The Pathology and Surgery of the Salivary Glands

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Chapter 5: Functional disorders: xerostomia and drooling

The amount of saliva secreted and to a large extent, its composition, are normally controlled by the autonomic nervous system. Other factors can also interfere with function, namely drugs affecting autonomic responses, deficiency of fluid, and disease or destruction of the gland parenchyma.

Autonomic Control of Salivary Gland Function

Garrett (1987) has carried out extensive animal experimentation in this field. His main conclusions are that normal reflex secretion of saliva depends on centrally coordinated parasympathetic and sympathetic activity.

--> Parasympathetic impulses
- usually evoke most of the fluid secreted;
- cause variable degrees of exocytosis of some cells;
- induce contraction of myoepithelial cells;
- cause vasodilatation as part of the secretory process.

--> Sympathetic impulses
- are more intermittent;
- act essentially on cells receiving parasympathetic impulses and tend to have synergistic effects;
- frequently do not cause much modulation of fluid;
- tend to modulate the composition of saliva by increasing exocytosis from certain cells;
- usually induce contraction of myoepithelial cells;
- some sympathetic fibres exercise tonic effects on blood vessels; these fibres are likely to be under separate central control and not involved directly in the reflex secretory pathway.

Mechanisms of Salivary Secretion

The mechanisms of saliva secretion have been reviewed in detail by Baum (1993) who emphasizes that much of the information (as presented below) comes from study of rat salivary glands. Little is known of the mechanisms of human salivary secretion but such data as exists generally conform to the broad principles discussed here.

As Baum (1993) has described, saliva is produced only in response to neurotransmitter stimulation. Neurotransmitters probably bind to specific receptors in the basolateral region of the acini. Noradrenaline binds to both alpha- and beta-adrenergic receptors while acetylcholine binds to cholinergic receptors. These receptors depend on G-protein (guanine nucleotide-binding regulatory protein) for transduction of the neurotransmitter stimuli. The G-proteins that carry the stimuli into the acinar cells are heterotrimeric molecules consisting of alpha-,

beta- and gamma-subunits. The alpha-subunit is the site of guanine nucleotide binding and probably conveys the functional specificity to a G-protein. Binding of a neurotransmitter to a receptor greatly strengthens the ability of a receptor to associate with a G-protein. The association with a G-protein stimulates in turn, replacement of GDP by GTP at the nucleotide binding site and promotes dissociation of the heterotrimer into a free alpha-subunit and a betagamma complex. The alpha-subunit can then activate the appropriate receptor molecule. Activation continues until the GTP is broken down into GDP by endogenous enzymic (GTPase) activity in the alpha-subunit. The GDP bound to the alpha-subunit then unites with the betagamma complex to regenerate the heterotrimeric molecule. There is also some evidence to suggest that the betagamma complex or even the beta-subunit alone may be able directly to activate some receptor molecules. The best recognized signal transduction processes in salivary glands are first, generation of cAMP as a result of beta-adrenergic receptor stimulation and second, formation of 1,4,5-inositol triphosphate (IP_3) after acetylcholine receptor stimulation. The latter leads to calcium-ion mobilization and, as a consequence, fluid secretion.

The specific mechanism by which cAMP acts a an intermediary in protein exocytosis remains unclear. Typically, cAMP elicits a response by activation of cAMP-dependent protein kinase and protein phosphorylation. In rat salivary gland, beta-adrenergic receptor stimulation leads to a rise in cAMP levels, activation of the A-kinase, phosphorylation or dephosphorylation of several cellular proteins and thus amylase or glycoprotein secretion by the parotid or submandibular glands, respectively. Indeed, treatment of rat parotid and submandibular gland cells with cAMP analogues also leads to protein secretion. Kinetic analyses suggest that the 24-26 kDa species protein is the most probable candidate for phosphorylation though its function in protein exocytosis is unknown. Moreover, the role of A kinase-dependent phosphorylation in rat parotid gland protein secretion has become somewhat controversial.

It is well established that Ca^{2+} plays a central role in fluid secretion by acinar cells in response to acetylcholine receptor stimulation. Activation of this receptor leads to fluid-related signal transduction via coupling to a G-protein often designated G_p. The latter is probably a member of the $G_{q/11}$ family which is known to couple to phospholipase C. Activation of phospholipase C results in hydrolysis of a minor membrane phospholipid, phosphatidylinositol 4.5-biphosphate, and formation of second messengers IP_3 and diacylglycerol. IP_3 is the main mediator of Ca^{2+} mobilization and thus of fluid secretion, but diacylglycerol can promote activation of protein kinase C and lead to stimulation of a minor exocytic pathway.

IP_3 binds to a receptor protein located on an intracellular Ca^{2+} storage pool that is probably related to, or part of, the endoplasmic reticulum. The IP_3 receptor also functions as a Ca^{2+} release channel and allows stored Ca^{2+} to move down a concentration gradient into the cytoplasm. These Ca^{2+} levels quickly rise approximately tenfold as a consequence of acetylcholine receptor stimulation. This reaction triggers a cascade of event which include sustained Ca^{2+} entry and activation of specific ion-transport pathways. Generation of fluid in the acinar lumen is the final result, by mechanisms discussed in detail by Turner (1993).

Extracellular Ca^{2+} and entry of Ca^{2+} sustain high levels of salivary fluid secretion over long periods. However, mechanisms of Ca^{2+} entry into acinar cells which are non-excitable and not voltage-activated, are poorly understood. Possible mechanisms for Ca^{2+} entry into acinar cells include a direct receptor-gated Ca^{2+} channel, a G-protein-activated Ca^{2+} channel, and a second messenger-activated Ca^{2+} channel. However, there is little evidence for the involvement of the first two of these mechanisms and only a few reports have suggested that Ca^{2+} entry may be activated by the synergistic action of IP_3 and its metabolite 1,3,4,5-inositol tetrakisphosphate (IP_4).

Currently, the most favoured explanation of the mechanism of Ca^{2+} entry appears to be the one termed 'capacitative Ca^{2+} entry'. This suggests that depletion of the Ca^{2+} storage pool provides the driving force for sustained Ca^{2+} entry. Support for this theory comes from studies that have shown that graded depletion of the Ca^{2+} store has led to similarly graded Ca^{2+} entry.

Use of non-receptor activation by, for example, thapsigargin, to deplete the Ca^{2+} store also leads to Ca^{2+} entry. Though the exact mechanism by which depletion of Ca^{2+} stores causes Ca^{2+} entry is uncertain, it is clear that it can be modulated by extracellular pH and cytoplasmic Ca^{2+}.

Acinar cells are responsible for production of the fluid component of saliva and most of the proteins that it contains. The acinar cells are water-permeable and derive fluid from the surrounding blood vessels. The fluid is carried by the duct system which is impermeable to water. During passage through the ducts, there is exchange of electrolytes. Most of the Na^+ and Cl^- ions are removed, but some K^+ and HCO_3^- ions as well as a little protein, are added.

Primary fluid secretion in salivary acini

As in all secretory epithelia, fluid transport in salivary gland cells is thought to be driven osmotically by transepithelial salt gradients. Turner (1993) has explained that studies on rat and rabbit salivary glands have suggested three mechanisms for primary salivary fluid secretion. Unexpectedly, these mechanisms do not appear to be alternative explanations for salivary fluid secretion, but appear to operate concurrently within the same gland or possibly within the same acinar cells.

The first mechanism, of which the other two can be regarded as variations, is that fluid secretion depends on the combined action of four membrane-transport systems, namely: (i) an Na^+-K^+-$2Cl^-$ cotransporter that is located in the basolateral membrane of the acinar cells; (ii) a basolateral Ca^{2+}-activated K^+ channel; (iii) an apical conductive pathway for Cl^- which is presumably a Ca^{2+}-activated Cl^- channel; and (iv) the Na^+/K^+ ATPase.

In the resting state, both K^+ and Cl^- are concentrated in the acinar cell above electrochemical equilibrium, with K^+ being concentrated by Na^+/K^+ ATPase and Cl^- by Na^+-K^+-$2Cl^-$ cotransporter. As discussed earlier, secretagogue stimulation leads to a rise in intracellular Ca^{2+} concentration and in turn, opening of the basolateral Ca^{2+}-activated K^+ channel and the apical Cl^- channel. Increases in K^+ and Cl^- conductance allow KCl to flow out of the cell and this results in accumulation of Cl^- ions and their associated negative electrical charge in the acinar lumen. As a consequence, electrical attraction causes Na^+ to

leak from the interstitium through the tight junctions to follow Cl^-. The resulting osmotic gradient for NaCl causes transepithelial movement of water from the interstitium to the lumen. Continued influence of an agonist results in a transepithelial chloride flux and concomitant fluid secretion. This is sustained by Cl^- entry via Na^+-K^+-$2Cl^-$ cotransporter and exit via the apical Cl^- channel. Removal of the stimulus is followed by a fall in intracellular calcium concentration to resting levels, closure of the K^+ and Cl^- channels and return of the cell to its resting state.

The second mechanism is similar except that the basolateral Na^+-K^+-$2Cl^-$ cotransporter is replaced by a $Cl^-/HC)_3^-$ exchanger acting in parallel with an Na^+/H^+ exchanger. A fall in intracellular Cl^- concentration as a result of secretagogue KCl loss, thus leads to entry of more Cl^- in exchange for HCO_3^- acidification of the cytoplasm. This HCO_3^- loss is buffered by the Na^+/H^+ exchanger which uses the extracellular-to-intracellular Na^+ gradient generated by Na^+/K^+-ATPase, to drive protons out of the cell.

Unlike the first two mechanisms in which Cl^- is the secreted ion, the third involves acinar HCO_3^- secretion. In this last mechanism, CO_2 enters the acinar cell across the basolateral membrane and is converted to HCO_3^- plus a proton, by intracellular carbonic anhydrase. The HCO_3^- is lost across the apical membrane via an anion channel which is possibly the same as that involved in Cl^- secretion. The proton is expelled by the basolateral Na^+/H^+ exchanger.

Antibacterial Substances in Saliva

Molecules such as lysozyme, thiocyanate-dependent factors and lactoferrin can be shown to have antibacterial activity *in vitro.* Other factors which might affect bacterial activity in the oral cavity are the pH and buffering capacity of saliva and its content of immunoglobulins.

Despite the presence of these substances, the oral cavity supports a flourishing population of microbes including a great variety of pathogens. Dental caries is usually also active on a high sugar diet and periodontal disease is likely to progress unless a high standard of oral hygiene is maintained. Despite great efforts to show protection against these diseases by saliva, the findings have been unconvincing. Moreover, salivary mucins for example, may have harmful effects by promoting adhesion of bacteria to the teeth. That aside, putative effects of protective substances are difficult to substantiate in humans because of the difficulty of performing critical experiments.

It is clear that the oral cavity has a high level of local immunity. The large bony wounds resulting from extraction of teeth normally heal rapidly despite contamination by the vast and pathogenic flora that proliferates in periodontal pockets. However, this fortunate outcome is likely to result from local tissue immunity rather than from salivary components.

Another factor which may affect the nature of the bacterial flora of the mouth is bacterial competition for nutrients in their individual ecological niches rather than antibacterial substances in the saliva. However, the bacterial flora of the mouth is undoubtedly affected by low salivary flow rates which, in particular, promote cariogenic activity and the proliferation of *Candida* species. Nevertheless, xerostomia does not appear to promote periodontal disease.

The only aspect of saliva production that can be reliably related to protection against infection is that of the overall flow rate. In conditions of xerostomia, the oral bacterial flora changes and in particular *Candida albicans* and staphylococci are likely to flourish. Dental caries activity and mucosal infections are thus promoted.

Collection of Saliva and Measurement of Salivary Flow Rates

Saliva may need to be collected to provide a sample for assays of various types but more frequently flow rates are required for objective confirmation of xerostomia. Both unstimulated (resting) and stimulated flow can be measured. A variety of apparatus has been devised to carry out these measurements and record them automatically, but are mainly for research purposes.

Many methods have been devised and may be summarized as follows:

--> Unstimulated flow of whole (mixed) saliva.
--> Stimulated flow of whole saliva.
--> Stimulated or unstimulated flow from individual glands.

Salivary stimuli

A variety of stimulants to salivary secretion have been recommended. These are either systemic or local sialogogues. The main systemic sialogogue that has been used is the parasympathomimetic drug, pilocarpine. Though effective, pilocarpine does not precisely reproduce the balance of sympathetic and parasympathetic activity responsible for normal secretion. One consequence is that it can alter the concentrations of normal constituents, particularly sodium and potassium ions. Pilocarpine can also cause systemic cholinergic effects such as colic, diarrhoea, bradycardia and sweating which can be troublesome.

Local sialogogues are convenient and generally more satisfactory. A good example is 5% citric acid solution which is a potent sialogogue and does not interfere with the composition of the final specimen. Five drops of this solution can be dropped from a pipette or disposable syringe onto the dorsum of the tongue. This may be used as a preliminary measure before collecting unstimulated saliva. The purpose of this manoeuvre is to flush out stagnant secretions that confuse the analysis. Thus, citric acid is applied to the tongue and saliva is collected for 15 min to eliminate rest transients.

Stimulates or unstimulated saliva can then be collected for periods of 15-30 min as necessary. However, Ericson (1969) has pointed out that the results obtained by different sialometric methods are not necessarily comparable because individuals with a vigorous secretory response to one stimulus do not necessarily have a similar response to another.

Collection of mixed whole saliva

A variety of methods of collecting saliva from individual glands or collecting mixed whole saliva is available. Many of these methods require specially made equipment and are mainly suitable for research purposes.

Mason and Chisholm (1975) described the following methods:

--> Spitting
--> Drainage
--> Suction
--> Cotton-wool rolls.

More recently, Navesh (1993) has reviewed methods for collecting saliva.

Spitting and drainage methods

The patient is put into a comfortable sitting position with the head inclined forward and encouraged to spit at one-minute intervals or to allow saliva to drain out of the mouth into a funnel draining into a sterile collecting vessel.

Suction

This method requires the equipment associated with a dental chair. The patient is put into a similar position as before to allow saliva to collect into the anterior floor of the mouth. A saliva ejector is placed behind the lower incisor teeth and the secretion is trapped in a bottle intervening between the ejector and the drainage system.

Cotton-wool rolls and other absorbent devices

Pre-weighed cotton-wool rolls are put under the tongue for a two-minute period then taken out and reweighed. This method allows quantitation only. More recently, proprietary devices such as OraSure have been introduced to simplify saliva collection and preserve it for analysis. Its use has been reported by Thieme *et al* (1994) for collection of saliva for determination of measles, mumps and rubella immunization status. OraSure is a cotton-fibre pad which can absorb 1 mL of oral fluid. It is saturated with hypertonic saline solution, dried and mounted on a plastic handle. It is placed between the gingiva and buccal mucosa for two minutes, then withdrawn, and placed in an antiseptic transport medium. In the laboratory, the saliva specimen is recovered by centrifugation.

While there appear to be advantages to the OraSure system for gathering oral fluid, it must be appreciated that by virtue of the filtering effect of the cotton-fibre pad, it does not collect whole saliva. Cordeiro *et al* (1993) found that the levels of IgG in OraSure oral fluid were three- to fourfold higher than those of saliva, and amylase levels were two- to fourfold higher. They suggested that the cotton-fibre pad might promote passage of crevicular gingival exudate and stimulate secretion of amylase as a result of the pad's buffer solution. However, North *et al* (1993) found that the OraSure system provided a reliable index of tobacco usage by means of cotinine salivary assay.

Collection of parotid gland saliva

Pure parotid saliva can be collected by cannulating the parotid duct with a polythene catheter or using a suction tip.

Catheterization of the duct allows collection of uncontaminated saliva but is uncomfortable for the patient and the tube has to be held in place.

For suction, the most widely used device is the Carlson-Crittenden/Lashley cup, the centre of which is placed over the parotid papilla. The cup consists of a central chamber into which the saliva flows and an outer chamber to which suction is applied to cause the cup to adhere to the buccal mucosa (Fig. 5.1).

Collection of submandibular gland saliva

In the past, submandibular gland saliva in particular was collected by catheterizing the submandibular duct after dilatation. This technique is neither very simple nor comfortable for the patient.

Segregator appliances require to be purpose-made to fit the patient's mouth and function in the same way as the Carlson-Crittenden cup. The device, which fits the anterior floor of the mouth, has a central collecting chamber, isolated by a surrounding ridge from the outer suction chambers.

Advantages and disadvantages of collection methods

The choice of method depends on the type of investigation being carried out. If for example, the aim is to investigate viral shedding from the parotid gland, then catheterization of the duct has to be carried out or a Carlson-Crittenden cup used. Mandel (1980) has listed the variations in concentrations of sodium, potassium, calcium, magnesium, bicarbonate and phosphate ions in the secretions of individual glands and whole saliva. But, overall, his findings suggest that collection of whole saliva is satisfactory for such measurements.

For straightforward measurement of salivary flow rate for confirmation of the diagnosis of Sjögren's syndrome, in particular, the simple spitting method for unstimulated saliva over a period of 10 or 15 min, requires minimal equipment and is satisfactory. In the past, it was thought that since the parotid glands were predominantly affected by this disease, it was necessary to collect parotid saliva. However, the total activity of all salivary glands including the minor glands make a greater contribution to the amount of saliva produced under normal conditions. Stimulated parotid flow is unsatisfactory because of difficulties that some patients have with the collection devices and because of the variety of stimulants used have cause confusion as to what is meant by abnormal flow rates.

Atkinson *et al* (1990) assessed salivary gland function and its clinical features in 64 patients with Sjögren's syndrome (SS). They found that stimulated salivary flow rates correlated inversely with the microscopic lymphocytic focus scores in labial gland biopsies and therefore suggested that stimulated flow rate studies were a particularly useful alternative to repeated lip biopsies in long-term studies of the progress of the disease. By contrast, Saito *et al* (1991) found that in 113 patients with dryness of both mouth and eyes, periductal lymphocytic infiltration of labial glands was found in only 50% of those with reduced salivary flow, but correlated better with xerophthalmia and SS-A and SS-B autoantibodies (see Chapter 4). Nevertheless, Speight *et al* (1992) found that a whole unstimulated salivary flow rate of

≤ 0.1 mL/min was 81% predictive of Sjögren's syndrome if other causes of xerostomia could be excluded. Saliva was collected by encouraging the patients to spit gently or drool into a beaker for 15 min.

Though controversy persists about methods of saliva collection and whether or not sialogogues should be used, there is a growing body of opinion as reflected by Speight *et al* (1992) that collection of unstimulated mixed saliva best reflects the normal resting state. The European Community Study Group on Diagnostic Criteria for Sjögren's Syndrome (1994) have also concluded that collection of whole unstimulated saliva is the best method for confirming the degree of xerostomia (Vitali *et al*, 1994).

In practice, therefore, an adequate indication of salivary flow rates is obtained by putting the patient in quiet surroundings and asking them not to swallow but to expectorate all saliva into a Universal or other container over a period of 15 min. The measurement may then be repeated after stimulating the flow with a few drops of lemon juice or 5% citric acid, dropped on the tongue. Typical normal flow rates are 0.1 mL/min (resting) and 1.5-2 mL/min after stimulation with lemon juice. In established Sjögren's syndrome the stimulated flow rate is typically about 0.25-0.5 mL/min.

Unstimulated whole saliva flow rates are not merely simpler to measure but probably give a better guide to the level of discomfort felt under normal conditions by the patient.

Changes in Composition of the Saliva

The composition of saliva changes in disease states, particularly those causing xerostomia and these changes have been reviewed by Mandel (1990). A variety of hormones, antibodies and drugs can also be assayed in this secretion.

Hormones that can be monitored in saliva include aldosterone, cortisol, dehydroepiandrosterone, testosterone, 5alpha-dihydrosterone, 17beta-hydroxyprogesterone, progesterone, 17beta-oestradiol, oestriol, oestrone, insulin and melatonin. Salivary assay of cortisol has been reported to be more reliable than serum levels for monitoring adrenal cortical function.

It must be emphasized that most studies on the composition of saliva have been based on relatively few subjects and may therefore be biased. Differences in laboratory methods may also result in discrepancies in the results which are not, in fact, real. Other complications are the presence of enzymes which are probably of bacterial origin.

The composition of saliva in health provides a baseline for variations resulting from disease or drug administration. Unfortunately, even in healthy persons, many variables such as the following can affect the composition of saliva:

--> Flow rate.
--> Source. Saliva specifically collected from the major gland differs in composition from whole saliva which includes that secreted by the minor glands.
--> Diurnal variation.
--> Duration and type of stimulus.

--> Rest transients. The concentration of ions such as K^+ may vary according to whether the saliva sample is taken shortly after stimulation or after the flow has been allowed to continue for several minutes.

--> Age and gender differences. Findings on differences in salivary flow and hence salivary composition, according to the age or gender of the subjects have been conflicting. Findings that, for example, salivary flow rates are lower in older females than males could conceivably be biased by the presence of unsuspected Sjögren's syndrome among the females.

--> Plasma levels. The concentration of many molecules in saliva is related to and varies with their plasma levels.

--> Diet. Findings suggesting that a predominantly carbohydrate diet, for example, leads to higher concentrations of salivary amylase or that high protein diets lead to higher concentrations of salivary amylase have not been widely confirmed or are conflicting.

--> Drugs. Any drug which affects salivary flow rates can affect the concentration of salivary constituents that are flow-dependent. Important drugs which affect salivary flow rates are shown in Table 5.1.

--> Hormonal effects. Mineralocorticosteroids affect both plasma and salivary concentrations of ions such as Na^2 and HCO_3^-. It has also been suggested that the concentration of some salivary constituents such as Ca^{2+} and Na^+ may fall and K^2 levels rise at the time of ovulation.

Constituents of saliva are shown in Table 5.2 but in view of the comments already made, these figures should be accepted with caution.

Table 5.1 Drugs liable to cause xerostomia

1. Drugs with antimuscarinic activity

- Atropine and analogues (hyoscine, ipratropium, etc)
- Tricyclic antidepressives
- Monoamine oxidase inhibitors
- Phenothiazines and related neuroleptics
- Orphenadrine, benzhexol and related anti-parkinsonian agents
- Antihistamines
- Ganglion blockers and clonidine
- Anti-emetics (antihistamines, hyoscine and phenothiazines)

2. Drugs with sympathomimetic activity

- 'Cold cures' and decongestants containing ephedrine or phenylpropylamine
- Bronchodilators (isoprenaline, orciprenaline, etc)
- Appetite suppressants, particularly amphetamines and diethylpropion.

Salivary assays in diagnosis

Saliva is undoubtedly a valid medium for many diagnostic assays. Collection of saliva is non-invasive, painless and obviates the risk of needle-stick injuries. Nevertheless, most clinicians are unused to collecting saliva but practised in collecting blood which can usually be done more quickly. Moreover, most laboratories use equipment for handling blood and are

unused to dealing with saliva with its mucins and other costituents or contaminants which may affect the assays. With regard to drug assays, few current pharmacology texts even mention the possibility of using saliva.

Mandel (1993) has provided an interesting brief history of the uses of saliva in diagnosis. He has described both the value of salivary assays in diagnosis and the difficulties in getting them accepted. As he had earlier stated (Mandel, 1990) 'Saliva is not one of the popular body fluids. It lacks the drama of blood, the sincerity of sweat and the emotional appear of tears'.

Inorganic ions

The findings of Mandel (1980) include the following:

Sialadenitis

Raised Na^+, K^+, Ca^{2+} and PO_4^- levels.

Radiation damage

Raised Na^+, Ca^{2+}, Mg^{2+} and Cl^- levels.

Sjögren's syndrome

Raised Na^+, Cl^- and PO_4^- in parotid gland saliva.

Cystic fibrosis

Raised Na^+, Ca^{2+}, and PO_4^- levels. Mandel (1980) suggested that the combined Ca^{2+} PO_4^- concentrations formed a useful diagnostic index.

Aldosteronism

Depressed Na^+ but raised K^+ levels. Mandel (1980) suggested that the ratio of Na^+/K^+ could form a useful diagnostic index.

Hypertension

Depressed Na^+ levels.

Alcoholic cirrhosis

Raised K^+ levels.

Hyperparathyroidism

Raised Ca^{2+} levels.

Diabetes mellitus

Depressed HCO_3^- levels.

Psychiatric illness (not otherwise specified)

Raised Na^+ and K^+ levels. Mandel (1980) suggested that the product Na^+ x K^+, could form a useful diagnostic index.

Organic components

Mandel's (1980) findings included the following:

Sjögren's syndrome

Raised total protein and $beta_2$-microglobulin levels in parotid gland saliva.

Cystic fibrosis

Raised total proteins, amylase, lysozyme in submandibular gland saliva and glycoproteins in parotid gland saliva.

Cirrhosis

Raised total protein and amylase in parotid gland saliva.

Hyperparathyroidism

Raised total protein.

Diabetes mellitus

Raised total protein, IgA, IgG and IgM, and raised glucose levels.

Sarcoidosis

Depressed amylase and lysozyme levels.

Two diseases that have been studied in terms of sialochemistry in particular detail are Sjögren's syndrome and cystic fibrosis.

Sjögren's syndrome

Considerable difficulties are sometimes found in confirming the diagnosis of Sjögren's syndrome because of the great variability in the abnormalities that may be detected. The problem is well illustrated by the necessity to institute the European Community Study Group on Diagnostic Criteria for Sjögren's Syndrome. The Group concluded in its 1994 report, that unstimulated whole saliva flow rate and minor salivary gland biopsy are two of the most

valuable diagnostic tests. Neither involves either exposure to X-rays or scintigraphy.

Mandel (1990) has noted that salivary function changes, in addition to a lowered secretion rate include raised Na^+ and Cl^+ but lowered PO_4^-, raised lactoferrin, raised $beta_2$-microglobulin, raised kallikrein concentrations and a 20-fold elevation in the concentration of phospholipids. Parotid gland lysozyme was also found to be raised in primary but not in secondary Sjögren's syndrome. Such changes could be useful as screening tests that could indicate whether labial gland biopsy or other tests were indicated but perhaps more important, for monitoring disease progress.

Table 5.2 Typical figures for important constituents of saliva

	Unstimulated		Stimulated	
Substance	Mean ± s. d.	Range	Mean ± s. d.	Range
Protein (g/L)		1.4-6.4	2.8	1.8-4.2
IgA (mg/L)	194.0			
IgG (mg/L)	14.4			
IgM (mg/L)	2.1			
Amylase (g/L)	0.38 ± 0.08			
Lysozyme (mg/L)			108.9 ± 12.9	3.7 ± 62.5
Carbonic anhydrase (K/L)			2100	
Histamine (mg/L)	0.15	0.11-0.18		
Glucose (mmol/L)	0.55±	0.048	0.056	0.02-0.17
Urea (mmol/L)	3.22 ± 2.5	2.33-12.5	2.17	0.1-4.8
Creatinine (mg/L)	0.09	0.05-0.18		
Cholesterol (mg/L)	0.2	0.07-1.3		
Sodium (mmol/L)	6.2 ± 0.46		26.4 ± 11.8	
Potassium (mmol/L)	21.6 ± 1.2		19.7 ± 3.9	
Calcium (mmol/L)	1.56 ± 0.06		1.48 ± 0.04	
Magnesium (mmol/L)	0.21 ± 0.01	0.15 ± 0.04		
Phosphate (mmol/L)	6.2			
Chloride (mmol/L)	17.4 ± 1.4		29.0 ± 8.8	
Iodide (microm/L)	0.8	0-3		1-3
Fluoride (microm/L)	15 ± 0.68	0.5-3	0.56 ± 0.25	0.25-1.2.

Cystic fibrosis

Although all exocrine gland function is affected the clinical effects on salivary gland function are minimal, but there are significant changes in salivary composition. In particular, there are dramatic elevations in salivary protein and Ca^{2+} concentrations. The complexing of these substances leads to obvious turbidity of the saliva. This is such as to obstruct the excretory ducts of the minor salivary glands. Their greatly depressed secretion rate can be measured in the accessible labial glands with a capillary tube.

Hormone monitoring

Lipid-soluble, unconjugated steroids pass readily into saliva and their concentrations are proportional to the concentrations of free, unbound steroids in plasma as discussed by Ferguson (1987). Read (1993) has discussed the current status of salivary oestrogen and androgen measurements. An informative light on the limitations of salivary hormone measurements is cast by the problems of salivary dehydroepiandrosterone sulphate (DHA-S) assay. The salivary concentration of this hormone is only about 0.1% of that in plasma and is a poor predictor of plasma levels in an individual or a particular plasma sample. The inconsistencies arise from the fact that the concentration in parotid fluid is particularly low. Most salivary DHA-S probably therefore comes from blood in gingival exudate. The fall in DHS-S concentrations with high salivary flow rates helps to confirm this possibility. If this is the case, concentrations of DHA-S in saliva depend largely on the degree of contamination and as has been shown are affected by the oral conditions in the person being tested and the method of saliva collection.

Read (1933) concluded that salivary assays for testosterone in the male, androstenedione and oestriol in particular were valuable but considered that the value of salivary testosterone and oestradiol in the female remained unproven.

Ellison (1993) has considered certain technical aspects of salivary progesterone assay and their interpretation, and reviewed the utility of these assays for clinical purposes, particularly for the diagnosis and treatment of infertility. Salivary progesterone levels are valid indicators of plasma levels and Ellison (1993) concluded that their assay was particularly valuable because of the ease of obtaining serial samples from the same individual. On a shorter time scale, salivary monitoring could provide samples at short intervals for characterization of pulsatile progesterone patterns without the inconvenience and expense of hospitalization. The ability to adapt salivary progesterone monitoring under field conditions also made possible basic research on a wide scale into human reproductive biology. Elisson's (1993) concerns were the need for standardized laboratory procedures, methods of data reduction and analysis, recognized reference ranges and statistics on diagnostic efficiency. Further the low absolute levels of steroids in saliva placed a premium on an unusually high level of quality control in the laboratory.

Testosterone is a hormone that may additionally affect social behaviour. Assay of testosterone in saliva provides a valuable method for field studies. Dabbs (1993) has summarized the preliminary findings on differences in salivary testosterone levels both between and within individuals. Studies on individual differences are being carried in relation to violent and anti-social behaviour. Studies on changing testosterone levels are being carried out on winning sports contests, winning non-athletic events, winning political contests, 'winning' games of sex, and vicarious winning (sports spectators). Broadly speaking, it appeared that salivary testosterone levels fell in losers and were unchanged or rose in winners according to the importance of the victory. While animal studies confirmed elevations of testosterone with real or anticipated sexual activity there were considerable difficulties in obtaining samples at appropriate times in humans. However, it appeared that testosterone levels were higher on evenings when there was sexual activity, particularly in females, and low in its absence. Studies on salivary testosterone levels under conditions of abuse, depression and suicide were also in progress.

Salivary monitoring of viral antigens and antibodies

Viral hepatitis

In view of the hazard to surgeons, a simple method of detecting carriers of hepatitis viruses has obvious benefits.

The presence of hepatitis B antigen in saliva was demonstrated by Broderson *et al* (1974). Shikata *et al* (1985) were able to detect the surface antigen (HBSAg) and the core antigen in hepatocytes but could not detect either in parotid gland parenchymal cells. However, in the patient with the highest serum titre of HBSAg immunoreactivity was detected in the vascular wall and luminal fluid of the parotid gland. Piecentini *et al* (1993) have reported high sensitivity and specificity in the diagnosis of hepatitis A, B and C using the OraSure collecting system, and that the samples had titres of antibodies to viral hepatitis were similar to those of the serum.

Hiv infection

Malamud (1992) has made a strong plea for use of saliva as a diagnostic fluid for the detection of antibodies to HIV among other purposes. For detection of carriage of antibodies to HIV, saliva testing as a simple non-invasive method presents enormous advantages. It removes the risk of needle-stick injuries and the emotional connotations of blood sampling in this alarming disease. Salivary sampling has special advantages when investigating children because of the great ease of collection. Archibald *et al* (1993) have described the practical applications for saliva testing for perinatal HIV diagnosis. Only a minority of infants born to HIV-positive mothers develop HIV infection, but in the neonatal period, usually carry passively transferred antibodies to HIV from the mother. However, it is believed that IgA and IgM antibodies do not cross the placenta. To assess the value of an IgA-specific Western blot assay, Archibald *et al* (1993) collected blood and saliva from 95 infants and children born to HIV-infected women. Saliva samples from infants were collected by gentle aspiration from the buccal sulcus. The total sensitivity of the salivary assay for detecting antibodies to HIV gp160 antigens was 50% of infants under 12 months old and 97.3% for infants over 12 months. The earliest age for detection of serum IgA antibodies to HIV is believed to be two months. Salivary IgA antibodies were detected by Archibald *et al* (1993) in an infected infant at six months but had been negative at four months.

Reliable salivary detection of HIV infection in the immediate neonatal period awaits more sensitive methods. However, if these can be found, saliva sampling for antibodies to HIV, particularly for infants and children, is a potentially valuable method. Saliva collection does not require skilled personnel, avoids the need for repeated venepunctures and is ideal for studies in developing countries.

Drug Monitoring in Saliva

Jusko and Milsap (1993) have discussed the pharmacokinetics of drug distribution in saliva. They showed that the primary properties of a drug determining its entry into saliva were molecular size, lipid solubility, pK_a, and protein binding. However, salivary flow rates, the time of sampling, and disease states which altered saliva composition could affect the

results.

Haeckel (1993) has summarized some of the reasons for the failure to use saliva to any great extent for therapeutic drug monitoring as follows:

--> Blood has to be sampled anyhow for other electrolytes.
--> There are technical difficulties with sampling saliva.
--> There are existing difficulties with the interpretation of salivary drug concentrations.

The remaining applications for saliva sampling were therefore:

--> When sampling at home is required.
--> Special cases where the sample is taken only for the monitoring of a particular drug.
--> Circumstances where the sample volume is critical, as for example in newborns, affect the results.

Nevertheless, if a constant saliva/plasma ratio can be established, use of saliva for therapeutic drug monitoring becomes a clinically useful possibility and Siegel (1993) has pointed out that the saliva/plasma ratios for at least 170 drugs have been established experimentally.

Drugs which can be monitored in saliva include digitalis, phenytoin, primidone, ethosuximide, carbamazepine, theophylline, caffeine, lithium, methadone, cyclosporin, marijuana, cocaine and alcohol.

A related use for salivary drug monitoring is for detection of drugs of abuse as described by Cone (1993) who reviewed the findings for alcohol, amphetamines, barbiturates, benzodiazepines, caffeine, cocaine, inhalants such as general anaesthetic agents as well as solvents, LSD, marijuana, opioids, phencyclidine and tobacco.

Xerostomia

Even in the absence of parenchymal salivary gland disease, function can be abnormal and result in xerostomia. However, it should not be assumed that ageing itself causes deterioration of salivary gland function. Several studies have been carried out and have yielded somewhat conflicting results. The most recent study (Ship *et al*, 1991) confirms at least one of the earlier studies that normal postmenopausal women, the main group thought to be at risk, have no deterioration of salivary gland function. However, postmenopausal women have the highest incidence of Sjögren's syndrome and are also the group who most frequently complain of symptoms such as sore mouth or even of dry mouth, though the later may not be confirmed by objective testing.

Aetiology

In assessing the cause of depressed salivary secretion a careful drug history is essential. Causes of xerostomia include the following:

--> Depression and chronic anxiety states.
--> Dehydration (fluid deprivation or excessive loss).
--> Drugs. See Table 5.1.
--> Salivary gland disease. See Table 5.2.
- Sjögren's syndrome and benign lymphoepithelial lesion (Chapter 4)
- radiation damage (Chapter 4)
- graft-versus-host disease (Chapter 4)
- sarcoidosis (Chapter 4)
- HIV infection
- iron and other infiltrations (Chapter 3)
- amyloidosis (Chapter 3)
- type V hyperlipoproteinaemia.

Depression and anxiety states

The rate of salivary secretion was measured in 42 untreated depressed patients by Busfield and Wechsler (1961a) who, in confirmation of the results obtained by earlier workers, found it to be decreased in comparison with non-depressed hospital patients and healthy controls. No correlation was found between the degree of xerostomia and the assessed severity of depression, nor was there any difference found in secretion rates in a later study by Busfield and Wechsler (1961b), between the different categories of depression or between those who complained of dry mouth and those who did not.

Chronic fatigue syndrome (myalgic encephalomyelitis)

In a survey on several hundred patients with chronic fatigue syndrome ('myalgic encephalomyelitis'), Komoroff and Buchwald (1991) noted that 30-40% complained of dry mouth. Whether or not this is related to the widely held view that this syndrome is a form of depression is also speculative. The question whether or not it has an organic basis also remains controversial, but the many suggested causes seem unlikely to have any organic effect on the salivary glands. However, this does not exclude the possibility of coincidental disease, especially as some organic disorders, such as the connective-tissue diseases in their early stages, can also give rise to vague and varied symptoms similar to those of chronic fatigue syndrome. Investigation may therefore be indicated.

Anxiety states may also be associated with significant sympathetic overactivity and drying of the mouth. When this affects actors or other public speakers, it can be a troublesome occupational hazard; in such patients, a beta-blocking agent such as oxprenolol may possibly be helpful.

The fact that xerostomia is associated with sympathetic overactivity, can be caused by sympathomimetic drugs and may be relieved by beta-blockers strongly suggests that the sympathetic supply to the salivary glands is inhibitory in humans. Nevertheless, Garrett (1987), in addition to the points made earlier in this section, strongly denies that sympathetic inhibitory fibres exist but that anxiety-induced xerostomia is due to central inhibition from higher centres. These in turn act on the salivary centres and thereby suppress reflex activity. The clinical implications of these findings are as yet unclear, especially as Garrett (1987) has emphasized that wide variations exist in the neuroeffector arrangements and in the cellular

responses of different glands from different species.

Dehydration

Drying of the mouth is an inevitable consequence of dehydration. It can therefore result from such causes as haemorrhage, loss of other body fluids secondary to diarrhoea or chronic vomiting, polyuria secondary to diabetes mellitus, hypovolaemia from any cause, restricted fluid intake or overdose of diuretics. As mentioned in Chapter 4, Raad *et al* (1990) found that acute bacterial sialadenitis was secondary causes such as these in approximately 50% of their patients.

Drug-induced xerostomia

Tricyclic antidepressants and phenothiazine neuroleptics are among the most troublesome as they have particularly strong antimuscarinic effects, are generally used over long periods and as a result of such side-effects as these, are disliked by most patients. Ganglion-blocking drugs are obsolete in the routine management of hypertension, and modern antihypertensives are considerably more selective in their action.

However, by simplistic analogies between quite different groups of drugs, which happen to be used for similar medical indications, widespread misunderstandings persist about the effects of different drugs on salivary gland function but few objective trials confirm these assertions. For example, antihypertensive drugs in general, are frequently said to cause xerostomia, but beta-blocking drugs which are currently among the most widely used for this purpose, have not been shown to decrease salivary secretion. Moreover, by blocking sympathetic activity, they are more likely to increase secretion and, as mentioned earlier, may be used beneficially by actors for example, whose mouths dry up on stage. Similarly, the commonly used benzodiazepines ('minor tranquilizers') do not cause dry mouth and may even relieve anxiety-induced xerostomia. By contrast, neuroleptic drugs (formerly termed 'major tranquilizers'), particularly the phenothiazines, have strong antimuscarinic side-effects and cause significant drying of the mouth.

In practical terms, the chief effects of prolonged xerostomia are the distressing symptom itself and the promotion of oral infections as discussed below.

HIV infection

The varied effects of HIV infection on the salivary glands are discussed in Chapter 4. It needs only to be noted here in that deterioration of function appears to have no consistent relationship with lymphoproliferative lesions of the glands in the various reports. By contrast, studies have shown progressive impairment of salivary flow rates associated with HIV infection in patients apparently without over salivary gland disease.

As yet, it is as difficult to correlate these apparently contradictory findings as to evaluate the effect of benign lymphoepithelial lesion (Chapter 8) on salivary function, despite their having the same microscopic appearances as Sjögren's syndrome.

Type V hyperlipoproteinaemia

Xerostomia, sometimes associated with parotid swelling, is sometimes a major complaint in type V hyperlipoproteinaemia. However, the pathogenesis is unclear. According to Reinertstein *et al* (1980) labial gland biopsies have shown no abnormality but salivary gland scintigraphy suggests that focal inflammatory, infiltrative or obstructive lesions may be present.

Clinical features

It is important to appreciate that, as confirmed by Fox (1987), Sreebny *et al* (1988) and others, patients with impaired salivary flow rates frequently make no spontaneous complaint of dry mouth and may not even admit to it if asked. However, they may admit to difficulties with eating dry foods unless taken with fluid. By contrast, patients who complain of dry mouth are not uncommonly found to have normal flow rates. In extreme cases dryness of the oral mucosa is obvious (Fig. 5.2), but even when the flow rate is significantly diminished, the mucosa usually appears moist. In such cases, diminished salivary flow is indicated by absence of pooling of saliva in the floor of the mouth and frequently by salivary froth adhering patchily to the mucosa (Fig. 5.3).

In severe cases, xerostomia can be recognized even before examination by the clicking quality of the patient's speech as a result of the tongue adhering to the palate.

Much also depends on the rest of the clinical picture. A patient complaining of dryness of the mouth and who has long-standing rheumatoid arthritis or a history of irradiation of the oral cavity, or is on tricyclic antidepressant treatment, is unlikely to need the complaint to be confirmed by testing.

Oral changes suggestive of a dry mouth are the onset of rapid dental decay which often affects unusual sites such as the lower anterior teeth (Fig. 5.), adherence of food debris to the teeth, or soreness and redness of the oral mucosa due to *Candida albicans* infection (Fig. 5.5). In extreme cases, the oral mucosa appears wrinkled and parchment-like, or rarely, sores may be seen on the palate or elsewhere (Fig. 5.6). Changes in the oral flora secondary to drying can also lead to complaints of unpleasant taste sensation or halitosis. A feature characteristic of Sjögren's syndrome is that the tongue becomes red, partially depapillated and lobulated. Occasionally the onset of suppurative parotitis is the first indication of impaired salivation.

Because of the erratic correlation between the complaint of dryness of the mouth with impaired salivation, objective confirmation of decreased flow rates may be necessary. It is particularly required in Sjögren's syndrome where a variety of abnormalities (Chapter 4) may be present but their association with the disease is inconstant. In the case of salivary lymphoepithelial lesion, flow rates are unlikely to be measured, because the disease is usually only recognized after parotidectomy.

It is also important to remember that dryness of the mouth is frequently associated with poor lacrimal gland function, particularly in Sjögren's syndrome. Conjunctivitis is occasionally obvious, but early keratoconjunctivitis sicca is asymptomatic.

Management

The distressing nature of this complaint should not be underestimated as it so frequently is by surgeons. Considerable effort to make the patients comfortable and their meals more pleasant is justified.

The two requirements are relief of the symptoms and control of the complications. The patient should *not* be told, as sometimes happens, to suck acid drops. The sugar and acid content of these sweets will quickly lead to destruction of any remaining teeth and probably also help to promote other infections.

Patients should take plenty of fluid in the form of frequent sips of water (or as Seifert *et al* (1986) suggest, low-alcohol beer), particularly with meals. Sugar-free chewing-gum is frequently also helpful as mastication reflexly increases salivary flow.

Artificial salivar are also beneficial. However, these preparations inevitably lack the same degree of lubricating and other essential properties of normal saliva and, in any case, cannot be used in quantities comparable to natural flow rates. If one assumes, for simplicity, an average flow rate of normal saliva, of 2 mL/min this represents 1.44 litres of saliva over a period of 12 h. The use of artificial salivas in such quantities as great as this is hardly feasible, but patients should be encouraged to use them as freely as possible.

Even if artificial salivas are used conscientiously and in as large quantities as possible, there is still unpleasantly impaired and abnormal taste sensation and frequently as a consequence, poor appetite.

Cholinergic drugs, such as pilocarpine, have been reported to be of value but functional salivary tissue must be present, and their other effects, particularly diarrhoea, may limit their usefulness.

Control of oral infection is important. Standing teeth rapidly decay and periodontal infection is accelerated unless stringent precautions are taken. If not, extraction of teeth becomes necessary and dentures are frequently then difficult to manage. Extractions are particularly hazardous after radiation damage to the tissues, as the risk of radiation-associated osteomyelitis is high. Meticulous preventive dental care is therefore essential; this includes attention to the diet, rigorous oral hygiene measures and application of fluorides.

In addition to dental decay, the mucosal flora changes and *Candida albicans* or staphylococci frequently proliferate. Candidosis should be controlled with an antifungal preparation such as amphotericin or nystatin suspension. Alternatively, antifungal imidazoles such as ketoconazole suspensions or miconazole gel may be more effective and may also help to control associated bacterial infections. There preparations are more suitable than tablets which may not dissolve, and the patient should be told to hold them in the mouth for as long as possible to achieve the maximal effect.

The changes in the oral flora are probably a major cause of the abnormal taste sensations in this condition. Lack of parotid salivary flow also increases the risk of bacterial (ascending) parotitis. This risk should also be lessened by maintenance of high standards of

oral hygiene.

Nevertheless, as mentioned earlier, the possibility of asymptomatic keratoconjunctivitis sicca should be excluded. If neglected it can lead to irreparable damage to sight. Paradoxically therefore, ocular examination may the most important aspect of the care of a patient with xerostomia. Even if salivary gland function is normal, slit-lamp examination may be desirable in the case of several of the diseases listed earlier.

Sialorrhoea

Table 5.3 Causes of ptyalism

Local reflexes
- Painful oral infections
- Oral wounds
- Dental procedures
- New dentures

Systemic
- Nausea
- Acid regurgitation (reflux oesophagitis)

Toxic
- Iodine
- Heavy-metal poisoning

False ptyalism
- Psychogenic
- Bell's palsy
- Parkinson's disease
- Stroke
- Cerebral palsy.

A few drugs or painful conditions in the mouth may increase salivation, but this is not a significant complaint as any excess of saliva can normally be swallowed. 'False ptyalism' is more common and is either delusional (a disturbed patient may suddenly become alarmed at 'water' or 'too much saliva' continually appearing in the mouth) or due to faulty neuromuscular control that leads to drooling despite normal salivary flow (Table 5.3).

If lip function and swallowing are normal, the complaint of hypersalivation ('too much saliva' or 'water coming into the mouth') is essentially neurotic.

Drooling is not usually due to excessive salivation but either to difficulty in swallowing or abnormal lip function; this is well shown in Parkinson's disease where drooling may persist even when antimuscarinic drugs (orphenadrine, benztropine, benzhexol or procyclidine) are being given and salivary secretion is impaired. Inadequate lip function and drooling is also common in mental defectives.

The surgical management of drooling is discussed in Chapter 9. Though hardly relevant in the present context, the importance of defective deglutition in severe sialorrhoea is exemplified by the sign traditionally known as 'foaming at the mouth', in the later stage of

rabies. Severe dysphagia results from brain-stem dysfunction and there is also excessive salivation due to autonomic overactivity. The defect in deglutition frequently leads to so-called 'hydrophobia', in which any attempt to swallow liquids, leads to violent, painful and terrifying contractions of the pharyngeal, laryngeal accessory respiratory muscles and diaphragm. Survival after the onset of such symptoms is usually less than a week. Rabies virus spreads centrifugally to the salivary glands from the central nervous system and is present in the saliva. It is the main vehicle for transmission of the infection from animals, but spread by this means from a patient to a health worker does not appear to have been reported.

Frey's Syndrome

Frey's syndrome (gustatory sweating, auriculotemporal syndrome) usually results from damage to the innervation of the salivary glands during parotidectomy and is probably caused by faulty regeneration of autonomic fibres secondary to the injury.

Clinically, Frey's syndrome is characterized by sweating, warmth and redness of the face as a consequence of autonomic stimulation of salivation by the smell or taste of food. The sweating can be demonstrated by coating the side of the face with starch, which turns blue on exposure to iodine in the sweat (Fig. 5.7).

Once established, Frey's syndrome is difficult to treat. Application of ointments, containing anticholinergic drugs, to the face to inhibit sweating, or commercial antiperspirants (usually based on astringents such as aluminium chloride) should be tried in the first instance. Denervation by tympanic neurectomy or auriculotemporal nerve avulsion has been advocated when simpler measures fail.

Note

1. Lucie Frey, Polish physician (1898-1944), murdered by the Nazis.