

Introduction: the anatomy and physiology of salivary glands

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

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

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

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Table 1.1: Functions of saliva

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

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

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

Functions of saliva

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

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

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

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

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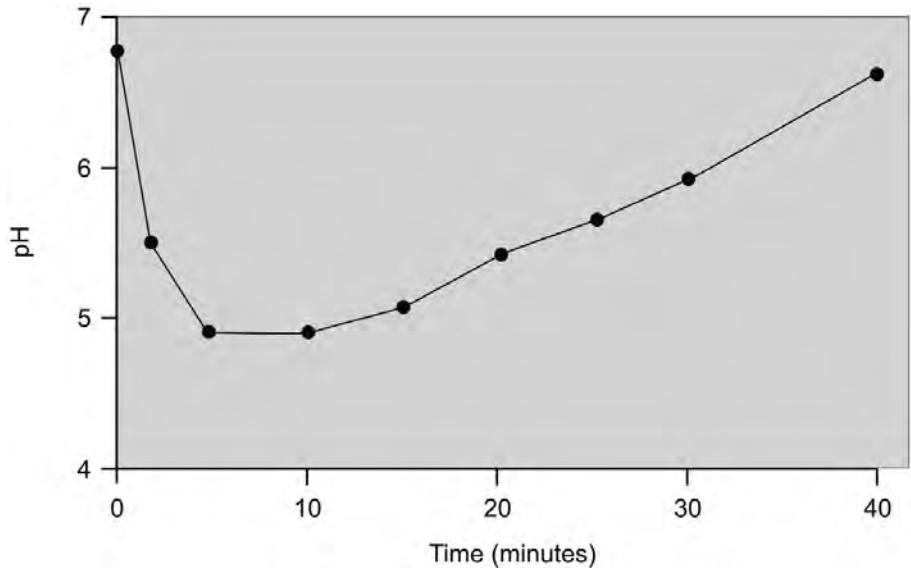


Figure 1.1 Stephan Curve illustrating the changes in plaque pH over time following a sucrose rinse



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

Anatomy and histology

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

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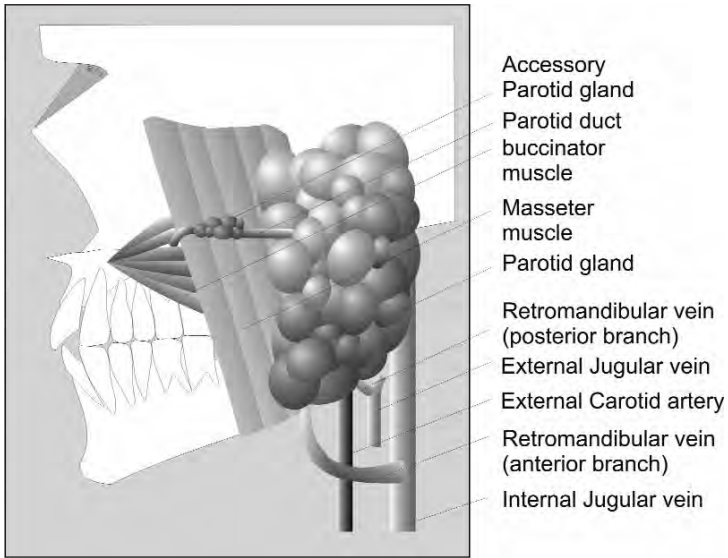


Figure 1.2a Anatomy of Parotid Gland

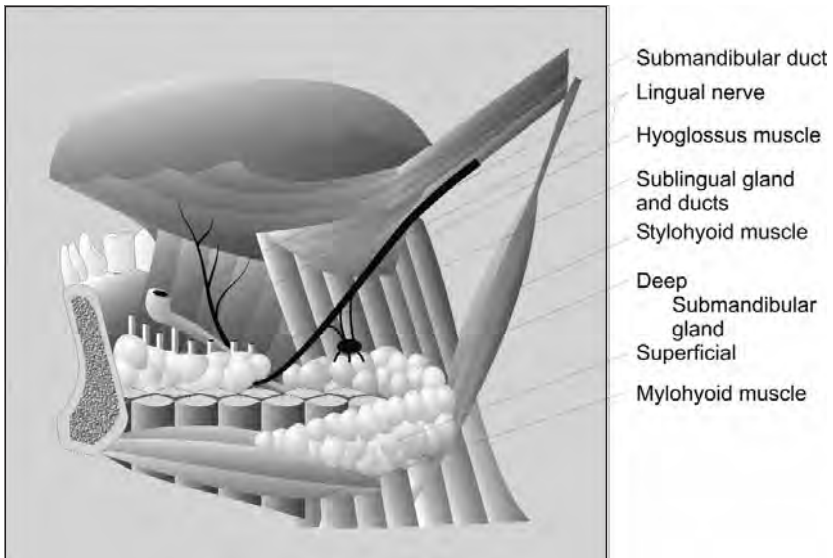


Figure 1.2b Anatomy of Sublingual and Submandibular Glands

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

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

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

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

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

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

Structure of salivary glands

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

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

Formulation of saliva

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

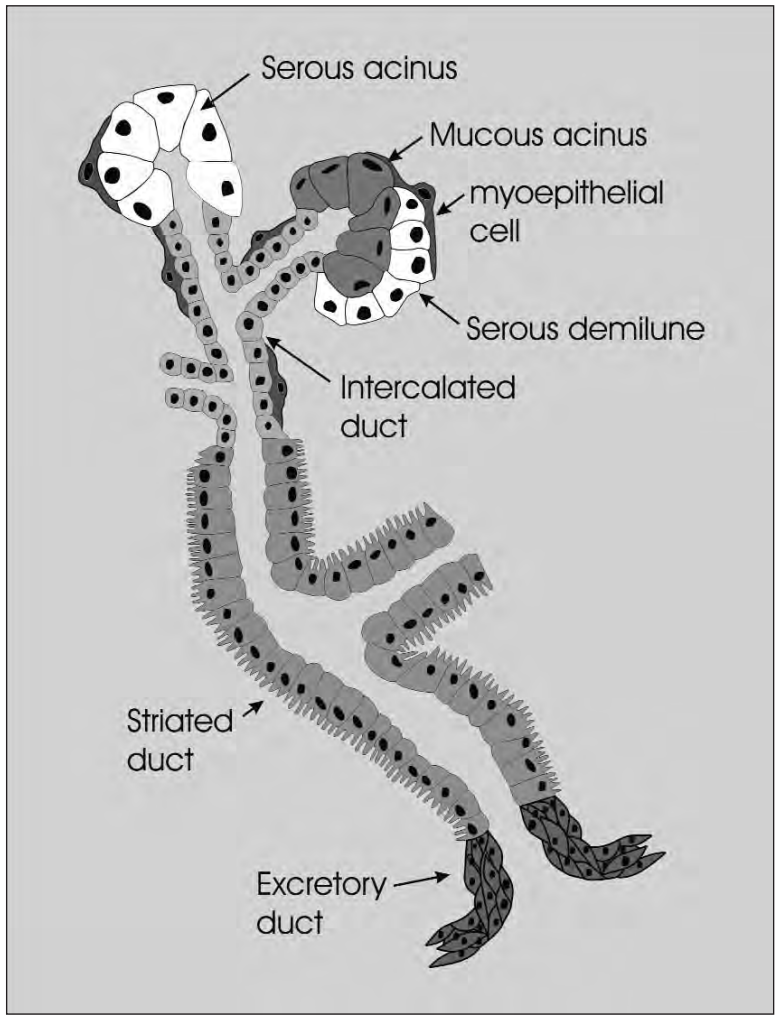


Figure 1.3 Salivary Glandular Tissue

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

Nerve supply

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

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

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

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

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

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

Blood supply

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

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

Physiology

Composition

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

Flow rate

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

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

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

Table 1.2: References

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

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

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

Effects of ageing

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

Saliva as a diagnostic fluid

Caries risk assessment

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

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

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

Table 1.3: Salivary variables measured for caries risk assessment

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

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

General diagnostics

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

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

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

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

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

Acknowledgements

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Further reading

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Mechanisms of salivary secretion

Peter M Smith

Introduction

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

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

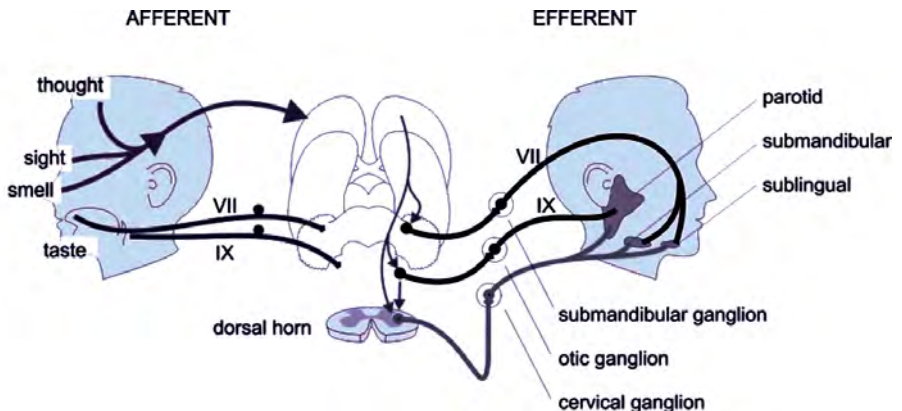
‘**Fluid**’, ‘**electrolytes**’ and ‘**macromolecules**’ describe defining components of saliva. The unique viscoelastic and antibacterial properties of saliva stem largely from its protein component. The electrolyte content adds acid buffering and remineralisation capabilities and the fluid vehicle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

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

Stimulation

Neural control of salivation

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



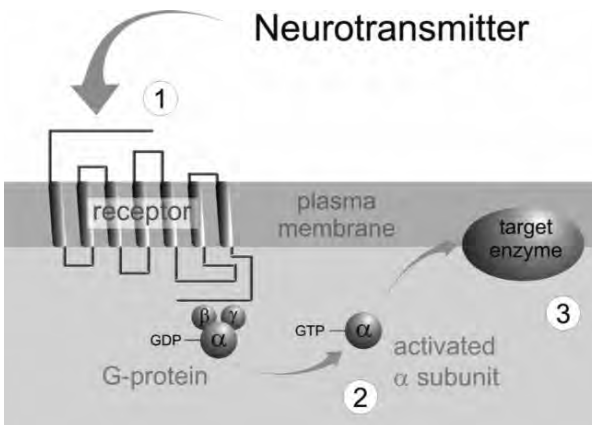
Afferent pathways: taste; facial (VII) and glossopharyngeal (IX) nerves to solitary nucleus in the medulla. Also input from higher centres in response to smell etc. Efferent pathways: Parasympathetic; sublingual and submandibular from facial nerve via submandibular ganglion. Parotid from glossopharyngeal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

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

Second messengers

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

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



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

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

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Adenylate cyclase and cyclic-AMP

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

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

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

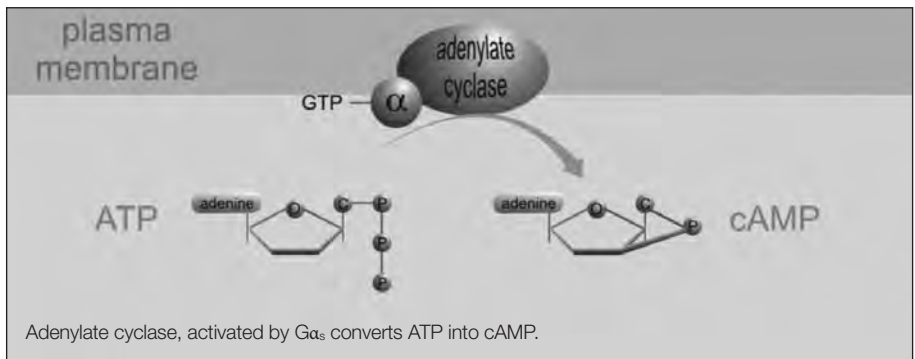


Figure 2.3 The third step in macromolecule stimulus-secretion coupling is production of cAMP

(Figure 2.4). IP_3 acts by binding to IP_3 receptors on endosomes, such as the endoplasmic reticulum (ER), and releasing the Ca^{2+} stored within. The Ca^{2+} content of the ER is maintained at a much higher concentration ($\approx 1 \text{ mM}$) than that of the cytoplasm ($\approx 100 \text{ nM}$) by Ca^{2+} ATPase activity so that activation of a Ca^{2+} channel is sufficient to raise cytosolic Ca^{2+} activity by diffusion from the Ca^{2+} stores. IP_3 receptors are Ca^{2+} channels, activated by IP_3 binding.¹⁰

IP_3 receptors are also sensitive to cytosolic Ca^{2+} activity and stay open for longer when $[\text{Ca}^{2+}]_i$ is raised. This property of the receptor can dramatically enhance the Ca^{2+} mobilising properties of IP_3 by positive feedback or Ca^{2+} -induced Ca^{2+} release (CICR).¹⁰ The Ca^{2+} signal may be further amplified by Ca^{2+} release through ryanodine receptors, a second Ca^{2+} channel also present on the ER of acinar cells.¹¹ Ryanodine receptors are also Ca^{2+} sensitive and contribute to CICR. The sensitivity of ryanodine receptors to Ca^{2+} may be 'set' by the cytosolic concentration of cyclic ADP ribose, a product of βNAD produced by ribosyl cyclase regulated by cyclic GMP and possibly Nitric Oxide levels.¹¹ The Ca^{2+} signal is therefore actively propagated through the acinar cell by an explosive release of Ca^{2+} from stores, triggered by IP_3 , amplified by Ca^{2+} and carried by both IP_3 and ryanodine receptors (Figure 2.5).¹²

In addition to mobilising stored Ca^{2+} , the secretory process can also utilise extracellular Ca^{2+} from influx across the plasma membrane through store-operated Ca^{2+} channels. Whilst the physiological characteristics of store-operated Ca^{2+} influx

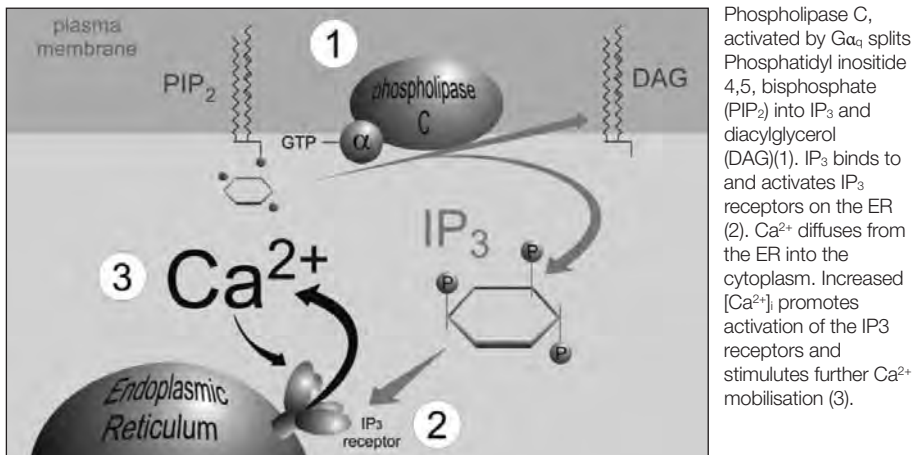
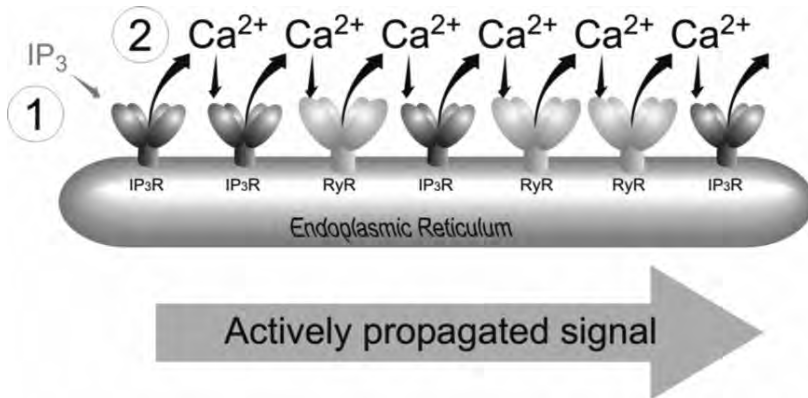


Figure 2.4 The third step in fluid and electrolyte stimulus-secretion coupling is an increase in intracellular Ca^{2+} activity



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

Figure 2.5 Actively propagated Ca²⁺ signal

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

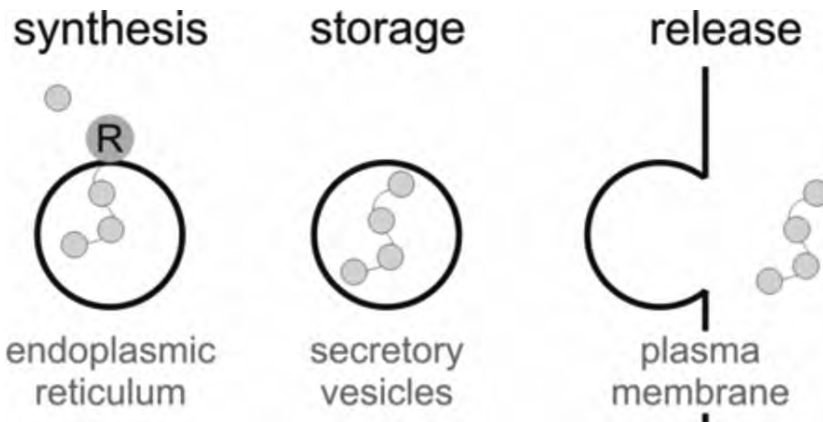
Macromolecules

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

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

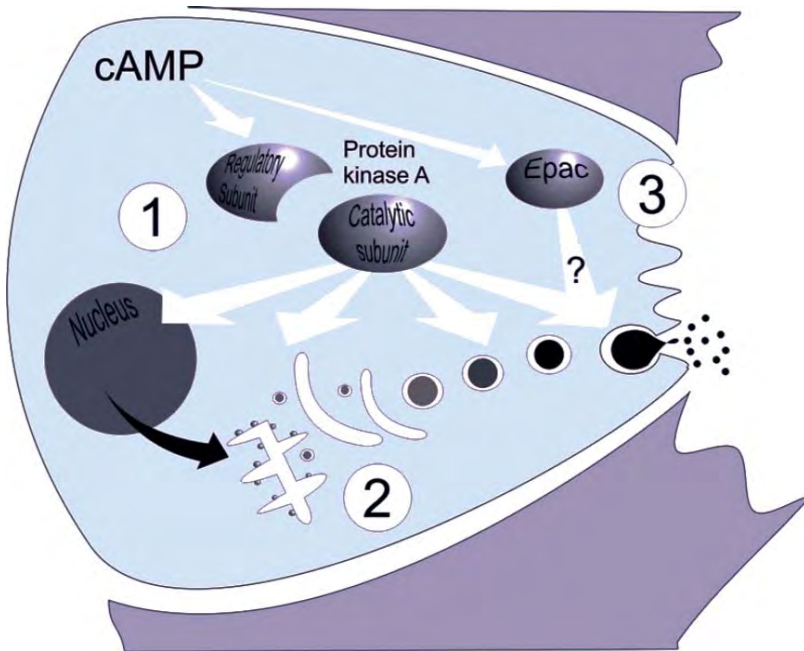
The secretory process may be divided into four stages. Synthesis, segregation and packaging, storage and release. Each of these stages is regulated by phosphorylation of target proteins by cAMP-dependent pKA. Therefore an increase in cAMP stimulates:

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



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

Figure 2.6 Secretory proteins are synthesised in endosomes

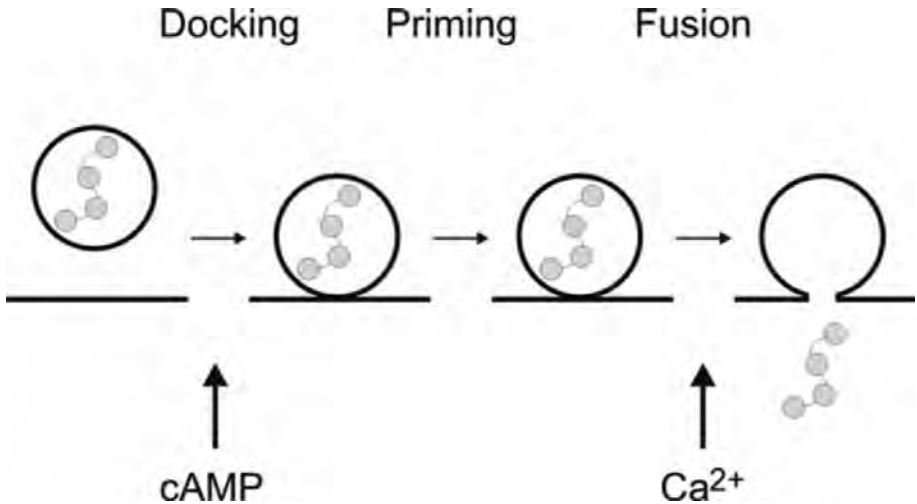


cAMP binding to the regulatory subunit of pKA releases and activates the catalytic subunit (1)
 The catalytic subunit phosphorylates and upregulates many components of the secretory pathway including exocytosis (2). Recent work has also identified an Epac-mediated pKA-independent mechanism that may also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

Figure 2.7 cAMP and PKA

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

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

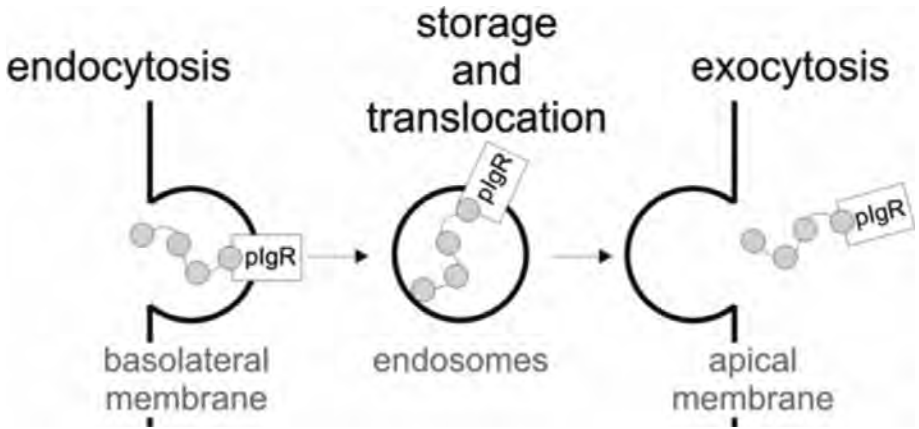


Exocytosis occurs in three stages; docking, priming and fusion. The fusion process itself is Ca^{2+} -dependent. However, earlier stages of the process, e.g. 'docking' could be cAMP-dependent. In salivary gland cells, this step is the rate-limiting 'brake' point in exocytosis.

Figure 2.8 A cAMP-dependent 'brake' point

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

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



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

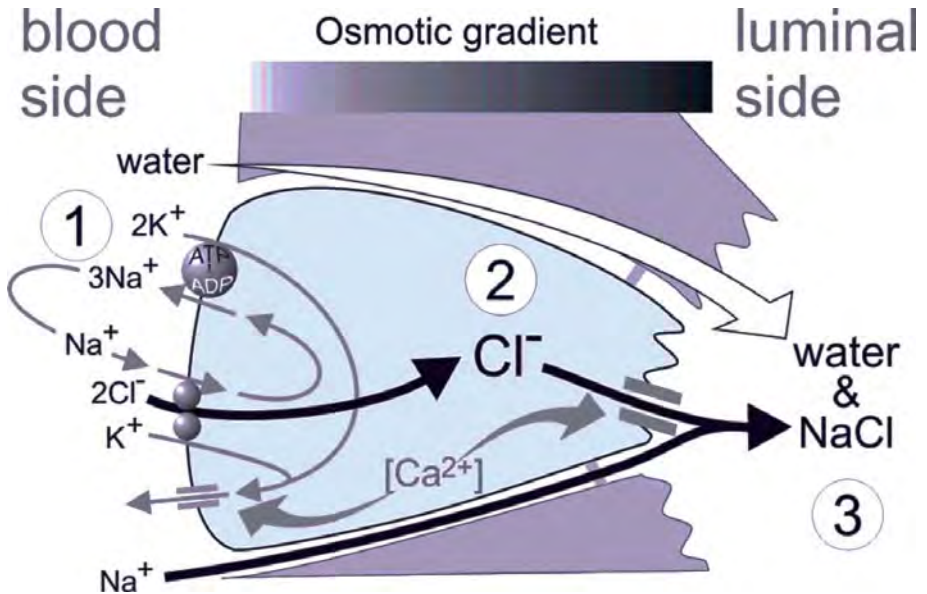
Figure 2.9 Transcellular protein transport

Fluid and electrolytes

Fluid secretion

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

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



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

Figure 2.10 Fluid secretion follows electrolyte secretion

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

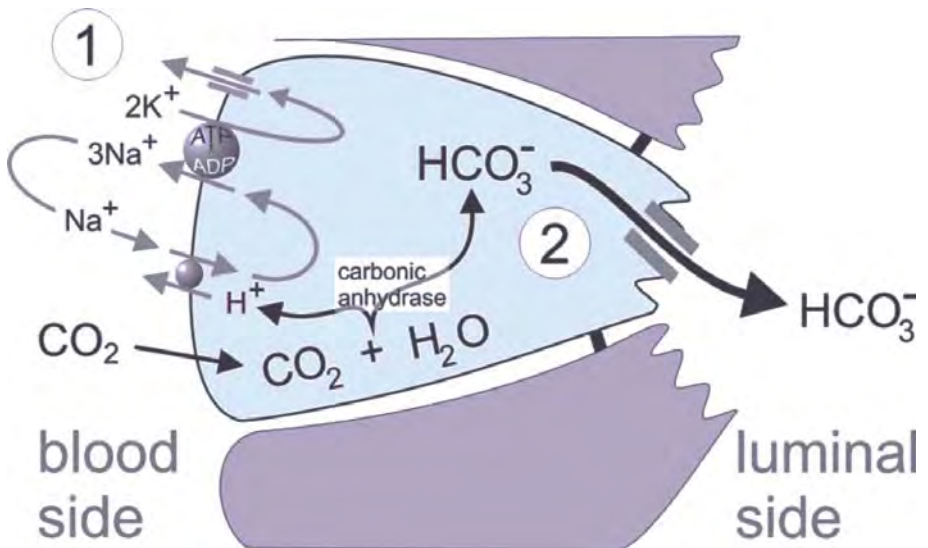
Electrolyte-led fluid transport movement is always isotonic. Once isotonicity is reached, there is no additional driving force for water movement. The ability of salivary glands to generate an hypotonic saliva lies with the striated ducts. Striated duct cells pump electrolytes from the primary saliva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

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

Bicarbonate secretion

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



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

Figure 2.11 Fluid secretion follows electrolyte secretion

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

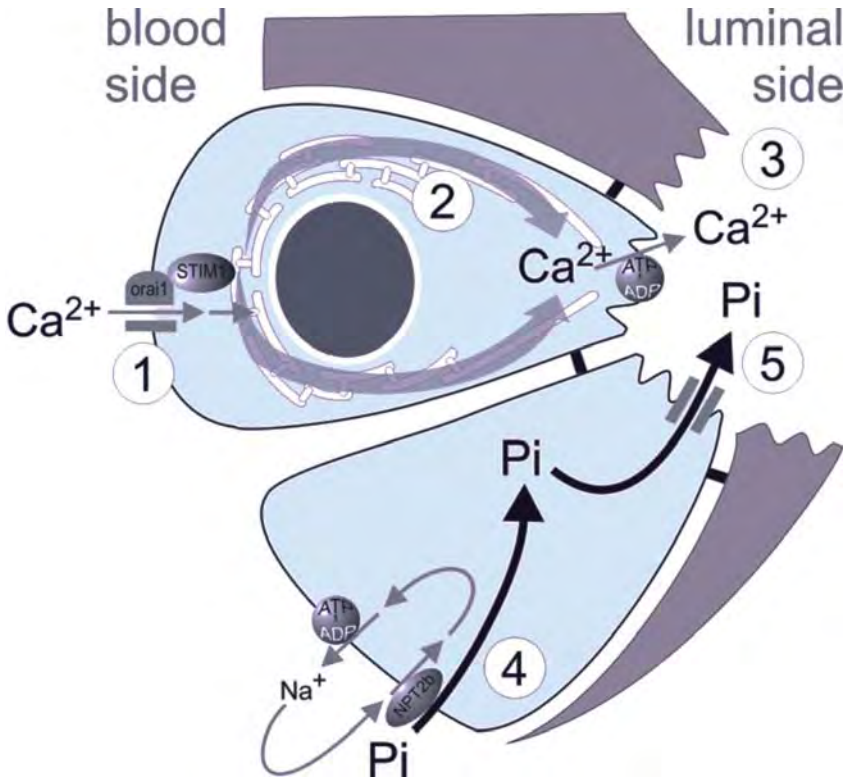
Bicarbonate is one of the electrolytes reabsorbed by the striated ducts and bicarbonate concentration in unstimulated saliva is consequently low. A combination of increased secretion and failure to reabsorb bicarbonate at high flow rates is the simplest explanation of the much higher bicarbonate concentration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

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

Calcium and phosphate secretion

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

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

Figure 2.12 Calcium and phosphate transport

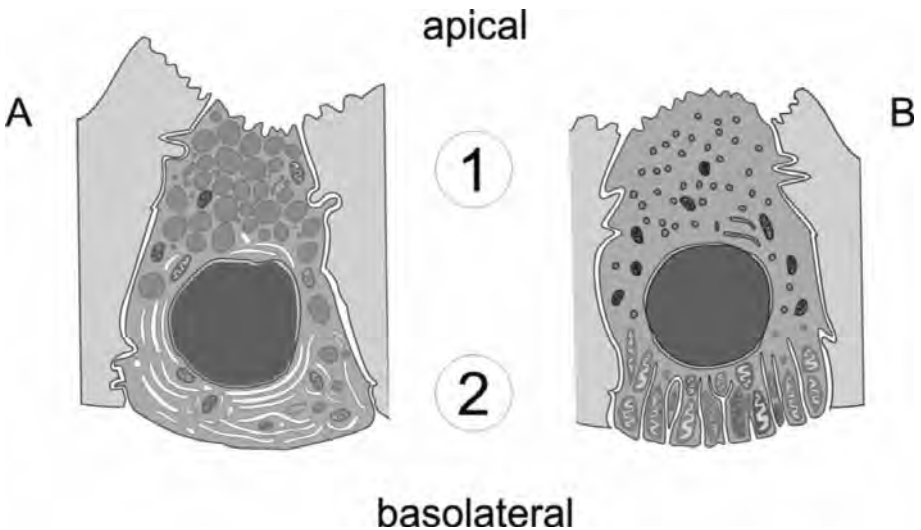
Water channels

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

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

Unidirectional

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



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

Figure 2.13 Histological polarity

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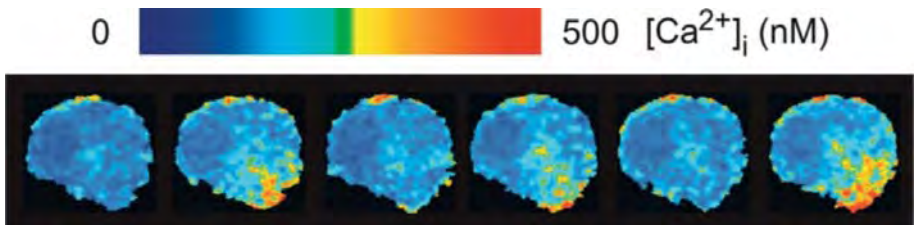
(Figure 2.13) and function. Every cell type involved in salivary secretion is polarised in one way or another. Acinar and duct cells are connected together by tight junctions, which also form the division between the apical membrane which faces into the lumen of the gland and the basolateral membrane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

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

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

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

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



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

Figure 2.14 Local Ca^{2+} signals

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

Pharmacological control of fluid and electrolyte secretion

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

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

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