Chapter 57: Physiology

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The study and elucidation of the physiology of salivary tissues have evolved more slowly than of other tissue/organ systems. Indeed, only since the late 1970s have the misconceptions and misinterpretations of earlier data been corrected; with these corrections has come an appreciation of the physiology's complex nature. The excretory-secretory functions of the salivary duct system rival those of the renal tubules in their metabolic activity, and only recently have the acinar cell processing and delivery of their products been postulated. Saliva, the medium produced by salivary tissue, also remains to be fully characterized despite investigations dating back to Greco-Roman times.

The information presented in this chapter is as contemporary as possible but should be regarded as only the beginning of our understanding of salivary physiology.

Normal Physiology

The salivary glands have a variety of functions. They provide (1) a lubricant to aid in the swallowing of food, mechanical cleaning, and immunologic defense; (2) digestion by an emulgation of food and enzymatic cleavage (primarily starch by alpha-amylase); (3) production of hormones or hormonelike products and other metabolically active compounds; (4) excretion of endogenous and exogenous materials, for example, antibodies, blood group-reactive substances, iodine, and viruses; (5) mediation of taste sensations; and (6) defense, which is pertinent only to subhuman species.

For their size the salivary glands produce a large volume of saliva: the maximal rate in humans is about 1 mL/min/g of glandular tissue. The rate of metabolism of salivary glands is also high, accompanied by a high blood flow - both proportional to the rate of saliva formation. The flow of blood to maximally secreting salivary glands is approximately 10 times that of an equal mass of actively contracting skeletal muscle.

Autonomic control

With the exception of the vasodilator action of bradykinin and the stimulation of ductal sodium ion and potassium ion transport by aldosterone, the physiologic control of salivary glands is almost solely effected by the autonomic nervous system (Argleve, 1981; Garrett, 1975). Stimulation o either sympathetic or parasympathetic nerves produces salivary secretion. The effects of the parasympathetic nerves are predominant; interruption of the sympathetic innervation causes little or no change in function. If interruption of the parasympathetic supply occurs, however, salivary glands undergo atrophy.

Probably there is no truly spontaneous secretion from either the parotid or the submandibular glands. All secretion of saliva results from stimulation of the glands' autonomic innervation or by the action of the substances mimicking the effects of such stimulation.

Functional studies with electrical stimulation of nerves continue to provide new information about the respective roles of the diferent nerves involved in salivary secretion. Garrett (1989) rightly points out that the different nerves and their various transmitters allow a harmonius interplay and the interaction is not "a contest between two solo performers" (Table 57-1). The principal stimulus for inducing flow of saliva is parasympathetic. Sympathetic impulses serve more as modulators of the composition of the saliva. There is also some indication that nerve impulses may have an important role in the resynthesis of intracellular secretory material (Garrett, 1989).

Table 57-1. Salivary gland innervations and functional correlates

Parasympathetic	Sympathetic
Principal stimulus for fluid formation	Weak-to-poor mobilizer of fluid
May be isolated	Additive to parasympathetic; may yield synergistic effect
Causes some exocytosis	Increase exocytosis and modulates composition of saliva
Induces contraction of myoepithelia	Usually induces contraction of myoepithelia
Causes vasodilatation as part of secretory process	Some fibers maintain vascular tone; believed to be anatomically separate from secretory nerves.&

The innervation patterns of the major salivary glands differ considerably, not only from species to species but also between glands within the same subject and between different cell types within a gland. The human parotid gland is furnished equally well with both sympathetic and parasympathetic nerve fibers (Chilla, 1981; Garrett, 1975). Preganglionic parasympathetic fibers from the ninth cranial nerve (CN XI) travel to the optic ganglion and from there as secretomotor fibers to reach the parotid gland via the auriculotemporal nerve. Sympathetic fibers from the cervical ganglia accompany the blood vessels to the salivary gland. Gangliocytes are not found in the gland itself. Once within the gland, the postganglionic sympathetic and parasympathetic fibers reach myoepithelial cells, intercalated ducts, and acinar cells. Cholinergic and adrenergic terminal axons innervate the same cells, with receptors found on the cell membranes (Garrett, 1975).

Electron-optic study shows two types of neuroeffector arrangements in the glands: epilemmal and hypolemmal (Chilla, 1981; Garrett, 1975). In the former the axon lies outside the epithelial basement membrane; in the latter the axon penetrates the masement membrane. Neuroeffector sites occur where a nonomyelinated axon is in intimate relationship with adjacent salivary cells, and neuroactivation takes place where sufficient transmitter is released to activate adjacent cell(s). This depends on the distances between axon and cells and also on the sensitivity of the cell membranes. Most hypolemmal axons are cholinergic. No pattern of order exists, however, and at certain sites hypolemmal axons have been found in the submandibular gland, whereas both adrenergic and cholinergic hypolemmal axons occur in relation to parotid acinar cells (Garrett, 1975).

The parasympathetic nerves are primarily responsible for the low of saliva during reflex secretion via acetylcholine released from nerves acting on the membranes of effect cells (Chilla, 1981; Garrett, 1975). The electrical changes and the movement of ions induced by acetylcholine are associated with permeability changes in activated cell membranes.

Electrophysiology

With a few (nonsalivary) exceptions, all stimulats to secretion cause membrane potential changes in their target cells, and this results at least in part from changes in membrane conductance to certain ions (Petersen, 1980). Two distinct factors contribute to the electrical properties of a cell surface. One is the presence of "fixed" negative charges associated with phospholipids, glycolipids, and glucoproteins that constitute the plasma membrane - the so-called surface potential; the other factor is the difference in charge between the inside and the outside of cells - the so-called transmembrane potential. The surface potential contributes little to transmembrane potential but it seems to affect the ability of certain stimuli to collapse the transmembrane potential in excitable cells; that is, the ability to generate an action potential. Electrogenic pumps in the plasma membrane, such as the sodium pump, contributes in varying amounts to the setting of membrane potential (Petersen, 1980; Shannon et al, 1974).

Neurotransmitters, hormones, and other regulatory molecules affect the function of salivary cells in a complex manner that is only beginning to be understood (Garrett, 1975; Petersen, 1980; Shannon et al, 1974; Williams, 1984). Nearly all of the effects result from a direct regulation of target cells. Recepts, second messengers, and effectors are essential to this regulation of cell function (Table 57-2). With the parotid gland as the prototype, the following is presented to illustrate the interactions of the various modules in the electrophysiologic patterns seen.

Receptors

The receptors are moieties that interact with ligands involving recognition and transfer of information (Lefkowitz et al, 1984a). The interaction initiates a biologic response or sequence of responses. The quality of the biologic response resides in the receptor-effector and not in the ligand. The binding of a ligand to a specific receptor is the first step in the regulation of cell function by extracellular factors. The majority of regulatory receptors are found in the plasma membrane (proteins or glycoproteins); a few (hormonal) are present within the cell. Once occupied by its specific regulatory molecule, the receptor complex activates an effector process to bring about the cellular response. Activation is either direct or by a second messenger. A receptor may be a single or composite membrane protein and may contain subunits with special functions.

Little is known of the molecular characteristics of salivary gland receptors. Probably, however, adrenergic, muscarinic-cholinergic, and other receptors are similar to those found on other cells. Receptors for polypeptide hormones and neurotransmitters are on the plasma membrane, and for exocrine glands they are on the basal or lateral membranes (Stumpf, 1984). For some cells, receptor occupancy and biologic effects follow saturation kinetics; for other cells, regulation does not require full occupancy (Lefkowitzx et al, 1984a).

Two major classes are alpha-adrenergic and beta-adrenergic receptors. Lefkowitz et al (1984b) have defined two subtypes of each receptor, termed alpha1 and alpha2 and beta1 and beta2. Table 57-3 outlines their properties. Three of the four subtypes of adrenergic receptors are linked to the same biochemical effect on the adenylate-cyclase system, which generates the second messenger, adenosine 3':5'-cyclic phosphate (c-AMP). The beta1 and beta2 receptors stimulate the enzyme, whereas the alpha2 receptors inhibit it. Rather than being coupled to adenylate cyclase, alpha1 receptors appear to be coupled to processes that regulate cellular calcium-ion fluxes (Lekowitz et al, 1984b).

Second messengers

Cyclic AMP and cytosol-free calcium ion have been the most intensively studied second messengers. Others, considered to be messenger-like systems, include guanylic acid (GMP), phospholipids, cytosol pH, and membrane depolarization (Williams, 1984). In the parotid gland or pancreas, intracellular calcium ion serves as a trigger for acutely stimulating acinar function. The relationship, however, between receptor occupancy, intracellular calcium ion, and the induced response is not known.

Williams (1984) has indicated that specific second messengers are not typically linked to specific biologic effects. In the parotid gland, calcium largely mediates stimulation of fluid secretion, whereas in the pancreas, calcium is the major messenger stimulating enzyme release from acinar cells. Conversely, cAMP in salivary glands mediates macromolecular secretion and fluid from the pancreas. Probably the two messengers potentiate each other. The messenger-like systems may be involved in the formation of second messengers, function as transduction mechanisms, or be induced as tertiary phenomena. Not all hormones or neurotransmitters possess known second messengers. They may act as their own second messengers through enzyme activation of their receptors (Williams, 1984).

Besides the generation of cAMP as a mechanism of neurotransmitter signal transduction, it is likely that a second signal transduction mechanism exists, and this relates to phsophatidylinosital-4, 5 biphosphate turnover (Michell, 1984).

Effectors

Far less is known about these systems than of receptors or second messengers. The protein-kinases in salivary tissue are, however, similar to those in the pancreas and other tissues (Williams, 1984). Both cAMP and calcium-activated kinases exist. All of the kinases phosphorylate different subset of proteins. Control of exocytotic secretion may involve phosphorylation of granule or plasma membrane or activation of contractility by phosphorylation of the light chain of myosin (Williams, 1984). A calcium-activated monovalent cation-selective channel is another effector system; that is, ion flux (influx of Na+ and Ca++ and efflux of K+) leads to a depolarization of the cell (Garrett, 1975; Williams, 1984).

Secretion

Saliva consists of two components: macromolecules and fluid. The fluid component is derived primarily from perfusing blood vessels; the macromolecules are primarily derived from secretory granules of the acinar cells. The fluid is produced at the secretory end-pieces and is currently thought to occur via an osmotic coupling (solute-solvent coupling) of trans-epithelial fluxes of sodium chloride and water (Izutsu, 1989). It is likely that the water and electrolyte fluxes occur transcellularly through acinar cells. Movement of sodium and chloride through the cell and into the acinar lumen provides an osmotic gradient to establish an accompanying water flow across the cell. The currently favored hypothesis to explain how sodium and chloride cross the basal cell membrane is that a co-transport of the two ions occurs with the movement of sodium down its electromechanical gradient driving the accumulation of chloride against its electrochemical gradient (Izutsu, 1989).

For the salivary glands the precise method by which secretion is brought about is still largely unknown. Cyclic AMP- and calcium-activated phosphorylation of cellular substances are currently believed to be the major effects leading to macromolecular secretion (Williams, 1984). In the case of electrolyte secretion, attention is directed primarily to ion channels and carriers by which ions enter the cells or to the energy-dependent Na+-K+ pump, which carries out ion extrusion. Petersen (1980) and Williams (1984) have postulated a calcium effector system and/or a channel linking extracellular and intracellular compartments permeable to calcium and capable of different conformations corresponding to different calcium permeabilities. Voltage-sensitive channels permeable to sodium, potassium, or calcium ions open after a depolarization of the plasma membrane (Findlay, 1984; Petersen, 1980). The sodium pump exists in all cells, and since more sodium ions are actively pumped out than potassium is taken up, it contributes directly to the membrane potential. The pump is primarily activated by an increase in sodium. Calcium-sensitive ion channels, permeable to sodium, potassium, or chloride ions, open when calcium increases (Petersen, 1980). This is in addition to a calcium-activated channel. Possibly, cyclic nucleotides (AMP, GMP) activate ion channels or pumps.

Despite the many gaps in our knowledge of the electrophysiology of salivary acinar cells, the action of the major secretagogues can be summarized as follows. In mammalian salivary glands, acetylcholine and epinephrine and/or nor-epinephrine acting on alpha-receptors cause an increase in potassium and sodium ion permeability of the plasma membrane. This results in a pronounced reduction of the surface membrane resistance and a loss of potassium from the cells, balanced by an uptake of sodium. An active electrogenic extrusion of sodium and accumulation of potassium follow. An increase in calcium permeability probably mediates the permeability of sodium and potassium. Epinephrine and/or norepinephrine, acting on receptors, produce only small potential and resistance changes. The most important effect of beta-adrenergic activation is an increase in intracellular cAMP, stimulating enzyme secretion. Cholinergic or alpha-adrenergic stimulation causes a marked fluid and some calcium-dependent enzyme secretion.

There is a dissociation of cellular secretion mechanisms underlying protein secretion and fluid/electrolyte secretion in the salivary glands. Of the three major cationic electrolytes - sodium, potassium, and chloride - sodium is the only one with a pattern of very low concentrations at lowest flow rates to high concentrations at the highest rates of flow. Sodium

is also the primary contributor to the increasing osmolality of the fluid as the level of secretion increases (Shannon et al, 1974). In human parotid saliva, sodium is the key in the secretion of fluid because local osmotic effects across the secretory luminal membrane influence the generation of the fluid (Shannon et al, 1974).

Acinar secretion

Secretory cells such as the acinar cells of the parotid gland discharge their products by a process of *exocytosis,* wherein fusion of secretory granules with a delimited portion of the plasmalemma at the apex of the acinar cell occurs. The membrane fusion is the last of a series required for the transfer of export proteins from their synthesis in the rough endoplasmic reticulum (RER) to the extracellular environment. Using the model of Palade (1975), the secretory process can be divided into six successive steps: (1) synthesis, (2) segregation, (3) intracellular transport, (4) concentration, (5) intracellular storage, and (6) discharge.

Synthesis of secretory proteins requires the uptake of amino acids by cells. Much of this synthesis is accomplished by an active transport from the extracellular pool via carrier systems located in the basolateral plasma membrane, with transfer-RNA molecules delivering the amino acids to the ribosomes of the RER (Palade, 1975). Production and processing occur in the RER and the Golgi complex, as they do in all eukaryotic cells (Hand and Oliver, 1984). Most of the polypeptides produced pass through the Golgi cisternae. Carbohydrate synthesis is also believed to take place in the Golgi complex, but it is more variable than polypeptide synthesis in the RER, which is strictly template controlled (Williams and Cope, 1981).

After synthesis, the secretory proteins are segregated in the cisternal space of the RER. Segregation is regarded as an irreversible step, and the vectorial transportation leading to it is probably obligate for all protein-secreting cells (Palade, 1975).

From the cisternal space of the RER the secretory proteins are transported to the Golgi complex. The intracellular transport requires energy (adenosine triphosphate synthesis). Condensation and maturation follow after the secretory proteins reach the condensing vacuoles in a dilute solution. The final result of the concentration step is the conversion of the condensing vacuoles into mature secretion granules. Concentration does not depend on a continuous energy supply but is highly sensitive to pH, with lysis occurring above pH 7.2 (Palade, 1975).

Terminal glycosylation of secretory proteins also occurs in the Golgi comlpex, and at that level the product is transferred from a high-permeability membrane to a membrane whose lipids approach that of the plasmalemma.

Temporary storage of secreting proteins in the cell is within secretion granules (condensing granules at the end of the concentration step). Their membranes come from the Golgi complex, and their content is an outcome of attached polysomes that have undergone modification (Palade, 1975; Williams and Cope, 1981).

Discharge of the secretion granules into the glandular lumina happens by a process of exocytosis (membrane fusion). Such a process is generally accepted for the discharge of macromolecular secretory products. Morphologically the membrane of the secretion granule fuses with the plasmalemma, with a fusion of the fused membranes within the area of fusion (Palade, 1975; Williams and Cope, 1981). A continuity is thereby established between the granule and the extracellular lumen and ensures maintenance of a continuous diffusion barrier between the interior of the cell and the extracellular medium. In exocrine cells the fuion site is limited to the luminal domain, and the consequent exocytosis requires calcium ions and energy (often a cyclic nucleotide-generating system and one or more protein kinases plus a depolarization of plasmalemma).

The procedures the cell uses to recover and redistribute membrane after exocytosis are not known. Recovery of membrane in the form of endocytic vesicles with translocations to Golgi vacuoles and cisternae is most likely. Appreciable amounts of membrane material are probably discharged into the lumen, but as Fig. 57-1 suggests, excess apical membrane, removed by interiorization, is then disassembled within the cell to provide new protein molecules via a "cryptic pool" (Williams and Cope, 1981).

Saliva

The variability of the composition of saliva can be accounted for by the fact that the several classes of salivary glands contribute different constituents and also because the final product depends on the stimuli evoking the secretion (Table 57-4). The concentration of many of the inorganic constituents further depends on flow rate.

Whole or mixed saliva is composed of approximately 99.5% water and has a specific gravity between 1.002 and 1.012. In humans the amount of saliva secreted in 24 hours is between 1000 and 1500 mL (Argleve, 1981; Mandel, 1980). The secretory rate is highest during meals; during sleep or in the absence of stimulation the secretory rate is low or nearly absent.

The pH of saliva primarily depends on the relative concentrations of free and combined CO_2: the ratio of H_2CO_3/$NaHCOO_3$ and the pH varies directly with the CO_2 content of blood. These bicarbonates (and to a lesser degree, phosphates) buffer saliva. In human mixed saliva the pH varies from 5.75 to 7.05 (Arglebe, 1981).

If data on flow and composition are to be meaningful, the saliva must be collected under standardized conditions (Argleve, 1981; Mandel, 1980). Resting and stimulated secretions should be evaluated, and because many constituents of saliva are circadian, the times of collection should be uniform.

The electrolyte composition of saliva from the parotid and submandibular glands shows differences between each source and is markedly different from plasma. In general, parotid-gland concentrations are higher than those from the submandibular glands (Arglebe, 1981; Mandel, 1980). Calcium is the main exception, with submandibular calcium nearly twice the concentration of parotid calcium. The electrolytes are relatively independent of plasma concentrations because they reflect an active transport system. Sodium and chloride are directly related to flow rate. Calcium concentrations appear to be flow dependent only at

high flow rates.

The organic (nonelectrolytic) analytes are passively diffused from plasma as opposed to actively transported and thereby reflect blood concentrations (Mandel, 1980).

Table 57-5 presents the composition of saliva in adults. The components are expressed in mean values. The flow-rate data were documented in mean values. The flow-rate data were documented after 2% citric acid was applied to the tongue as the stimulus. After such stimulation, sodium, chloride, bicarbonate, and calcium concentrations increase with an increase in the rate of flow, but magnesium, phosphates, urea, ammonia, and uric acid decrease. Protein is variable in the parotid saliva but increases in saliva from the submandibular gland. The pH increases with flow rate.

The flow rate in nonstimulated parotid glands is about 0.04 mL/min/gland; submandibular saliva has a somewhat higher resting flow rate, 0.05 mL/min/gland (Mandel, 1980). This decreases markedly during sleep: the sleeping flow rate from the parotid gland is nearly nil.

Flow rates after stimulation vary with the stimulus. With usual gustatory stimulation a 0.6 mL/min/gland flow rate is obtained. A ranges of 0.4 to 1.0 is not uncommon. Discomfort from dryness is usually not a complaint until the rate of flow is below 0.2 mL/min/gland (Mandel, 1980).

Proteins or protein-containing moieties make up the majority of the organic components of saliva. Of the two major salivary glands, the concentration of protein is higher in stimulated parotid than is stimulated submandibular secretions (Arglebe, 1981; Mandel, 1980). Albumin passively diffuses into the glandular secretions from plasma. The major salivary glands are regarded as principal sources of salivary antimicrobial substances such as secretory immunoglobulin-A (IgA), lactoferrin, lysozyme, and peroxidases (Moro et al, 1984). Salivary and other epithelial IgA differ from that of serum by possessing an additional "secretory component", which is free in saliva as well as bound to dimers of serum-type IgA. Secretory component can be immunoreactively identified as being on the basolateral surface of the epithelial cells, where it serves as a receptor for polymeric J chain-containing immunoglobulins (Moro et al, 1984). A partial listing of protein analytes in saliva follows:

Albumin
Globulin
Enzymes (alpha-amylase, peroxidases, muramidase (lysozyme), phosphates, hydrolase, dehydrogenases, arginase, esterases)
Glycoproteins (blood grou-reactive substances, other glycoconjugates, and so forth)
Proline-rich proteins
Histidine-rich proteins
Tyrosine-rich proteins
Amino acids
Polypeptides.

Plasma cells in the connective tissues of all salivary glands produce salivary IgA, and ductal and acinar epithelial cells selectively transport it into the secretions. Secretory component is synthesized separately from IgA ni the glandular cells and moves to the basal and lateral plasma membranes. Polymeric IgA from the blood may also contribute a small amount of salivary IgA and is also transported by the secretory component - IgA complexes (Moro et al, 1984).

Blood group-reactive substances, as are found in the ABO and Lewis systems, are found on the surface of salivary cells and in saliva. The concentration of the substances in saliva is low (10 to 130 mg/L), with labial salivary glands being the richest source (Arglebe, 1981). Parotid saliva contains only small amounts, if any, of the blood group-reactive substances.

A number of enzymes can be identified in saliva, but alpha-amylase is quantitatively the most prominent (Arglebe, 1981). It has been estimated that the total daily secretion of amylase in humans is approximately 1.6 g (Arglebe, 1981): 60% is excreted by the pancreas, the remainder by salivary tissues. Seventy percent of the salivary contribution is from the parotid gland. Like all enzyme proteins, amylase exists in many molecular forms; that is, isoenzymes distinct from other alpha-amylases (Arglebe, 1981; Mandel, 1980).

The biosynthesis of the individual salivary glycoproteins is incompletely understood, but their biologic functions are believed to be related to discrete structural domains. The salivary glycoproteins are involved in tissue coating, lubrication of dental and soft tissues, microbial clearance and antimicrobial activity, digestion, buffering capacity, post-translational processing, and heterotypic complexing (Cohen and Levine, 1989). The purpose of kallikreins in salivary tissues appears to be to process a number of hormones, notably epidermal growth factor and nerve growth factor (Bothwell et al, 1979).

The box above separates the salivary glycoproteins according to their cellular origin and molecular families. The mucin glycoproteins have at least two members in their family: a multisubunit species that has a higher molecular weight and a single subunit species that has a lower molecular weight. Examples of the serous glycoproteins are proline-rich glycoproteins, alpha-amylases, and salivary peroxidase. Single-species glycoproteins (lactoferrin, kallikrein, fibronectin) are those which do not occur as component members of structurally related families, with secretory IgA being the exception.

Salivary polypeptides. Since the discovery of kallikreins in submandibular glands, an increasing number of polypeptides have been either isolated and purified from, or claimed to be present in, the submandibular glands of mammals, primarily mice (Barka, 1980). Most are androgen dependent and have their secretion mediated primarily by alpha-adrenergic mechanisms. Nearly all are found in or considered to be synthesized by the granular convoluted tubules/striated duct cells where they are localized in secretory granules. Most are found in saliva, and several in both saliva and blood. The presence in the latter inevitably raises the question as to the "endocrine or endrocrine-like" function of the submandibular gland (Barka, 1980). Several of the polypeptides certainly fulfill the biochemical properties of a hormone, even though excretion rather than secretion is the dominant mode of release. Barka suggests that they may play important roles in developmental and homeostatic mechanisms, basing this assumption on their phylogenetic and structural conservations. For

the submandibular gland at least, an expansion of the diffuse neuroendocrine system would accommodate its putative paracrine-endocrine-neuroendocrine-neural regulatory mechanisms.

The known and putative polypeptides in submandibular glands, modified from Barka (1980), are listed as follow:

1. Known, purified, and characterized

a. Nerve growth factor (NGF)

b. Epidermal growth factor (EGF)

c. Renin

d. Kallikrein

e. Some peptidases

2. Probably present, not purified

a. Erythropoietin

b. Glucagon and glucagon-like substances

c. Angiotensin II

d. Certain other enzymes

3. Possibly present

a. Mesoderma growth factor

b. Lymphoid (thymotropic) factors

c. Somatostatin

d. Gastrin

e. Other miscellaneous growth factors (epithelial, endothelial, and neural tube).

Epidermal growth factor

One of the most extensively studied factors found in the submandibular gland, EGF appears to have important physiologic roles (Barka, 1980; Murphy et al, 1980). It is a potent mitotic stimulator, and it enhances keratinization and inhibits gastric acid secretion. Human EGF is considered to be nearly identical to urogastrone. The factor is localized in the granular convoluted tubular (GCT) cels and confined largely to the secretory granules of these cells, where it is also synthesized. This localization is similar in the submandibular glands of

mouse, rat, and man. The level of the factor is androgen dependent, and alpha-adrenergic mechanisms mediate secretion in saliva. To date, EGF has been singled out as one of the several factors that may regulate cell replication of a variety of normal and perhaps neoplastic cells (Barka, 1980; Murphy et al, 1980).

Nerve growth factor

NGF plays an important role in the development and maintenance of the functional integrity of sympathetic and some sensory neurons (Barka, 1980). The mouse's submandibular gland is unique in its capacity to *synthesize, store,* and *secrete* large amounts of NGF (Barka, 1980). The factor is unequivocally localized in the secretory granules of the granular convoluted tubules of the duct system (Murphy et al, 1980). The concentration of NGF manifests a prominent sexual dimorphism, being higher in male mice, and the level is androgen dependent. NGF's role in saliva is unknown despite its high concentration in saliva from the submandibular gland. Its secretion is mediated primarily by alpha-adrenergic mechanisms. Submandibular gland NGH undoubtedly contributes to the steady state of the factor in blood, where it maintains the functional integrity of the sympathetic nervous system (Murphy et al, 1980).

Renin

Like NGF and EGF, submandibular gland renin is confined to the secretory granules of GCT cells (Bing et al, 1980). A similar sexual dimorphism and androgen dependence on the concentration also exist. Secretion into saliva, where its physiologic role is unkown, is stimulated by both alpha-adrenergic and cholinergic mechanisms. In contrast, release of renal renin is mediated by beta-adrenergic receptors and inhibited by alpha-adrenergic receptors. Plasma angiotensin II concentrations do not have any impact on the release of submandibular-gland renin (Bing et al, 1980).

The presence of renin in the submandibular glands certainly implies a role in the renin-angiotensin-aldosterone system, despite observations that removal of the gland has no significant effect on plasma concentrations of renin (Bing et al, 1980). The findings of other angiotensin-forming enzymes, pseudorenin, and tonin in the submandibular gland are further related implications (Bing et al, 1980).

Kallikreins

Kallikreins, which are serine proteases, have been found in and isolated from submandibular glands of many mammalian species - including man, where they exist in many molecular forms (isoenzymes).

The kallikreins are localized in duct cells (secretory granules of the GCT or striated ducts) (Barka, 1980; Kimura and Moriya, 1984; Orstavik, 1980). No sexual dimorphism exists in kallikrein contents. In the rat the submandibular gland is the richest source of glandular kallikrein, and it may contribute to the blood concentration of the enzymes.

The glandular kallikreins' physiologic role is poorly understood. They may be involved as mediators of functional vasodilation, and an interrelation between the renin-angiotensin and

kallikrein-kinin systems has been suggested (Orstavik, 1980).

Peptide hydrolases

The submandibular glands contain a number of peptidases and proteases that are androgen dependent and nearly exclusively found in the GCT cells (Barka, 1980). These enzymes may also exhibit kininogenase activity and may be related to kallikrein. They may perform important regulatory functions in the submandibular glands by serving as processing enzymes to form stable complexes with biologically active polypeptides. Other suggested biologic functions are vascular effects and stimulation of growth (Barka, 1980).

Glucagon or glucaogon-like substances

Current information about the role of salivary tissues in glucose homeostasis is far from clear despite the presence of glucagon-like immunoreactive substances in the submandibular glands of several species and effective competition with pancreatic glucagon for specific receptors on rat liver cell plasma membrane (Barka, 1980). There is a reported sexual dimorphism. The substances have not been found in the saliva of man and do not contribute to plasma levels.

Altered Physiology of Salivary Tissues

Changes in the composition of saliva may occur in various diseases affecting salivary glands or other tissues in the body. In mucoviscidosis, for example, the concentrations of sodium, calcium, phosphorus, urea, and uric acid are increased. The thyroid and salivary glands share a similar iodide-concentrating mechanism, with the inorganic iodide being secreted in saliva because conjugation with proteins does not take place in salivary glands (Chisholm and Mason, 1975). A decrease in salivary sodium occurs in primary hypertension, and protein alterations are seen in diabetes mellitus and Sjögren's syndrome, among other diseases. Drug-induced alterations are also well known (Setser et al, 1979; Simson et al, 1974).

All or nearly all of the causes of an altered physiologic response by salivary glands have either a defective (increase or decrease) stimulation of or obstruction of (change in low rate) secretion, or physical-chemical changes of product as their underlying bases. Sialadenosis, sialolithiasis, radiation sialadenitis, and Sjögren's syndrome illustrate this.

Sialoadenosis

Sialoadenosis, or sialosis, is a noninflammatory, parenchymatous disease of the salivary glands whose origins are based in metabolic and secretory disorders of the functional salivary parenchyma and are clinically manifested by recurrent, painless swelling of the salivary glands, principally the parotid (Seifert, 1971; Seifert and Donath, 1975).

The association of sialadenosis with disorders of endocrine glands, malnutrition, and several neuropathic disorders has led to its classification into three types (Chilla, 1981).

1. *Hormonal.* Parotid enlargement has been described in connection with nearly all endocrine disorders. That accompanying diabetes mellitus is the most common.

2. *Dystrophic-metabolic.* Primarily nutritional in basis, this form is seen in protein deficiency, the malnutrition of alcoholics, and vitamin deficiencies such as beriberi and pellagre.

3. *Neurogenic.* This type of sialadenosis is an outcome of dysfunction of the autonomic nervous system.

Morphologic and biochemical investigations indicate that changes or disorders of the salivary glands' acinar protein secretion are responsible for sialadenosis. Changes in the secretory behavior can be the result of either an excessive stimulation or inhibition of secretion, and both of these are probably aberrations of the autonomic nervous system (Chilla, 1981).

Light and electron-optic study of parotid tissue from patients with sialadenosis demonstrates enlarged acinar cells filled with zymogen granules. The granules, however, are different in different subjects. Donath and Seifert (1975) have distinguished the following three different types of sialadenosis based on the ultrastructural appearance of the granules:

1. *Dark granule type,* in which densely packed, protein-rich secreting granules are found. No evidence of increased protein synthesis exists.

2. *Light granule type,* in which the acinar cells contain low-optical-density secreting granules. The acinar cells in this form manifest signs of an increased synthesis of proteins.

3. *Mixed granule type,* in which there is a simultaneous presence of light and dark acinar cells.

These types of human sialadenosis can be induced experimentally in the rat by isoproterenol (light granule type) or guanacline (dark and mixed type) (Chilla, 1981).

Pathogenetically, all of the varied clinical associations, as well as the wide variation in the results of sialochemical and sialometric determinations observed in the sialadenoses, can be explained using peripheral autonomic neuropathology as the fundamental basis.

Sialolithiasis

Sialoliths (calculi) constitute one of the most common causes of salivary gland dysfunction. The composition of salivary calculi has been well studied with methods including histochemical studies, chemical analysis, and electron-optic examinations (Anneroth et al, 1978; Blatt, 1964; Harrill et al, 1959). The calculi consist of a laminated structure of concentric shells of calcareous materials alternating with layers of organic resinous material (El Deeb et al, 1981). Carbohydrates and amino acids compose the organic matrix; the crystalline components consist of calcium phosphate in the form of hydroxyapatite with small amounts of magnesium, carbonate, and ammonium ions. The distribution of the mineral elements varies extensively from one calculus to another. Some are lamellar; others are

homogeneous (Hiraide and Nomura, 1980).

The process of stone formation is clearly enhanced in the presence of stasis of salivary flow within the duct of the salivary gland. The submandibular gland is the most susceptible for salivary calculi because of the anatomic arrangement of its principal duct and the physiochemical characteristics of its secretions. The nonstimulated saliva from the submandibular gland has a higher pH, higher mucin content, and about twice as much calcium as has parotid saliva. All of these favor mineralization of a mucoid gel formed in the duct system of the submandibular gland (Anneroth et al, 1978). The following, modified from El Deeb (1981), lists physiochemical and anatomic factors predisposing to calculi in the submandibular gland.

1. Submandibular saliva

a. High mucin content

b. Alkaline pH

c. High percentage of calcium and phosphate salts

d. Concentration of calcium and phosphate salts

e. Low carbon dioxide level

f. High phosphatase enzyme content

2. Anatomy

a. Length and irregular course of Wharton's duct

b. Dependent position of gland and its duct system

c. Position of ductal orifice

d. Size of orifice smaller than duct lumen.

Whereas knowledge of salivary calculi in the major salivary glands is relatively well documented, information concerning those in minor salivary glands is limited. Reports indicate that 92% of salivary calculi occur in the submandibular gland, 6% in the parotid gland, and 2% in both the sublingual and minor salivary glands (El Deeb et al, 1981). Probably, however, minor salivary calculi are more common than the literature presents (Anneroth and Hansen, 1983). The principal locations are in the buccal and upper labial mucosa (Table 57-6). The lower lip is seldom involved, and the alveolar and palatal mucosae are even less often afflicted. The preponderant localization of major salivary gland calculi to the submandibular gland and of the minor salivary gland calculi to the buccal mucosa and upper lip suggests that local factors such as trauma and duct morphology may be important. It should be noted that the duct lengths in the labial and buccal mucosal glands are longer than those in the palate and that biochemical and biophysical properties of the secretions are

more favorable to stone formation in the lip and buccal mucosa (Jensen et al, 1979).

Sjögren's syndrome

Current evidence indicates that Sjögren's syndrome is the result of a lymphocyte-mediated destruction of exocrine glands, which in turn leads to diminished or absent glandular secretion and mucosal dryness (Batsakis, 1982; Moutsopoulos et al, 1980). The syndrome is a consequence of altered immunoregulation in which destruction of the salivary exocrine parenchyma occurs. Pathogenetically, the syndrome and the observed salivary and extrasalivary lesions possibly represent a graft-versus-host diseaselike process, in which the histocompatibility antigens of ductal epithelium or lymphoid cells are changed, so that self-recognition does not occur. Patients at risk are those with accompanying genetic abnormalities of T-cell and B-cell cooperation. In vitro tests, in vivo tests, and the high incidence of non-Hodgkin's lymphomas in patients with the syndrome provide clinical and laboratory support for this reasoning (Batsakis, 1982).

Sialochemistry in patients with Sjögren's syndrome also appears to be characteristic and distinguishable from that in patients with parotitis. Stuchell et al (1984) indicate that the parotid saliva in patients with the syndrome manifests increased sodium, chloride, IgA, IgG, lactoferrin, and albumin. Phosphates are decreased. The increase in IgA is almost all 11S in type - not 7S, which is the major component in patients with parotitis. This finding suggests that the increase in IgA is a result of local synthesis and not a reflection of the serum increase of IgA, as is the case in parotitis.

Cystic fibrosis

Salivary tissue involvement in this recessively inherited disease is preponderantly of the mucous-type glands, especially as manifested by a change in the composition and flow rate of salivary fluid in the labial minor salivary glands. These glands have a disease-related increase in sodium concentration and a decrease in flow rate. According to Izutsu (1989), there are three principal hypotheses for cystic fibrosis-related changes in salivary gland function: (1) alterations in intracellular calcium concentration, (2) autonomic dysregulation of the secretory process, and (3) decreased chloride permeability.

The histomorphologic changes are nonspecific and consist of duct ectasia, inspissation of secretions, and acinar atrophy.