Paparella: Volume I: Basic Sciences and Related Principles

Section 2: Physiology

Part 2: Head and Neck

Chapter 16: Physiology of the Salivary Glands

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The salivary glands function as organs responsible for the maintenance of an oral environment conducive to the mastication of food. Studies of the components of saliva have also demonstrated a protective role in maintaining oral and dental hygiene and in the prevention of dental caries. Multiple antibacterial systems have been described and their roles in the prevention of oral and systemic diseases continue to be elucidated. The discovery of biologically active polypeptides in salivary glands with properties as cytoprotective agents, growth factors, and homestatic factors has opened a new field of investigation into the organic consitutents of saliva.

The production of saliva and its role in the oral phase of digestion have been the subject of extensive study since the 19th century when such great physiologists as Mueller, Baylis, Bernard, Pavlov, Ludwig, and Heidenhain used their investigations of the salivary glands to contribute to the understanding of cellular biology. Current investigations into the production of saliva have greatly expanded our knowledge concerning the physiology of the autonomic nervous system.

Saliva is formed in the paired major salivary glands, consisting of the parotid, submandibular, and sublingual glands, as well as the minor salivary glands scattered throughout the oral cavity. Secretion of saliva results from tactile, mechanical, and gustatory stimulation of intraoral reflexes; olfactory stimuli; and direct stimulation of the sympathetic and parasympathetic nervous systems. Study of the mechanism of secretion is made difficult by the heterogenous nature of saliva and by the differences in cellular composition of the various salivary glands. Furthermore, many factors influencing the production of saliva, including the method of collection, the time of day, and the control of hormonal influences, must be considered in planning these studies.

Several devices have been described for the collection of separate salivary secretions. These involve either the cannulation of the duct or a suction cup placed over the ductal orifice. An acrylic device was devised to separate submaxillary from sublingual secretions (Schneyer, 1955). This allowed an estimate of the relative salivary contributions of the thre major salivary glands (Schneyer and Levine, 1955) (see later).

Production of Saliva

Anatomy of the Secretory Unit

The salivary gland unit consists of an acinus, a secretory tubule, and a collecting duct (Fig. 1). The structural relationships and secretory capabilities of these units within each salivary gland differ widely among the several salivary glands. The major salivary glands exhibit a specialized and branched duct system. The parotid and submandibular glands have a single, elongated, large-caliber collecting duct with only a few major branches, the interlobular ducts. These ducts are connected to many intralobular ducts, each of which transports saliva from several acini through the small intercalated ducts. The intralobular and proximal interlobular ducts are designated as the secretory tubules in this chapter in order to emphasize their important role in salt and water transport. The sublingual gland secretions are discharged through 10 to 12 separate collecting ducts. The parotid acini are composed solely of serous cells, the sublingual acini are predominantly mucous cells, and the submandibular acini contain both cell types. The myoepithelial cells surround the acini and proximal ducts. They have been shown to possess high energy metabolism as well as contractile elements consistent with their proposed role in expelling preformed secretions (Shear, 1966). The minor salivary glands are small groups of secreting units that are distributed throughout the submucosa of the oral cavity. Their acinar cells are serous, mucous, or both, and their collecting ducts are short and convoluted. Little is known about the secretory capabilities of the minor salivary gland ductal system.

Ultrastructural studies support the secretory nature of acinar cells. Secretory vesicles are intracellular containers of protein that is manufactured by the ribosomes, transported apically by the endoplasmic reticulum, and packaged by the Golgi bodies (Fig. 2). Ductal cells contain prominent basal striations formed by infoldings of the plasma membrane that enclose columns of rod-shaped mitochondria. The high energy characteristics of these cells suggests that they are involved with the transport of ions and water (Tandler, 1963) (Fig. 3).

Secretory Processes

The formation of saliva was originally thought to be a passive process, involving the ultrafiltration of plasma. It is now appreciated that active transport processes occur throughout the salivary gland unit and that these processes are under complex hormonal and neural control. At low salivary flow rates, aerobic metabolism predominates, but at high flow rates, anaerobic conversion of pyruvate to lactate occurs, resulting in an increase in the lactic acid and a decrease in the glucose content of saliva (Chauncey and Shannon, 1965).

Saliva formation can be studied at two distinct levels: the proximal, or acinar level, and the distal, or tubular level. Proximally, a primary secretion is formed whose osmolality and major electrolyte composition are similar to plasma. In the tubule, hypertonic resorption of electrolytes secondarily modifies the primary secretion and a hypotonic fluid results. Electrolyte concentrations are seen to be influenced by the rate of salivary flow. Sodium, chloride, bicarbonate, and calcium concentrations are directly related to flow, whereas potassium content

is independent (Wotman and Mandel, 1976). The common conception that the acinar cells are the principal source of salivary secretion is no longer tenable in view of considerable evidence that the events occurring in the tubule primarily determine the final composition of saliva. Salivation has been demonstrated in the newborn puppy and rat before acini develop; ductal secretion alone produces as abundant a saliva as that in the mature animal (Schneyer and Schneyer, 1961). These findings were reproduced in animals made deficient in acini by ligation of their excretory ducts. The saliva was produced at high flow rates with an increased potassium concentration (Schneyer and Schneyer, 1961). The study conducted by Schneyer and Schneyer demonstrated the secretory capacity of ductal elements and specifically their ability to secrete sodium, potassium, and water at rates similar to that of the adult gland.

Primary Secretion

Histologically, the acini cells resemble other protein-secreting cells. The secretory granules have been isolated and have been shown to contain amylase and deoxyribonuclease (Schramm and Danon, 1961). Immunohistochemical techniques have confirmed the presence of amylase in the acinar cells, as well as in the intercalated duct cells (Kraus and Mestecky, 1971). Glynn and Holbrow (1959) studied the distribution of blood group-specific substances in human salivary tissue and demonstrated strong staining in the mucous acini of submandibular salivary glands but not in serous acini or duct cells. Glycoprotein molecules have been identified by electron microscopy in submandibular gland acini (Gallagher et al, 1969).

Primary secretion refers to the fluid produced by secretion of the secretory granules into the acinar lumen. It is plasma-like in composition and is probably formed by the acini and the intercalated duct (Young et al, 1967). Theories regarding the formation of the primary fluid include pinocytosis (Lewis, 1931) and active ion transport. Pinocytosis is the process whereby interstitial fluid imbibed by basal pseudopods is transported through the cell in vesicle form, absorbing potassium and organic solutes along the way, and is expelled apically into the acinar lumen and intracellular spaces (Yoshimura, 1967). Oliver (1982) described a unique system of endocytosis located in the lateral and basal cell surfaces of resting and stimulated pancreatic and parotid acinar cells. Oliver used horseradish peroxidase to detect uptake by the acinar cell and showed that the rate of endocytosis increased markedly after stimulation by a secretagogue. Furthermore, Oliver detected the intravenously administered horseradish peroxidase in the same golgi apparatus as ferritin that was intraductally administered, suggesting that there are two distinct systems of endocytosis, one located in the apical region and the other located in the basal and lateral regions.

The electrical properties of the acinar cell indicate that active ion transport is involved in the secretory process. Unlike nerve or muscle cells that depolarize on stimulation, salivary acinar cells respond to autonomic stimulation by hyperpolarization of the basal membrane (Lundberg, 1957). The inward transport of chloride provides the intracellular negativity, and the hyperpolarization of the glandular membrane with stimulation is due to the outward flux of potassium (Lundberg, 1958). This outward diffusion of potassiuim was postulated to be secondary to a stimulation-induced increase in cell membrane permeability (Yoshimura, 1967). This concept

is supported by the observations of Burgen (1956) concerning the marked depletion of intracellular potassium during stimulation and the hypertonic potassium concentration in primary saliva of certain glands.

The mechanism of potassium release and uptake has been extensively studied. The presence of a sodium-potassiuim ATPase has been demonstrated (Filsell and Jarrett, 1965). Furthermore, the inhibition of this enzyme-transport system has been shown to lead to increased potassium and decreased sodium secretion from rat submaxillary gland cells (Schneyer and Schneyer, 1965). This finding is most consistent with the prevention of reuptake of potassium by the salivary gland cells. This is supported by Selinger's observation that potassium secretion in rat parotid slices was increased by alpha-adrenergic stimulation or by specific inhibition of active cation transport (Selinger et al, 1973). A calcium-activated potassium channel has recently been demonstrated in acinar cell membranes to mediate the release of potassium upon stimulation (Maruyama et al, 1983). These observations support the hypothesis that potassium efflux is mediated by the alpha-adrenergic system through a calcium dependent channel and that influx is mediated by energy requiring sodim-potassium ATPase.

It would, therefore, appear that both active transport of cations and anions as well as stimulation-induced changes in cell membrane permeability are important determinants of salivary secretion. The dualistic effect of secretory stimuli upon the volume and solute concentration of saliva is mediated by the physiologic differences in acinar and tubular secretion. Net fluid transfer into the saliva probably occurs only into the primary secretion, which remains isotonic even at different flow rates (Young et al, 1967), whereas in the ductal system the principal effect is upon tonicity, which varies significantly with flow rate.

Ductal Secretion

The functional capabilities of the secretory tubule cells of the proximal ductal system are complex, varied, and only partially understood. Secretion of electrolytes, water, and organic solutes as well as resorption of electrolytes and water has been demonstrated through both direct and indirect means. The relationship between secretion and resorption, which presumably proceed simultaneously at varying rates in different levels of the ductal system, are not well defined, and, in general, only the net effect on saliva has been measured. Localization studies have been used to identify protein components in the ductal system. Gresik (1980) studied the granular convoluted tubule in rat and mouse submandibular glands. This ductal component is located between the intercalated and striated ducts. Several alkaline proteolytic enzymes are located in these cells, including proteases and esteroproteases. The differentiation of these cells is seen to be under complex hormonal control (Gresik, 1980). The biologically active salivary gland polypeptides, which have received increasing attention in recent literature, are also located in these cells (Barka, 1980).

The net change in electrolyte concentration at the tubular level is closely related to the rate at which the precursor fluid flows past the luminal face of the striated cells. At low flow rates, there is a relatively long period of time in which ion transfer across the tubular cell can

alter the luminal fluid. At higher flow rates, the contact time is shortened, thereby diminishing the effect of tubular cell secretion and resorption upon solute concentration.

Figure 4 demonstrates the change in osmolality as well as sodium and potassium concentrations as measurements are taken at various locations along the salivary ducts. This experiment was conducted by Young and co-workers (1967) and demonstrates that the concentration of sodium falls from 150 mEq/L in the primary secretion to 5 mEq/L at the duct opening. Potassium concentration rose from 10 mEq/L to 40 mEq/L, and the total osmolality decreased from 300 mOsm/microg to 100 mOsm/microg. Hypertonic resorption of sodium occurred primarily at the intralobular duct level. The rise in sodium concentration at higher flow rates reflects the decreased contact time in the resorptive segments. At very low flow rates, steady-state potassium concentration levels as high as 130 mEq/L were seen; but at higher flow rates, the concentration decreased. This may be due to the release of large quantities of intracellular potassium at low flow rates or at the initiation of stimulation. As stimulation increases and the flow continues, the initial intracellular store is depleted and a steady-state concentration may exist between the plasma, the intracellular pool, and the saliva (Burgen, 1956).

The intraductal localization of electrolyte secretion and several organic solutes was demonstrated by Burgen and co-workers, who studied the salivary outflow patterns following the intra-arterial injection of radioactive isotopes (Burgen and Emmelin, 1961). The appearance times of water, sodium, potassium, chloride, iodide, and urea are shown in Figure 5. Since the blood flow in the periductal capillaries runs countercount to the direction of salivary flow, the isotope reaches the distal ductal segments before the proximal ducts and acini. It is seen that water enters the saliva most distally, followed by the anions, the cations, iodide, and finally urea. These data suggest that a considerable functional specialization exists in the intralobular ducts.

Whereas the leading edge of the outflow curve indicates the most distal sites of ion entry, the slope of their trailing edge of the curve may indicate a more proximal process. The long duration of the potassium curve suggests that acinar secretion is also involved. The rapid decline in the radioactivity in the water is most likely secondary to rapid dilution or to exchange of the labeled water in the proximal saliva by nonradioactive water from the bloodstream. This factor may reflect the free permeability of the ducts to water. The secretory ducts are also responsible for the uptake and secretion of iodide by the salivary glands. The mechanism of uptake of the salivary glands is similar to that of the thyroiod glands (Myant, 1960).

Regulation of Secretion

General Aspects

The daily volume of saliva produced by normal humans has been estimated as 1000 to 1500 mL, which corresponds to an average flow of 1 mL/min. The basal flow rate in the awake individual has been reported as low as 0.33 mL/min (Becks and Wainwright, 1943). Schneyer and Levin (1955) place this value at 0.65 mL/min. During sleep, the salivary flow from the major salivary glands falls to zero (Schneyer et al, 1956). The minor salivary glands, however, continue

to exhibit spontaneous secretions (Ostlund, 1953). The relative contribution of the major salivary glands to total salivary flow was estimated in normal adult males (Schneyer and Levin, 1955). At rest, the submaxillary glands contributed 69 per cent of salivary flow, followed by the parotid glands with 26 per cent and the sublingual glands with 5 per cent. This relationship was confirmed by Enfors (1962), who adds that under stimulatory conditions, the ratios are reversed, with the parotid gland contributing two-thirds of the total saliva. The contribution made by the minor salivary glands has been estimated as 8 per cent at rest and 7 per cent under maximal stimulation (Dawes and Wood, 1973).

The rate of salivary secretion has a direct influence on the concentration of its constituents. Shannon and Prigmore (1960) found a positive correlation between the flow rate in the human parotid gland and the concentration of sodium, chloride, bicarbonate, pH, calcium, and total protein. They found no significant correlation between flor rate and potassium. Siegel (1972) studied this correlation in the baboon parotid gland and confirmed the direct relationship between flow rate and sodium concentration. He reported a negative correlation, however, between flow rate and potassium and chloride concentrations.

The regulation of salivary flow and composition is under control of the autonomic nervous system. Many factors have been shown to influence this control. These include psychologic factors, circadian rhythm, age, hormones, diet, and drugs.

Psychological Factors

Brown (1970) discusses the psychologic factors involved in salivation. One interesting anecdote that he relates is an ancient lie detector test, in which the suspect was given a cup of dried rice to hold in the mouth. If guilty, then there was believed to be decreased salivation, and the person would be able to expel all of the rice. If innocent the saliva would transform the rice into a gummy mass.

The question of psychologic influence on salivary secretion has been studied since Langley's work in the late nineteenth century, when he reported that the sight of food did not increase salivary flow rate. This was supported by Kerr's observations that there was no increase in salivation when subject talked about food, when the subject watched as scrambled eggs and bacon were being cooked, or when a subject watched a lemon being sucked (Kerr, 1961). Conversely, the thought of food was reported to statistically increase salivation in 160 dental students (Jenkins and Dawes, 1966).

Winer and colleagues (1965) used hypnosis and told subjects that a sour stimulus was either tasteless or sweet, and that a sweet stimulus was either tasteless or sour. They found that the suggestion that any stimulus was sour created the largest secretory response, and the suggestion that a stimulus was tasteless created the lowest response.

Depression leads to a decrease in salivary flow rate (Brown, 1970). Patients in a manic phase still secrete at a lower rate than normal but at a higher rate than bipolar patients in their

depressive phase. Depressed patients react normally with weak acid stimulation. There is a definite correlation between salivary flow rates and the prognosis of these patients. It is suggested that salivary secretion is the most accessible measurement of the activity of the digestive tract in patients with depressive psychoses.

Circadian Rhythm

A diurnal variation has been reported in salivary flow. Dawes (1972) measured unstimulated salivary flow rates and electrolyte concentrations at various times during the day. The peak flow rate occurred at 15.26 hours. Peak sodium concentration occurred at 5.00 hours. This is 12 hours out of phase with flow. The authors cite studies that show that there is an increased taste sensitivity for sodium in the afternoon, which corresponds to the time of lowest concentration (Dawes, 1972). Interestingly, rhythms in sodium concentration were not seen in patients with Addison's disease, suggesting that variations in aldosterone levels might be responsible for this.

Age

Salivary flow has been shown to be age dependent. Becks and Wainwright (1943) measured salivary flow in 661 healthy individuals from 5 to 96 years of age. Although there was a wide scatter in the distribution, there was an increase in flow between the ages of 5 and 29, with a decrease in flow thereafter. This decrease may be secondary to atrophy of acinar cells, with their replacement in later ages with fat (Manson and Chisholm, 1975). Age also appears to play a factor in the maturation of the salivary gland unit. Using histochemical studies in rats and mice, the secretory granules of the striated duct cells first appear at 20 days after birth (Gresik, 1980). This parallels the secretion of amylase into the saliva. Several biologically active peptides and proteolytic enzymes contained within these secretory granules are also observed only after maturation of these cells. Age-related changes in salivary composition has had limited study in man, although there does appear to be an increase in its calcium content between the ages of 5 and 49 (Becks, 1943).

Hormones

Hormones have been shown to affect both the size and function of salivary glands. The submaxillary glands of cats atrophy after bilateral adrenalectomy or hypophysectomy in cats (Kahlson and Renvall, 1956). Aloxan-induced diabetes in rats is accompanied by a decrease in growth of the submaxillary glands. With insulin replacement, this growth retardation was not seen (Liu and Lin, 1969). Thyroid hormone also appears to play a role in salivary gland regulation. Salivary flow is decreased and viscosity is increased after thyroidectomy (Shafer and Muhler, 1960). The administration of 131I in a dose high enough to cause atrophy of the thyroid gland induces a similar atrophy of the granular tubules of the salivary glands of the mouse. A direct effect of thyroid hormone on beta-adrenergic receptors in the submaxillary glands of thyroidectomized rats has been demonstrated (Pointon and Banerjee, 1979). Conversely, sialoadenectomy reduces thyroid activity (Wase and Feng, 1956). It has been postulated that the

salivary glands have an important role in the extrathyroid metabolism of iodide (Fawcett and Kirkwood, 1954).

The electrolyte composition of saliva is also seen to be partially under hormonal control. The sodium-sparing effect of mineralocorticosteroids has been demonstrated in salivary secretions (Grad, 1952). Wotman and co-workers (1973) suggest that sodium secretion in the saliva is under the control of the renin-aldosterone system. Salivary electrolyte composition during pregnancy and at midcycle have demonstrated a decrease in calcium and sodium concentration (Marder et al, 1972; Puskulian, 1972).

Diet

Dietary effects on salivation may be discussed at two levels: First is the effect of different foodstuffs on reflex salivation. The rate and composition of salivary secretion appears to be dependent on the nature of the stimulus. This adaptation appears to be physiologically useful; eg meat placed in the mouth evokes a viscous mucin-rich secretion that facilitates deglutition, whereas inedible material such as sand results in a thin, watery secretion that helps to irrigate the oral cavity. In general, foodstuffs elicit a protein-rich secretion, whereas the material secreted in response to nonfoods is a protein-poor fluid. The type of carbohydrate in the diet does not affect protein secretion or concentration of amylase in the saliva (Behall et al, 1973). Caldwell and Pigman (1966) measured protein and glycoprotein valies in saliva at rest and when any of the basic four gustatory stimuli were presented. Low flow rates were associated with the lowest concentration of protein and protein-bound carbohydrates. These concentrations increased as the flow rate increased, but they were independent of the nature of the gustatory stimulus.

The effect of diet upon salivation can also be studied in terms of long-term changes in salivary physiology. Squires (1953) measured salivary amylase in different geographical populations with varying diets. The highest amylase contents were seen in the population with the highest carbohydrate diet. The lowest level was observed in an African population whose diet consisted of minimal carbohydrates. Dawes (1970) reviewed the effect of diet on salivary secretion and composition. He concludes that there is insufficient evidence to conclude that diet has a significant influence on either parameter. Data are available, however, that suggest that a high-protein diet may have an anticariogenic effect by elevating salivary urea nitrogen levels (Dawes, 1970).

Drugs

The salivary glands are sensitive to a variety of chemical agents that stimulate or inhibit the salivary reflex by their actions at the peripheral, ganglionic, or central levels (Table 1). Stimulation of salivation results from agents that affect the sensory receptors, act centrally, mimic the pharmacologic action of the autonomic neural mediators, or prolong the action of these mediators. Inhibition results from certain central nervous system depressant drugs, peripheral autonomic blocking agents, or ganglionic blocking compounds.

Table 1. Factors Influencing Salivation

Increase	Decrease
Citric acid	Sjögren's syndrome
Ether	Irradiation
Chloroform	Dehydration
Cyclopropane	Antihistamines
Mercury poisoning	Antihypertensives
Cholinergic drugs	Diuretics
Anticholinesterases	Antiparkinson druugs
Mucous membrane irritants:	Antinauseants
Peppermint	Muscle relaxants
Ammonia	Phenothiazines
Quinine	Monoamine oxidase inhibitors.
Ulceration	
Rabies	

Peripheral Agents. Chemical agents that irritate taste or pain receptors can initiate reflex secretion. Six per cent citric acid sprayed into the mouth is an effective salivary stimulant (Enfors, 1962). Inhalation anesthetics, such as ether, chloroform, and cyclopropane, stimulate salivation by irritating the oral mucosa. Hypersalivation is a prominent feature of mercury poisoning; the mercury is excreted into the saliva in high levels and causes a chemical stomatitis. Topical anesthetization of the sensory receptors prevents reflex stimulation of salivation.

Central Nervous System Agents. Central stimulants, such as picrotoxin, induce salivation as a part of their generalized effect. Hypersalivation occurs in the nausea syndrome and may be induced by drugs that stimulate the chemoreceptor emetic trigger zone, such as apomorphine, morphine, cardiac glycosides, and Veratrum alkaloids (Goodman and Gilman, 1965). General anesthetics and barbiturates depress salivation. Antiemetic drugs, such as the antihistamines and the phenothiazine compounds, reduce the hypersalivation component of the nausea syndrome by depressing the chemoreceptor zone.

Autonomic Nervous System Agents. Drugs that affect the autonomic nervous system have been extensively studies. The maximum pharmacologic stimulation of saliva occurs with those drugs that possess the muscarinic properties of acetylcholine, such as pilocarpine and methacholine. These drugs stimulate the secretory cell directly. The nicotinic properties of acetylcholine can induce salivation by ganglionic stimulation, but with the large doses required, muscarinic effects predominate. Anticholinesterases produce their effect by prolonging the action of endogenously generated acetylcholine. Adrenergic agents are also stimulatory to salivary flow, but their effects are less potent than parasympathomimetic drugs. Autonomic agents are rarely used clinically to stimulate salivation because of the widespread and often unpleasant side effects from stimulation of other receptor sites.

Autonomic blocking agents have been demonstrated to decrease salivation. Atropine, which competes with acetylcholine for cell receptor sites, is widely used as an antisialogogue, although scopolamine and methscopolamine are more effective (Domino and Corssen, 1967). Reserpine leads to decreased salivary volume with increased calcium and proteiin (Martinez et al, 1975). On a cellular level, it has been shown to increase beta receptor concentration in acinar cells as well as to lead to the accumulation of secretory granules. Its effects are thought to be mediated by norepinephrine depletion (Bylund et al, 1981). An opposing effect is seen with isoproterenol (Roscher and Wiesmann, 1981). Bylund and Martinez (1980) have also demonstrated a reserpine-induced increase in alpha-2 receptors, which are believed to regulate presynaptic alpha responses. Mood-elevating drugs such as imipramine (Tofranil) and amitriptyline (Elavil) and, to a lesser extent, many of the monoamine oxidase inhibitors exert an atropine-like side effect on the salivary glands (Goodman and Gilman, 1965), which occasionally precludes their use because of secondary stomatitis. The mechanism of inhibition may result from the competition for acetylcholine receptors at the cell membrane level (Yu and Schneyer, 1982).

Autonomic Innervation

Anatomy. The salivary glands contain both parasympathetic and sympathetic nerve fibers. The latter arise in the superior cervical ganglion and enter the gland with its arterial supply. The former airce in the salivary nuclei of the brain stem and enter the gland in sensory nerves - the auriculotemporal nerve to the parotid gland, and the chorda tympani - lingual nerve to the submandibular and sublingual glands. The parasympathetic fibers are postganglionic from the otic ganglion, and the sublingual fibers are postganglionic from the submandibular ganglion, but the submandibular fibers are preganglionic and synapse in ganglion cells within the hilum of the gland (Langley's ganglion).

Garrett (1967) described the distribution of nerve fibers in the human parotid and submandibular glands using cholinesterase and catecholamine stains. Cholinergic and adrenergic fibers of both the parotid and submandibular glands are distributed in a similar fashion about the acini, intercalated ducts, and striated ducts, with cholinergic fibers being more numerous. The collectiing ducts are sparsely innervated; the myoepithelial cells are liberally innervated. Neuroeffectos synapses or specialized nerve ends are not present; rather, bared axons frequently lie in close proximity to the secretory cells. Garrett assuumes that the neurohumoral transmitter is produced by these axons, many of which contaiin mitochondria and vesicles, and reaches the cells by diffusion.

Neurotransmitters. Both sympathetic and parasympathetic stimulation lead to salivary gland stimulation. Lundberg (1958) provided evidence that acetylcholine is the cholinergic mediator. Oborin (1954) demonstrated release of both epinephrine and norepinephrine from the submandibular gland in the cat following sympathetic stimulation. Bylund and Martinez (1982) reviewed the function of the autonomic receptors observed in salivary glands. Adrenergic receptors are divided into alpha and beta receptors. The former are then subdivided into alpha-1 and alpha-2 receptors, based on the differential potency of various stimulatos. Alpha-1 receptors mediate postsynaptic alpha effects; alpha-2 receptors mediate presynaptic effects. The alpha-2

receptors are differentially increased after treatment with reserpine. The beta receptors are divided into beta-1 and beta-2 receptors, although only the former are found in rat submandibular glands (Bylund et al, 1982). Cholinergic receptors are divided into muscarinic and nicotinic, with the former providing the dominant role in salivary stimulation. Vasoactive intestinal peptide (VIP) response fibers have been described in close association with secretory acini, ducts, and blood vessels. There is a similarity between the VIPergic system in salivary glands and that described in the gut and pancreas (Polak and Bloom, 1980). Baseline concentrations of VIP in the salivary glands are similar to those found in the exocrine pancreas. It is postulated that this system might act as an intrinsic neuronal control innervating glandular structures (Polak and Bloom, 1980). Demonstrated neurophysiologic effects of VIP include alteration of electrolyte concentration in saliva and mediation of the atropine-resistant vasodilation, which results from parasympathetic stimulation. Increased local concentration of VIP has been observed following stimulation of the chorda tympani nerve (Polak and Bloom, 1980). Recently, purinergic receptors have been descriibed on acinar cells, the stimulation of which is similar to acetylcholine and alpha-adrenergic agents (Gallacher, 1982).

The multiple receptor systems have complex interactions at the glandular and cellular level that are still incompletely understood. Both the rate of flow and the salivary composition have been studied in relation to autonomic stimulation.

Rate of Flow. In most species, both sympathetic and parasympathetic stimuli result in saliva flow. The latter is generally copious and watery and persists as long as the stimulus is applied, whereas saliva produced by sympathetic stimuli is scant, rich in organic and non-organic solutes, and ceases entirely with prolonged stimulation. In humans, stimulation of the chorda tympani nerve results in a profuse secretion from the submandibular gland (Diamant et al, 1959). Stimulation of the cervical sympathetics (Folkow and Laage-Hellman, 1961) and epinephrine administration (Emmelin and Stomblad, 1954) elicit secretion from the submandibular gland but not from the parotid gland. Electrical responses from a single cell occur with either parasympathetic or sympathetic stimulation, although the responses to each differ in magnitude, latency, and duration (Lundberg, 1955). The synergism with which the sympathetic and parasympathetic system works in the salivary glands is different from their antagonistic interactions elsewhere in the body. This was described by Langley (1889), who found that after stimulation of the chorda tympani nerve, a follow-up stimulation of the sympathetic nervous system elicited a further increase in the flow rate. With increased stimulation, however, an antagonism between these two systems develops, which is believed to be related to sympathetic-induced vasoconstriction. Emmelin (1955) found that with strong sympathetic stimulation, the secretion was less than that produced by stimulation of the chorda tympani alone. This diminution in secretion is proportional to the amount of vasoconstriction in the gland (Emmelin, 1955).

The secretory status is integrally associated with the vascular bed of the gland, sympathetic stimulation causing vasoconstriction, and parasympathetic stimulation causing vasodilatation. Under physiologic conditions, it is difficult to distinguish between the purely vascular and the purely secretory components. Parasympathetic vasodilatation occurs even when secretion is blocked by atropine. Hilton and Lewis (1956) proposed that this blockage was

mediated by kinin. Schacter (1967) attributed this phenomenon to atropine-resistant dilator fibers. These fibers may be mediated by the VIPergic system (Edwards and Bloom, 1982), whose fibers are observed in close proximity to blood vessels (Polak and Bloom, 1980). Stimulation of the chorda tympani in the presence of physostigmine or large doses of acetylcholine has been shown to result in vasoconstriction. Conversely, sympathetic vasoconstriction is followed by vasodilatation after prolonged stimulation. This latter effect is mediated by the release of vasodilating enzymes from the secretory cell. It may also result from beta-adrenergic stimulation, since it is abolished by propranolol administration (Schacter, 1967). It is likely that "dry mouth" associated with anxiety or fear states is secondary to vasoconstriction from increased sympathetic discharge.

Composition of Saliva. The difference in composition between saliva induced by parasympathetic stimulation and that observed after sympathetic stimulation has been investigated. Schneyer and Schneyer (1960) showed that amylase secretion was increased with parasympathetic activity, although its major stimulation is probably under control of beta-adrenergic receptors (Batzri and Selinger, 1973). Both alpha-adrenergic and cholinergic stimulation lead to release of potassium from salivary glands in vitro (Arnett and Davis, 1979; Martinez and Quissell, 1976; Strittmatter et al, 1977; Batzri et al, 1973). Isolated alpha-adrenergic stimulation without concomitant beta-adrenergic stimulation leads to the secretion of a similar ionic fluid with small amounts of organic material, as is seen with parasympathetic agents (Martinez and Quissell, 1976; Batzri et al, 1973). The effects on potassium secretion appear to be mediated by different mechanisms, however, since potassium secretion can be specifically desensitized to alpha stimulation while maintaining its response to cholinergic stimulation (Strittmatter et al, 1977).

Beta-adrenergic agents act by increasing intracellular cAMP concentrations. The cAMP concentrations, in turn, mediate the release of enzymes from the gland. Enzyme secretion is independent of the sodium potassium ATP pump, and it is increased by agents specific for beta-adrenergic stimulation (Batzri and Selinger, 1973). Repeated beta-adrenergic stimulation leads to increased DNA synthesis and gland hypertrophy (Burke and Barka, 1978).

Autonmic Denervation

After secretomotor nerve interruption, a phenomenon of denervation supersensitivity develops. Two phases have been described (Emmelin, 1967). Denervation secretion follows postganglionic section of the parasympathetic and, to a lesser extent, the sympathetics. This process lasts only 2 days and is the result of leakage of transmitter substance from the degenerating nerve ends. Paralytic secretion occurs after either preganglionic or postganglionic denervation or following prolonged administration of blocking drugs such as atropine (pharmacologic denervation). This form of secretion is the result of changes that occur in the nerve or the cell that render it more sensitive to circulating transmitter substances or their chemical analogues. Maximum supersensitivity develops at about 3 weeks (Cannon's law of denervation) and persists for years or until regeneration occurs (Emmelin, 1967). Section of either the sympathetic or parasympathetics causes the gland to become supersensitive to both epinephrine and acetylcholine. On a cellular level, controversy still exists over the mechanism

of denervation supersensitivity. Arnett and Davis (1979) found an increased beta-adrenergic response with a concomitant increase in beta-adrenergic receptor density in rat submandibular gland cells after denervation. Alpha-adrenergic response and receptor density was unaltered by denervation. They suggested that denervation was mediated by the beta-adrenergic receptors. Pointon and Banerjee (1979) found an increase in both receptor levels after chemical sympathectomy. Talamo and associates (1979) studied parasympathetic denervation, and they found no increase in muscarinic binding sites up to 16 days after denervation. They postulate that some other mechanism is involved with this phenomenon. Levin (1984) described a phenomenon that he terms the "atropine paradox", in which, during the late stages following denervation, atropine cause a paradoxic increase in parotid secretion.

Schneyer (1972) found that, in adult rats, sympathectomy of the parotid resulted in a 10 per cent reduction in gland weight. When both sympathectomy and parasympathectomy were performed simultaneously, a 4 per cent reduction in gland weight occurred. Atrophy of the parotid is more pronounced following hypophysectomy or adrenalectomy (Kahlson and Renvall, 1956).

Functions of Salivary Glands and Saliva

Functions of salivary glands and saliva can be divided into digestive, protective, homeostatic, and hormonal. Traditionally, the digestive function is thought of as the prime function of saliva, but work in recent years has led to the hypothesis that the salivary glands may play a role in homeostasis and in synthesizing biologically active peptides related to growth and development.

Digestive Function

Saliva aids in the mastication of food through its lubricating actions. Soluble food substances dissolved by saliva are able to act chemically on the taste receptors. Subsequent taste stimuli can be detected because the flow of saliva from the minor salivary glands of the tongue continually irrigates the taste buds. Prolonged gustatory stimulation results from those substances such as iodides and saccharin that are secreted into the saliva. Fortunately, the salivary concentrations of glucose and sodium are normally below taste thresholds.

Carbohydrate digestion is initiated by salivary amylase and continues for some time in the inner parts of the gastric bolus until inhibited by gastric acidity. The importance of salivary amylase to carbohydrate breakdown has been debated. The rapid transit time of ingested food through the mouth and the inactivation of amylase activity by low pH suggest that salivary degradation of starch can occur to only a limited extent. Amylase provides the major electrophoretic peak in parotid saliva. Like pancreatic amylase, it is an alpha-1,4-glucan 4-glucanohydrolase thatsplits the alpha-1,4-glucosidic bonds of starch in a random fashion to produce maltose and a variety of dextrins. Amylase is the major protein fraction of parotid saliva, and its concentration is independent of flow rates (Ferguson et al, 1958). It is also present in submandibular saliva. It is a carbohydrate-free protein with an optimum pH of 6.9 (Muus, 1954)

and a molecular weight of 45,000. Electrophoresis of crystalline salivary amylase shows from 4 to 8 bands of amylolytic activity, which represent isoenzymes of amylase (Lamberts and Meyer, 1967; Muus and Vnenchak, 1964; Wolf and Taylor, 1967). The nonidentity of pancreatic and salivary amylase was demonstrated by Norby (1964), who found that pancreatic amylase is a single rapidly moving band compared with the several heavier bands of salivary amylase.

The absence of nutritional problems with carbohydrates in patients without saliva suggests that sufficient pancreatic amylase is available for the degradation of starch. Digestion of starch by salivary amylase does continue in the stomach up to several hours after ingestion, however, because the inner layers of the gastric bolus remain alkaline. Under these circumstances, conversion of starch to maltose in the stomach may be 75 per cent complete (Bergheim, 1926). The commonly observed hypertrophy of the salivary glands of patients with excessive carbohydrate intake indicates that salivary amylase does play an active role in starch digestion. Although other digestive enzymes have been reported in salivary secretions, they have not been as extensively studied as salivary amylase and their physiologic roles are not yet understood.

Protective Function

The protective function of saliva and the salivary glands can be divided into four areas: Mechanical protection, anticariogenic activity, antibacterial activity, and antineoplastic activity. Mechanical protection is best understood and results from the dilution and irrigation of retained foodstuffs, epithelial debris, and bacteria. Also, the glycoproteins produced serve as a protective coating to the mucosa and guard against the irritant effects of chemical agents or desiccation (Wotman and Mandel, 1976).

The anticariogenic properties of saliva have been extensively studied. Desalivation caused increased incidence of caries in hamsters given a high-sucrose diet, but not a carbohydrate-free diet (Klapper and Volker, 1953). Fanning and associates (1954) postulated a role for saliva in enamel formation, since they demonstrated that rats that were desalivated earlier in development had the greatest propensity for caries formation. Incorporation of salivary inorganic ions such as calcium, fluoride, and phosphates by immature teeth contributes to the maturation of and subsequent decreased solubility of enamel (Leung, 1965). Incipient or early dental caries become filled with saliva-derived accumulations, which tend to prevent further enamel dissolution. Whether these accumulations actually remineralize the defect or act as an occlusive plaque is debated, but it is well recognized that a reparative process occurs. Attempts to correlate the concentration of inorganic compounds and enzyme substances in saliva with caries susceptibility and caries resistance generally have been inconclusive. The most convincing evidence relating salivary flow to the development of dental caries is in disease states such as Sjögren's syndrome, radiation therapy, stone formation, and chronic use of para-sympatholitic agents, all of which are associated with an increased incidence of dental caries (Mandel, 1974).

Antibacterial functions of saliva are directly related to its anticariogenic potential. Four antibacterial components have been described: lysozyme; a thiocyanate-dependent factoor, probably a glycoprotein; bacteriolysin, or Green factor; and immunoglobulins. Bibby and co-

workers (1938) tested the antibacterial activity of saliva by placing it in wells in a bacteria-inoculated agar plate. They reported zones of inhibition of varying diameters around 110 oof 169 organisms tested. The least affected organisms were those present as normal oral flora. Therefore, a role is postulated for saliva in determining the composition of normal oral flora. Lysozyme was identified as one of the factors active in the saliva (Kesteren et al, 1942). Green (1959) detected a bacteriolytic factor in the saliva of caries-immune individuals that was absent in caries-susceptible individuals. This element was characterized as a protein in the globulin factor. This factor was shown to be different than lysozyme (Green, 1966) and was termed bacteriolysin. Dogon and Amdur (1965, 1970) describe two thiocyanate-dependent antibacterial systems in parotid secretions. The first consists of hydrogen peroxide, thiocyanate, and a peroxidative component. The second consists of thiocyanate and a salivary protein. Whereas lysozyme acts by destroying the cell walls of bacteria, this latter system is postulated to attack an unknown element in the bacterial cytoplasm (Dogon and Amdur, 1970).

Much recent attention has been focused on the role of secretory immunoglobulin in oral defense. Gamma A immunoglobulin (IgA) has been demonstrated to be synthesized locally in human parotid and submandibular tissues (Hurlimann and Zuber, 1968). Its synthesis has been identified in the plasma cells in the subepithelial connective tissue (Tomasi, 1972). Secretory immunoglobulin-A (SIgA) is the major immunoglobulin found in exocrine secretions (tears, respiratory mucus, colostrum, and saliva). The secretory component, a polypeptide with a molecular weight of about 60,000, differentiates salivary IgA from serum IgA. Lehner and Caldwell (1967) found a significantly elevated level of serum IgA and a significantly decreased level of salivary IgA in caries-prone individuals. They discuss the salivary clearance of IgA, which appears to be deficient in these individuals. Zengo (1971) confirmed these observations and localized the difference in SIgA secretion to the submandibular glands.

The antineoplastic activity of the salivary glands has gained recent attention. Gasic and associates (1983) demonstrated the inhibition of mediastinal and lung metastases in a mouse sarcoma model using salivary gland extract from the leech. Although it is possible that this is due to the anticoagulation effects of saliva, these findings could not be reproduced wiith intravenous heparin. O'Connell and co-workers (1983) studied the effect of salivary gland removal on the development of skin tumors in mice. They performed a controlled study in which mice underwent either total submandibular and sublingual gland excision or a sham operation. They then applied skin carcinogens and studied the growth of tumors. Sialadenectomized mice developed more tumors and developed them earlier than the control group. Attempts to isolate the factor or factors responsible for this observation have not yet been reported.

Homeostatic Function

The main recognized role of salivary physiology in homeostasis is related to thirst regulation. With loss of body fluids through hemorrhage, evaporation, and sweating or from the lungs, gastrointestinal tract, and urinary system or from decreased water intake, the salivary glands, like other tissues, become dehydrated, and salivary flow becomes diminished or absent. Dryness of the mouth follows and leads to the sensation of thirst and the desire to replace fluid

losses by ingestion of water. Salivary excretion of metabolites is not essential for homeostasis, but many substances appear in saliva following ingestion: mercury, lead, sulfur, iodides, morphine, and many antibiotics. The deposit of lead in the gingiva and the stomatitis of mercury poisoning are manifestations of salivary secretion of these compounds.

Hormonal Function

Extensive literature exists concerning the identification and activity of salivary gland hormones. Parotin was described as a parotid hormone that lowered serum calcium by 15 per cent and enhanced calcification of teeth and bones. The identification of this calcium-lowering property could not be reproduced by others (Lazarus and Shephard, 1969). Fleming (1960) studied the effect of injection of this substance on enamel protein matrix in mice. It was shown to increase vascularization at the growth center. An analogous effect was seen in the epiphyseal plates of the femur. Ito (1960) describes several disease states including chondrodystrophia fetalis, which might represent a deficiency of this protein.

A factor demonstrated to promote growth of sensory and sympathetic nerve cells was isolated from the salivary glands of mice, hamsters, and mammals (Cohen, 1960). This polypeptide, which had originally been described in snake venom, was called nerve growth factor. It caused overgrowth of the sympathetic ganglia and hyperneurotization of the viscera (Levi-Montalcini and Cohen, 1960). Also, there was an increase in both cell number and size. Antiserum to this factor was injected in various animals, and the antiserum caused atrophy of the sympathetic nerve cells. Extirpation of the salivary glands does not have a significant effect on the development of the sympathetic nervous system. The authors explain this by postulating that nerve growth factor is synthesized elsewhere and is then stored in and released from the salivary glands. It is felt that this factor plays a significant role in the development and function of the sympathetic nervous system (Barka, 1980).

A second polypeptide growth factor was isolated shortly after nerve growth factor. This factor was shown to accelerate incisor eruption and eyelid opening when injected into newborn rats or mice (Levi-Montalcini and Cohen, 1960; Cohen, 1962). This protein, which was called epidermal growth factor, has been characterized as a polypeptide of 53 amino acids. Its known functions include proliferation and differentiation of epithelial tissues, inhibition of gastric acid secretion, generation of hepatic hypertrophy, and stimulation of macromolecular synthesis (Carpenter and Cohen, 1979; Barka, 1980). A similar protein has been isolated from human urine and serum (Gregory et al, 1979). Much research has centered around the role of epidermal growth factor in the prevention of peptic ulcers. It has been shown to inhibit histamine-induced gastric acid stimulation (Bower, 1975). Desalivated rats showed increased susceptibility to the development of bile salt-induced gastric ulcers (Skinner and Tepperman, 1981). Intraluminally placed epidermal growth factor significantly reduced the ulcerogenic effect of cysteamine (Kirkegaard et al, 1983). This effect was independent of any effect on gastric acid secretion or bicarbonate secretion by the duodenum. It was postulated that epidermal growth factor has a cytoprotecitve effect on duodenal mucosa (Kirkegaard et al, 1983; Olsen, 1984). The mechanism of action of epidermal growth factor is unknown, but it appears to be independent of

prostaglanding metabolism (Konturek et al, 1981). Other growth factors have been isolated from salivary glands. These include neural tube growth factor, mesodermal growth factor, endothelial growth factoor, epithelial growth factor, and wound contraction factor (Adler and Narbaitz, 1965; Barka, 1980; Greene et al, 1971; Hoffman et al, 1967; Hutson et al, 1979).

A role has been postulated foor the renin-kallikrein system in the salivary glands of various animals including humans. Renin was isolated from the submandibular gland by Werle and colleagues (1957). Takeda and associates (1969) studied the effect of submandibular gland excision on blood pressure and plasma renin level in mice. He found that there was a significant effect only in male mice. This finding was reported to be testosterone dependent (Oliver and Gross, 1967). The renin-kallikrein system may play a role in blood vessel contraction (Barka, 1980). Furthermore, it has been demonstrated to produce sodiuim secretion into the saliva (Wotman et al, 1973). In fact, the submaxillary electrolyte concentration has been shown to provide a useful indicator of aldosterone secretion in certain high-aldosterone states (Wotman and Mandel, 1973). After surgical treatment of primary aldosteronism, parotid and submaxillary sodium concentrations rise and the submaxillary potassium level falls. The secretion of renin by the submaxillary glands is under control of alpha-adrenergic receptors as opposed to the beta-adrenergic stimulation of renal renin release (Garrett et al, 1982; Lin et al, 1983; Menzie et al, 1974; Michelakis et al, 1976). There is a postulated role for salivary renin during stress (Menzie et al, 1974). Whether the renin-kallikrein system in human salivary glands plays a physiologic role in homeostasis remains to be determined.

Other homeostatic factors have been reported to be produced by the salivary glands. Glucagon-like material (IRG), which elevates rat serum glucose concentrations in vivo, has been isolated (Lawrence et al, 1975, 1976, 1977). Erythropoietin, bone marrow colony-stimulating factor, granulocytosis-inducing factor, nonsuppressible insulin-like activity, lymphoid factor, anticomplementary factor, gastrin, and somatostatin have been reported in salivary gland extracts (Barka, 1980).

These polypeptide hormones have been located in the granular convoluted tubules of the mouse salivary gland. The fact that these substances are present in secretory granules in exocrine glands implies that they are secreted (Barka, 1980). However, the role of these factors in growth and homeostasis is still unknown.