>
<td>79 ± 34.3</td>
<td>4</td>
<td>18 ± 1.5</td>
<td>2</td>
</tr>
<tr>
<td>Acetamide-¹⁴C</td>
<td>50.0</td>
<td>60 ± 10.8</td>
<td>2</td>
<td>9 ± 1.0</td>
<td>2</td>
</tr>
<tr>
<td>Glycerol-¹⁴C</td>
<td>18.0</td>
<td>27 ± 7.8</td>
<td>2</td>
<td>24 ± 15.8</td>
<td>3</td>
</tr>
<tr>
<td>Fructose-³H</td>
<td>25.0</td>
<td>24 ± 4.7</td>
<td>2</td>
<td>8 ± 3.4</td>
<td>2</td>
</tr>
<tr>
<td>Sodium butyrate-¹⁴C</td>
<td>50.0</td>
<td>16 ± 3.2</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Glucose-¹⁴C</td>
<td>2.5</td>
<td>11 ± 4.1</td>
<td>2</td>
<td>10 ± 1.9</td>
<td>2</td>
</tr>
<tr>
<td>Mannitol-¹⁴C</td>
<td>14.7</td>
<td>11</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( \rho = \text{Permeability coefficient.} \quad * \text{Values are means} \pm \text{sd.} \)

The results of the experiments reported here are presented as permeability constants or coefficients, \( \rho \). The \( \rho \) is calculated according to Fick's law: \( \frac{ds}{dT} = k\rho \Delta C_a \). In this equation, \( k\rho \) is the permeability constant, \( \frac{ds}{dT} \) is the rate of penetration, and \( \Delta C_a \) is the difference in penetrant concentration on two sides of the epithelium. In the experiments described here, \( \Delta C_a \) can be considered equal to the concentration of penetrant applied to the dorsal surface of the epithelium. This assumption is valid since the penetrant is collected on the dermal side in a constantly flowing Ringer solution.

The \( \rho \) value used here is obtained by simply rewriting Fick's law as:

\[ \rho = \frac{r}{C} = \frac{\text{mmoles/cm}^2 \cdot \text{min}}{\text{cm}} \]

and

\[ \rho = \frac{r}{C} = \frac{\text{mmoles/cm}^2 \cdot \text{min}}{\text{cm}} \]

### RESULTS

Figure 3 is a typical curve for penetration of a nonelectrolyte through rat tongue epithelium or abdominal skin. The amount of substance penetrating the tissue plotted against time gives a straight line once a steady penetration rate has been reached. The slope of the straight line is therefore the rate of penetration. By extrapolating from the straight-line portion of the curve to the abscissa, one obtains a delay time. The delay time is the amount of time which lapses before a steady rate of penetration is reached.

Table 1 is a summary of the data. Permeability coefficients for both tongue epithelium and belly skin are presented.
TABLE 2. Permeability coefficients for rat belly skin compared to permeability coefficients for rabbit belly skin

<table>
<thead>
<tr>
<th>Penetrant</th>
<th>$p$, cm/min $\times 10^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rat belly</td>
</tr>
<tr>
<td>Methanol</td>
<td>119</td>
</tr>
<tr>
<td>Ethanol</td>
<td>28</td>
</tr>
<tr>
<td>Glycerol</td>
<td>24</td>
</tr>
<tr>
<td>Thiourea</td>
<td>18</td>
</tr>
<tr>
<td>Glucose</td>
<td>10</td>
</tr>
</tbody>
</table>

$p =$ Permeability coefficient. * Data from Treherne (18).

Alcohols are among the nonelectrolytes that penetrate tongue and belly most rapidly. Ethylene glycol, ethyl carbamate, and propionamide are also rapid penetrators. Of substances tested, sodium butyrate and sugars are the slowest penetrants.

The composite graph shown in Fig. 4 further illustrates the differences in penetration rates for various chemicals. For clarity, alcohols have been excluded from this figure, but if shown they would fall in the penetration range of ethyl carbamate.

Treherne (18) investigated the penetration of some low-molecular weight nonelectrolytes through rabbit belly skin. Table 2 presents a comparison of results for rabbit and rat skin. When the permeability coefficients for five chemicals are arranged in order of decreasing magnitude, the ranking is similar for both sets of data.

The permeability curve for butanol on rat tongue epithelium is presented in Fig. 5. The shape of the penetration curves for all three alcohols studied is markedly different from the typical curve presented in Fig. 3. A break occurs in each of the alcohol curves between 90 and 120 min, after which the penetration rate decreases. Also, high variability was observed in measurements with the alcohols. This may be due to the fact that the alcohols are affecting the integrity of the tissue.

A correlation exists between the ether-water partition coefficients of the chemicals studied (6, 7) and the permeability constants. Figure 6 shows clearly that those compounds with a high coefficient penetrate the tongue most rapidly. A greater scatter of the data is seen in Fig. 7 which plots the same relationship for rat belly skin. This scatter may be due to the greater variation in the thickness of the rat belly skin for different experiments.

**DISCUSSION**

During the course of a lifetime, the skin comes into contact with many insulting stimuli and, therefore, it must function as a barrier in order to protect deeper tissues. The
epidermis of the tongue is no exception; it is constantly exposed to stimuli of differing temperatures, textures, and chemical composition. The primary question to be discussed here is the effectiveness of the lingual epithelium as a barrier to chemicals.

The results of these permeability experiments indicate that lingual epithelium is similar to external body skin in its capabilities to prevent penetration of chemicals. Even a cursory examination of the histological section of rat tongue epithelium (Fig. 1) leaves one impressed by the heavy cornification that exists on the tongue. It is now well accepted that the stratum corneum is the skin’s principal permeation barrier (3). Therefore, one would expect any cornified epithelium to prevent rapid chemical penetration.

The lingual dorsum has a complex topography. Areas of light keratinization (the fungiform papillae) are interspersed with areas of heavy keratinization (the filiform), and where taste buds occur the keratin layer is interrupted by a pore. In contrast, the rat belly skin (Fig. 2) presents a more uniform structure to an external chemical. Hair follicles are the only structure which complicate the picture. Yet, even in the light of these extreme anatomical differences, consideration of Table 1 indicates that the two tissue types present equally effective barriers to chemicals. Rapid penetrants for both tongue and abdomen are methanol, ethanol, butanol, ethylene glycol, and propionamide; slower penetrants are thiourea, acetamide, glycerol, fructose, mannitol, glucose, and sodium succinate. Ethyl carbamate penetrates tongue epithelium rapidly, but abdominal tissue slowly.

Although all substances penetrate tongue epithelium more quickly than abdominal skin, the permeability coefficients are all certainly of the same order of magnitude. It should be recalled that the belly tissue used in these experiments was much thicker than the tongue epithelium (see Figs. 1 and 2). According to Scheuplein (16), penetration through epidermis varies inversely with thickness. If corrections were made for the differences in thickness between tongue and belly tissues, the belly values would be much closer to those for tongue epithelium. Some belly values would even be greater than those for tongue. However, since two such different types of tissues are being considered here, a simple correction for thickness would still not allow direct quantitative comparison. The results do give a clear indication, though, that the tongue and belly are comparable barriers.

It seems necessary to discuss the possibility that some disruption of the tongue epithelium occurs as a result of using collagenase. Damage seems unlikely, since after collagenase injection the tongue epithelium slips off very easily; no pulling or tearing is necessary to remove it. Even if damage did occur, it would be unlikely to affect measurements reported here. As mentioned earlier, it is the keratinized tissue that presents the main barrier to penetration and it is seemingly unaffected by collagenase. Einbinder, Walzer, and Mandl (8) have mentioned that dermal-epidermal separation of mouse skin cannot be accomplished after topical application of collagenase. This is attributed to the inability of the enzyme to penetrate or digest the stratum corneum. Tregear (17) gives references to numerous experiments that demonstrate that human epidermis isolated by various means retains its integrity as a barrier.

Within the range of non-electrolytes studied here, no clear correlation was found between molecular size and penetration rate. It has been found that, within a given molecular series, the penetration of compounds into rat, human, and ox red cells decreased with increase in molecular size (19). Certainly, though, other factors have been shown to have more influence on the penetration of chemicals through complex tissues.

One of the clearest relationships found in work on the permeability of the skin is between the ether/water partition coefficient of the penetrant and the permeability coefficient. Treherne (18) noted this relation in his work on the permeability of whole rabbit belly skin to various non-electrolytes. Also, Ross (15) studied the permeability of the blood-aqueous barrier and found this correlation, as did Collander and Barslund (7) with Chara cells. In Fig. 6 the relation between the ether/water partition coefficient of penetrants and the permeability coefficients is presented for rat tongue epithelium. The same relationship is shown in Fig. 7 for rat belly skin. For both tissues the permeability increases with increasing ether/water partition coefficient. These data again emphasize the similarity between lingual epithelium and skin from other body areas as barriers to chemicals.

Application of data on permeability of tongue epithelium to study of taste. Although it had been suggested that the taste response is related to permeability (10), Beidler (3) pointed out that the diffusion time contributes no significant portion to the latency of the chorda tympani response to taste stimuli. Certainly there is no apparent relation between permeability values reported here and the response of the chorda tympani to these chemicals. It has been suggested that familial dysautonomia patients, who lack lingual taste buds, may be able to taste with the free nerve endings in the tongue (12). The permeability data presented here make this seem unlikely unless the lingual epithelium of these patients is abnormally permeable. The impermeability of the tongue compares well with that of the belly, confirming Beidler's original findings (4) that chemicals do not readily penetrate the lingual epithelium. Indeed, only a few chemicals have been found which stimulate the trigeminal portion of the lingual nerve.

Using cats, different workers have located the free endings of the lingual nerve at depths varying from 55 to 180 µ (11, 13). Certainly, to reach these endings, a chemical must penetrate some depth of tongue epithelium a good part of which is keratinized. It is interesting to note that one of the few chemicals which has been found to stimulate free trigeminal endings, ethyl alcohol (11), is also one of the rapid penetrants of tongue epithelium.

The discussion of the permeability of rat tongue epithelium has emphasized that this tissue serves as an effective barrier against chemical diffusion. One then wonders how the tongue epithelium can function as both a barrier and as a sensory epithelium, providing information about the environment to an animal. In studies on cutaneous absorption, Scheuplein (16) distinguishes between penetration through hair follicles and sweat ducts and penetration through the unbroken stratum corneum between the follicles and ducts. By mathematical analysis, he shows that transient diffusion, occurring shortly after application of a penetrant,
PERMEABILITY OF TONGUE EPITHELIUM

is potentially greater through hair follicles and sweat ducts. On the other hand, once steady-state diffusion has been established, penetration occurs predominantly through the matrix of the stratum corneum.

A chemical therefore encounters different resistances to its penetration through epidermis. For the tongue, it would seem that three different routes are presented to chemicals. 1) The taste pore, literally a channel, presents the route of least resistance. (However, the tight junctions observed between the apical portions of taste cells have led to the suggestion that chemicals cannot penetrate into the taste bud to stimulate nerves directly (9).) 2) Regions of light keratinization, the fungiform papillae, present a greater resistance. 3) The heavily keratinized filiform papillae, which occupy by far the greatest area of the tongue surface, would seem to present the highest resistance to chemical penetration.

It may be that in the tongue the taste pore is the primary route for the early, transient stages of diffusion. Steady-state penetration would then occur chiefly through the highly keratinized filiform papillae. The pieces of tongue epithelium used in the present experiments contained an average of four fungiform papillae or four taste buds. The area occupied by the taste pores of these buds is only about 0.0004% of the total area of epithelium studied. Thus, a reasonable change in the number of taste buds would not have affected the steady penetration measurements which were obtained here. This method is therefore not sensitive enough to determine the role played by the taste pore in the penetration of chemicals through the tongue dorsum. Permeability measurements through a single fungiform compared to penetration through a region of filiform might help to clarify the picture of chemical passage through the tongue epithelium. In any case, the experiments presented confirm Beidler's earlier suggestion (4) that chemicals do not easily penetrate the lingual epithelium.

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REFERENCES