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The Permeability of Oral Mucosa

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ABSTRACT: In discussing permeability, we are describing one of the fundamental barrier functions of oral mucosa. Despite assumptions to the contrary, the oral mucosa is not a uniformly, highly permeable tissue like gut, but shows regional variation. The keratinized areas, such as gingiva and hard palate, are least permeable and nonkeratinized lining areas are most permeable. This variation appears to reflect differences in the types of lipid making up the intercellular permeability barrier in the superficial layers of the epithelium. Differences in permeability may be related to regional differences in the prevalence of certain mucosal diseases and can be utilized to advantage for local and systemic drug delivery.

KEY WORDS: mouth mucosa, oral mucosa, permeability, membrane coating granules, epithelial barrier lipids, mucosal disease, drug delivery.

I. INTRODUCTION

In 1879, in a letter to *The Lancet*, William Murrell, a lecturer at Westminster Hospital in London, first described the effects of oral nitroglycerin in relieving the pain of angina pectoris.¹ Today, the drug is still ingested by dissolving a tablet sublingually or in the buccal pouch. The ease with which certain compounds can be absorbed across the oral mucosa and the convenience of this route as a means of systemic drug delivery has led to development of a number of therapeutic substances for oral or buccal administration. The use of the oral mucosa for drug delivery and the erroneous belief that it is a nonkeratinized tissue² has sometimes given rise to the belief that oral mucosa is a highly permeable tissue.^{3,4} Such an assumption is not supported by clinical experience; for example, despite an abundant oral flora containing many opportunistic organisms, inflammatory lesions are relatively infrequent in the oral mucosa except at the marginal gingiva. A permeable mucosa would also permit transudation of fluid in order to maintain a moist oral lining even in individuals with salivary insufficiency. The presence of xerostomia

under such conditions is evidence of the relative impermeability of the oral mucosa. Clearly, the permeability of oral mucosa is a complex phenomenon and reflects the structure and pathologic status of the tissue as well as the nature of the penetrants.

There is currently much interest in oral mucosal permeability because of the possibility of utilizing the tissue for controlled delivery of drugs for both local and systemic purposes; in addition, permeability might play a role in the etiology of certain oral mucosal diseases, including premalignant conditions and cancer. This article will review information on the permeability of oral mucosa and the nature of the permeability barrier, and then examine the implications of this for theories of the etiology of oral disease and for therapy involving drug delivery across the oral mucosa.

II. THE STRUCTURE OF ORAL MUCOSA

The permeability of a tissue is related to its structure; permeable membranes such as gut, where absorption is an important function, tend

to be single-layered epithelia. Tissues like skin, which represents the principal barrier between the organism and the environment, are stratified and keratinized. The lining mucosa of the oral cavity is covered by a stratified epithelium and three different types of oral mucosa are recognized. These reflect the functional demands put upon different regions of the oral cavity^{5,6} and are classified accordingly. Masticatory mucosa covers the gingiva and hard palate, regions that are subject to mechanical forces of mastication, causing abrasion and shearing. It consists of a keratinized epithelium that closely resembles the epidermis of the skin in its pattern of maturation (Figure 1A),^{5,7} and is usually tightly attached to underlying structures by a collagenous connective tissue. Lining mucosa covers the remaining regions, except for the dorsal surface of the tongue, and provides an elastic, deformable surface capable of stretching with movements such as mastication and speech. It is covered with a stratified squamous epithelium that is nonkeratinized (Figure 1B) and can vary considerably in thickness in different oral regions. Lining mucosa is attached by a loose, elastic connective tissue to underlying structures. A similar nonkeratinized tissue lines the human esophagus⁸ and uterine cervix.⁹ A specialized mucosa, with characteristics of both masticatory and lining mucosa, is found on the dorsum of the tongue. It has a surface consisting of areas of both keratinized and nonkeratinized epithelium;⁶ these are tightly bound to the underlying muscle of the tongue. The various types of oral mucosa differ in their relative extent in the oral cavity. From measurements made by Collins and Dawes,¹⁰ it can be calculated that the masticatory mucosa represents approximately 25%, the specialized mucosa (dorsum of tongue) approximately 15%, and the lining mucosa approximately 60% of the total surface area of the oral lining. Both the structure and the relative area of the different types of mucosa will influence the permeability of the oral lining.

III. MEASUREMENT OF ORAL MUCOSAL PERMEABILITY

A. *In Vivo* Methods

Much of the literature dealing with oral mu-

cosal permeability has been concerned with drug absorption and the relationship between the structure of a compound and its ability to penetrate the tissue, and there have been a number of studies on the kinetics of drug absorption across oral mucosa (see Beckett and Hossie,¹¹ Moffat,¹² Siegel et al.,¹³ and Siegel¹⁴ for reviews).

The earliest studies on the oral absorption of drugs compared systemic effects after oral and cutaneous delivery. Walton and Lacy¹⁵ were able to show that some compounds were as effective when applied sublingually as when injected subcutaneously. In 1967 Beckett and Triggs¹⁶ described the "buccal absorption test" in which a known quantity of a drug in solution is taken into the mouth, swirled around, and then expectorated. By measuring the drug concentrations in the solution before use and after expectoration the amount of absorption can be calculated. Although this has provided much useful information on oral mucosal absorption,¹¹ there are drawbacks, such as the change in concentration of the drug in the mouth as the result of salivary secretion. Modifications have been described to overcome this problem,¹⁷ but, despite its name, the method is not able to provide information on absorption in a specific mucosal region, because the test solution comes in contact with all parts of the oral mucosa.

The limitations of the buccal absorption test have led to attempts to better control localization of the compound on the oral mucosa and to increase the sensitivity of detection. Bergman et al.^{18,19} placed radiolabeled lidocaine on the floor of the mouth of anesthetized animals and monitored the appearance of isotope in either plasma or urine. Although this method has considerable sensitivity there is a risk that the compound can spread over different mucosal regions, and the use of radioactive labels are not appropriate in human studies. More recently, Pimlott and Addy²⁰ placed tablets of isosorbide dinitrate on the hard palate, buccal mucosa, and sublingually in human volunteers and measured the plasma levels of the compound using gas liquid chromatography. Significantly higher plasma levels were obtained after placing the compound sublingually than buccally, but the method was insufficiently sensitive to detect any palatal absorption. Using a different approach, Kaaber²¹ measured the gain in weight of filter paper disks placed on the sur-

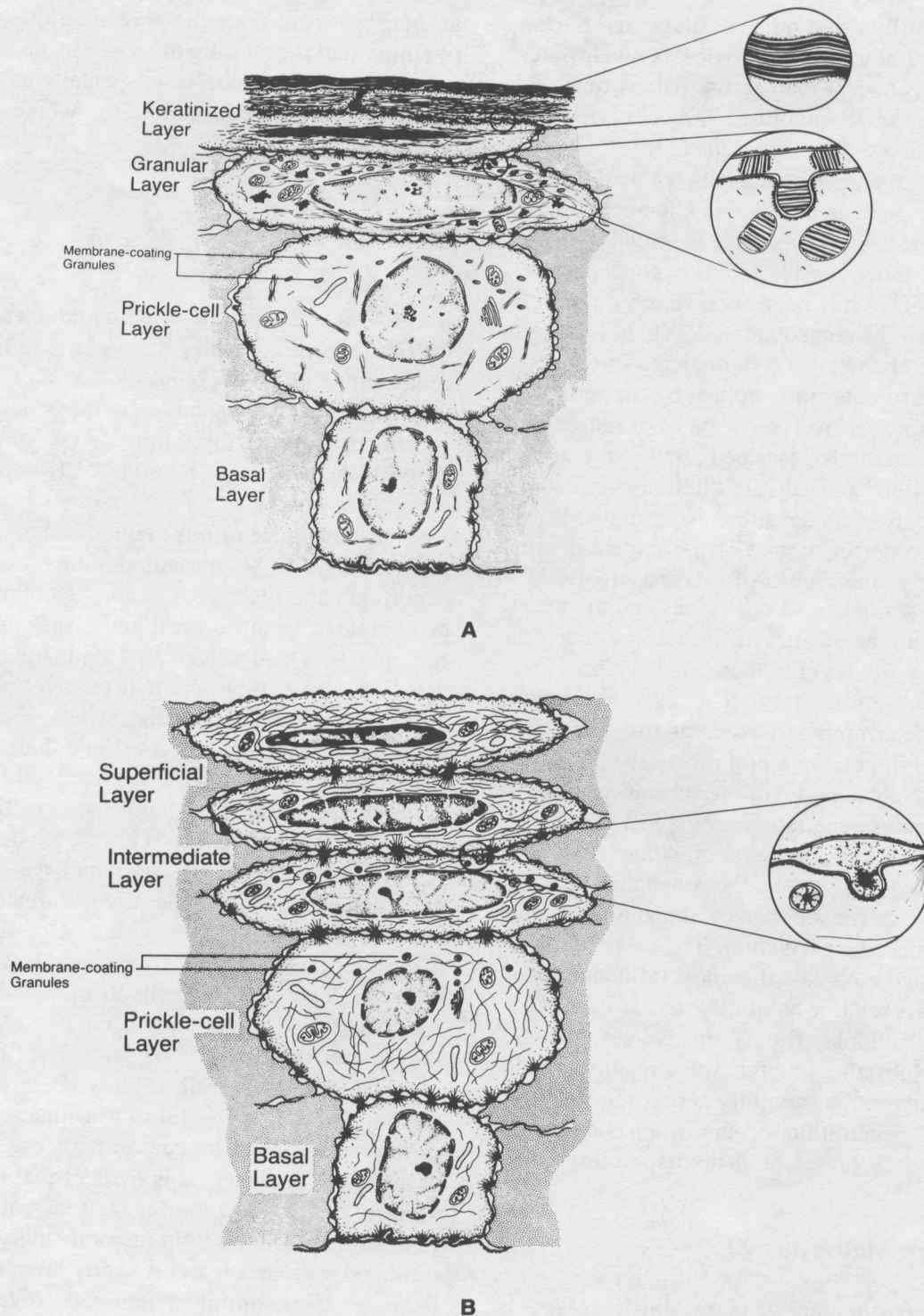


FIGURE 1. Diagram of events occurring during differentiation of a keratinized stratified squamous epithelium. The lamellae of the membrane-coating granules are discharged into the intercellular space at the junction of the granular and keratinized layers (lower inset). The stacks of lamellae rearrange to form extensive intercellular sheets (upper inset). Diagram of events occurring during differentiation of a nonkeratinized stratified squamous epithelium. The amorphous contents of the membrane-coating granules are extruded into the intercellular space in the upper third of the epithelium (inset).

face of carefully dried human palatal and buccal mucosa. He found that over twice as much water passed across buccal than across palatal mucosa, but only very small quantities of electrolytes (sodium and potassium) were detected.

Ritschel et al.²² have described a method that involves the placement of small chambers on the surface of the oral mucosa and are filled with the compound under study and the concentration monitored. This has been modified by Veillard et al.²³ so that the compound under study is passed through the chamber and the change in concentration used to determine uptake by the mucosa. These approaches overcome many of the objections to the methods described above, in that the region is defined and salivary dilution eliminated. However, extremely sensitive assay methods are necessary to detect uptake of a compound over the relatively small areas of mucosa involved.

Despite the practical difficulties and the many potential sources of error in measuring mucosal permeability *in vivo*, the methods have provided much useful information. The most important factor in determining the extent to which any substance will penetrate oral mucosa is the physical and chemical nature of the substance. In general, molecules penetrate more rapidly than ions, and small molecules more rapidly than large molecules. A factor governing the penetration of ionic compounds is the degree of ionization at any particular pH (the pK value). The property of an undissociated molecule that most influences penetration is its relative solubility, or partition coefficient, in nonpolar (lipid) and polar (aqueous) solvents. Substances that dissolve readily in both types of solvent pass rapidly across mucosa, but maximum penetration occurs when substances have a slightly preferential lipid solubility.^{24,25}

B. In Vitro Methods

The measurement of permeability *in vitro* is a standard approach in dermatology²⁶ and has been applied to oral mucosa.^{18,19,27-29} The usual procedure is to clamp an excised sheet of mucosa between two half-perfusion chambers (Figure 2). Each is filled with a physiological solution, such as phosphate-buffered saline, and the compound to be studied is added to one side, usually as a radioisotope. Samples of the solutions are taken

at intervals from both chambers and the amount of compound penetrating the tissue at steady state can then be calculated. This is usually expressed as a permeability constant (Kp), which can be calculated as follows:¹⁴

$$Kp = \frac{Q}{A \cdot t \cdot (C_o - C_i)}$$

where Q is the quantity of compound traversing the tissue in time t (min), C_o and C_i are the concentration 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 cm². The units of Kp are centimeters per minute.

The advantage of this method is that it allows permeability to be measured under controlled conditions and the Kp value that is obtained can be compared to those for other tissues and other compounds. The disadvantages are that tissue may deteriorate over time and that relatively large (7 mm²) specimens are required, which limit its application for human subjects. Nevertheless, there is evidence (see below) that permeability is not metabolically linked, so that tissue viability may not be critical, and methods have been developed to utilize tissue samples as small as 2 mm in diameter.³⁰ This makes the use of human biopsy specimens possible. In a series of comparisons using skin, Franz³¹ has shown good agreement between permeability constants obtained *in vitro* for a variety of compounds when compared with the *in vivo* values.

The method described above has been used to compare the permeability to tritiated water of different regions of human oral mucosa obtained at autopsy (Table 1). It is evident that skin provides a better water barrier than any of the oral regions, although, within the oral cavity, the keratinized regions are significantly less permeable than the nonkeratinized regions. Nevertheless, there are significant differences within these latter regions, the floor of the mouth and the lateral border of the tongue being more permeable than buccal mucosa.

Examination of the kinetics of penetration of a variety of compounds across oral mucosa under different conditions *in vitro* has led to the conclusion that the process is most likely to be one

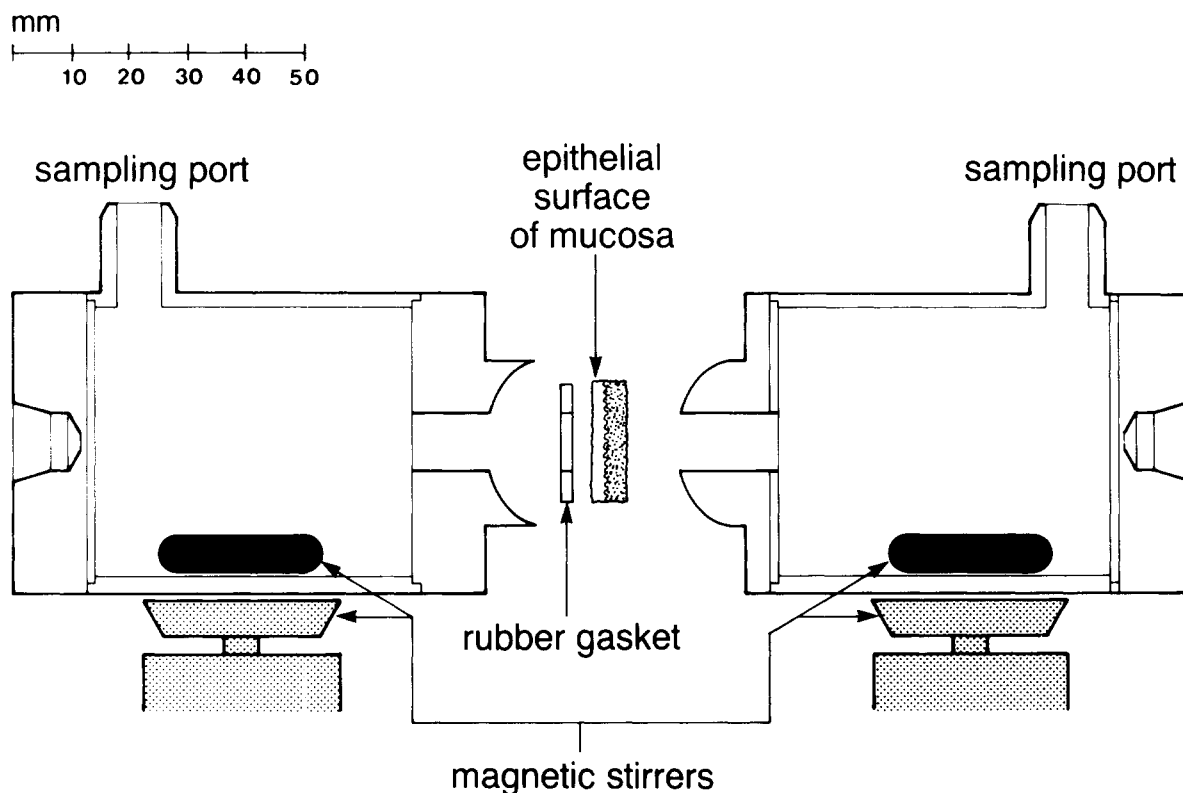


FIGURE 2. Diagram of perfusion chamber used to determine permeability constants for oral mucosa.

of simple diffusion. It has been shown that penetration occurs at the same rate from surface to connective tissue as in the opposite direction and that the rate is not affected by the presence of a metabolic inhibitor such as cyanide.²⁹ Thus, active transport is unlikely to be involved, although Siegel¹⁴ has pointed out that facilitated diffusion (carrier-mediated transport) cannot be totally ruled out for certain compounds. More recently, it has been shown that a potential difference can be detected across oral mucosa *in vitro*³³ and active transport of sodium has been demonstrated from the surface to the connective tissue in human and canine buccal mucosa.³⁴ However, given the relatively small amounts of electrolyte that penetrate oral mucosa,²¹ this may not be a quantitatively important process.

Studies such as those mentioned above have been important in defining the parameters that enable a compound to penetrate the oral lining as well as in explaining the process by which it may occur. This information is of particular value in formulating new therapeutic compounds for delivery across oral mucosa. However, a com-

plete understanding of oral mucosal permeability requires a detailed knowledge of the possible pathways of penetration and of the location and nature of any barriers within the tissue. Similarly, in considering the penetration of substances such as toxins or carcinogens that may be implicated in the development of disease, it is important to know whether a specific barrier exists in the tissue, and how it might vary in different oral regions, some of which are clearly more susceptible to disease than others. These questions will be considered in the next section.

IV. THE LOCATION AND NATURE OF THE PERMEABILITY BARRIER IN ORAL MUCOSA

A. Superficial Barriers

In 1969, Schreiner and Wolff³⁵ used the protein horseradish peroxidase (mol wt 40 kDa) as a tracer to demonstrate the location of a permeability barrier in human epidermis. This tracer

TABLE 1
Permeability of Human Skin and
Oral Mucosa to Water Kp values
($\times 10^{-7} \pm \text{SEM cm/min}$) (n = 58)

Region	Kp ^a
Skin	44 $\pm$ 4
Palate	470 $\pm$ 27
Buccal mucosa	579 $\pm$ 16
Lateral border of the tongue	772 $\pm$ 23
Floor of the mouth	973 $\pm$ 33

^a Regional values were significantly different ($p < 0.05$).

Modified from Lesch et al., *J. Dent. Res.*, 68, 1345, 1989.

can be visualized in the light and electron microscope by virtue of its enzymatic properties. After being injected intradermally, the peroxidase molecule was seen to have crossed the basal lamina and penetrated through the intercellular spaces of the epidermis as far as the boundary of the granular and cornified layers; no tracer was seen in the intercellular region of the stratum corneum. The level at which the penetration of peroxidase ceased corresponded with the site where small intracellular organelles, the membrane-coating granules, extrude their lamellate contents as stacks of membranous disks into the intercellular space (see Figure 1A). It was suggested that these lamellae occluded the intercellular pathways so as to constitute a barrier to the peroxidase. Subsequently, Elias and Friend³⁶ used freeze fracture preparations to show that the disks underwent rearrangement in order to form lamellate sheets that extended throughout the intercellular region of the stratum corneum.

In a series of studies, Squier and co-workers³⁷⁻⁴⁰ used horseradish peroxidase and lanthanum, an inherently electron dense element with a smaller particle size than peroxidase, to demonstrate the location of a permeability barrier in oral mucosa. In keratinized mucosa from several oral sites, the limit of penetration of the tracers was at the boundary of the granular and keratinized layers, as in epidermis.^{37,38} At this level in the tissue, the contents of the membrane-coating granules, which have a similar structure to those

of epidermis,⁴¹ are extruded. When the procedure was carried out using nonkeratinized oral mucosa, the tracers failed to penetrate the outer one third to one quarter of the epithelium.^{37,38} This coincides with the level at which small intracellular vesicles appear to fuse with the superficial cell membrane and extrude their contents into the intercellular space (see Figure 1B). These granules differ morphologically from membrane-coating granules of keratinized epithelium in lacking lamellate contents and instead having an amorphous core. However, in their location and behavior they appear to be homologous with the granules of keratinized epithelia.⁴² Granules with the same morphology have been observed in a variety of human nonkeratinized epithelia, including various regions of oral mucosa,⁴³⁻⁴⁵ uterine cervix,⁴⁶ and esophagus.⁸

If the presence of membrane-coating granules in a stratified squamous epithelium is a prerequisite for the formation of a permeability barrier, then tissues from which they are absent might be expected to lack such a barrier. There are observations from *in vitro* and *in vivo* systems to support this proposition. Epithelial cells from skin and keratinized oral mucosa maintained *in vitro* in a submerged culture show poor differentiation, and presence of membrane-coating granules has rarely been observed in ultrastructural studies.^{47,48} When such cultures are treated topically with horseradish peroxidase it readily penetrates between the cells of the superficial layer; this finding indicates that a permeability barrier is not present.⁴⁹ This is an important consideration if cultured tissue is to be used as a model system for permeability measurements. The use of raised (interface) cultures has been found to facilitate epithelial differentiation, including the development of membrane-coating granules in epidermis,^{50,51} and oral epithelium.⁵² The permeability of such a system is 2 to 10 times higher than that of skin *in vivo*.^{53,54} For oral mucosa, the only report of permeability measurements *in vitro* has been that of Tavakoli-Saberi and Andus,⁵⁵ who used keratinized hamster cheek pouch maintained in a submerged culture. They reported permeability values similar to those of nonkeratinized buccal epithelium of man and rabbit, suggesting that a normal keratinized barrier had not developed under these culture conditions.

The junctional epithelium is a normal component of the gingival attachment and links the oral sulcular epithelium to the tooth surface. The tissue shows little differentiation and does not possess membrane-coating granules.⁵⁶ When horseradish peroxidase is introduced into the gingival sulcus *in vivo*, it is able to penetrate the intercellular regions of the junctional epithelium and enter the underlying connective tissue. It does not penetrate the adjacent sulcular epithelium, where membrane-coating granules are present⁵⁷ (see Section V.A for further discussion). Thus, in two very different situations, the absence of membrane-coating granules is related to the lack of an intercellular permeability barrier in the superficial layers of the tissue.

It is evident that in a variety of stratified squamous epithelia there is an intercellular permeability barrier in the superficial layers of the tissue. This location has been confirmed by experiments that demonstrate an increase in permeability on stripping the surface layers of epidermis⁵⁸ or nonkeratinized oral mucosa (floor of the mouth⁵⁹), as well as by the morphological localization of tracers, as described above.

B. Other Permeability Barriers in Oral Mucosa

Although the superficial layers of the oral epithelium represent the site of the primary barrier to the entry of substances from the exterior, it is evident that the basement membrane also plays a role in limiting the passage of materials across the junction between epithelium and connective tissue.⁶⁰ For example, intravenously injected horseradish peroxidase can enter the intercellular spaces of the epidermis, but the passage of the larger protein, Thorotrast, is restricted.⁶¹ A similar mechanism appears to operate in the opposite direction. When labeled, albumin is applied to the surface of the oral mucosa of animals sensitized to this protein, immune complexes that are formed in the epithelium are trapped above the basement membrane, suggesting that immunoglobulins traverse this region, but larger molecules do not.⁶²

C. Pathways of Epithelial Penetration

Studies with microscopically visible tracers, such as those described above, would indicate that a major route across stratified epithelium for many compounds is via the intercellular space, and that there is a barrier to penetration as a result of modifications of the intercellular substance in the superficial layers. However, it is clear from measurements of permeability that this barrier is not absolute, even for a relatively large molecule such as horseradish peroxidase.²⁹ Almost all compounds can penetrate epithelium, but the rate at which they do so will depend on their size, chemical nature, and the type of tissue that is being traversed. This has led to the suggestion that substances with different chemical properties traverse the barrier region by different routes, some crossing the cell membrane and entering the cell (a transcellular or intracellular route), others passing between the cells via an intercellular route.⁶³⁻⁶⁵ Obviously, if the nature of the permeability barrier is to be identified, then it is necessary to determine the routes taken by compounds in traversing the epithelium. Elias et al.⁶⁶ have suggested that, for epidermis, the major factor regulating permeability is intercellular lipid, and that the intercellular route is the principal pathway for substances penetrating the stratum corneum. Direct evidence for this assertion has been provided by visualizing the pathway taken by butanol in penetrating human stratum corneum.⁶⁷ For oral mucosa, Squier and Lesch⁶⁸ have used light and electron microscopic autoradiography to examine the route taken by isotopically labeled compounds applied to the surface of different oral regions. Compounds with a range of water/lipid solubilities, including water and cholesterol, were applied to epidermis and keratinized and nonkeratinized oral epithelium. They were subsequently shown to be predominantly localized in the intercellular regions of the superficial layers of the tissues, suggesting that this compartment represents an important route across the barrier region of oral epithelium. The nature of the intercellular material will, therefore, be a major determinant of the permeability of oral epithelium.

D. Chemical Nature of the Permeability Barrier

A considerable amount of evidence indicates that the water permeability barrier in epidermis is of a lipid nature.⁶⁹⁻⁷² Recent studies have suggested that the major components are neutral lipids, consisting principally of ceramides and acylceramides and derived from the lamellae of membrane-coating granules.^{73,74} When these are extruded from the cell at the junction of the granular and keratinized layers, there is hydrolysis of glucose moieties from acylglycosylceramides permitting alignment of the ceramide to form sheets within the intercellular zone.⁷⁵ These provide a continuous lipid phase throughout the surface layer that will be impermeable to hydrophilic compounds.

In order to examine the chemical nature of the permeability barrier in oral mucosa, epithelium from different oral regions of pig was separated and the lipids extracted and identified.^{76,77} This involves analysis of the total epithelial lipids without regard to spatial relationships in the tissue. Several methods have been used to determine the morphological location of the different lipid classes; histochemistry was carried out on frozen and routinely processed histological sections so as to reveal the distribution of the major classes of lipid.^{73,77} It was also possible to extract lipids from consecutive, horizontal, frozen sections, representing the histological strata of the tissue.⁷⁸ This provides a quantitative profile of lipid classes in different epithelial layers.

When the data from lipid analysis are examined, it is found that keratinized oral regions, such as gingiva and palate, contain acylceramides and ceramides, which are believed to represent the major barrier components in epidermis (Table 2). The similarities in the structure and fate of the membrane-coating granules in keratinized oral epithelium and epidermis and the presence of the same neutral lipids suggest that the permeability barrier is formed in a similar way in both tissues. However, the total quantities of acylceramides and ceramides in keratinized oral epithelium are 25 to 50% less than in epidermis, which might explain the relatively greater permeability to water of keratinized oral epithelium (see Table 1 and References 77 and 78).

The epithelium from nonkeratinized oral regions (the floor of the mouth and buccal mucosa) contains no acylceramides or acylglycosylceramides and very small amounts of ceramide but relatively high quantities of glycosylceramide. However, this does not appear to be the same as the glycolipids isolated from keratinized regions, which are glucosylceramides, and the nature of the carbohydrate moiety is unknown. As ceramides are present only in minute amounts in nonkeratinized epithelia, it would appear that there is no mechanism for converting the glycosylceramide to ceramide, as occurs in keratinized epithelium, so that it persists unchanged in the epithelium. The amorphous contents of the membrane-coating granules of nonkeratinized epithelium may represent this glycosylceramide, which, on extrusion from the cell, forms an amorphous intercellular barrier material (see Figure 1B). Such glycolipids, although not forming an efficient water barrier, would limit the penetration of larger molecules, such as toxins and enzymes, across the epithelium. This would explain the relatively greater permeability of nonkeratinized epithelium to water as well as the inability of tracer proteins to significantly penetrate the tissue.

Apart from the presence of a barrier material between the cells of the superficial layer, the surface of the oral epithelium is normally bathed in saliva. The role of this fluid in diluting and removing surface materials has been claimed as the reason why topical application of a carcinogen is less successful in inducing cancer in oral mucosa than in skin.⁷⁹ However, saliva provides more than just a washing action and salivary mucin may contribute to the barrier layer of the oral mucosa. Anticholinergic drugs, which decrease salivary flow, increase permeability,¹⁴ and Adams⁸⁰ has reported a transitory decrease in water permeability when saliva is added to human oral mucosa *in vitro*. Recently, Levine, Tabak and co-workers^{81,82} have identified a high-molecular-weight mucin (MG1), which may bind covalently to the surface of the oral epithelium. This may be able to concentrate protective molecules, such as secretory immunoglobulins and lysozyme, as well as limit the attachment of microorganisms to the mucosal surface. It also maintains hydration and provides lubrication of the mucosal surface. Treatment of the mucosal

TABLE 2
Lipids of Epidermis and Oral Epithelia

Region	Epidermis	Gingiva	Palate	Floor of the mouth	Buccal mucosa
Phospholipids	24.1(3.9)	42.3(5.6)	39.1(2.9)	44.2(2.6)	38.2(3.7)
Cholesteryl sulfate	0.2(0.1)	2.0(0.3)	1.7(0.3)	3.2(0.2)	7.8(0.8)
Glycosylceramides	2.3(0.6)	2.1(0.7)	1.8(0.3)	5.8(2.2)	16.5(4.2)
Acylglycosylceramides	3.2(0.9)	2.1(0.7)	2.8(0.5)	0.0	0.0
Acylceramides	1.7(0.3)	0.4(0.1)	0.2(0.2)	0.0	0.0
Ceramides	12.2(2.7)	6.6(2.6)	3.3(1.2)	0.7(0.4)	0.9(0.4)
Cholesterol	15.4(6.0)	21.0(2.9)	33.6(3.1)	19.5(1.2)	13.6(2.2)
Fatty acids	13.6(2.4)	5.0(1.9)	1.3(0.4)	0.6(0.5)	1.6(0.5)
Triglycerides	24.7(3.5)	16.9(2.3)	15.9(1.9)	11.1(1.0)	15.7(1.9)
Cholesteryl esters	2.6(1.3)	1.1(2.2)	0.2(0.2)	15.0(1.5)	5.9(0.4)

Note: Values represent weight percent of total lipid ($\pm$ SD).

From Wertz et al., *Comp. Biochem. Physiol.*, 83B, 529, 1986. With permission.

surface with 0.1% sodium dodecylsulfate, which might be expected to distort or remove the mucin layer, increases the water permeability of the tissue,⁸³ providing further evidence for a barrier function of the salivary coating on oral mucosa.

V. IMPLICATIONS OF PERMEABILITY FOR ORAL MUCOSAL DISEASE AND THERAPY

This section intends to explore some of the ways in which impairment, or absence, of a permeability barrier may be associated with oral disease. Much of the discussion is speculative, because the etiology of many of the conditions to be described is unclear. Nevertheless, this is an area where new research approaches are needed and where mechanistic studies must replace descriptive accounts.

A. Periodontal Disease

The relationship between the differentiation of a stratified squamous epithelium, the presence of membrane coating granules, and the formation of a superficial permeability barrier has already been discussed (Section IV.A). The junctional epithelium of the tooth represents the unusual example of an undifferentiated tissue persisting

into maturity and even old age.⁸⁴ Lack of differentiation may be a biologic mechanism by which epithelial attachment to the enamel surface is possible⁸⁵ or it may serve to limit apical migration of the adjacent oral epithelium.⁸⁶ Regardless of the reason, the absence of differentiation results in a tissue that lacks a superficial permeability barrier and that has been shown to be permeable to a variety of materials ranging from carbon particles⁸⁷ to protein.⁸⁸ Attempts to keratinize the adjacent oral sulcular epithelium,^{89,90} which is nonkeratinized or parakeratinized in most primates, does not alter the status of the junctional epithelium. Indeed, in situations where the oral sulcular epithelium is orthokeratinized, as in the rodent, exogenous material placed in the sulcus can still enter the underlying tissues.⁵⁷ The junctional epithelium is therefore a route by which plaque-derived toxins can enter the subepithelial connective tissue and set up a cycle of inflammation and tissue destruction that will facilitate the entry of further material from the sulcus and so exacerbate the damage. From the point of view of periodontal therapy, procedures that remove plaque and bacteria by mechanical and chemical means will reduce the amount of material able to enter the gingival tissues and so tend to promote clinical health. Nevertheless, the intrinsic permeability of the junctional epithelium will always provide

access to some exogenous materials, which probably account for the persistent subclinical inflammation observed in this region.⁹¹

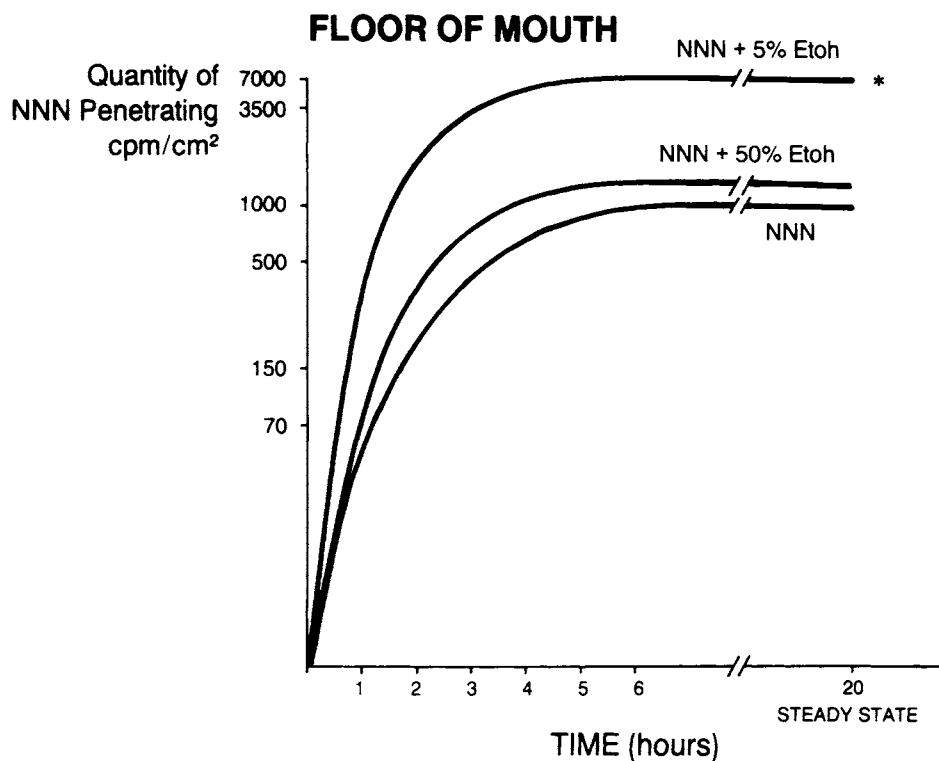
B. Oral Mucosal Diseases

It is clear from measurements of the permeability of oral mucosa already presented that there are significant differences between the various oral regions. As might be expected, a keratinized masticatory region such as palate has a significantly lower permeability than any of the lining regions. Nevertheless, there are also significant differences between lining regions, buccal mucosa being the least permeable and the floor of the mouth the most permeable. This raises the question as to whether the etiology of mucosal disease may be related to permeability, particularly when that etiology is associated with habits such as the oral use of tobacco and alcohol, as is the case for oral cancer. Cawson⁹² has pointed out that, in pipe smokers, a hyperkeratotic lesion can often be found on the hard palate, but that carcinoma tends to develop in the floor of the mouth, where saliva and tobacco carcinogens may pool. Lining regions such as the floor of the mouth and the lateral border of the tongue have been designated as "high-risk" areas for squamous cell carcinoma,⁹²⁻⁹⁴ and also turn out to be regions of high permeability³² (Table 1). Although the regional permeability values given are for water, it is likely that some important oral carcinogens are water soluble. For example, the only known organic carcinogens in processed tobacco are the tobacco-specific nitrosamines,⁹⁵ and at least one of these, nitroso-nornicotine (NNN), is present in smokeless tobacco. When smokeless tobacco is extracted with saliva, higher levels of NNN are obtained than have been reported for any other environmental nitrosamine.⁹⁶

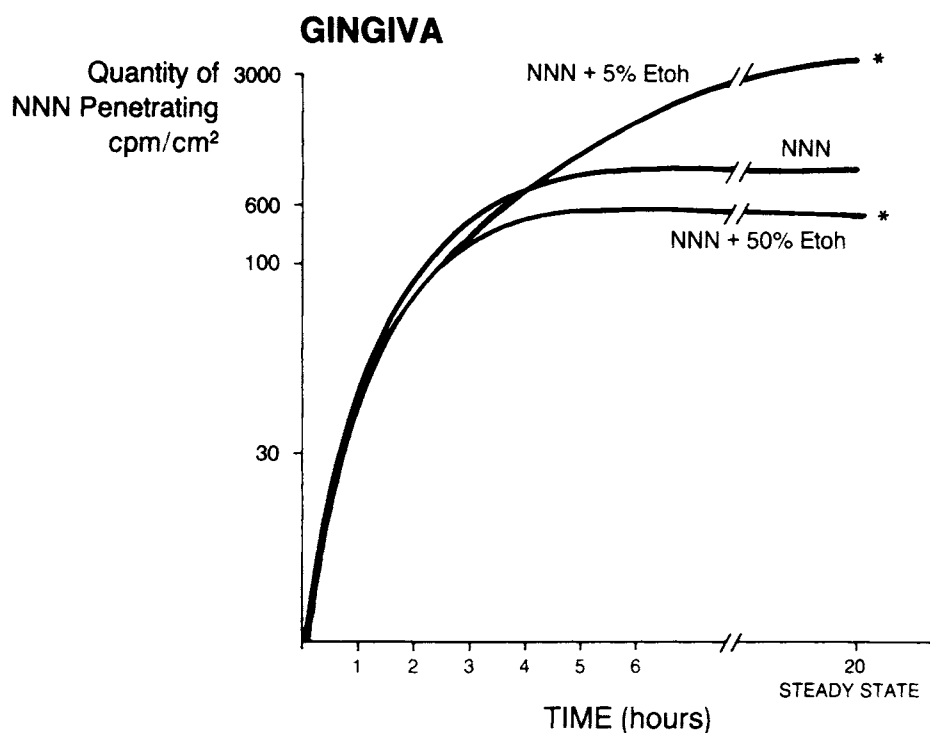
There is considerable evidence for a synergistic or multiplicative effect between tobacco and alcohol in the etiology and pathogenesis of oral cancer. The relative risk for oral and pharyngeal cancer increases from 2.5 in nonsmokers who consume 7 oz or more of alcohol per day to between 24 and 35 if they also smoke two or more packs of cigarettes.^{97,98} The mechanism by which alcohol and tobacco interact to increase

the risk of oral cancer is unclear; ethanol may inhibit first-pass hepatic clearance of carcinogens⁹⁹ that are subsequently activated by ethanol-induced enzymes.¹⁰⁰ Alternatively, the presence of ethanol may enhance the penetration of tobacco carcinogens across the oral mucosa.^{101,102} Such a local effect is suggested by the increased relative risk of oral cancer among individuals using mouthrinse solutions containing alcohol.^{103,104} Unlike alcoholic beverages, mouthwash is rarely swallowed and its action would be primarily local. There is some experimental evidence to support this concept.¹⁰⁵ Regions of pig oral mucosa were exposed to labeled NNN in the presence of 5 or 50% ethanol, and the penetration of NNN measured *in vitro*. A significant increase in penetration of NNN occurred in gingiva and the floor of the mouth mucosa in the presence of 5% ethanol (Figure 3). However, this increase was achieved far more rapidly for floor of the mouth mucosa (1 h or less) than for gingiva (more than 8 h). Such a result is in accord with data from Mashberg and Meyers,¹⁰⁶ which indicate that in a population of heavy drinkers and smokers squamous cell carcinoma is more prevalent in the floor of the mouth and contiguous regions than in the buccal mucosa. The greater effect of 5 over 50% ethanol is also consistent with etiological data that indicate a higher relative risk of oral cancer among beer or wine drinkers (relative risk: 20.4) than among whiskey drinkers (relative risk: 7.3¹⁰⁶). This may indicate the effectiveness of dilute alcohol as a vehicle for carcinogens. Higher concentrations of alcohol might act as a chemical fixative and reduce permeability of some oral tissues.

Superficial, asymptomatic candidal infections of the oral mucosa offer an interesting example of the role of the barrier function in normal oral mucosa. Histological sections will often reveal hyphae and spores in the superficial layers of keratinized or nonkeratinized epithelium accompanied by a slight, acute, subepithelial inflammatory response.¹⁰⁷ It is likely that in these circumstances *Candida* can secrete soluble toxins capable of penetrating the epithelial barrier and eliciting a mild inflammatory response without threatening the integrity of the tissue, as demonstrated for skin.^{108,109} However, the location of the organism in the cells above the permeability



A



B

FIGURE 3. Effect of alcohol on penetration of labeled nitrosonornicotine (NNN) across oral mucosa. In the presence of 5% ethanol (asterisk), there is significantly ($p < 0.05$) higher permeability to NNN than to NNN alone or with 50% ethanol. This effect is evident after 1 h for the floor of the mouth (3a), where the value of the permeability constant is also twice that for gingiva (3b). (Modified from Squier et al.¹⁰⁵)

barrier will isolate it from larger molecules such as anticandidal antibodies or complement that are confined to the epithelial regions beneath the barrier. There is thus an equilibrium so long as the organism does not acquire greater virulence or the host defenses are not compromised.

There are several oral mucosal diseases of uncertain etiology, such as recurrent aphthous stomatitis, lichen planus, and erythema multiforme, where antigens derived from the local oral flora or from food have been implicated as possible causes of immunologically mediated mucosal damage.^{110,111} Similarly, the concept of a common mucosal immune system in which antigens that penetrate the surface can be transported to gut mucosa-associated lymphoid tissue for processing and presentation to antibody-secreting cells (reviewed by Michalek and Childers¹¹²) raises the question of the importance of oral mucosal permeability to proteins. Tolo⁶² has shown that albumin can penetrate the oral mucosa of guinea pigs. In animals immunized with albumin it was found that there was a decreased permeability to this protein but increased penetration of an unrelated macromolecule, transferrin was increased.¹¹³ This was attributed to the binding of albumin by intraepithelial immune complexes that damaged the integrity of the epithelial barrier so as to permit increased penetration of the bystander protein, transferrin. It is possible that the mechanism of mucosal damage in patients with conditions such as those mentioned above involves complexing of food or bacterial-derived antigens by antibodies that would lead to activation of complement. This causes chemotaxis of polymorphonuclear leukocytes and release of activated lysosomal enzymes that bring about tissue damage. The critical event that initiates this sequence is the penetration of the epithelial barrier by an antigen. However, apart from the studies mentioned, there has been little work on mucosal penetration by macromolecules. Despite the demonstration of a permeability barrier in oral mucosa to horseradish peroxidase in histological preparations, small amounts of this protein can cross the tissue,²⁹ and Tolo and Jonsen¹¹⁴ have demonstrated the penetration of dextrans with a molecular weight as large as 70 kDa. In a recent series of studies examining the permeability of human skin and

oral mucosa to ovalbumin (mol wt 34 kDa), similar relative differences in permeability between skin and oral regions were evident for this molecule as for water, although the absolute rate of penetration (Kp) was considerably less (Table 3). Clearly, sufficient amounts of a potential antigen would be able to pass the mucosal barrier in order to elicit an immune response; such a mechanism could offer an explanation for the etiology of several mucosal diseases.

TABLE 3
Permeability of Human Skin and Oral Mucosa to Ovalbumin

Region	Kp
Skin	25.5 ± 3.2
Palate	186.3 ± 27.4
Buccal mucosa	177.9 ± 8.7
Lateral border of the tongue	301.4 ± 33.1
Floor of the mouth	426.2 ± 53.3

Note: Kp values ($\times 10^{-7} \pm$ SEM cm/min) (n = 58).

From Lesch, Squier, and Williams, unpublished data.

C. Permeability of Altered Oral Epithelium

1. Hyperplasia and Hyperkeratosis

One of the reactions of skin and oral mucosa to mild irritation is a hyperplastic change characterized by acanthosis and hyperkeratosis. This can be induced in oral mucosa by chemical irritants such as tobacco and tobacco smoke¹¹⁵ or by mechanical irritation from cheek biting and dental restorations or tooth brushing.^{116,117} Such changes in response to irritation are clearly protective and it is often assumed that a thicker, hyperkeratotic epithelium will offer an improved barrier function. However, studies of the permeability of hamster cheek pouch in which hyperplasia was induced by mechanical or chemical means showed that such epithelium was either no different or significantly more permeable than untreated controls.¹¹⁸ Such results may seem sur-

prising but are consistent with data from skin that show that the thicker palmar and plantar regions are more permeable than thin skin.¹¹⁹ Similarly, a variety of hyperplastic epidermal lesions such as psoriasis, epidermolytic hyperkeratosis, and ichthyosis show a greater water permeability than normal skin does.¹²⁰ The increased permeability of a hyperplastic epithelium probably reflects the increased cell division and transit time of such tissue.¹²¹ Under these conditions, membrane-coating granules are often retained in the keratinized layer^{122,123} so that the intercellular barrier would be deficient in the neutral lipids derived from these organelles (see Section IV).

A hyperplastic epithelium is a component of many lesions occurring in the oral mucosa and particularly of those characterized clinically as "white lesions" or leukoplakia. One of the concerns about the presence of leukoplakia is that approximately 4% of these lesions undergo malignant transformation.¹²⁴ As these lesions are frequently seen in tobacco users, there is a likelihood that such an area of increased permeability would permit greater access by tobacco carcinogens (such as nitrosamines) and thus facilitate malignant transformation of the tissue.

2. Inflammation and Atrophy

Inflammation represents a common pathologic condition affecting the oral mucosa. Surprisingly, there have been few studies of the effect of inflammation on epithelial permeability. Riber and Kaaber¹²⁵ found that inflamed palatal mucosa under dentures was nonkeratinized and had a permeability almost three times that of normal palatal tissue and twice that of normal, nonkeratinized, buccal mucosa. In a group of 18 denture wearers followed over 12 months, one third showed a reduction in barrier function; this was always associated with inflammatory changes of the mucosa.¹²⁶ Epithelium shows a bimodal response to inflammation; a mild degree of inflammation stimulates proliferation, severe inflammation depresses it.¹²⁷ Inflammation tends to reduce keratinization, as observed by Riber and Kaaber,¹²⁵ and this change in differentiation is presumably accompanied with an alteration in the permeability barrier toward the type found in

nonkeratinized tissue. In normal oral mucosa, nonkeratinized epithelium may be more than twice as permeable as keratinized regions (see Table 1), which is the same order of difference found by Riber and Kaaber.¹²⁵

One of the changes often described clinically in the oral mucosa of the elderly is atrophy. However, although a thinner epidermis has been reported in the skin of older individuals,¹²⁸ there is no clear evidence of this association in human oral epithelium.¹²⁹ There is also confusion as to whether rates of proliferation alter with age, although some agreement that the rate of epithelial replacement decreases.¹²⁹ If hyperproliferation leads to increased permeability, as discussed above, then the changes associated with aging might be expected to lead to a decrease in permeability. For skin, this tends to be supported by the few published studies, although much of the data are ambiguous.^{130,131} There are no data for alterations in oral mucosal permeability with age; given the tendency for mucosa to show even fewer age-related changes than skin¹²⁹ such differences might be expected to be slight.

Atrophy and necrosis of oral epithelium may occur in some forms of lichen planus, pemphigus, viral infections, and allergic reactions so that upper cell layers are damaged or lost. In such situations, the major permeability barrier between the superficial cells is destroyed and the tissue will be more permeable. Overtly ulcerated tissue would be freely permeable to exogenous substances, but the fibrin clot forming on the surface would provide a partial barrier.

Changes in the mucosal barrier after anticancer therapy are of increasing concern to those involved with patient management. The chemotherapeutic agents and radiation used in such treatments not only limit the proliferative capacity of the epithelium so that it becomes thinner or ulcerated but may reduce the production of salivary mucins so that the barrier function is seriously compromised.¹³² Once the integrity of the epithelial barrier is compromised, the underlying tissues are exposed to the risk of infection from organisms in the oral cavity. This not only exacerbates the local mucosal lesion but predisposes the individual to systemic infections, because of indirect effects, such as leukopenia and neutropenia, following the administration of

chemotherapeutic agents and myeloablative radiation.¹³³ Therapeutic solutions might include the development of artificial saliva with synthetic mucins that can restore barrier function.

3. Effect of Surfactants

Toothpastes and mouthwashes frequently contain surface-active agents (detergents) that improve the solubility of their constituents and increase foaming. Siegel has examined the effect of such agents on permeability using animal tissue.^{14,134,135} Ionic surfactants, such as sodium lauryl sulfate, caused a greater increase in permeability than nonionic agents like Tween-80. This difference seemed to be related to the greater tissue damage caused by the former group, ranging from damage to the surface layers to loss of the whole epithelium. Such results are in accord with clinical studies that indicate that these compounds may affect the mucosal surface sufficiently to cause sloughing.¹³⁶

VI. DRUG DELIVERY ACROSS ORAL MUCOSA

A. Local Delivery

Although the oral mucosa has been used for the systemic delivery of drugs for over a century, there is little information about mechanisms by which therapeutic compounds can be made available for treatment of local oral infections and inflammatory conditions. This is unfortunate, because topical therapy provides a unique opportunity to deliver drugs directly to a disease site at optimum concentrations and with minimal risk of systemic side effects. Since their discovery, synthetic corticosteroids such as hydrocortisone and triamcinolone acetonide have been used for the management of acute and chronic inflammatory mucosal disease because of their antiinflammatory and immunosuppressive effects. For dermatological use, corticosteroids are made up in an ointment base that provides ready adherence to the skin surface and facilitates penetration of the epidermal barrier. Ointment does not readily adhere to the moist oral mucosa, and in 1959 Orabase was developed as an adhesive that could

retain topically applied drugs at the surface of mucous membrane for 2 h or longer.¹³⁷ Orabase consists of a lipid soluble component (polyethylene in mineral oil) and hydrophobic emulsifying agents (carboxymethylcellulose, pectin, and gelatin) that swell on contact with water. It provides more efficient release of incorporated drug at an oil-water interface than from a lipid vehicle alone. Addy¹³⁸ has shown that isotopically labeled triamcinolone acetonide in Orabase can penetrate into the connective tissue of buccal mucosa, but gave no information on the kinetics of the process. If the penetration of triamcinolone in Orabase across mucosa is measured *in vitro*, it is found that the rate reaches a maximum value 2 h after application and then steadily declines to a lower value. However, if the material on the mucosal surface is then stirred, there is again an increase in the rate of penetration followed by another decline (Figure 4). These results suggest that the rate-limiting step in the system may be the diffusion of drug through the vehicle to the mucosa surface. Once the drug at the interface had been exhausted the rate of mucosal penetration decreased, stirring the mixture brought in new drug and the rate of penetration increased until this was in turn depleted. From a practical point of view, this suggests that the best way of ensuring drug deliver *in vivo* is to apply thin layers of the vehicle frequently to the surface of the mucosa.

B. Systemic Delivery

The delivery of compounds such as small peptides and proteins for systemic therapy demands routes other than the gastrointestinal tract, where such compounds will be hydrolyzed by gut enzymes or by first-pass hepatic metabolism. Oral mucosa offers excellent accessibility and is more acceptable to the patient than the rectal or vaginal mucosal routes for systemic drug delivery. While not as effective as the nasal mucosa, the oral mucosa is less sensitive and shows fewer side effects.¹³⁹ The development of mucosal adhesives that can provide controlled release of compounds across the oral lining has become an area of particular interest to the pharmaceutical industry interested in delivering compounds across the oral mucosa for systemic therapeutic pur-

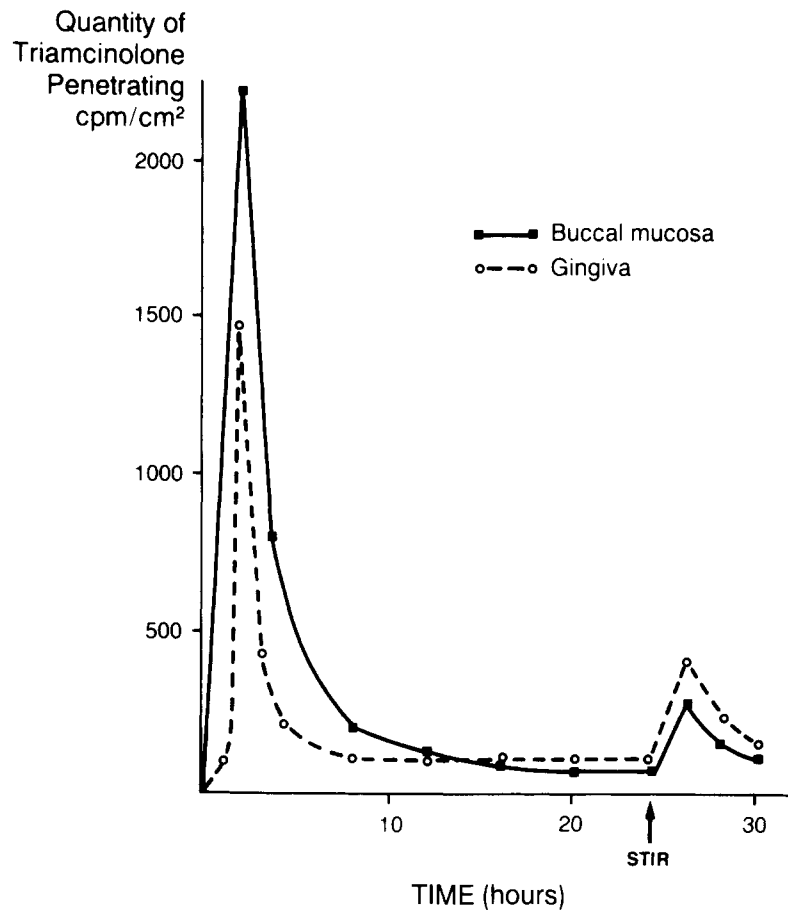


FIGURE 4. Time course for penetration of tritiated triamcinolone acetonide across pig buccal mucosa (solid line) and gingiva (dotted line) after topical application in Orabase. Penetration reaches a maximum at about 2 h after which there is a continuous decline that can be reversed by stirring (arrow). (Modified from Reid et al.¹⁴³)

poses. Hydrogels, consisting of acrylic acid and butyl acrylate, have high flexibility and biocompatibility and are sufficiently adhesive to attach to the mucosal surface.¹⁴⁰ Ritschel et al.¹⁴¹ have recently described the use of a gel based on gelatine, Carbopol P934 (a carboxy-vinyl polymer manufactured by Goodrich), glycerol and triethanolamine to deliver insulin buccally in order to achieve a systemic effect equivalent to 11 to 15% of that achieved by intravenous injection. Different designs of adhesive patches have also been described.¹⁴¹ These can provide bidirectional release, so that a drug diffuses both into the mucosal surface and into saliva, where it is available for absorption over the total mucosal surface. Alternatively, an impermeable backing can be incorporated into the adhesive patch so that ab-

sorption is restricted to the mucosal area covered by the device. These methods should also lend themselves to local treatment of periodontal disease and mucosal lesions, including candidiasis, by the drug delivery of chemotherapeutic and antimicrobial agents. Such possibilities make mucosal delivery a subject of considerable importance for the future in medicine and dentistry.

VII. CONCLUSIONS

In a review of oral mucosal permeability published almost 20 years ago, Siegel and co-workers¹³ pointed out that it was almost impossible to calculate a permeability constant for any one compound across any particular area of oral

mucosa. Not only do we now have data for a variety of compounds and several areas of mucosa, but there is a far better understanding of the mechanisms of penetration and of the nature of permeability barriers. This knowledge can facilitate both local and systemic drug therapy as well as clarify the etiology of a number of mucosal diseases that are, as yet, poorly understood. On the one hand, it may be possible to develop delivery systems that will maintain therapeutic compounds in contact with, and at the same time permeabilize, regions of mucosa in order to improve drug delivery. On the other hand, if susceptibility to certain mucosal conditions is a reflection of increased permeability, or where habits such as alcohol and tobacco use may compromise barrier function, it may be possible to augment the epithelial barrier, as has been accomplished in the epidermis.¹⁴²

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