

Saliva and oral health

fourth edition

an essential overview for the health professional

Michael Edgar, Colin Dawes & Denis O'Mullane

WRIGLEY
Oral Healthcare
Programme



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Preface

A fourth edition of *Saliva and Oral Health* seemed overdue, as the third edition was published in 2004 and considerable advances in several aspects of salivary research have occurred since that time.

Six of the eight chapter authors in the previous edition remain the same in the fourth edition. The untimely demise of Professor Jonathon Ship has necessitated a replacement and we are delighted that Professor Mahvash Navazesh has agreed to contribute the chapter on xerostomia. Professor Jorma Tenovuo indicated that, because of his impending retirement, he wished to be replaced and we are fortunate that Dr. Eva Helmerhorst agreed to write a chapter on salivary proteins, an area in which she has made several important contributions.

Although the eight chapters of the book concentrate on the effects of saliva on oral health, Drs. Michael Dodds and Taichi Inui, from our Sponsor, The Wrigley Company, have contributed a commentary on other functions of saliva of particular interest to the food industry. These include the role of saliva in ‘mouth feel’ and the sensory perception of foods and beverages and also the potential use of assays of salivary components in the diagnosis of a number of systemic as well as oral diseases.

As in previous editions, the book is aimed not only at undergraduate and postgraduate students but also at dental practitioners and other health professionals who deal with oral diseases.

We are very grateful for the cooperation of all involved in the production of the new edition, including the new publisher. As ever, we are indebted to the Wm. Wrigley Jr. Company, in the person of Dr. M.W.J. Dodds, for underwriting the costs of producing this fourth edition.

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Saliva: functionality beyond oral health. Comments from the Sponsor

Michael Dodds and Taichi Inui

We are delighted to have had the opportunity to support the publication of *Saliva and Oral Health* through all four editions, since the first one appeared in 1990. Over the subsequent editions we have observed the addition of new research findings as they pertain to fundamental understanding of physiological secretory mechanisms, along with greater understanding of the roles of the organic components of saliva in protecting the oral cavity against insult, to cite just two examples. It is particularly encouraging to note that this book serves as a resource not just for researchers in the field of saliva physiology and biochemistry, but also dental students, dental practitioners and other professionals with an interest in the health of the oral cavity, and we hope this trend continues. In this context, the importance of saliva as a fluid that is crucial for the maintenance of both oral and systemic health is a central dogma, and this aspect is comprehensively covered by the various chapter authors, who are all well respected and highly published experts in their fields. However, beyond the function of saliva in contributing to the maintenance of both oral and systemic health, at the local level it also plays fundamental roles in delivering mouth comfort and oral sensations, as well as in food processing in the oral cavity, that also contribute to the overall health and wellbeing of the body. As scientists with a keen interest in the functionality of saliva, but approaching this also from a food industry perspective, we would like to provide some additional thoughts on the importance of saliva beyond its local effects on the mouth, teeth, and oral tissues, and briefly consider some areas of saliva function that may not be covered by the main chapter topics.

Saliva is an easily accessible medium which can be collected without the need for needles, aseptic or sterile conditions, or other specialised apparatus, and commercial kits are currently available for measuring hormone levels, such as cortisol or testosterone, illegal drugs and other substances in saliva. Recently, there has been increasing interest in, and support for, the use of saliva as a diagnostic fluid for screening not only oral conditions, such as caries, periodontal diseases or oral cancer, but also

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for other systemic conditions, such as breast and pancreatic cancers.¹ It is also possible that in the future the specific organic composition of saliva could function as an indicator of disease risk elsewhere in the body; for example, higher levels of the salivary mucins, MUC5B and MUC7 have been found in individuals who are suffering from gastric diseases and are infected with *Helicobacter pylori*, the organism associated with gastric ulcers.² In addition, there has been recent interest directed at salivary exosomes as a means to gain better understanding of cell-cell communications mediated by body fluids.³ Many studies in the psychology and exercise literature have used salivary cortisol or α -amylase as indicators of psychosocial or physical stress, as sympathetic nerve activity stimulates salivary acinar cell release of macromolecules, including α -amylase.^{4,5} However, this approach has been criticised due to methodological concerns, such as lack of control of salivary flow rate, as well as physiological complications, such as the fact that amylase secretion is partially controlled by parasympathetic stimulation (described in Chapter 2), as well as the dissociation between serum and salivary cortisol levels.^{6,7} Thus, an understanding of saliva composition, secretory physiology and the impact of functional stimulation is an important aspect of undertaking any type of work that involves objective measurement of saliva or any of its components.

Although not specifically a health-related issue, the treatment and prevention of extrinsic dental staining is almost an industry in itself, both for the profession, and also from the consumer goods stand point (*i.e.*, toothpastes and over-the-counter whitening treatments). The mechanism of tooth staining is in part mediated by saliva composition. Specifically, certain salivary proteins, including the proline-rich proteins (PRPs) have been shown to bind salivary polyphenols but not lower-molecular-weight salivary proteins, such as the histatins.⁸

The perception of astringency that is typically associated with consumption of polyphenol- and tannin-rich foods has a specific mechanism, involving binding and precipitation of PRPs, as well as another salivary protein, statherin.^{9,10} Although astringency may be perceived to be a negative sensation in response to consuming foods or beverages, a balanced level of astringency may also be seen to be an inherent aspect of the enjoyment of others, such as red wine or tea. It has been proposed that salivary PRPs, by binding dietary tannins, constitute a protective mechanism against their health-detrimental effects. The parotid salivary glands of rats and mice increased in size and PRP content in response to being fed a high tannin diet, while these rodents maintained normal weight gains. Conversely, hamsters fed a similar diet did not show this pattern of protein up-regulation and failed to demonstrate normal growth, suggesting that, at least in rodents, the PRPs constitute an inducible protective mechanism against excessive consumption of dietary components with a potential negative health impact. It has been argued that, from an

evolutionary standpoint, the constitutive presence in human saliva of high levels of PRPs and other tannin-binding proteins is a remnant of an earlier stage of human development when hominid populations relied on a diet rich in fruits, vegetables and nuts.⁹

The effect of saliva composition on mouth feel also seems to have been an historically under-researched topic. This could relate both to health, through the maintenance of a thin film of saliva to keep the oral tissues hydrated and lubricated, as well as to other aspects of oral function. For example, saliva plays an important role in facilitating the swallowing of food, by influencing food particle size and lubrication of the food bolus. Salivary mucins and other glycoproteins help to form food into a coherent and slippery bolus which can be transported smoothly down the oesophagus. The cohesiveness of the food bolus, rather than its water content, is considered to play a more important role in determining whether the bolus can be swallowed, indicating the relative importance of the organic content.¹¹ While it is generally accepted that the various glycosylated proteins and mucins in saliva form a well hydrated film on the mouth tissues to provide oral lubrication, facilitate food bolus formation and swallowing, and help to prevent desiccation of the mucosal tissues, much less is known about how minor alterations or polymorphisms in the macromolecular composition of saliva could alter oral perception. The rheology, or viscoelasticity, of saliva has been shown to vary under different conditions of stimulation, and it has been suggested that the inability of artificial salivas to be well tolerated by patients suffering from xerostomia is due to their failure to mimic effectively the unique and complex rheological properties of human saliva.¹² There is also increased interest from scientists working in the food industry in understanding how saliva can influence mouth feel, food processing and sensory perception of foods and beverages. For example, salivary protein concentration was found to be correlated with mouth feel with semi-solid foods.¹³ Beyond this non-specific effect, the high molecular weight mucin, MUC5B, and α -amylase specifically adsorb to lysozyme-stabilised emulsions that have the sensory perception of dryness (astringency), in contrast to the creamy, β -lactoglobulin-stabilised emulsions, that attract the lower molecular weight mucin, MUC7, but not α -amylase.¹⁴ This effect is probably mediated by charge differences, but provides an interesting insight into how salivary composition may help modify how pleasant (creamy or rich) versus unpleasant (dry, or astringent) food textures are perceived. While an increasing number of studies have been conducted around saliva and oral perception, other attributes of mouth feel and food texture still remain to be explained by physical and chemical properties of saliva, such as melting and stickiness.

Saliva is the medium for dissolving food taste molecules and carrying them to the taste buds. However, rather than acting as a simple aqueous solvent, components of saliva may modify how tastants are perceived. Saliva stimulated by different tastants (pungent,

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tingling, and sour) has different protein profiles as assessed by electrophoresis and mass spectrometry,¹⁵ and different tastants have been shown to affect the protein composition of the saliva, which could affect subsequent perception of bitter or astringent stimuli.^{16,17} The salivary enzyme carbonic anhydrase VI, formerly known as gustin, is a zinc-binding enzyme that has been shown to be associated with taste sensitivity to bitter stimuli, as well as demonstrating an intriguing association with body mass index.¹⁸

In addition to the well known action of salivary α -amylase in initiating the process of starch digestion, saliva may also help stabilise, protect, and transport critical nutrients so that they can be absorbed from the gastro-intestinal system. An example of this is haptocorrin. This glycosylated salivary protein binds to vitamin B12 in the mouth, protecting it from digestion in the stomach, but is then cleaved off in the duodenum and replaced by intrinsic factor, finally facilitating absorption of the vitamin B12/intrinsic factor complex in the proximal ileum.¹⁹

Summary

As described throughout this book, saliva plays a pivotal role in the overall maintenance of a healthy homeostatic condition in the oral cavity, which from the dental perspective is usually considered to be related to protection of the teeth and mucosal surfaces. The oral cavity is also the starting point of the digestive system, so saliva has advantages in being easily accessible for sampling as well as providing an impact on systemic health. With the increasing volume of biological data becoming available due to newer analytical methodologies offered by proteomics and other so-called 'omics' approaches, the contribution of saliva to overall human health and wellbeing is being explored from an holistic, or systems biology approach, emphasising interactions among constituents, that is opening up novel aspects of the importance of this body fluid to the human condition.

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Introduction: the anatomy and physiology of salivary glands

Helen Whelton

Saliva is the mixed glandular secretion which constantly bathes the teeth and the oral mucosa. It is constituted by the secretions of the three paired major salivary glands; the parotid, submandibular and sublingual. It also contains the secretions of the minor salivary glands, of which there are hundreds contained within the submucosa of the oral mucosa and some gingival crevicular fluid.

The presence of saliva is vital to the maintenance of healthy hard (teeth) and soft (mucosa) oral tissues. Severe reduction of salivary output not only results in a rapid deterioration in oral health but also has a detrimental impact on the quality of life for the sufferer. Patients suffering from dry mouth can experience difficulty with eating, swallowing, speech, the wearing of dentures, trauma to and ulceration of the oral mucosa, taste alteration, poor oral hygiene, a burning sensation of the mucosa, oral infections including *Candida* and rapidly progressing dental caries. The sensation of dry mouth or xerostomia is becoming increasingly common in developed countries where adults are living longer. Polypharmacy is very common among the older adult population and many commonly prescribed drugs cause a reduction in salivary flow. Xerostomia also occurs in Sjögren's syndrome, which is not an uncommon condition. In addition to specific diseases of the salivary glands, salivary flow is usually severely impaired following radiotherapy in the head and neck area for cancer treatment in both children and adults of all ages. Clearly oral dryness is a problem which faces an increasingly large proportion of the population. An understanding of saliva and its role in oral health will help to promote awareness among health care workers of the problems arising when the quantity or quality of saliva is decreased; this awareness and understanding is important to the prevention, early diagnosis and treatment of the condition.

There is an extensive body of research on saliva as a diagnostic fluid. It has been used to indicate an individual's caries susceptibility; it has also been used to reflect systemic physiological and pathological changes which are mirrored in saliva. One of the major

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Table 1.1: Functions of saliva

Fluid/Lubricant	Coats hard and soft tissue which helps to protect against mechanical, thermal and chemical irritation and tooth wear. Assists smooth air flow, speech and swallowing.
Ion reservoir	Solution supersaturated with respect to tooth mineral facilitates remineralisation of the teeth. Statherin and acidic proline-rich proteins in saliva inhibit spontaneous precipitation of calcium phosphate salts (Ch. 8).
Buffer	Helps to neutralise plaque pH after eating, thus reducing time for demineralisation (Ch. 6).
Cleansing	Clears food and aids swallowing (Ch. 5).
Antimicrobial actions	Specific (e.g. sIgA) and non-specific (e.g. Lysozyme, Lactoferrin and Myeloperoxidase) anti-microbial mechanisms help to control the oral microflora (Ch. 7).
Agglutination	Agglutinins in saliva aggregate bacteria, resulting in accelerated clearance of bacterial cells (Ch. 7). Examples are mucins and parotid saliva glycoproteins.
Pellicle formation	Thin (0.5 μm) protective diffusion barrier formed on enamel from salivary and other proteins.
Digestion	The enzyme α -amylase is the most abundant salivary enzyme; it splits starchy foods into maltose, maltotriose and dextrins (Ch. 7).
Taste	Saliva acts as a solvent, thus allowing interaction of foodstuff with taste buds to facilitate taste (Ch. 3).
Excretion	As the oral cavity is technically outside the body, substances which are secreted in saliva are excreted. This is a very inefficient excretory pathway as reabsorption may occur further down the intestinal tract.
Water balance	Under conditions of dehydration, salivary flow is reduced, dryness of the mouth and information from osmoreceptors are translated into decreased urine production and increased drinking (integrated by the hypothalamus, Ch. 4).

benefits of saliva is that it is easily available for non-invasive collection and analysis. It can be used to monitor the presence and levels of hormones, drugs, antibodies, micro-organisms and ions.

This chapter will provide an overview of the functions of saliva, the anatomy and histology of salivary glands, the physiology of saliva formation, the constituents of saliva and the use of saliva as a diagnostic fluid, including its role in caries risk assessment. Much of the material in this chapter will be covered in more detail in later chapters.

Functions of saliva

The complexity of this oral fluid is perhaps best appreciated by the consideration of its many and varied functions. The functions of saliva are largely protective; however, it also has other functions. *Table 1.1* provides an overview of many of these functions. More detail is provided in subsequent chapters as indicated.

Changes in plaque pH following sucrose ingestion and buffering capacity in the presence of saliva

The changes in plaque pH following a sucrose rinse are illustrated in Figure 1.1. The graphs are referred to as Stephan curves after the scientist who first described them in 1944 when he measured changes in plaque pH using antimony probe micro-electrodes in a series of experiments.

As can be seen in *Figure 1.1* the unstimulated plaque pH is approximately 6.7. Following a sucrose rinse the plaque pH is reduced to less than 5.0 within a few minutes. Demineralisation of the enamel takes place below the critical pH of about 5.5. Plaque pH stays below the critical pH for approximately 15-20 minutes and does not return to normal until about 40 minutes after the ingestion of the sucrose rinse. Once plaque pH recovers to a level above the critical pH, the enamel may be remineralised in the presence of saliva and oral fluids which are supersaturated with respect to hydroxyapatite and fluorapatite. The shape of the Stephan Curve varies among individuals and the rate of recovery of the plaque pH is largely determined by the buffering capacity and urea content of saliva, the degree of access to saliva and the velocity of the salivary film (see Chapters 5 and 6). The buffering capacity of saliva increases with increasing flow rate as the bicarbonate ion concentration increases. The carbonic acid / bicarbonate system is the major buffer in stimulated saliva.

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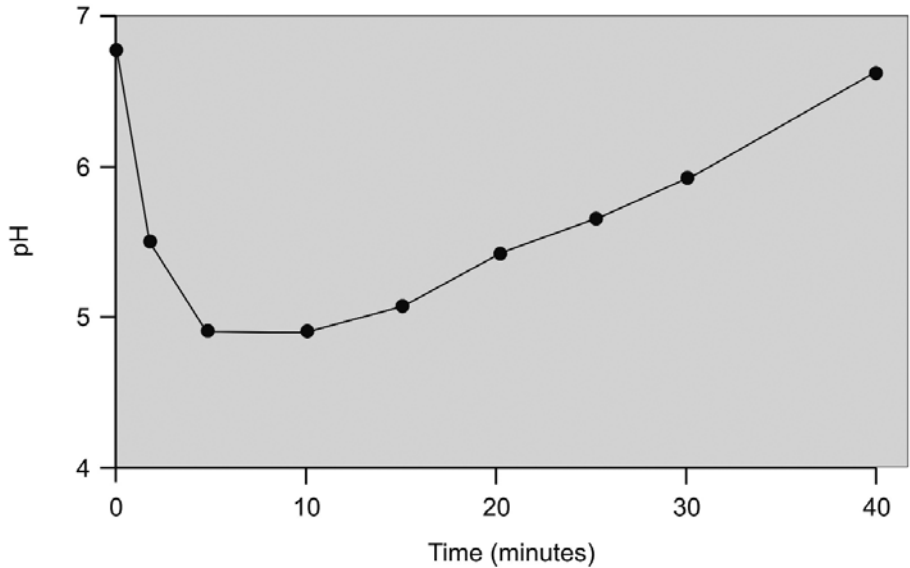


Figure 1.1 Stephan Curve illustrating the changes in plaque pH over time following a sucrose rinse



Hydrogen and bicarbonate ions form carbonic acid, which forms carbon dioxide and water. Carbon dioxide is exhaled and thus the acid is removed.

Anatomy and histology

The type of salivary secretion varies according to gland. Secretions from the parotid gland are serous or watery in consistency, those from the submandibular and sublingual glands, and particularly the minor mucous glands, are much more viscous, due to their glycoprotein content. The histology of the gland therefore varies according to gland type.

INTRODUCTION: THE ANATOMY AND PHYSIOLOGY OF SALIVARY GLANDS

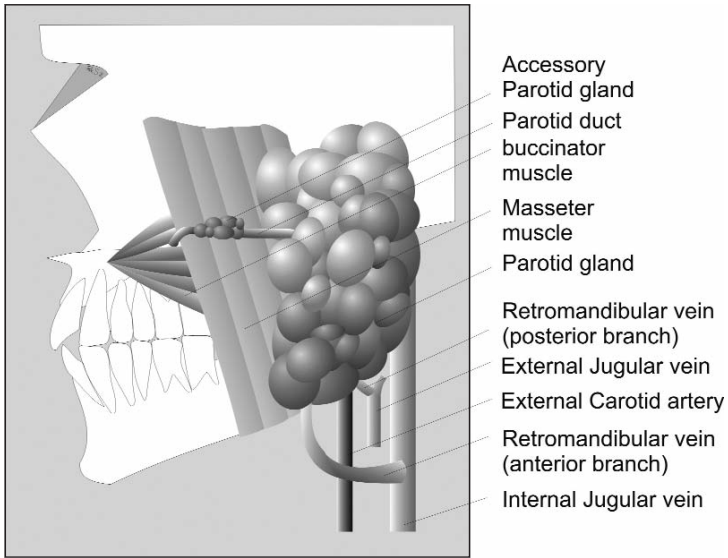


Figure 1.2a Anatomy of Parotid Gland

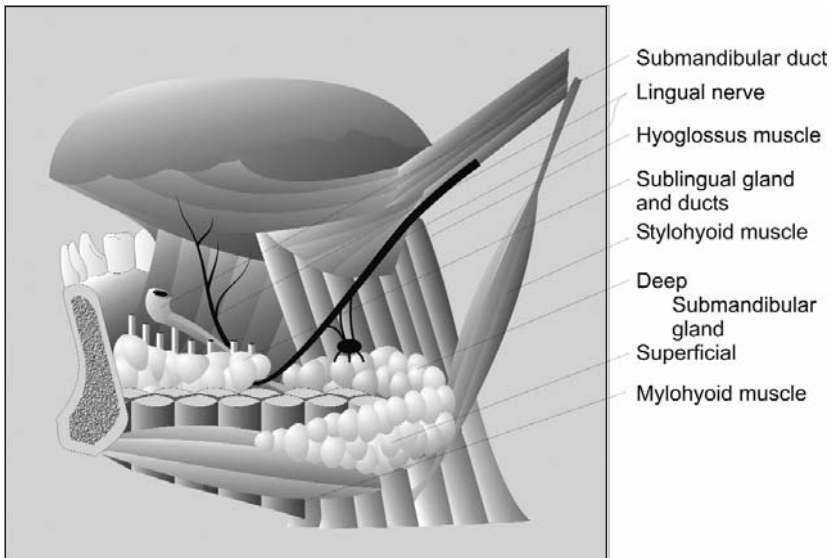


Figure 1.2b Anatomy of Sublingual and Submandibular Glands

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All of the salivary glands develop in a similar way. An ingrowth of epithelium from the stomatodeum extends deeply into the ectomesenchyme and branches profusely to form all the working parts of the gland. The surrounding ectomesenchyme then differentiates to form the connective tissue component of the gland i.e. the capsule and fibrous septa that divide the major glands into lobes. These developments take place between 4 and 12 weeks of embryonic life, the parotids being the first and the sublingual and the minor salivary glands being the last to develop. The minor salivary glands are not surrounded by a capsule but are embedded within the connective tissue. *Figure 1.2* shows some of the relations of the parotid, the submandibular and the sublingual glands.

The parotids are the largest salivary glands. They are wedge-shaped with the base of the wedge lying superficially covered by fascia and the parotid capsule. They are situated in front of the ear and behind the ramus of the mandible. The apex of the wedge is the deepest part of the gland. The gland is intimately associated with the peripheral branches of the facial nerve (CN VII). This relationship is particularly noticeable when an inferior alveolar nerve block is inadvertently administered too high up in a child. In this situation the anaesthetic is delivered into the parotid gland and the facial nerve is anaesthetised, thus resulting in an alarming appearance of a drooping eyelid, which is of course temporary.

The parotid duct is thick-walled, formed by the union of the ductules which drain the lobules of the gland. It emerges at the anterior border of the gland on the surface of the masseter muscle and hooks medially over its anterior border. It can be felt at this point by moving a finger over the muscle with the jaw clenched. The duct opens into the oral cavity in a papilla opposite the second upper molar tooth. The parotid secretions are serous.

The submandibular gland is variable in size being about half the size of the parotid. Its superficial part is wedged between the body of the mandible and the mylohyoid muscle (which forms the floor of the mouth). The gland hooks around the sharply defined posterior border of the mylohyoid muscle and its smaller deep part lies above the mylohyoid in the floor of the mouth. The thin-walled duct runs forward in the angle between the side of the tongue and mylohyoid. It opens into the floor of the mouth underneath the anterior part of the tongue, on the summit of the sublingual papilla lateral to the lingual fraenum. The secretions are a mixture of mucous and serous fluids.

The sublingual is the smallest of the paired major salivary glands, being about one fifth the size of the submandibular. It is situated in the floor of the mouth beneath the sublingual folds of mucous membrane. Numerous small ducts (8-20) open into the mouth on the summit of the sublingual fold or, in some people, join the submandibular duct. It is predominantly a mucous gland.

Minor salivary glands are found throughout the oral cavity; these small glands include the buccal, labial, palatal, palatoglossal and lingual glands. The buccal and labial glands contain both mucous and serous components, the palatal and palatoglossal glands are mucous glands, the lingual glands are mucous except for the serous glands of Von Ebner, which are found around the circumvallate papillae (conspicuous dome-shaped papillae on the posterior dorsum of the tongue).

Structure of salivary glands

The working parts of the salivary glandular tissue (*Figure 1.3*) consist of the secretory end pieces (acini) and the branched ductal system. In serous glands (e.g. the parotids) the cells in the end piece are arranged in a roughly spherical form. In mucous glands they tend to be arranged in a tubular configuration with a larger central lumen. In both types of gland the cells in the end piece surround a lumen and this is the start of the ductal system. There are three types of duct present in all salivary glands. The fluid first passes through the intercalated ducts which have low cuboidal epithelium and a narrow lumen. From there the secretions enter the striated ducts which are lined by more columnar cells with many mitochondria. Finally, the saliva passes through the excretory ducts where the cell type is cuboidal until the terminal part which is lined with stratified squamous epithelium.

End pieces may contain mucous cells, serous cells or a mixture of both. A salivary gland can consist of a varied mixture of these types of end pieces. In mixed glands, the mucous acini are capped by a serous demilune. In addition, myoepithelial cells surround the end piece, their function being to assist in propelling the secretion into the ductal system. The gland and its specialised nerve and blood supply are supported by a connective tissue stroma.

Formulation of saliva

The fluid formation in salivary glands occurs in the end pieces (acini) where serous cells produce a watery seromucous secretion and mucous cells produce a viscous mucin-rich secretion. These secretions arise by the formation of interstitial fluid from blood in capillaries, which is then modified by the end piece cells. This modified interstitial fluid is secreted into the lumen. From the lumen it passes through the ductal system where it is further modified. Most of the modification occurs in the striated

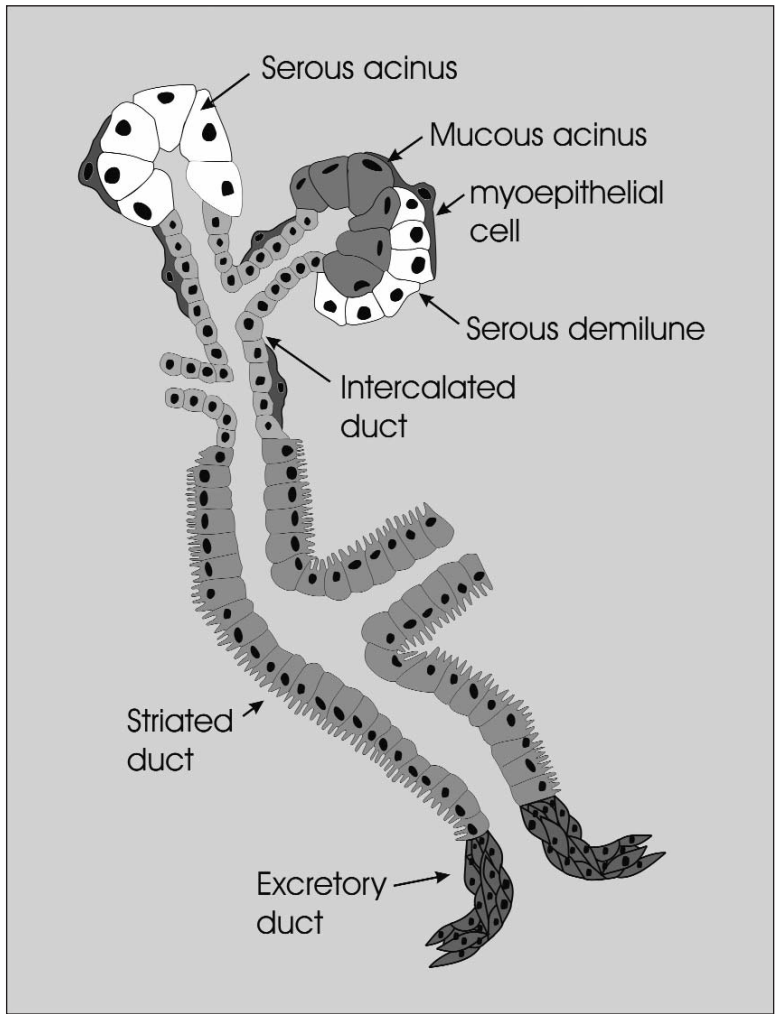


Figure 1.3 Salivary Glandular Tissue

ducts where ion exchange takes place and the secretion is changed from an isotonic solution to a hypotonic one. The composition of saliva is further modified in the excretory ducts before it is finally secreted into the mouth (see Chapter 2 for a detailed account of saliva secretory mechanisms).

Nerve supply

Secretion of saliva is a nerve-mediated reflex. The volume and type of saliva secreted is controlled by the autonomic nervous system.

The glands receive both parasympathetic and sympathetic nerve supplies. The reflex involves afferent receptors and nerves carrying impulses induced by stimulation, a central hub (the salivary nuclei), and an efferent part consisting of parasympathetic and sympathetic autonomic nerve bundles that separately innervate the glands.

Taste and mastication are the principal stimuli (unconditioned reflex) but others such as sight, thought and smell of food (conditioned reflex) also play a role. Taste and mechanical stimuli from the tongue and other areas of the mouth excite parasympathetic nerve impulses in the afferent limbs of the salivary reflex which travel via the glossopharyngeal (CN IX), facial (CN VII), vagal (CN X) (taste) and the trigeminal (CN V) (chewing) cranial nerves. These afferent impulses are carried to the salivary nuclei located approximately at the juncture of the pons and the medulla. In turn impulses from the salivary centres can be modulated i.e. stimulated or inhibited by impulses from the higher centres in the central nervous system; for example, the taste and smell centres in the cortex and the lateral hypothalamus where the regulation of feeding, drinking and body temperature occurs. Also, in stressful situations dry mouth sometimes occurs, as a result of the inhibitory effect of higher centres on the salivary nuclei. The secretory response of the gland is then controlled via the glossopharyngeal nerve synapsing in the otic ganglion, the postganglionic parasympathetic fibres carrying on to the parotid gland and via the facial nerve synapsing in the submandibular ganglion and carrying on to the sublingual and submandibular glands. Parasympathetic stimulation also increases the blood flow to the salivary glands, increasing the supply of nutrition.

Other reflexes originating in the stomach and upper intestines also stimulate salivation. For example, nausea or swallowing very irritating foods initiates reflex salivation which serves to dilute or neutralise the irritating substances.

Sympathetic stimulation can also increase salivary flow to a moderate extent but much less so than parasympathetic stimulation. Sympathetic impulses are more likely to influence salivary composition by increasing exocytosis from certain cells and inducing changes in the reabsorption of electrolytes. The relevant efferent sympathetic nerves originate in the spinal cord, synapse in the superior cervical ganglia and then travel along blood vessels to the salivary glands.

Hormones such as androgens, oestrogens, glucocorticoids and peptides also influence salivary composition.

Blood supply

The blood supply to the glands also influences secretion. An extensive blood supply is required for the rapid secretion of saliva. There is a concentration of capillaries around the striated ducts where ionic exchange takes place whilst a lesser density supplies the terminal secretory acini. The process of salivation indirectly dilates the blood vessels, thus providing increased nutrition as needed. Salivary secretion is usually accompanied by a large increase in blood flow to the glands.

The main arterial supply to the parotid gland is by the superficial temporal and external carotid arteries. Venous drainage is provided by numerous veins which drain into the retromandibular and external jugular veins. Lymph drainage goes mainly via the superficial and deep parotid nodes to the deep cervical nodes. The submandibular gland takes its arterial blood supply from branches of the facial artery and a few branches of the lingual artery. Venous drainage is via the common facial and lingual veins and lymph drainage goes via the submandibular lymph nodes and the deep cervical and jugular chains. The sublingual gland is served by the sublingual branch of the lingual artery as well as the submental branch of the facial artery and drainage is by the submental branch of the facial vein. Lymph drainage goes to the submandibular lymph nodes.

Physiology

Composition

The composition of saliva varies according to many factors including the gland type from which it is secreted. The average compositions of both unstimulated and chewing-stimulated whole saliva are shown in *Table 1.2*.

Flow rate

Salivary flow rate exhibits circadian variation and peaks in the late afternoon; the acrophase. Normal salivary flow rates are in the region of 0.3-0.4 ml/min when unstimulated and 1.5-2.0 ml/min when stimulated, although both rates have wide normal ranges (see Chapter 3). Approximately 0.5 – 0.6 litres of saliva is secreted per day. The contribution of the different glands to whole saliva varies according to the level of stimulation. For unstimulated saliva, about 25% comes from the parotid glands, 60% from the submandibular glands, 7-8% from the sublingual gland and 7-8% from the minor mucous glands. During sleep, flow rate is negligible. For highly stimulated

INTRODUCTION: THE ANATOMY AND PHYSIOLOGY OF SALIVARY GLANDS

Table 1.2: The composition of unstimulated and chewing-stimulated whole saliva (Courtesy of C Dawes). Cells are blank where quantitative data are not available

	Unstimulated	Stimulated
Water	99.55 %	99.53% ¹
Solids	0.45%	0.47% ¹
	Mean ± S.D.	Mean ± S.D.
Flow Rate	0.32 ± 0.23 ²	2.08 ± 0.84 ³
pH	7.04 ± 0.28	7.61 ± 0.17 ⁴
Inorganic Constituents		
Sodium (mmol/L)	5.76 ± 3.43	20.67 ± 11.74 ⁴
Potassium (mmol/L)	19.47 ± 2.18	13.62 ± 2.70 ⁴
Calcium (mmol/L)	1.32 ± 0.24	1.47 ± 0.35 ⁴
Magnesium (mmol/L)	0.20 ± 0.08	0.15 ± 0.05 ⁵
Chloride (mmol/L)	16.40 ± 2.08	18.09 ± 7.38 ⁴
Bicarbonate (mmol/L)	5.47 ± 2.46	16.03 ± 5.06 ⁴
Phosphate (mmol/L)	5.69 ± 1.91	2.70 ± 0.55 ⁴
Thiocyanate (mmol/L)	0.70 ± 0.42	0.34 ± 0.20 ⁶
Iodide (µmol/L)		13.8 ± 8.5 ⁷
Fluoride (µmol/L)	1.37 ± 0.76 ⁸	1.16 ± 0.64 ⁹
Organic Constituents		
Total protein (mg/L)	1630 ± 720	1350 ± 290 ¹⁰
Secretory IgA (mg/L)	76.1 ± 40.2	37.8 ± 22.5 ⁶
MUC5B (mg/L)	830 ± 480	460 ± 200 ¹⁰
MUC7 (mg/L)	440 ± 520	320 ± 330 ¹⁰
Amylase (U = mg maltose/mL/min)	317 ± 290	453 ± 390 ¹¹
Lysozyme (mg/L)	28.9 ± 12.6	23.2 ± 10.7 ⁶
Lactoferrin (mg/L)	8.4 ± 10.3	5.5 ± 4.7 ⁶
Statherin (µmol/L)	4.93 ± 0.61 ¹²	
Albumin (mg/L)	51.2 ± 49.0	60.9 ± 53.0 ¹¹
Glucose (µmol/L)	79.4 ± 33.3	32.4 ± 27.1 ¹³
Lactate (mmol/L)	0.20 ± 0.24	0.22 ± 0.17 ⁴
Total Lipids (mg/L)	12.1 ± 6.3 ¹⁴	13.6 ¹⁵
Amino Acids (µmol/L)	780 ¹⁶	567 ¹⁷
Urea (mmol/L)	3.57 ± 1.26	2.65 ± 0.92 ¹⁸
Ammonia (mmol/L)	6.86 ¹⁹	2.57 ± 1.64 ²⁰

Table 1.2: References

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saliva the contribution from the parotids increases to an estimated 50%, the submandibulars contribute 35%, the sublinguals 7-8% and 7-8% comes from the minor mucous glands.

Many drugs used for the treatment of common conditions such as hypertension, depression and allergies (to mention but a few), also influence salivary flow rate and composition. Factors influencing salivary flow rate and composition are considered in more detail in Chapter 3.

The determination of a patient's salivary flow rate is a simple procedure. Both unstimulated and stimulated flow rates can be measured and changes in flow can be monitored over time to establish a norm for that patient. Measurement of salivary flow is considered further in Chapters 3 and 4. Other clinical investigations of salivary function such as sialography and scintiscanning require referral for specialist evaluation.

Effects of ageing

Although dry mouth is a reasonably common complaint of older adults, the total salivary flow rate is independent of age; reduced salivary flow rate does not occur primarily as a result of the ageing process but is secondary to various diseases and medications, the reduction in salivary flow being related to the number of medications taken simultaneously. Acinar cells, however, do degenerate with age. The submandibular gland is more sensitive to metabolic/physiological change, thus the unstimulated salivary flow, the majority of which is contributed by the submandibular gland, is affected more by physiological changes.

Saliva as a diagnostic fluid

Caries risk assessment

A number of caries risk assessment tests based on measurements in saliva have been developed. Examples are tests which measure salivary mutans streptococci and lactobacilli and salivary buffering capacity. High levels of mutans streptococci, i.e. $>10^5$ colony forming units (CFUs) per ml of saliva, are associated with an increased risk of developing caries. High levels of Lactobacilli ($>10^5$ CFUs per ml saliva) are found amongst individuals with frequent carbohydrate consumption and are also associated with an increased risk of caries. Buffering capacity is a measure of the host's ability to neutralise the reduction in plaque pH produced by acidogenic organisms. Salivary tests are useful indicators of caries susceptibility at the individual level where they can be

SALIVA AND ORAL HEALTH

used for prospective monitoring of caries preventive interventions and for profiling of patient disease susceptibility. Although many efforts have been made to identify a test or combination of tests to predict caries development, no one test has been found to predict this multifactorial disease accurately. In fact, past caries history in the primary and permanent dentitions is presently the best indicator of caries susceptibility.

A number of salivary variables measured for caries risk assessment in dentistry are listed in *Table 1.3*. Some of these variables are more accessible to the practitioner for measurement than others. Whole salivary flow rates are easily measured although due attention must be paid to the conditions under which saliva is collected. Either unstimulated or stimulated flow rate can be measured. Unstimulated flow is of interest because the usual state of the glands is at rest. For stimulated flow, various stimuli such as gustatory (citric acid) and mechanical (chewing) stimulation will produce different results. Because of the circadian rhythm of salivary flow rate, repeated measurements should be made at the same time of day (for details of method of measurement of unstimulated and stimulated flow rates see Ch. 3). Buffering capacity is easily measured at the chairside using a commercially-available kit and may be measured on unstimulated or stimulated saliva; the buffering capacity of the former is usually lower. Paraffin-wax-stimulated saliva samples are used for bacteriological tests as chewing dislodges the flora into the saliva. Mutans streptococci and Lactobacilli may both be cultured from stimulated saliva samples. Commercially-available chairside tests also

Table 1.3: Salivary variables measured for caries risk assessment

Variable	Caries Risk Assessment
Flow rate	At extremes of flow, flow rate is related to caries activity. Low flow rate is associated with increased caries and high flow rate is related to reduced caries risk.
Buffering capacity	Higher buffering capacity indicates better ability to neutralise acid and therefore more resistance to demineralisation.
Salivary mutans streptococci	$>10^5$ CFU/ml saliva indicates increased risk.
Salivary Lactobacilli	$>10^5$ CFU/ml saliva indicates frequent carbohydrate consumption and therefore increased risk.
Fluoride ions	Higher ambient levels of fluoride ions in saliva are associated with use of fluoride products or with water fluoridation.
Ca and P ions	Higher levels associated with less caries.

facilitate their measurement. The biochemical measurement of fluoride, calcium and phosphate requires special laboratory facilities which are not readily available to the practitioner.

General diagnostics

As increasingly sophisticated techniques are available for the study of genes, proteins and bacteria, their application to saliva promises to extend the scope of oral diagnostics to the study of systemic disease as well as oral disease and metabolism. Saliva is easily available for non-invasive sampling and analysis and with careful collection and handling presents possible opportunities for the identification of biomarkers for the two major oral diseases, periodontal disease and dental caries. As the concept of personalised medicine has grown, the use of saliva for pharmacogenomics has also received attention. Pharmacogenomics studies the impact of genetic variation on drug response in patients. It correlates gene expression with a drug's toxicity or efficacy. A pharmacogenomic test result can be used by physicians to select the most effective drug and dose with the least side effects in many different situations; it has the potential to reduce adverse reactions or even death through accidental overdose. The use of oral mucosal swabs instead of blood to collect DNA from cell samples for pharmacogenomics would be far less invasive for physicians and patients and is a natural development in oral diagnostics research. Other future areas for development include the use of saliva or oral swabs to study cancer biomarkers, not only for local but also systemic disease.

Saliva can be used to monitor the presence and level of hormones, drugs, antibodies, and micro-organisms. It can be particularly useful where there are problems with venipuncture; for example where study logistics require repeated sampling, which makes venipuncture uncomfortable or unacceptable. In many instances collections of whole saliva could be easier and more acceptable.

Developments in this important area of diagnostics are still at an early stage and many of the uses listed below are in the early stages of development. In addition to showing promise for the prediction of periodontal disease progression, caries levels, systemic cancer biomarkers and pharmacogenomics, analysis of saliva has been employed in:

- Pharmacokinetics, therapeutic drug monitoring of some drugs and metabolic studies
- Monitoring of a number of drugs: Theophylline, Lithium, Phenytoin and Carbamazepine, Cortisol, Digoxin and Ethanol
- Testing for drugs of abuse

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- Evaluation and assessment of endocrine studies
- Testosterone in the male
- Progesterone in the female
- Diagnostic Immunology - virus diagnosis and surveillance (e.g. antibodies against the measles, rubella and mumps viruses)
- Diagnosis of graft versus host disease
- Screening tests.

Acknowledgements

My thanks to Colin Dawes for compiling *Table 1.2* and to Mairead Harding for her comments on the draft.

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Mechanisms of salivary secretion

Peter M Smith

Introduction

Salivary secretion may be defined as “*A unidirectional movement of fluid, electrolytes and macromolecules into saliva in response to appropriate stimulation*”. This simple statement encapsulates most aspects of the secretory process. The critical words in the statement are **stimulation**, **fluid** and **electrolytes**, **macromolecules** and finally **unidirectional**.

‘**Stimulation**’ encompasses the neural mechanisms that integrate the response to salivary stimuli, such as taste and mastication, and the processes within each salivary acinar cell that communicate between the nervous system and the secretory machinery. All important aspects of salivation are regulated by nerves and this regulation is mediated through G-protein coupled receptors.

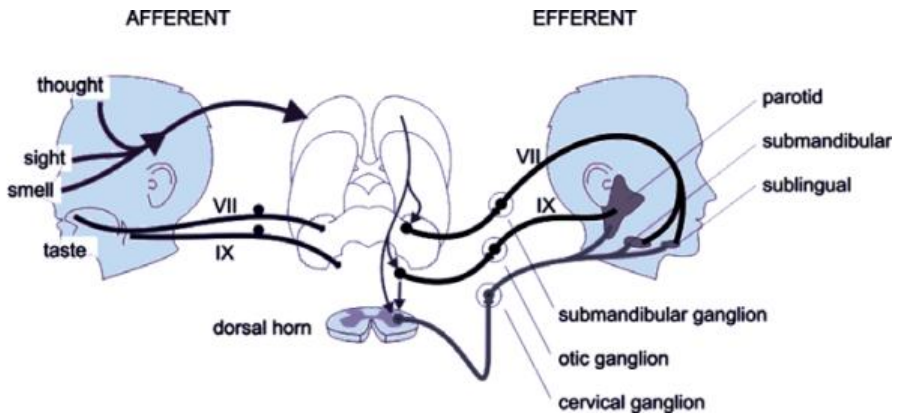
‘**Fluid**’, ‘**electrolytes**’ and ‘**macromolecules**’ describe defining components of saliva. The unique viscoelastic and antibacterial properties of saliva stem largely from its protein component. The electrolyte content adds acid buffering and remineralisation capabilities and the fluid vehicle dilutes and clears the oral environment (see Chapters 5, 7, and 8). Fluid and electrolyte secretion are functionally entwined, one is not possible without the other and both are largely separate from the processes by which proteins are synthesized and secreted.

The only way to achieve a ‘**Unidirectional**’ movement of fluid, electrolytes and macromolecules across a cell is if one end of the cell behaves differently from the other. It has always been obvious that one end of a secretory acinar cell looks different from the other; what is equally true, but much less obvious is that this polarity extends to every aspect of cell function, including the control of secretion.

Stimulation

Neural control of salivation

The neural control of secretion is outlined in *Figure 2.1*. The primary stimulus for salivation is taste¹ and afferent input is carried to the solitary nucleus in the medulla via the facial (VII) and glossopharyngeal (IX) nerves. Input from mastication and from other senses, such as smell, sight and thought are also integrated in the solitary nucleus. In man, taste and mastication are by far the most important stimuli of salivary secretion. Parasympathetic efferent pathways for the sublingual and submandibular glands are from the facial nerve via the submandibular ganglion and for the parotid gland from the glossopharyngeal nerve via the otic ganglion. These pathways regulate fluid secretion by releasing acetylcholine (ACh) at the surface of the salivary gland acinar cells. Macromolecule secretion is regulated by noradrenaline (NorAd or norepinephrine, US) release from sympathetic nerves. Sympathetic post-ganglionic pathways are from the cervical ganglion of the sympathetic chain. The division between parasympathetic and sympathetic control of different aspects of the secretory process is blurred slightly because parasympathetic nerves may also release peptides, such as substance P and Vasoactive Intestinal Polypeptide (VIP) and NorAd will also bind to Ca²⁺-mobilising α -adrenergic receptors.²



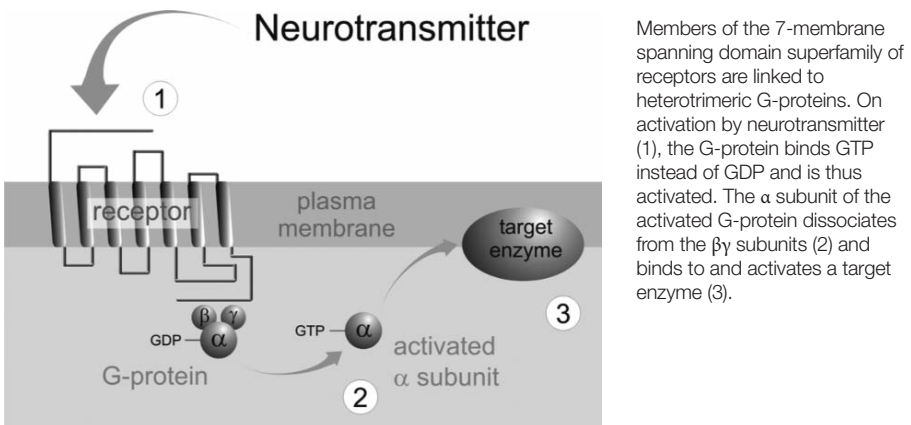
Afferent pathways: taste; facial (VII) and glossopharyngeal (IX) nerves to solitary nucleus in the medulla. Also input from higher centres in response to smell etc. Efferent pathways: Parasympathetic; sublingual and submandibular from facial nerve via submandibular ganglion. Parotid from glossopharyngeal via otic ganglion. Sympathetic post-ganglionic from cervical ganglion of sympathetic chain.

Figure 2.1 The first step in stimulus-secretion coupling is release of a neurotransmitter

Second messengers

Second messengers carry the secretory stimulus from the nerves into the secretory cells and provide a flexible coupling between the intracellular and extracellular environments with built in amplification. Amplification is one of the most significant aspects of 2nd messenger signalling because it transduces a very small extracellular stimulus into a large intracellular event.³

As shown in *Figure 2.2*, fluid secretion is activated by binding of ACh to muscarinic M3 receptors and macromolecule secretion by binding of NorAd to β -adrenergic receptors. Both of these receptors belong to the very large and diverse G-protein-coupled receptor (GPCR) superfamily now known to mediate most responses to hormones and neurotransmitters.⁴ The wide diversity of responses controlled by GPCRs stems from the unique combinations of G-proteins coupled to the receptors. Ligand binding to a GPCR leads to activation of the associated heterotrimeric G-protein by replacement of bound GDP with GTP. The activated α -subunit of the G-protein dissociates from the $\beta\gamma$ subunits and in turn activates a target enzyme⁴. The target enzyme in fluid secretion is phospholipase C (PLC, activated by $G\text{-}\alpha_q$) and in protein secretion adenylate cyclase (activated by $G\text{-}\alpha_s$). The G-protein α subunit is self inactivating because it has an intrinsic GTPase activity. Once GTP is hydrolysed to GDP, the α subunit and the enzyme it has activated switch off again. Nevertheless, the relatively slow rate of GTP hydrolysis means that a single activated target enzyme can process many molecules of substrate before it inactivates.



Members of the 7-membrane spanning domain superfamily of receptors are linked to heterotrimeric G-proteins. On activation by neurotransmitter (1), the G-protein binds GTP instead of GDP and is thus activated. The α subunit of the activated G-protein dissociates from the $\beta\gamma$ subunits (2) and binds to and activates a target enzyme (3).

Figure 2.2 The second step in stimulus-secretion coupling is binding of neurotransmitter to receptor and activation of an intracellular enzyme

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Adenylate cyclase and cyclic-AMP

The next and all subsequent steps in macromolecule secretion are regulated by cyclic-AMP (cAMP). Where *Figure 2.2* shows the general process whereby receptor activation is linked to activation of a 'target enzyme', *Figure 2.3* shows a specific example where the 'target enzyme' is adenylate cyclase. Adenylate cyclase converts ATP into cAMP. Cyclic AMP was the first 2nd messenger to be identified: in fact the term '2nd Messenger' was coined to describe the actions of cAMP. Until relatively recently, cAMP-dependent protein kinase A (pKA) was thought to be essentially the sole mediator of the actions of cAMP.⁵ At rest, pKA is a tetramer composed of 2 catalytic subunits and 2 regulatory subunits. When cAMP binds to pKA, the catalytic subunits separate from the regulatory subunits and become active.⁶ Phosphorylation is a very common mechanism of upregulating the activity of cellular proteins. Protein kinase A phosphorylates and activates the cellular proteins responsible for the synthesis and secretion of salivary macromolecules. A characteristic of cAMP-dependent cellular processes is that upregulation depends not on increased activity of a single enzyme or process but rather on increased activity of many processes. Downregulation of cAMP-dependent processes, including macromolecule secretion, is accomplished by a reduction in cAMP levels mediated by the enzyme cAMP phosphodiesterase.⁷ Phosphodiesterase activity is itself subject to many regulatory factors, including G-protein coupled receptor activation.⁸

Phospholipase C, inositol 1,4,5 trisphosphate and calcium

The third step for stimulus fluid secretion coupling is activation of PLC by G- α_q and production of the soluble second messenger, inositol 1,4,5 trisphosphate (IP₃)⁹,

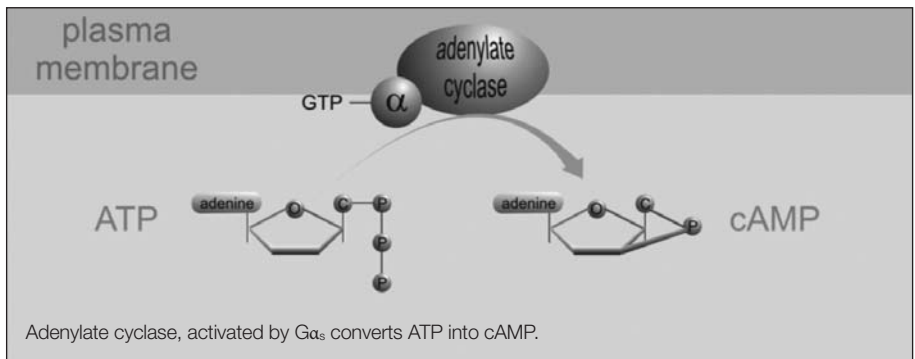


Figure 2.3 The third step in macromolecule stimulus-secretion coupling is production of cAMP

(Figure 2.4). IP₃ acts by binding to IP₃ receptors on endosomes, such as the endoplasmic reticulum (ER), and releasing the Ca²⁺ stored within. The Ca²⁺ content of the ER is maintained at a much higher concentration (≈1 mM) than that of the cytoplasm (≈100 nM) by Ca²⁺ ATPase activity so that activation of a Ca²⁺ channel is sufficient to raise cytosolic Ca²⁺ activity by diffusion from the Ca²⁺ stores. IP₃ receptors are Ca²⁺ channels, activated by IP₃ binding.¹⁰

IP₃ receptors are also sensitive to cytosolic Ca²⁺ activity and stay open for longer when [Ca²⁺]_i is raised. This property of the receptor can dramatically enhance the Ca²⁺ mobilising properties of IP₃ by positive feedback or Ca²⁺-induced Ca²⁺ release (CICR).¹⁰ The Ca²⁺ signal may be further amplified by Ca²⁺ release through ryanodine receptors, a second Ca²⁺ channel also present on the ER of acinar cells.¹¹ Ryanodine receptors are also Ca²⁺ sensitive and contribute to CICR. The sensitivity of ryanodine receptors to Ca²⁺ may be 'set' by the cytosolic concentration of cyclic ADP ribose, a product of βNAD produced by ribosyl cyclase regulated by cyclic GMP and possibly Nitric Oxide levels.¹¹ The Ca²⁺ signal is therefore actively propagated through the acinar cell by an explosive release of Ca²⁺ from stores, triggered by IP₃, amplified by Ca²⁺ and carried by both IP₃ and ryanodine receptors (Figure 2.5).¹²

In addition to mobilising stored Ca²⁺, the secretory process can also utilise extracellular Ca²⁺ from influx across the plasma membrane through store-operated Ca²⁺ channels. Whilst the physiological characteristics of store-operated Ca²⁺ influx

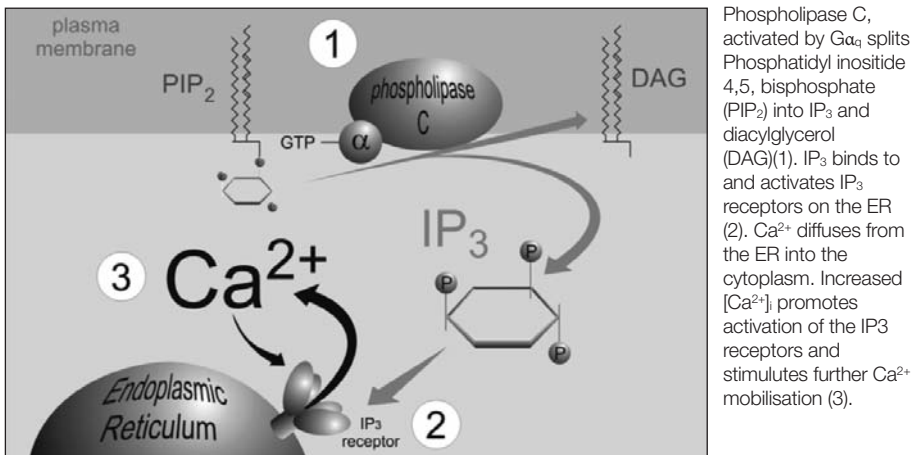
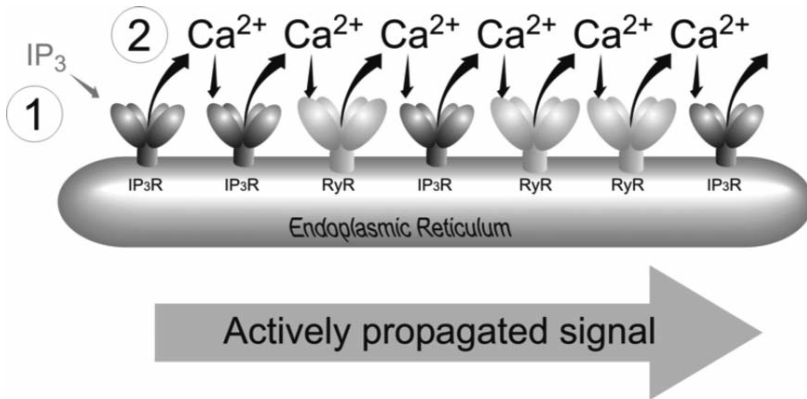


Figure 2.4 The third step in fluid and electrolyte stimulus-secretion coupling is an increase in intracellular Ca²⁺ activity



IP₃ stimulates Ca²⁺ release from IP₃ receptors (IP₃R). Ca²⁺ stimulates further Ca²⁺ release from IP₃R and from ryanodine receptors (RyR). Release of Ca²⁺ by one receptor triggers activation of the next and thus actively propagates the signal. Thus a Ca²⁺ signal may start at the apical pole of the cell and then rapidly become cell-wide

Figure 2.5 Actively propagated Ca²⁺ signal

have been extensively studied over the last 20 years,¹³ it is only recently that the molecular identity of both the Ca²⁺ sensor¹⁴ and the Ca²⁺ channel itself¹⁵ have been discovered. The former is now known to be the Ca²⁺ binding protein STromal Interaction Molecule (STIM)1, situated in the membrane of the endoplasmic reticulum.¹⁶ Dissociation of Ca²⁺ from the EF-hand (a finger or hand-shaped Ca²⁺ binding domain) Ca²⁺ binding region of STIM1 following depletion of the Ca²⁺ stores is the trigger for oligomerisation of STIM1 into a complex capable of interaction with the plasma membrane Ca²⁺ channel protein Orai1 (named for the gatekeepers of heaven in Greek mythology and not for the city in Uttar Pradesh) which opens to allow Ca²⁺ influx.^{13,17} Down-regulation of the Ca²⁺ signal, following closure of both intracellular and extracellular channels, depends mainly on Ca²⁺ ATPase activity to pump the Ca²⁺ back into the stores or out of the cell.

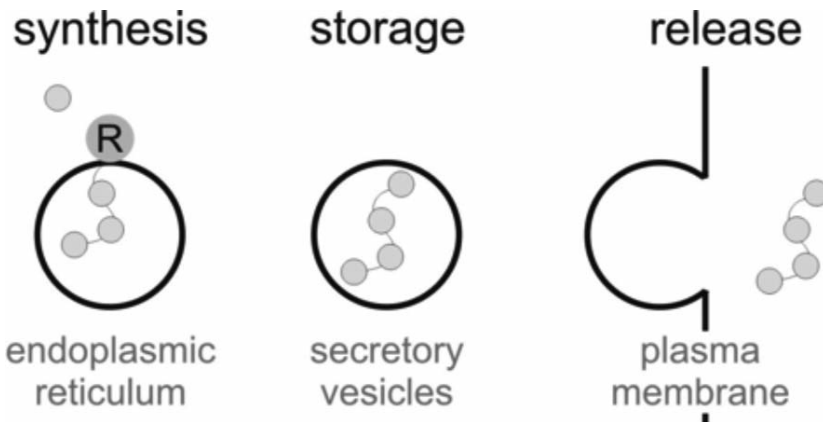
Macromolecules

Macromolecules cannot cross the plasma membrane. At first sight, this might seem to be an insurmountable problem for a protein-secreting cell but the secret to protein secretion is to synthesise proteins for export within endosomes (Figure 2.6). Topologically at least, these proteins are never inside the cell and so do not have to cross the cell membrane to get out. Proteins are secreted when the endosome or vesicle into which they were synthesised fuses with the plasma membrane in the process of exocytosis.

Synthesis of secretory proteins begins with gene transcription and manufacture of messenger RNA to carry the sequence information from the nucleus to ribosomes in the cytoplasm. Secretory proteins start with a 'signal sequence' which targets the developing polypeptide to the ER where it is N-glycosylated and folded into the correct three-dimensional structure. Small membrane vesicles carry proteins from the ER through several layers of the Golgi apparatus for additional processing and 'packaging' for export. Proteins move by default onwards from the ER; those destined to remain in the cell contain specific 'retention sequences' to segregate them from secretory proteins. Secretory proteins are concentrated within Golgi condensing-vacuoles and stored in secretory vesicles. As these mature they are transported close to the apical membrane. In response to a secretory stimulus, secretory vesicles fuse with the plasma membrane and discharge their contents outside the cell.^{1,18}

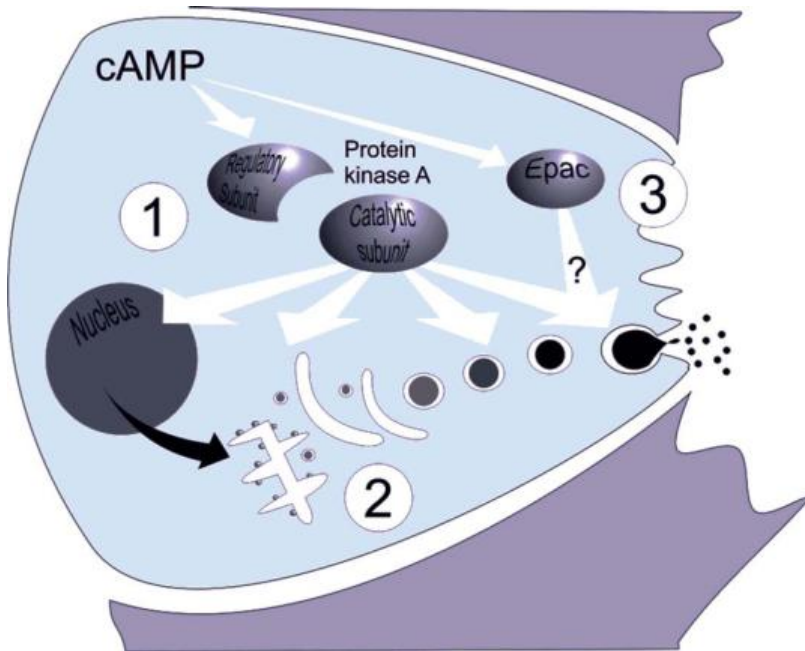
The secretory process may be divided into four stages. Synthesis, segregation and packaging, storage and release. Each of these stages is regulated by phosphorylation of target proteins by cAMP-dependent pKA. Therefore an increase in cAMP stimulates:

- Transcription of genes for salivary proteins (e.g. proline-rich proteins).
- Post-translational modification (e.g. glycosylation)
- Maturation and translocation of secretory vesicles to the apical membrane
- Exocytosis.



Proteins are synthesized inside secretory vesicles by ribosomes (R). Secretory vesicles mature and are stored until a secretory stimulus is received.

Figure 2.6 Secretory proteins are synthesised in endosomes

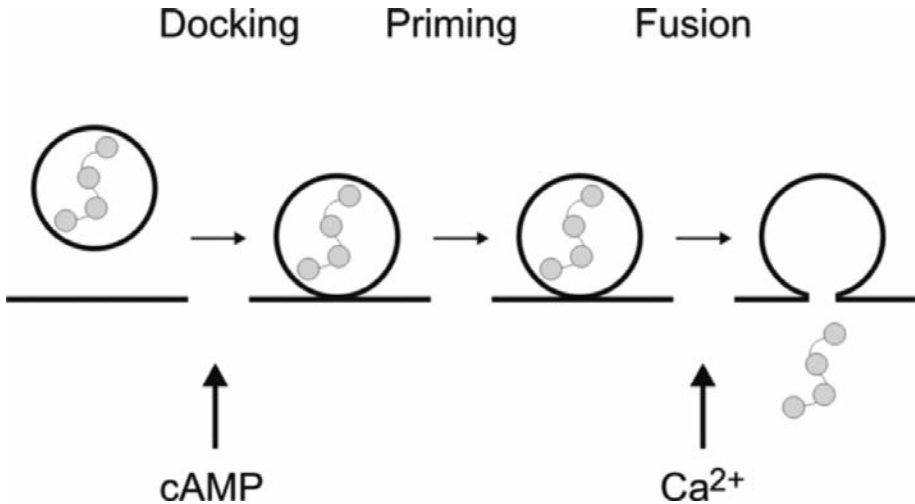


cAMP binding to the regulatory subunit of pKA releases and activates the catalytic subunit (1) The catalytic subunit phosphorylates and upregulates many components of the secretory pathway including exocytosis (2). Recent work has also identified an Epac-mediated pKA-independent mechanism that may also be implicated in the regulation of exocytosis (3)

Figure 2.7 cAMP and PKA

Thus, an increase in the level of cAMP within the cell will stimulate every step involved in the secretion of protein (Figure 2.7).^{19,20}

The molecular components of exocytosis have been extensively studied and the key players, soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors (SNAREs) are present in salivary gland acinar cells. In most secretory cells, unlike salivary acinar cells, an increase in $[Ca^{2+}]_i$ is the proximal trigger for exocytosis. Secretory vesicles have v-SNAREs which recognise plasma membrane t-SNAREs and the two form tight complexes that link the two membranes and mediate the three steps in regulated exocytosis; docking, priming and fusion²⁰ (Figure 2.8). In neuronal cells, for example, secretory vesicles are docked and primed and await a Ca^{2+} signal to trigger exocytosis. Perhaps in salivary gland acinar cells, secretory vesicles wait for a secretory

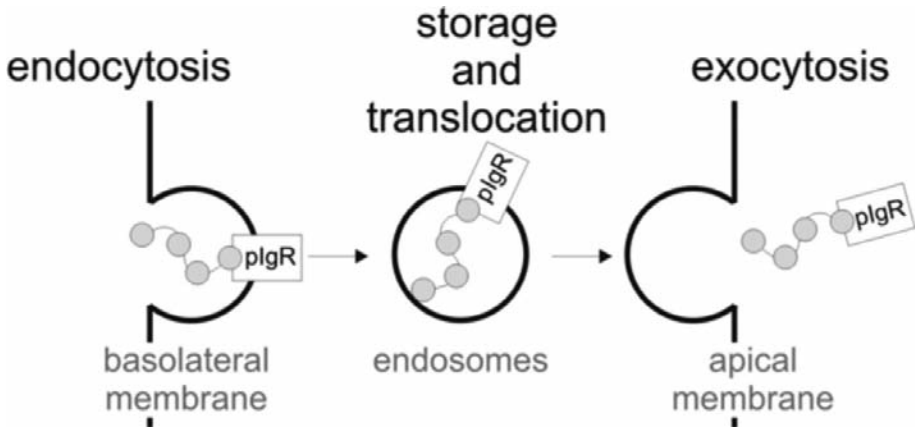


Exocytosis occurs in three stages; docking, priming and fusion. The fusion process itself is Ca^{2+} -dependent. However, earlier stages of the process, e.g. 'docking' could be cAMP-dependent. In salivary gland cells, this step is the rate-limiting 'brake' point in exocytosis.

Figure 2.8 A cAMP-dependent 'brake' point

stimulus at an earlier cAMP-dependent 'brake' point.²⁰ The role of other intracellular signals in the control of exocytosis is becoming better understood and both pKA-dependent and pKA-independent mechanisms have been identified that may be responsible for the control of exocytosis in salivary acinar cells.⁷ Most recently, a role for an exchange protein directly activated by cAMP (Epac) has been identified in insulin secretion in pancreatic β cells. In this relatively novel pKA-independent cAMP-mediated signaling pathway,⁵ Epac functions as guanine nucleotide exchange factor for members of the ras family of small G-proteins and also binds directly to Rim2, a key component of the exocytotic machinery. Whilst details of the mechanism remain unclear, preliminary studies allow the possibility of a similar mechanism in salivary gland cells.²¹

Not all secreted proteins originate in salivary gland cells. Saliva also contains plasma proteins, for example the immunoglobulin, IgA. IgA is no more able to cross the plasma membrane than any other protein and so crosses acinar cells in a membrane vesicle. Receptors for IgA on the basolateral membrane of the acinar cells bind IgA which is taken 'within' the cell by endocytosis. Following transcytosis of the vesicles containing IgA, the immunoglobulin is released into the saliva by exocytosis (*Figure 2.9*).²²



Polymeric IgA and IgM are transported across salivary gland cells by the polymeric immunoglobulin receptor (plgR). The plgR binds its ligand at the basolateral surface and is internalized into endosomes. Here it is sorted into vesicles that transcytose it to the apical surface. At the apical surface the plgR is proteolytically cleaved, and the large extracellular fragment is released together with the ligand.

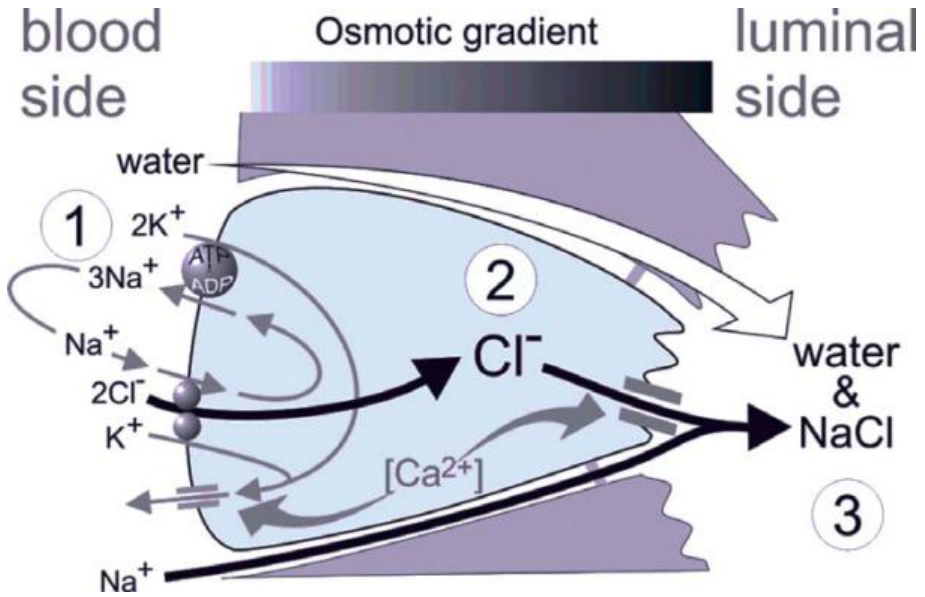
Figure 2.9 Transcellular protein transport

Fluid and electrolytes

Fluid secretion

The fluid secretion is inevitably a process with multiple steps because biological systems cannot actively transport fluid as such. The only way of moving fluid rapidly across a tissue is by osmosis. Therefore, as shown in *Figure 2.10*, fluid-secreting tissues, including salivary acinar cells, concentrate electrolytes by active transport and the concentration gradient forces water to move. Throughout the salivary glands, there is in general only a single cell layer between the extracellular fluid and the lumen of the acinus or duct. Therefore, the processes of secretion and absorption involve transport across a single cellular layer.

Acinar cells utilise the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ triple cotransporter (NKCC1) to actively concentrate Cl^- inside the cell so that activation of an apical membrane Cl^- channel allows Cl^- to leave down its electrochemical gradient into the lumen of the acinus. Na^+ crosses the acinar cells to maintain electroneutrality and the movement of Na^+ and Cl^- create the osmotic gradient across the tissue and water follows. The pivotal step, the single step that determines whether or not a cell is secreting is activation of the apical membrane Cl^- channel whose precise molecular identity has yet to be determined. This step is regulated by increased $[\text{Ca}^{2+}]_i$. A cell-wide increase in $[\text{Ca}^{2+}]_i$ will also activate



The Na^+/K^+ ATPase makes direct use of ATP to pump Na^+ out of the cell and create an inwardly directed Na^+ gradient. This energises the $\text{Na}^+/\text{K}/2\text{Cl}^-$ (NKCC1) cotransport system (1) which in turn concentrates Cl^- above its electrochemical potential (2). Increased $[\text{Ca}^{2+}]$ opens the Ca^{2+} -dependent K^+ and Cl^- channels and Cl^- crosses the apical membrane into the lumen of the acinus (2). Na^+ follows Cl^- across the cell to maintain electroneutrality and the resultant osmotic gradient moves water (3)

Figure 2.10 Fluid secretion follows electrolyte secretion

the basolateral K^+ channel (SLO), which keeps the membrane potential at a high negative value and thus preserves the driving force for Cl^- efflux.

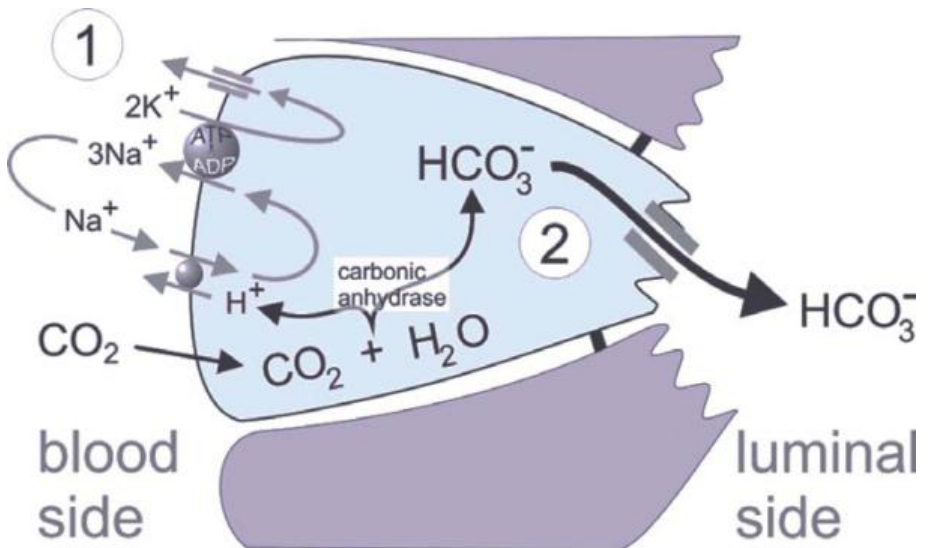
Electrolyte-led fluid transport movement is always isotonic. Once isotonicity is reached, there is no additional driving force for water movement. The ability of salivary glands to generate an hypotonic saliva lies with the striated ducts. Striated duct cells pump electrolytes from the primary saliva by active transport. At first sight, it might seem that this will simply reverse the secretory process, but the striated ducts are impermeable to water, so there can be no osmotically driven water reabsorption. The basic outline of this secretory process was identified as the 'two-stage hypothesis' by Thaysen *et al*^{22a} in 1954. The fluid secretory process in the acinar cells has a much greater capacity than the electrolyte reabsorptive process in the ducts. This is why the composition of saliva changes with flow rate. At low, unstimulated, flow rates, saliva

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moves slowly through the ducts and the striated ducts are able to substantially modify the composition of the saliva. At high, stimulated, flow rates, the saliva passes rapidly through the ducts with little alteration. The composition of saliva at high flow rates more closely resembles that of the primary saliva produced by the acinar cells.

Bicarbonate secretion

The secretory process for bicarbonate is similar to that for Cl^- inasmuch as bicarbonate is concentrated within acinar cells and released following receipt of a secretory stimulus. Details of the process for bicarbonate are much less well understood than for Cl^- . In most salivary glands, uptake is probably via a carbonic anhydrase mediated process that depends ultimately on Na^+/H^+ exchange and the Na^+ gradient. Efflux is probably



Carbon dioxide inside cells is converted to HCO_3^- and H^+ by carbonic anhydrase. HCO_3^- is secreted across the apical membrane of the cell through an anion channel (2). H^+ are actively extruded across the basolateral membrane by Na^+/H^+ exchange energised by the Na^+ gradient which is created by the action of the Na^+/K^+ ATPase (1). If protons were not lost from the cell, carbonic anhydrase would be unable to generate HCO_3^- .

Figure 2.11 Fluid secretion follows electrolyte secretion

via a bicarbonate-permeable channel (*Figure 2.11*). The Ca^{2+} -dependent Cl^- channel is bicarbonate permeable and bicarbonate efflux via this channel would be the most simple mechanism for bicarbonate secretion. Qualitatively at least, bicarbonate secretion will be as effective as Cl^- secretion as a mechanism for driving fluid movement.¹⁹

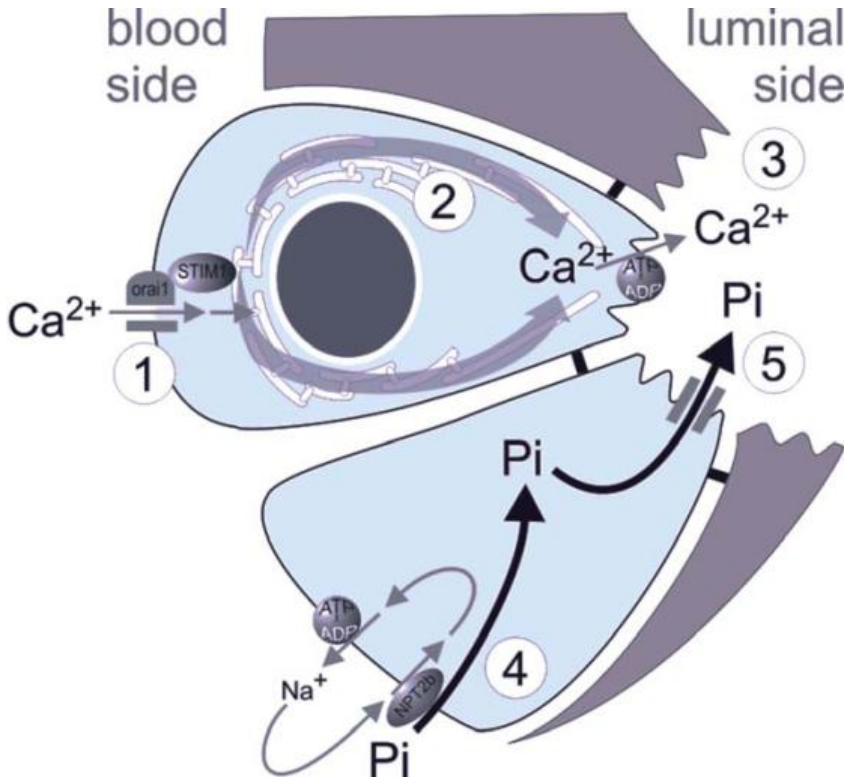
Bicarbonate is one of the electrolytes reabsorbed by the striated ducts and bicarbonate concentration in unstimulated saliva is consequently low. A combination of increased secretion and failure to reabsorb bicarbonate at high flow rates is the simplest explanation of the much higher bicarbonate concentration in stimulated saliva.

Acinar cells secrete macromolecules and fluid and electrolytes. Striated duct cells reabsorb electrolytes. Intercalated ducts lie between the acini and the striated ducts and seem to function more like acinar cells than striated duct cells. They probably make little contribution to protein secretion but may have an important role in bicarbonate and fluid secretion.

Calcium and phosphate secretion

Calcium not only has a pivotal role in the control of secretion, it also has, along with phosphate (Pi), an important role in oral homeostasis, in particular with respect to the teeth. The mineral content of teeth is water soluble and teeth would demineralise in a simple bicarbonate-rich NaCl solution. Saliva contains, in addition, sufficient Ca^{2+} and Pi to prevent demineralisation (*Figure 2.12*). There is therefore a unidirectional transport of Ca^{2+} and Pi across the acinar epithelial cells and into the saliva. All the components for Ca^{2+} translocation are present in salivary acinar cells.^{23,24} Ca^{2+} pumped out of cells across the apical membrane of the cells would be replaced by Ca^{2+} that had 'tunnelled' across the cells within the endoplasmic reticulum (see below and ²⁵). Ca^{2+} influx across the basolateral membrane via store-operated Ca^{2+} influx will replenish the Ca^{2+} within the stores. Thus Ca^{2+} may be translocated across cells without deranging the cellular processes which depend on low $[\text{Ca}^{2+}]_i$. Despite the probable involvement of primary active transport in the Ca^{2+} translocation process, Ca^{2+} activity in saliva is similar to that of the blood. This is not the case for Pi, which may be concentrated several fold in saliva. Translocation of Pi therefore involves transepithelial active transport. The uphill, energy-dependent, step of Pi transport is across the basolateral membrane by the well characterised Na^+ -dependent Pi transporter, NPT2b.²⁶ Phosphate exits the acinar cells down its concentration gradient through an as yet unidentified pathway. The phosphate concentration, like that of HCO_3^- , is modified by the passage of saliva through the ducts. NPT2b is also expressed on the apical membrane of ductal cells and it has been proposed that here it functions to reabsorb Pi from the primary saliva.²⁶

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A possible mechanism for Ca^{2+} translocation. Ca^{2+} enters across the basolateral membrane through Orai1 Ca^{2+} channels (1) and tunnels across the cell in the ER (2) to be released at the apical pole and extruded by the PMCA (3). The active step of phosphate translocation is uptake across the basolateral membrane by the Na^+ -coupled Pi transporter NPT2b which utilizes the inwardly directed Na^+ gradient to concentrate Pi inside the cells (4). Pi exits across the apical membrane down its electrochemical gradient through an as yet unidentified mechanism (5).

Figure 2.12 Calcium and phosphate transport

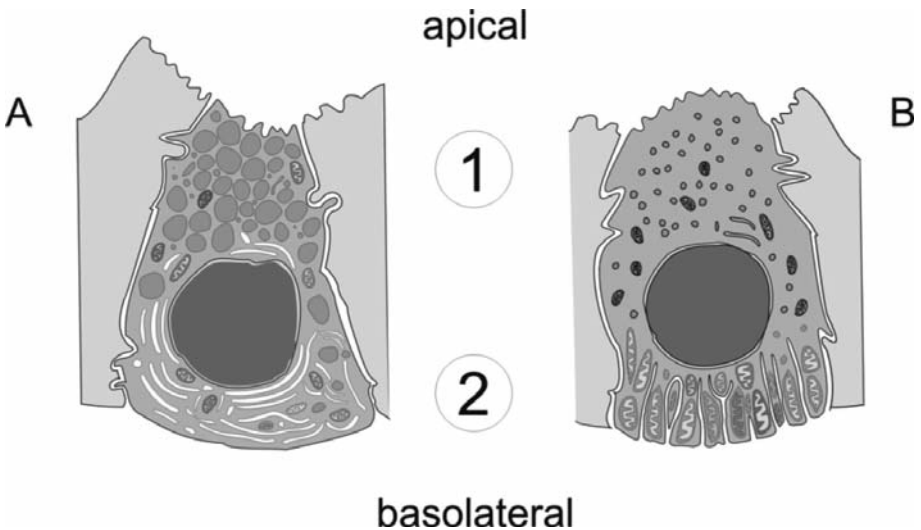
Water channels

There are two possible routes for water to take across the cell, either through the tight junctions between the cells (paracellular) or across both the apical and basolateral membranes (transcellular). There has been much discussion as to which is the dominant route and little evidence to distinguish absolutely between them.²⁷ The

intrinsic water permeability of the plasma membrane is very low and both apical and basolateral membranes must therefore contain water channels to facilitate transcellular water transport. Water channels in salivary acinar cells are members of the aquaporin (AQP) family. Aquaporins are membrane proteins composed of 4 subunits, each of which has 6 membrane-spanning domains that form a water-permeable pore. Aquaporins come in two types, one of which transports only water and another which is also permeable to glycerol. Neither type conducts ions.²⁸ There are at least 10 mammalian aquaporin isoforms and AQP5 has been localised to the apical membrane of salivary gland acinar cells. AQP5 knockout mice (Genetically modified mice that cannot produce AQP5) show a 60% reduction in stimulated flow in airway mucosal glands which would suggest that at least this proportion of water flow is transcellular.²⁹

Unidirectional

In normal circumstances the secretory process works only one way. The unidirectionality of secretion is achieved by the barrier function of the acinar and duct cells in separating blood from saliva and, at a cellular level, by polarisation of structure



Acinar and striated ducts are very obviously polarised. Acinar cells (A) have a high density of secretory vesicles at the apical pole (1) and striated duct cells (B) have basal infoldings and a high density of mitochondria (2).

Figure 2.13 Histological polarity

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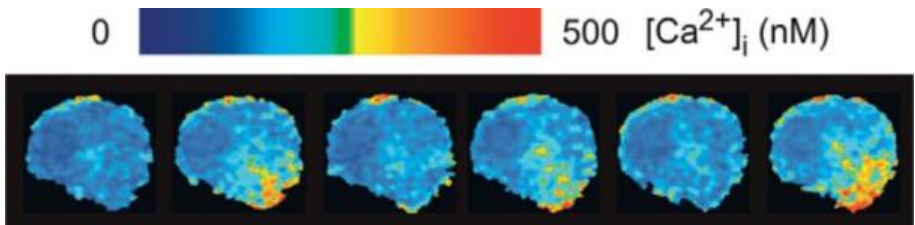
(Figure 2.13) and function. Every cell type involved in salivary secretion is polarised in one way or another. Acinar and duct cells are connected together by tight junctions, which also form the division between the apical membrane which faces into the lumen of the gland and the basolateral membrane which faces the extracellular fluid. The different properties of these two membranes are fundamental to the polarisation of cell function necessary for unidirectional secretion.

Striated ducts are so called because in longitudinal section, their basolateral side has a striped appearance. The stripes are caused by many infoldings of the basal membrane, crammed full of mitochondria (Figure 2.13). A high density of mitochondria, close to the plasma membrane is usually indicative of primary active transport, in this case the Na^+/K^+ ATPase. The most obviously defining feature of acinar cells is the apical pole of the cell, densely packed with secretory vesicles.

From a functional perspective, the apical pole of the acinar cells is where all the most critical events occur. Secretory vesicles are directed by the actin cytoskeleton towards the apical pole of the cell and exocytosis occurs almost exclusively at the apical pole.

The key event in fluid secretion, activation of the Ca^{2+} -dependent anion channel also occurs at the apical pole. There is growing evidence to indicate that the controlling Ca^{2+} signal originates at the apical pole of the cell and in certain circumstances, may be restricted to this pole of the cell (Figure 2.14).³⁰

Calcium signals are very 'expensive' in terms of the metabolic cost of holding $[\text{Ca}^{2+}]_i$ at nanomolar levels and, because sustained elevated Ca^{2+} levels are cytotoxic, potentially dangerous to the cell. Spatially restricted Ca^{2+} signals may be an elegant resolution to both of these problems. It has proved very challenging to elucidate the mechanisms



Sequential Ca^{2+} image maps taken over 20 s of a single mouse submandibular acinar cell loaded with the Ca^{2+} -sensitive dye fura-2 and stimulated with 20 nM ACh. The Ca^{2+} signal manifests only at the apical pole, at the bottom of the image. Each Ca^{2+} response lasted < 500 ms

Figure 2.14 Local Ca^{2+} signals

underlying local Ca^{2+} signals, not least of all, how the cell stops the signal from propagating across the cell by Ca^{2+} -induced Ca^{2+} release. A partial answer to this question may simply be that spatially restricted Ca^{2+} signals are very brief. The apical origin of the Ca^{2+} signal is slightly odd, given that the ER, which is thought to be the primary source of stored Ca^{2+} , is almost exclusively distributed through the basolateral region of the cell. Secretory vesicles, which have a very obvious apical location, have been proposed as a possible Ca^{2+} store.³¹ An alternative, and more widely accepted, mechanism depends on the reticulate nature of the ER. In this model, Ca^{2+} is stored at the basolateral pole of the cell and ‘tunnels’ through the ER to the apical pole where it is released (*Figure 2.14*).²⁵ This last mechanism also offers the intriguing possibility that the actin cytoskeleton that shapes the dynamic structure of the ER and regulates transport of secretory vesicles to the secretory pole of the cell, might also have a role in the control of fluid secretion.²⁴ Ca^{2+} tunnelling also offers a mechanism of transepithelial translocation of Ca^{2+} that is intrinsically coupled to the process of fluid secretion, because the apical Ca^{2+} activity is greatest when secretion is triggered.

Pharmacological control of fluid and electrolyte secretion

Every step of stimulus-secretion coupling is potentially vulnerable to dysfunction under pathological conditions. The challenge for secretory physiologists studying autoimmune hyposalivation conditions, such as Sjögren’s syndrome, is to find the points at which the immune response could damage the secretory process.^{32,33} There is a growing body of evidence to suggest that severe glandular atrophy is the end-stage of Sjögren’s syndrome and that glandular hypofunction occurs much earlier in the pathology of the condition. Contrariwise, every step of stimulus-secretion coupling is a potential point for therapeutic intervention. Stimulation of fluid secretion by activation of muscarinic receptors is one of the more obvious and accessible entry points. Pilocarpine, a naturally occurring alkaloid, is probably the best known therapeutic cholinomimetic agent and is distributed under the trade name ‘Salagen.’ Cevimeline (evoxac) is another cholinomimetic agent used therapeutically in the US which may have a higher specificity for muscarinic M3 receptors than pilocarpine and potentially, therefore fewer side effects. The side effects of therapeutic application of salagen (15-30 mg/day) or evoxac (90 mg/day), sweating etc. are usually tolerated in preference to dry mouth (see Chapter 4). ACh itself is of little use therapeutically because it is so rapidly metabolised. Saliva production may be blocked by cholinergic receptor antagonists, such as atropine, which compete with ACh for muscarinic receptors and prevent the effects of parasympathetic stimulation on fluid and electrolyte secretion. The most common cause of dry mouth is as a side effect of xerogenic drugs used to treat other conditions.

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Microfluorimetry, electrophysiology and molecular biology are proving to be a powerful combination with which to study secretory mechanisms. For example: fluorescent probes for subcellular components, such as the ER, mitochondria or the nucleus may be used to visualise these organelles in living cells and determine their role in signal transduction. Caged agonists or second messengers may be used to provide precise spatial mapping of intracellular responses and so further refine our understanding of cellular polarisation. Genes for key elements of the secretory machinery can be linked to fluorescent markers and expressed and visualised in isolated acinar cells by transient transfection. Gene knockouts can help pinpoint the function of specific proteins, such as AQP5 or ACh M3 receptors. There is now great scope and great potential in turning these powerful techniques towards understanding glandular pathologies

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Factors Influencing Salivary Flow Rate and Composition

C. Dawes

Introduction

This chapter covers the differences in flow rate and composition between unstimulated saliva (secreted continuously in the absence of exogenous stimulation) and stimulated saliva (secreted usually in response to masticatory or gustatory stimulation), the factors influencing salivary flow rate and composition, and their physiological importance.

Unstimulated saliva

Unstimulated whole saliva is the mixture of secretions found in the mouth in the absence of exogenous stimuli such as tastants or chewing. It is composed of secretions from the parotid, submandibular, sublingual, and minor mucous glands but it also contains gingival crevicular fluid, desquamated epithelial cells, bacteria, leucocytes (mainly from the gingival crevice), and possibly food residues, blood, and viruses. Unstimulated whole saliva is usually collected with the patient sitting quietly, with the head down and mouth slightly open to allow the saliva to drip from the lower lip into a beaker or similar receptacle over a given time, or the patient can spit out the saliva at regular intervals, while not swallowing. However, when saliva is spat out rather than drooled, the number of bacteria and desquamated epithelial cells is increased. The measured flow rate is actually the difference between the volume secreted by the different salivary glands and the volumes which may be lost by evaporation, if there is mouth-breathing, or mucosal absorption over the collection period.¹

Several large studies of unstimulated salivary flow rates in healthy individuals (*Table 3.1*)² have found the average value for whole saliva to be about 0.3-0.4 ml/minute, but the normal range is very large and includes individuals with very low flow rates who do not complain of a dry mouth. Such a broad normal range makes it difficult to say whether or

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not a particular individual has an abnormally low flow rate. Unless saliva is almost completely absent, patients can be said to have a dry mouth (xerostomia) only on the basis of their subjective symptoms. However, a flow rate of <0.1 ml/min is considered objective evidence of hyposalivation.

Whether the flow rate is high or low is much less important than whether it has changed adversely in a particular individual. Physicians will often take a patient's blood pressure as a yardstick for future measurements. Dentists, however, do not routinely measure the salivary flow rate, so that when a patient complains of having a dry mouth, it is impossible to judge whether or not a genuine reduction in flow has taken place. It would therefore be very advantageous if dentists included measurement of salivary flow as part of their regular examination. Just as there are individuals with very little saliva but without discomfort, so there are others with flow rates within the normal range who feel that their mouth is drowning in saliva. This problem is often due to difficulty in swallowing, rather than to a genuinely high flow rate.

Factors affecting the unstimulated salivary flow rate (Table 3.2)

Degree of hydration

This is potentially the most important factor. When body water content is reduced by 8%, the salivary flow rate decreases to virtually zero. For a person of about 70 kg,

Table 3.1: Unstimulated salivary flow rate (ml/minute) in healthy individuals (for references see Dawes²)

Studies	Type of saliva	Sample number	Mean (ml/minute)	SD*
Andersson <i>et al.</i> (1974)	Whole	100	0.39	(0.21)
Becks and Wainwright (1943)	Whole	661	0.32	(0.23)
Heintze <i>et al.</i> (1983)	Whole	629	0.31	(0.22)
Shannon and Frome (1973)	Whole	50	0.32	(0.13)
Shannon (1967)	Parotid	4589	0.04	(0.03)
Enfors (1962)	Submandibular	54	0.10	(0.08)

*Note: the very high standard deviations (SD), indicating a very wide range of values covering normality.

FACTORS INFLUENCING SALIVARY FLOW RATE AND COMPOSITION

Table 3.2: Factors affecting the unstimulated salivary flow rate in healthy subjects

Major factors*	Minor factors
Degree of hydration	Gender
Body position	Age (above 15 years)
Exposure to light	Body weight
Previous stimulation	Gland size
Circadian rhythms	Psychic effects - thought/sight of food
Circannual rhythms	Functional stimulation?

*Note: Most factors listed in the first column should be standardised during saliva collection

comprising about 50 kg of water, 8% dehydration means a loss of 4 litres. Smaller degrees of dehydration also decrease salivary flow while, in contrast, hyperhydration will increase the salivary flow rate.

Body posture and lighting conditions

Flow rate varies with position and a person when standing or lying will have a higher or lower flow rate, respectively, than when seated. Flow rate also decreases by 30-40% when subjects are blindfolded or in the dark. However, a study has shown that salivary flow is not less in blind subjects than in those with normal sight, which suggests that blind individuals eventually adapt to the lack of light entering the eyes.

Biological rhythms

Circadian rhythms are rhythms with a period of about 24 h and include the rhythms in body temperature and in salivary flow.³ The body temperature and the flow rate of saliva peak during the late afternoon (the acrophase) but the flow rate drops to almost zero during sleep (*Figure 3.1*). It may therefore be important to standardise the time of day at which saliva is collected. This circadian rhythm also has important clinical implications for the timing of oral hygiene. The most important time to clean the teeth is probably at night before going to sleep, since the presence of plaque and food debris and a greatly reduced salivary flow during sleep provide optimum conditions for progression of dental caries.

A study has also shown a circannual (about-yearly) rhythm in the flow rate of parotid saliva, with a peak value in the winter. This study, carried out in Texas, tested over 300

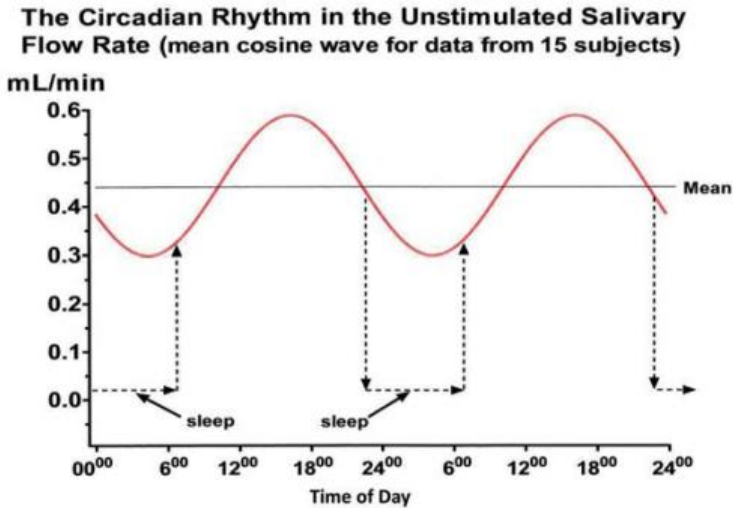


Figure 3.1 The circadian rhythm in unstimulated salivary flow rate and the idealised effect of sleep (dashed line) from 2300 hours to 0700 hours (from Dawes²).

subjects per month but each subject provided only one sample of parotid saliva for the entire study. About 35% lower flow rates were found in the summer and it was assumed that the reduction then was due to dehydration. More recently, a circannual rhythm in unstimulated salivary flow rate was found in 46 subjects tested monthly in Sri Lanka, where the monthly ambient temperature varies by only 2°C. The lowest flow rate also occurred at the time of the peak temperature although the amplitude of the rhythm was lower than in the Texas study. Whether these findings mean that people are more susceptible to caries in the summer than in the winter would be very hard to determine because full development of a caries lesion is such a long process, often taking several years.

Drugs

Many classes of drugs cause a reduction in salivary flow as a side effect. They may act centrally on the salivary nuclei in the pons and medulla or directly on the salivary glands (see Chapter 4).

Psychic stimuli

Thinking about food or seeing food are poor stimuli for salivation in humans. It may appear that one salivates at the thought of food but it is more likely that one merely becomes aware of the pool of saliva present in the floor of the mouth between swallows.

FACTORS INFLUENCING SALIVARY FLOW RATE AND COMPOSITION

Table 3.3: Stimulated salivary flow rate (ml/minute) in healthy individuals (for references see Dawes²)

Authors	Type of saliva	Stimulus	Sample number	Mean (ml/min)	SD
Heintze <i>et al.</i> (1983)	Whole	Paraffin wax	629	1.6	(2.1)
Shannon and Frome (1973)	Whole	Chewing gum	200	1.7	(0.6)
Shannon <i>et al.</i> (1974)	Parotid	Grape candy	368	1.0	(0.5)
Mason <i>et al.</i> (1975)	Parotid	Lemon juice	169	1.5	(0.8)
Ericson <i>et al.</i> (1972)	Subman-dibular	1% citric acid	28	0.8	(0.4)

Although some researchers have measured a small rise in salivary flow with visual stimuli, others have found no effect. In general, therefore, thinking about or seeing food has little effect in stimulating salivary flow.

Functional stimulation

Further studies are needed to clarify whether regular stimulation of salivary flow, as by use of chewing gum, leads to an increase in the unstimulated flow rate, although there is evidence that it increases the stimulated flow rate (see later).

Stimulated saliva

This type of saliva is secreted in response to masticatory or gustatory stimulation, or to other less common stimuli such as certain drugs (e.g. pilocarpine) or to activation of the vomiting centre. Several studies of stimulated salivary flow rates have been done in healthy populations and show a wide variation among individuals (*Table 3.3*). The studies used a variety of stimuli, however, and international agreement on a suitable stimulus for experimental use would greatly help comparison of results from different studies.

Factors affecting the stimulated salivary flow rate

Many factors (*Table 3.4*) influence the stimulated salivary flow rate which, for whole saliva, has an average maximum value of about 7 ml/minute.

Table 3.4: Factors affecting the flow of stimulated saliva

Nature of stimulus	Gland size
- mechanical	Unilateral stimulation
- gustatory	Vomiting
- pharmacological	Olfaction
- food intake	Smoking
	Gag reflex

Mechanical stimuli

The action of chewing, in the absence of any taste (see results for gum-base in *Figure 3.2*), will itself stimulate salivation but to a lesser degree than maximum gustatory stimulation with citric acid. Surprisingly, empty clenching of the teeth does not lead to an increase in salivary flow rate, although rhythmic clenching on rubber blocks which separate the teeth by a very small amount does increase flow. Mastication also serves to mix the contents of the mouth, thus increasing slightly the distribution of the different types of saliva around the mouth. Mechanical stimulation of the fauces (the gag reflex) leads to increased salivation.

Vomiting

Salivary flow is increased just prior to and during vomiting. Unfortunately, the increased buffering power of the saliva secreted at the increased flow rate is inadequate to protect the teeth against the erosion caused by the acid gastric juice, particularly in individuals with chronic bulimia or gastro-oesophageal reflux disease (GORD).

Gustatory and olfactory stimuli

Acid is the most potent of the five basic taste stimuli, the other four being salt, bitter, sweet and umami. The latter taste is due to activation of receptors for monosodium glutamate. A study⁴ done with various concentrations of citric acid found that 5% citric acid stimulated a mean maximum salivary flow rate of about 7 ml/minute. The citric acid was continuously infused into the mouth, and the teeth were covered with a paraffin film to protect them against the acid. For a clinical evaluation of the residual secretory capacity in patients with hyposalivation, a 3% citric acid solution can be applied to the patient's tongue at regular intervals, so that the degree of stimulation is relatively standardised. If a gustatory stimulus is held in the mouth without movement, salivary flow decreases to

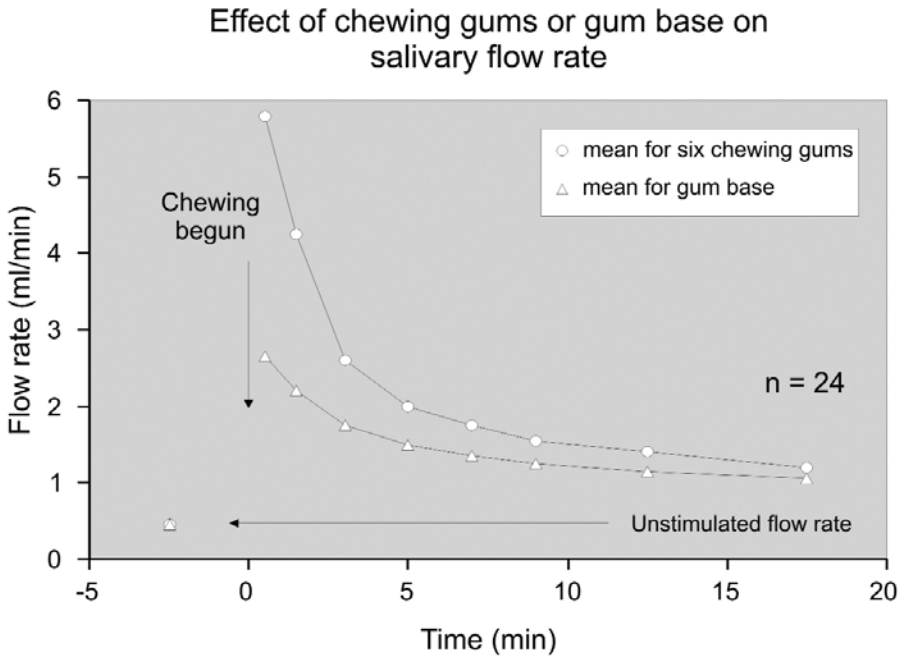


Figure 3.2 Effect of six chewing gums and gum-base on the flow rate of whole saliva. Unstimulated saliva was collected for 5 minutes prior to chewing gum or gum-base stimulation, which began at time zero.

the unstimulated rate with a half-time of about 11 s. However, if the gustatory stimulus is moved around to activate fresh taste receptors, the higher flow rate can be maintained.

For research purposes, sour candies (sweets) can be used to standardise the stimulated flow rate from cannulated individual glands. The patient collects saliva into a graduated test tube in front of a mirror. With the aid of a stopwatch, the patient can calculate the salivary flow rate, and adjust it by changing the intensity of sucking on the candy. The ability to standardize flow-rate at values up to the physiological maximum for the gland has allowed study of the effect of many other variables on salivary composition.^{3,5}

Olfactory stimuli and tobacco smoking, in comparison with gustatory stimuli, have relatively small effects in stimulating salivary flow.

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Gender, gland size and unilateral stimulation

Most studies have found that females have lower salivary flow rates than males and a recent study,⁶ which used three-dimensional magnetic resonance imaging, showed that in females, the sizes of the major salivary glands are smaller than in males. The maximum stimulated flow rate from a single gland is directly related to gland size but it is unknown why females need less unstimulated saliva than males (see Chapter 5). If one habitually chews on one side of the mouth (for instance with chewing gum), most of the saliva will be produced by the glands on that side after the initial tastants in the gum have been leached out.

Age

Salivary flow is unrelated to age above 15 years. For a long time it was believed that salivary flow decreased with age, because such studies had been done on institutionalised, medicated patients. More recent research has shown that ageing has little effect on either the unstimulated or stimulated flow rate in normal healthy people who are not on medication. This is surprising because histological studies of salivary glands have shown a reduction in the proportion of secretory cells with age. Presumably there is normally a surplus of secretory tissue. However, many elderly people receive medication and the greater the number of drugs taken, the greater is the tendency for reduction in salivary flow.

Food intake

Surprisingly, very few studies have been carried out with food as the secretory stimulus. A study⁴ tested the effects of seven foods. Even the most bland food (boiled rice) elicited 43% of the maximum flow rate produced by 5% citric acid. Rhubarb pie, which is both acidic and sweet, elicited 70% of the maximum flow rate. Further study showed that it was the gustatory stimulus provided by the food, rather than the mechanical stimulus of chewing, which was mainly responsible for these relatively high flow rates.

With chewing gum (*Figure 3.2*), the flow rate is high initially but after about ten minutes, as the flavour and sweeteners leach out and only the gum-base remains, it falls to the rate obtained by chewing gum-base alone, namely to two to three times the unstimulated rate. This increase in salivary flow during gum chewing can be maintained for as long as two hours and this may be very beneficial to those with a dry mouth. Even after two hours of gum chewing, the salivary glands do not become 'exhausted' and introduction of a fresh piece of gum causes a secretory response similar to that initially (*Figure 3.3*).

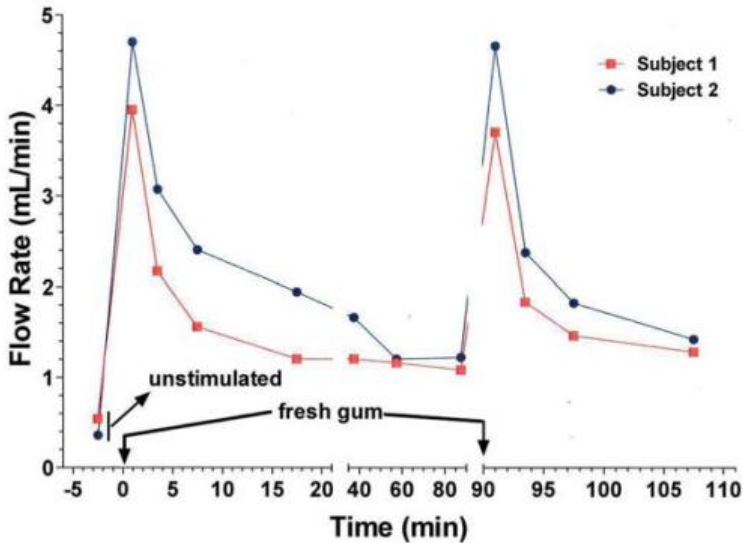


Figure 3.3 The effect of fresh gum on salivary flow rate after 90 minutes of gum chewing.

Salivary flow rate and oral health

The unstimulated flow rate is more important than the stimulated flow for oral comfort, since only a small fraction of the day (54 minutes in a group of dental students) is spent eating.⁴ However, stimulation of the glands through mastication is beneficial in terms of promoting clearance of food from the mouth (see Chapter 5) and may help by causing an increase in the unstimulated flow rate, although further studies of this are needed. A study has shown that two sugar-free chewing gums, one containing chlorhexidine, used by a group of 'frail, elderly', dentate subjects over a one-year period, led to improved oral health and a statistically significant 55-100% increase in their stimulated flow rate.⁷ This suggests that if the glands are stimulated regularly, their secretory ability may increase. Unfortunately, unstimulated flow rates were not measured in that study.

Carbohydrate clearance from the oral cavity

One major role of saliva is the clearance of carbohydrate from the mouth (see Chapter 5). The more rapid the flow, the faster the carbohydrate is cleared. This is true whether the saliva is unstimulated or stimulated, for example by chewing gum. If the gum contains sweeteners such as xylitol or sorbitol, which are minimally metabolised by

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plaque bacteria, then the increased salivary flow will be very effective in clearance of cariogenic carbohydrates remaining from previously consumed food.

Total daily salivary flow

If the average unstimulated flow rate over a waking period of 16 hours is about 0.3 ml/minute, the total volume will be about 300 ml of saliva. During sleep, the maximum flow will fall to less than 0.1 ml/minute, producing less than 40 ml of saliva in 7 hours. The average time spent eating each day has been estimated as 54 minutes and studies with various foods suggested that during eating the average stimulated flow rate is about 4 ml/minute.⁴ So about 200 ml of saliva per day will be produced during meals. Thus the total daily flow of saliva amounts to about 500-600 ml/24 hours,⁴ which is much less than the 1500 ml/24 hours quoted in many textbooks.

The composition of saliva

The composition of saliva is affected by many factors (*Table 3.5*), such as the type of salivary gland producing the saliva. For example, most of the amylase in saliva is produced by the parotid glands while blood-group substances are derived mainly from the minor mucous glands.

Table 3.5: Factors affecting salivary composition

Species	Hormones
Glandular source	Pregnancy
Flow rate	Genetic polymorphisms
Duration of stimulation	Antigenic stimuli
Previous stimulation	Exercise
Biological rhythms	Drugs
Nature of stimulus	Various diseases
Plasma composition (diet)	

Factors affecting salivary composition

Contribution of different glands

The parotid glands normally contribute about 25% of the total volume of unstimulated whole saliva, while the submandibular glands contribute 60%, the sublingual 7-8%, and the minor mucous glands 7-8%. At very high stimulated flow rates, the parotid becomes the dominant gland, contributing about 50% of the whole saliva.

Flow Rate

The main factor affecting the composition of saliva is the flow rate (*Figure 3.4*). As the flow rate increases, the pH and concentrations of some constituents rise (e.g. protein, sodium, chloride, bicarbonate), while those of others fall (e.g. magnesium and phosphate). The fluoride concentration in saliva is about 1 $\mu\text{mol/l}$ (0.019 ppm) and is relatively independent of flow rate but with a slight increase at low unstimulated flow rates. *Table 1.2* shows many of the differences in composition between unstimulated and chewing-stimulated whole saliva, although most of the stimulated samples were collected only over the first five minutes of chewing.

Duration of stimulation

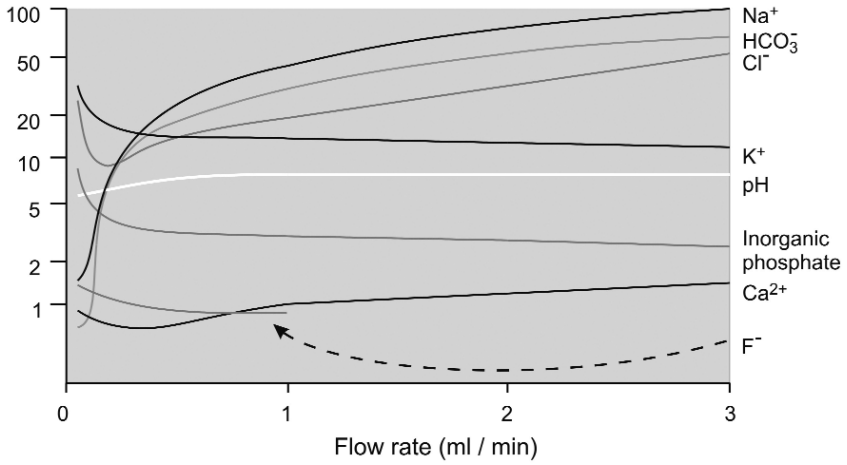
When the salivary flow rate is held constant, the composition of the saliva depends on the duration of stimulation.⁵ So saliva collected at a constant flow rate for 2 minutes will have a different composition from saliva collected at the same flow rate for 10-15 minutes. For instance, the bicarbonate concentration increases progressively with duration of stimulation, whereas the chloride level, after an initial rise, falls in a reciprocal manner. The salivary composition will also vary depending on whether the gland has been stimulated during the previous hour.

Nature of the stimulus

Different stimuli have an effect on salivary composition, mainly because of their effect on the rate of flow. When four of the basic taste stimuli (salt, acid, bitter, and sweet) were tested under constant flow conditions, the type of stimulus used had virtually no effect on the electrolyte composition of parotid saliva, but the taste of salt elicited significantly higher protein content than did the other stimuli. There does not seem to be any physiological reason why this should be so. The increase occurred with all protein components; different stimuli did not elicit secretion of different proteins.

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Effect of flow rate on the composition of parotid saliva



Effect of flow rate on the composition of submandibular saliva

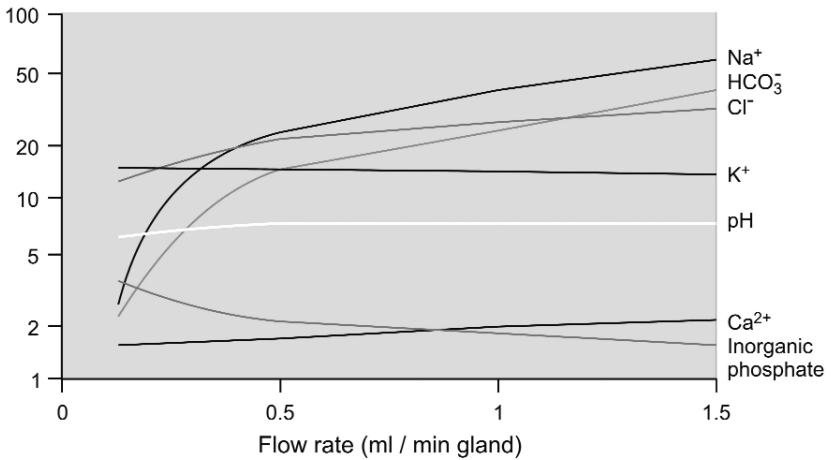


Figure 3.4 The effects of flow rate on the concentrations of some components of (a) parotid saliva and (b) submandibular saliva.

Acid is the most potent stimulus for salivary secretion and leads to production of an alkaline saliva. At one time it was thought that this was a beneficial adaptation to the nature of the stimulus. However, it is now known that the pH of saliva is dependent mainly on the flow rate and is independent of the nature of the stimulus.

Circadian rhythms

As with flow rate, salivary composition shows rhythms of high amplitude.³ For instance, sodium and chloride levels peak in the early morning, while the rhythm in potassium concentration is 12 hours out of phase. The peak protein concentration is in the late afternoon. Thus for longitudinal studies, it may be important to standardise the time of saliva collection.

Saliva and taste

When saliva is first secreted by the acinar cells of the salivary glands, its electrolyte composition resembles that of an ultrafiltrate of plasma. As the saliva passes down the salivary duct, the gland expends energy to reabsorb virtually all the sodium chloride and most of the bicarbonate, while secreting potassium (*Figure 3.5*). By the time the salivary secretion reaches the opening of the main excretory duct into the mouth its osmotic pressure is only about one sixth of that in plasma and in the acinar cells.

Why do the salivary glands go to so much trouble to produce hypotonic saliva? The probable reason is to facilitate taste. Taste buds rapidly adapt to the taste of any solution in the mouth including, of course, saliva. Thus, if saliva had the same salt concentration as plasma (which is very high), we would be unable to taste salt concentrations lower than that in plasma. Hence the reabsorption of sodium and chloride during saliva production, and the resultant hypotonicity of saliva, facilitate our ability to taste salt.

Unstimulated saliva is particularly well adapted to facilitate our ability to taste low concentrations of substances with one of the five taste qualities of salt, sweet, acid, bitter or umami. Besides being low in sodium and chloride (salt), unstimulated saliva is also low in glucose (sweet), bicarbonate (for buffering of acid), and urea (bitter). Taste recognition threshold concentrations for NaCl, HCl, NaHCO₃, sucrose, and urea are compared with the appropriate concentration levels in plasma and in unstimulated saliva in *Table 3.6*. It is only the concentrations of sodium and chloride in plasma that are higher than the taste recognition thresholds.

Unstimulated Saliva

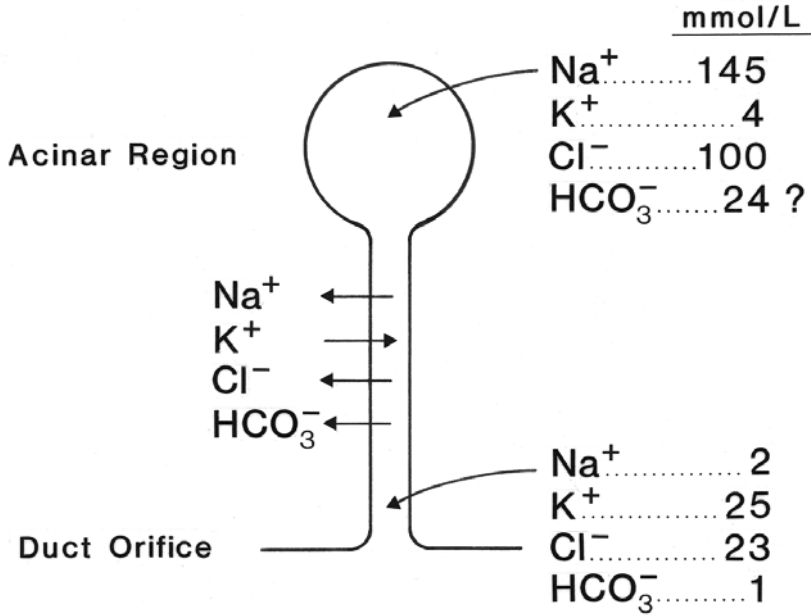


Figure 3.5 Changes in some electrolyte concentrations as unstimulated parotid saliva moves down the salivary duct.

Table 3.6: Table 3.6 Relation of plasma and unstimulated whole saliva compositions* to taste thresholds

	Salt Na ⁺	Cl ⁻	Sour H ⁺	HCO ₃ ⁻	Sweet Glucose	Bitter Urea
Plasma	145	101	4 x 10 ⁻⁵	24	4.5	6
Saliva	6	16	1 x 10 ⁻⁴	5	0.08	4
Taste recognition threshold	(NaCl)		(HCl)	(NaHCO ₃)	(Sucrose)	(Urea)
	12	-	0.8	10	30	90

*Note: All concentrations are in mmol/l.

The buffering ability of saliva

Proteins

The concentration of protein in saliva is only about one-thirtieth of that in plasma and too few amino acids with acidic or basic side chains are present to have a significant buffering effect at the usual pH of the oral cavity. The buffering of dental plaque is discussed in Chapter 6 and the different proteins in saliva are discussed in Chapter 7.

Phosphate

Although the phosphate concentration in unstimulated whole saliva is about 5-6 mmol/l, compared with a level of about 1 mmol/l in plasma, there is still too little phosphate in saliva to act as a significant buffer. The pH of unstimulated saliva is less than the pK_2 value of 7.2 for phosphate, so that most of the phosphate is present as $H_2PO_4^-$ and cannot accept another hydrogen ion until the pH is close to 2.1, the pK_1 for phosphate.

Bicarbonate

This is the most important buffering system in saliva but only at high flow rates, when it is an important buffer against acid produced by dental plaque. Its concentration varies from less than 1 mmol/l in unstimulated parotid saliva to almost 60 mmol/l at very high flow rates, with whole saliva elicited by chewing gum having a bicarbonate concentration of about 15 mmol/l. Thus, in unstimulated saliva, the level of bicarbonate ions is too low to be an effective buffer. The bicarbonate in saliva though will facilitate the clearance of acid from the oesophagus in those with gastro-oesophageal reflux disease.

pH

Salivary pH is dependent on the bicarbonate concentration, an increase in which results in an increase in pH. The relationship between the pH and the bicarbonate concentration is given by the Henderson-Hasselbalch equation, $pH = pK + \log[HCO_3^-]/[H_2CO_3]$, in which the pK (about 6.1) and $[H_2CO_3]$ (about 1.2 mmol/l) are virtually independent of the flow rate. The latter is in equilibrium with the pCO_2 which, in saliva, is about the same as that in venous blood. When the pH of saliva is to be measured, it is important to avoid exposure of the saliva to the atmosphere, as CO_2 will be released and the pH will be artificially elevated. At very low flow rates, the pH of parotid saliva can be as low as 5.3, rising to 7.8 at very high flow rates. Individuals with hyposalivation will thus have a low salivary pH and a low salivary buffering capacity because of the low bicarbonate concentration (*Figure 3.4*).

Urea

The urea concentration in saliva (about 4 mmol/l) is only slightly lower than that in plasma. Urea can diffuse from saliva into dental plaque, where bacterial ureases convert it into carbon dioxide and ammonia, the latter causing an increase in pH. Computer simulation suggests that in the absence of salivary urea, the minimum pH of the Stephan curve (see Chapter 6) would be deeper by about 0.5 pH units. Patients with uraemia develop less caries, more calculus, and the resting pH of their plaque may be as high as 9.

Calcium and phosphate concentrations

Calcium and trivalent phosphate (PO_4^{3-}) ions, along with hydroxyl ions, maintain the saturation of saliva with respect to tooth mineral, and are therefore important in calculus formation and in protecting against the development of both caries and erosion (see Chapter 8).

Saliva (*Table 1.2*) contains less calcium but more phosphate than does plasma ($\text{Ca} = 2.5$ mmol/l; Inorg. P = about 1 mmol/l). The mechanisms responsible for a higher concentration of phosphate in saliva than in plasma are uncertain. In addition, secretions from different salivary glands have different concentrations of calcium and phosphate. For example, parotid saliva contains less calcium but more inorganic phosphate than does submandibular saliva (*Figure 3.4*), while the minor mucous gland secretions are very low in phosphate (about 0.4 mmol/l).

A decreasing total phosphate concentration at high flow rates (*Figure 3.4* and *Table 1.2*) would seem to be bad for teeth, as it might result in undersaturation of the saliva with respect to tooth mineral. However, as the flow rate increases, so does the bicarbonate concentration and therefore the pH of saliva. A high pH alters the proportions of the four different phosphate species (H_3PO_4 , H_2PO_4^- , HPO_4^{2-} , and PO_4^{3-}) such that, along with the fall in total phosphate concentration, there is a fall in H_2PO_4^- , a slight increase in HPO_4^{2-} , but a dramatic increase in PO_4^{3-} . It is the PO_4^{3-} which is the important ionic species with respect to the solubility of tooth mineral (see Chapter 8). Thus, although the total level of phosphate falls with increasing flow rate, the concentration of PO_4^{3-} actually increases (*Figure 3.6*) as much as forty-fold when flow rate increases from the unstimulated level to high flow rates.⁵

If, therefore, we consider the components of the ion product determining the solubility of tooth mineral in saliva, all three (Ca^{2+} , PO_4^{3-} , OH^-) increase with salivary flow. Thus the higher the flow rate, the more effective is saliva in reducing demineralisation and promoting remineralisation of the teeth. This also means,

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however, that the higher the flow rate, the greater is the potential for calculus formation to occur (see Chapter 8 for further information).

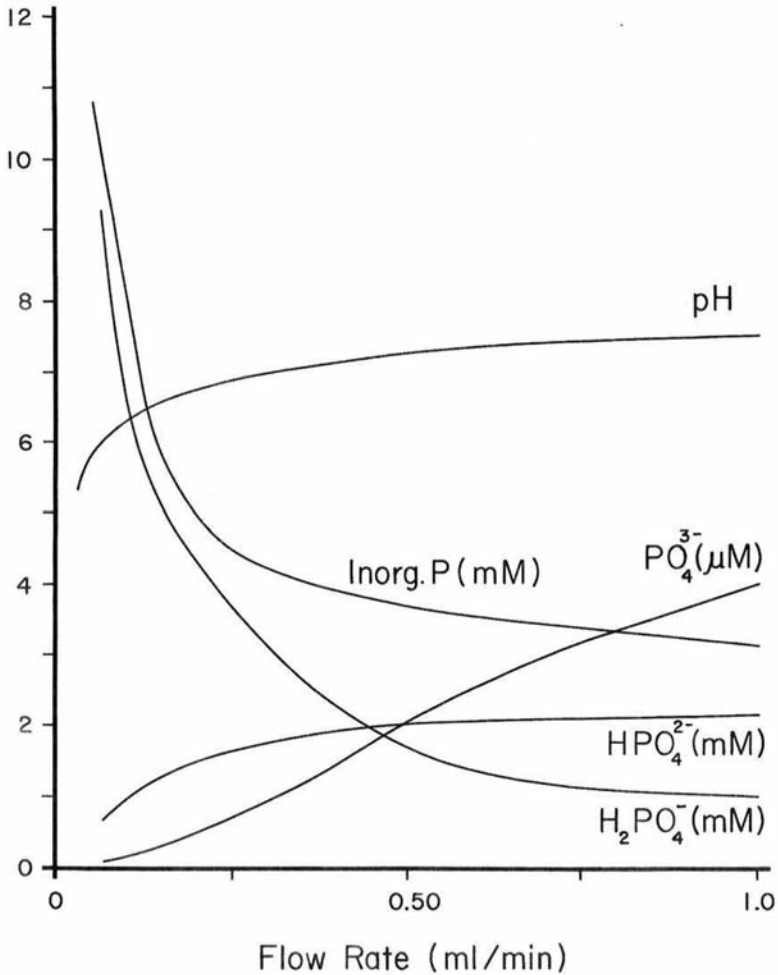


Figure 3.6 The effect of salivary flow rate on the concentrations of the different species of inorganic phosphate in parotid saliva. Note that the PO_4^{3-} concentration is in $\mu\text{mol/l}$, whereas the concentrations of the other species are in mmol/l .

Minor mucous gland secretions

These differ in several ways from the secretions of the major glands (submandibular, sublingual, and parotid). They are extremely viscous because of a high mucin content, very low in phosphate, they contain virtually no bicarbonate, so they have a low buffer capacity, and their pH is about neutrality. The main ions are sodium, potassium, and chloride, and they are the main source of secretory IgA in the mouth. Their fluoride concentration has been reported to be several times higher than that in whole saliva or in secretions from the major glands. It is unfortunate that, mainly because of collection difficulties, these secretions have received little study despite their being in intimate contact with most of the oral mucosa and hard tissues.

Summary - clinical highlights

Salivary flow rate is nearly zero in sleep. Maximum cariogenic activity is likely to occur when people eat carbohydrate at night and then do not brush their teeth before going to sleep.

Dentists should be aware that many patients are taking medications (for example beta blockers) that have a tendency to reduce salivary flow, making the patient more susceptible to dental caries.

When salivary flow rate increases, this results in a higher salivary pH and bicarbonate content which have beneficial effects on plaque pH if the stimulus to salivation does not include acid or additional sugar. The increased flow rate will itself help to remove carbohydrate from the mouth, and stir up the very thin film of saliva (see Chapter 5) which covers the oral surfaces. The bicarbonate will tend to diffuse into plaque and act as a buffer by neutralising acids present in the plaque, allowing more time for remineralisation of early caries.

Dentists should measure the unstimulated salivary flow rate of patients at appropriate intervals, as this would provide baseline values for future comparison. A very low salivary flow rate is an indication of caries susceptibility and should influence the preventive treatment provided by the dentist.

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Xerostomia and salivary gland hypofunction; aetiology, diagnosis, clinical implications and management

Mahvash Navazesh

Introduction

Saliva plays a significant role in the maintenance of oral-pharyngeal health. Subjective complaints of a dry mouth (xerostomia) and objective evidence of diminished salivary output (salivary gland hypofunction) are common conditions, particularly in medically compromised older adults. They can result in impaired food and beverage intake, a sundry of oral disorders, and diminished host defence and communication (*Table 4.1*). Persistent salivary gland hypofunction can produce permanent oral and pharyngeal disorders and impair a person's quality of life.^{1,2}

Global estimates of xerostomia and salivary gland hypofunction are difficult to ascertain due to varying study design, differences in study populations, usage of the terms xerostomia and salivary gland hypofunction interchangeably, utilisation of different diagnostic criteria and saliva collection methods, and small sample sizes. Overall, the prevalence of xerostomia and salivary gland hypofunction increases with age and affects >30% of the population aged 65 years and older.

There are multiple causes of xerostomia and salivary gland hypofunction (*Table 4.2*), the most common being drug-induced, since most older adults are taking at least one medication that causes salivary gland hypofunction. It is difficult, however, to estimate the true prevalence of xerostomia in older adults taking medications. The prevalence of xerostomia is nearly 100% among patients with Sjögren's syndrome, an autoimmune exocrinopathy affecting between 1-4% of older adults. Radiation of the head and neck for the treatment of cancer causes permanent xerostomia, which has a 100% prevalence rate if the dose is >25 Gy, but the numbers affected are relatively small compared with those older adults susceptible to medication-induced xerostomia. For example, in the

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USA, 36,540 cases of head and neck cancers were diagnosed in 2010, and most of these patients required radiotherapy which leads to permanent salivary hypofunction and xerostomia. Estimates of the prevalence of xerostomia in adult ambulatory and nursing home populations range from 16-72%.³ Combining the prevalence of xerostomia-associated conditions with the percentage of adults with these conditions who complain of xerostomia yields the above-mentioned general estimate of approximately 30% xerostomia prevalence among adults 65 years and older.

In 2011, the first members of the Baby Boom population in the United States reached age 65. The last members of this population will reach age 65 in 2029. The adults 65 years or older represented 12.4% of the population in 2000 but are expected to grow to be 19% by 2030.

Table 4.1: Oral and pharyngeal complications associated with salivary gland hypofunction

Dental caries	Mastication difficulty
Dry lips	Oral-pharyngeal candidiasis
Dry mouth	Poorly fitting prostheses
Dysgeusia	Sleeping difficulty
Dysphagia	Speech difficulty
Gingivitis	Traumatic oral lesions
Halitosis	

Table 4.2: Aetiology of xerostomia and salivary gland hypofunction

Condition	Examples
Medications	Anticholinergics, tricyclic antidepressants, sedatives, tranquilizers, antihistamines, antihypertensives, cytotoxic agents, anti-Parkinsonian drugs, anti-seizure drugs, skeletal muscle relaxants
Oral diseases	Acute and chronic parotitis, sialolith, mucocele, partial/complete salivary obstruction
Systemic diseases	Mumps, Sjögren's syndrome, diabetes, HIV/AIDS, scleroderma, sarcoidosis, lupus, Alzheimer's disease, dehydration, graft versus host disease, Hepatitis C infection
Head and neck radiotherapy	

Approximately 80% of all persons over age 65 have at least one chronic condition and 50% have at least two. Hypertension and heart diseases, diabetes, arthritis and cancer are the most frequently occurring conditions among older adults. These conditions, and the medications often prescribed for their management, could impact the structure and function of salivary glands leading to complaints of xerostomia or clinical evidence of salivary gland hypofunction. More than 400 medications list dry mouth as a potential adverse effect. In May 2011 the United States Food and Drug Administration (FDA) added dry mouth to its consumer health information.

Aetiology of xerostomia and salivary gland hypofunction

Salivary gland pathology

Intraoral sources of salivary gland pathology can be divided into three broad classifications: infectious, non-infectious, and neoplastic (*Table 4.3*).⁴ Bacterial infections are more common in older adults who experience salivary gland hypofunction secondary to medications, head and neck radiation, systemic diseases, or dehydration. Acute parotitis was commonly seen before the antibiotic era in terminally ill and dehydrated patients and contributed to mortality by sepsis. Now, acute parotitis is observed infrequently. Chronic parotitis is not unusual and it follows obstruction of a parotid duct with subsequent bacterial colonisation and infection. Signs and symptoms of bacterial salivary infections include swelling, purulence from the major salivary gland duct, and pain.

Viral infections occur in persons of all ages, particularly in immunocompromised patients, and preferentially involve parotid glands. Mumps is caused by paramyxovirus, and presents as bilateral parotid gland swelling in children. Cytomegalovirus infections tend to be mild with non-specific findings, and are observed primarily in adults.

Non-infectious (reactive) causes of salivary pathology are most commonly due to obstruction of a salivary gland excretory duct and can be divided into acute and chronic conditions. Acute sialadenitis usually results from an immediate partial or complete ductal obstruction (i.e. sialolithiasis), whereas chronic recurrent sialadenitis occurs as a result of prior infection and/or ductal scarring.

Mucocele are the most common reactive lesion of the lower lip and are caused by local trauma. When a minor salivary gland duct is severed mucin leaks into the surrounding connective tissue resulting in a smooth-surfaced painless nodule in the submucosal tissues. Mucous cysts of the sublingual gland, and, less frequently, the submandibular gland, are referred to as ranulas. They present as either unilateral

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circumscribed lesions (subsequent to ductal obstruction and cystic dilation) or plunging lesions (following extravasation of saliva herniating through the tissues of the floor of the mouth and the mylohyoid muscle). Both types of ranulas require surgical excision and possible marsupialisation of the cyst.

Most calculi (sialoliths, stones) develop in the submandibular duct system and are caused by calcification of mucous plugs and cellular debris, typically as a result of dehydration and glandular inactivity. Sialoliths occur infrequently in the parotid duct system and are considered rare in the sublingual and minor salivary glands.

Most salivary gland tumours are benign, arising from epithelial tissues; however, neoplasms may originate from any adjacent tissue or structure (adipose, nerves, blood vessels, lymph nodes, lymphatics). The preponderance of benign salivary gland neoplasms occurs within the parotid gland, with the majority (80%) being pleomorphic adenomas. These tend to be unilateral and most commonly present as an asymptomatic mass in the tail of the parotid gland. They are slow growing, well delineated and encapsulated. Malignant salivary gland tumour incidence increases with age, and these tumours are more common in the submandibular and sublingual glands compared with the parotid gland. When epithelial neoplasms arise in the submandibular or sublingual glands, only 50% are benign.⁵

Mucoepidermoid carcinoma is the most common malignant salivary gland tumour, followed by adenoid cystic carcinoma (cylindroma), acinic cell carcinoma, adenocarcinoma, squamous cell carcinoma, and carcinoma arising in a pleomorphic adenoma. The most commonly affected intraoral site is the palate followed by the upper lip. Adenoid cystic carcinomas are aggressive tumours that undergo perineural invasion. They have good 10-year survival rates, but long-term mortality is likely. The signs and symptoms of a malignant salivary gland tumour include a swelling with facial nerve paralysis, pain, or facial paresis.

Systemic diseases

There are numerous systemic conditions that have been associated with xerostomia and salivary gland hypofunction (*Table 4.2*), the most common being Sjögren's syndrome. Sjögren's syndrome is primarily a disease affecting women with a typical onset during the fourth or fifth decade of life.⁶ Clinically, Sjögren's syndrome presents in either primary or secondary forms. Primary Sjögren's syndrome is characterised by xerostomia and xerophthalmia (dry eyes) that are the result of a progressive loss of salivary and lacrimal gland function. Secondary Sjögren's syndrome includes involvement of one or both of these exocrine sites in the presence of another connective tissue disease such as rheumatoid arthritis, systemic sclerosis, or systemic lupus

XEROSTOMIA AND SALIVARY GLAND HYPOFUNCTION

Table 4.3: Classifications of intraoral salivary gland pathologies

Pathology	Etiology
Infectious	
Acute sialadenitis	Salivary gland hypofunction: secondary to dehydration, debilitation, medications. Bacterial species: <i>Staphylococcus aureus</i> , <i>S. pyogenes</i> , <i>Streptococcus pneumoniae</i> , <i>E. coli</i>
Chronic recurrent sialadenitis Viral sialadenitis	Bacterial species (see acute sialadenitis) Paramyxovirus, Cytomegalovirus
Non-infectious	
Sialectasis	Salivary gland hypofunction: secondary to dehydration and post-general anaesthesia
Sialolithiasis	Salivary gland hypofunction: secondary to dehydration, debilitation, medications, metabolic disorders, poor oral hygiene
Sialadenosis	Malnutrition, alcoholic cirrhosis, diabetes mellitus, hyperlipidemia
Mucous cyst	Blockage of an excretory duct
Mucocele	Traumatic severance of a minor salivary gland duct, producing spillage of mucin into surrounding connective tissue
Neoplastic	
<i>Benign Tumours</i>	
Pleomorphic adenoma Monomorphic adenoma	
<i>Malignant Tumours</i>	
Adenoid Cystic Carcinoma Mucoepidermoid Carcinoma Acinic Cell Carcinoma Malignant Mixed Tumour*	

*Note: Carcinoma arising in a pleomorphic adenoma, and squamous cell carcinoma.

erythematous. Lymphocytic infiltrates of salivary glands increase as the inflammatory disease progresses, ultimately producing acinar gland degeneration, necrosis, atrophy, and complete destruction of the salivary gland parenchyma. Diagnosis requires a combination of objective salivary, lacrimal, and serological criteria and subjective complaints of xerostomia or xerophthalmia.⁷

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Other autoimmune conditions associated with Sjögren's syndrome and causing salivary gland hypofunction include rheumatoid arthritis, scleroderma, and lupus. HIV+ infected individuals and those with AIDS frequently experience salivary gland hypofunction from lymphocytic destruction of the glands and as a sequela of medications. Diabetes may cause changes in salivary secretions, and associations have been made between poor glycemic control, peripheral neuropathies, and salivary hypofunction. Alzheimer's disease, Parkinson's disease, strokes, cystic fibrosis, Hepatitis C and dehydration will also inhibit salivary secretion.

It was previously thought that salivary function declined with greater age, yet it is now accepted that output from the major salivary glands does not undergo clinically significant decrements in healthy older adults.⁸ There are reports of age-related decrements in several salivary constituents, whereas other studies report age-stable production of salivary electrolytes and proteins in the absence of major medical problems and medications. It is likely that numerous systemic diseases (e.g. Sjögren's syndrome) and their treatments (medications, head and neck radiation, chemotherapy) contribute significantly to salivary gland hypofunction in the elderly (*Table 4.2*).^{9,10} It has been demonstrated that the salivary glands of older adults are more vulnerable to the deleterious effects of medications compared with those of younger individuals,¹¹ confirming the finding of greater xerostomia prevalence among older adults, particularly those taking medications.

Medications

The most common causes of salivary gland hypofunction and xerostomia are prescription and non-prescription medications. For example, 80% of the most commonly prescribed medications have been reported to cause xerostomia,¹² with over 400 medications causing a side effect of salivary gland hypofunction.¹⁰ The intake of prescription medications increases with age, and more than 75% of persons over the age of 65 years take at least one prescription medication. Further, with the increased intake of prescription medications, there is an increase in xerostomia.

The most common types of medications causing salivary hypofunction have anticholinergic effects *via* inhibition of acetylcholine binding to muscarinic receptors on the acinar cells. This prevents initiation of the cascade of physiological events that ultimately result in water movement through acinar cells, into the ductal system, and ultimately into the mouth. Importantly, any medications that inhibit neurotransmitter binding to acinar membrane receptors, or that interfere with ion transport pathways, may also adversely affect the quality and quantity of salivary output. These medications include tricyclic antidepressants, sedatives and tranquilisers, antihistamines,

antihypertensives (alpha and beta blockers, diuretics, calcium-channel blockers, angiotensin converting enzyme inhibitors), cytotoxic agents, anti Parkinsonian, and anti-seizure drugs.¹⁰

Chemotherapy for cancer treatment has also been associated with salivary gland hypofunction. These changes appear to occur during and immediately after treatment. Most patients experience a return of salivary function to pre-chemotherapy levels, yet long-term changes have been reported. Finally, radioactive iodine (I^{131}) used in treatment for cancers of the thyroid gland may cause parotid, but not submandibular, salivary gland hypofunction in a dose-dependent fashion, since the salivary glands concentrate iodide to levels much higher than those in the blood.

Head and neck radiotherapy

Radiation therapy (RT), a common treatment modality for head and neck cancers, causes permanent salivary gland hypofunction and xerostomia.¹³ The serous acini of salivary glands are considered to be the most radiosensitive cells, followed by mucous acini. Experiments with rhesus monkeys suggest that irradiated serous salivary glands undergo interphase cell death by apoptosis. There is an increase in the intensity of degenerative changes with dose and time in serous acinar cells, which produces apoptosis at low doses and necrosis at high doses. Within one week of the start of irradiation (after 10 Gy have been delivered) salivary output declines by 60-90% with later recovery only if the total dose to salivary tissue is <25 Gy.¹⁴

After the first week of RT, patients will experience viscous saliva, because serous cell loss results in diminished water secretion. Eventually, mucous cells are also affected, decreasing the overall volume of saliva produced.¹³ As indicated above, there is a dose-dependent relationship between the amount of radiation delivered to oral tissues and the damage that eventually occurs.

Diagnosis of xerostomia and salivary gland hypofunction

Subjective responses and questionnaires

The establishment of a diagnosis of xerostomia may be initiated with patients' complaints and can be advanced with the use of questionnaires (*Table 4.4*). It should be noted that a patient's presenting complaint may not be dry mouth in spite of the presence of salivary gland hypofunction. Therefore, lack of complaint should not be perceived as presence of adequate saliva secretion. Many of the common oral symptoms of dry mouth are associated with mealtime: altered taste, difficulty eating, chewing, and

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Table 4.4: Table 4.3 Classifications of intraoral salivary gland pathologies

Measure	Response	Reference
Do you have difficulties swallowing any foods?	Yes/no	(16)
Does your mouth feel dry when eating a meal?	Yes/no	(16)
Do you sip liquids to aid in swallowing dry foods?	Yes/no	(16)
Does the amount of saliva in your mouth seem to be too little, too much, or you don't notice it?		(16)
Rate the difficulty you experience in speaking due to dryness	0-10 scale 1	(18)
Rate the difficulty you experience in swallowing due to dryness	0-10 scale 1	(18)
Rate how much saliva is in your mouth	0-10 scale 2	(18)
Rate the dryness of your mouth	0-10 scale 3	(18)
Rate the dryness of your throat	0-10 scale 3	(18)
Rate the dryness of your lips	0-10 scale 3	(18)
Rate the dryness of your tongue	0-10 scale 3	(18)
Rate the level of your thirst	0-10 scale 4	(18)
Dryness of lips	Present/absent	(17)
Dryness of buccal mucosa	Present/absent	(17)
I sip liquids to aid in swallowing food	1-5 scale 5	(19)
My mouth feels dry when eating a meal	1-5 scale 5	(19)
I get up at night to drink	1-5 scale 5	(19)
I have difficulty in eating dry foods	1-5 scale 5	(19)
I suck sweets or cough lollies to relieve dry mouth	1-5 scale 5	(19)
I have difficulties swallowing certain foods	1-5 scale 5	(19)
I have a burning sensation in my gums	1-5 scale 5	(19)
I have a burning sensation in my tongue	1-5 scale 5	(19)
My gums itch	1-5 scale 5	(19)
My tongue itches	1-5 scale 5	(19)
The skin of my face feels dry	1-5 scale 5	(19)
My eyes feel dry	1-5 scale 5	(19))
My lips feel dry	1-5 scale 5	(19)
The inside of my nose feels dry	1-5 scale 5	(19)

¹ From "Not difficult at all" to "Very difficult" ² From "A lot" to "None" ³ From "Not dry at all" to "Very dry"

⁴ From "Not thirsty at all" to "Very thirsty".

⁵ 1 = "never", 2 = "hardly ever", 3 = "occasionally", 4 = "fairly often", 5 = "very often"

swallowing, particularly dry foods, and especially without drinking accompanying liquids (*Table 4.4*). Patients complain of impaired denture retention, halitosis, stomatodynia, and intolerance to acidic and spicy foods.⁹ Night-time xerostomia is also common, since salivary output normally reaches its lowest circadian level during sleep and may be exacerbated by mouth breathing.¹⁵

General oral examination

Extraoral findings associated with salivary gland hypofunction may include dry and cracked lips that are frequently colonised with *Candida* species (angular cheilitis). Visible and palpable enlarged major salivary glands occur secondary to salivary infections and obstructions (e.g. bacterial parotitis, mumps, and Sjögren's syndrome). A swollen parotid gland can displace the earlobe and extend inferiorly over the angle of the mandible, whereas an enlarged submandibular gland is palpated medial to the posterior-inferior border of the mandible.

There are numerous intraoral complications associated with salivary gland hypofunction. Oral mucosal surfaces become desiccated and easily friable. The tongue can lose its filiform papillae and will appear dry, erythemic, and raw with an irritated dorsal surface. Mucosal tissues are susceptible to developing microbial infections, the most common being candidiasis. This intraoral fungal infection manifests itself as erythematous candidiasis beneath prostheses and as pseudomembranous candidiasis, which produces a white plaque that can be removed from mucosal surfaces. Clinicians can also observe a decrease or an absence of saliva pooling in the anterior floor of the mouth.

A second frequent problem is dental caries that occurs both on coronal and root surfaces. New caries lesions can develop on surfaces not normally affected (e.g. incisal edges of anterior teeth), and recurrent lesions are prevalent on the margins of existing restorations. Edentulous and partially dentate adults using removable prostheses have diminished denture retention, which will adversely impact chewing, swallowing, speech, and nutritional intake. Denture-bearing tissues can develop erythematous candidiasis and traumatic and painful lesions due to tissue trauma.

Saliva collection

Numerous investigators have attempted to define the lower limits of 'normal' salivary flow rates. However, there is substantial variability in flow rates that makes it difficult to define diagnostically useful ranges of glandular fluid production. In studies of healthy persons across the lifespan, unstimulated fluid secretion varies 10-100 fold, while stimulated secretion varies 10-20 fold.^{20,21}

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In patients considered to be at risk for developing salivary gland hypofunction, it would be useful to monitor salivary flow rates over time. Most investigators consider a diagnosis of salivary gland hypofunction if the unstimulated whole salivary flow rate is less than 0.1 ml/min using standardised techniques. Unstimulated secretions are probably more indicative of salivary gland hypofunction compared with stimulated secretions, since saliva is produced under unstimulated conditions during most of the hours a person is awake. The most common collection technique for unstimulated whole saliva is to have a patient refrain from eating, drinking, smoking, or performing oral hygiene for at least 60 minutes prior to saliva collection. The patient is seated in a quiet environment with the head tilted forward. Immediately before the test begins the patient should swallow any residual saliva that may be in the mouth. The time is recorded and the person is instructed to allow saliva to flow gently into a pre-weighed test tube or other container placed under the chin for five minutes without swallowing or spitting. At five minutes the person is instructed to expectorate the remaining saliva into the container. The volume is recorded gravimetrically and expressed as ml/min.

Stimulated whole salivary flow rates of less than 0.5 ml/min are also considered to be suggestive of salivary hypofunction. The most common technique for collecting this form of saliva is with the use of a standard piece of paraffin wax or unflavoured gum base (typically 1-2 g). A test tube or similar container with the paraffin or gum base is weighed prior to saliva collection. The person is instructed to swallow any residual saliva that may be in the mouth before the saliva collection begins. A timer begins and the person is instructed to chew the wax or gum base at a rate of 60 chews/minute. Without swallowing, the patient expectorates all saliva into the pre-weighed container placed under the chin at each 60 second interval. At five minutes the person is instructed to expectorate the remaining saliva and wax into the container and the collection is completed. The volume is recorded gravimetrically, and expressed as ml/min.

Values below 45% of normal levels can be used to define salivary gland hypofunction. It is also generally accepted that when glandular fluid production is decreased by about 50%, patients will begin to experience xerostomia.²² The best strategy is simply to monitor a patient's salivary health (both objectively and subjectively) over time to determine whether there are demonstrable changes.²⁰

Histopathology

The treatment of lesions and tumours associated with the salivary glands starts with an appropriate diagnosis and frequently, this includes examination of histopathological tissue. Incisional or excisional biopsies and histopathological evaluation are critically important. For Sjögren's syndrome, minor salivary glands are biopsied to examine for

a focal lymphocytic sialoadenitis. A focus score ≥ 1 is used as part of the objective criteria in the Revised European Classification Criteria for Sjögren's syndrome.⁷ A focus score is defined as the number of lymphocytic foci that are adjacent to normal-appearing mucous acini and contain >50 lymphocytes per 4 mm^2 of glandular tissue.²³

Imaging

Occlusal intraoral radiographs, conventional sialography, computed tomography (CT) with or without contrast, magnetic resonance imaging (MRI) with or without enhancement, MR sialography, salivary scintigraphy, and diagnostic ultrasound are available technologies for the diagnosis of salivary gland disorders.²⁴

Sialograms can identify changes in salivary gland architecture and are performed with radio-opaque iodine and extraoral radiographs (lateral cephalograms, panographs, etc.). Radioactive isotope scintiscans (e.g. Tc^{99} pertechnetate) can provide a qualitative functional assessment of the major salivary glands. Decreased uptake of radio-isotopes and delayed expulsion are associated with salivary gland hypofunction. MRI and CT scans will help rule out salivary gland tumours and other pathoses associated with the craniofacial region that may adversely affect salivation. Positron Emission Tomography/CT can be used to stage malignant salivary gland neoplasms.

Serology

Serological studies are critically important for the diagnosis of Sjögren's syndrome. Autoantibodies (particularly anti-Ro/SSA, anti-La/SSB and rheumatoid factor) are frequently present in Sjögren's syndrome and tend to be higher in family members than in the general population.⁷ Caucasian patients with primary Sjögren's syndrome also often have the HLA-DR3 and HLA-DQ2 alleles. Other serological factors, such as an elevated erythrocyte sedimentation rate and antinuclear antibody (ANA) levels greater than 1:160, tend to be positive in patients with autoimmune diseases who may have secondary Sjögren's syndrome. Abnormal levels of anti-SM and anti-RNP are suggestive of systemic lupus erythematosus, while Scl-70 can be positive in progressive systemic sclerosis (scleroderma).

Clinical implications of xerostomia and salivary gland hypofunction

The structural changes commonly associated with chronic salivary gland hypofunction are shown in *Figures 4.1- 4.4*. Clinical manifestations are described below.

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Figure 4.1 Clinical manifestations of salivary gland hypofunction involving palatal mucosa and tongue in a patient with Sjögren's syndrome before and after antifungal therapy.



Figure 4.2 Bilateral salivary gland enlargement in a patient with Sjögren's syndrome.

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Figure 4.3 Recurrent dental caries associated with salivary gland hypofunction.

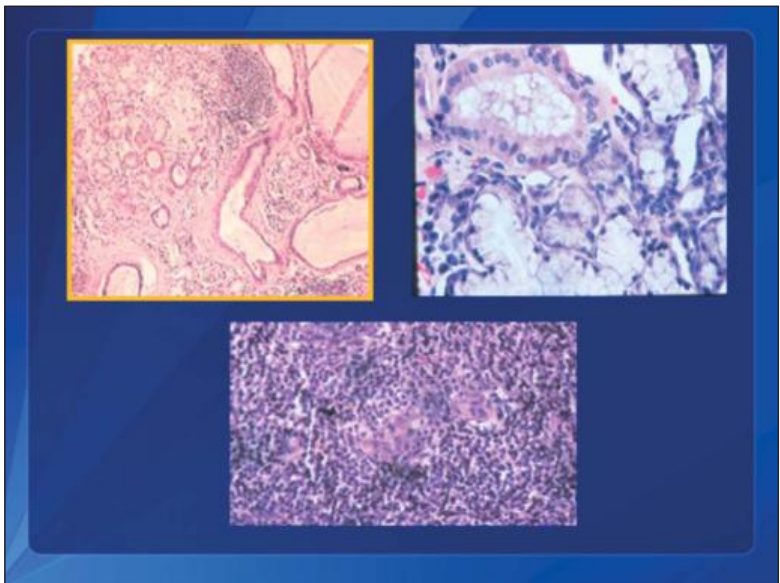


Figure 4.4 Chronic sialadenitis (upper left). Normal minor salivary gland tissue (upper right) and severe lymphocytic infiltration of minor salivary gland caused by Sjögren's syndrome (lower centre).

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Dental caries and dental erosion

One of the most common oral conditions that develop as a result of salivary gland hypofunction is new and recurrent dental caries. In the presence of persistent salivary gland hypofunction, the inability of the salivary system to restore oral pH towards neutrality and inhibit certain bacteria after food and beverage ingestion leads to an oral environment conducive to microbial colonisation with caries-associated micro-organisms and enamel demineralisation. The margins of existing restorations are also vulnerable to recurrent decay. Salivary hypofunction-associated root surface caries is a particularly difficult condition to diagnose and treat and, therefore, identification of patients at risk will allow measures to be taken to preserve the dentition.

With deficient remineralisation, dental erosion is a more frequent occurrence in patients with salivary gland hypofunction. The cervical regions of teeth occasionally receive greater abrasion from tooth brushes and are susceptible to dental erosion. Occlusal and incisal surfaces exposed to attritional and traumatic forces can also undergo greater loss of enamel and dentine when there is insufficient saliva to permit remineralisation.

Gingivitis

The increase in salivary output during and immediately after the consumption of foods and fluids assists in the lavage of the oral cavity and the removal of food particles from oral surfaces. Conversely, salivary gland hypofunction is frequently associated with retained food particles, particularly in interproximal regions and beneath denture surfaces, and can cause gingivitis. Long-standing gingivitis may develop into periodontal loss of attachment, so patients with chronic hyposalivation are at risk for developing gingival and periodontal problems.

Interestingly, most studies have not demonstrated significantly greater levels of periodontal disease in patients with Sjögren's syndrome compared with healthy controls,²⁵ which may be due to greater attention to oral health and more frequent use of professional dental services. In addition, while several studies have demonstrated significantly greater numbers of caries-associated mutans streptococci and lactobacilli in patients with salivary gland hypofunction compared with healthy controls, similar levels of micro-organisms associated with gingival inflammation were detected in both populations.²⁶ Therefore, the primary dental problem in patients with salivary gland hypofunction is dental caries, with less risk (but greater than that for healthy individuals) for developing gingival and periodontal problems.

Impaired use of removable prostheses

Removable intra-oral prostheses depend upon a thin film of saliva on mucosal surfaces in order to enhance adhesion. Accordingly, changes in saliva quantity and quality have been associated with impaired use of intra oral prostheses. Most commonly, salivary gland hypofunction leads to trauma to the desiccated, friable tissues and an increased rate of oral microbial infections. Patients with removable prostheses frequently require denture adhesives to assist in retention of their appliances in the presence of salivary gland hypofunction.

Oral fungal infections

Decreased salivary output leads to oral mucositis, pain, and increased susceptibility to developing microbial infections, the most prevalent of which is candidiasis. This fungal infection is caused by *Candida albicans*, a commensal organism that normally resides in the oral cavity. There are five clinical manifestations of oral candidiasis: angular cheilitis of the lips, erythematous candidiasis (denture stomatitis), atrophic candidiasis, hyperplastic candidiasis, and pseudomembranous candidiasis.

Dysgeusia

Saliva plays a critical role in taste function as a solvent for food, a carrier of taste-eliciting molecules, and through its composition. Accordingly, when salivary output is reduced, taste function can be adversely affected (dysgeusia).²⁷ Head and neck radiation has deleterious effects on both taste cells and salivary glands and thus directly and indirectly affects gustation. Other diseases and medical problems adversely affect the composition of saliva (e.g. adrenergic blocking agents), which could influence taste.

Dysphagia

Salivary gland hypofunction also contributes to difficulty with chewing and swallowing (dysphagia). Swallowing occurs in four stages: oral preparatory, oral, pharyngeal, and oesophageal (see Chapter 5). Each stage requires adequate lubrication of mucosal tissues to ensure a safe and efficient swallow. It requires greater time to perform a single swallowing movement, as well as repeated multiple swallowing movements, in subjects with salivary gland hypofunction. Therefore, dysphagia is more prevalent among patients with salivary gland hypofunction. Importantly, dysphagia is a significant risk for developing aspiration pneumonia, a condition which carries great morbidity and mortality, particularly in those inhabiting long-term care institutions.

Impaired quality of life

Many of the oral-pharyngeal sequelae of salivary gland hypofunction and chronic xerostomia lead to an impaired quality of life. Dentoalveolar and oropharyngeal infections can rapidly lead to systemic disease, particularly in medically complex patients. Desiccated and friable oral mucosal tissues are more likely to develop traumatic lesions, especially in denture-wearing older adults, which cause pain and interfere with nutritional intake. Also, dysgeusia, dysphagia, and difficulty chewing food secondary to salivary gland hypofunction can lead to changes in food and fluid selection that compromise nutritional status. The speech and eating difficulties that develop can impair social interactions and may cause some patients to avoid social engagements. Dysphagia increases susceptibility to aspiration pneumonia and colonisation of the lungs with Gram-negative anaerobes from the gingival sulcus.²⁸

Management of xerostomia and salivary gland hypofunction

Overview

The initial step in the management of xerostomia is the establishment of a diagnosis. This frequently involves a multidisciplinary team of health care providers who communicate effectively, since many patients have concomitant medical conditions and frequently experience complications of polypharmacy. The second step is scheduling frequent oral health evaluations due to the high prevalence of oral complications.⁹ Maintenance of proper oral hygiene and hydration (water is the drink of choice) are helpful. Several habits, such as smoking, mouth breathing, and consumption of caffeine-containing beverages, have been shown to increase the risk of xerostomia. Limiting or stopping these practices should lessen the severity of dry mouth symptoms. A low-sugar diet, daily topical fluoride use (e.g. fluoride toothpaste and mouth rinses), anti-microbial mouth rinses, and use of sugar-free gum or candy to stimulate salivary flow, help to prevent dental caries.

There are many biomaterials available for the restoration of dental caries, and glass ionomer resins and liners are well-suited for patients with salivary gland hypofunction who are caries-susceptible. These materials provide sustained fluoride release that reduces the incidence of recurrent caries, and the fluoride is considered 'rechargeable' when the patient uses a fluoridated rinse or toothpaste

Dry mucosal surfaces and dysphagia are managed with oral moisturisers, lubricants, and saliva substitutes, as well as careful use of fluids during eating. The night-time use of bed-side humidifiers can assist in reducing nocturnal xerostomia.

Patients must be instructed on the frequent use of fluids during eating, particularly for dry and rough foods. Eating and swallowing problems secondary to salivary gland hypofunction can impair the intake of fibre-rich foods, restricting some older adults to a primarily soft and carbohydrate diet. Accordingly, patients must be counselled on a well-balanced, nutritionally adequate diet and the importance of limiting sugar intake, particularly between meals.

Gustatory and masticatory stimulation

If there are remaining viable salivary glands, stimulation techniques using sugar-free chewing gum, candies (sweets), and mints can stimulate salivary output. Chewing sugarless gum is an extremely effective and continuous sialogogue, since it increases salivary output and increases salivary pH and buffer capacity. Citric acid, which is present in fruits as well as sour and sugarless candies or lozenges, may also be used to stimulate salivary output. However, dentate individuals should refrain from excessive use of acid-containing substances, since they could cause dental erosion. Buffered xylitol-containing chewing gums or mints are often recommended, because xylitol has an anti-cariogenic effect. Cinnamon or strong mint-flavoured sialogogues should be avoided, because chronic usage can lead to soft tissue irritation. Recent investigations have demonstrated that sucking on lemon candy prior to or within the first 24 hours of therapy may induce a significant increase in salivary gland damage in patients who have received radioactive iodine for thyroid cancer. Therefore, lemon candy should not be given until 24 hours after therapy.²⁹

Pharmacological stimulation

Treating xerostomia with medications that enhance salivation is another therapeutic option, particularly in a relatively healthy person for whom polypharmacy may not be a critical concern. Secretagogues such as pilocarpine (a non-specific muscarinic agonist) can increase secretion rates and diminish xerostomic complaints in patients with sufficient remaining exocrine tissue.³⁰ Pilocarpine has been approved by the US Food and Drug Administration (FDA) for the treatment of xerostomia and salivary gland hypofunction in patients with Sjögren's syndrome as well as in patients who have received head and neck radiotherapy for cancer. Pilocarpine is typically given in a dosage of 5 mg orally three times a day and before bedtime. When taken approximately 30 minutes before mealtime, patients may benefit from the increased salivation in eating their meals. The total daily dose should not exceed 30 mg. Adverse effects include increased perspiration, greater bowel and bladder motility, and feeling hot and flushed. Patients with a history of bronchospasm, severe chronic obstructive pulmonary disease,

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congestive heart disease, and narrow angle glaucoma should not take pilocarpine. Another pharmacological sialogogue is cevimeline, which has FDA approval for the treatment of dry mouth in Sjögren's syndrome in a dosage of 30 mg orally three times daily. Like pilocarpine, it is a muscarinic agonist that increases the production of saliva. Pilocarpine is a non-selective muscarinic agonist, whereas cevimeline reportedly has a higher affinity for M1 and M3 muscarinic receptor subtypes. Since M2 and M4 receptors are located on cardiac and lung tissues, cevimeline can enhance salivary secretions while minimising adverse effects on pulmonary and cardiac function. Patients with uncontrolled asthma, significant cardiac disease, and narrow angle glaucoma should not take cevimeline.

Drug substitution and deletion

Instead of prescribing xerostomia-associated drugs, substitution with similar types of medications with fewer xerostomic side effects is preferred. For example, serotonin-specific reuptake inhibitors (SSRIs) have been reported to cause less xerostomia and salivary gland hypofunction than tricyclic antidepressants. If anticholinergic medications can be taken during the daytime nocturnal xerostomia can be diminished, since salivary output is lowest at night. Furthermore, if drug dosages can be divided, unwanted side effects from a large single dose can be avoided. Scrutiny of drug side effects can assist in diminishing the xerostomic potential of many pharmaceuticals used by patients presenting with xerostomia or salivary gland hypofunction.

Polypharmacy is a common problem among the elderly, who can be treated concomitantly by multiple health care providers. Occasionally, medications with xerostomic sequelae may no longer be required, but the patient continues to take them. Other times multiple drugs are prescribed for similar medical conditions by different health care providers. Under these conditions it is advisable to recommend a critical review of all medications. Perhaps some can be substituted or even deleted from the daily regimen to diminish unwanted side effects including xerostomia and salivary gland hypofunction.

Salivary replacements

Saliva substitutes and oral lubricants may ameliorate some xerostomic symptoms in patients who have remaining salivary tissue as well as those who have no viable salivary glands. These products tend to diminish the sensation of oral dryness and improve oral functioning. The choice of product depends on effect duration, lubrication, taste, delivery system, and cost; many patients nevertheless primarily use water.³¹ Several products currently available without a prescription include Biotene (mouth rinse, toothpaste and chewing gum), Saliva Orthana (a mucin-based artificial saliva), Freedent

(a low-tack, sugar-free chewing gum), Saliva Sure (buffered xylitol-containing lozenges) and Oralbalance gel.

Acupuncture

There are data suggesting that acupuncture therapy can increase the flow rate of stimulated saliva for up to six months after the completion of radiotherapy.³² Although this treatment modality is not commonly utilised, it presents an option for patients who respond well to muscarinic agonists (e.g. pilocarpine, cevimeline) yet have difficulty taking these medications due to secondary adverse effects.

Salivary gland sparing radiotherapy

Three-dimensional treatment planning and dose delivery techniques have been devised to limit radiation exposure to salivary glands in an attempt to preserve salivary function after radiotherapy. Significant dose reductions have been achieved to parotid glands on the contralateral side of the primary tumor, resulting in retention of secretory ability, reduction of xerostomia and salivary gland hypofunction, and improvement of xerostomia-related quality of life.³³ Further, it appears that reducing the dose to the salivary glands does not impair radiation efficacy with respect to tumours and lymph nodes considered to be at risk for cancer spread, and that long-term survival may not be reduced with these radiation-sparing techniques.

Cytoprotectants

A new category of drugs has been developed that may protect oral mucosal and salivary gland tissues during chemotherapy and head and neck radiotherapy. The most commonly used is Amifostine, a broad-spectrum cyto- and radio-protectant that provides mucosal and organ protection against myelotoxicity, nephrotoxicity, mucositis, and xerostomia associated with various chemotherapy and radiation modalities. Specifically, Amifostine has received FDA approval for reduction of xerostomia in patients receiving head and neck radiotherapy,³⁴ and it has demonstrated efficacy in reducing mucositis and candidiasis in these patients. Amifostine is given intravenously (200 mg/m²) or subcutaneously (500 mg) 30-60 minutes prior to each dose of external beam RT. Common side effects include hypotension, nausea, and vomiting. Accordingly, fluids and anti-nausea medications are required daily to prevent serious side effects.

Salivary gland surgical transfer

Surgical techniques have been used to spare salivary glands from head and neck radiotherapy. One technique involves transferring the contralateral submandibular

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gland to the submental region, which can shield the gland from the damage induced by external beam radiation.³⁵ Post-radiotherapy follow up data suggest fewer complaints of xerostomia and salivary gland hypofunction with few surgical complications. Therefore this and other surgical techniques could be combined with chemotherapeutic agents to spare salivary glands from toxicity and stimulate existing secretions to compensate for radiotherapy-destroyed glands.

Gene therapy

Ongoing research in the field of gene therapy may make it possible to prevent damage to, as well as correct, already damaged salivary glands.³⁶ Transfer of genes to salivary glands has already been demonstrated using viral and non-viral vectors in animal models. The close access to salivary gland cells via the intraoral cannulation of the main excretory ducts permits relatively non-invasive delivery of vectors and transferring genes. With increased pathobiological understanding and biotechnological improvements, it is believed that gene transfer may become a common modality for treating certain salivary gland disorders in the future.³⁶

Clinical highlights

1. The prevalence of xerostomia and salivary gland hypofunction increases with advancing age and significantly impacts the quality of life.
2. There are multiple causes of xerostomia and salivary gland hypofunction, the most common being polypharmacy, medications with anti-cholinergic adverse effects, Sjögren's syndrome, and radiotherapy for head and neck cancer.
3. There are multiple intra-oral causes of salivary gland hypofunction, and they are classified into three categories; infectious (bacterial, viral), noninfectious (obstructions) and neoplastic.
4. Patients with dry mouth often complain of altered taste, difficulty eating and/or chewing and swallowing dry foods, especially without drinking liquids.
5. The diagnostic work up for salivary gland hypofunction includes a comprehensive medical history and review of systems, a thorough head and neck examination, collection of whole saliva, histopathological examination of biopsy specimens, serology, culture, and imaging.
6. The most common complications associated with chronic salivary gland hypofunction include: dental caries, gingivitis, fungal infection, impaired prosthesis retention, dysphagia and dysgeusia.

7. The management of salivary gland hypofunction, if there is remaining viable salivary gland tissue, requires masticatory or gustatory stimulation techniques, such as sugar-free chewing gum or candies, and utilisation of systemically administered cholinergic agonist medications (pilocarpine hydrochloride and cevimeline hydrochloride).
8. The management of drug-induced xerostomia should include medication substitution (exchanging one medication for another with fewer adverse effects) and deletion of unnecessary medications with xerogenic effect.
9. Saliva substitutes and oral lubricants may ameliorate some xerostomic symptoms and improve oral function in patients with or without remaining viable salivary gland tissue.
10. Gene therapy may in the future be used for the prevention and treatment of radiation-induced salivary gland hypofunction and, hence, improve the quality of life for patients.
11. A systematic approach to data collection, clinical, imaging and laboratory evaluations, as well as close collaboration among different health care providers should lead to accelerated diagnosis and more timely management of patients.

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Salivary clearance and its effects on oral health

C. Dawes

Differences in the rates of salivary clearance of carbohydrates from food, acids from plaque, and therapeutic substances (for example fluoride) help to explain differences in disease susceptibility among individuals and at various sites within a single mouth.

A large number of substances pass through the oral cavity every day, some of which, such as sucrose or acids, are a threat to the health of the mouth, with its unique and vulnerable tissues. Other substances, such as fluoride, may act as a defence, promoting oral health. Many substances will dissolve in saliva, from which they may then diffuse into, or react with, the oral tissues. The effect of incoming freshly secreted saliva, together with the swallowing process, is to reduce the concentration of exogenous substances, a process that is described as salivary clearance.

Thus, a rapid salivary clearance of harmful substances is beneficial for oral health, while the reverse is true for protective substances.

Models of salivary clearance

The Swenander Lanke model

The first model of salivary clearance was a simple one suggested by Swenander-Lanke¹ in the mid 1940s. In her model, a chemical sucrose dissolves in saliva (of volume V) to create an initial concentration (C_0). Saliva flows at a constant rate (F) into the mouth and is continuously removed (swallowed) at the same rate. The sucrose concentration (C_t) at a later time (t) can be shown to be given by $C_t = C_0 \cdot e^{-Ft/V}$. In experimental studies, a graph of the logarithm of the sucrose concentration versus time usually forms a straight line, but only if the plot is begun after the salivary flow rate has returned to the unstimulated rate following its initial rise due to the gustatory stimulation. The rate of decrease in concentration can also be described by using the time taken for the sucrose concentration to decrease by half, or the time taken for the concentration to fall to a given low level.

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The Dawes model

A more recent model² describes the swallowing process as being equivalent to the action of an incomplete siphon (Figure 5.1). After a swallow, the mouth retains a minimum volume of saliva, called the residual volume (Resid). Saliva then flows into the mouth at a rate dependent initially on the stimulating effect of the ingested substance but later, once the concentration is below the taste threshold, or after taste adaptation has occurred, on the unstimulated flow rate. The volume of saliva in the mouth thus increases until a maximum volume (V_{max}) is reached. This stimulates the subject to swallow, which clears some of the substance from the oral cavity. The remainder (dissolved in the residual volume of saliva) is then progressively diluted by more saliva entering the mouth until V_{max} is reached again, and another swallow occurs. The

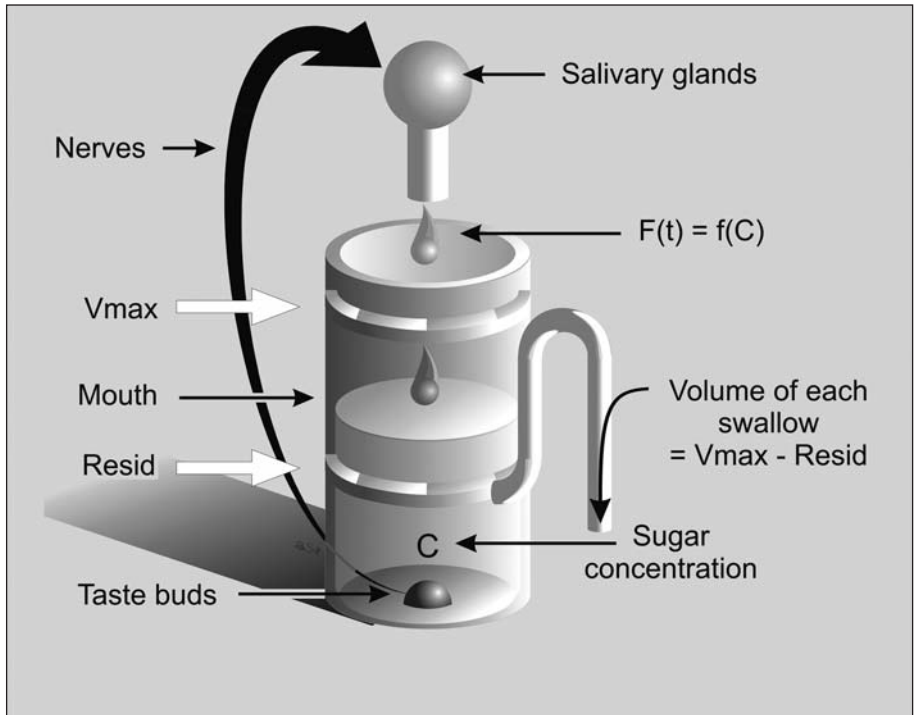


Figure 5.1 The Dawes (1983) model of oral clearance. Saliva is produced at a rate dependent on the concentration of sugar in the saliva. When a maximum volume of saliva (V_{max}) is reached, a swallow occurs and the salivary volume decreases to a residual volume (Resid), thereby eliminating some of the sugar

SALIVARY CLEARANCE AND ITS EFFECTS ON ORAL HEALTH

Dawes model has been used to describe with considerable accuracy the clearance of substances, including sucrose, which do not bind to oral surfaces.

Other studies have indicated that with some substances, clearance may occur in two stages, rapidly from the bulk of the saliva, and more slowly from stagnation areas.

Swallowing

The swallowing process requires extremely complex neuromuscular control, since not only is there the transfer of food or drink from the mouth to the digestive tract (oesophagus) but the respiratory and speech-producing structures must be protected against aspiration of food, drink or saliva, which can lead to severe pulmonary infections.

With voluntary swallowing the oral preparatory phase involves the formation of a bolus from food and saliva and its positioning on the dorsum of the tongue, while the oral phase is characterised by elevation of the tongue to transfer the bolus into the pharynx. The sequence of events in the pharyngeal phase is largely under the control of the swallowing centre in the pons, which is activated by contact of the food bolus with receptors in such areas as the anterior pillars of the fauces. The swallowing centre coordinates the cessation of respiration, contractions of the pharyngeal musculature for transfer of the bolus into the oesophagus, the elevation of the soft palate to block off the nasal cavity, the movement of the larynx superiorly and anteriorly into the base of the tongue and the downward movement of the epiglottis to protect the lower airway. The oesophageal phase involves peristaltic waves which transfer the bolus toward the stomach and finally relaxation of the gastro-oesophageal sphincter.

The mechanism by which swallowing of saliva is initiated when food or drink are not being consumed (spontaneous swallowing) is uncertain but it is most probably due to activation of laryngeal receptors from saliva which has passed over the posterior dorsal surface of the tongue.

Dysphagia,³ or difficulty in swallowing, can be a direct result of the ageing process in which there may be deterioration in fine motor control or it can accompany many diseases such as Parkinson's disease, multiple sclerosis, stroke, or trauma. A major problem then may be the aspiration of saliva which enters the pharynx without eliciting the normal spontaneous swallowing process. Dysphagia has been treated by injection of botulinum toxin type A into the submandibular glands (the main source of unstimulated saliva) but the patient may then experience a dry mouth.

Clearance of substances with binding properties

Fluoride

For fluoride, which is a natural component of saliva, and which reacts with the teeth and with plaque, the Dawes model requires further refinement, since plaque fluoride levels can be elevated for several hours following a fluoride rinse or intake of a fluoride tablet and can constitute a 'reservoir' of fluoride.⁴ During the early phase of clearance, when the salivary concentration is still high, some of the fluoride will diffuse into dental plaque, or bind to the oral mucosa, from which it is later redistributed back into the bulk saliva. This will delay clearance of fluoride, as will the formation of calcium fluoride deposits on the teeth. These can be formed at higher fluoride concentrations and they will later dissolve slowly. Also, most of the fluoride which is swallowed will be absorbed from the gastrointestinal tract into the blood and then a very small fraction of this (<0.2%) will be recycled via the salivary glands. When all of these factors are built into a computer model, it is possible to evaluate the effects of a single variable on clearance while keeping other variables constant.⁵

Chlorhexidine

Chlorhexidine, in the form of rinses, gels, or varnishes, is an antibacterial agent used for plaque control and for the prevention of both dental caries and periodontitis. An important property (termed substantivity) of chlorhexidine is its ability to bind more strongly than other antibacterial substances to the oral surfaces. This greatly delays its clearance from the mouth, thereby prolonging its effectiveness.

Micro-organisms and epithelial cells

Saliva, when secreted by the salivary glands, is sterile but whole saliva in the mouth may contain bacteria at levels up to 10^9 /ml. For bacteria to survive in the mouth they must be able to attach to and proliferate on oral surfaces since the unstimulated salivary flow rate is too high for saliva to act as a continuous culture system.⁶

Epithelial cells are continually being shed from the oral mucosa into saliva and it has been estimated that the surface cells stay attached for only about 3 hours before being desquamated⁶. Each epithelial cell has about 100 bacteria attached to its surface and in saliva there are about three times as many bacteria bound to epithelial cells as are unattached⁶. Most of the bacteria in saliva appear to be derived from mucosal tissues rather than from the teeth. However, after a prophylaxis, and in the subsequent absence of oral hygiene, the amount of plaque on the teeth will gradually increase until an equilibrium is reached when the rate at which bacteria are being shed from plaque into

saliva is equal to the rate of their proliferation on the teeth. Thus salivary clearance plays an important role in removing bacteria and epithelial cells from the mouth and individuals with hyposalivation will have higher salivary bacterial and epithelial cell counts. In addition, salivary flow is very low during sleep (see Chapter 3), which explains why the salivary bacterial and epithelial cell counts are greatest before breakfast. Halitosis is thus usually most marked at this time of day since epithelial cells are one of the substrates from which some Gram negative anaerobes are able to form volatile sulphur compounds with an unpleasant odour.

Some factors influencing salivary clearance

The most important variables are the residual and maximum volumes, the unstimulated and stimulated flow rates, and the extent to which the substance being cleared binds to oral surfaces.⁷

The volume of saliva left in the mouth after swallowing (Resid)

As measured in studies on 40 normal individuals,⁸ the mean residual volume is about 0.8 ml, but the large range (0.4-1.4 ml) suggests that variations in Resid may be responsible for some individual differences in clearance patterns. According to the Dawes computer model, the effect of varying Resid on clearance half-time (the time for the salivary sucrose concentration to decrease by half) after a 10% sucrose mouthrinse is very large (*Figure 5.2*). In fact, the difference in concentration obtained with the lowest residual volume (0.4 ml) and the highest volume tested (1.4 ml) was more than 50 fold after only ten minutes. Thus individuals who swallow more effectively (i.e. have a low residual volume) will clear substances from the mouth more quickly.

The volume of saliva in the mouth just prior to swallowing (V_{max})

Another important physiological variable affecting salivary clearance is the maximum volume of saliva allowed to accumulate in the mouth before swallowing is initiated. The mean value in 40 individuals was 1.1 ml, but as with the residual volume, a wide range (0.5-2.1 ml) was found, those with larger residual volumes naturally having larger maximum volumes.⁸ Again, a large effect on the clearance half-time is seen (*Figure 5.2*), and individuals who do not allow as much saliva to accumulate in the mouth before swallowing will clear substances more rapidly.

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The unstimulated salivary flow rate

This is normally about 0.3-0.4 ml/minute but may vary a great deal among individuals (see Chapter 3). Since the swallowing frequency is dependent on the rate of entry of saliva into the mouth, it is obvious that the salivary flow rate is an extremely important variable. According to the Dawes model, the lower the unstimulated salivary flow rate, the more prolonged will be the clearance half-time for sucrose (*Figure 5.3*). Since individuals with severe hyposalivation may have unstimulated flow rates even lower than the minimum value of 0.05 ml/minute shown in *Figure 5.3*, the delayed clearance of carbohydrate may help to account for their high susceptibility to dental caries.

It should be noted that for two subjects having identical unstimulated salivary flow rates, their clearance half-times could be very different if one has low values of Resid and Vmax while the other has high values.

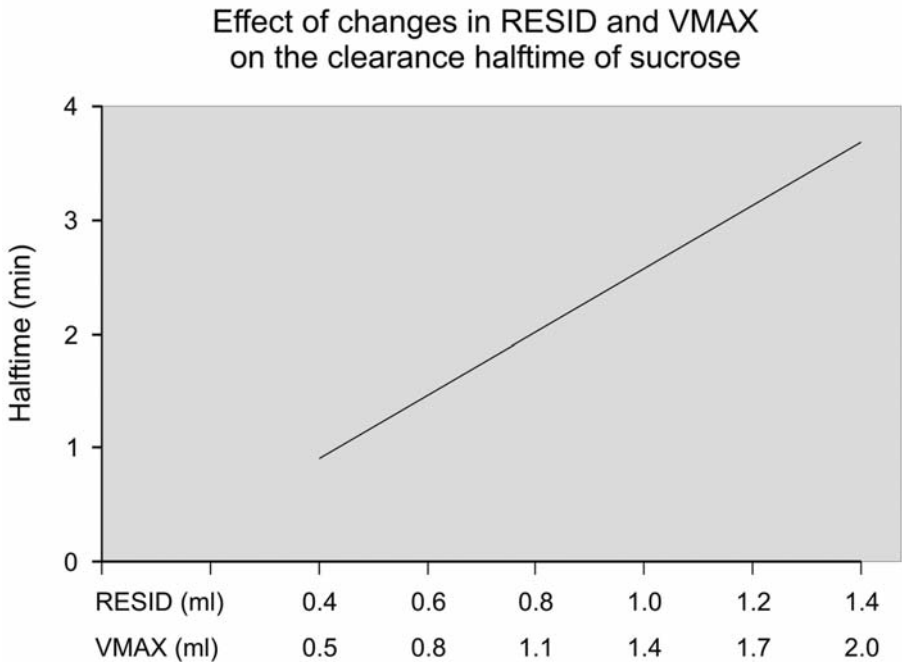


Figure 5.2 A computer simulation of the effect of changes in the residual volume after swallowing (Resid) and the maximum volume before swallowing (Vmax) on the clearance half-time of sucrose after a 10% sucrose mouthrinse. The clearance half-time is the time for the concentration to fall by half

Although there are many substances, such as antimicrobial peptides, which appear to have potential for inhibiting the metabolism of oral micro-organisms, *Figure 5.3* shows that with normal values for Resid, V_{max} and the unstimulated salivary flow rate, the clearance halftime is very short, only about 2.2 minutes.

The stimulated salivary flow rate

Although salivary flow may remain above the unstimulated rate for only about a minute after food consumption or after a sucrose rinse, the initial rate at which the sucrose is diluted plays a critical role in determining how much sucrose diffuses into dental plaque. The longer the salivary sucrose concentration remains high, the more sucrose will diffuse into dental plaque. In individuals with a normal unstimulated flow rate, the salivary sucrose concentration will have fallen so low within the first two or three

**Effect of changes in the UNSTFR on
on the clearance halftime of sucrose**

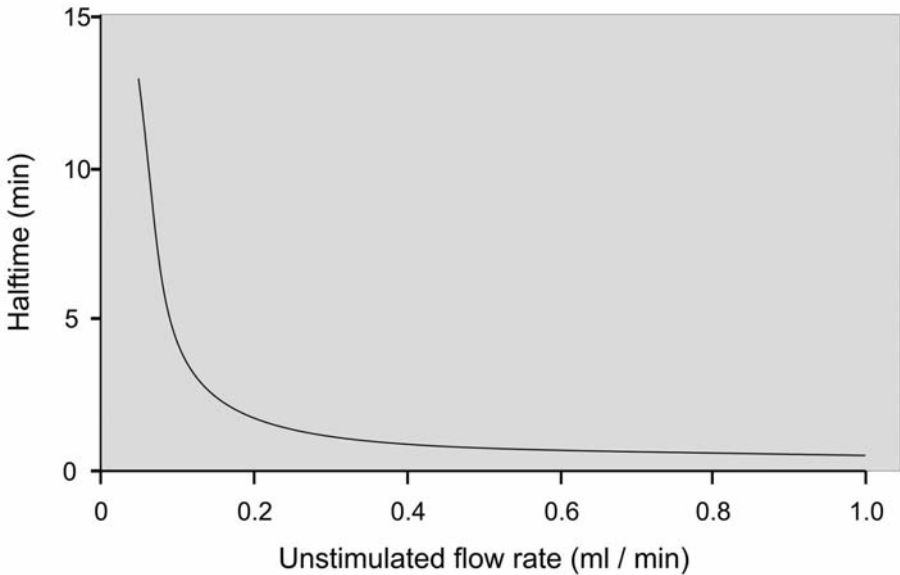


Figure 5.3 A computer simulation of the effect of changes in the unstimulated flow rate on the clearance of sucrose after a 10% sucrose mouthrinse. The simulation assumed average values for the unstimulated flow rate (0.32 ml/min), Resid (0.8 ml), and V_{max} (1.1 ml). The curve approximates a rectangular hyperbola so that clearance is greatly prolonged at low flow rates

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minutes after a sucrose rinse that a rinse with water at that time would have little influence on acid production in plaque (see later).

Lagerlöf *et al.* have shown, in a computer model, that the stimulated flow rate can also have a great effect on the clearance pattern of fluoride⁵. As with sucrose, the faster clearance rate caused by stimulation of salivary flow reduces the amount of fluoride diffusing into dental plaque. In formulating cariostatic topical fluoride products, such as fluoride tablets, it thus seems advisable to use agents which do not stimulate salivation: in other words, ones that are tasteless. Tablets should be allowed to dissolve slowly in the mouth rather than be chewed.

Many investigators measure bacterial counts on saliva samples elicited by chewing on paraffin wax but with inadequate control of the flow rate or duration of chewing. However, as seen in *Figure 5.4*, when sequential saliva samples are collected, there is an enormous peak in the output of both bacteria and epithelial cells at the initiation of

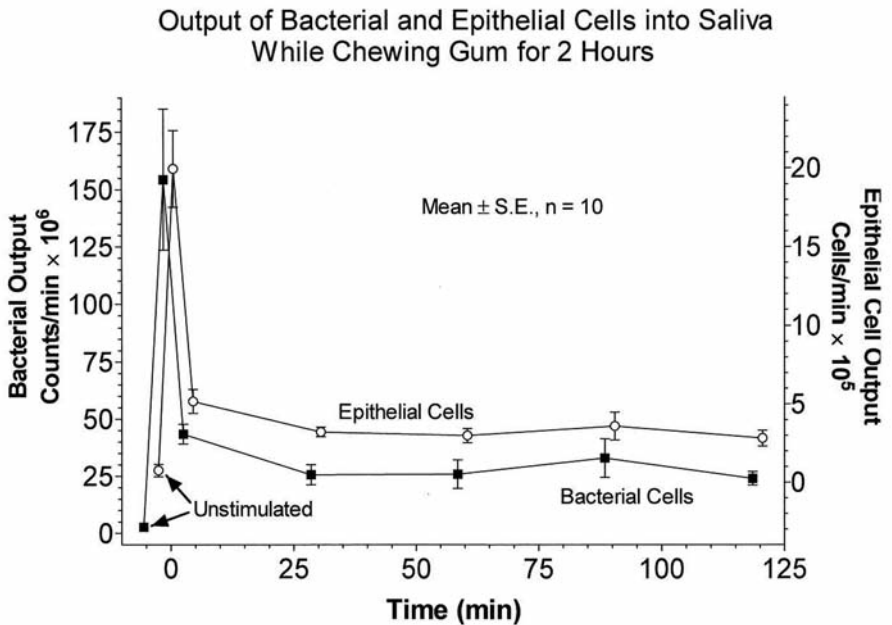


Figure 5.4 Output of bacteria and epithelial cells into saliva while chewing gum for 2 hours. To avoid overlap of data, the results for bacterial cells over the -5 - 0 minute period (unstimulated) and the 0 - 1 minute period of chewing have been set back on the abscissa by 1 minute

the chewing process⁹. Lack of control of the saliva collection conditions thus makes interpretation of salivary bacterial counts very difficult.

Use of products such as chewing gum causes an initial increase in salivary flow rate to about 12 times the unstimulated rate and with prolonged chewing the flow rate remains at two to three times the unstimulated rate. Surprisingly, the use of chewing gum does not greatly increase the degree of mixing of the different salivary secretions but the increased flow rate does speed up the clearance process.

Saliva as a film

Oral biologists have generally thought of dental plaque as being covered by a large volume of saliva, the composition of which remains essentially constant unless the flow rate changes. In fact, for most of the time, saliva is present as a very thin film whose composition changes locally when materials diffuse into or out of dental plaque. Given an average volume of saliva in the mouth of about 1 ml, and that the surface area of the adult mouth is just over 200 cm², the saliva must be present as a film averaging about 0.1 mm or less in thickness between adjacent surfaces.¹⁰

Recent studies^{11,12} have shown marked site-specificity in the thickness of the salivary film with values on individual surfaces ranging from 70 µm on the posterior dorsum of the tongue to 10 µm on the anterior hard palate. Film thicknesses of <10 µm in the latter location are associated with reports of mouth dryness. In patients who report that their mouth is very dry, the residual volume still averages 71% of normal, which suggests that dry mouth is not due to complete oral dryness but to localised areas of dryness, notably on the anterior hard palate and anterior tongue.

When flow is unstimulated the film has been estimated to move at different rates (0.8-8.0 mm/minute) in different regions of the mouth¹³. These extremely low velocities have important implications for the clearance of ingested carbohydrate and topical fluoride, but particularly for clearance of acid from dental plaque.

Figure 5.5 shows the postulated directions of flow of the salivary film.

Clearance of substances from local sites

Ingested carbohydrate

Dental caries is caused by the demineralising effects of organic acids produced in dental plaque by micro-organisms that ferment carbohydrates, most notably sucrose. The

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Stephan curve is the fall and subsequent rise in plaque pH which occurs after exposure of dental plaque to fermentable carbohydrate (see *Figure 6.1*). In regions of the mouth where clearance is rapid, less sucrose will be available to diffuse into dental plaque, and thus less acid will be formed.

Studies have shown that sucrose ingested in several different forms is distributed very unevenly around the mouth and is cleared at very different rates in different locations.¹⁴⁻¹⁸ In general, clearance is more rapid from lingual than from buccal tooth surfaces, except buccal to the upper molars where parotid saliva enters the mouth. Apart from that region, buccal tooth surfaces are mostly exposed to the extremely viscous secretions from the minor mucous glands. In contrast, lingual surfaces are exposed mainly to the secretions from two of the major salivary glands, namely the submandibular and sublingual.

Figure 5.6 illustrates mean results on 10 subjects for the clearance of sucrose from whole saliva and from six specific oral sites after a 10% sucrose mouthrinse.¹⁷ Because the ordinate is a logarithmic scale, a change of one unit represents a ten-fold change in concentration. The higher the salivary film velocity in a given region, the lower is the initial concentration of sucrose and the more rapid is its clearance. Compare, for

Probable net directions and volumes of saliva flow around the teeth in different regions of the mouth

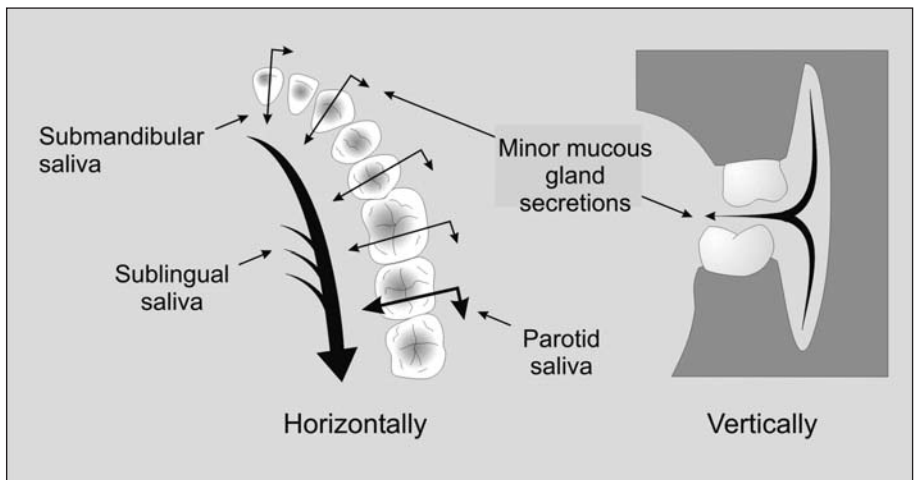


Figure 5.5 Diagrammatic representation of the anticipated directions and volumes of salivary flow in different locations in the mouth

instance, clearance from the lingual of the lower incisors with that from the facial of the lower molars, where the salivary film velocities, when flow is unstimulated, have been estimated to average 8 mm/minute and 1 mm/minute, respectively.

Sucrose concentrations at different oral sites after a sucrose mouthrinse

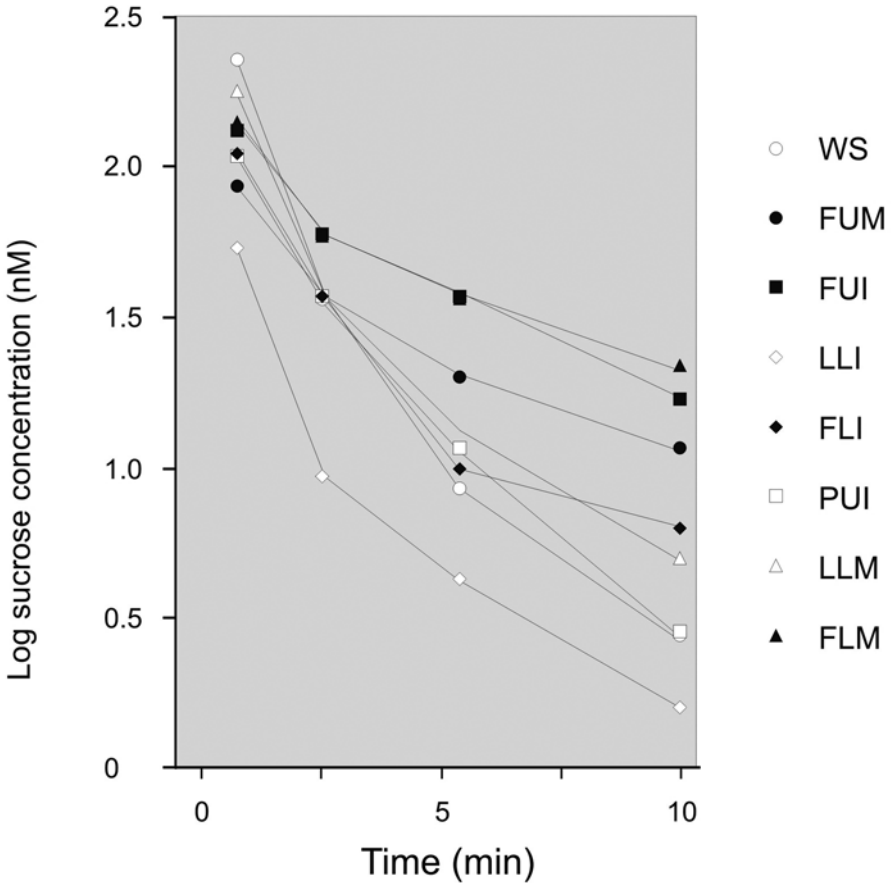


Figure 5.6 Sucrose concentrations in saliva at different sites and times after a 10% sucrose mouthrinse. WS = whole saliva; FUM = facial upper molars; FUI = facial upper incisors; LLI = lingual lower incisors; FLI = facial lower incisors; PUI = palatal upper incisors; LLM = lingual lower molars; FLM = facial lower molars

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Topical fluoride

The current view of fluoride's role in demineralisation and remineralisation of enamel stresses the value of raising slightly the fluoride level in the liquid surrounding the enamel crystals for prolonged periods of time (see Chapter 8). To be effective, only a small increase in salivary fluoride concentration is needed above the normal value of about $1 \mu\text{mol/l}$ (0.019 ppm). This certainly occurs during, and for a certain time after, the use of dentifrices, which usually contain about 1000 ppm F, and during other preventive applications of fluoride. Those sites where the salivary film velocity is low have been shown to clear fluoride more slowly,¹⁹ which will facilitate its anticariogenic action at the sites most susceptible to caries.

Acid from dental plaque

When plaque is exposed to sugar, the bacteria in plaque form acid which will tend to diffuse toward the tooth surface but also out of the plaque, down its concentration gradient into saliva. A computer model of this process²⁰ suggested that if the salivary film is moving slowly over plaque, acid will accumulate in the film and reduce the concentration gradient between the plaque and the saliva, which will retard the diffusion of acid out of the plaque (*Figure 5.7*). These predictions of the computer model

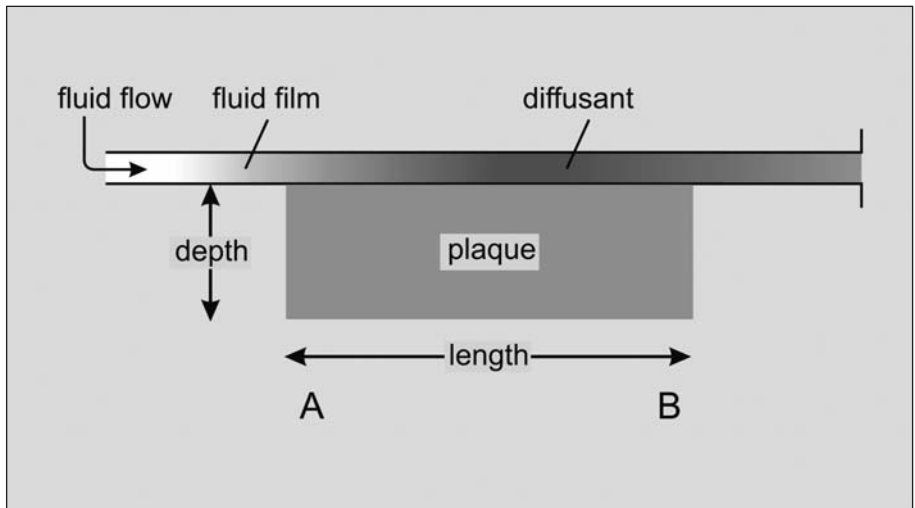


Figure 5.7 Diagrammatic representation of the flow of a slowly-moving film of saliva over dental plaque and the accumulation of a diffusant (such as organic acid) in the film at the distal edge (B) of the plaque

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were tested in a physical model²¹ in which 10% sucrose was passed for 1 minute over a 0.5 mm-deep artificial plaque of *Streptococcus oralis* (with the same acid-forming ability and buffering capacity as natural plaque), followed by unstimulated saliva at one of three different film velocities and with a film thickness of 0.1 mm. The film velocities of 0.8 and 8 mm/min are, respectively, the lowest and highest mean values estimated in the mouth when salivary flow is unstimulated, while 86 mm/min is about ten times higher than the latter. The resulting Stephan curves (Figure 5.8) at positions A and B (see Figure 5.7) on the undersurface of the plaque were much deeper and more prolonged at the lowest film velocity, especially at position B. Thus oral regions with a more slowly moving salivary film (e.g. buccal to the upper incisors - velocity 0.8 mm/minute) might be more susceptible to caries than regions with a faster moving salivary film (e.g. lingual to the lower incisors - velocity 8 mm/minute) because acid is cleared away from plaque more slowly at a low film velocity.

The effect on the Stephan curve of a mouthrinse with water

It has often been reported that rinsing the mouth with water after eating or drinking sugary items does not significantly reduce the fall in plaque pH, suggesting perhaps that the role of saliva in the clearance of sugar and in the clearance of plaque acid, by

Effects of salivary film velocity on pH changes at A and B (fig 6) in plaque (6 x 6 x 0.5 mm) after a 1 min exposure to 10% sucrose

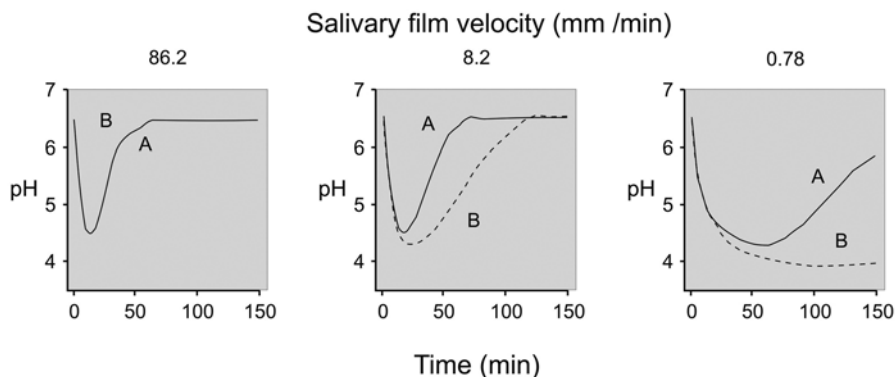


Figure 5.8 Effect of fluid film velocity on the pH at position A (see fig. 5.7), where the plaque is first contacted by fresh saliva, and at position B (see fig. 5.7), where the salivary film leaves the plaque, under an artificial plaque 6 mm square and 0.5 mm deep after exposure to a 10% sucrose solution for 1 minute

diffusion into saliva, has been overestimated. By contrast, stimulation of salivary flow by the chewing of paraffin wax, which increases the bicarbonate concentration in saliva, is very effective in restoring plaque pH to neutrality (see *Figure 6.7*). This could suggest that the effect of saliva is due only to the buffering power of the bicarbonate in saliva, rather than to the enhanced clearance of sugar or acid.

However, the lack of effect of mouthrinsing with water on the Stephan curve may be partly because it is generally done too late: a few minutes after a sucrose challenge, the sugar concentration in saliva is usually lower than that in plaque, so rinsing with water at that time would not be expected to reduce the diffusion of sugar into plaque, unless the sugar clearance were excessively slow, as in patients with hyposalivation. As far as the removal of acid is concerned, outward diffusion of hydrogen ions alone does not adequately explain plaque neutralisation. Shellis and Dibdin²² have shown that most of the H⁺ ions in dental plaque are fixed to bacterial surface proteins and other fixed buffers. That is why mobile salivary buffers such as bicarbonate and phosphate, which are present in the salivary film (but not in a water rinse), are so important - they are able to diffuse into plaque as HCO₃⁻ and HPO₄²⁻ ions, capture the hydrogen ions from the fixed buffers, and diffuse out into the saliva as H₂CO₃ and as H₂PO₄⁻ ions.

The advantage of mouthrinsing after meals then is that it helps to remove food debris as well as sugars in solution and this may be particularly valuable in individuals with hyposalivation in whom sugar clearance may be greatly delayed (*Figure 5.3*).

The site-specificity of dental caries and calculus deposition

In a fasted subject, the extracellular fluid phase of dental plaque (plaque fluid) is normally supersaturated with respect to several calcium phosphates, such as those in teeth and dental calculus (see Chapter 8). This condition favours remineralisation of early caries lesions and deposition of calculus. However, when plaque is exposed to fermentable carbohydrate, the bacteria form acid, and if the pH falls below a critical value (probably about 5.1-5.5), which is inversely proportional to the calcium and phosphate concentrations in plaque fluid, the latter becomes unsaturated. At such times, the teeth will tend to dissolve (dental caries), as will the calcium phosphate crystals present in early calculus.

Dawes and Macpherson¹⁷ have postulated that supragingival calculus forms most readily on the lingual surfaces of the lower anterior teeth and the buccal surfaces of the upper molars because these are sites with a high salivary film velocity. This will promote the development of shallow Stephan curves, because of the rapid clearance of sugar from the adjacent saliva and because of the rapid clearance of acid from dental plaque,

and there will be little opportunity for calcium phosphate crystals in early calculus to dissolve during meals or snacks. They have also suggested that smooth-surface caries is much more prevalent buccally than lingually because salivary film velocity is much lower buccally than lingually. A low film velocity buccally will slow the rate of sucrose clearance from the saliva and the clearance of acid from dental plaque, which will promote the development of deep and prolonged Stephan curves and enamel dissolution, leading to dental caries.

The mouth is clearly not a uniform environment but contains many distinct microenvironments, some of which are more conducive than others to the development of oral disease.

Summary clinical highlights

Rapid oral clearance of micro-organisms, of sucrose and other carbohydrate substrates, and of acid from plaque metabolism, will be of clinical benefit to oral health. However, for protective agents like fluoride or chlorhexidine, a slow clearance is preferable. Knowledge of the factors determining clearance rates in different locations is leading to a more detailed picture of the reasons for the site-specificity of dental caries and supragingival calculus deposition and suggesting ways to maximise some benefits for example, the avoidance of salivary stimulation during topical fluoride applications and by allowing fluoride tablets to dissolve slowly in the mouth rather than be chewed.

Acknowledgements

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Saliva and the control of plaque pH

Michael Edgar and Susan M Higham

The Stephan curve

Acidogenic bacteria in dental plaque can rapidly metabolise certain carbohydrates to acid end-products. In the mouth, the resultant change in plaque pH over time is called a Stephan curve (*Figure 6.1*). Under resting conditions the pH is fairly constant although differences among individuals and among sites in one individual are found. Following exposure of the plaque to fermentable carbohydrate the pH decreases rapidly to reach

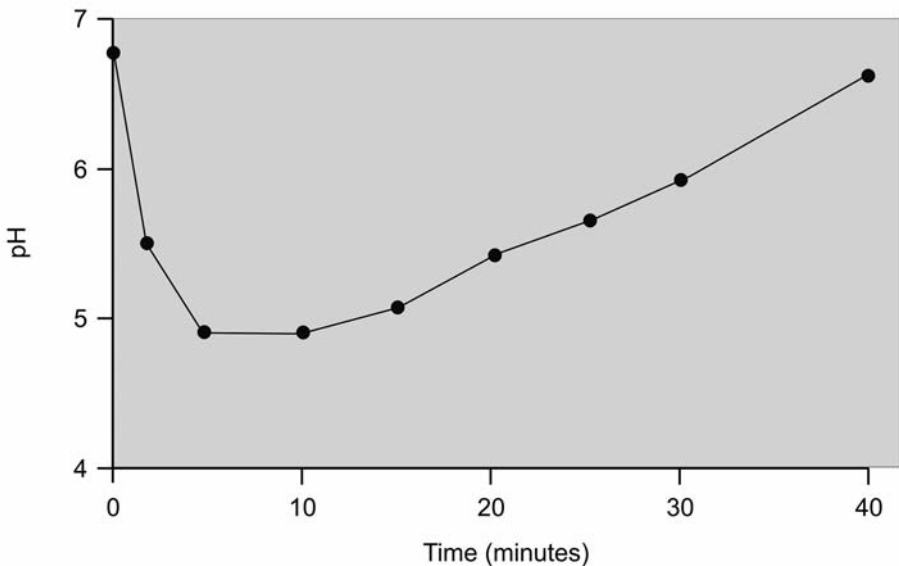


Figure 6.1 Diagram of a Stephan curve – the plaque pH response to a 10% glucose solution (Redrawn from G N Jenkins, *The physiology and biochemistry of the mouth* (4th Edition). Blackwell, London, 1978)

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a minimum after approximately 5-20 minutes before slowly returning to its starting value over 30-60 minutes or even longer.

Resting plaque pH

The term 'resting plaque' refers to plaque 2-2.5 hours after the last intake of dietary carbohydrate as opposed to 'starved plaque' which has not been exposed to carbohydrates for 8-12 hours. Resting plaque pH is usually between 6.0 and 7.0 whereas the starved plaque pH is normally between 7.0 and 8.0. A large range of plaque pH values seem to be compatible with oral health, but due to the multifactorial nature of dental caries, what may be healthy for one individual may be unhealthy for another.

Resting plaque contains relatively high concentrations of acetate anion compared with lactate. The predominant free amino acids in plaque are glutamate and proline, with ammonia also found at significant levels.¹ The presence of elevated levels of acetate is due to the accumulation of end products of amino acid breakdown as well as those of carbohydrate metabolism. These metabolic products are present at much higher concentrations than in saliva. This is partly due to the fact that they are constantly produced from the metabolism of intracellular and extracellular bacterial carbohydrate stores, and from the breakdown of salivary glycoproteins. Their diffusion out of plaque is hindered by the slow salivary film velocity (Chapter 5) under 'resting' conditions when saliva is unstimulated.

The decrease in plaque pH

Two main factors affect the rate at which the plaque pH decreases:

1. The presence of exogenous, rapidly fermentable carbohydrate, usually sugars
2. Low buffering capacity of saliva at unstimulated flow rates.

The fall in pH has been related to the production of principally lactic acid.² Simultaneously, acetate and propionate anions are lost from the plaque. These acids were assumed to be lost to the saliva but there are indications that they may diffuse also from the plaque into the tooth.³ In terms of the pH change in plaque, the amount of a low pK acid such as lactate relative to a higher pK acid such as acetate is very important. The high pK acids can provide a buffering system because they can absorb the hydrogen

ions generated by dissociation of the low pK acids. The fall in pH could thus be enhanced by a reduction of the buffering power of plaque acetate. The nature of the acids in plaque may be important because they differ in their ability to attack enamel.

As the pH of plaque decreases, the concentrations of amino acids and ammonia in plaque also fall rapidly.¹ This fall may be due to the bacterial uptake and utilisation of nitrogenous material for anabolic reactions, stimulated by the availability of energy from carbohydrate fermentation.

The minimum plaque pH

The minimum value of plaque pH and how long the pH stays at that minimum are determined by several factors:

1. Whether any fermentable carbohydrate remains in the mouth, and whether the carbohydrate has been cleared from the mouth e.g. by swallowing, rather than being metabolised by plaque bacteria
2. The pH may fall to values at which bacterial enzyme systems are no longer functioning properly
3. The buffering capacity, both in plaque and saliva but particularly in stimulated saliva, may be critical
4. The velocity of the salivary film flow over the plaque will affect the minimum pH.

The minimum pH corresponds with the greatest concentration of lactic acid produced during a Stephan curve and with a reduction in acetate, succinate, and propionate anions, most of the amino acids, and ammonia.

The length of time that the pH remains at its minimum is important since if it reaches the so-called 'critical pH', which is the pH at which saliva and plaque fluid cease to be saturated with respect to enamel mineral, then the dissolution of enamel may ensue (Chapter 8). The pH minimum usually occurs after salivation ceases to be stimulated, and although the buffering power of post-stimulated saliva remains higher than that of unstimulated saliva for some minutes, it eventually falls: this fall in salivary buffering may coincide with the minimum pH in plaque, thus allowing it to remain low. The benefit of continued stimulation of saliva throughout the Stephan curve is discussed below.

The rise in plaque pH

The steady rise in pH back to the resting value is influenced by all the factors mentioned above, including diffusion of acids out of the plaque into the salivary film. It is also influenced by base production in plaque. Ammonia is highly alkaline and can thus neutralise acid and cause a rise in pH. It is derived mainly from the breakdown of salivary urea (Chapter 1) but also from the deamination of amino acids. Another group of basic products in plaque are amines – formed from amino acids by decarboxylation. These bases are thought to have an important neutralising action in plaque, especially under conditions of moderate carbohydrate intake.

Glutamate is the predominant amino acid in plaque during the Stephan curve.¹ It is an extremely important amino acid since it is able to act as an amino donor in the synthesis of many amino acids from organic acid precursors - these amino acids are all less acidic than the corresponding organic acid. Delta-amino n-valeric acid (DAVA) has been shown to be present in plaque: its concentration is lowest around the plaque pH minimum after a sucrose challenge.¹ It is formed by the reduction of proline in the Stickland reaction (Figure 6.2). Its importance as a pH regulator, in addition to its basicity due to the amino group, is because its formation utilises reduced NAD from the breakdown of lactate. The pH rise may also be assisted by the removal of acids. Bacteria of the genus *Veillonella* metabolise lactate to less acidic products. Acids may also diffuse into enamel and thus no longer influence plaque pH.³

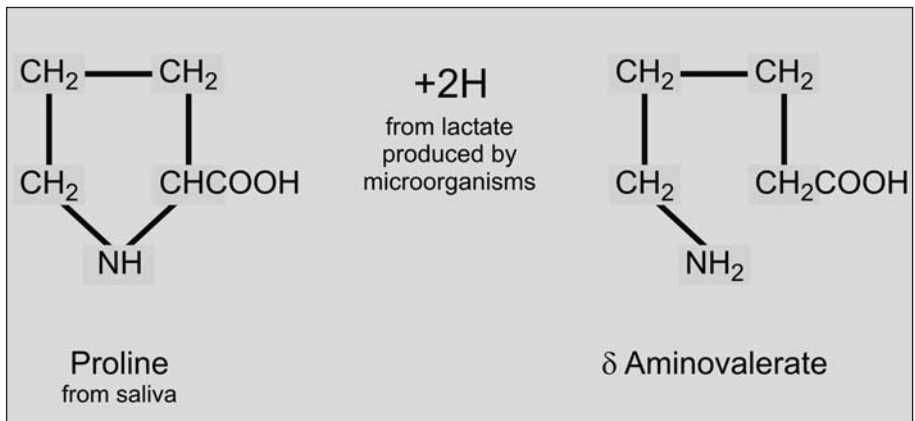


Figure 6.2 The Stickland reaction

Any residual dietary carbohydrate, as well as bacterially stored carbohydrate, may be broken down during the pH rise phase, thus slowing the process. Although the pH approaches the resting value after 30-60 minutes, the organic acid profile does not return to the resting state for several hours.

Maintenance of plaque pH by saliva

Many years ago, researchers compared the Stephan curves produced following a sucrose rinse, with and without salivary restriction. The results showed that by excluding saliva, by cannulating the ducts of the major glands and diverting their secretions out of the mouth, the minimum pH was lower and the return to the resting pH value delayed (*Figure 6.3*).

Regulation of the intraoral pH by saliva can be largely attributed to the neutralising and buffering actions of its bicarbonate content, with smaller contributions from phosphate, and other factors.

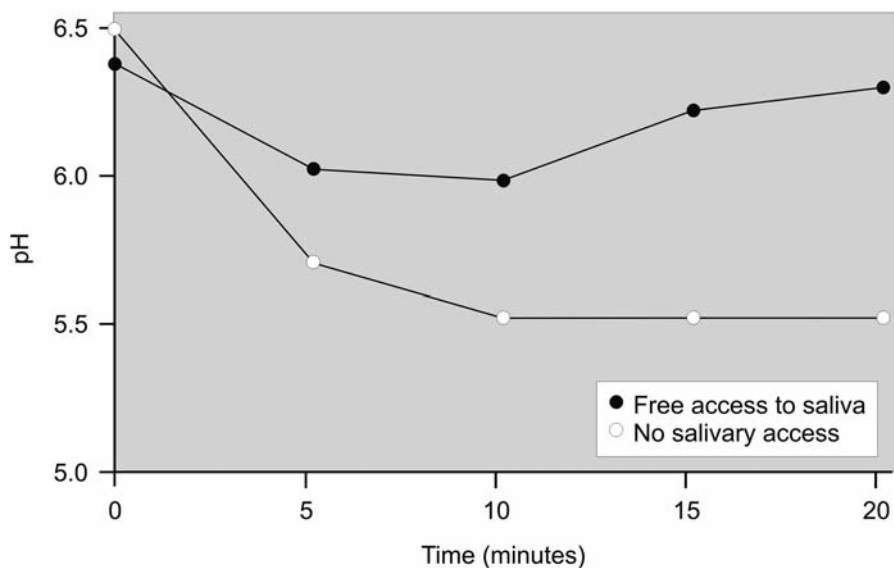


Figure 6.3 The effect of restricting the access of saliva to plaque upon the shape of the Stephan curve (redrawn from G N Jenkins, *The physiology and biochemistry of the mouth (4th Edition)*. Blackwell, London, 1978)

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Bicarbonate

This is the most important buffering system in stimulated saliva. Metabolically-derived bicarbonate increases in concentration with increased salivary gland activity, so that bicarbonate provides an increasingly effective buffer system against plaque acid, especially at high flow rates when concentrations may reach up to 60 mmol/L. The rise in bicarbonate concentration also leads to a rise in salivary pH, which directly neutralises the plaque acidity.

Phosphate

In unstimulated saliva, concentrations of phosphate may peak at around 10 mmol/L, higher than in plasma (see Chapter 3). Despite this relatively high concentration, the buffering due to phosphate is not important in the control of plaque pH, as the pH minimum is below the pK_2 for phosphate (7.2). At higher rates of flow the phosphate concentration falls, and the phosphate buffering system is unimportant in stimulated saliva. The protective role of salivary phosphate is due more to its contribution to the saturation of saliva with respect to enamel mineral (Chapter 8).

Other factors

Saliva contains urea at concentrations slightly lower than those in blood. Many plaque bacteria possess urease activity, converting urea to ammonia, thus raising plaque pH. Saliva also contains peptides, known as 'pH rise factors', which have been suggested to maintain plaque pH. The best-established of these is a basic peptide containing arginine which has been named 'sialin'.⁴ Some bacteria can decarboxylate the amino acids from such peptides to form basic amines. Base production in the form of ammonia and amines is responsible for the fact that the pH of starved plaque is often higher than that of the saliva bathing it.

Urea has been added to chewing gum to increase salivary concentrations and raise the pH of plaque. Salivary concentrations of urea after chewing urea-containing gum have been measured, and their effect on an artificial Stephan Curve evaluated. The beneficial effect of urea was shown to occur only after a sucrose challenge - when the gum was chewed before the challenge there was no reduction in plaque pH fall.⁵

Buffering capacity of plaque

Plaque has intrinsic 'fixed' buffering capacity due mainly to bacterial proteins and other macromolecules in plaque. These fixed buffers are in equilibrium with 'mobile' buffers

– phosphates and bicarbonate – which exchange with those in saliva (Chapter 3). Calcium phosphate crystals are thought to be present even in young plaque and can dissolve under acid conditions to increase greatly the buffering capacity. This can also raise the concentrations of calcium and phosphate ions, and thus help to oppose the demineralisation of the tooth. A negative correlation exists between calcium phosphates in plaque, and caries activity.⁶

Age and site of plaque

The age and site of plaque in the mouth are important considerations in plaque pH studies since they influence the chemical and microbial composition and thickness of plaque, and the access of saliva to the plaque. The age of plaque is usually defined as the time elapsed since plaque was last removed, for example, by professional or by very thorough home tooth-cleaning. This definition is limited, however, because plaque is constantly being disturbed and removed by the action of tongue, lips and cheeks, and by foods. The thickness of plaque is therefore a more rational parameter, although difficult to measure. Thickness affects microbial composition, and the velocity of diffusion of substances through plaque. Thicker plaques are more anaerobic, and so in their inner layers will favour the growth of more strictly anaerobic bacterial species. The rate of penetration of nutrients into, and metabolic products out of plaque will vary inversely with the square of the thickness of plaque, and also with the molecular size and charge of the diffusing substance. Calcium and phosphate levels in plaque increase with time; 10-day-old plaque has about 25% of the mineral content of calculus. Most plaque pH studies have used plaque in subjects who have refrained from oral hygiene procedures for 24 or 48 hours.

It is sometimes suggested that tooth brushing may be more effective before meals, as the residual plaque is too thin to lead to a large drop in pH, and with fluoride dentifrice the metabolism of plaque bacteria will be inhibited. However, the salivary stimulation during eating is known to accelerate the clearance of fluoride from the mouth, and this disadvantage may outweigh the advantages of brushing before meals.

Diet history

The dietary history of plaque is one of the most important factors affecting the Stephan curve. Even a modest restriction of sugar intake for 1-2 days will considerably influence

the shape of the curve. For example, when plaque pH in humans is compared before and after a sequence of sucrose rinses over 3 weeks, there is a decrease in both resting and minimum pH. Many oral bacteria produce extracellular polysaccharides in the presence of excess sucrose. These include glucans which are thought to increase plaque adhesion and thickness, as well as fructans which are subsequently broken down to acid. Some bacteria form intracellular polysaccharide stores, the breakdown of which is an ongoing contribution to acid production in resting plaque.

Plaque pH and salivary clearance

Salivary clearance refers to the dilution and removal of substances from the mouth (Chapter 5). The flow rate of saliva has the greatest influence on the rate of clearance - the faster the flow, the faster the clearance rate. Subjects with rapid clearance rates have a shallow Stephan curve, whereas those whose clearance is slow have deeper curves as lower pH values are reached.⁷

Studies have shown that the labial and upper anterior region is a site of slow clearance, the lingual and lower anterior region a site of rapid clearance, and the buccal area a site of intermediate clearance. The plaque pH in these regions relates well to the rate of clearance. The approximal surfaces of the upper anteriors have the lowest plaque pH, since clearance is slower from these sites. This also relates to the caries prevalence in anterior teeth, being higher in upper than lower approximal surfaces.

The residual volume of saliva after swallowing has been found to be important in determining the clearance rate: the smaller the residual volume, the faster the clearance (Chapter 5). A significant positive correlation between the residual volume of saliva and the caries experience of an individual has been found.⁸

Plaque pH in renal dialysis patients

Children on renal dialysis have high concentrations of ammonia and urea in saliva compared with normal children. In one study it was found that although the children on dialysis ate many sweets, they had a lower caries experience than control children. It is likely that this is due to a direct effect of salivary urea and ammonia on plaque pH, as plaque from these children was capable of forming acid when incubated with sugars after removal from the oral cavity. A study has shown significantly higher plaque pH in children with chronic renal failure compared with controls, in parallel with the raised

salivary urea concentration (mean, 11.6 mmol/L compared with 3.6 mmol/L). In addition, the number of mutans streptococci isolated from the renal patients was significantly lower.⁹

Plaque pH and fluoride levels

Salivary fluoride levels, even in a fluoridated area and with the use of fluoride dentifrice, are quite low, about 0.5-2.0 $\mu\text{mol/L}$ (0.01-0.04 mg/L) although immediately after brushing the salivary concentration derived from dentifrice is much higher (100-200 mg/L) and a small increase (around 0.05 mg/L) is detectable in the saliva of fluoride dentifrice users 18 hours after brushing. These minor changes in salivary fluoride can lead to increased levels of fluoride in plaque. Plaque fluoride concentrations are high for up to 8 hours after a fluoride rinse – i.e. fluoride is retained directly from the rinse and not recycled via saliva. Fluoride levels in plaque are usually 50-100 times higher than those in whole saliva.

Systemic fluorides have only a small effect on plaque acid production, but their effect may be great enough to tip the scales between demineralisation and remineralisation of the tooth enamel. Part of the fluoride in plaque is in bound form, but is released into the plaque fluid when the pH falls. This can be potentially beneficial in favouring remineralisation and modifying subsequent bacterial metabolism.

Topically administered fluorides have antibacterial actions but this is a direct effect and not mediated by saliva. However, fluoride from dentifrices, gels and other vehicles may bind to the soft tissues or precipitate on the tooth surface as calcium fluoride, which then slowly dissolves into the saliva leading to the raised concentrations noted above.

Fluoride-containing chewing gum has been investigated as an anti-caries product for daily use; doses range from 0.1-0.5 mg F as sodium fluoride. Salivary fluoride concentrations are elevated, especially on the chewing side, and may promote increased remineralisation of enamel and dentine.¹⁰ Because of the uncontrolled use of gum by individuals, there is a possibility of over-dosage of fluoride. However, it may be that much lower levels of fluoride in the gum may be sufficient to benefit remineralisation.

Salivary stimulation and plaque pH

The knowledge that saliva is beneficial in terms of plaque pH and buffering following consumption of a cariogenic food has provoked much interest in agents which stimulate

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an increase in salivary flow. Chewing gum has been tested in plaque pH studies, with sugar-free gum producing a rise in plaque pH reflecting the raised pH of stimulated saliva. With a sugar-containing gum, a fall in plaque pH occurred which lasted for 20 minutes (Figure 6.4). The use of chewing gum would seem to have a beneficial effect on plaque pH by stimulating salivary flow, but this effect may be reduced by the presence of fermentable carbohydrates. If sugar-containing gum is chewed after meals or snacks which contain fermentable carbohydrate, it can exert a pH raising effect, but this is smaller than with sugar-free gum (Figure 6.5). Chewing sugar-free gum to stimulate salivation not only raises plaque pH after carbohydrate intakes, but may increase stimulated saliva flow, pH and buffering power, and both resting and post-sucrose plaque pH when chewed vigorously every hour for 2 weeks, suggesting an increase in gland function¹¹.

Chewing an unflavoured material such as paraffin wax following consumption of a fermentable carbohydrate leads to a marked rise in plaque pH, similar to that with sugar-free chewing gum, accompanied by consistent decreases in the plaque concentrations of lactate and acetate anions and increases in many amino acids (Figure 6.6). The rise in pH during chewing was closely associated with the increase in saliva flow and bicarbonate buffering but may also involve other factors including an increased supply of nitrogenous

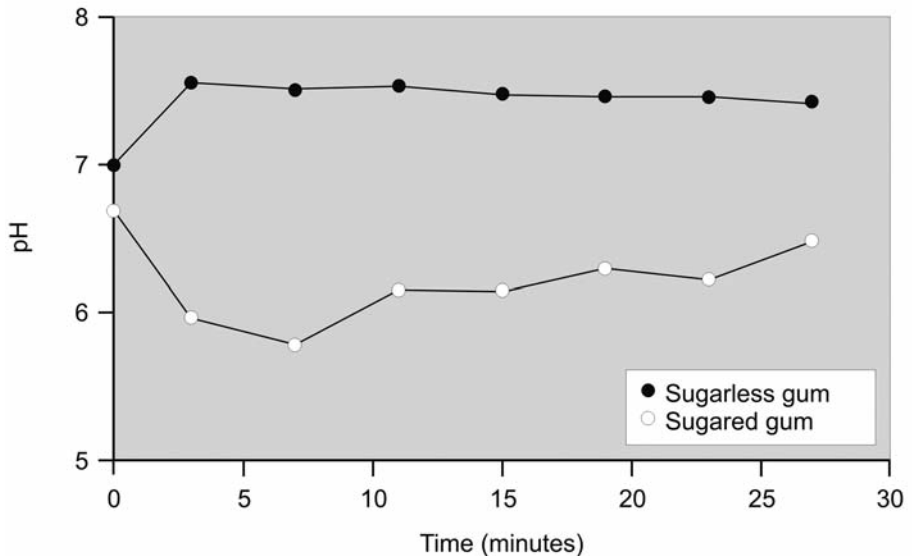


Figure 6.4 Plaque pH responses to chewing sugarless or sugared chewing gum (redrawn from Rugg-Gunn *et al.*, *Br Dent J* 1978; 145: 95-100)

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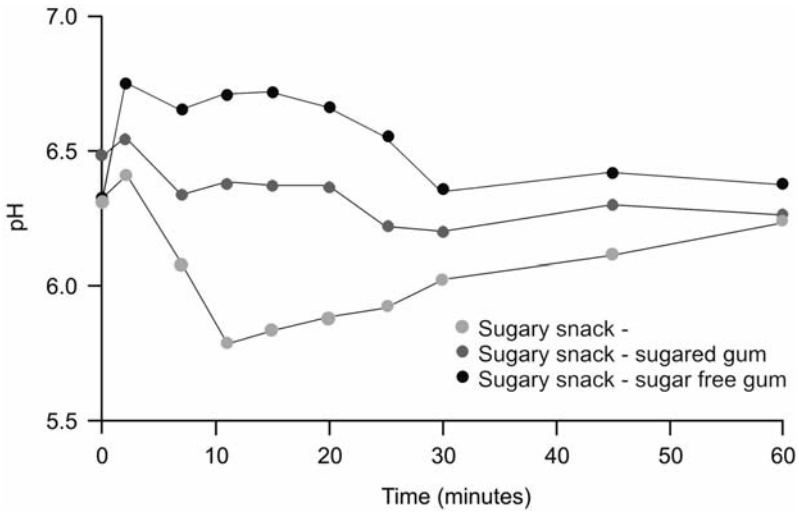


Figure 6.5 Plaque pH responses to a sugary snack alone, and followed by sugared or sugar-free gum (redrawn from Manning and Edgar, *Br Dent J* 1993; 174: 241-244).

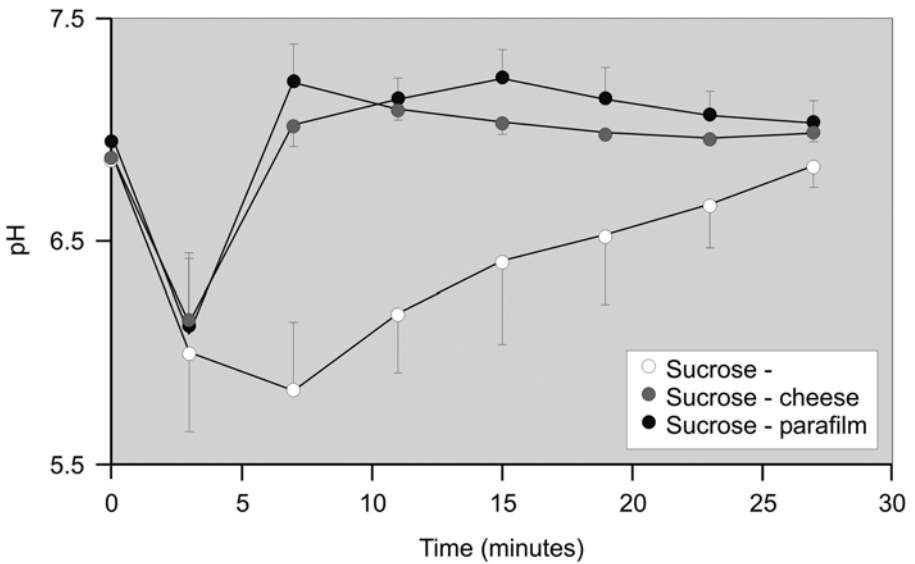


Figure 6.6 Plaque pH responses to a sucrose mouthrinse alone, and followed by paraffin or cheese (redrawn from Higham and Edgar, *Caries Res* 1989; 23: 42-48)

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material for base production. The chewing of cheese, a food rich in nitrogenous substrates, also elicits a rise in plaque pH similar to that with paraffin wax (*Figure 6.6*) despite the pH of the cheese bolus being acidic. Not only is the pH of plaque raised to less damaging levels in terms of enamel demineralisation, but also the plaque fluid concentrations of strong acids fall and those of neutral and basic products rise. It is likely that part of the effect of cheese can be explained by the breakdown of cheese proteins, notably casein, but other factors may be involved including the fact that cheese is a strong sialogogue. Cheese also raises the plaque concentrations of calcium and phosphate.

Similar plaque pH effects have been found with chewing gum containing urea, and urea rinses. Some studies of plaque pH use the technique of chewing paraffin wax or using a urea rinse to bring the plaque pH back up to resting levels after a carbohydrate challenge. *Figure 6.7* shows the effect of a range of sucrose concentrations (0.05-10%) on plaque pH. At 0.05%, there was a small decrease in plaque pH which returned to resting values quite quickly, even without the aid of a paraffin chew. However, at higher sucrose concentrations, the pH only returned to normal after a urea rinse, indicating that continuing sugar metabolism was taking place in the plaque.

These results show that simple measurement of pH does not necessarily indicate the metabolic processes going on in the plaque; just because the plaque pH is 7 does not mean that there is no carbohydrate breakdown occurring in the plaque. This implies that the pH should be monitored over an extended period. Determination of the

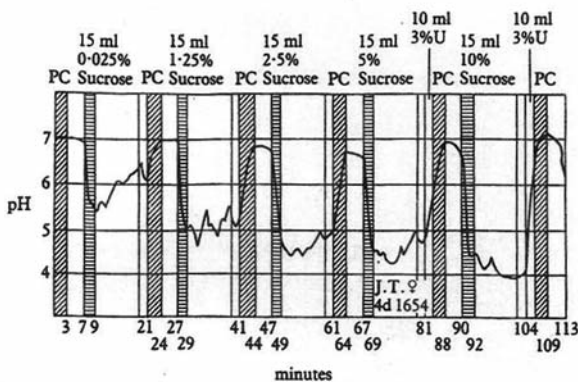


Figure 6.7 Plaque pH recordings from an interdental electrode in a partial denture after rinsing with increasing concentrations of sucrose solution. PC=paraffin chewing; U=urea 3% rinse. (Reproduced from Imfeld, *Schweitz Monats Zahn* 1977; 87: 448)

concentrations of the acid end-products of metabolism gives a more direct indication of plaque activity in the mouth.

Salivary pH can show a fall after a carbohydrate challenge, as well as plaque pH. Although stimulated saliva is normally more alkaline than unstimulated, after some challenges, the formation of acid in the mouth, not only by plaque organisms but also by bacteria on soft tissues e.g. the tongue, can be so rapid that the buffering capacity of the saliva is overcome and the salivary pH can fall to as low as 6.0.¹² This will reduce the protective buffering of plaque by saliva; however, use of a sugar-free salivary stimulant such as chewing gum after such a challenge would restore the protective salivary buffering.

Plaque pH in caries-free and caries-susceptible subjects

Caries-free subjects, or those with minimal caries, tend to have a slightly higher resting plaque pH, a higher minimum pH following consumption of fermentable carbohydrate, and a faster return to resting levels, when compared with caries-susceptible subjects. When saliva is excluded, however, the differences between caries-free and caries-susceptible subjects are less marked and the minimum pH values reached are lower (*Figure 6.8 - overleaf*). These findings indicate the importance of saliva as a factor determining caries susceptibility by modifying the plaque pH response.

Plaque from caries-free subjects produces more base than plaque from caries-susceptible subjects: these bases include polyamines and ammonia. The levels of free arginine and free lysine in stimulated parotid saliva from caries-free adults have been found to be significantly higher than those from subjects with caries experience.¹³ Recently, urease activity in plaque (but not saliva) has been shown to be negatively associated with caries status in cross-sectional¹⁴ and prospective studies.¹⁵

Clinical implications of salivary stimulation

There is a wealth of evidence supporting the benefits of stimulating saliva following eating to enhance its protective role in dental health. In recent years the use of chewing gum as a salivary stimulant has received much attention since it has been shown to produce a continued flow of saliva during prolonged mastication.

The use of sugar-free chewing gum has been found to be particularly valuable.¹⁶ Clinical evidence suggests that these products are non-cariogenic, and that when they are chewed

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after meals they may reduce the cariogenic effects of other foods. Sugar-free gum can be chewed for a prolonged period without increasing the calorific content of the diet. Using an intra-oral caries model system, artificial enamel lesions were remineralised more effectively when subjects chewed sugar-free gum after meals and snacks than in controls who did not chew gum. Clinical trials of the use of sorbitol-sweetened gum after meals and snacks have revealed reductions in caries incidence of between 10 and 40%.

In most clinical caries studies, gum sweetened with xylitol has produced greater reductions in caries incidence than sorbitol-sweetened gum, although this is not a universal finding. While part of the beneficial effect of xylitol gum can be attributed to salivary stimulation, its superiority over sorbitol gum found in most studies has been attributed to the antibacterial effects of xylitol, especially on mutans streptococci. After two weeks of gum chewing, the plaque pH response to sucrose has been shown to be smaller with xylitol gum than with sorbitol gum. Maternal use of xylitol gum was associated with reduced caries incidence in their offspring during their first five years

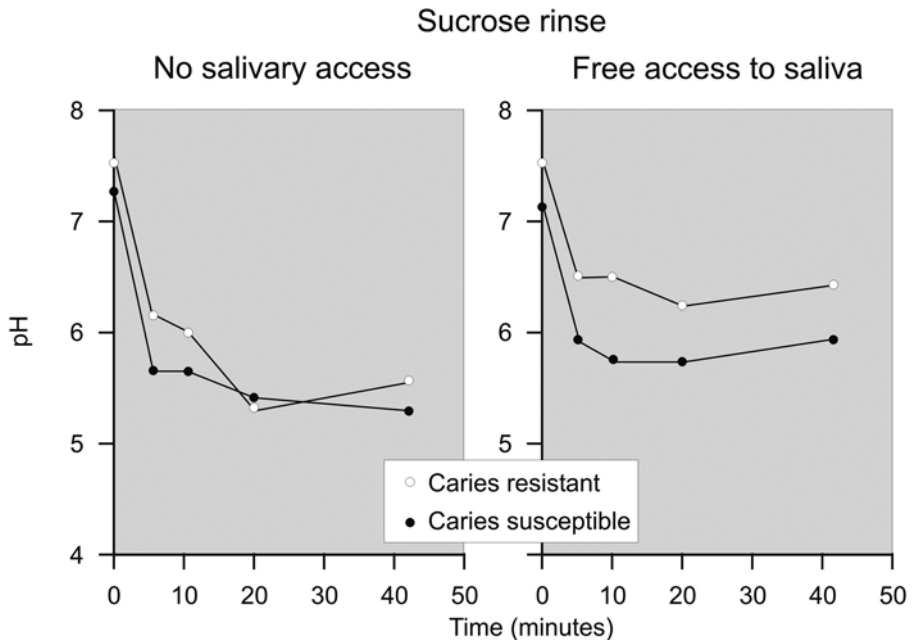


Figure 6.8 Stephan curves from caries-free and caries-susceptible subjects when saliva is excluded or not excluded. (Redrawn from Abelson and Mandel, *J Dent Res* 1981; 60: 1634-1638)

of life; this effect cannot be attributed to salivary stimulation and was associated with reduced mutans streptococcal infection of the neonates by their mothers.¹⁷

Plaque pH and salivary gland hypofunction

Patients suffering from xerostomia due to salivary gland hypofunction are often recommended to chew sugar-free gum, partly to relieve their symptoms but also to stimulate the function of the residual active secretory tissue (Chapter 4). Studies have shown that subjects with salivary hypofunction could still benefit from chewing sorbitol-sweetened gum after a 10% sucrose challenge through the protective effects of the saliva produced by stimulation of their residual gland function.¹⁸

The protective effects of saliva can be clearly observed when comparisons are made between subjects with normal and those with low salivary secretion rates, following consumption of fermentable carbohydrates. A low salivary flow rate accentuates the pH decrease in dental plaque (*Figure 6.9*).

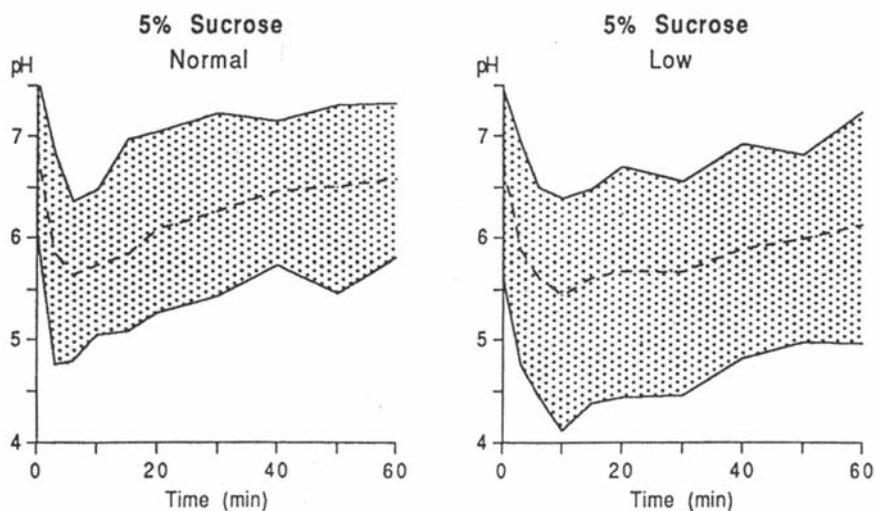


Figure 6.9 Individual variations in plaque pH in subjects with normal and low salivary secretion rates after sucrose mouthrinsing. Shaded areas represent inter-subject variation. (Reproduced from Lingström and Birkhed, *Acta Odont Scand* 1993; 51: 379-388)

Summary – clinical highlights

1. Plaque pH is a major factor controlling the equilibrium between demineralisation and remineralisation of the teeth, the balance of which determines the progression or repair of initial caries.
2. Plaque pH reflects the balance between the production of acids (mainly from dietary carbohydrates) and bases (mainly from salivary urea and amino acids).
3. Caries susceptibility and plaque pH at different sites around the mouth are related – the higher the pH, the lower the susceptibility.
4. Caries-free individuals have a higher plaque pH; this appears to be a result of more active base production from salivary substrates, as well as a less-acidogenic plaque flora.
5. Continued stimulation of saliva following a meal or snack, e.g. by chewing sugar-free gum, raises plaque pH, increases enamel remineralisation, and reduces caries incidence.
6. Patients with salivary gland hypofunction, provided they retain some secretory function, may benefit from saliva stimulation by chewing sugar-free gum.
7. Understanding by patients of the significance of the Stephan curve, and of ways by which it can be controlled, helps them to know how best to look after their teeth.

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Protective functions of saliva

Eva J. Helmerhorst

Human saliva not only lubricates oral tissues, making oral functions such as swallowing and speaking possible, but it also protects teeth and mucosal surfaces in many ways. The main protective factor is the constant flow of saliva from the mouth into the gut, and this 'flushing effect' transfers, for example, food debris as well as many endogenous and exogenous agents into the gut. These comprise both oral and exogenous, often noxious, micro-organisms. Some of the exogenous bacteria and viruses as well as food-borne mutagens are detoxified or killed by innate components of human saliva. Also, some members of the commensal oral microflora and many of its harmful metabolic products are inhibited or neutralised by salivary components. Thus a proper amount of saliva, with its antimicrobial agents, is a requirement for a healthy balance between host defence and microbial attack in the human mouth.¹

Micro-organisms in whole saliva

The human mouth is almost perfect for bacterial growth because bacteria appreciate its temperature, its humidity, the large surface areas for attachment, and various nutrients (growth-stimulating effects) presented through saliva. This perfect environment is made possible by the constant presence of saliva, which dissolves the nutrients and also provides some substances, for example amino acids and endogenous carbohydrates, for bacterial growth.

Saliva is sterile when it enters the oral cavity through the glandular excretory ducts. Once released into the mouth, mixing of the glandular secretions occurs with the non-exocrine components in saliva, together forming what is called 'whole saliva'. Whole saliva is a non-sterile fluid and contains a wide variety of micro-organisms. Commensal micro-organisms as well as epithelial cells are shed from various oral surfaces where endogenous bacteria are attached and where they multiply to be detached into whole saliva, for example during chewing. The major sites of origin are tooth surfaces (dental

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plaque, oral biofilms), tongue surface and tonsils. However, micro-organisms exist over the entire intraoral surface area. Gingival, periodontal, tonsillar or mucosal inflammations may increase both the number of species and the total number of salivary micro-organisms remarkably. It must be emphasised that while micro-organisms may multiply in saliva, the salivary flow rate is higher than oral bacterial doubling times, such that newly formed cells are removed by swallowing more quickly than they are generated by cell division. Therefore, the salivary microflora is primarily a reflection of the overall composition of the attached microflora in the mouth. Considering the multitude of microbial species, the large surface areas and other growth-supporting properties in this saliva-moistened environment, it is not surprising that the number of micro-organisms in whole saliva is high. It has been estimated that each millilitre of whole saliva (oral fluid) contains 10^8 - 10^9 micro-organisms and the amount of bacteria swallowed per day is in the range of 1-3 grams! Based on these facts, it is very understandable that clearance of bacteria from the mouth into the gut is essential to prevent microbial overgrowth in the mouth – which often exists in cases of hyposalivation. In a healthy situation a dynamic equilibrium exists between oral micro-organisms and us. It has been estimated that oral bacteria multiply once in approximately 3-4 hours, which emphasises the need of salivary clearance of microbial cells into the gut (see Chapter 5).

The micro-organisms harboured in the oral cavity have been characterised by culture-dependent approaches and more recently by culture-independent molecular methods based on 16S rDNA sequencing. The oral microbiome was the first human body-associated microbiome to be characterised in great detail. More than 800 different oral taxa belonging to 13 different phyla have been identified. Over 92% of these taxa group into five phyla: *Firmicutes* (42%), *Proteobacteria* (20%), *Bacteroidetes* (13%), *Actinobacteria* (11%) and *Spirochaetes* (6%). The numbers of taxa that account for 90%, 95% and 99% of microbial biomass in the oral cavity are approximately 259, 413 and 875 taxa, respectively. Streptococci are the most abundant species in the mouth, followed by members of the genera *Abiotrophia*, *Gemella* and *Granulicatella*.² Besides microbe identification, the 16S rDNA technology has also provided new ways of microbe visualisation through Fluorescence In Situ Hybridization (FISH). Bacteria in typical brush-like structures, previously observed in dental plaque, have now been speciated. In these organised aggregates bacteria from the *Cytophaga-Flabobacterium-Bacteroides* (CFB) cluster (most likely *T. forsythia*) and *F. nucleatum* are arranged perpendicularly around lactobacilli, forming the fine test tube brush-like appearances³ (Figure 7.1). In dispersed dental plaque, species of *Veilonella*, *Prevotella* and *Actinomyces* are most frequently engaged in interactions with other species.⁴ Defining bacterial interactions and unravelling the biofilm architecture at the species level are important

steps forward allowing the design of strategies to interfere specifically with such associations as a means of plaque control. In this context, it must be noted that bacterial colonisation is not necessarily disadvantageous to the host when it concerns commensal microbes that in fact may prevent, by occupying docking sites, colonisation with more harmful bacteria, a process known as colonisation resistance.

Transmission of micro-organisms by saliva contacts

The first micro-organisms colonise infants' oral cavities immediately after delivery and the quality of flora during the first days of life is rather similar to that of the mother's vaginal flora. However, increasing exposure to external sources widens the number of species and increases the total quantity of oral micro-organisms - of course, depending on the available species. The most important source of transmission into the baby's

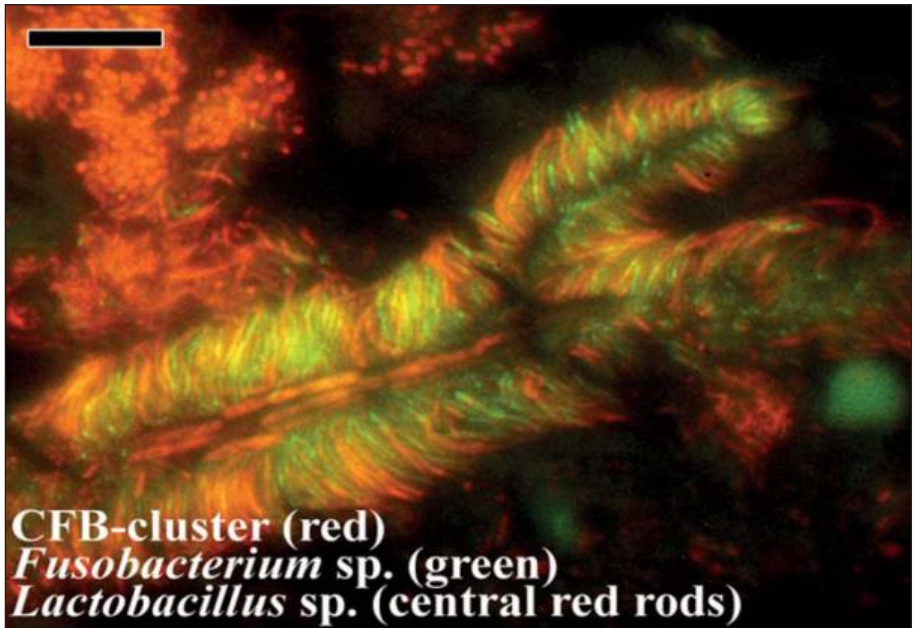


Figure 7.1 Identification of bacterial species in aggregates in dental plaque. The 'test tube brush' structure contains *Lactobacillus* sp. (red rods, in centre), *F. nucleatum* (green) and filamentous bacteria belonging to the CFB cluster. Bar = 10 μm . Adapted from Zijngel *et al.*, 2010³

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mouth is his/her mother. There are a number of ways by which mothers (and also other family members) can supply new micro-organisms into the infant's developing oral flora. These include e.g. 'cleaning' the pacifier (dummy) in the mother's own mouth before giving it to the baby (*Figure 7.2*), tasting the food in her own mouth before feeding the baby, and kissing the baby on the lips. It would be of no concern if the mother's own (oral) health is qualitatively good and she does not carry abundant cariogenic micro-organisms in her own saliva.

A typical example of this salivary transmission of cariogenic species is the group of mutans streptococci. Mothers with high salivary counts of mutans streptococci often infect their children's dentitions via salivary contacts by the age of 1-2 years and reliable scientific evidence shows that the earlier and the more abundant the transmission of mutans streptococci to the baby's dentition, the higher is the caries incidence in later childhood. This information is clinically highly relevant, since chair-side test methods can be used to screen mothers (or other family members/caretakers) with high salivary counts of mutans streptococci. High numbers can be reduced temporarily with e.g.



Figure 7.2 A pacifier (dummy) that has been contaminated by saliva for 10 seconds by a mother whose salivary counts of mutans streptococci are high ($>10^6$ CFU/ml). The dummy has been incubated in a selective growth medium for 3 days to show the adsorbed colonies of mutans streptococci

chlorhexidine or xylitol to prevent, or at least delay and diminish, the salivary transmission of these harmful bacteria from mothers to their babies.

Strong evidence also exists that periodontal pathogens, mainly anaerobic bacteria, are transferred via salivary contacts from person-to-person, often already by preschool age. More familiar infections from saliva are, for example, viruses – such as herpes simplex type 1, Epstein-Barr virus ('kissing disease') and influenza viruses.

Growth of bacteria in saliva

Saliva not only inhibits but also selectively supports the growth of certain bacterial species. This has been proven by studies of humans (and animals) who have received their nutrition by stomach tube (gavage) and they still harbour large numbers of micro-organisms in their mouth. This is mainly due to the presence of saliva-borne glycoproteins, which provide carbohydrates, proteins and amino acids for bacteria. However, the microbial flora in these cases is usually low in lactobacilli, mutans streptococci and yeasts but their numbers increase as soon as oral intake of fermentable sugars is frequent.

In the salivary ecosystem the micro-organisms first metabolise glycoprotein-derived carbohydrates and later the proteins. Individual species grow in different types of saliva but in most cases submandibular/sublingual secretions rich in mucins are the best growth medium. To be able to grow in saliva, bacteria need to produce glycosidases and/or proteases to get nutrients to survive without external supply. Mucins are mucoglycoproteins containing protein cores with a number of attached oligosaccharide side chains. There are two mucins in saliva,⁵ high molecular weight mucin glycoprotein 1 (MUC5B, previously designated MG1) and lower molecular weight mucin glycoprotein 2 (MUC7, formerly MG2). They are products of different cell populations within the submandibular, sublingual and minor salivary glands. Among salivary streptococci there is large variation in their ability to grow in the presence of mucins (*Figure 7.3 - overleaf*) and this difference is related to their capacity to hydrolyse oligosaccharide side chains by glycosidases. These enzymes are seldom found in mutans streptococci. Because mucins form an integral part of biofilms on tooth surfaces, the relative proportion of various streptococci reflects the amount of mucins in salivary pellicles, i.e. early colonisers – such as *S. oralis* (formerly *S. mitior*), *S. mitis* and *S. sanguinis* – are dominant in early dental plaque rich in mucins. Saliva alone, without an external source of fermentable sugars, selects for a non-cariogenic microflora with low levels of mutans streptococci.

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S. mutans and *S. sanguinis* both possess proteolytic activity which enables them to use the nitrogen sources in saliva (see Chapter 6). Urea and free amino acids in saliva may serve as substrates for ammonia production by salivary micro-organisms; ammonia is the major base in dental plaque. *S. sanguinis* can also digest arginine peptides to release arginine, which is a good source of ammonia production in the vicinity of tooth surfaces. Thus, salivary energy sources for different bacteria produce different components, which partly determine the microbial composition of oral biofilms.

Salivary components in oral biofilms

In the mouth, salivary glycoproteins are found either as a mucosal film, or on the tooth surfaces as the acquired enamel pellicle. The acquired enamel pellicle is a cell-free proteinaceous layer formed immediately after tooth enamel is exposed to saliva⁶. The adsorption of salivary proteins onto enamel is a specific process; the composition of pellicle differs significantly from the overall protein composition of glandular secretions or whole saliva⁷. Pellicle precursor proteins primarily constitute phosphorylated salivary proteins with demonstrated high affinity for hydroxyapatite, i.e. acidic proline-rich

Growth of various streptococci in mucin as sole source of carbohydrate

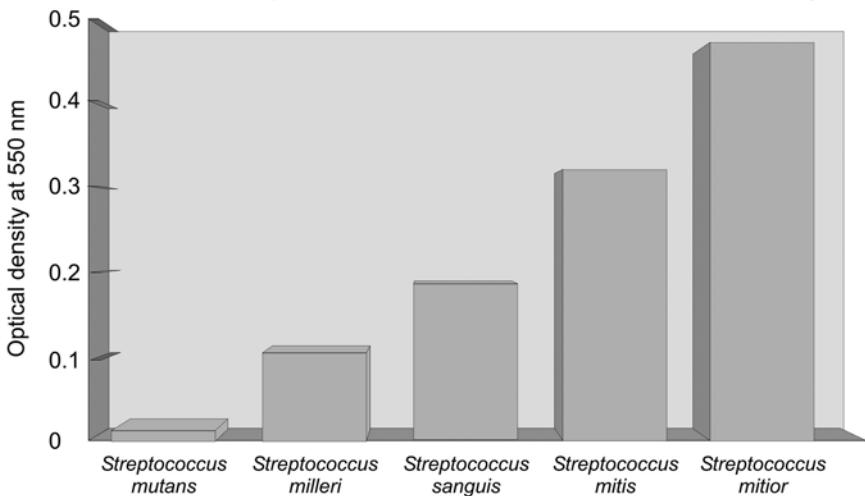


Figure 7.3 Growth of various oral streptococci in mucin as a sole source of carbohydrate

proteins, statherin, histatin 1 and cystatin. Besides phosphoproteins, dental pellicle also contains glycosylated proteins such as mucins, amylase, and sIgA.^{8,9} Protein compositional analysis of dental pellicle has been hampered by the small amounts of this integument that can be harvested from the tooth surface. One of the techniques to collect pellicle uses PVDF membranes soaked in sodium bicarbonate buffer (*Figure 7.4*). With the advent of mass spectrometry it has become feasible to identify proteins at sub- μg levels and to simultaneously identify proteins in complex mixtures, such as in oral specimens.^{7,10} Shotgun proteomics approaches have catalogued over 2000 proteins in human saliva, with 20% representing cytoplasmic cell-derived components¹¹. Substantially fewer proteins, 130, have been documented in the acquired enamel pellicle¹², consistent with the selective adsorption concept.

The pellicle and the attached biofilm are dynamic structures. Attachment, growth, removal and reattachment of bacteria may occur at the same time. Because of its significant content of salivary glycoproteins and its rapid rate of formation on cleaned tooth surfaces, pellicle can be considered as a renewable lubricant, which helps to protect the teeth from attrition and abrasion. In addition, protective functions of pellicle pertain to maintenance of mineral homeostasis by protection of demineralisation and facilitation of remineralisation. Indeed, acidic PRPs, as well as lipocalin, cystatin SN and cystatin S are more abundant in dental pellicle obtained from caries-free subjects than in pellicle from individuals with high levels of decayed, missing or filled teeth (DMFT). On the other hand, amylase, immunoglobulin A and lactoferrin are correlated

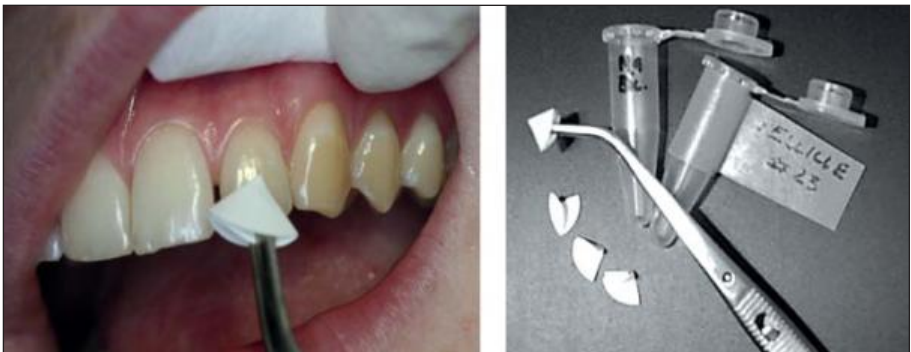


Figure 7.4 Collection of acquired enamel pellicle formed *in vivo*. Tooth surfaces were cleaned with a rubber cup and fine grade pumice. Subjects were asked to refrain from eating and drinking (except water) for 2 h. Pellicles formed during this incubation time period were subsequently harvested by swabbing the tooth surfaces with PVDF membranes that had been soaked in bicarbonate buffer. Adapted from Yao *et al.*, 2001.¹⁰

with high DMFT levels.¹³ Relationships between oral disease and proteins in the dental pellicle may be explained by the direct protective functions of pellicle proteins towards enamel. Alternatively, or additionally, the oral disease relationship can be attributed to differences in the levels of cariogenic bacteria that are attached to the pellicle structure. Pellicle serves as a layer to which oral bacteria bind. As such, pellicle influences early bacterial colonisation of the tooth surface and may even determine the composition of the ultimate bacterial biofilm formed. Thus, dental pellicle may dictate, to some extent, the composition of dental plaque. This interesting concept has not been proven *in vivo* yet, but with the available proteomics and 16S rDNA technologies it is anticipated that pellicle signatures will be identified that are associated with ‘good’ or ‘bad’ biofilms.

Biological activities of salivary proteins

The salivary protein concentration is rather low, only approximately 2 mg/ml as compared with about 70 mg/ml in human serum. Many salivary proteins have important antimicrobial, lubricative and digestive functions. They are also involved in modulating microbial colonisation of teeth and soft tissues, in providing a barrier against entry of exogenous toxins, even carcinogens, through the oral mucosa, and in modulating salivary calcium phosphate chemistry. The latter function is important for maintaining salivary supersaturation, i.e. maintaining the integrity of the tooth surfaces and also for preventing adventitious calcifications in the salivary glands and in the mouth (see Chapter 8).

As stated previously, salivary proteins also participate in the formation of the acquired pellicle, which is not only protective but also influences the initial microbial colonisation on the teeth. Base production from basic amino acids and peptides in saliva may help to neutralise plaque acids. Collectively, salivary proteins display a wide variety of functional activities (*Table 7.1*), which help to maintain the integrity of the mouth and also provide protection against oral and non-oral microbial infections.¹⁴

Protective components and systems in saliva

The main protective factor against noxious agents, endogenous and exogenous, is the salivary flow rate. Constant flow of saliva flushes untoward components from the mouth into the gut, i.e. clears away excess components and cells which are not able to attach to oral surfaces (see Chapter 5). This clearance is enhanced by salivary agglutinins³,

Table 7.1: Salivary protein functions in the oral cavity (modified from Bowen, 1996; 2nd edition of this book)

Oral function/activity	Associated problem	Protein function
Acts as an airway	Air-borne micro-organisms Dehydration	Antimicrobial systems Water-retaining glycoproteins
Entry for food	Food-borne micro-organisms Food toxins Soft & hard tissue abrasion	Antimicrobial systems Antimicrobial systems Lubrication, mucins
Speech & swallowing	Need for lubrication	Lubrication systems, mucins
Taste & digestion	–	Gustin, α -amylase, lipases
Control of endogenous and exogenous micro-organisms	Colonisation & infection Controlling pathogens and commensals	Innate antimicrobial systems Salivary immunoglobulins
Protection of soft tissues	Toxins, carcinogens, degradative proteases	Mucin-rich protective barrier Cystatins
Protection of hard tissues	Acid damage	Inorganic compounds, fluoride, Statherin, PRPs, pellicle
Plaque acid production	Plaque pH control	Basic amino acids, urea & peptides. Buffer effect

which are glycoproteins with a capacity to clump bacteria into large aggregates, which may be more easily flushed away by saliva and swallowed. Therefore, the term aggregation is often used synonymously with agglutination. The most potent agglutinin is a high molecular weight glycoprotein,¹⁵ which has been found in human saliva secreted from all major salivary glands: as little as 0.1 μg of it can agglutinate 10^8 - 10^9 bacteria. Other known salivary agglutinins are mucins (particularly MUC5B), fibronectin and β_2 -microglobulin.

Major antimicrobial proteins in human saliva are listed in *Table 7.2 (overleaf)*. These are usually divided into non-immune (innate) and immune (acquired) factors, the latter representing antigen-stimulated immunoglobulins. Four major types of interactions exist between salivary antimicrobial agents and oral micro-organisms. These include agglutination, inhibition of adherence, bacteriostatic or bacteriocidal activity, and interference with nutrition. Although much is known of the functions of the antimicrobial proteins *in vitro*, rather limited information of their possible clinical relevance exists¹⁶. It seems, however, that they are important for the control of microbial

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Table 7.2: Major antimicrobial proteins in human whole saliva

Protein	Major target or function
Non-immunoglobulin proteins	
Lysozyme Lactoferrin Peroxidases Agglutinins Amylase Histidine-rich proteins (histatins) Cystatins	Gram-positive bacteria, Candida yeasts Gram-positive and -negative bacteria Bacteria, viruses, yeasts, decomposition of H ₂ O ₂ Oral bacteria Clearance of starch Antibacterial, antifungal Antiviral
Immunoglobulins	
Secretory IgA IgG IgM	Inhibition of adhesion Enhancement of phagocytosis Enhancement of phagocytosis (?)

overgrowth in the mouth but how selective they are against pathogens is not yet fully understood.

Lysozyme

Lysozyme is secreted into whole saliva from major and minor salivary glands, gingival crevicular fluid and salivary leukocytes. Lysozyme is present in saliva of newborn babies at levels equal to those of adults and can thus exert antimicrobial functions before tooth emergence. The classical concept of lysozyme action is based on its muramidase activity, i.e. the ability to hydrolyse the $\beta(1-4)$ bond between *N*-acetylmuramic acid and *N*-acetylglucosamine in the peptidoglycan layer of the bacterial cell wall. Gram-negative bacteria are more resistant to lysozyme because of their protective outer lipopolysaccharide layer.¹⁷ Gram-positive bacteria, such as mutans streptococci, may be protected by cell-produced extracellular polysaccharides. In addition to the muramidase activity, lysozyme is a strongly cationic protein which can activate bacterial autolysins - ‘suicide packages’, which can destroy the bacterial cell walls. Salivary lysozyme concentration is not related to caries incidence or prevalence.

Lactoferrin

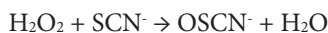
Lactoferrin is a non-enzymatic glycoprotein which is secreted by major and minor salivary glands. Oral leukocytes also release lactoferrin into whole saliva. The biological activity of lactoferrin is attributed to its high affinity for iron (Fe³⁺) and the consequent

deprivation of this essential metal from pathogenic micro-organisms. This leads to a bacteriostatic effect which is lost if the lactoferrin molecule is saturated with iron. In its iron-free state, lactoferrin (called apo-lactoferrin) also has a bactericidal, irreversible effect against a number of oral bacteria. This killing effect requires direct binding of apo-lactoferrin to bacteria and it is not blocked by excess iron. There is evidence that both partly iron-saturated and iron-free forms of lactoferrin may exist simultaneously in human saliva. Recently, it has been demonstrated that within the lactoferrin molecule there are antimicrobial domains (called lactoferricins) which may be released by host or microbial proteases. Furthermore, fragments inhibiting adherence of *S. mutans* to saliva-coated hydroxyapatite have been identified. It is likely that the lactoferricin domains are released also during digestion of lactoferrin in the gastro-intestinal tract. This supports the idea that salivary proteins can also be involved in the antimicrobial protection of the upper gastro-intestinal tract.¹⁸ Lactoferrin has bacteriostatic, bactericidal, fungicidal, antiviral and anti-inflammatory activity.

Peroxidases

There are two different peroxidase enzymes in whole saliva: salivary peroxidase (sometimes called sialoperoxidase) and myeloperoxidase. The former is secreted by the parotid and submandibular glands; the latter is a leukocyte-derived protein entering the mouth mainly via gingival crevices. In whole saliva the proportion of myeloperoxidase of the total peroxidase activity varies from 30 to 75 % depending on the extent of inflamed sites in periodontal and mucosal tissues.¹⁹

Both enzymes catalyse the oxidation of salivary thiocyanate ions (SCN^-) by H_2O_2 to the antimicrobial component, hypothiocyanite (OSCN^-).²⁰



At $\text{pH} < 6.0$, hypothiocyanous acid (HOSCN) is the main form of the oxidation and it is an even more powerful agent against micro-organisms than the ionic form. $\text{OSCN}^-/\text{HOSCN}$ is a normal component of human whole saliva and plaque fluid and in predentate individuals it exists already at adult concentrations.

Salivary peroxidases have two major functions: antimicrobial activity by $\text{OSCN}^-/\text{HOSCN}$ and protection of host proteins and cells from the toxicity of H_2O_2 . Peroxidase systems display antimicrobial activity against a variety of micro-organisms, such as mutans streptococci (*Figure 7.5 - overleaf*), lactobacilli, yeasts, many anaerobes (periodontal pathogens) and even some viruses (herpes simplex type 1, human immunodeficiency virus). Of course, these activities depend on the concentration of

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OSCN⁻/HOSCN, pH and exposure time. In the human mouth, the antimetabolic activity is likely to be more important than the bactericidal effect since increasing concentrations of hypothiocyanite are known to decrease the acid production by dental plaque after stimulation by sugars. Interestingly, if SCN⁻ ions are replaced by I⁻ ions (or I⁻ ions are in large excess), the peroxidase-I-H₂O₂ system is much more powerful against oral and gastrointestinal anaerobes (such as *Helicobacter pylori*) than is hypothiocyanite.²¹ Because H₂O₂ is constantly generated in the mouth by aerobic bacteria and H₂O₂ as such is toxic to mucosal and gingival cells,²² saliva provides peroxidases to consume H₂O₂ by peroxidation. Also, bacterial catalase enzymes can destroy excess H₂O₂.

α -Amylase

α -Amylase is the most abundant salivary enzyme, accounting for approximately 40-50 % of the total salivary-gland-produced protein. Amylase mainly (80%) originates from the parotid glands, the rest from the submandibular glands. The biological role of amylase is to split starch into maltose, maltotriose and dextrans. Maltose can be further fermented by oral bacteria to glucose. Salivary amylase clears food debris (containing starch) from the mouth. It also interacts with specific oral bacteria, obviously to some extent modulating their adhesion to pellicles. Swallowed salivary α -amylase is

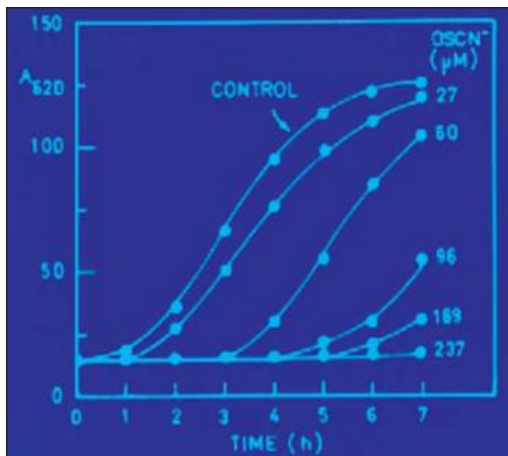


Figure 7.5 Growth curves of *Streptococcus mutans* in the presence of increasing concentrations of salivary peroxidase-generated hypothiocyanite (OSCN⁻) ions at pH 6.5. The normal salivary values of hypothiocyanite range from 10 to 50 μM

inactivated in the acidic environment of the stomach while pancreatic amylase completes the degradation of starch after the acid gastric juice has been neutralised by the bicarbonate secreted by the pancreas.²³

Histatins

Histatins are a group of histidine-rich proteins secreted by the parotid and submandibular glands. The most abundant histatin family members are histatins 1, 3, and 5, the latter arising from post-translational processing of histatin 3. Histatins exhibit strong *in vitro* killing and growth inhibitory activities towards *C. albicans*, an opportunistic oral fungus.²⁷ Furthermore, due to the high histidine content, histatins are capable of complexing copper and zinc ions but a clear biological function for this property has not yet been assigned. More recently it was discovered that histatins can promote cell migration suggesting they contribute to the rapid wound healing observed in the oral cavity.²⁸

Cystatins

Cysteine-containing phosphoproteins, cystatins, are ubiquitous, being present in a wide range of body fluids and tissues.^{29,30} They are considered protective by inhibiting untoward proteolysis. Of many isoforms of cystatins, cystatin C displays the most avid inhibition of proteases. In the mouth cystatins are present both in saliva and in salivary pellicle where they may selectively inhibit proteases originating from bacteria and/or leukocytes. As with PRPs, cystatins also show multifunctional properties such as antiviral and -bacterial activity.

Salivary immunoglobulins

Salivary antibodies to oral or orally transmitted micro-organisms are induced by the common mucosal immune system, which produces protective antibodies on various mucosal surfaces, including the oral cavity (*Figure 7.6 - overleaf*). The gut-associated lymphoid tissue (GALT) is important since it contains precursor IgA B-lymphocytes with a potential to populate mucosal tissues. Orally administered antigen stimulation induces migration of these IgA-committed B-cells to glandular epithelia, e.g. in mammary, lacrimal and salivary glands (where they mature to IgA-producing plasma cells) and this is how IgA-antibodies appear in human whole saliva.³¹ Small amounts of salivary IgA are also produced in minor salivary glands.

Salivary IgA antibodies are dimeric molecules linked together by a joining carbohydrate chain (J-chain). The complex also comprises a small glycoprotein, called 'secretory component' (= sIgA), which makes the molecule more resistant to proteases in the oral environment than single-molecule serum IgA.

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Salivary sIgA antibodies to mucosal bacteria, such as *Escherichia coli*, *S. mitis* and *S. salivarius*, begin to appear as early as the first weeks of life and approach adult levels by 1 to 2 years of age. The appearance of new antibodies and the overall increase in salivary sIgA levels parallel quite well with the colonisation of the oral cavity by the respective micro-organisms. For example, a great majority of children over 3 years have already developed antibodies reactive with mutans streptococci.

Although children gradually develop salivary sIgA antibodies against mutans streptococci, little evidence exists that these naturally evoked IgA antibodies really protect against dental caries.¹⁴ These surprising results may be explained by differences in study populations, sample collections and assays and variable degrees of binding of antibodies by oral bacteria. Further evidence that naturally occurring sIgA antibodies

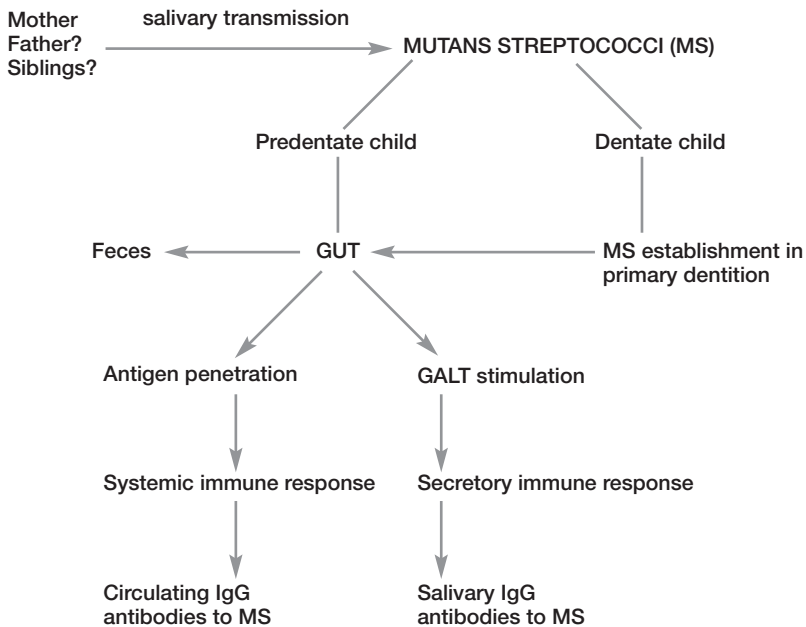


Figure 7.6 Proposed scheme for the development of specific salivary and serum antibodies against mutans streptococci in children. After intrafamilial transmission, swallowed streptococcal cells induce low serum IgG response due to antigens penetrating immature gut mucosa. During mucosal maturation a higher proportion of swallowed antigens stimulate gut-associated lymphoid tissue (GALT), which generates IgA-committed B-lymphocytes in exocrine glands, such as salivary glands

do not protect against dental caries comes from studies with IgA-deficient subjects (frequency 1:300 to 1:3000 in different populations). There is no strong evidence that these persons are more prone to dental caries (or other oral infections) than those with normal levels of sIgA but this may be due to the fact that most of these subjects compensate for the hereditary lack of IgA with increased levels of IgM and, particularly, with enhanced non-immunoglobulin defense.³²

Human whole saliva also contains serum-derived antibodies, mainly IgG but also some IgM. These antibodies leak into the oral cavity via gingival crevices and also to some extent through the surface of the tongue. Serum antibodies are often specific against oral pathogens, such as mutans streptococci, but again, no clear-cut evidence of their protective role against dental caries exists.

Vaccines against dental caries

Three major approaches to develop a vaccine against dental caries have been explored. First, one line of research is focusing on enhancing the common mucosal immune system to produce a high amount of MALT (mucosal-associated lymphoid tissue)-derived sIgA antibodies against mutans streptococci in whole saliva.³¹ This enhancement is done by application of high numbers of *S. mutans* antigens in a short time, intra-orally or -nasally, to stimulate the MALT-system. Also, delivery of antigens in liposomes or co-administered with mucosal adjuvants has been used to enhance the antibody response and related immunological memory. Animal studies have clearly proven some protection against colonisation and re-colonisation with mutans streptococci as well as some reduction of caries incidence. However, human studies have been only short-term and although effects on bacterial numbers have been observed, no evidence yet exists of a caries preventive effect.

Another approach under active investigation is so-called passive immunisation where antibodies are produced outside the body, then enriched and applied to the dentition to protect from mutans streptococci. As 'antibody-fermenters', cow's milk³³ and hen's eggs have proven suitable but also genetic engineering of human-like sIgA antibodies in plants³⁴ has proven effective. However, no long-term clinical trials of the possible protective effects of these passively administered antibodies have been published. The third approach, now with the most remote possibility of becoming a reality, is the induction of a systemic immune response (IgG) against mutans streptococci. Although successful in monkeys, the potential side effects of active immunisation – such as cross-reactivity of the antigens with heart tissues – makes this approach least tempting.

Effect of aging on protective functions of saliva

If the flow rate of stimulated saliva remains stable, there is no proof of any decline in the output of salivary sIgA with age. If the person is dentate, the same is true also for IgG and IgM levels in saliva. Thus, the total numbers of antibody molecules do not seem to decline with age. However, the antibody response to an antigenic challenge is impaired by age. There is notable evidence that antibody response e.g. against mutans streptococci, poliovirus and *Candida albicans* is weakened after the age of 60-65 years. This might contribute to the rather frequent oral yeast infections among the elderly.

The non-immunoglobulin salivary agents seem to work at full capacity throughout life. Also the functional activity of polymorphonuclear leukocytes in saliva remains stable with age.

Acidic proline-rich proteins and statherin

Human saliva is supersaturated with respect to most calcium phosphate salts but salivary proteins are important in inhibiting spontaneous precipitation of these salts. Such proteins are proline-rich proteins (PRPs) and statherin, which bind calcium and maintain the supersaturated state.^{24,25}

Acidic PRPs comprise as much as 25-30 % of all proteins in saliva and they form a complex group with a large number of genetic variants. Some of these inhibit the spontaneous precipitation of calcium phosphate salts, while others adhere to salivary pellicles and selectively promote the adhesion of some bacteria, e.g. *Streptococcus gordonii* and *Actinomyces viscosus*, to tooth surfaces. Acidic PRPs, as well as basic PRPs and histatins have the ability to bind tannins, present in such beverages as tea and red wine, and reduce their toxicity.

Statherin, a small protein with only 43 amino acids, originates from both parotid and submandibular glands. The molecule inhibits precipitation of calcium phosphates in spite of the fact that many oral proteases (from bacteria) can degrade statherin. *In vivo*, however, the concentration of statherin is high enough to act as an inhibitor of spontaneous calcium salt precipitation.²⁶ As with PRPs, statherin may also promote the adhesion of *A. viscosus* to tooth surfaces.

Clinical applications of salivary antimicrobial agents

Because of the deepened knowledge of the chemical and functional properties of many host proteins in human saliva, some commercial applications for their use in preventive

clinical dentistry have been made. Apart from the short-term vaccination experiments to enhance salivary sIgA (see above), much more interest has been focused on several innate salivary proteins, i.e. lysozyme, lactoferrin and peroxidases. The idea of their clinical applications seems sound: to add physiological antimicrobial agents into a mouth that lacks saliva-mediated protection (patients with dry mouth), or to enhance saliva's own antimicrobial capacity in infection-prone individuals, such as cancer patients. Many products, comprising one or all of the above components, are already on the market but clinical documentation of their efficacy is rather limited.¹⁶ There are reports showing a positive response, particularly among patients with xerostomia or cancer treatment but the exact role of the antimicrobial proteins is still unclear. The proteins for these products (toothpastes, mouth rinses, gels, chewing gums) are purified from cow's milk or colostrum because these milk proteins are both structurally and catalytically almost identical to those in human saliva. No adverse effects have been reported. Based on a rather long clinical experience, i.e. over 15 years, these products can be recommended by dentists or physicians to relieve some of the subjective oral symptoms of xerostomia or cancer treatment.

Summary – clinical highlights

1. An understanding of the multifunctional role of salivary proteins and particularly of their interactions with oral micro-organisms is a necessary prerequisite for their clinical applications. Technologies such as mass spectrometry and 16S rDNA sequencing facilitate comprehensive characterisations of oral protein and microbial species, respectively, and their interactions. The clinical application of such knowledge may include modifying bacterial adhesion to oral surfaces, eliminating specific pathogens and designing artificial saliva for patients suffering from dry mouth. A good basic understanding of salivary proteins' mechanisms of action furthermore offers opportunities to combine protective proteins to achieve the best possible concerted action
2. Assays of individual salivary proteins are of limited or no diagnostic value. This is mainly because they interact in many ways and other proteins often compensate for deficiency in one factor. There is no evidence from a diagnostic point of view that any single salivary protein would constitute a reliable diagnostic marker. More promising are approaches that pursue a panel of salivary diagnostic candidates, e.g. in microarray formats

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3. Significant achievements have been made in developing multifunctional, genetically engineered proteins, which carry many of the biological activities described in this chapter. Progress has also been made in the introduction of gene therapy to compensate for salivary gland deficiencies, which tend to become more and more prevalent in aging populations.

Acknowledgement

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The role of saliva in mineral equilibria - caries, erosion and calculus formation

Bob ten Cate

Introduction

The importance of saliva in the prevention of dental caries is dramatically shown in patients with impaired salivary function. When, as a result of medication or radiation in the oro-facial region, salivary flow is reduced (see Chapter 4), the dentition may be completely destroyed within a short period of time. Unlike 'normal' caries, caries as a result of xerostomia is often seen at the incisal or occlusal edges of the teeth and in the cervical region.¹ This can sometimes lead to entire layers of enamel being chipped off even on the smooth surfaces (*Figures 8.1, 8.2*). Caries due to impaired saliva quantity has an appearance and location different from 'general' caries (*Figure 8.3*). The same is true for the loss of tooth mineral due to 'dental erosion' which develops as a result of frequent acid intake (*Figure 8.4*).



Figure 8.1 Caries in a patient with impaired salivary function as result of radiation therapy (courtesy of Drs. Jansma and Vissink, UMCG, The Netherlands).



Figure 8.2 Electronmicroscopic picture of enamel surface with radiation caries, showing the characteristic chipping of the layers of enamel (courtesy of Drs. Jansma and Vissink, UMCG, The Netherlands).

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Figure 8.3 Patient with severe dental caries (courtesy of Dr. Gambon, Rotterdam, The Netherlands).



Figure 8.4 Patient with severe dental erosion due to excessive acid exposure in the oral cavity (courtesy of Dr. Gambon, Rotterdam, The Netherlands).

Saliva-pellicle-plaque

Saliva is never in direct contact with the dentition. Even at sites where the plaque is removed by the mechanical cleansing effect of the mucosa or the antagonistic teeth, a thin layer of salivary origin (the acquired enamel pellicle) covers the enamel (Figure 8.5).² This layer of proteins and lipids forms immediately after a surface has been

completely cleaned, and it has been shown that the pellicle adheres so strongly to the enamel that it is not totally removed during tooth brushing or prophylaxis. The pellicle protects the enamel to some extent from severe mechanical and chemical damage, the latter, for instance, imposed by acids in the oral environment. The acquired pellicle has been shown to contain 130 different proteins derived from cellular (68%), plasma (18%), and salivary (14%) proteins³.

Laboratory experiments have shown that the pellicle delays the initiation of caries and the dissolution of enamel when teeth are placed in low pH soft drinks.⁴ At retention sites dental plaque forms the second layer separating the tooth surface from saliva. Plaque is mainly composed of bacteria in a polysaccharide matrix. Much attention has



Figure 8.5 Schematic representation of the interface between enamel and the oral fluids, showing the acquired enamel pellicle and the plaque with bacteria and plaque matrix.

recently been given to the liquid phase of plaque (the 'plaque fluid'), as this is the solution often in closest contact with the tooth surface. Mineral dissolution and (re)precipitation processes, as they occur during caries and calculus formation, are directed by the composition of the plaque fluid more than by the composition of saliva, although the two are related (see below). In the recent decade much attention has been given to the biofilm properties of dental plaque, which explain the resilience of bacteria in plaque to antimicrobial treatments and antibiotics.^{5,6}

Enamel composition

The calcified tissues in the body are composed of a calcium phosphate mineral phase and an organic matrix. The latter has different roles, such as forming the 'cement' which holds the mineral crystals together, and regulating their formation and regeneration. In enamel, the tissue-forming cells (ameloblasts) secrete an organic matrix onto which crystallites are laid down. This is a process which takes place prior to the eruption of the tooth into the oral cavity. Once erupted, the ameloblasts (being on the outside of the tooth) are worn off and the fate of the enamel is no longer determined by cellularly driven mechanisms but by the interactions between the oral fluids (the term 'oral fluid', in this chapter, refers to saliva and plaque fluid) and the enamel.

In dentine, on the other hand, the odontoblasts, being on the pulpal side, remain active and deposit secondary dentine after eruption, or tertiary dentine as a result of a chemical or mechanical insult to the teeth. This can be seen as a natural defence

Table 8.1: Calcium phosphates occurring in the body

Mineral	Chemical formula	Calcium concentration at equilibrium at pH=7, Ca/P=0.16 and $\mu=0.06$.
Hydroxyapatite	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	0.105 mmol/l
Brushite	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	0.560 mmol/l
B tricalcium phosphate	$\text{Ca}_3(\text{PO}_4)_2$	0.165 mmol/l
Octacalcium phosphate	$\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$	0.369 mmol/l
Fluorapatite	$\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$	0.013 mmol/l at [F]=0.2 ppm 0.018 mmol/l at [F]=0.02 ppm

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mechanism of the body against caries and mechanical trauma. For enamel the body has to rely on saliva as a protective substance.

Saliva contains a number of components which have a specific role in this respect. The above-mentioned organic constituents, proteins and lipids, form the enamel pellicle which is a diffusion barrier to acids formed in the dental plaque, and in general regulates dissolution and precipitation processes. Of similar importance are the inorganic components, especially calcium and phosphate ions. In its composition saliva possesses features similar to those of the other body fluids, although the degree of saturation with respect to minerals is different.

The mineral phase of enamel consists of an impure hydroxyapatite, HAP (*Table 8.1*). This mineral is the least soluble in a range of calcium phosphates which are found in nature, and more specifically in the body.⁸ Two characteristics of this substance need to be discussed in relation to their importance in the oral environment. Firstly, hydroxyapatite is very permissive in incorporating foreign ions in the crystalline lattice. These may be either positively charged (sodium, potassium, zinc or strontium ions) or negatively charged (fluoride or carbonate ions). The concentrations of these impurities in the tissue are influenced by their presence during its formation. These mineral modifications have either a positive or a negative effect on the solubility: carbonate incorporation makes the apatite more soluble, while fluoride incorporation makes it less soluble.

Secondly, the solubility of the apatite mineral depends highly on the pH of the environment. In an acid environment (low pH), the concentration of ions in the liquid phase surrounding the crystallites necessary to maintain saturation is higher than at high pH. pH is therefore the driving force for dissolution and precipitation of hydroxyapatite. Apart from such physico-chemical considerations, other regulatory mechanisms exist, also in saliva. One example of this is 'nucleators' for precipitation: solutions which are supersaturated with respect to a given mineral do not necessarily precipitate unless this precipitate can form onto a surface. For calculus formation these nucleators are the plaque bacteria, which facilitate mineralisation of the plaque. In enamel in contact with saliva or plaque fluid, mineral deposition may occur onto the hydroxyapatite crystallites.

In its most simple form the dissolution and reprecipitation can be described as:

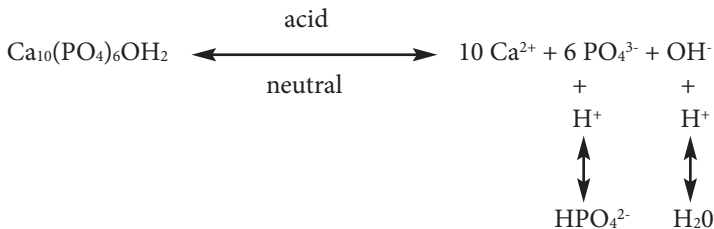


Table 8.2: Calcium, phosphate and fluoride levels in human stimulated whole saliva and plaque fluid

Saliva:	Approximate concentration ranges	
	mmol/l	ppm
Calcium	0.75-1.75	30-70
Phosphate	2.0-5.0	60-155
Fluoride	0.0005-0.005	0.01-0.10

Plaque fluid ⁵ :	Mean (SD) values	
	mmol/l	ppm
Calcium ion	0.85 (0.52)	34 (21)
Phosphate	11.5 (3.3)	356 (102)
Fluoride	0.0049 (0.0027)	0.09 (0.05)

Saliva and the Stephan curve

The mineral composition of saliva and plaque fluid is given in *Table 8.2*. These data show that differences in composition exist between saliva and plaque fluid, even though they are presumed to be in equilibrium. At this stage one can only speculate about the causes of this observation. Possibly the 'solid' phase of the plaque exchanges ions with the plaque fluid very slowly, which, due to its capillary nature, is never in true equilibrium with the saliva. The calcium and phosphate content and in particular the pH of these liquids determine whether enamel will dissolve (leading to caries) and whether mineral may be precipitated (which would result in calculus formation). *Figure 8.6* (overleaf) shows the relationship between the saliva and plaque fluid calcium and phosphate levels and the saturation lines for enamel and dentine. It should be noted that the degree of saturation differs between the saliva secreted from the various glands and with secretion rate. For instance, saliva is more supersaturated (with respect to HAP and fluorapatite = FAP) at a higher secretion rate.

Caries and calculus formation may be explained from *Figure 8.6*. At physiological pH, saliva and plaque fluid are supersaturated with respect to the hydroxyapatite phase of enamel. This implies that HAP will precipitate if a suitable precipitation nucleus is available. However, after eating foods or drinks containing fermentable carbohydrates, acids are formed in the plaque leading to a fall and subsequent rise in pH called a

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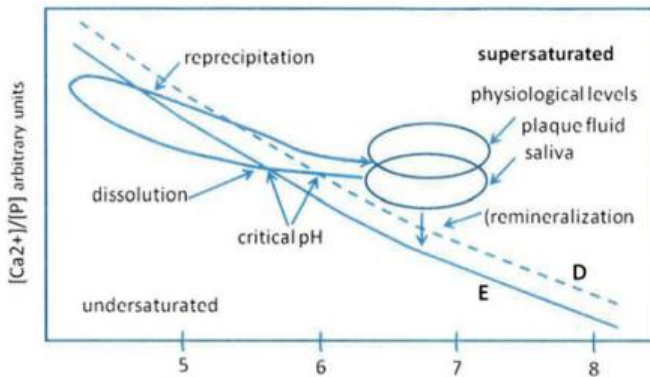


Figure 8.6 Solubility isotherms (schematic) for enamel (E) and dentine (D) in relation to the levels of calcium and phosphate of saliva and plaque fluid. The change in pH after carbohydrate consumption and the consequences for saturation of the oral fluids are also indicated.

‘Stephan curve’ (see *Figure 6.1*). When the pH is lowered, the concentration of ions needed for saturation increases and in the pH range around 5.6 (the ‘critical’ pH) the tissues will start to dissolve to maintain saturation. The lower the pH, the faster this demineralisation. As a result, the phosphate and hydroxyl ions released will take up protons (H^+) thus slowing down or reversing the fall in pH. Consumption of foods or drinks containing fermentable carbohydrates also increases salivary flow; the increased buffering power of saliva, and the washing out of remaining sugars and acids from plaque, contribute to the pH-rising phase of the Stephan curve.

During the recovery phase the plaque gradually becomes saturated and later supersaturated with HAP, and after the critical pH value is exceeded mineral may reprecipitate. Ideally, this occurs at the sites ‘damaged’ during the demineralisation. As mentioned before, the exact composition of the apatite formed depends on the composition of the solution from which it is precipitated, in this case the plaque fluid. If, for instance, fluoride is present, this will ‘co-precipitate’ to form a fluoridated hydroxyapatite. In short, this periodic cycling of pH results in a step-by-step modification of the chemical composition of the outer layers of enamel which become somewhat less soluble with time. This process is known as the post-eruptive maturation of the enamel.

It has been argued that some demineralisation is beneficial because it will remove the more soluble components of enamel, rich in carbonate, which may be replaced with a fluoride rich component, making the enamel more resistant to subsequent demineralisation.

Caries and remineralisation

If frequency of carbohydrate consumption is too high, the redeposition of mineral (during the recovery phase of the Stephan curve) is far from complete and there is a cumulative loss of enamel substance. Then a caries lesion will be formed, which is often the 'forerunner' of the caries cavity. A caries lesion is characterised by subsurface loss of mineral while the surface, due to its lower solubility, remains apparently intact. Only small pores are 'etched' in the surface layer at sites corresponding with the interprismatic regions.⁹ These enable the transport of acids into the deeper layers of the tissue and of dissolved ions out of the tissue (*Figure 8.7*).

Even when a lesion has been formed, saliva can play an important role in preventing excessive decay. With improved oral hygiene or other preventive measures (e.g. fluoride), deposition of mineral from saliva or plaque fluid may take place instead of further tissue loss. In a laboratory model, remineralisation can be illustrated when early enamel lesions are immersed in saliva. From the radiographic pictures (*Figure 8.8 - overleaf*) the disappearance of the radiopacities is evident.

Clinically, remineralisation was documented in a longitudinal study of drinking water fluoridation¹⁰ and more recently in various toothpaste clinical trials.¹¹ In the Dutch Tiel

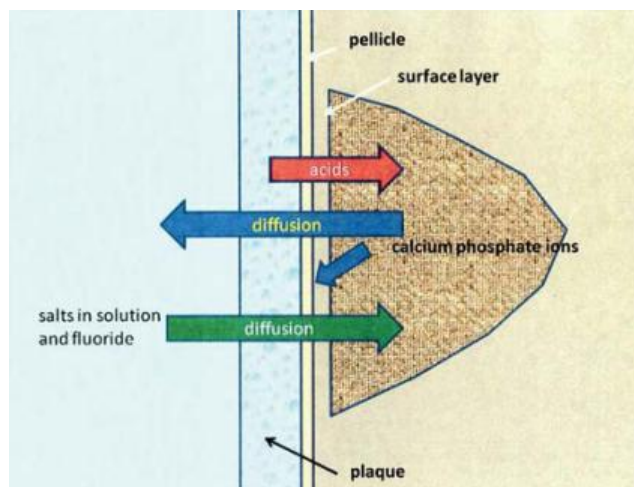


Figure 8.7 Schematic cross-section of the enamel-pellicle-plaque interface with the diffusion, dissolution and precipitation processes occurring during caries development and regression. (*redrawn from reference 7*)

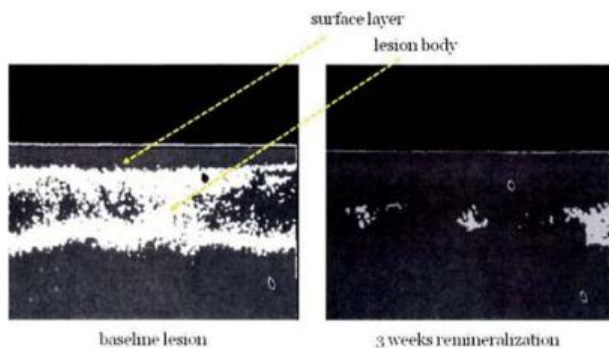


Figure 8.8 Microradiographs of sections taken from lesions before and after a 3-week immersion in saliva, showing the disappearance of the lesion as a result of saliva-induced remineralisation. (ten Cate, original data).

Culemborg study the investigators noted that 50% of the lesions seen at the first molar buccal surfaces of 8-year-old children disappeared during the next seven years. A factor put forward to explain this finding was the further eruption of the teeth which brought the lesions out of the area at risk and in direct contact with saliva from which the remineralisation took place. A closer look at the data revealed that the lesions were seen at rather different stages. In some cases the surfaces appeared chalky and dull, while others were yellowish and shiny. It was concluded that the first type (found more often in the non-fluoridated town) indicated active caries lesions. The dull appearance was due to recently exposed (non light reflecting), acid 'treated' enamel, a phenomenon also seen after the deliberate acid etching of enamel prior to placing sealants or composites. The shiny appearance of 'arrested' lesions (found more often in the fluoridated town) was due to the deposition of mineral and organic components from saliva in the porous carious enamel (Figure 8.9). With time such lesions will also accumulate dyes from food and, unless completely remineralised, eventually develop into 'brown spots'.

Erosion

Acids formed in dental plaque are the cause of dental caries. Recently, attention has also been given to a different group of acids, those which are present in foods and drinks and which have a direct eroding effect on the dentition.¹² Various reviews report that around 30% of adolescents show signs of erosion in their dentition and that erosion is also often seen in very young patients. In erosion, tissue loss does not occur by subsurface

demineralisation but by a layer-wise removal of enamel or dentine. The pH in such cases is substantially lower than in dental plaque, with values between pH 2 and 3 not being uncommon in beverages. Apart from being caused by so-called extrinsic sources, erosion may result from gastric fluids (at pH values just above 1) when patients suffer from eating disorders resulting in frequent vomiting or gastroesophageal reflux. Not only is erosion different from caries at the microscopic level, also the distribution around the oral cavity is typical and indicates where acids first come in contact with the dentition and where protective factors are present. The most common site for erosion in some patients is the lingual aspect of mandibular molars. This points to the role of serous saliva and salivary pellicle in protecting the dentition against erosion.¹³ Studies to correlate saliva properties with susceptibility to erosion have shown that a low buffer capacity will make individuals more prone to erosion. Salivary flow rates have not been shown to be related to erosion at the individual level. Also in laboratory studies saliva, probably in particular salivary mucins and pellicle, has been shown to slow down the rate of tissue loss.¹⁴ As dental erosion is caused by fluids with very low pH it is easy to explain why fluoride, calcium and phosphate additions have little or no effect in its aetiology. It would require massive amounts of one of these ions to come near to saturation for either HAP or FAP. Recent studies to increase both pH and ion composition have resulted in a beverage with reduced risk for erosion.¹⁵ Obviously such changes to the gastric fluids which cause erosion are not possible.



Figure 8.9 Examples of active (top) and arrested (bottom) lesions. (*ten Cate, original data*).

Calculus

Plaque fluid is supersaturated with respect to many of the calcium phosphate minerals listed in *Table 8.1*. Mineralisation inhibitors present in plaque prevent these minerals from

precipitating unless the inhibitors are degraded by enzymes or nucleators for precipitation are present. It has been shown that dead or 'dying' bacteria (or components from bacterial cell walls) serve as nuclei for precipitation. Unlike in enamel, where the calcium phosphate mineral is present as HAP, in calculus four different calcium phosphates may be found, with the distribution of minerals being determined by the age of the deposit.¹⁶

Because salivary secretions are the main sources of calcium and phosphate in the oral cavity, calculus forms most abundantly on the tooth surfaces opposite the orifices of the main salivary glands. Saliva secretion from the parotid glands may lead to calculus formation on the buccal surfaces of the maxillary molars, while submandibular saliva may contribute to calculus deposition on the lingual surfaces of the mandibular anterior teeth (for more details see Chapter 5). In addition to the difference between calculus at various sites around the mouth, the variation in calculus from supra- and subgingival parts of the tooth should be mentioned. Both are formed as a result of the mineralisation of dental plaque, but for subgingival calculus, crevicular fluid and exudate from infected periodontal tissue substitute for saliva in providing the materials from which calculus is formed. Subgingival calculus develops from subgingival plaque, a process that is not necessarily related to a prior formation of supragingival calculus. Chemical analyses have shown that the mineral density of subgingival calculus is higher, which makes it even harder to remove by a dentist or hygienist. The rate at which calculus forms is variable among individuals. In general, supragingival calculus forms first on the lingual aspects of the lower anterior teeth.

Individual variations in plaque fluid and saliva saturation and caries

Variation in the composition of the oral fluids occurs between different sites in the mouth as well as between individuals.¹⁷ The resulting differences in degree of saturation are very small compared with the dramatic changes occurring after acid formation in the plaque. Nevertheless, researchers have for many years been seeking to identify correlations between one individual's caries experience and his/her calcium and phosphate levels or resting pH of saliva and plaque. Over the last two decades, techniques have become available for the analysis of very small fluid volumes (nanoliters), which has made plaque fluid the focus of this research. For that fluid a difference in degree of supersaturation (with respect to apatite) has been observed between caries-susceptible and caries-free subjects. These data revealed that this difference in supersaturation is primarily caused by a 0.3 unit higher pH value for the plaque fluid of the caries-free individuals.¹⁸ Apparently, although very small, the difference in saturation has clinical implications,

presumably by the difference in remineralisation potential between the respective plaque fluids. Studies of indicators for patients at risk for caries have similarly found that the degree of supersaturation of saliva with respect to fluorapatite showed a high correlation with caries progression. It is argued that, in particular, fluoride levels in the oral fluids are an important prognostic tool.

Modifying saliva to favour caries prevention and prevent calculus formation

Caries and calculus formation are both caused by dental plaque. The most obvious direct method of their prevention would therefore be effective mechanical plaque removal (e.g. tooth brushing and flossing) or antimicrobial therapy. However, neither has been very effective. It seems that effective plaque removal is almost impossible to achieve by patients.¹⁹ Likewise, antimicrobial approaches in caries prevention have, so far, not been very successful. Prevention therefore relies mainly on changing the physico-chemical mechanisms of caries and calculus, in other words once the bacteria have done their job! In the intrinsic mechanisms of caries prevention saliva has an important part, with its capacity of buffering the acid and clearing the oral cavity of foods or drinks containing fermentable carbohydrates and acids (see Chapter 5). Also, saliva affects bacterial growth and metabolism (Chapter 7).

Caries

The presence of fluoride, derived from topical applications, water, toothpastes, varnishes, rinses or tablets, in the oral fluids has a significant depressing effect on the initiation and progression of dental caries, as shown in many epidemiological and clinical studies. The smooth and interproximal surfaces benefit the most from fluoride as a caries preventive agent. During any kind of fluoride usage, fluoride is deposited at retention sites in the oral cavity. These can be porous regions (such as caries lesions) in the dentition, or the soft tissues. Fluoride, when given in sufficiently large concentrations, may also be laid down on the teeth as globular calcium fluoride deposits.²⁰ While in aqueous solutions pure calcium fluoride is fairly soluble, in the oral cavity it is surprisingly stable. This is thought to be due to the presence of a protective outer layer on the globules, formed by a reaction between calcium fluoride and phosphate and proteins from saliva. These globules may then act as fluoride slow-release devices.

Saliva also serves as a carrier for fluoride ions from the various depots to the sites at risk for caries in the oral cavity. In clinical studies on the effects of fluoride dentifrices

it was observed that the depot formation resulted in an elevation of the fluoride levels in plaque and saliva throughout the day.²¹ After cessation of the use of fluoride dentifrices it took about two weeks for the fluoride in plaque and saliva to return to 'baseline' levels. Now more attention is given to the patient's usage of toothpaste, which apart from brushing methods and frequency, considers what should be done after tooth brushing to guarantee optimal retention of the fluoride in the oral cavity. One set of advice given is to minimise the amount of water used to rinse out the mouth, or to use the dentifrice (tap water diluted) as a mouthrinse.²² Even low fluoride levels in plaque or saliva are effective in caries inhibition, because they inhibit the demineralisation of enamel and enhance the remineralisation, by increasing the rate of mineral deposition. Equally important to note is that this deposition then occurs as a fluoridated apatite, which is less susceptible to demineralisation during subsequent acid challenges. The success of fluoride has initiated studies to increase the salivary levels of other 'common' ions of apatite, calcium and phosphate or the pH. Phosphate has been widely studied as a food additive and although it was quite effective in reducing caries in rats, it was never found to be beneficial in humans, probably because its concentration in saliva from the major salivary glands in humans is already much higher than that in plasma, whereas rat saliva is relatively deficient in phosphate.

Many currently available types of toothpaste contain calcium, which is reported to have an additional preventive effect. Also xylitol is now added to chewing gum and to some dentifrices (see Chapter 6). The working mechanism relies on the xylitol-enhanced salivary flow and on its antibacterial properties. In recent years the effects of xylitol and sorbitol, in particular in chewing gum, have been confirmed in a number of independent randomized clinical trials, which on average showed that xylitol-containing chewing gum use resulted in 30-70% lower DMFS increments. The corresponding data obtained for sorbitol chewing gums varied between 10 and 60%.^{23,24}

Root surface caries

Caries of the root surface has received increasing attention due to the longevity of the teeth.²⁵ As a result of medication and of diseases or surgery of the periodontal tissues, the root surface often becomes exposed to the oral cavity when the patient gets older. The tissues of the root surface are particularly vulnerable to acid attacks and subsequently to proteolytic breakdown of the collagen matrix. Fluoride treatments have been shown to prevent this type of caries. When root lesions have formed it is now advised to improve first the local oral hygiene and give fluoride applications. This leads to a saliva-induced rehardening of the dentine; restorative treatment can follow if indicated.

Calculus

Agents contained in dentifrices are now available which interfere with calculus formation. Crystal growth inhibitors (such as pyrophosphate and zinc citrate) are very effective in reducing the rate of supragingival calculus formation, while the calculus that does form can be more easily removed.

Saliva stimulation

Stimulated saliva contains higher levels of bicarbonate buffer and is more supersaturated with respect to hydroxyapatite than unstimulated saliva. If, after sugar intake, saliva stimulation is prolonged (e.g. by chewing sugar-free gum) two beneficial effects may follow: the increased bicarbonate prevents the pH of plaque from falling as much and thus reduces the potential for hydroxyapatite dissolution, and the increased saturation raises the potential for remineralisation of any damaged crystallites (see also Chapter 6). These effects have been demonstrated experimentally using pieces of enamel from extracted teeth attached to the dentitions of volunteers and are consistent with the results of clinical trials noted above.

Concluding remarks

As outlined above, components from saliva interact in different ways with the dentition to protect the teeth from becoming carious or from excessive calculus formation. In addition, saliva is the oral transport medium by which preventive agents are distributed around the mouth. Patients who lack sufficient saliva suffer from many oral diseases, of which caries is only one. To alleviate the discomfort they are advised to use saliva stimulants and substitutes which have the function of lubricating the oral surfaces. Most substitutes are developed for their rheological and wetting properties, more than for their chemical composition (buffer capacity, calcium, phosphate, fluoride). It is recommended therefore that these products should be better formulated for their potential to mimic natural saliva also in its caries preventive properties.

Clinical highlights

1. The arrest and/or reversal of early caries lesions is a natural and very important means of caries prevention which can be enhanced by intervention.
2. Saliva contains calcium and phosphate at concentrations such that it is supersaturated with respect to hydroxyapatite. As a result, saliva reduces the dissolution of tooth mineral in caries, and replaces mineral (that is, remineralises the crystals) in early lesions. Salivary hypofunction will eliminate both these functions. Salivary stimulation increases its potential for remineralisation.
3. Fluoride in the mouth inhibits demineralisation if it is present in the aqueous phase between the enamel crystals at the time of an acid challenge.
4. Fluoride enhances remineralisation of early lesions by helping calcium and phosphate, derived primarily from saliva, to regrow the surfaces of partially dissolved crystals. This will produce a fluorapatite-like surface which is more resistant to subsequent acid attack. Hence strategies which maintain the ambient level of fluoride in saliva can help control caries.
5. From a clinical viewpoint, a continual supply of elevated levels of fluoride in the mouth is a very effective preventive measure against caries.
6. Methods which deliver fluoride to the mouth (water, toothpaste, mouth rinses or professionally applied topicals) are very effective in caries prevention, even in patients with severely reduced salivary flow. In fact, fluoride is essential in these patients.
7. Because of the supersaturation of saliva, calculus formation would occur much more generally were there no inhibitors of calcification present in saliva and plaque.
8. Teeth in direct contact with strong acids (food or gastric) will be eroded. This process is somewhat delayed by saliva-derived pellicle or salivary mucins.

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Saliva and oral health

Saliva and oral health has continued to be such a popular text on our evolving knowledge of saliva and its functions that this 4th edition has been prompted in order to bring the reader fully up to date. The editors and authors have taken the opportunity to fully revise the text to make it an essential guide to the role of this vital body fluid and the secrets it is still revealing.

This edition is still aimed primarily at the 'progressive and inquisitive practitioner' but will doubtless find favour with undergraduate and postgraduate dental students as well as other members of the dental team and health professionals.

Chapters include: *The anatomy and physiology of the salivary glands, xerostomia, salivary clearance and its effects on oral health and The role of saliva in caries, erosion and calculus formation.*



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