Biology of Oral Mucosa and Esophagus

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The mucosal lining of the oral cavity and esophagus functions to protect the underlying tissue from mechanical damage and from the entry of microorganisms and toxic materials that may be present in the oropharynx. In different regions, the mucosa shows adaptation to differing mechanical demands: Masticatory mucosa consists of a stratified squamous keratinized epithelium tightly attached to the underlying tissues by a collagenous connective tissue, whereas lining mucosa comprises a nonkeratinized epithelium supported by a more elastic and flexible connective tissue. The epithelium is constantly replaced by cell division in the deeper layers, and turnover is faster in the lining than in the masticatory regions. Chemotherapeutic agents and radiation limit proliferation of the epithelium so that it becomes thin or ulcerated; this will first occur in the lining regions. The principal patterns of epithelial differentiation are represented by keratinization and nonkeratinization. As keratinocytes enter into differentiation, they become larger and begin to flatten and to accumulate cytokeratin filaments. In addition to the keratins, the differentiating keratinocytes synthesize and retain a number of specific proteins, including profilaggrin, involucrin, and other precursors of the thickening of the cell envelope in the most superficial layers. The concept of epithelial homeostasis implies that cell production in the deeper layers will be balanced by loss of cells from the surface. There is a rapid clearance of surface cells, which acts as a protective mechanism by limiting colonization and invasion of microorganisms adherent to the mucosal surface. [J Natl Cancer Inst Monogr 2001;29:7–15]

Introduction

The oral cavity has sometimes been described as a mirror that reflects the health of the individual. Changes indicative of disease are seen as alterations in the oral mucosa lining the mouth, which can reveal systemic conditions, such as diabetes or vitamin deficiency, or the local effects of chronic tobacco or alcohol use. Modern anticancer therapy represents a significant challenge to the integrity of the oral mucosa. Chemotherapeutic agents and radiation therapy limit the proliferative ability of the epithelium so that it becomes thin or ulcerated. This is manifest first in the more rapidly proliferating tissues, such as gastrointestinal and oral lining mucosae. There may also be indirect effects, such as damage to the salivary glands, that will reduce salivary production and impair barrier efficiency and a reduction in immunocompetence as a result of myeloablative therapy. This will increase the risk of local infection from oral organisms.

This article will first describe the organization of the oral mucosa and esophagus, then examine important functional aspects of the covering epithelium, including epithelial proliferation, differentiation, turnover, and barrier function, all of which have important implications for the maintenance of the integrity of this tissue in the face of anticancer therapy. Finally, since most cancer is a disease of the elderly, there will be a brief consideration of changes caused by the aging of the tissue.

Organization and Function of the Oral and Esophageal Mucosa

The mucosa of the mouth and esophagus may appear to differ little from the rest of the moist lining of the gastrointestinal tract, with which it is continuous. In fact, with the notable exception of the uterine cervix, this tissue is remarkably different from other mucosae of the body and has more in common with skin, with which it forms a junction at the lips, than with the intestinal mucosa.

The soft tissues of the human oral cavity and esophagus are covered everywhere by a stratifying squamous epithelium *(1).* In regions subject to mechanical forces associated with mastication (i.e., the gingiva and hard palate) there is a keratinizing epithelium resembling that of the epidermis covering the skin. In these masticatory mucosae, the keratinized epithelium is tightly attached to the underlying tissues by a collagenous connective tissue, or lamina propria. The floor of the mouth, buccal regions, and esophagus, which require flexibility to accommodate chewing, speech, or swallowing of a bolus, are covered with a nonkeratinizing epithelium. The connective tissue of lining mucosae is more elastic and flexible than the connective tissue in the masticatory mucosa. The dorsum of the tongue is covered by a specialized epithelium, which can be represented as a mosaic of keratinized and nonkeratinized epithelium. This epithelium is attached tightly to the muscle of the tongue.

Fig. 1 illustrates diagrammatically the distribution of the different types of mucosa within the oral cavity *(2).* From measurements made by Collins and Dawes *(3),* 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.

The esophagus extends from the upper esophageal sphincter, which delineates it from the oropharynx, to the lower esophageal sphincter, representing the junction with the gastric mucosa *(4).* The organization of the tissues reflects their function—that of transporting ingested food from the oral cavity to the stomach. The process of peristalsis, which is initiated by swallowing and involves rhythmic contractions of the muscular walls, accomplishes this transportation. The extensibility and motility of the mucosal lining are reflected in the presence of a nonkeratinized mucosal surface resembling that of the oral lining mucosa (Fig.

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See "Note" following "References."

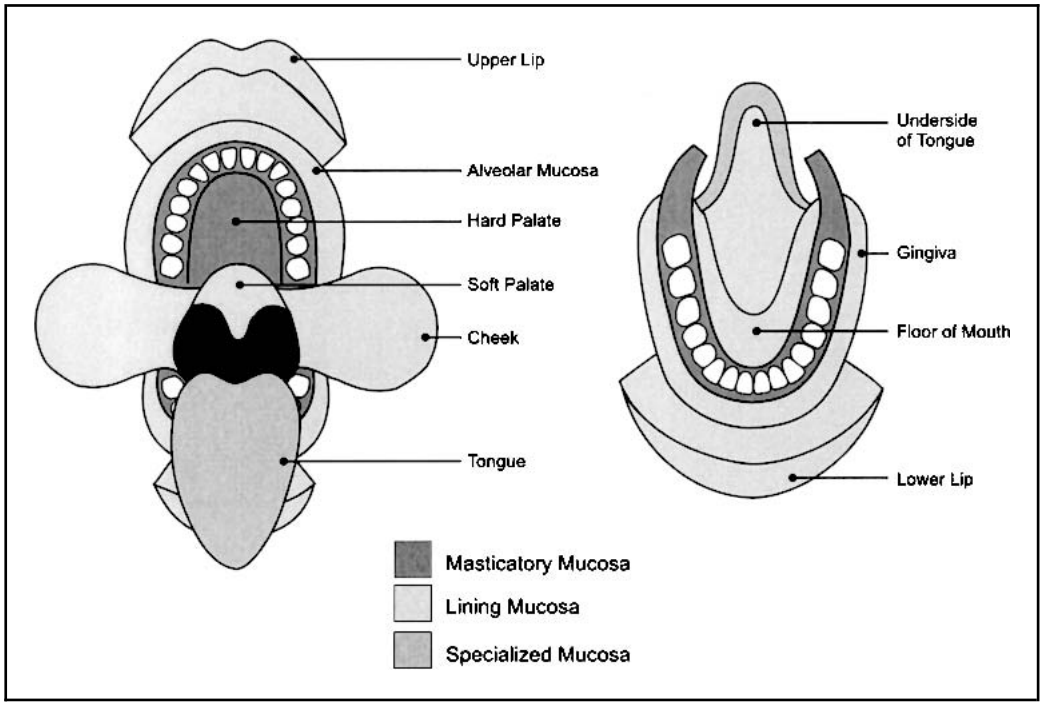

Fig. 1. Diagram to show the anatomic location and extent of masticatory, lining, and specialized mucosa in the oral cavity. [Modified from reference *(2).*]

2). This surface is separated from the submucosa by a muscularis mucosa, consisting of a smooth muscle and elastic fiber layer, which may serve to reduce the excursion of the luminal lining mucosa as a result of the contractions of the external esophageal muscle, consisting of circular and transverse layers of striated or smooth muscle.

The primary function of oral and esophageal epithelium is the protection of the underlying tissue *(1).* In the masticatory regions, the mechanically tough stratum corneum serves to dissipate shearing forces, and in the lining areas, including the esophagus, there is a distensible and flexible surface layer. In both regions, lipid-based permeability barriers in the outer epithelial layers protect the underlying tissues against fluid loss and against the ingress of a range of potentially harmful environmental agents. These include microbial toxins and enzymes and antigens and carcinogens from foods and beverages.

Structure of the Oral and Esophageal Mucosa

All covering and lining tissues of the body consist of a surface epithelium supported by a fibrous connective tissue. Epithelium, by virtue of the close packing and constant turnover of cells, is well adapted to protect underlying tissues and organs against mechanical and chemical insult, whereas the connective tissue, consisting of relatively few cells in an extensive matrix, provides mechanical support and nutrients for the epithelium. In comparing the structure of skin and oral mucosa to the gastrointestinal tract, a major difference emerges in the organization of the epithelium, which reflects the different functions of these regions. The lining of the stomach and small and large intestine consists of a simple epithelium composed of only a single layer of cells, which facilitates absorption across the tissue. Skin, oral mucosa, and esophagus are covered by a stratified epithelium (Fig. 3) composed of multiple layers of cells that show various patterns of differentiation (or maturation) between the deepest cell layer and the surface.

Features that distinguish the oral and esophageal mucosa from skin are its moist surface and the absence of appendages. The skin contains numerous hair follicles, sebaceous glands, and sweat glands, whereas the glandular component of oral and esophageal mucosa is represented primarily by the minor salivary glands. These glands are concentrated in the submucosa, and the secretions reach the mucosal surface via small ducts. The salivary glands have an important role in maintaining a moist surface containing mucins and a variety of antimicrobial substances as well as epidermal growth factor (EGF). In the esophagus, the minor salivary glands can produce a secretion with high bicarbonate concentration to neutralize refluxing stomach acid *(5).* Sebaceous glands are present in the upper lip and buccal mucosa in about three quarters of adults. Unlike the esophagus,

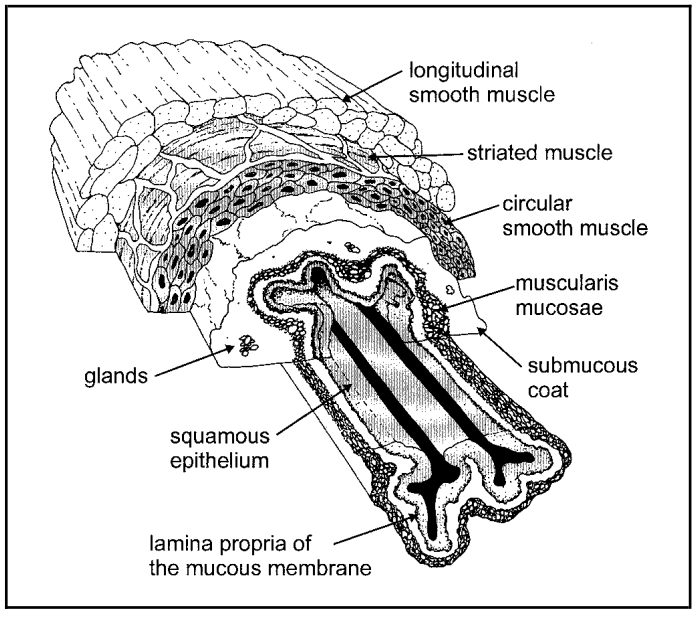

Fig. 2. The organization of the tissues of the human esophageal lining. [Modified from reference *(4).*]

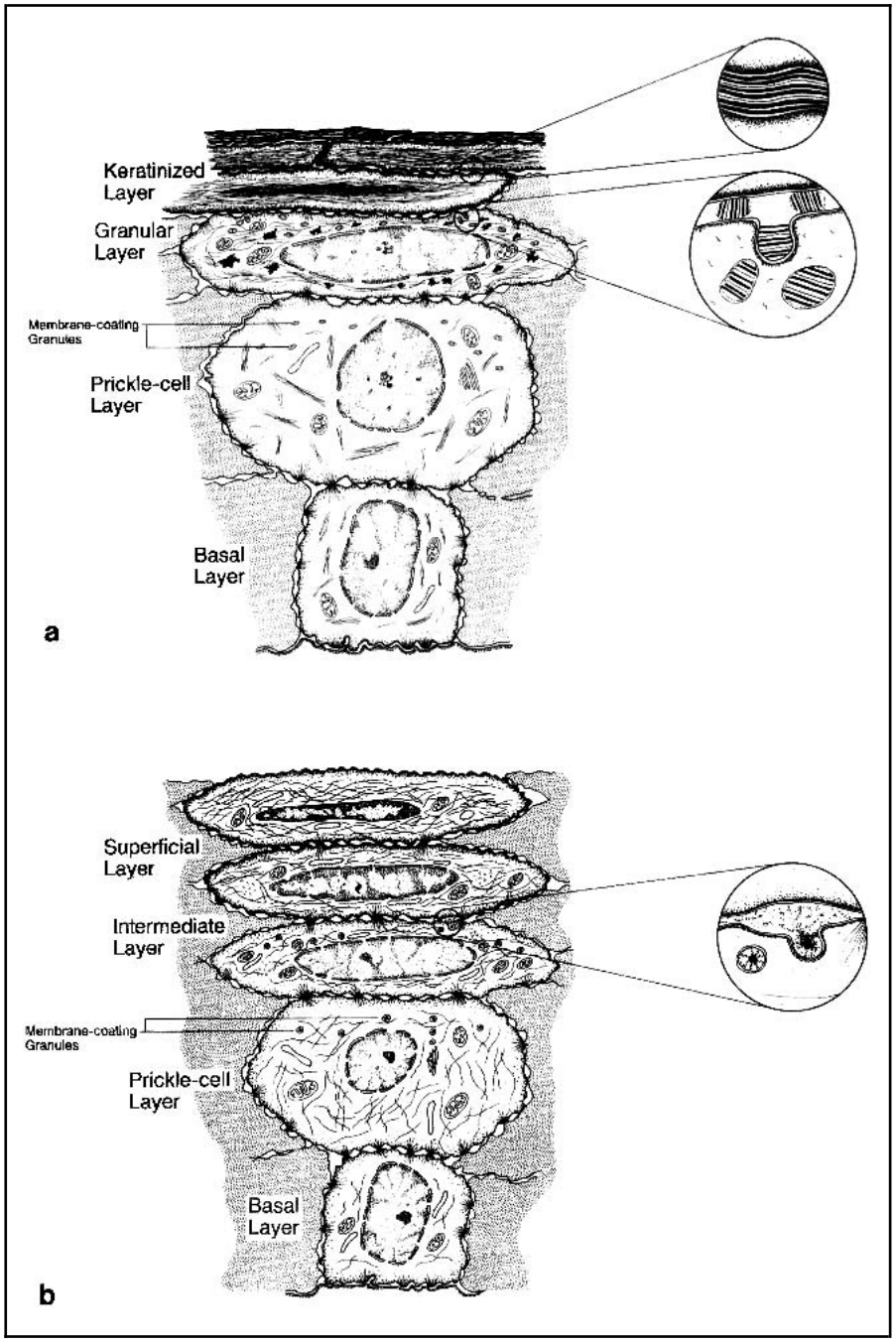

Fig. 3. Principal ultrastructural features of differentiation in (**a**) keratinized oral epithelium and (**b**) nonkeratinized oral and esophageal epithelium. [Modified from reference *(48).*]

the oral mucosa has no muscularis mucosae, and, consequently, it is difficult to identify clearly the boundary between it and the underlying tissues. In many regions, such as the cheeks, the lips, and parts of the hard palate, a layer of loose fatty or glandular connective tissue containing the major blood vessels and nerves supplying the mucosa separates the oral mucosa from underlying bone or muscle. This represents the submucosa in the oral cavity, and its composition determines the flexibility of the attachment of the oral mucosa to underlying structures. A similar organization is seen in the esophagus. In regions of the oral mucosa, such as the gingiva and parts of the hard palate, the oral mucosa is attached directly to the periosteum of underlying bone with no intervening submucosa. This arrangement is called a mucoperiosteum and provides a firm, inelastic attachment. In several regions of the oral cavity, there are nodules of lymphoid tissue consisting of crypts formed by invagination of the epithelium into the lamina propria. These areas are extensively infiltrated by lymphocytes and plasma cells. Because of their ability to mount immunologic reactions, such cells play an important role in combating infections of the oral regions.

The mucosal lamina propria consists of cells, blood vessels, neural elements, and fibers embedded in an amorphous ground substance. The lamina propria shows regional variation in the proportions of its constituent elements, particularly in the concentration and organization of the fibers. Cancer therapies will tend to lower cell proliferation and turnover in connective tissue; ionizing radiation has a direct effect on large molecules that make up the ground substance, so that depolymerization occurs, vascular permeability increases, and there will be tissue edema and an inflammatory infiltrate *(6).* Damage to fibroblasts will

result in cell loss and the appearance of abnormal cells leading to fibrosis after about 6 months *(7).* Similarly, damage to blood vessels will lead to hypovascularity and tissue ischaemia *(6).* Together, these changes will reduce the ability of the tissue to heal and resist infection *(8).*

Cellular and Molecular Events in Differentiation in Oral and Esophageal Epithelium

The effects of cancer therapy primarily manifest in the oral and esophageal mucosae as changes in the epithelium that reflect damage to proliferating and differentiating cells. This section will describe keratinocyte structure and function in normal tissue. Chemotherapeutic agents and radiation therapy limit the proliferative ability of the epithelium so that it becomes thin or ulcerated. Basal keratinocytes are cuboidal or columnar cells with a bounding plasma membrane and a full complement of the normal intracellular organelles (Fig. 3). These cells are capable of division so as to maintain a constant epithelial population as cells are shed from the surface. Tissue homeostasis requires differentiation and desquamation at the epithelial surface to be matched by cell division. Many factors, including aging and disease, can alter this balance so that an epithelium may become thicker (hyperplastic) or thinner (atrophic) than normal.

The progenitor cells are situated in the basal layer in thin epithelia, such as the floor of the mouth, and in the lower two to three cell layers in thicker epithelia, such as the cheek, esophagus, and palate. Dividing cells tend to occur in clusters so that more are seen at the bottom of epithelial ridges than at the top. The progenitor compartment is not homogeneous but consists of two functionally distinct subpopulations of cells. A small population of progenitor cells cycles very slowly and is considered to represent stem cells whose function is to produce basal cells and retain the proliferative potential of the tissue *(9–11).* Because it divides infrequently, the epithelial stem cell may be important in preserving the genetic information of the tissue, since DNA is most vulnerable to damage during mitosis. While the position of stem cells can be related to anatomic structure in some tissues, such as intestine, tongue papillae, and hair follicles, the cells are not morphologically identifiable in most areas of skin and oral mucosa. There have been many attempts to develop specific stem-cell markers, including the presence of adhesion molecules, such as the β1-integrins, β-catenin, and cytokeratins 15 and 19 which some have claimed can be used to identify these cells in skin and oral mucosa *(12–15).* The larger portion of the progenitor compartment is composed of amplifying cells whose function is to increase the number of cells available for subsequent maturation by entering into mitosis.

The control of epithelial proliferation and maturation is the subject of extensive research, and there are a large number of biologically active substances, most of which are peptide growth factors that are collectively termed cytokines and that may stimulate or suppress epithelial cell proliferation. Those that stimulate keratinocyte proliferation include epidermal growth factor (EGF), transforming growth factor-α (TGF-α), platelet-derived growth factor (PDGF), and interleukin 1 (IL-1) *(16–18).* The rate of proliferation is the result of interaction between positive and negative regulators, which act via a complex control system involving the binding of peptide factors to cell surface receptors, a cascade of cytoplasmic elements regulated by the activities of kinases and phosphatases, and transcriptional activity in the nucleus leading to expression of proteins involved in cell cycle regulation *(18,19).*

Mitotic activity can also be affected by a number of factors, such as time of day, stress, and inflammation. For example, the presence of a slight subepithelial inflammatory cell infiltrate stimulates mitosis, while severe inflammation causes a marked reduction in proliferative activity. It has recently been demonstrated that, for buccal epithelium, there is a clear circadian rhythm, with most cells being in the mitotic (M) phase at 2100 hours *(19).* Since the M phase represents one of the most radiosensitive stages of the cell cycle, radiation therapy involving the oral mucosa should optimally be administered in the morning.

The use of different techniques has led to a wide range of estimates of the rate of cell proliferation in the various epithelia, but, in general, the rate is highest for cells in the thin nonkeratinized regions, such as floor of mouth and underside of tongue, than for the thicker keratinized regions, such as palate and gingiva *(20)* (*see* Table 1). Apart from measuring the number of cells in division, it is also possible to estimate the time necessary to replace all of the cells in the epithelium. This is known as the turnover time of the epithelium and is derived from knowledge of the time it takes for a cell to divide and pass through the entire epithelium. Published human data for turnover times range from a median value of 34 days for epidermis to 4 days for the small intestine, with the values for oral and esophageal epithelium falling between *(21,22)* (*see* Table 1). The regional differences in the patterns of epithelial maturation appear to be associated with different turnover rates; for example, nonkeratinized buccal epithelium turns over faster than keratinized gingival epithelium. Such differences can have important implications for healing and for the rate of recovery of the tissue from damage, which is of particular relevance in considering the effects of cancer therapy on these regions. Clinically, these differences are reflected both in the more rapid appearance of therapy-induced mucositis than in dermatitis and in the prevalence of damage to nonkeratinized rather than to keratinized surfaces.

After cell division, each daughter cell either recycles in the progenitor population or enters the maturing compartment. The switch between proliferation and differentiation is modulated by the presence of factors, such as extracellular calcium, phorbol esters, retinoic acid, and vitamin D3 *(23).* Cells in the basal layer are attached by integrin-containing focal adhesions, and differentiation involves migration with a loss of integrin expression and an increase in cadherin-mediated adhesion via close intercellular junctions or desmosomes. There are also changes in the

Table 1. Epithelial cell proliferation and turnover in selected tissues

Tissue region	Mean labeling index, %*	Median turnover time, days†
Small intestine	—	4
Floor of mouth	12.3	20
Labial mucosa	11.8	—
Buccal mucosa	10.2	14
Ventral tongue	10.1	—
Esophagus	—	21‡
Gingiva	9.1	—
Hard palate	7.2	24
Dorsal tongue	4.3	—
Skin	—	27

*Reference *(19).*
†Reference *(20).*
‡Reference *(21).*

composition of intracellular proteins, termed cytokeratins, and in the development of new ones, including involucrin, loricrin, and filaggrin *(24,25).*

The principal patterns of differentiation are represented by keratinized and nonkeratinized epithelia. Differentiation in keratinized epithelia (Fig. 3, a) leads to production of the stratum corneum. The cornified cells making up this layer are flat and hexagonal in shape *(26),* filled with a compact array of condensed cytokeratin filaments *(27),* bounded by a thickened cell envelope *(28),* and surrounded by an external lipid matrix *(29,30).*

As cells leave the basal layer and enter into differentiation, they become larger and begin to flatten and accumulate cytoplasmic protein filaments, representing the cytokeratins. Keratins represent 30 different proteins of differing molecular weights; those with the lowest molecular weight (40 kd), such as keratins 8 and 18, are found in glandular and simple epithelia; keratins of intermediate molecular weight are found in stratified epithelia; and the largest keratins (approximately 67 kd) are found in keratinized stratified epithelium. All stratified oral epithelia possess keratins 5 and 14 in the undifferentiated basal cells, but differences emerge in the suprabasal layers with differentiation. Ortho-keratinized oral epithelium, such as the palate, contains keratins 1 and 10, whereas gingiva and parakeratinized palatal epithelium contains keratins 1 and 10 or keratins 4 and 13. Nonkeratinized epithelium, including esophagus, contains keratins 4 and 13 *(31,32).*

As the cells enter the prickle cell layer, small organelles known as membrane-coating granules or lamellar granules representing accumulating lipid become evident (Fig. 3, a) *(33).* In addition to the accumulation of lipids and keratins, the differentiating keratinocytes synthesize and retain a number of specific proteins, including profilaggrin *(34,35),* involucrin *(36),* and other precursors of the thickening of the cell envelope *(37).* At the boundary between the granular and cornified layers, the membrane-coating granules migrate to the superficial (apical) aspect of the keratinocyte, where the bounding membrane of the organelle fuses with the cell plasma membrane so that the lipid lamellae are extruded into the extracellular spaces of the surface layer *(28,29).* Thus, the membrane-coating granules are believed to be responsible for the formation of a superficial, intercellular, permeability barrier in stratified squamous epithelium. After the granules are extruded, the interior of the cell becomes filled with aggregated cytokeratin filaments, and involucrin, loricrin, and other proteins are deposited on the inner aspect of the plasma membrane as a thick band of protein that becomes covalently cross-linked *(24,25).*

In keratinized oral epithelium, about 50% of the intercellular space of the stratum corneum is occupied by desmosomes *(38),* and the interdesmosomal regions are frequently dilated. Although the extruded membrane-coating-granule contents fuse to form multiple broad lipid sheets in the intercellular spaces of the stratum corneum of this tissue, the number of individual lamellae in oral tissue is less than that observed in epidermis.

In nonkeratinizing epithelia (Fig. 3, b), the accumulation of lipids and of cytokeratins in the keratinocytes is less evident and the change in morphology is far less marked than in keratinizing epithelia. The mature cells in the outer portion of nonkeratinized epithelia become large and flat and possess a cross-linked protein envelope, but they retain nuclei and other organelles, and the cytokeratins do not aggregate to form bundles of filaments, as seen in keratinizing epithelia. As cells reach the upper one third to one quarter of the epithelium, membrane-coating granules become evident at the superficial aspect of the cells and appear to fuse with the plasma membrane so as to extrude their contents into the intercellular space. The membrane-coating granules found in nonkeratinizing epithelia are spheric in shape and membrane bounded and measure about 0.2 μm in diameter *(39).* They have often been referred to as cored granules because of their appearance in transmission electron micrographs. Such granules have been observed in a variety of human nonkeratinized epithelia, including oral mucosa *(40–42),* esophagus *(43),* and uterine cervix *(44).* Studies employing ruthenium tetroxide as a postfixative have indicated that a small proportion of the granules in nonkeratinized epithelium do contain lamellae, which may be the source of short stacks of lamellar lipid scattered throughout the intercellular spaces in the outer portion of the epithelium *(45).* In contrast to the appearance of the intercellular spaces of the surface layer of keratinized epithelia, those of the superficial layer of nonkeratinizing epithelia contain electron lucent material, which may represent nonlamellar phase lipid, with only occasional short stacks of lipid lamellae. It is the absence of organized lipid lamellae in the intercellular spaces that accounts for the greater permeability of this tissue.

The concept of epithelial homeostasis implies that cell production in the deeper layers will be balanced by loss of cells from the surface. While there has been much focus on programmed cell maturation and death (e.g., apoptosis) in other systems, comparatively little is known about the events determining desquamation in skin and mucosa. The available evidence suggests a programmed breakdown of cell adhesion molecules, involving both lipids and proteins, probably by intercellular enzymes that might originate in the extruded membrane-coating granules *(46).* Regardless of the nature of the process, the rate at which cells leave the surface represents a defense mechanism by rapidly clearing the substrate to which many microorganisms adhere so that they are unable to produce toxic effects or to invade. Data for murine oral mucosa from Kvidera and Mackenzie *(47)* suggest a clearance of surface cells in 2–4 hours, depending on the region. While these rates are likely to be lower in humans, the process will clearly limit colonization and invasion.

Nonkeratinocytes in Oral and Esophageal Epithelium

Many histologic sections of oral and esophageal epithelium contain cells that differ in appearance from the other epithelial cells, and it is obvious from ultrastructural and immunochemical studies that they represent a variety of different cell types, including pigment-producing cells (melanocytes), Langerhans' cells, Merkel cells, and inflammatory cells such as lymphocytes, which together can make up as much as 10% of the cell population in the oral epithelium *(48).* All of these cells except Merkel cells lack desmosomal attachments to adjacent cells, so that during histologic processing, the cytoplasm shrinks around the nucleus to produce the clear halo. None of these cells contain the large numbers of tonofilaments and desmosomes seen in the epithelial keratinocytes nor do they participate in the process of maturation seen in oral epithelia; therefore, they are often collectively called nonkeratinocytes.

Melanocyte and Pigmentation

The endogenous pigments most commonly contributing to the color of the oral mucosa are melanin and the hemoglobin in the blood. Melanin is produced by the specialized pigment cells called melanocytes, which are situated in the basal layer of the oral epithelium and the epidermis. Melanocytes lack desmosomes and tonofilaments but possess long dendritic processes that extend between the keratinocytes, often passing through several layers of cells. Melanin pigment is synthesized within the melanocytes as small structures called melanosomes. These are inoculated or injected into the cytoplasm of adjacent keratinocytes by the dendritic process of the melanocyte. Similar cells have been described in the esophageal epithelium and can give rise to melanotic lesions *(49).*

Another type of dendritic cell sometimes seen in the suprabasal layers of epidermis and oral and esophageal epithelium is the Langerhans' cell *(48,50).* It is usually demonstrated by specific immunochemical reactions that stain cell surface antigens. Langerhans' cells may be capable of limited division within the epithelium, but it is clear both that they can move in and out of the epithelium and that the source of these cells is the bone marrow. This is in accord with evidence suggesting that they have an immunologic function, recognizing and processing antigenic material that enters the epithelium from the external environment and presenting it to helper T lymphocytes. It also seems likely that Langerhans' cells can migrate from epithelium to regional lymph nodes.

The Merkel cell is situated in the basal layer of the oral and esophageal epithelium and epidermis *(48,51).* It possesses keratin tonofilaments and occasional desmosomes linking it to adjacent cells, but the characteristic feature of the Merkel cell is the presence of small, membrane-bound vesicles in the cytoplasm, sometimes situated adjacent to a nerve fiber associated with the cell. These granules may liberate a transmitter substance across the synapselike junction between the Merkel cell and the nerve fiber and, thus, trigger an impulse. This arrangement is in accord with neurophysiologic evidence suggesting that the Merkel cell is a sensory cell responding to touch. Merkel cells may arise from division of an epithelial cell (keratinocyte).

Inflammatory Cells

When sections of epithelium taken from clinically normal areas of mucosa are examined microscopically, a number of inflammatory cells can often be seen in the nucleated cell layers. These cells are transient, and the cell most frequently seen is the lymphocyte, although the presence of polymorphonuclear leukocytes and mast cells is not uncommon. Lymphocytes are often associated with Langerhans' cells, which are able to activate T lymphocytes.

It is becoming evident that the association between nonkeratinocytes and keratinocytes in skin and oral mucosa represents a subtle and finely balanced relationship in which cytokines represent the controlling factors *(16).* Thus, keratinocytes produce interleukins (1, 6, 7, 8, 10, 11, and 12), colony-stimulating factors (GM, G, and M), and tumor necrosis factor-α, all of which modulate the function of Langerhans' cells. In turn, Langerhans' cells produce IL-1, which can activate T lymphocytes, which secrete IL-2, thus bringing about proliferation of T cells capable of responding to antigenic challenge. IL-1 also increases the number of receptors to melanocyte-stimulating hormone in melanocytes and so can affect pigmentation. The influence of keratinocytes extends to the adjacent connective tissue where cytokines produced in the epithelium can influence fibroblast growth and the formation of fibrils and matrix.

Epithelial Surface Barrier

In a variety of stratified squamous epithelia, there is an effective permeability barrier in the tissue. For example, present in the oral mucosa and esophagus is an abundant flora containing many opportunistic organisms, yet inflammatory lesions are relatively infrequent, except around the teeth. The location of this barrier in the superficial layers of the epithelium has been confirmed by experiments that demonstrate an increase in permeability when the surface layers are removed by stripping *(52).* Studies with microscopically visible tracers, such as small proteins *(53)* and dextrans *(54),* suggest that the major pathway across stratified epithelium of large molecules is via the intercellular spaces and that there is a barrier to penetration as a result of modifications to the intercellular substance in the superficial layers, described in the previous section. However, it is clear from measurements of permeability that different compounds may penetrate an epithelium at different rates, depending on the chemical nature of the molecule and the type of tissue being traversed. This has led to the suggestion that materials with different chemical properties cross the barrier region by different routes, some crossing the cell membrane and entering the cell (transcellular or intracellular route) and others passing between the cells (intercellular route). For oral mucosa, Squier and Lesch *(55)* have used light and electron microscopic autoradiography to show the route taken by isotopically labeled compounds applied to the surface of the tissue. Compounds ranging from water to cholesterol, applied to either keratinized or nonkeratinized oral epithelium, could be subsequently localized in the intercellular regions of the superficial layer of the tissues, suggesting that this compartment is the predominant route for compounds moving across the barrier layer of oral epithelium. However, Zhang and Robinson *(56)* have pointed out that the pH dependency that is evident in absorption of ionizable compounds reflects their partitioning into the epithelial cell membrane, so it is likely that such compounds will tend to penetrate transcellularly. Finally, from the point of view of delivering bioactive peptides that might protect the epithelium during cancer therapy, it is worth noting that the superficial layers of the tissue may act as a reservoir for topically applied compounds. Although this phenomenon has been inferred from kinetic studies in oral mucosa *(57,58),* it is poorly understood. As we have already mentioned, the permeability barrier in nonkeratinized epithelia consists of groups of lipid lamellae located in the intercellular spaces of the superficial epithelial layer *(45,59).* These limit the penetration of nonpolar compounds, which may become trapped in a nonlipid or fluid lipid intercellular compartment of the barrier layer. Thus, the surface layer of the epithelium may take up a compound relatively rapidly (depending on its lipophilicity and the nature of the vehicle). Once saturated, this layer cannot adsorb any more material, regardless of the duration of exposure. Subsequently, the adsorbed material diffuses into the deeper layers of the tissue at a fairly constant rate that is more dependent on the capacity (or loading) of the reservoir than on the duration of surface exposure.

The constancy of the oral environment is ensured to a large extent by the continual secretion of saliva into the oral cavity

from the three major salivary glands and numerous minor salivary glands located in, or beneath, the mucosa. Saliva, by continually bathing the surface of the oral mucosa, maintains a moist atmosphere and a stable, but slightly acidic, pH. Compared with the secretions of the gastrointestinal tract, saliva is a relatively mobile fluid with less mucin, limited enzymatic activity, and virtually no proteases. The mucosal surface has a salivary coating that has been estimated to be 70 mm thick *(3)* and that may act as an unstirred fluid layer. Several independent lines of evidence suggest that saliva and salivary mucin contribute to the barrier properties of oral mucosa *(60).* Within saliva there is a high-molecular-weight mucin named MG1 *(61)* that can bind to the surface of the oral mucosa so as to maintain hydration, provide lubrication, concentrate protective molecules such as secretory immunoglobulins, and limit the attachment of microorganisms. Histatins are small salivary-derived histidine-rich polypeptides with marked antifungal activity *(62).* These may be augmented by the activity of a recently discovered class of antimicrobial peptides, the defensins, that are expressed by oral epithelium *(63).*

Aging of Oral Mucosa

Skin shows well-documented changes in structure and function with age, most of which arise from chronic exposure to UV radiation (i.e., photoaging). The oral mucosa, being protected from such environmental effects, shows few changes that can be unambiguously ascribed to aging. In some regions, there is a slight thinning of the epithelium with a concomitant flattening of the epithelial–connective tissue interface *(64).* Despite claims of a reduction in the rate of cell proliferation with age, there are no clear data to support this for human tissue, although there may be some increase in turnover time *(65).*

The limited information available on the permeability of oral mucosa indicates that there is a trend toward decreased permeability to water with age, which is statistically significant for floor-of-mouth mucosa from females *(66).* It is of interest to note that, in skin, where the morphologic changes with age are more marked than in oral mucosa, there have been a number of reports that demonstrate a significant decrease in permeability with age; Squier et al. *(66)* discussed the reasons for this.

Among the age changes evident in the lamina propria are those affecting the vascular system. Although there is some evidence for a reduction in the number of individual vessels with active flow *(67),* it is not known whether this reduction affects overall blood flow and perfusion. Systemic conditions encountered in the elderly that can affect the oral vasculature include diabetes and atherosclerosis *(68,69).* In a study of blood flow in atherosclerotic monkeys, Goodman and Squier *(70)* reported a 50% reduction in flow in the oral mucosa. However, given the ample blood supply to the oral tissues, it appears that perfusion is still sufficient to tissue viability, even in the presence of these vascular alterations *(71).*

Relationship to Mucosal Injury in Cancer

Anticancer therapy represents a significant challenge to the integrity of mucosal tissues. Chemotherapeutic agents and radiation limit proliferative ability so that the overlying epithelium becomes thin or ulcerated. This effect is first seen in the more rapidly proliferating tissues, such as gastrointestinal and oral lining mucosa, where atrophy and ulceration can represent a dose-limiting and potentially serious complication of treatment. In the oral mucosa, lesions first appear on the soft palate, tongue, and cheeks; as they enlarge, they lead to extreme pain and dysphagia. As a consequence, there may be dehydration, a compromised nutritional status because of painful chewing, and a decreased quality of life.

The effect of cancer therapies is not limited to epithelia and will tend to lower cell proliferation and turnover in connective tissue; ionizing radiation has a direct effect on the tissue matrix leading to an increase in vascular permeability, tissue edema, and an infiltration of inflammatory cells. Damage to fibroblasts will result in cell loss and fibrosis; similarly, damage to blood vessels will lead to hypovascularity and tissue ischemia. Together, these changes will reduce the ability of the tissue to heal and resist infection. There may also be indirect effects, such as damage to the salivary glands, which will reduce salivary production and impair barrier efficiency, and a reduction in immunocompetence as a result of myeloablative therapy. This will increase the risk of local infection from oral organisms.

Future Research Directions

The treatment of mucosal injury in cancer patients has tended, in the past, to focus on palliation. Apart from effective combinations of antimicrobials, local anesthetics, and, possibly, anti-inflammatory agents, nonirritative vehicles that will coat the mucosa to enhance lubrication and provide some degree of occlusion so as to relieve acute symptoms have also been included. The discovery of potent agents that might protect or promote healing of the mucosal lining leads to the possibility of therapy rather than palliation. Most of the candidates are cytokines, with an effect on epithelial proliferation that has already been mentioned ("Cellular and Molecular Events in Differentiation in Oral and Esophageal Epithelium," *above*). While intuitively, it might be assumed that agents that increase epithelial proliferation would protect the epithelium during anticancer therapy, animal studies suggest the opposite effect—increasing proliferation sensitizes epithelial cells to the effects of chemotherapy and results in increased mucositis. This discovery has led to increased interest in cytokines that act by arresting epithelial cell division, thus sparing the cells from the effects of anticancer therapy. After release from arrest, there is rapid proliferation and repopulation of the tissue so as to restore normal mucosal function. Studies to identify other compounds with these effects and to characterize their behavior will be critical if management of mucosal injury is to progress from palliation to therapy.

The profound effect of cytokines on cell proliferation makes it essential that they be delivered locally to the mucosa, so as not to interfere with anticancer therapy. In practice, this demands topical application. To exert an effect after topical application, such compounds must pass across a surface permeability barrier (described in "Cellular and Molecular Events in Differentiation in Oral and Esophageal Epithelium," *above*) to reach the proliferative (basal) compartment of the epithelium. These requirements demand the maintenance of high local concentrations at the mucosal surface, so as to maintain a concentration gradient, and the presence of permeabilizers to ensure penetration of large molecules across the epithelial permeability barrier.

A major challenge in formulating topical agents for the oral cavity is the need for adhesion to the moist surface of the mucosa and the need to resist the flushing action of saliva. The use of bioadhesive gels reduces the frequency of application and the amount of drug administered and can also improve patient com-

pliance and acceptance. Optimizing the retention time of the drug is important in improving its clinical effectiveness. Finally, for the mucositis patient, the occlusion and lubrication of a bioadhesive gel reduce the discomfort of the lesion.

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