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Natural and Synthetic Saliva: A Stimulating Subject

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Introduction

Saliva performs many important functions in the mouth and upper aerodigestive tract. Only those unfortunate individuals who suffer from xerostomia (a dry mouth due to impaired salivary secretion) can fully understand such functions at a basic physiological level – and indeed the impaired quality of life which such xerostomia brings. The mouth and pharynx become dry, uncomfortable and overtly sore; eating and speaking are difficult and painful processes; the sense of taste is diminished and the wearing of dentures may be an ordeal. Those with natural dentition suffer from increased gingivitis and an aggressive level of dental caries. The oral musoca is vulnerable to damage and open to infection. The mouth painfully and progressively breaks down.

This chapter aims to present a review of saliva composition, function and its role in maintaining oral health. This will lead to a brief discussion of the conditions that cause xerostomia and our current knowledge of artificial saliva preparations and stimulants.

Saliva: its origin

The human salivary glands consist of three pairs of major glands (parotid, submandibular and sublingual) and multiple minor glands (buccal, palatal, labial and lingual) dispersed throughout the mouth (Mason and Chisholm, 1975). Also present in the mouth are bacteria and their products of degradation, food debris and exfoliated epithelial cells. In addition, in dentate individuals there is gingival exudate (or crevicular fluid). This is a serum-like fluid which seeps from between the teeth and gingival margins. The mixed saliva present in the oral cavity at any one point in time comes from these various sources and is termed whole saliva.

The three pairs of major glands produce around 95% of the total saliva volume – the

Abbreviations: EGF, epidermal growth factor; NGF, nerve growth factor.

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parotid glands providing 60–65%, the submandibular glands 20–30% and the sublingual glands 2–5%. The minor glands produce relatively small amounts of saliva but their important role is increasingly recognized in contributing to musocal integrity by way of the hydrated mucosal surface film (Ferguson, 1989).

CONTROL OF SALIVARY SECRETION

Much work has been done on the control of salivary secretion (Emmelin, 1972) and it is now firmly established that the autonomic nervous system is fundamental and central to the control process (Garrett, 1982). The sympathetic and parasympathetic nerves are not simply opposite in nature but work together in a complex manner to stimulate, antagonize and control secretion (Garrett, 1987). The autonomic output to the glands is influenced by taste and tactile sensation from the mouth, with taste the most potent stimulus (Watanube and Dawes, 1988a) – in particular the acidic taste (Watanube and Dawes, 1988b). Visual and olfactory stimuli and inputs from higher cortical centres (e.g. fear and anxiety) also influence the control of saliva secretion via the autonomic nervous system. Mastication is also a potent stimulus to salivary flow. The composition is modulated on stimulation and also subject to circadian variation (Edgar, 1992).

Each salivary gland is made up of a number of acini (sing. acinus). These acini comprise the terminal or secretory end-piece of the gland, situated furthest away from the oral environment. Each acinus is composed of a number of acinar cells on a basement membrane and positioned around a central duct lumen. Each acinus may have cells which are exclusively serous or mucous in secretion type or contain a combination of both cell types. The acinar cells of the submandibular gland and sublingual gland and many minor glands are predominantly mucus secreting. Serous cells form the bulk of acinar cells in the parotid glands and in the minor glands found around the sulci of the circumvallate papillae on the tongue (serous glands of von Ebner). Serous cells also occur in the submandibular gland and in the minor glands anteriorly on the tongue (Waterhouse, Beeley and Mason, 1990).

Each acinus, regardless of predominant cell type, opens into a central duct lumen, which opens into an intercalated duct, lined by cuboidal epithelium with small projecting villi. The intercalated duct opens into a short, wider striated duct, lined by columnar cells with cell-surface projections. The striated duct opens into an excretory duct and on into the terminal duct, lined by stratified squamous epithelium in continuity with the oral cavity. The functions of each part of this duct system are discussed below.

A further cell population identified around the acini and ducts is the myoepithelial cell, which contains myofibrils and is contractile – compressing the acini and duct tissue to facilitate salivary flow.

MECHANISM OF SALIVARY SECRETION

Thaysen and co-workers proposed a two-stage hypothesis of saliva formation in 1954. They suggested that saliva was formed initially as an isotonic secretion in salivary gland acini and that it was subsequently modified by the addition or removal of specific ions as it passed through the ductal system. This hypothesis was confirmed

by duct micropuncture techniques (Martinez, Holzgrove and Frick, 1966). Further details have emerged more recently on the two main stages of water and electrolyte secretion in saliva, i.e. acinar secretion and ductal modification (Martinez, 1987):

1. The primary acinar secretion is isotonic and similar to plasma.
2. Stimulation of salivary glands alters the rate of formation of this primary secretion, but not its composition or osmolarity.
3. The duct cells act to alter the electrolyte content, but not the water content, of the primary secretion.
4. Autonomic stimulation of the duct system modifies the ion transport function of the duct cells.

Saliva: composition

Duct saliva is an aqueous hypotonic solution consisting of 99% water and containing inorganic ions, low molecular weight organic constituents and proteins (concentra-

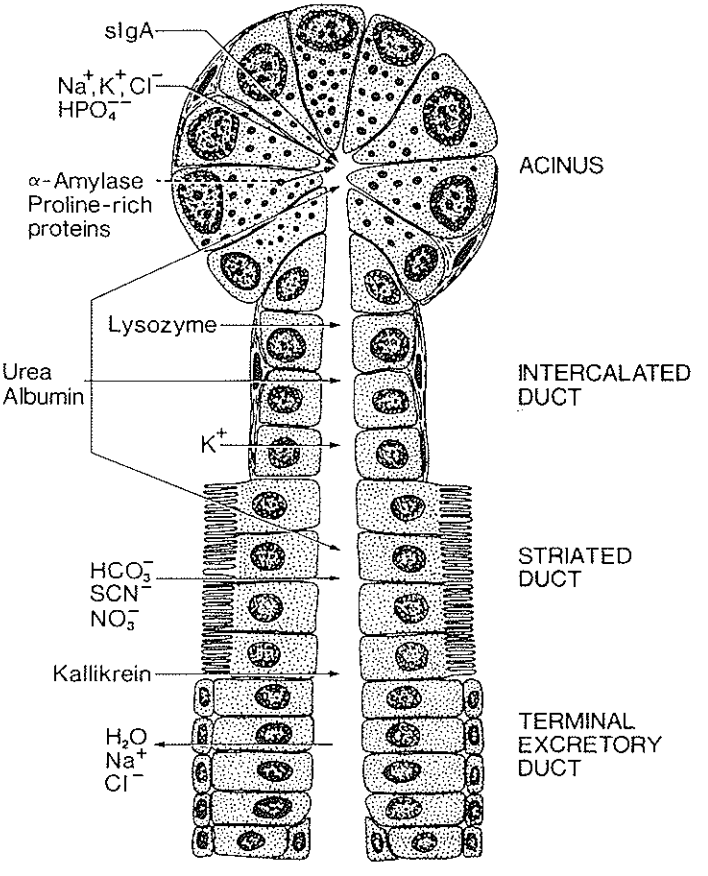

Figure 1. Sites of secretion within the gland of the constituents of saliva (modified from a diagram kindly provided by Dr D.B. Ferguson; Waterhouse, Beeley and Mason, 1990).

tion ~1–3 mg ml^{-1}). The sites of secretion within the gland of some of these components are shown in *Figure 1*. The inorganic ions include sodium, potassium, calcium, zinc, magnesium, phosphate, bicarbonate, chloride, fluoride, thiocyanate, iodide and nitrate, and their concentrations have been published elsewhere (Mason and Chisholm, 1975; Young, 1979; Waterhouse, Beeley and Mason, 1990). The major monovalent cations are sodium and potassium, with the concentration of potassium far exceeding that of sodium. The major divalent cation is calcium, which occurs both as the free ion and bound to proline-rich proteins. Phosphate also occurs in the free and bound state, with about 25% being covalently bound in phosphoproteins. Changes in the levels of the inorganic components are associated with certain pathological disorders (Ferguson, 1987).

The low molecular weight organic compounds include urea (levels of which may rise in patients on renal dialysis), uric acid, creatinine, glucose, amino acids, lactate and some steroid hormones; drugs may also be secreted in saliva.

The major proteins in duct saliva are the fascinating polymorphic families of polyfunctional proteins, α-amylases, proline-rich proteins and histatins (Beeley, 1993; Lamkin and Oppenheim, 1993). These, together with mucins, constitute about 90% or more of the total protein in saliva. The major proteins in parotid saliva, as revealed by electrophoretic analysis, are shown in *Figure 2*. Curiously, however, although proline-rich proteins are the major proteins in duct saliva, their levels in mixed saliva are very low (Beeley *et al.*, 1991); although the mechanism is still unclear, interaction with bacteria is probably partially responsible (Newmann *et al.*, 1993).

α-Amylase occurs in two forms, glycosylated and non-glycosylated; there is familial inheritance of the different types which probably results from post-translational modification (glycosylation and deamidation) of the products of a single gene (Eckersall and Beeley, 1981).

The major proteins in parotid and submandibular saliva are the proline-rich proteins (Williamson, 1994). These belong to a unique multigene superfamily of proline-, glycine- and glutamate-rich salivary specific proteins which are quite unusual in their amino acid compositions. There are three types – basic, acidic and glycosylated, with substantial genetic variation from individual to individual (*Figure 2*). Basic proline-rich proteins are, however, not expressed in the submandibular glands. Proline-rich proteins have unusual properties in that they stain poorly with conventional electrophoretic staining procedures and because of their low levels of aromatic amino acids, they do not absorb at 280 nm; for these reasons they were overlooked until relatively recently and their functions are still unclear (Waterhouse, Beeley and Mason, 1990).

Another major group of proteins with an unusual amino acid composition and multiple molecular forms is a group of histidine-rich proteins known as histatins, which have antifungal properties and constitute a non-humoral defence mechanism. The viscosity of saliva results from submandibular and sublingual mucins, which are glycoproteins containing 70–80% carbohydrate and may also be sulphated. Two types have been studied extensively, MG1 (M_r>1 000 000) and MG2 (M_r 200 000–250 000).

A variety of other proteins of salivary gland origin also occurs in human saliva. These include a tyrosine-rich polypeptide, statherin, a group of cysteine protease

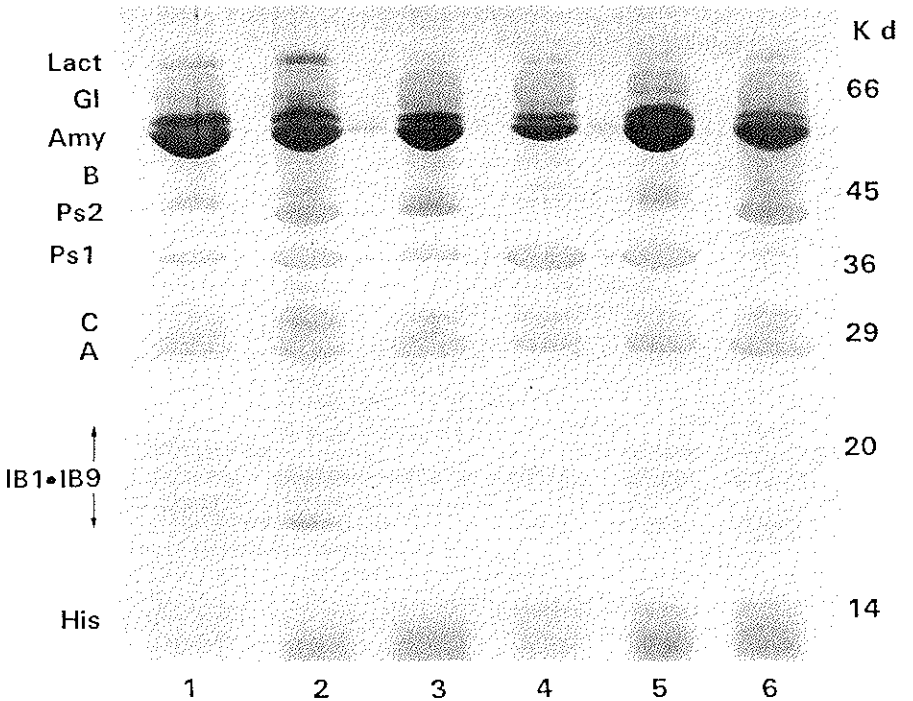

Figure 2. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (Beeley *et al.*, 1991) of parotid salivary proteins from six different individuals. Lact, lactoferrin; Gl, major glycosylated proline-rich protein; Amy, α-amylase; Ps1 and Ps2, basic proline-rich proteins; B, glycosylated proline-rich proteins; A and C, acidic proline-rich proteins; IB1-IB9, basic proline-rich proteins; His, histatins. The gel was stained with Coomassie Blue R-250; in this system proline-rich proteins stain pink-violet and all other proteins stain blue.

inhibitors known as cystatins and the zinc-containing protein, gustin, which is necessary for taste sensation. The major immunoglobulin in duct saliva is IgA, 85% of it is made up of sIgA and a third of this originating from the minor glands. The main source of IgG in whole saliva is gingival exudate. Free secretory component is also present in duct saliva but levels of albumin are extremely low, except in inflammatory disorders. Lactoferrin may also be secreted by salivary glands. Blood group substances (ABO and Lewis antigens) are present in the saliva of 'secretors' and are associated with the high molecular weight mucins which originate from the submandibular and minor glands (Prakobphol, Leffler and Fisher, 1993). Saliva is also an important source of polypeptide hormones such as EGF (epidermal growth factor) and NGF (nerve growth factor). Other enzymes present include carbonic anhydrase, lysozyme, lactoperoxidase, kallikrein and lipase (from minor glands) (Castle, Arvan and Cameron, 1987; Waterhouse, Beeley and Mason, 1990). Variations in organic components may also be associated with disease states (Ferguson, 1987). A list of the proteins of gland origin present in saliva is shown in *Table 1*.

Saliva: functions

Saliva is the fluid that keeps our mouths healthy. Its complexity, both in terms of

composition and formation, would suggest myriad functions and, interestingly, many of its components are polyfunctional.

Table 1. Human salivary proteins

Protein	M_r	pI	Function
[a] Proline-rich proteins			Binding to hydroxyapatite
acidic	16 300, 9000	3.5–4.5	Ca^{2+} binding
basic	6000–1200	>8.0	Inhibition of crystal growth
glycosylated	38 900	>8.0	Adhesion of micro-organisms
			Protection against dietary tannins
			Mucosal pellicle and enamel pellicle formation
[a] α-Amylase		6.5–7.5	Digestion
glycosylated	63 000		Antibacterial
non-glycosylated	59 000		Adhesion of micro-organisms
			Antibacterial
[a] Histatins	4500–7000	7.0, >9.5	Antifungal (non-immune)
			Pellicle formation
			Regulation of mineralization
			Buffering
[a] Statherin	5380	4.2	Inhibition of $CaPO_4$ precipitation
			Regulation of mineralization/inhibition of crystal growth
			Binding to hydroxyapatite
			Lubrication
[a] Cystatins	14 000		Cysteine and serine protease inhibitors
			Inhibition of $CaPO_4$ precipitation
			Binding to hydroxyapatite
			Antibacterial, antiviral
[a] Acid phosphatase	—		—
Mucins			Lubrication (speech, chewing and deglutition)
MG1	>1000 000		Bacterial agglutination/antibacterial
MG2	200 000–250 000		Pellicle formation
sulphated	15 000–300 000		Antiviral
blood group substances (ABO and Le)			Blood group antigens
Kallikrein	9600	3.8–4.5	Protease
			? Activation of polypeptide hormones
Immunoglobulins (sIgA, etc.)	405 000		Antibacterial
Lactoferrin	77 000		Antibacterial
Lysozyme	15 000		Anibacterial
Lactoperoxidase	75 000–78 000		Antibacterial
Gustin	37 000		Taste sensation
Albumin	68 000		?
Secretory component	67 000		
Carbonic anhydrase	42 000–45 000		Maintenance of bicarbonate buffering
Lipase			? Digestion
Polypeptide hormones, e.g. epidermal growth factor, nerve growth factor, etc.			Maintenance of oral and gastrointestinal mucosa
Transcalciferin	—		—

Modified from Beeley (1993).
[a] Polymorphic proteins.
Le, Lewis antigens.

FOOD-RELATED ROLES

Cleansing the mouth

This aqueous secretion both washes the oral tissues and contains components that assist in this action. α-Amylase (activated by chloride ions in saliva), generally considered to be a digestive enzyme, also has a key role in removal of residual dietary carbohydrate from the mouth. Indeed, as its pH optimum is 6.8, on passing into the acid environment of the stomach it will quickly become inactivated, although some activity may remain in the centre of a food bolus until inward seeping of gastric juice occurs.

The digestion of fat may well also begin in the oral cavity, with a lipase secreted by the minor salivary glands around circumvallate papillae (Hamosh and Burns, 1977), but a cleansing role for this enzyme may also be involved. However, despite the proteolytic activity of gastric juice, salivary lipase appears to continue acting in the acidic environment in the stomach but its activity is miniscule in comparison with pancreatic lipase.

Taste

A very common complaint of patients with xerostomia is that food has become bland and tasteless. Saliva acts to solubilize food particles to allow interaction with taste bud receptors; it also avoids competition with food substances due to its low salt and sugar concentrations. Shatzman and Henkin (1981) suggested the presence in saliva of gustin, a zinc-containing protein, as a mediator of taste sensation, and, indeed, individuals who are zinc deficient may also have impaired taste sensation.

Mastication and deglutition

The ease with which food is transferred around the oral cavity during mastication and subsequent swallowing is due in no small part to the properties of saliva. Food is wetted by the serous secretions of the parotid gland, secretion of which is stimulated on taking in food, and the lubricant properties of the viscous mucins from the submandibular, sublingual and minor glands facilitate movement during mastication and adhesion to form a bolus (Hatton *et al.*, 1985).

Although these 'food related' functions of saliva are essential, its 'protective' functions are more numerous.

MAINTENANCE OF ORAL HEALTH

Dentition

The buffering properties in saliva minimize pH fluctuations in the mouth, thereby protecting the tooth mineral from solubilization when the pH falls and deposition of calculus when the pH rises. Although both phosphate and bicarbonate, together with proteins (especially the basic histatins), act as buffers, the major salivary buffer is bicarbonate. Because the concentration of bicarbonate increases substantially when

flow is stimulated, the saliva produced while chewing has a much higher buffering capacity than that produced when resting (Edgar, 1976; Shellis and Dibdin, 1988; Watanube and Dawes, 1988b; Wikner and Nedlich, 1988). Carbonic anhydrase helps to maintain this high bicarbonate level in solution. In addition to buffering the pH, saliva can also help to raise the pH of the mouth as a result of the formation of ammonia by bacterial action on urea or degradation of amino acids.

Saliva is also a potent remineralization fluid. Indeed, as the pH of the mouth fluctuates, dental enamel may loose mineral ions as the pH falls and subsequently be remineralized as the pH rises again. Only when excessive remineralization has occurred does the process become irreversible and a carious lesion is formed. Because calcium phosphate is very insoluble, the effective calcium reserve of saliva is substantially increased by the presence of acidic proline-rich proteins which bind large numbers of calcium ions. Furthermore, precipitation of calcium phosphate is inhibited by statherin, proline-rich proteins and histatins, hence saliva is supersaturated with respect to calcium phosphate. However, remineralization must be a regulated process because the topography of the tooth does not change; for example, one never gets 'spikes' forming on the teeth. Presumably salivary components are involved in this regulation process.

The protective role of saliva is seen as the tooth erupts into the mouth. At this stage, the enamel is hypomineralized and relatively porous, explaining the susceptibility of newly erupted teeth to dental caries. However, the enamel undergoes post-eruptive maturation by diffusion of calcium phosphate, magnesium and fluoride into its surface layer. Thus the surface exhibits increased hardness and decreased permeability, which are essential features in a hostile oral environment.

Similarly, the erupting tooth has an enveloping protective pellicle which is soon removed by masticatory activity. This pellicle is constantly replaced by a coating of proteins absorbed from the saliva – the so-called acquired pellicle – which acts as a diffusion barrier to acid ingress and limits dissolved mineral egress (Slomiany *et al.*, 1986). Although proline-rich proteins, and more recently α-amylase also, are frequently regarded as being involved in pellicle formation, the role of crevicular fluid, a rich source of protein, must not be overlooked. However, the protective role of pellicle is debatable, because acquired pellicle also facilitates the adhesion of plaque bacteria to the tooth surface, especially by the early colonizers of the tooth surface in plaque formation.

Some of the most important work in recent years in the sphere of caries research has revealed the significance of fluoride in remineralization of early carious lesions. Early work by McCann and Brudevold (1966) has been built upon to reveal the positive influence of fluoride ion concentrations in promoting remineralization and to encourage the formation of an enamel surface which is progressively more resistant to acid attack during the demineralization–remineralization cycles occurring during bacterial digestion of fermentable carbohydrate in plaque.

Antimicrobial activity

Saliva has an array of antimicrobial activities: antibacterial, antifungal and antiviral.

The mechanisms of antibacterial action are diverse (Waterhouse, Beeley and Mason, 1990). The major immunoglobulin is sIgA together with low concentrations

of IgA, IgG and IgM. In addition to antibodies, it contains other antibacterial constituents, including lactoferrin, lactoperoxidase and lysozyme. Lactoferrin is a ferric ion-binding bifunctional glycoprotein; the apo-(iron-free) form occurs in saliva and binds ferric ions, thereby depriving micro-organisms of this nutrient and inhibiting their growth. Lactoperoxidase utilizes microbially produced hydrogen peroxide to oxidize salivary thiocyanate (SCN^-) to hypothiocyanite ($OSCN^-$) which inactivates the bacterial hexokinase (Adamson and Pruitt, 1981). Lysozyme (muramidase), which acts on the peptidoglycan of Gram-positive bacterial cell membranes, causing increased permeability or lysis, is also active against some oral bacteria. α-Amylase, together with cystatins, histatins and mucins, may also have antibacterial functions (MacKay *et al.*, 1984). Mucins, too, play an antibacterial role in causing agglutination and thereby facilitating elimination of bacteria from the mouth by swallowing.

Antifungal activity is displayed by the histidine-rich peptides, histatins, which at very low concentration are able to inhibit growth and kill off *Candida albicans* (Pollock *et al.*, 1984; Sabatini *et al.*, 1989).

Although saliva also has antiviral activity, including anti-HIV activity, the nature of this is still unclear (Malamud and Friedman, 1993). Although it may be modulated via secretory IgA (e.g. oral polio vaccine), activity has been associated with mucins (Heineman and Greenberg, 1980) and, more recently, with cystatins.

Although saliva is generally considered to have a variety of antimicrobial properties, bacteria are normal inhabitants of the mouth. Whereas some species colonize the oral surfaces, other are actively expelled. Perhaps, therefore, 'antimicrobial' is a misnomer and 'microbial modulating' would be more accurate.

Oral soft tissues

Saliva is essential for maintaining the normal properties and health of the soft tissues of the mouth. They are kept moist by water and lubricated by mucins and statherin (Douglas *et al.*, 1991). Especially important in this role are the secretions of the minor glands. These features are essential for speech as well as mastication, deglutition and the retention of dentures. A suitable osmotic environment for the cells of the soft tissues is provided largely by the relatively high levels of sodium, potassium and chloride, along with bicarbonate and phosphate.

Also important is the mucosal pellicle, which results from the formation of a surface coating of proteins, probably involving proline-rich proteins linked by the action of transglutaminase (Bradway *et al.*, 1992); presumably this also plays a protective role.

The polypeptide hormones EGF and NGF are important in maintaining the integrity of the oral mucosa, a function which may be extended to the oesophageal and gastric mucosa too. These polypeptide hormones are secreted as inactive precursors and are activated by the trypsin-like protease kallikrein.

Every day, the oral and oesophageal mucosa is exposed to potentially harmful substances in the diet. High among the list of toxins/potential carcinogens/antidigestive compounds in the human diet are tannins, which occur in tea, coffee, red wine and a variety of fruits. Recent work has shown that proline-rich proteins bind to dietary tannins to form insoluble complexes, a process which may protect us from the harmful effects of these compounds (Mehansho, Butler and Carlson, 1987).

Saliva indeed has myriad functions, but it also contains myriad polyfunctional components to enable it to perform these roles.

Xerostomia: perception and aetiology

This article is at pains to detail the complexity of saliva in terms of composition and function in an attempt to highlight the serious physiological and pathological disturbances that ensue when an individual suffers from long-standing xerostomia (FDI, 1992). Since the salivary flow rate falls to virtually nil during sleep (Schneyer *et al.*, 1956), it is likely that most of us have experienced, many on a regular basis, the discomfort of wakening with a profoundly dry mouth. For a significant number of individuals with poorly or non-functioning salivary glands for whatever reason, that discomfort continues for months, years or a lifetime.

Xerostomia is a clinical sign (the patient often refers to the symptom of 'dry mouth') which implies a decreased quantity of saliva in the mouth, often related to salivary gland dysfunction. Xerostomia itself is not a disease entity. It was first described by Bartley in 1868 and has therefore been recognized for over 100 years (Bertram, 1967). It is clearly outwith the remit of this article to consider the numberous ways of assessing human salivary flow, either as whole saliva or individual (or paired) glands, and unstimulated or stimulated. For further information on this area of clinical interest, the reader is referred to a review article by Dawes (1987) where it is suggested that the sensation of dry mouth is reported when the normal flow rate of unstimulated saliva is reduced by 40–50%.

The sensation of dry mouth is related to dehydrated areas of oral mucosa and/or the rubbing together of such adjacent layers.

Clearly, saliva can be removed from the mouth by swallowing, absorption from the oral mucosa and evaporation. Since saliva entering the mouth is thought to induce swallowing (Mansson and Sandberg, 1975), it is difficult to understand why anyone with even a minimal quantity of saliva would report a dry mouth. Some individuals who complain of a dry mouth have normal parotid salivary flow rates on investigation and it may well be that minor gland hypofunction is more important in giving a sensation of 'dryness' than is generally recognized.

There is a sizeable volume of evidence to suggest that saliva may be absorbed from oral mucosa (in particular from areas of inflammation) but, at present, there is doubt as to the rate or significance of this.

It seems likely that evaporation of saliva from the mouth may also be important in creating the sensation of dryness. Around 10–15% of the population are mouth-breathers at rest and in such individuals, about 50% of inspired air passes through the mouth (Niinimaa *et al.*, 1981). Levine (1989) has calculated that an obligate mouth-breather would lose up to 350 ml day^{-1} of water by this route.

It is therefore evident that 'physiological' xerostomia may occur even in individuals with normal salivary gland function, but there are many other causes of xerostomia and a brief overview of some of these will be given here.

PSYCHOLOGICAL INFLUENCES

It has been recognized for some years now that emotional state may influence salivary

flow rates (Bates and Adams, 1968). Depression and anxiety are implicated in xerostomia and this is often complicated by drug treatment, which itself may cause further inhibition of salivary flow (see below).

SALIVARY GLAND AGENESIS

The failure of one or more major salivary glands to develop is a rare but recognized condition of unknown aetiology.

SALIVARY GLAND AND DUCT CALCULI

The formation of mucus plugs or calcified stones in the duct of a major gland may produce swelling, discomfort and reduced or absent salivary flow. However, xerostomia is unlikely to result if all other glands are functionally intact.

Similar problems may ensue following salivary gland surgery or in trauma, where the nerve supply to the gland may be damaged.

RADIOTHERAPY

Post-radiation atrophy of salivary tissue is a well recognized complication of radiotherapy to the head and neck. This atrophy is due in part to direct radiation damage to acinar tissue; and in part to decreased blood supply to the gland as a result of vascular fibrosis.

The resultant xerostomia is often permanent, but regeneration of acinar tissue may result in a partial return of salivary flow in some patients.

DRUG THERAPY

Drugs are probably the most common cause of salivary gland hypofunction (Schubert and Izutsu, 1987). Documentation of drug-induced salivary gland dysfunction goes as far back as 2000BC, with the Assyrians writing of belladonna being used to stop the flow of saliva (Michei-Pellegrini and Polayes, 1976). Modern-day surgeons and physicians may well use drugs specifically targeted at drying up secretions in the upper aerodigestive tract (e.g. anticholinergic agents) in general anaesthesia, endoscopic procedures or in sialorrhoea. However, the side-effects of many prescribed drugs result in troublesome xerostomia for some individuals. Lavelle (1988) lists over 50 such drugs and Bahn (1972) lists 250, although many of these are combination drugs.

Regardless of absolute numbers, it is clear that a significant number of drugs cause troublesome xerostomia as a side-effect. These include: antidepressants, antipsychotics, antihypertensives, diuretics, antispasmodics, antihistamines, anxiolytics, decongestants and antiparkinsonism drugs. Cancer chemotherapy agents may also cause xerostomia by direct action on the gland acinar and duct cells.

SJÖGREN'S SYNDROME

This is a relatively common chronic inflammatory autoimmune exocrinopathy characterized by decreased lacrimal and salivary gland function, leading to dry eyes

and dry mouth. It is now generally agreed that two forms of the syndrome exist (Scully, 1986):

1. *primary*: where the patient has dry eyes and dry mouth and no clinical evidence of any underlying connective tissue disorder but may have positive serological markers; and
2. *secondary*: where the patient has an underlying connective tissue disorder (e.g. rheumatoid arthritis or systemic lupus erythematosus) and dry eyes and/or dry mouth.

Diagnosis of Sjögren's syndrome is mainly on clinical grounds with important laboratory confirmation. The criteria include: serum immunological markers, sialometry (the measurement of salivary flow rates), sialography (the visualization of gland structure using radiopaque dye techniques), histological examination of minor gland tissue from the lip and scintiscanning. Salivary protein abnormalities are also associated with this disorder (Beeley, Khoo and Lamey, 1991).

The complexity of immunological status in Sjögren's syndrome is outside the scope of this article, but the reader is referred to one of many standard texts on the subject (Talal, Moutsopoulos and Kassan, 1987).

In the analysis of salivary flow rates, some authors use whole saliva (Ben-Aryeh *et al.*, 1984) while others use parotid saliva (Mason and Chisholm, 1975). Reference data are available to compare normal age- and sex-matched controls for both the stimulated and unstimulated situation (Heft and Baum, 1984).

Sialography is performed on parotid or submandibular glands, using either oil- or water-based contrast media. The presence of the 'characteristic pattern' of sialectasis is reported to vary with the type of media used, and sialectasis has been found in 15–20% of normal individuals anyway (Dijkstra, 1980).

Labial gland biopsy has been in use for over 25 years (Chisholm and Mason, 1968) and assumes that changes in minor glands are reflections of the changes in major glands (Bertram and Hjorting-Hansen, 1970). The diagnosis of Sjögren's syndrome centres on the finding of a chronic focal lymphocytic sialadenitis in the minor gland tissue.

Scintiscanning studies of major salivary glands uses a radionuclide (^{99m}Tc sodium pertechnetate) injected intravenously and selectively taken up by the glands. At the time of presentation, around 50–70% of patients with Sjögren's syndrome have some gland function (Stuchell, Mandel and Baurmash, 1984) but gland uptake of the radionuclide is delayed, reduced or absent (Daniels *et al.*, 1979). It has been shown that there is good correlation between stimulated parotid flow rates and scintigraphic findings (Lamey, 1989).

The impact of newer functional scanning techniques (e.g. single photon emission computed tomography – SPECT) on salivary gland disease remains to be seen.

Clearly, evidence from scintiscanning for the presence or absence of functional salivary tissue has enormous implications for patient management, and this concept is central to the next part of our discussion.

With current knowledge, patients with no functional salivary gland tissue require provision of an artificial saliva, whereas patients with some residual functioning tissue may be treated successfully by drug manipulation (Fox *et al.*, 1986), e.g. the parasympathomimetic agent, pilocarpine (Greenspan and Daniels, 1987).

Therapy for xerostomia

The basic principles of management apply to all cases of xerostomia, regardless of cause. However, all reversible aetiological factors should be dealt with, and where the dryness is thought to be drug induced, the possibility of changing to a structurally unrelated drug should be discussed with the patient's physician.

The following basic principles of management are important in xerostomia:

EDUCATION

The patient should be given time with an experienced clinician to discuss, in clear and simple terms, the diagnosis, prognosis and proposed management. This will be followed up appropriately at regular review appointments.

ORAL HYGIENE

A good, understandable oral hygiene regime should be established. Photographic material depicting the effect of xerostomia on the teeth (caries), tooth-bearing tissues (gingivitis and periodontitis) and oral soft tissues (fungal infection and traumatic erosions) is often helpful as an educational tool.

Dentate patients should be encouraged in the regular use of fluoride toothpaste (a preparation specially formulated for dry mouth should be used, if available), together with a daily rinse of a fluoride mouthwash (e.g. 0.05% NaF). The consumption of refined carbohydrate should be discouraged. The use of a chlorhexidine mouthwash as a means of chemical plaque control should also be encouraged.

Patients with dentures should be encouraged in meticulous denture hygiene measures – particularly removing the dentures at bed-time. The use of sucrose- and glucose-containing sweets to keep the mouth moist is to be discouraged as the constant bathing of the oral mucosa with a sugar-rich solution, particularly in denture wearers, may predispose to oral fungal infections.

MONITORING

Routine clinical examination coupled with microbiological sampling is helpful to detect overt or latent oral infection (e.g. *Staphylococcus aureus* or *Candida* sp.).

SALIVARY GLAND HYPOFUNCTION

An educated decision must be made on appropriate management of the dry mucous membrane, i.e. stimulation of natural salivary flow or its replacement with an artificial saliva or combination therapy. This is discussed more fully below.

REGULAR REVIEW

Regular review of patients with xerostomia is essential, as encouragement is a constant feature of management strategy.

Stimulants for salivary gland hypofunction

Stimulants for salivary gland hypofunction may be broadly categorized as 'topical' agents and systemic agents.

'TOPICAL' AGENTS

It has been suggested elsewhere in this article that the act of chewing itself reflexly leads to increased salivary flow and this can be augmented by the use of a flavoured chewing gum. A sorbitol, or xylitol, non-cariogenic formulation is the gum of choice. There may also be another benefit here as the use of sorbitol-containing chewing gum has proven beneficial after a sugary meal or snack in normalizing plaque pH (Jensen, 1986).

Citric acid has been recognized to be one of the most effective salivary stimulants over the years (Watanube and Dawes, 1988b). The use of citric-acid flavoured boilings, sweets or the new chewing gums may well provide effective symptomatic relief from xerostomia, but consideration must always be given to their cariogenic potential in dentate patients, especially as citrate also chelates calcium ions.

SYSTEMIC AGENTS

Systemically administered agents for salivary gland hypofunction have chiefly centred upon Sjögren's syndrome. Numerous anecdotal reports can be cited to suggest that drugs affecting the underlying immune response may lead to an improvement in salivary gland function, e.g. gold (Godfrey *et al.*, 1983) and prednisolone (Talal *et al.*, 1975; Tabbara and Frayha, 1983).

Several agents have been proposed as systemic agents in the treatment of salivary gland hypofunction, often as case reports with a lack of adequate objective measurements or control subjects (Fox, 1987). These include pyridostigmine, bethanechol chloride, pilocarpine hydrochloride, bromhexine, trithio-paramethoxyphenylpropene (also known as anethole trithione), nicotinic acid and vitamin A. The last two of these agents have not been studied in sufficient depth as salivary stimulants to merit any mention.

Bromhexine

This is a mucolytic agent used in chronic bronchitis. Its mode of action is one of increasing the quantity of secretions produced but at a lower viscosity. However, most studies (e.g. Manthorpe *et al.*, 1981; Prause *et al.*, 1984) recorded no change in salivary flow but, interestingly, an increase in lacrimal flow. Avisar *et al.* (1981) reported a subjective improvement in salivary flow but it seems that bromhexine is of little benefit in the treatment of xerostomia.

Trithio-paramethoxyphenylpropene

The mechanism of action of this agent is really unknown, but it has been used, with reported improvement, in drug-induced xerostomia (De Buck, Titeca and Pelc,

1973), chemotherapy (Falkson, 1975), post-radiotherapy (Pazat, 1971) and in Sjögren's syndrome (Epstein, Decoteau and Wilkinson, 1983; Schiodt, Oxholm and Jacobsen, 1986). It has also been of significant reported benefit in combination with pilocarpine (Epstein and Schubert, 1987). This latter study used a group of nine post-radiotherapy patients on a combined regime of pilocarpine, 4 mg four times daily, and trithio-paramethoxyphenylpropene, 25 mg three times daily. Significant increases in resting and stimulated whole saliva flow rates were reported. This suggests a possible synergistic effect between the two drugs.

Paramethoxyphenylpropene has no intrinsic cholinergic activity but it may increase the number of membrane muscarinic receptors or increase their sensitivity to intrinsic acetylcholine or exogenous pilocarpine (Ukai *et al.*, 1984, 1988).

Bethanechol

This agent is an analogue of acetylcholine with both muscarinic and nicotinic cholinergic activity. Given orally at a dose of 25 mg three times daily, it has been effective in counteracting xerostomia caused by tricyclic antidepressants (Everett, 1975) and phenothiazines (Schubert, 1979). Information on use of the drug in other conditions causing xerostomia is strictly limited.

Pilocarpine hydrochloride

The Indians of Paraguay and Brazil were aware for centuries that chewing leaves from the jaborandi plant caused sweating and salivation. This was investigated by missionaries and the plant was introduced to Europe in the late 19th century (Ferguson, 1993). Pilocarpine was isolated from jaborandi (Gerrard, 1875a) and its hydrochloride and nitrate salts were subsequently prepared (Gerrard, 1875b). Pilocarpine is a tertiary amine with cholinergic agonist action at the muscarinic receptors. A β-adrenergic action on the salivary glands is also known, stimulating protein secretion (Zelles, Blazsek and Keleman, 1990).

Numerous trials now point to pilocarpine as an effective agent against xerostomia in various conditions. The use of pilocarpine in this sphere of clinical practice has been suggested for many years (Mason and Glen, 1967), but it is only relatively recently that clinical trials have progressed our theoretical knowledge in any way.

Szabo has used pilocarpine to treat xerostomia in over 500 patients – post-radiotherapy, drug-induced, gland morphology defects and Sjögren's syndrome (Szabo, 1985). A high level of success with minimal side-effects is reported, accomplished by adjusting the pilocarpine dose to each patient's needs. Fox, Atkinson and Macynski (1991) reported the use of pilocarpine capsules, 5 mg three times daily, in a double-blind study for 6 months in 31 patients. Subjective improvement was reported by almost 90% of patients, but side-effects were noted by many (including sweating, flushing, micturition disturbance and gastrointestinal upset).

Ferguson *et al.* (1991) reported the use of pilocarpine solution in doses ranging from 1 to 15 mg, four times daily for up to 2 years in 100 patients with xerostomia. Side-effects were minimized by starting at low doses and increasing gradually to individually titrated quantities of the drug. They reported significant improvement in both salivation and lacrimation.

It would seem, therefore, that pilocarpine is an effective and relatively safe drug to stimulate residual functioning salivary gland tissue and that side-effects, although potentially serious, can be minimized by cautious prescribing and individual patient titration of drug dose. There is considerable variation in response between patients.

Pyridostigmine

This agent is a cholinesterase inhibitor and has a long duration of action. It exhibits both muscarinic and nicotinic activity. Teichman *et al.* (1987) reported a double-blind cross-over study with pyridostigmine at dose of 180 mg twice daily in the treatment of disopyramide-induced xerostomia. Significant symptomatic improvement in xerostomia was reported over a placebo and there was a clear increase in tear production.

OTHER CONSIDERATIONS

Numerous other therapies have been tired in xerostomia, with variable success, e.g. electrical stimulation (Steller, Chou and Daniels, 1988), reflexotheraphy (Pierminova, Goidenko and Rudenko, 1981) and acupuncture (Goidenko, Pierminova and Sitiel, 1985).

A more recent study (Blom, Dawidson and Angmar-Mansson, 1992) showed that patients with xerostomia of whatever cause who received acupuncture showed improved salivary flow rates during and after treatment, with some attaining normal flow rates. Improvement persisted for the follow-up year. Patients who received placebo acupuncture showed positive changes in flow rates during treatment but the changes reverted after placebo treatment was over.

Saliva substitutes

From previous discussion, it is apparent that natural saliva is a complex fluid with multiple functions and any currently available artificial saliva is a poor substitute. The ideal artificial saliva would be long-lasting, lubricant, protective of the oral mucosa to drying and inhibitory to undesired micro-organisms (Levine *et al.*, 1987). Many research centres are presently analysing the structural components of the salivary molecules responsible for various functions and it seems likely that genetic engineering and computer-assisted molecular design will provide a near-ideal saliva substitute in the not too distant future (Levine, 1993).

Currently available saliva substitutes fall into two main categories: those containing carboxymethylcellulose; and those containing natural mucins.

Sorbitol or xylitol is added as a sweetening agent. Electrolytes are added to mimic those in natural saliva, and calcium, phosphate and fluoride may be included to provide remineralization potential (Gelhard *et al.*, 1983). Various preservative agents (e.g. benzoate salts), flavourings (e.g. lemon or peppermint) and colourings may also be added.

Natural mucins are currently prepared from pig gastric mucosa or bovine submandibular gland – a fact that may be offensive to certain religious groups. Formulations containing natural mucins have been used in clinical studies (Vissink *et al.*, 1987; Blixt-Johansen *et al.*, 1992) and have been shown to exhibit relative

lubricating values comparable with those of human saliva, while saliva substitutes with no mucin display very low lubricating properties (Hatton *et al.*, 1987; Levine *et al.*, 1987). Visch *et al.* (1986) also showed that mucin-containing artificial salivas were preferred to those containing carboxymethylcellulose by patients with xerostomia of various causes. Duxbury, Thakker and Wastell (1989) demonstrated that a mucin-containing preparation was the most preferred artificial saliva tested, although not all subjects found it totally satisfactory. The effect of both mucin-based and carboxymethylcellulose-based preparations, assessed either objectively by friction measurement or subjectively, is similar for both preparations and lasts little more than 15 minutes (Olsson and Axéll, 1991). A new thermogelling system, based on ethyl (hydroxyethyl) cellulose and an ionic surfactant, which increases in viscosity on warming, was effective for no longer time periods than carboxymethylcellulose preparations (Olsson *et al.*, 1993). Unfortunately, the electrolytes in saliva probably affect the thermogelling properties of this preparation.

Other artificial saliva agents available for treatment of xerostomia are glycerine and lemon mouthwash (Weisenfeld, Stewart and Mason, 1983) where the glycerine acts as a surface-wetting agent and 2.5% citric acid as a gland stimulant; glycerine lemon swabs may be used in nursing care. Use of polyethylene oxide solutions has also been reported (Vissink *et al.*, 1984). Preparations containing acids such as citric acid and malic acid are, however, limited in their usefulness due to their erosive potential on enamel and dentine in dentate individuals (Weisenfeld, Stewart and Mason, 1983). Whereas the rheological properties of mucins and human saliva are similar, carboxymethylcellulose and polyethylene oxide solutions are non-Newtonian liquids and therefore somewhat different. The addition of albumin to mucin solutions results in a preparation with rheological properties even more similar to human whole saliva (Mellema *et al.*, 1992).

Other ideas for the treatment of xerostomia with artificial saliva have included the construction of a reservoir in a denture which can be filled with an artificial saliva from a syringe. The patient then moistens the mouth by sucking some artificial saliva out of the reservoir (Vissink, Huisman and S'-Gravenmade, 1986; Hirvikangas, Posti and Makila, 1989).

Currently available proprietary artificial salivas which can be prescribed in the UK are (British National Formulary, 1994):

1. Luborant® (Antigen, Trafalgar House, Union Street, Southport PR9 0QS, UK) which is licensed for any condition giving rise to a dry mouth and contains carboxymethylcellulose and fluoride.
2. Glandosane® (Frensenius Ltd, 6–8 Christleton Court, Stuart Road, Manor Park, Runcorn, Cheshire WA7 1ST, UK) which is licensed only for dry mouth associated with radiotherapy or sicca syndrome and contains carboxymethylcellulose. It comes in neutral, lemon or peppermint flavours.
3. Saliva Orthana® (Nycomed (UK) Ltd, Nycomed House, 2111 Coventry Road, Sheldon, Birmingham B26 3EA, UK) which is licensed only for dry mouth associated with radiotherapy or sicca syndrome. It contains porcine gastric mucin and is available in two forms: aerosol spray and lozenges. The spray is available with and without fluoride; the lozenges have no fluoride content.
4. Salivix® (Thames Laboratories Ltd, Abbey Road, The Industrial Estate, Wrexham,

Clywd LL13 9PW, UK) which contains acacia and malic acid. It is available only as a pastille and is licensed for use in radiotherapy-induced xerostomia or sicca syndrome.

The future

The complexity of natural saliva, in terms of composition and function, makes finding the ideal substitute very difficult. Current formulations have aimed only to simulate the inorganic composition and rheological properties of saliva. This is not unreasonable because it is only within the past decade that the nature and unusual polyfunctional properties of the families of proteins in saliva have become apparent. Future preparations will therefore need to contain active components which modulate the oral microflora by mechanisms analogous to natural constituents, as well as by regulating remineralization and protecting the dentition and the soft tissues.

But the genes that code for these bioactive proteins are already being cloned and it seems reasonable to assume that the recombinant gene technology already exists which would enable them to be synthesized in the same way as human growth hormone and insulin are now being prepared. But would such a saliva need to contain the normal range of proteins and peptides? Or could it be possible that because many of the proteins are polyfunctional, a selection would be adequate? Could such proteins be of use in maintaining oral health in patients with normal salivary flow rates, perhaps in the prevention of dental caries or treatment of oral soft-tissue infections?

As the three-dimensional structures and the nature of bioactive domains of salivary proteins are also now beginning to be investigated, the possibility of computer-designed salivary molecules with specific amino acid sequences and carbohydrate side-chains which can be chemically synthesized is no longer in the realms of fantasy. Might it be possible to design molecules which are more effective than those that have evolved over the millennia?

The vehicle or mode of delivery of saliva substitutes, however, continues to present a particular problem. How can compounds be retained in a cavity, one biological function of which is the reflex removal by swallowing of substances ingested? The answer may ultimately lie with tissue transplants of biocompatible cells.

A vast cohort of individual patients suffering from the very handicapping condition of xerostomia deserve the best efforts of science, medicine and dentistry to design and develop a saliva substitute which will overcome so many of the problems encountered daily by them. Artificial saliva – a stimulating subject, indeed.

Acknowledgements

Figure 1 is reproduced by permission of Baillière Tindall. The authors thank Mrs F. Newman and Mr K. Hunter for preparation of the gel shown in *Figure 2*. Our sincere thanks are due to Miss Grace Martin for secretarial assistance.

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