1 THE NORMAL SALIVARY GLANDS

The anatomic establishment of the major salivary glands occurred between 1650 and 1685, toward the end of the Renaissance. Nahlieli et al. credit Andreas Vesalius (1514–1564) as the first to use the term "salivary gland" in his magnum opus, De humani corporis fabrica (1543) (1).

The major salivary glands (parotid, submandibular [submaxillary], and sublingual glands), consisting of three paired, large collections of exocrine glandular tissue, and the minor salivary glands, consisting of numerous, nonuniformly distributed, small aggregations in the mucosa of the oral cavity, constitute the salivary gland system. All salivary glands share a basic structure, but have site-specific variations in function, secretions, and gross and microscopic features that influence frequency and types of neoplasms (2). The seromucinous glands of the nasal cavity, pharynx, larynx, and bronchi are morphologically and functionally similar to many of the oral minor salivary glands but are not salivary glands because they do not contribute to the saliva.

The basic structure of salivary gland tissue is a branching tubule or duct that has the principal secretory cells, the acinar cells, at one end of these branches and an opening into the oral cavity at the other end (3). The basic salivary gland unit has morphologically and functionally varying segments that are identified as acinus, intercalated duct, striated duct, and excretory duct (fig. 1-1). All segments have a bilayered cellular composition of luminal cells and abluminal peripheral cells. Abluminal cells surrounding the acinus and intercalated duct are myoepithelial cells and in the proximal portion of the gland (excretory duct) are basal cells. Acinar cells are divided into three types: serous, which secrete amylase; mucous, which secrete sialomucin; and mixed types. The relative proportion of each type dictates the chemical composition of saliva, which varies by anatomic site. A list of the distribution of acinar types among various salivary glands is in Table 1-1.

DEVELOPMENT

Salivary gland organogenesis involves multiple cell types, including epithelial, myoepithelial, mesenchymal, neuronal, lymphatic, and endothelial cells. Complex interactions among these cell types occur during the initiation

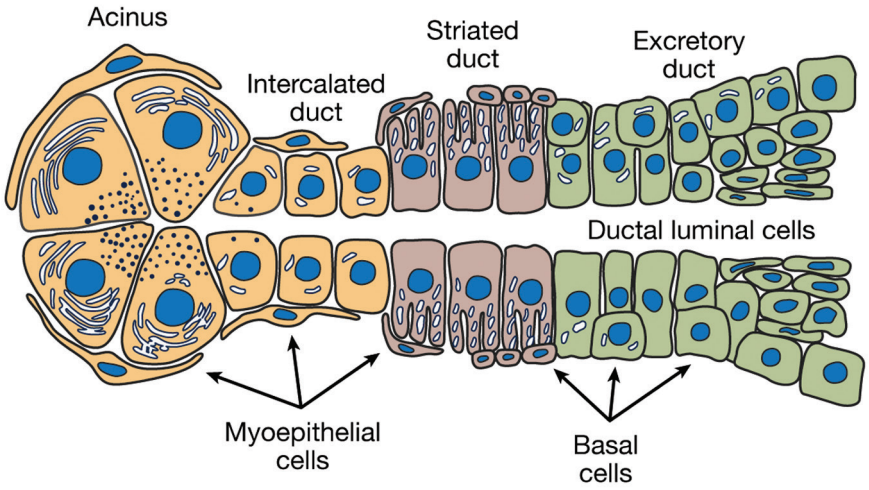


Figure 1-1

BASIC SALIVARY GLAND UNIT

Schematic of the salivary gland unit showing its various portions from the secretory end piece (left) to the oral cavity (right).

Table 1-1

ACINAR CELL TYPES IN VARIOUS SALIVARY GLANDS

Salivary Gland	Cell Type
Parotid	serous
Submandibular	serous > mucous
Sublingual	mucous > serous
Palate	mucous
Tongue	mucous > serous
Lip	mucous > serous
Buccal mucosa	mucous > serous

and development of the salivary epithelium, although the precise timing and sequence of these events are not completely understood (4).

The major and minor salivary glands develop as buds of proliferating epithelial cells originating from the primitive stomodeum (oral cavity) mucosa. The major glands form by a developmental process known as *branching morphogenesis* (5). This process involves interplay between glandular epithelium and the extracellular matrix surrounding it. Proliferating cell cords form terminal bulbs that then develop clefts and further proliferate as branches from the original cords, leading to the development of the gland. These branching epithelial cords become excretory ducts and their distal ends differentiate into acini. Around the forming lumens, epithelial cells actively proliferate, while those at the center of the presumptive lumens undergo apoptosis, producing a hollowing out of these branches and terminal bulbs to form the presumptive ducts and acini (5). Coordinated cell proliferation, clefting, differentiation, migration, apoptosis, and reciprocal interactions between the epithelial, mesenchymal, neuronal, and endothelial cells are required for normal development.

The parotid gland anlage arises at the angle between the maxillary process and mandibular arch from the primitive oral ectodermal layer as an epithelial bud during the 4th to 6th weeks of embryogenesis. It is subsequently entrapped in an incomplete capsular fascia, along with lymphoid tissue. As a result of this developmental process, the parotid gland is the only major salivary gland that contains intraglandular lymph nodes. The parotid gland moves dorsally and laterally by the 7th week of embryogenesis to the preauricular region (6). The facial nerve penetrates the gland by approximately the 10th week (7). Intralobular and duct and acinar cell differentiation begin about the 8th month of gestation and myoepithelial cell differentiation between the 19th and 24th week (8).

The submandibular (arising at the end of the 6th week of embryonic growth) and sublingual (appearing at about the 7th to 8th week) gland primordia, in contrast, arise from endoderm only and therefore normally lack intraglandular lymph nodes. The primordia of the submandibular glands grow from the floor of the oral cavity between the lower jaw and tongue, the so-called linguogingival groove, on each side of the midplane. The sublingual glands are the last major salivary gland to appear. Their primordia arise as several epithelial buds adjacent and lateral to the developing submandibular glands. They grow downward from the linguogingival groove between the lower jaw and tongue. The intraoral minor salivary glands develop during the 3rd month of gestation (9). The epithelial buds continue to proliferate as strands into the underlying oral ectomesenchyme, which increases in cellularity around the developing glands and has a role in the lobular organization of the glands and encapsulation of the parotid and submandibular glands (fig. 1-2).

For all three glands, the process is similar. At the end of each solid cord of epithelium of the developing salivary glands, a cluster of epithelium forms the terminal bulb. Clefts develop in the terminal bulbs, and these newly formed bulbs proliferate into the ectomesenchyme as branches from the original cord. This repetition of cord-like proliferation, bulb formation, bulb clefting, and further proliferation continues with growth. Fibronectin, laminin, gamma-2, and TIMP-3 proteins participate in this branching morphogenesis and probably in vascular and neural development (10). Each new cord of epithelium remains contiguous with the preceding cord so that continuity with the oral epithelium is maintained. Initially, the epithelial cords and bulbs are without lumens. Subsequent microlumen formation occurs via apoptosis, first in the epithelial cords and progresses to the terminal bulbs. These microlumens eventually coalesce to form a continuous ductule, and the

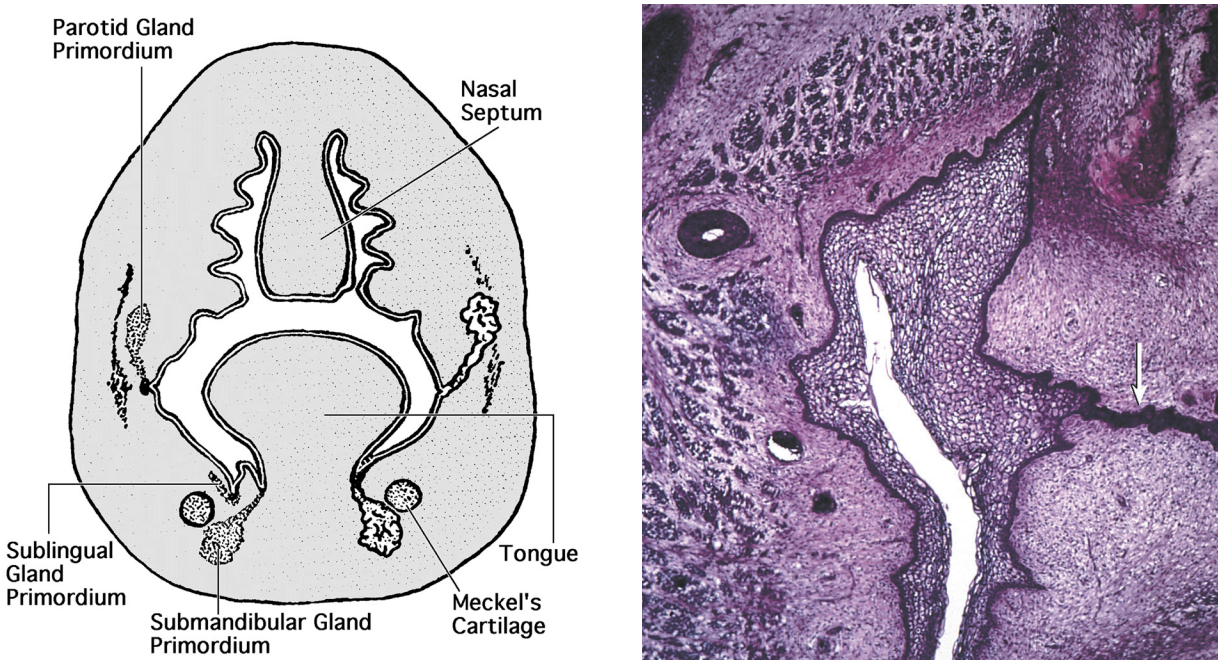


Figure 1-2

FETAL DEVELOPMENT OF SALIVARY GLANDS

Left: Origin of the parotid gland, submandibular gland, and sublingual gland from the epithelial lining of the primitive stomodeum is illustrated in this schematic drawing of the oral cavity region from a 9-week-old embryo.

Right: Section through fetal tongue, linguogingival groove, and buccal mucosa shows the proliferative epithelial cord of the developing parotid gland (arrow).

surrounding epithelial cells constitute a branch of the overall duct system (fig. 1-3).

Along with lumen development, cellular differentiation results in the characteristic features of excretory ducts, striated ducts, intercalated ducts, and acini. The proximal branched cords become excretory and main ducts, the distal branched cords become striated ducts, and the lumenized terminal bulbs become intercalated ducts and acini. After lumen formation, but before cellular differentiation, the terminal bulbs are referred to as terminal tubules and saccules. The epithelium of the terminal tubules and saccules has two layers (fig. 1-4). The inner, luminal layer of cells differentiates into acinar and intercalated duct cells, and the outer layer differentiates into myoepithelial cells. Complete maturation of the salivary glands does not occur until after birth.

At about the 3rd month of gestation, the parotid gland is colonized by lymphocytes, which eventually develop into several intraparotid and periparotid lymph nodes as well as lymphoid nodules. Lymphoid nodules lack the complete organization of a lymph node.

ANATOMY

Parotid Gland

The parotid gland consists of paired glands that are the largest of the major salivary glands. In adults, each gland weighs 15 to 30 g and most of the gland (about 80 percent) is superficial to the masseter muscle (6,11). These wedge-shaped glands are bounded at their base superiorly by the zygomatic arch, with the gland apex just inferior to the angle of the mandible (12). They extend inferiorly to the anteromedial margin of the sternocleidomastoid muscle (fig. 1-5). The anterior portion of the parotid gland lying against the mandibular ramus slightly covers the posterior edge of the masseter muscle. Posteriorly, the gland is bounded by the ear, the mastoid process, and the anterior edge of the sternocleidomastoid muscle. The deep portion of the gland extends into the retromandibular

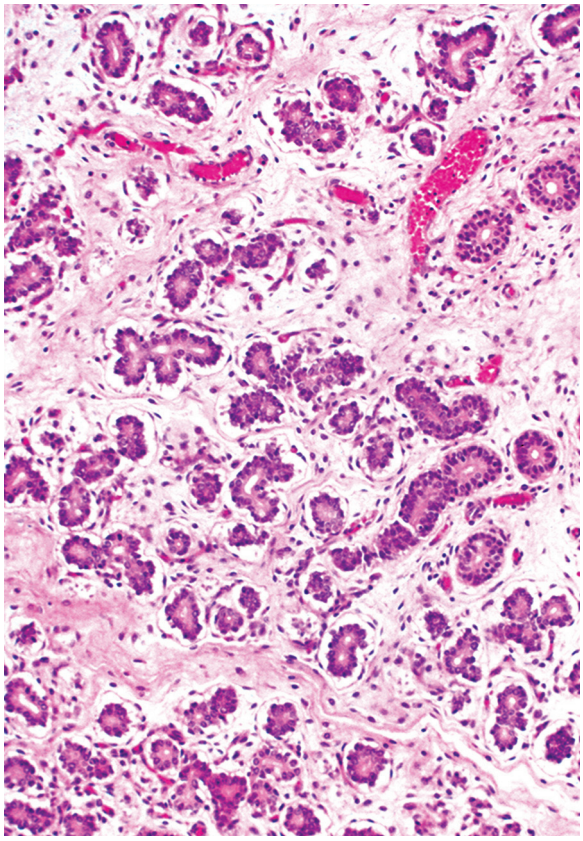


Figure 1-3

FETAL PAROTID GLAND

Many tubules of epithelium within a loose connective tissue stroma are in the early stages of luminization, but cellular differentiation into acini, intercalated ducts, and striated ducts is not evident at this stage of development.

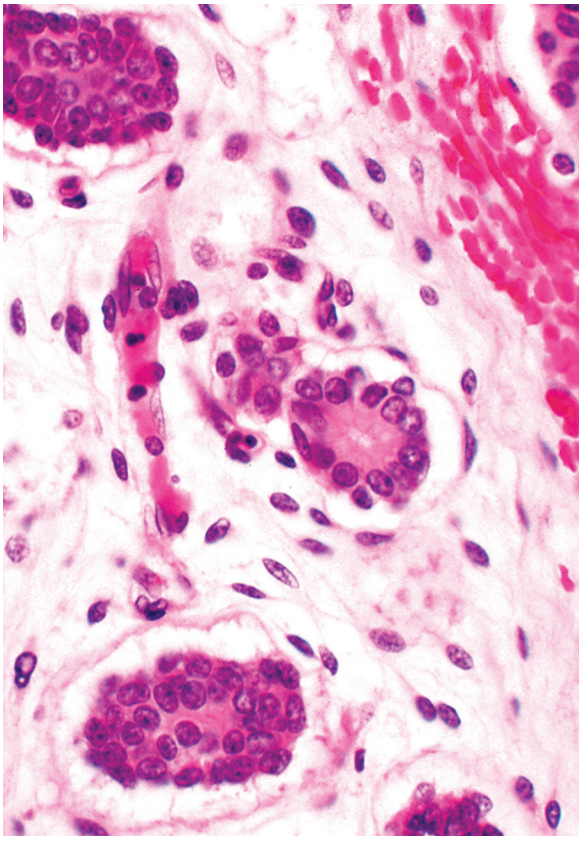


Figure 1-4

FETAL PAROTID GLAND

The epithelial tubules contain a double layer of cells. The inner cells differentiate into duct-luminal and serous acinar cells while the peripheral layer differentiates into myoepithelial and basal cells.

parapharyngeal area and is confined by the styloid process and stylomandibular ligament; the styloglossus, stylohyoid, stylopharyngeal, and digastric muscles; and the carotid sheath. Anteriorly, the deep portion lies against the medial pterygoid muscle. Masses that develop in the deep lobe typically expand into the parapharyngeal space since this is the path of least resistance (fig. 1-6). Laterally, the parotid gland is covered by skin and subcutaneous adipose tissue. The fascia that surrounds the parotid is derived from the superficial layer of the deep cervical fascia, and is densest over the lateral and inferior portions. No natural plane exists between the parotid gland and the overlying skin. Several critical structures including the internal jugular vein and its branches, external carotid arteries and their branches, auriculotemporal branch of the trigeminal nerve, and the facial nerve are in close proximity to the deep lobe (11,13).

Parotid gland secretions flow through a duct system that converges into a single duct, the Stensen duct, which is 4 to 7 cm in length. This duct exits the gland along its anterior edge, traverses laterally around the masseter muscle and anterior to the buccal fat pad, where it turns medially to pierce the buccinator muscle (12). It briefly runs caudally before entering the oral cavity opposite the second maxillary molar (3). Accessory parotid gland tissue is found in about 20 percent of individuals, typically superficial to the masseter muscle just cranial to the Stensen duct (6,14).

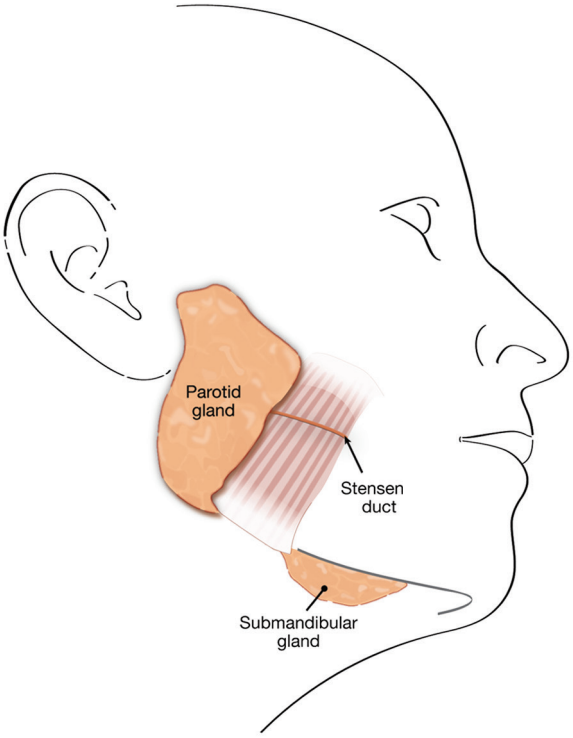


Figure 1-5

ANATOMIC RELATIONSHIP OF PAROTID AND SUBMANDIBULAR GLANDS

The anatomic position and relationship of the parotid and submandibular glands to the ear, zygomatic arch, mandible, and masseter muscle in a lateral view of the head are illustrated. The parotid gland duct (Stensen duct) crosses the masseter muscle and penetrates the buccal tissues. Lobules of accessory parotid tissue are located along the course of the duct.

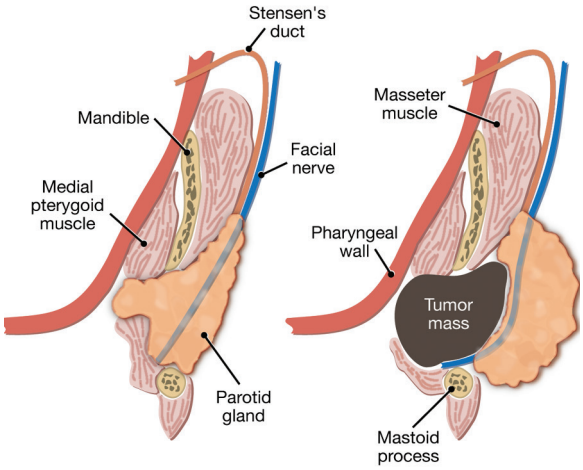


Figure 1-6

ANATOMIC RELATIONSHIP OF PAROTID GLAND

This horizontal section is through the lateral portion of the pharynx and mandible at the level of the mastoid process. The parotid is traversed by the facial nerve, and the deep portion of the gland narrows and is bounded by the posterior of the ramus of the mandible, muscles of the styloid process, and medial pterygoid muscle.

Although the parotid gland is a single contiguous structure, most pathologists and surgeons conceptualize it as having a superficial (lateral) and deep (medial) lobe, using the plane of the facial nerve (cranial nerve VII) as a dividing line. After exiting the brainstem, the facial nerve follows a circuitous route through the internal auditory canal into the inner ear, middle ear, and mastoid bone to emerge from the skull at the stylomastoid foramen (15). After departing this bony canal, the nerve immediately gives off small branches to supply the postauricular muscles, posterior belly of the digastric muscle, and the stylohyoid muscle. The main trunk then enters the substance of the parotid gland to divide at the pes anserinus, 1.5 cm distal to the foramen. At this point, the nerve divides variably into its five terminal divisions: a temporal branch courses superficially to the zygomatic arch, a zygomatic branch courses toward the lateral canthus innervating the orbicularis oculi, a buccal branch supplies the muscles of the midface, a mandibular division dips below the angle of mandible and back again to supply the orbicularis oris and mentalis muscles, and a cervical branch courses into the neck to innervate the platysma muscle. The facial nerve exits the substance of the parotid gland and generally follows a more superficial course distally to lie in the subdermal plane at the level of the muscles it innervates. The nerve is superficial to both arterial and venous blood vessels (16,17).

The parotid gland is innervated by parasympathetic nerve fibers via the glossopharyngeal nerve (cranial nerve IX), although the nerve fibers enter the gland by way of the otic ganglion and auriculotemporal nerve after a complicated course (3). Sympathetic fibers accompany the blood vessels. Interruption of the parasympathetic nerve pathways results in atrophy of the gland.

Lymphoid tissue in the parotid gland varies from well-defined nodes with capsules, sinuses,

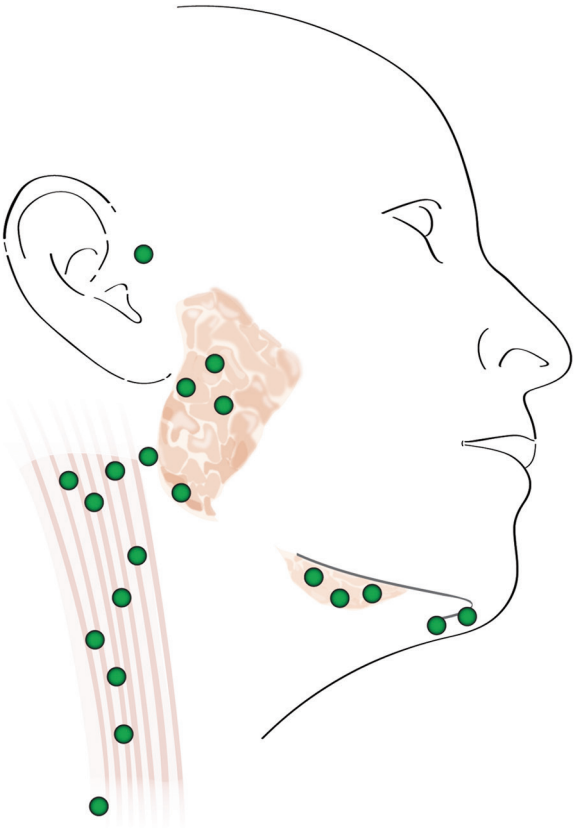


Figure 1-7

GLANDULAR LYMPH NODES

The parotid gland has several periparotid and intraparotid lymph nodes that drain portions of the ear, temporal region, lateral face, eyelids, and conjunctiva, and they in turn drain into the internal jugular lymph nodes. Submandibular gland lymph nodes are all extraglandular.

and germinal centers to small nodules without definitive structure. As mentioned, because of its ectodermal origin and entrapment within capsular fascia along with lymphoid cells, the parotid gland is unique in that it is seeded with lymphocytes during embryogenesis. These develop into lymph nodes both within and surrounding the gland (fig. 1-7).

Intraparenchymal lymph nodes are intimately associated with the parotid tissue, and thus benign intranodal salivary tissue is a frequent finding. About 90 percent of intraparenchymal lymph nodes are located in the superficial lobe (18,19). A study of 20 parotid glands from adult cadavers found that the number of parotid lymph nodes varied from 3 to 24 per gland (19). A larger, more recent study of 84 cadavers with total parotidectomy performed shortly after death found lymph nodes in all but 5 percent of superficial lobes, but a complete absence of nodes in 69 percent of deep lobes (18). The mean number of nodes was 2.8 to 3.1 for the superficial lobe, and 0.3 to 0.4 for the deep lobe.

Lymph node count is dependent on how one defines a lymph node since not all parotid lymphoid tissue contains the complete structural organization of a lymph node. In the above study (18), all groups of lymphocytes that were found with or without a germinal center, surrounded by a capsular structure, and associated with peripheral and central sinuses were accepted as a lymph node. No significant difference in node number between right and left glands or between male and female cadavers was found.

The parotid space harbors three groups of lymph nodes as determined by the embryologic development. The first group is embedded within the parotid fascia, the second within the parotid parenchyma, and the third, such as the preauricular lymph nodes, remain extrafascial and extraglandular. The lymphatic system of the neck develops after encapsulation of submandibular and sublingual glands, but before encapsulation of the parotid glands. For this reason the other major salivary glands do not have a lymphatic system, whereas the parotid gland has its own lymphatics due to relatively late embryologic encapsulation (20). The anterolateral portion of the auricle, external auditory meatus, temporal and parietal scalp, ipsilateral forehead, and cheek are the primary sites that drain to these parotid lymph nodes, which in turn drain into the deep cervical lymph nodes of the upper neck (21).

The external carotid artery supplies arterial blood to the parotid gland where it divides into maxillary and superficial temporal branches. Venous outflow occurs through the retromandibular vein which is formed by the maxillary and superficial temporal veins. The retromandibular vein courses through the parotid gland just deep to the facial nerve to join the external jugular vein. The anterior branch of the retromandibular vein joins the posterior facial vein to form the common facial vein, while the posterior branch of the retromandibular vein may also combine with the postauricular vein to drain into the external jugular vein (3,15).

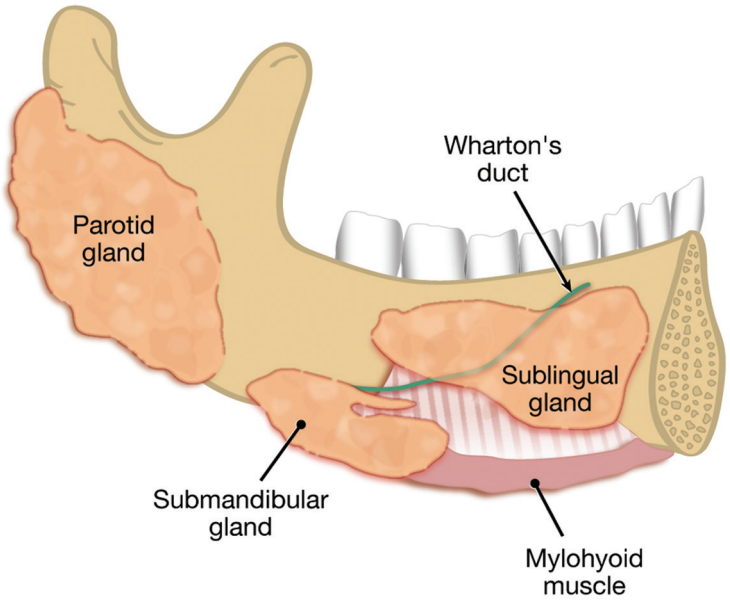


Figure 1-8

THE MAJOR SALIVARY GLANDS

The medial surface of the mandible and mylohyoid muscle showing the relationship of the submandibular, sublingual, and parotid glands. The submandibular duct (Wharton's duct) runs anteriorly to the anterior floor of the mouth.

Submandibular Gland

The submandibular gland is the second largest salivary gland; it weighs approximately 7 to 8 g (6). It occupies a large portion of the submandibular triangle formed by the inferior border of the mandible and the anterior and posterior bellies of the digastric muscle. It is finely encapsulated (fig. 1-5). Superiorly, the submandibular space is bounded by the mylohyoid muscle. The posterior extension of the submandibular gland rises upward and around the posterior edge of the mylohyoid muscle and into the sublingual space of the floor of the oral cavity (fig. 1-8). Posteriorly, the stylomandibular ligament separates it from the parotid gland (22). Similar to the parotid gland, the duct system converges into a single main excretory duct (Wharton duct) which extends about 5 cm from the superior portion of the gland in an anterior direction in the lingual sulcus to an opening, the sublingual caruncula, on each side of the frenulum linguae. A submandibular gland with three ducts opening separately into the oral cavity has been reported (6). The openings of the paired submandibular glands are only a few millimeters apart (fig. 1-9).

There are no lymph nodes within the fibrous capsule of this gland, but three to six level IB nodes lie in close proximity to the gland just outside the capsule. Metastases to these nodes often do not involve the gland proper (fig. 1-7). Afferent lymphatics draining into submandibular lymph nodes derive from the skin and nasal mucous membranes, anterior face including lips, buccal mucosa, lateral floor of mouth, and anterior tonsillar pillars. These nodes send

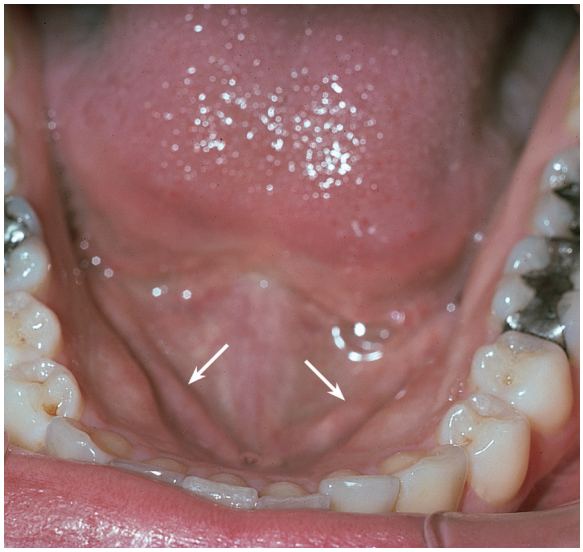


Figure 1-9

WHARTON DUCTS

Right and left submandibular ducts course anteromedially in the floor of the mouth to openings at the lingual carunculae (white arrows), which are only a few millimeters apart.

efferent vessels into the cervical lymph nodes along the internal jugular vein and sternocleidomastoid muscle. Blood supply and drainage of the gland is by the lingual and facial arteries and the anterior facial vein. The gland is innervated by the parasympathetic branch of the facial nerve; the vasomotor nerves derive from the superior cervical ganglion (6). Unlike the parotid gland, no large nerves course through the gland, but the lingual nerve lies above it and the hypoglossal nerve lies below (22).

Sublingual Gland

The smallest of the major salivary glands, the sublingual gland, weighs 2 to 4 g. It lies above the mylohyoid muscle in the lingual sulcus of the floor of the mouth between the tongue and the sublingual fossa of the mandible (fig. 1-8). Its superior aspect is covered only by oral mucosa. Unlike the parotid and submandibular glands, the sublingual gland has several ducts that connect to the oral cavity. The largest of these, the Bartholin duct, opens into the submandibular duct just posterior to the sublingual caruncula. Several smaller ducts (Rivinus ducts) open directly into the mouth in the plica sublingualis (6).

Vascular supply is from the sublingual and submental arteries, and venous drainage is via tributaries of the external jugular vein. Innervation is similar to that of the submandibular gland.

Minor Salivary Glands

Between 500 and 1,000 lobules of minor salivary gland tissue are dispersed within the head and neck submucosa. Most lobules (70 to 90 percent) are located in the oral cavity and oropharynx, including the palate, tongue, lips and buccal mucosa, and retromolar trigone. Those seromucous glands in the nose, paranasal sinuses, pharynx, and larynx are not salivary glands because they do not contribute to the production of saliva, but since some tumors in these sites are similar or identical to those arising in the minor salivary glands of the oral cavity, they are typically grouped along with them. Minor salivary gland tissue is more numerous than major gland tissue, but with a reduced volume, an abbreviated ductal system, and a paucity of capsular tissue (23,24).

Intraoral minor salivary glands usually are not clinically evident, although salivary lobules often can be palpated in the lips. The gland lobules range from 1 to 5 mm and are separated from one another by connective tissue; glands in the posterior hard palate are more numerous and more confluent. Most lobules have individual excretory ducts that open into the oral cavity, but these duct orifices are not usually perceptible in normal mucosa.

HISTOLOGY

Parotid Gland

Fascial extensions subdivide the parotid gland into lobules. The parotid ductal system is one of continuous partitioning: this begins proximally with the main excretory duct, which continues branching distally toward interlobular ducts, which subdivide into intralobular striated ducts, and finally into intercalated ducts that terminate as secretory acini. During this proximal to distal subdivision, ducts become more numerous and progressively smaller in caliber. Parotid gland acini are nearly all serous, although rare mucous acini can be found. Serous acinar cells are arranged in small, roughly spherical, three- to six-cell clusters (fig. 1-10). Each acinus surrounds a tiny lumen, although it is frequently not discernible in the plane of section, and the acinus itself is surrounded by a basement membrane. Serous cells are roughly pyramidal or trapezoidal, with the narrowest part of the cells at the luminal surface.

Acinar cell cytoplasm is filled with basophilic secretory zymogen granules (whose major enzyme is amylase); their number, however, varies with the secretory cycle phase of the cell. Cytoplasmic granules stain with periodic acid–Schiff (PAS) and are resistant to diastase digestion, but are unreactive with mucicarmine or Alcian blue (fig. 1-11). Cell nuclei are uniform in size, rounded, and located in the basal half of the cells. Secretion drains from acini into intercalated ducts.

Intercalated ducts are longer and more conspicuous in the parotid gland than in any other salivary gland, but their small size makes them more difficult to discern microscopically. Intercalated ducts are lined by low cuboidal cells with scant cytoplasm and uniform, round nuclei (fig. 1-12). Since their nuclei are about the same size as those of acinar cells, the nuclear to

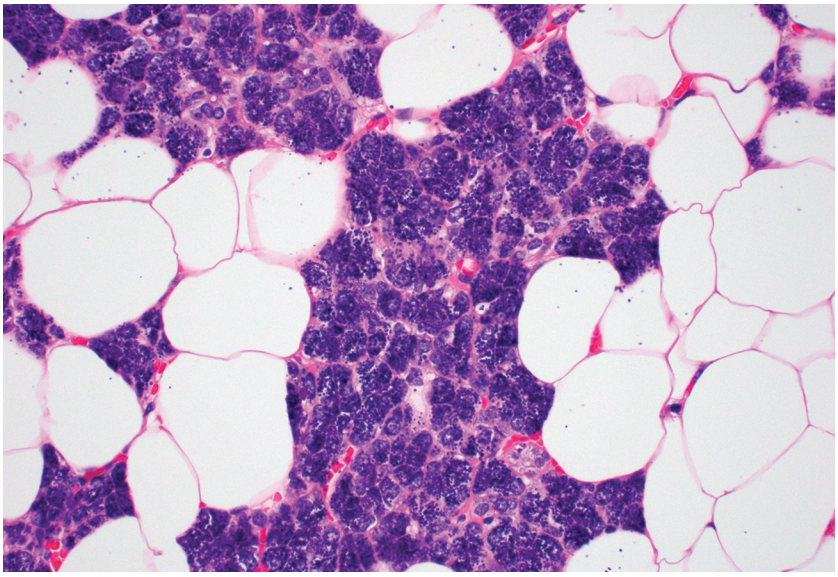


Figure 1-10

PAROTID GLAND ACINI

Rounded acini are serous type and composed of several pyramidal-shaped cells with basal nuclei and bluish-purple, granular cytoplasm.

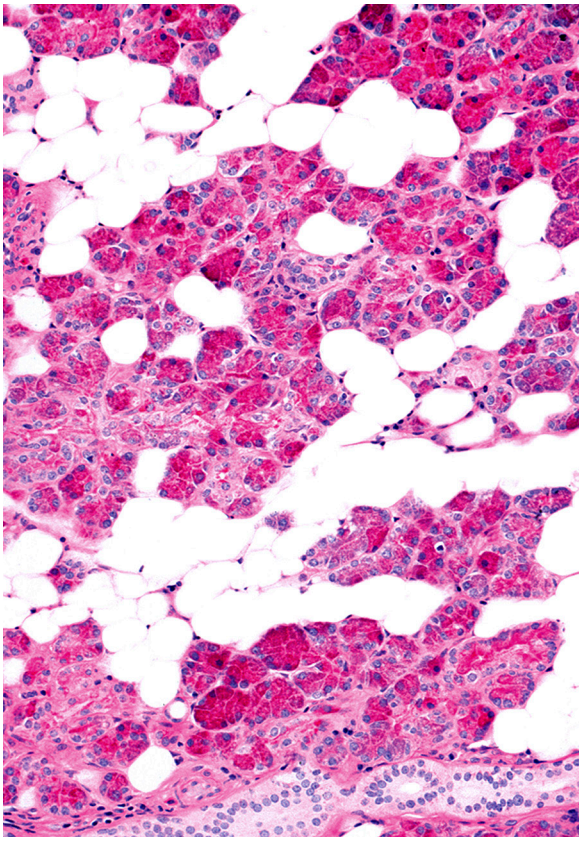


Figure 1-11

PAROTID GLAND ACINI

Cytoplasmic zymogen granules stain with PAS and resist diastase digestion, but are negative with mucicarmine stains (PAS stain with diastase).

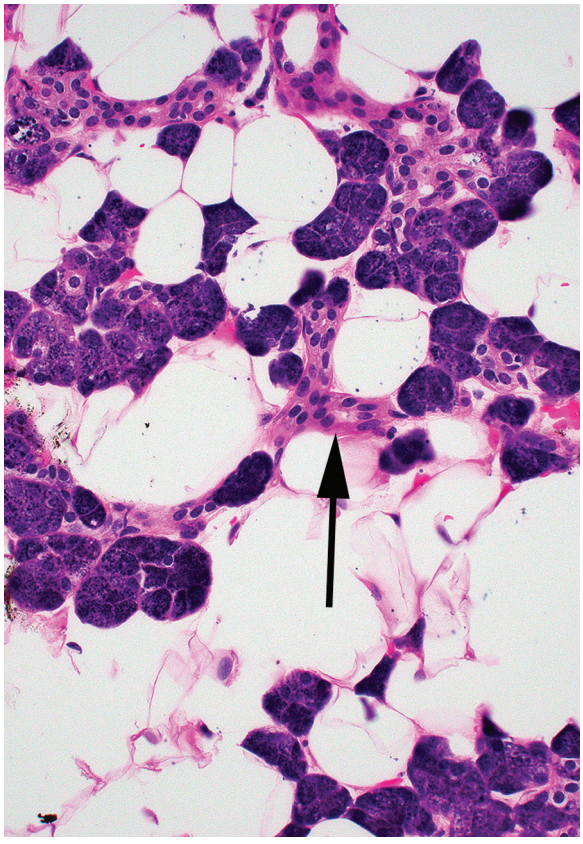


Figure 1-12

INTERCALATED DUCT

Luminal cells of the intercalated duct (arrow) surround a small lumen. These cells are smaller than acinar cells, are cuboidal, eosinophilic to amphophilic, and have centrally placed nuclei.

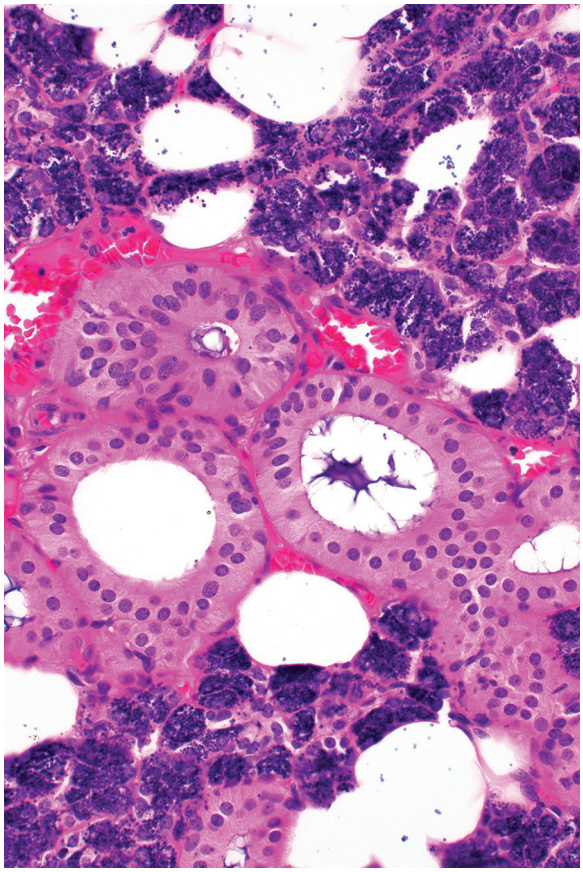


Figure 1-13

STRIATED DUCTS

The striated duct is much larger than the intercalated duct. Columnar luminal cells have bright eosinophilic cytoplasm, central nuclei, and vertical cytoplasmic striations in the basal half due to folds in the basal plasma membranes (H&E stain).

cytoplasmic ratio is much greater in intercalated duct cells than acinar cells. The cytoplasm is amphophilic to eosinophilic. Unlike the water-permeable cells of the acinus, ductal cells are impermeable to water.

Myoepithelial cells were first discovered in breast tissue by Krause in 1865 (25). Since then they have been observed in the terminal end pieces and ducts of most exocrine glands (salivary, mammary, sweat, lacrimal, and bronchial glands). Myoepithelial cells (basket cells) have histologic and ultrastructural features of both epithelium and smooth muscle (26). They differentiate from pluripotential duct cells by the 10th week of gestation. Since they are positioned between the basal lamina of acinar and intercalated duct cells, and contain myofilaments arranged in a pattern similar to that of smooth muscle, they are thought to have contractile properties that aid in the secretion of saliva (27). In addition, there is probably an association of myoepithelial cells with striated ducts and interlobular ducts (28). Myoepithelial cells are flattened, stellate, and spindle shaped, with cellular processes that surround and embrace the acini and intercalated ducts (26). Myoepithelial cells are best seen microscopically using specific immunohistochemical stains. In addition to contraction, myoepithelial cells participate in the elaboration of protein constituents of basal lamina, such as fibronectin, laminin, and type III collagen (29).

Larger than intercalated ducts, striated ducts are three to six times larger than an acinus. Cells of this duct are columnar and intensely eosinophilic due to their high content of mitochondria. Cell nuclei are uniform, rounded, and located in the center or luminal half of the cells. Fine, parallel vertical striations (due to invaginations in the plasma membranes) for which these cells are named are seen at high magnification light microscopy in the basal half to two thirds of the cells (fig. 1-13). Striated duct cells react more intensely with phosphotungstic acid-hematoxylin stain than other cellular constituents because they contain a large number of mitochondria (fig. 1-14). Occasional myoepithelial cells and scattered basal cells are located between the striated cells and basement membrane.

Striated ducts connect with interlobular (excretory) ducts, which are lined by pseudostratified columnar epithelium (fig. 1-15). Small basal cells are distributed along the basement membrane, and occasional goblet-type mucous cells are intermingled among the pseudostratified columnar cells. As the excretory duct merges with the oral mucosal epithelium, the luminal epithelium becomes stratified squamous in type.

The glandular parenchyma is segregated into varying sized lobules by septa of fibrous tissue, which also forms a capsule around the entire gland. A variable amount of fat is found in both intralobular and extralobular locations. Acinar cell clusters are closely apposed and dense in young persons, but the amount of interstitial

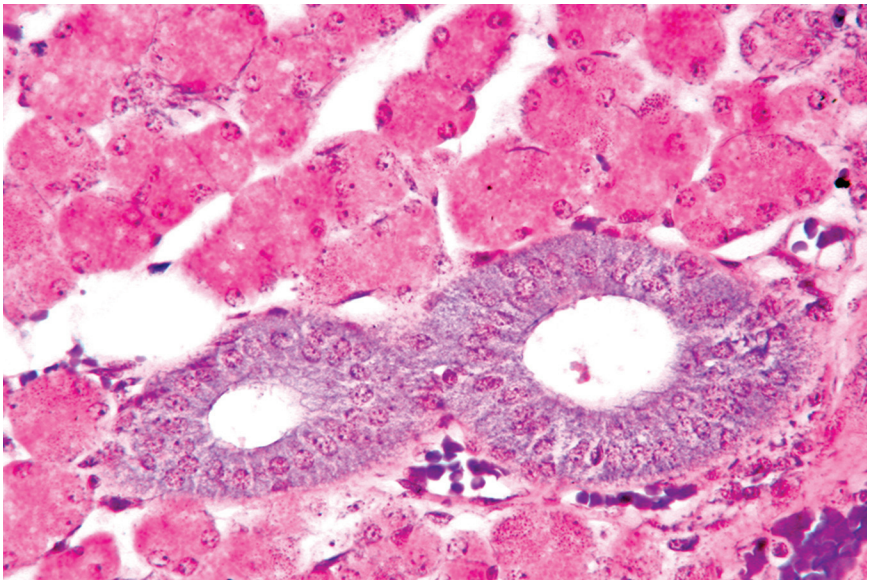


Figure 1-14

STRIATED DUCTS

Striated ducts stain with phosphotungstic acid-hematoxylin stain because of numerous cytoplasmic mitochondria.

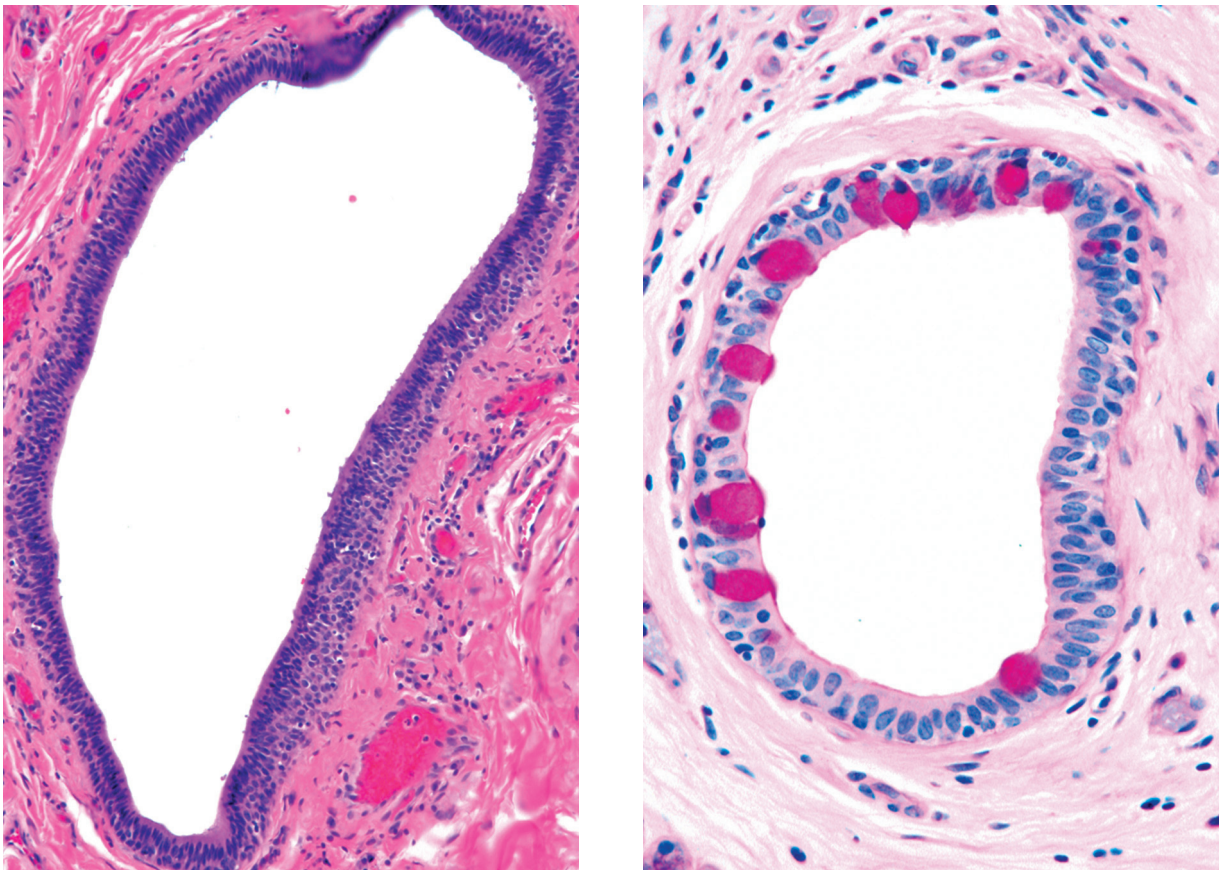
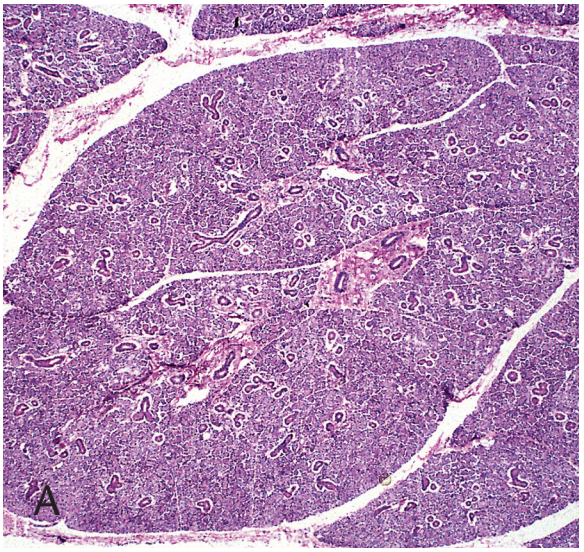
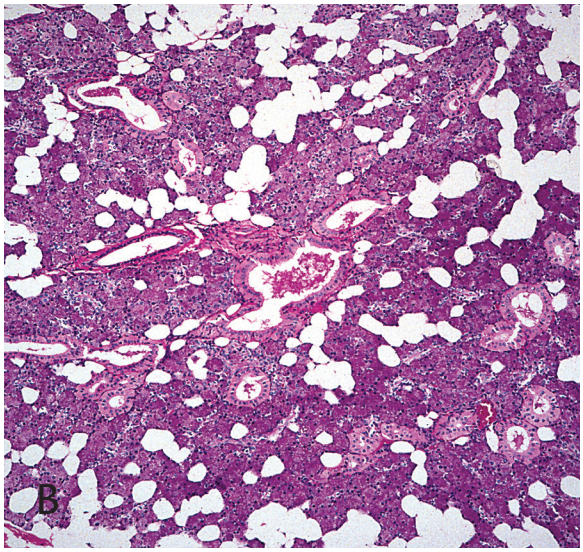


Figure 1-15

INTERLOBULAR EXCRETORY DUCT

Left: This large excretory duct is lined by pseudostratified columnar epithelium and supported by dense fibrous connective tissue. Right: Excretory ducts sometimes contain goblet-like mucous cells (PAS stain).





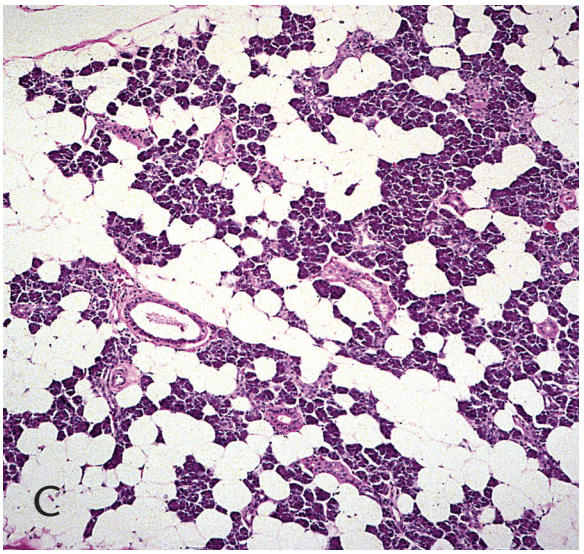


Figure 1-16

ADIPOSE TISSUE IN PAROTID GLAND

A: The parotid gland from a neonate contains no discernible adipose tissue.

B: A moderate amount of intralobular adipose tissue from the parotid gland of a middle-aged adult.

C: Prominent intraparotid adipose tissue from an elderly person.

mature adipose tissue increases with advancing age (fig. 1-16). Parotid fat content is higher in men (by about 10 percent) than in women and increases with age and body mass index for both sexes, consistent with an age-related reduction of acinar cells followed by subsequent replacement with adipose tissue (30). Since the secretory capacity of the parotid gland exceeds normal requirements, the decreasing parenchyma to fat ratio rarely results in xerostomia.

A detailed discussion of lymph nodes is found in the anatomy section of the parotid gland. Lymphoid tissue in the form of small, unstructured nodules and lymph nodes is found scattered within and around the parotid gland (fig. 1-17). As previously mentioned, some lymphoid aggregates lack true lymph node capsules as well as subcapsular and medullary sinuses. Periglandular nodes lie within the capsule of the gland. Heterotopic salivary gland tissue consisting of both glands and ducts is frequently entrapped in the medullary region of these lymph nodes. Heterotopic salivary gland tissue is also seen in several head and neck sites outside the salivary glands as well as in the mediastinum and thorax (6,31).

Sebaceous glands or small collections of sebaceous cells occur in most parotid glands, usually in the wall of a duct, but due to their scarcity are only infrequently encountered in routine examination of surgical pathology specimens

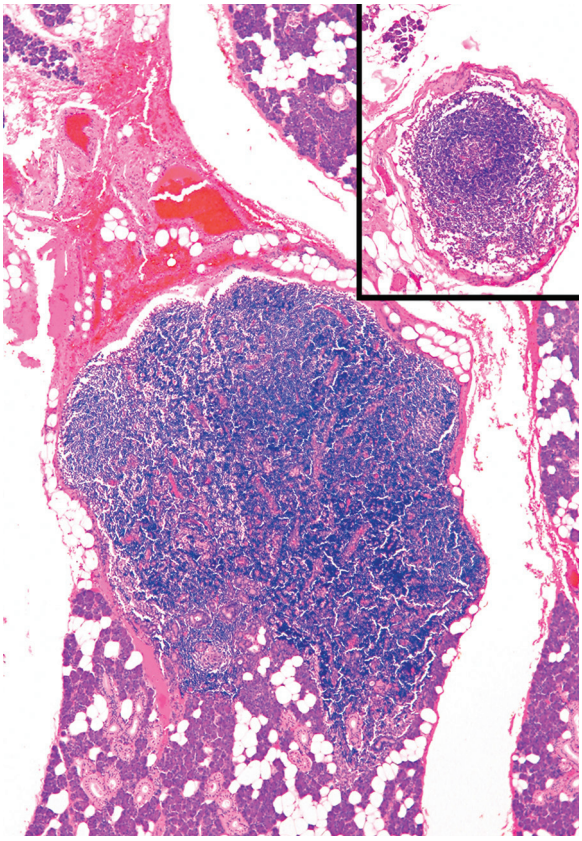


Figure 1-17

PAROTID LYMPH NODES

Intraparotid and periparotid lymphoid tissue varies from developed lymph nodes to nodules with little structure (inset).

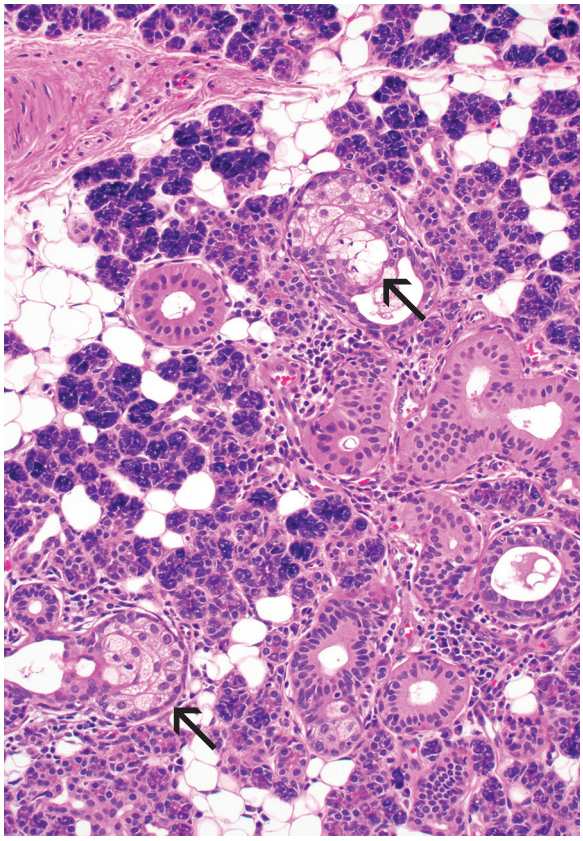


Figure 1-18

SEBACEOUS CELLS IN PAROTID GLAND

Foci of sebaceous cells occur in most parotid glands, but are so few in number that they are uncommonly noticed in tissue sections from surgical excision specimens.

(fig. 1-18). However, Martinez-Madrigal et al. (6) argue that their incidence is actually more common than thought, having found them in 42 percent of 100 parotid glands examined, but in only 5 percent of 100 submandibular glands. Unlike mucous cells, sebaceous cells are unreactive with the mucicarmine stain, but stain with androgen receptor. While sebaceous cells are a normal constituent of parotid glands, their presence and functional significance have not been satisfactorily explained. Branches of the facial nerve are often in contact with or in close proximity to parotid parenchymal tissue (fig. 1-19).

Submandibular Gland

The lobular architecture and parenchyma of acini, intercalated ducts, striated ducts, interlobular ducts, and main excretory duct of the submandibular gland are similar to those of the parotid gland (fig. 1-20). In contrast to the near universal serous nature of parotid acini, about 5 to 10 percent of submandibular acini are mucous. Acini with mucous cells are generally not purely composed of mucous cells but are a mixture of mucous and serous cells. Serous cells are typically arranged as crescent-shaped caps (demilunes) at the periphery of the mucous acinar cells, which have abundant, clear to faintly basophilic, finely granular to furrowed cytoplasm with basally oriented, round nuclei.

Mucous cells are reactive with PAS, mucicarmine, and Alcian blue stains (fig. 1-21). When compared to the parotid gland, intercalated ducts are shorter and less discernible, while striated ducts are longer and more conspicuous. Myoepithelial cells are obscure in routinely

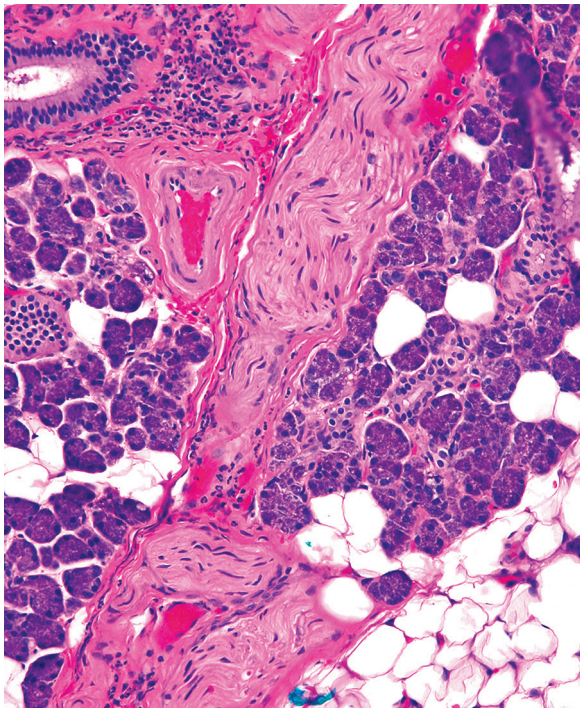


Figure 1-19

FACIAL NERVE BRANCHES IN PAROTID GLAND

Segments of peripheral nerve often are in close proximity to parotid parenchyma.

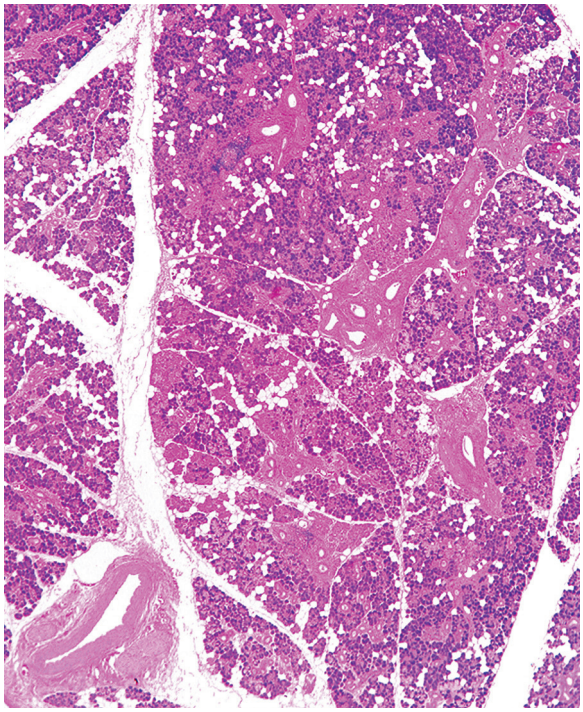


Figure 1-20

SUBMANDIBULAR GLAND

Although there is less fat in the adult submandibular gland than in the parotid gland, the lobular architecture is similar.

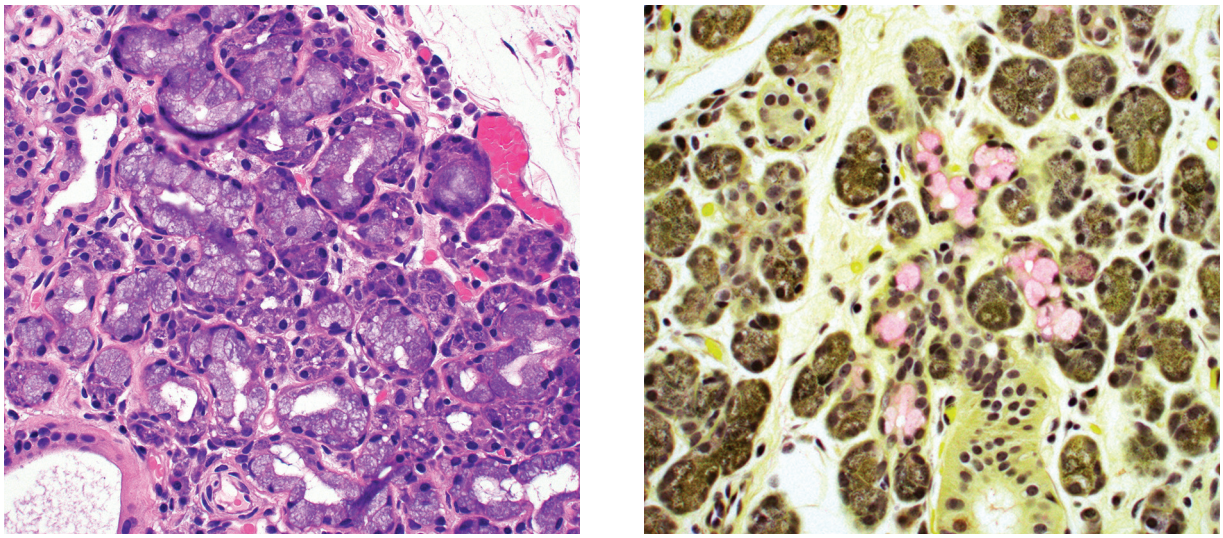


Figure 1-21

SUBMANDIBULAR GLAND ACINI

Left: Mucous acini comprise 5 to 10 percent of the acinar tissue. Serous cells are frequently located at the periphery of mucous acini. Striated ducts are more prominent and the intercalated ducts are shorter than those in the parotid gland.

Right: Mucicarmine stain highlights the mucous cells while the serous cells are unreactive.

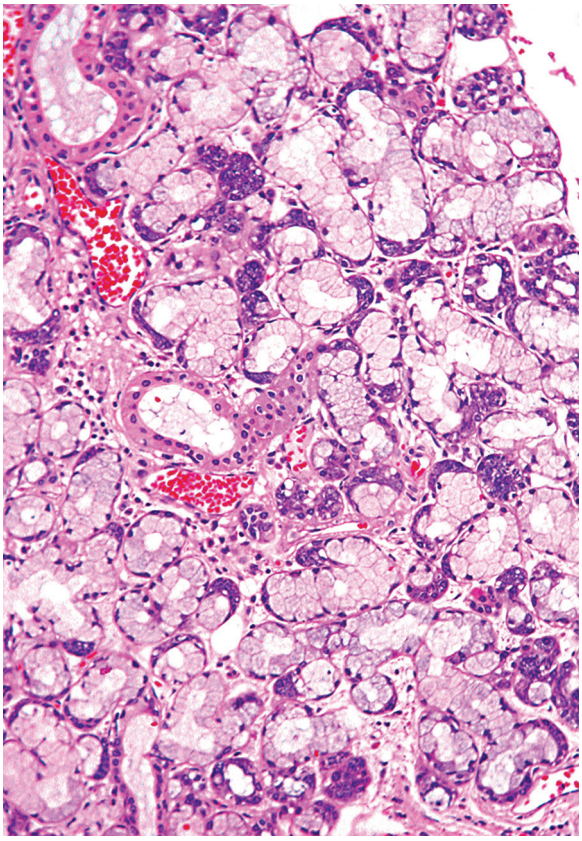


Figure 1-22

SUBLINGUAL GLAND

Sublingual gland acini are typically more elongated than those of either parotid gland or submandibular gland. Acini are predominantly mucous cells with serous cell demilunes.

stained tissue sections. For any specific age group, the amount of intraglandular adipose tissue is less than in the parotid gland. Lymph nodes, lymphoid nodules, and large peripheral nerves are not present within the gland.

Sublingual Gland

Most acini in the sublingual gland are mixed, composed of mucous cells with serous cell demilunes (fig. 1-22). These secretory end pieces are frequently elongated rather than spherical, like the acini seen in the parotid and submandibular glands. The lobular architecture is less organized, and both intercalated and striated ducts are shorter than in either of the other two major salivary glands. Similar to the submandibular gland, lymphoid tissue and large peripheral nerves are

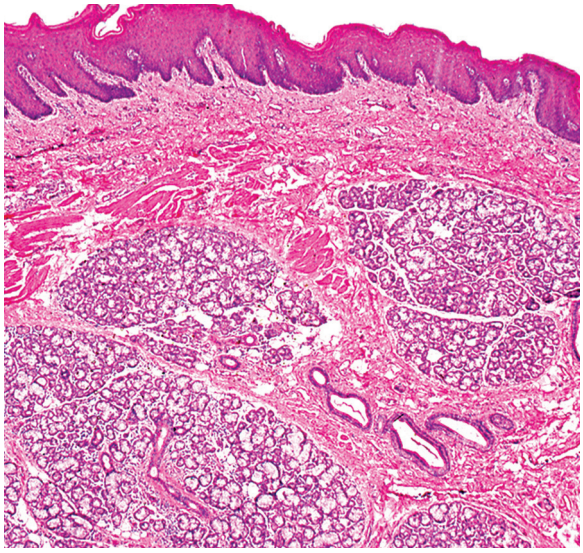


Figure 1-23

MINOR SALIVARY GLANDS IN TONGUE

In the tongue, lips, and buccal mucosa, lobules of salivary gland tissue are located beneath the mucosal epithelium and within the deeper skeletal muscle. These glands are unencapsulated and in contact with the adjacent muscle tissue.

not usually evident. There does not appear to be an age-related increase in intraglandular adipose tissue, as occurs in the parotid gland.

Several collecting ducts connect to the oral mucosa and submandibular gland duct. Azevedo et al. (32) found gender neutral significant differences with increasing age in sublingual glands, starting with acinar atrophy, followed by the presence of duct-like structures and ending with the replacement of the parenchyma by fibrous and/or adipose tissue. A mononuclear infiltrate progressed from focal to diffuse with increasing age.

Minor Salivary Glands

The intraoral minor salivary glands are scattered as small lobules within the oral mucosal and submucosal tissues of the buccal mucosa, lips, floor of mouth, hard and soft palates, tonsillar pillars, and tongue. Mostly unencapsulated, they lie in close contact with the muscles of the tongue and lips (fig. 1-23). The anterior hard palate and gingiva are generally devoid of salivary glands, but the retromolar mandibular ridge does contain salivary glands.

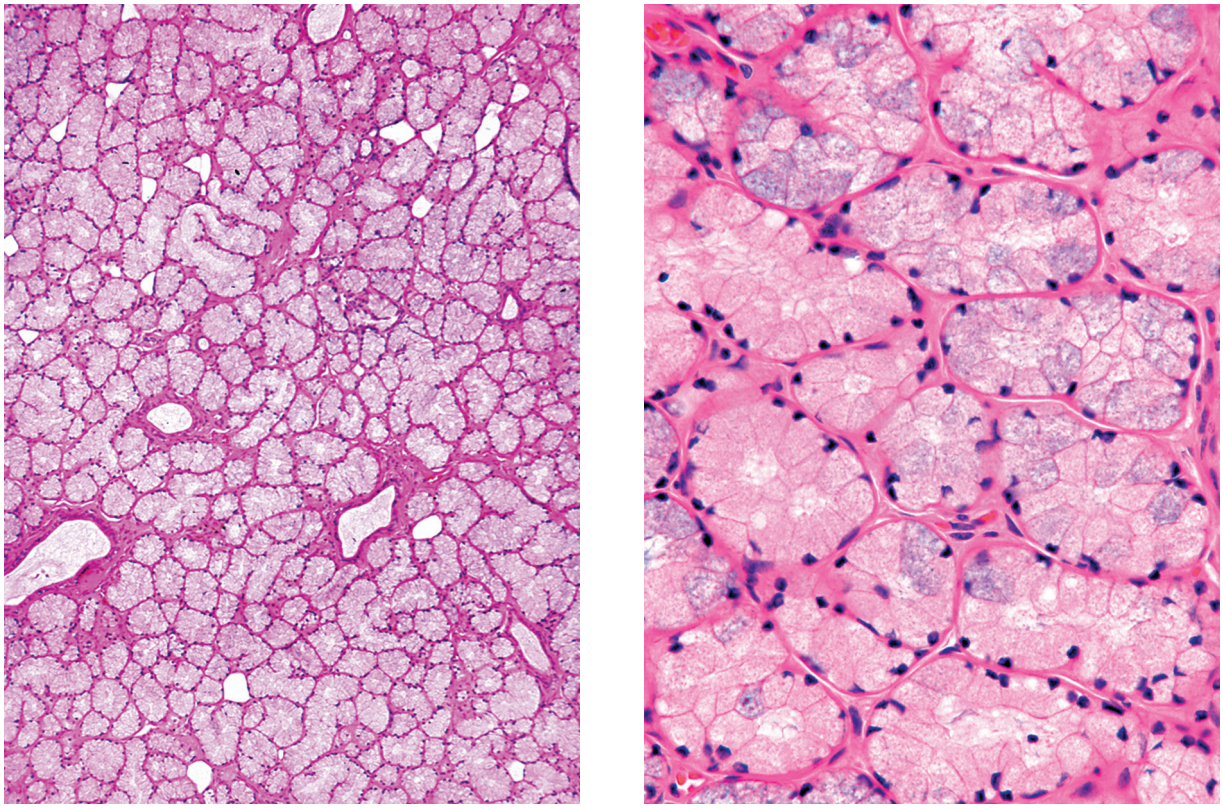


Figure 1-24

MINOR SALIVARY GLANDS OF PALATE

Left: The palate contains the largest foci of intraoral minor salivary gland tissue. These glands are composed only of mucous acini without serous acini.

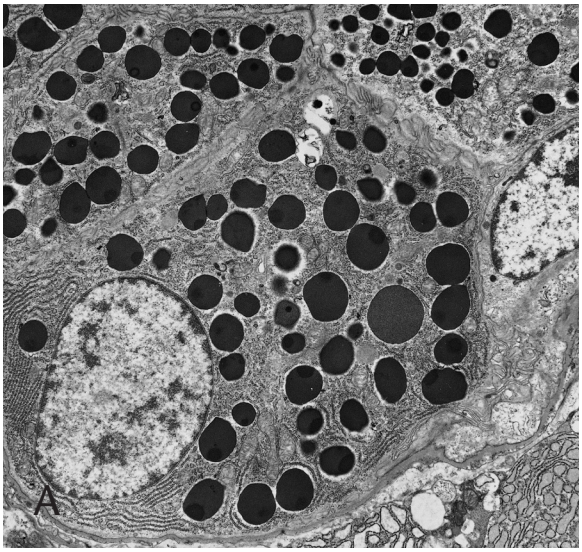
Right: Each rounded acinus is surrounded by a thin layer of connective tissue. The mucous cells surround small central lumens and are pyramidal shaped, with pale, granular cytoplasm and basally located nuclei.

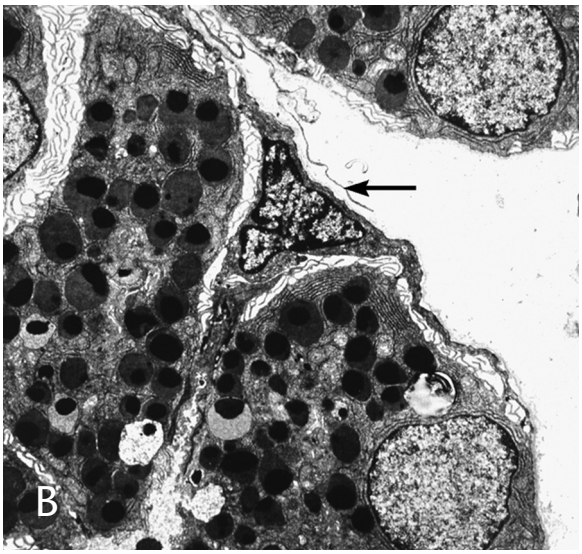
Glands in the posterior hard palate are pure mucous type without serous cells (fig. 1-24). In the tongue, salivary gland tissue is located on the anterior ventral portion, called Blandin and Nunn glands, which are mucous type, and in the region of the circumvallate papillae on the posterior dorsal and lateral portion, called von Ebner glands, which are serous type. Most of the other minor salivary glands are mixed mucoserous glands in which mucous cells predominate.

ULTRASTRUCTURE

Acinar Cells

Ultrastructurally, serous acinar cells contain innumerable rounded, membrane-bound cytoplasmic secretory granules of varying electron density and diameter, known as zymogen granules, located predominantly in the apical portion of the cell (fig. 1-25A). Their number depends upon the phase of secretory activity. Rough endoplasmic reticulum and Golgi complexes are abundant, and mitochondria are common. Basal lamina envelops the basal surface except where myoepithelial cells are interposed between acinar cells and the basal lamina. When the cell is not distended with secretory granules, the basal plasma membrane shows abundant infolding. Microvilli extend from the luminal surface into the intercellular space that is continuous with the lumen. In the intercellular space are complex interdigitations of plasma membranes of adjacent cells. Junctional complexes at the apical end of the cells and desmosomal attachments at sites along the lateral borders are seen (33).





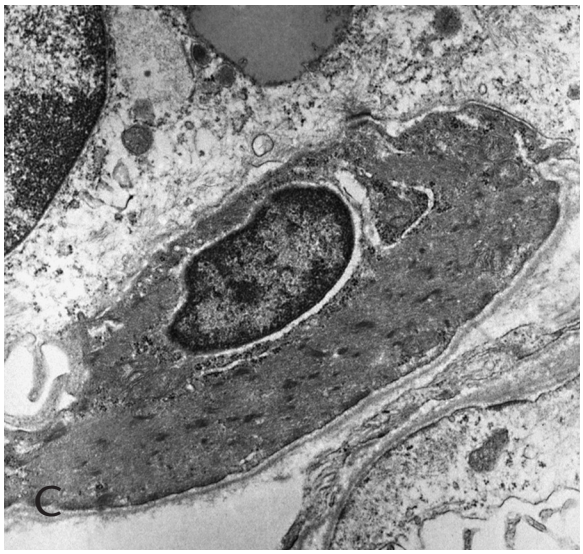


Figure 1-25

ULTRASTRUCTURE OF SEROUS ACINAR, MYOEPITHELIAL, INTERCALATED, AND STRIATED DUCT CELLS

A: Variable sized secretory granules occupy most of the apical portion of the cytoplasmic compartment of an acinar cell. Parallel arrays of rough endoplasmic reticulum lie adjacent to the basally located, round nucleus. The intercellular space contains interdigitations of adjacent cells and represents a canaliculus that is connected to the acinar lumen and is actually the beginning of the duct system.

B: A myoepithelial cell (arrow) is situated between the basement membrane and basal plasma membrane of the serous acinar cells. It has elongated cytoplasmic processes that extend over the surface of the acinar cells.

C: This myoepithelial cell lies adjacent to an intercalated duct cell, and contains numerous cytoplasmic fine filaments with focal dense bodies and focal densities along the basal plasma membrane adjacent to the basement membrane.

Mucous acinar cells are similar to serous cells but their secretory droplets of mucigen (precursor of mucin) are typically larger, more irregularly shaped, and more electron lucent. The number and size of secretory droplets and the prominence of the Golgi apparatus and endoplasmic reticulum vary with the functional stage of the cell. Secretory droplets often fuse to form very large droplets. Rough endoplasmic reticulum, Golgi apparatus, and mitochondria are mostly located in the basal portion of the cell. The lateral intercellular interdigitations are less complex than those of serous cells. The basal plasma membrane folds are more complex in mucous cells of the submandibular and sublingual glands than in mucous cells of the minor salivary glands, but minor salivary gland mucous cells have more complex lateral interdigitations (25,33).

Myoepithelial Cells

Abluminal myoepithelial cells (interposed between the plasma membrane of acinar and intercalated duct cells and their corresponding basal lamina) have a flattened, elongated contour, with cytoplasmic processes that spread over the outer surface of acinar and duct cells. Those adjacent to intercalated duct cells have fewer cytoplasmic processes (fig. 1-25B). Cell nuclei are elongated or irregularly shaped. Some of

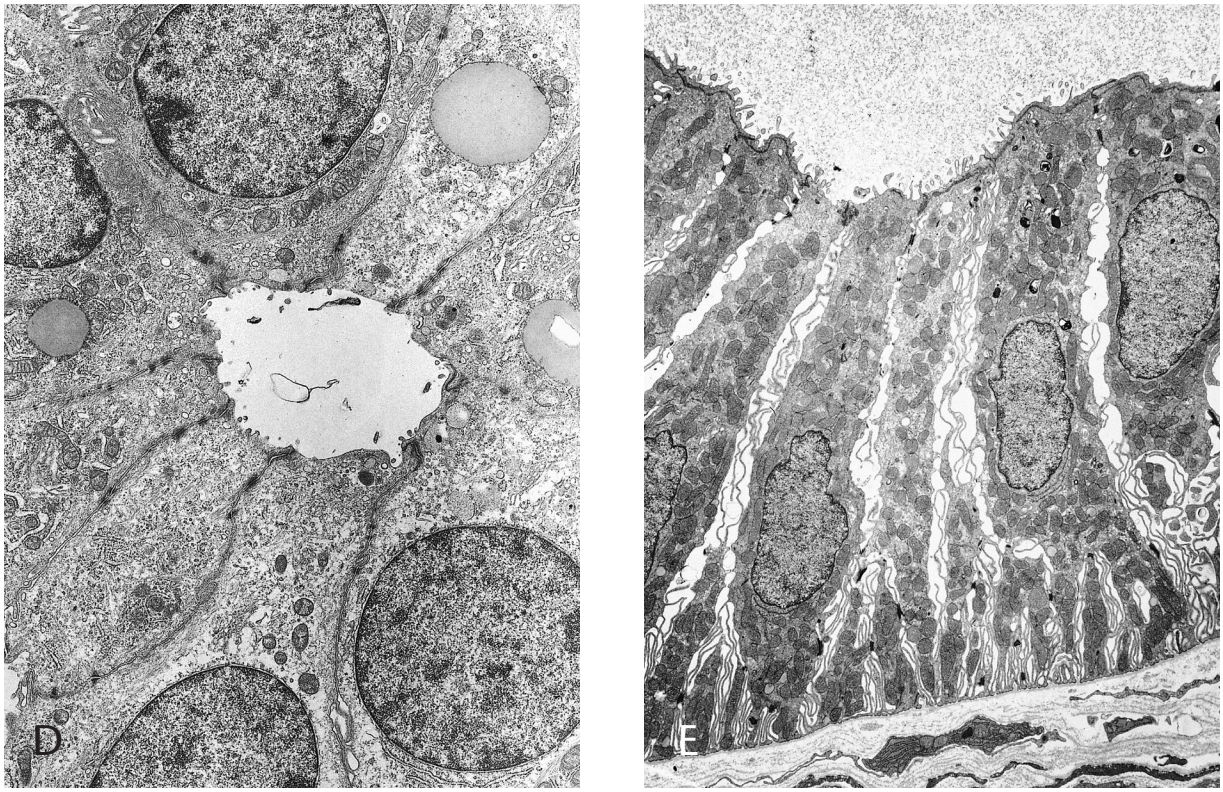


Figure 1-25, continued

D: The small cytoplasmic compartment contains a round nucleus, mitochondria, lipid vacuoles, and endoplasmic reticulum. A few short microvilli are on the luminal surface. Intercellular connections are apical junctional complexes with several desmosomes.

E: The basal plasma membranes have prominent vertical folds, and there are numerous mitochondria. The lateral surfaces of striated duct cells have processes that interdigitate with adjacent cells, and apical junctional complexes and desmosomes connect adjoining cells.

the shared features with smooth muscle include parallel arrays of filaments, which are gathered in "dense bodies" and are anchored to the basal plasmalemma in attachment plaques and focal densities and pinocytotic vesicles that align against the plasma membrane (fig. 1-25C) (26). Mitochondria, endoplasmic reticulum, Golgi vesicles, and lysosomes concentrate near the nucleus. Desmosomes attach myoepithelial cells to acinar and ductal cells, and tonofilaments are present in some cells (34).

Intercalated Duct Cells

Intercalated duct cells are distinguished by their simplicity and absence of special ultrastructural features. Sometimes there are ducts of intermediate morphology, usually at the edge of a given lobule, and continuing between lobules. A few cells in proximity to acinar cells may contain some secretory granules; otherwise, the scant cytoplasm is nonspecific, with rough endoplasmic reticulum, Golgi, and mitochondria. The cells are connected by junctional complexes and a few desmosomes (fig. 1-25D) (33).

Striated Duct Cells

Light microscopic cytoplasmic striations are seen as an extensive vertical folding of the plasma membranes ultrastructurally. Laterally, the basal folds form a complex interdigitation with the plasma membrane folds of adjacent cells. The cytoplasm is rich in mitochondria, and small amounts of endoplasmic reticulum and Golgi apparatus are present (fig. 1-25E). The transition between striated ducts and excretory ducts is not necessarily abrupt (35). Junctional complexes with desmosomes on lateral surfaces and short microvilli on luminal surfaces are present (33).

Table 1-2

SELECTED IMMUNOHISTOCHEMICAL MARKERS IN NORMAL SALIVARY GLANDS

	Acinar Cells	Intercalated Duct Cells	Striated Duct Cells	Excretory Duct Cells	Myoepithelial Duct Cells	Basal Cells
CK[a] AE1/AE3	+	++	++	++	weak +	weak +/ neg.
CK7	weak +/neg.[b]	++	++	++	weak +/neg.	weak +/ neg.
CK8	weak +/ neg.	++	++	++	weak +/ neg.	weak +/ neg.
CAM5.2	++	++	++	++	weak +/neg.	weak +/ neg.
EMA	++	++	++	++	neg.	neg.
HMW CK CK34βE12, CK5/6]	neg.	weak +	weak +	++	+/++	++
p63	neg.	neg.	neg.	neg.	+/++	+/++
p40	neg.	neg.	neg.	neg.	+/++	+/++
calponin	neg.	neg.	neg.	neg.	+/++	neg.
S-100 protein	neg.	+ or neg.	neg.	neg.	+/ neg.	neg.
SMA	neg.	neg.	neg.	neg.	+/++	neg.
GFAP	neg.	neg.	neg.	neg.	+/ neg.	neg.
CEA	++	+	neg.	neg.	neg.	neg.
DOG1	+	weak +/ neg.	neg.	neg.	neg.	neg.
SOX10	++	+	neg.	neg.	++	neg.

[a]CK = cytokeratin; SMA = smooth muscle actin; EMA = epithelial membrane antigen; HMWCK = high molecular weight cytokeratin; CEA = carcinoembryonic antigen; GFAP = glial fibrillary acidic protein.
[b]Neg. = negative.

IMMUNOHISTOCHEMISTRY

Since the publication of the previous Fascicle, immunohistochemistry has evolved as a powerful adjunctive tool in the identification of cellular differentiation and the correct classification of salivary gland neoplasms. This section only discusses immunohistochemistry as applied to normal salivary glands; subsequent chapters highlight immunohistochemical staining as applied to a variety of salivary gland neoplasms. Table 1-2 lists some of the more commonly used immunohistochemical markers for the different histologic components of normal salivary gland.

Acinar cells stain intensely with epithelial membrane antigen (EMA), low molecular weight cytokeratins 7, 8, and 19, and gross cystic disease fluid protein (GCDFP) (36–39). SOX10 is another marker of acinar cells as well as intercalated duct cells (40). Its expression in mucinous acini is weaker than in serous acini (41). DOG1 expression in salivary tissues is localized mainly to serous acini where it displays an apical staining pattern of moderate intensity, while mucous acini show a similar pattern, but of lesser intensity (fig. 1-26A) (42,43). Enzymatic acinar cell markers include alpha-amylase, lactoferrin, and lysozyme. Markers of ductal and acinar cells used in the past (α1-antichymotrypsin, α1-antitrypsin, transferrin, lactoferrin, secretory component, and lysozyme) have been supplanted in daily use by the other markers listed in Table 1-2.

Myoepithelial cell immunohistochemical stains focus principally on myogenic proteins. Although myoepithelial cells may coexpress CK14, CK17, and CK19, more specific markers include calponin, p63, p40, CD29, podoplanin (D2-40), h-caldesmon, muscle-specific actin, alpha-smooth muscle actin, and myosin heavy chain (fig. 1-26B) (39,44–47). Smooth muscle myosin heavy chain and h-caldesmon have poor sensitivity (39). Ianez et al. (46) report that alpha-smooth muscle actin, calponin, S-100 protein, and p63 are present from the earliest stages of salivary gland maturation (fig. 1-26C). Glial fibrillary acidic protein (GFAP) is variably expressed by myoepithelial cells; it is usually

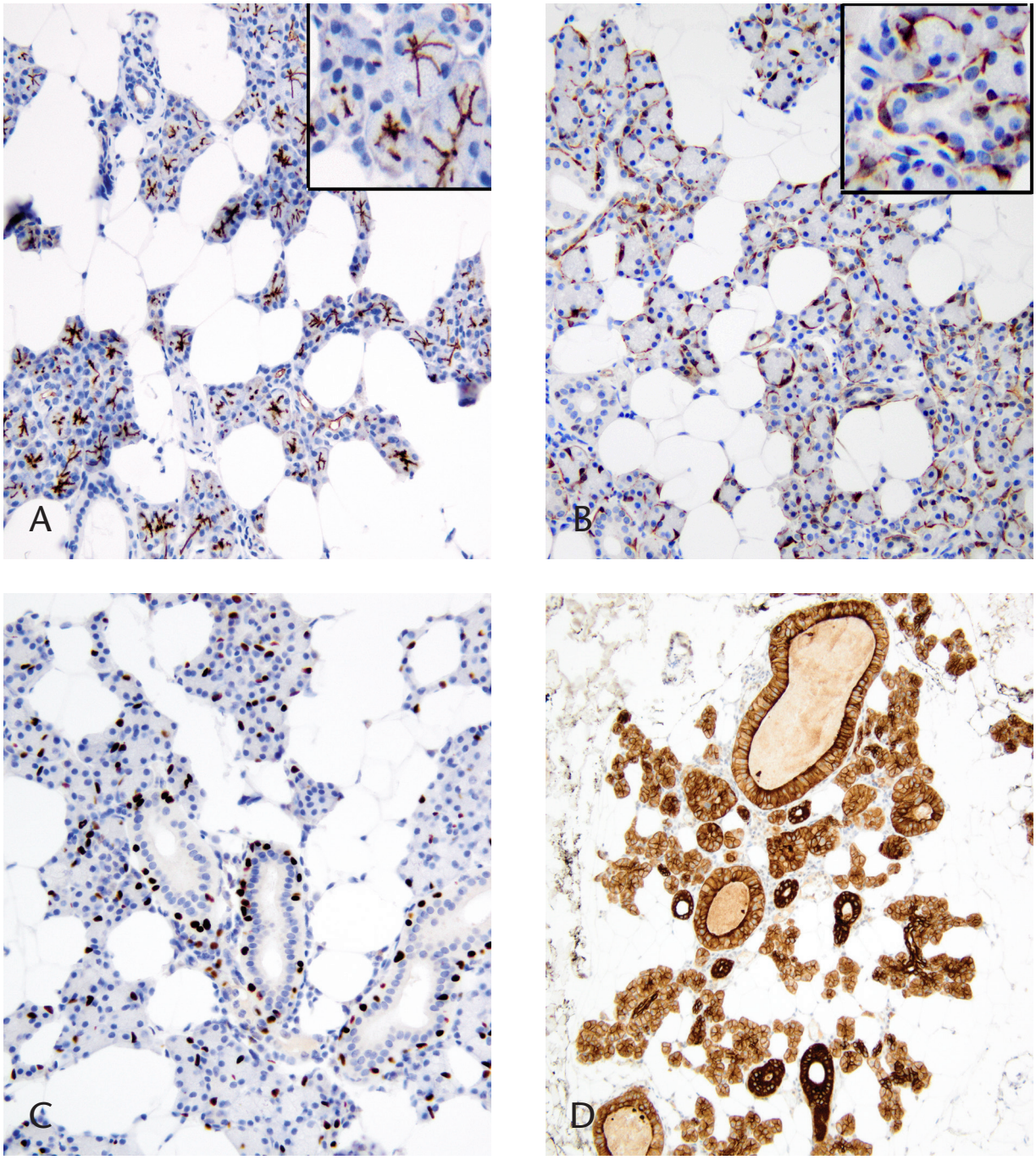


Figure 1-26

IMMUNOHISTOCHEMISTRY OF NORMAL SALIVARY GLAND

A: The apical luminal membranous pattern of staining with DOG1 is seen within parotid acinar cells, with no expression in the striated duct (lower left).

B: Abluminal calponin stained myoepithelial cells, many exhibiting a stellate shape, extend their delicate cytoplasmic process to envelop luminal acinar cells.

C: p63 staining occurs both in nuclei of myoepithelial cells and basal cells surrounding the striated ducts in this field.

D: Acinar cells, striated duct cells, and excretory duct cells all express strong cytoplasmic and membranous staining with CAM5.2. A nearly identical staining pattern is seen with pancytokeratin AE1/AE3.

not expressed in normal myoepithelial cells, but is seen in many neoplastic myoepithelial cells (39,41,46). The myoepithelial markers maspin, CD10, and podoplanin are infrequently applied to routine diagnostic work due to low specificity (48–50).

S-100 protein expression in myoepithelial cells in normal salivary glands and neoplastic myoepithelial cells in various salivary gland tumors has produced variable results (25). Work by Dardick et al. (51) has shown that S-100 protein is not expressed by normal salivary gland myoepithelium. SOX10, a well-known nuclear marker of neural and melanocytic cells, is also expressed in normal myoepithelial cells of the salivary glands, as well as by acinar cells and in intercalated ducts (both luminal and abluminal cells) (41,52). Basal cells stain with CK14, CK17, CK18, CK19, p40, and p63 (39).

Luminal ductal cells of the intercalated, striated, and excretory ducts all stain with pancytokeratin AE1/AE3, CAM5.2, CK7, CK8, CK18, CK19, and EMA (fig. 1-26D). Staining also occurs with high molecular weight cytokeratins such as CK5/6 and 34βE12 (53,54).

Extracellular matrix proteins, such as fibronectin, laminin, tenascin, and type IV collagen, and fibroblast growth factors are found in the basement membrane region of acinar and ductal salivary tissues. Laminin and type IV collagen occur close to glandular epithelium at all stages of salivary gland development, from bud formation to the acinar differentiation stage (55). In the normal salivary gland, the expression of tenascin is restricted to stromal tissue around the intercalated ducts, striated ducts, nerve bundles, and blood vessels, with interruptions of staining around some ducts (56). Tight junction proteins claudins 1, 2, 3, 4, and 16, occludin, and junctional adhesion molecules are expressed in major salivary glands and claudins 1, 3, 4, 7, and 11 are expressed in minor salivary glands (57). Their diagnostic utility remains to be determined.

PHYSIOLOGY

Our current understanding of salivary gland physiology is largely derived from the study of nonhuman salivary glands (58). The exocrine glands of the three major salivary glands contribute approximately 90 percent of the daily 1.0 to 1.5 L of saliva production (averaging 1 mL/minute) with 600 to 1,000 minor salivary glands serving to moisten the mucosal lining of the upper aerodigestive tract. The level of production is cyclical, with less than 5 percent of saliva produced during sleep (thus reducing the need to swallow during that time), and about 80 to 90 percent in response to gustatory and masticatory stimuli.

In a 1992 study, the critical range separating persons with normal gland function from those with hypofunction was more precisely identified as unstimulated whole salivary flow rates between 0.12 and 0.16 mL/min (59). Other factors that influence salivary flow include circadian rhythm, psychic factors such as depression, anticipation of food, and medications. Salivary flow can be augmented by the stimulus of chewing as well as by the muscular activity of the lips and tongue. Salivary flow does not occur evenly throughout the mouth. Regional variation in intraoral flow is site specific, with the mandibular lingual being a site of high volume and the maxillary anterior and interproximal regions being sites of low volume flow (60).

Contributions to salivary flow vary among the different salivary glands. During unstimulated flow, only 20 to 25 percent of saliva originates from the parotid gland, while 65 to 70 percent is from the submandibular, 7 to 8 percent from the sublingual, and only trace amounts from the minor salivary glands. Once stimulated, however, the flow rates drastically change among glands, with the parotid gland contributing 50 to 70 percent of total salivary secretion (61). Regarding flow rates from minor salivary glands, Wang et al. (62) found the buccal glands to have the highest flow rate, consistent with that reported in the literature. Flow rates of palatal glands were similar to those of the upper and lower labial glands in contrast to other reports that showed palatal flow rate to be lower than that from labial glands (63). The exact role of advancing age on average daily production of saliva is unknown, and is complicated by the multipharmaceutical intake, nutritional status, and presence of systemic disease more often associated with the elderly.

Among its multiple functions, saliva serves to lubricate the oral cavity and enable speech, swallowing, eating, tasting, dental health, and

maintaining oral hygiene and homeostasis, while also providing protective functions and aiding in digestion (61,64). It accomplishes the latter through the action of alpha-amylase and lipase which facilitate the breakdown of starches and fat in food during mastication. Amylase is primarily present in parotid saliva; concentrations in both submandibular and sublingual saliva are less than one quarter of those in parotid saliva (65). Because of the high concentration and activity of salivary amylase, a fraction of ingested starch is digested before the food bolus reaches the stomach. Although serous cells produce a watery fluid that is high in amylase, mucous cells generate a more viscous substance that is higher in heavily glycosylated glycoproteins and provides a tenacious, viscoelastic coating of all surfaces in the oral cavity and acts as an important lubricant between opposing surfaces during such processes as mastication, swallowing, and speaking (65).

By neutralizing acids, saliva plays a role in taste perception, rendering foods less sour, and protects taste receptors of the tongue from desiccation by bathing and hydrating them. The hypotonicity of unstimulated saliva allows the taste buds to perceive different tastes without being masked by normal plasma sodium levels (61). For example, if unstimulated saliva had the same concentrations of sodium and chloride as those in plasma, one would be unable to taste salty solutions more dilute than that of plasma. Similarly, unstimulated saliva also contains glucose, bicarbonate, and urea (the main bitter-tasting substance in saliva), but all at concentrations below their taste recognition thresholds, thereby facilitating the sensation of taste (65).

Immunoglobulin A (IgA), enzymes, glycoproteins, and peptides contained in saliva, including lactoferrin, lysozyme, lactoperoxidase, statherin, and histatins, serve as lines of defense against infection. IgA (produced by plasma cells in the interstitial tissue of the salivary glands and actively transported into the salivary secretions by the acinar and ductal epithelial cells) serves to aggregate bacteria and prevent their adhesion to oral and hard surfaces (66). Lubrication also creates a protective barrier against hydrolytic and proteolytic enzymes produced in plaque, and from chemical agents.

MUC5B, the primary gel-forming mucin in the oral cavity, which is secreted by mucous cells in the submandibular, sublingual, palatal, and labial salivary glands, and MUC7 both influence the composition of the oral microbiota. MUC7 is less efficient as lubricant, but more efficient in bacterial agglutination and clearance than MUC5B and therefore an important part of the salivary nonimmune defense system (66). The buffering action of bicarbonates helps to counteract the acids that accumulate in plaque thereby assisting in the prevention of dental caries (67). Statherin, a salivary peptide, contributes to the stabilization of calcium and phosphate salts solution, serves as a lubricant to protect the tooth from wear, and may initiate the formation of the protective pellicle by binding to hydroxyapatite (67). Saliva contributes to the formation of the acquired enamel pellicle and mucosal pellicle, which cover the oral hard and soft tissues, respectively, and thereby helps to modulate the initial adhesion and colonization of microorganisms and shape the composition of the resident oral microbiota (66). Other crucial roles of saliva include assistance in the excretion of endogenous substances such as antibodies and blood-group reactive substances (58,65–68).

Saliva is about 99.5 percent water, hypotonic with a specific gravity of 1.002 to 1.012, and has a pH that varies from 5.75 to 7.05 which hinders the growth of pathogenic oral bacteria. The remaining constituents are inorganic electrolytes (calcium, sodium, bicarbonates, potassium, etc.), organic analytes including immunoglobulins (primarily immunoglobulin A), enzymes (lysozyme, lactoferrin, peroxidase, and alpha-amylase), mucins, and macromolecules.

As a watery substance, saliva's composition varies from gland to gland, with the parotid glands producing mostly serous fluid, the submandibular glands mostly viscous and mucinous fluid, and the sublingual glands producing slightly more viscous fluid. Acinar cells produce either serous or mucous secretion, which contains water, salts, and proteins, while the ductal cells modify the secretion, primarily by reabsorbing the salt. The stellate myoepithelial cells, which surround the acini and intercalated ducts are innervated and facilitate secretion by contraction (69).

Salivary gland cells are intimately associated with the autonomic nervous system. The glos-

sopharyngeal nerve provides innervation to the parotid gland for the secretion of saliva (70). Parasympathetic and sympathetic nerves run together with Schwann cells to the target cells in salivary glands (68). Innervation of both the submandibular gland and the sublingual gland occurs via the parasympathetic fibers carried by the facial nerve. Postganglionic parasympathetic and sympathetic nerves are in contact with many cell types in salivary glands, including acinar, ductal, and myoepithelial cells and blood vessels. The secretion of saliva is controlled by a salivary center composed of nuclei within the medulla (inferior salivatory nucleus). Neuronal control of secretion occurs in response to stimulation by the autonomic nervous system or the action of substances that can mimic the effects of this stimulation.

Although both sympathetic and parasympathetic nerves innervate the salivary glands, the effects of the latter predominate, and are the principal stimulus for salivary gland secretion. Parasympathetic supply eventually reaches the parotid gland from postganglionic fibers by the auriculotympanic branch of the trigeminal nerve, while those to the submandibular gland travel via the nervus intermedius and chorda tympani to the submandibular ganglion. Sympathetic supply originates from the superior cervical ganglion. When sympathetic innervations dominate, secretions contain high levels of protein from acinar cells, whereas predominant parasympathetic innervations produce a more watery secretion. Sympathetic stimulation also causes myoepithelial contraction (58,60,71).

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