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A Comparison of Various Methods of Assessing Salivary Flow in Normal and Xerostomic Subjects

Submitted for the degree of Doctor in Philosophy

University of Dublin

2006

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Declaration

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Summary

Saliva is produced by the three major paired salivary glands and minor salivary glands. Alterations in the quantity and quality of saliva have adverse effects on the oral cavity and influence patient quality of life. How much saliva is needed to differentiate between a normal salivary flow and salivary hypofunction is debatable and the literature indicates contradicting results.

Dry mouth is a common clinical problem, especially among elderly patients. However, it may also be found in younger individuals suffering from a variety of diseases and in patients on various medications or who have had radiotherapy of the oral cavity and neck region.

The present study was designed: i) to examine the reproducibility of methods of salivary flow measurements for minor salivary glands and for whole saliva, both stimulated and unstimulated, in normal healthy adults, ii) to examine the relationships between different methods for the measurement of salivary flow, for minor salivary glands and for whole saliva, both stimulated and unstimulated, in normal healthy adults, iii) to examine the relationships between salivary flow measurements for minor, parotid and whole saliva, both stimulated and unstimulated, in normal healthy adults, iv) to examine the relationships between different methods for measurement of unstimulated salivary gland flow in a cohort of patients with severely reduced salivary flow following head and neck radiotherapy, and v) to examine the relationship between unstimulated minor salivary gland flow and subjective xerostomia, as measured by a conventional questionnaire, in the same patient cohort.

Unstimulated and stimulated saliva were collected with different techniques from young healthy adults. Collection of whole saliva was carried out by spitting and with Salivettes®. Saliva from labial minor salivary glands was collected using Sialopaper® (Periotron®) and filter paper (weighing). Paired measurements were
made at two time points. The weighing technique had poor reproducibility and spitting had the best.

Salivary flow measurements were made on a separate group of young healthy adults, using the same methods plus parotid saliva collection with modified Carlson-Crittenden cups. There was no correlation between different flow rates measured by different techniques. Paired unstimulated/stimulated measurements for each technique showed no correlation except for the spitting technique for whole saliva. Intra-individual variations were apparent and a wide range of salivary flow rate was observed. No correlation was found between parotid salivary flow, whole salivary flow rates and flow rate of labial minor salivary glands.

Patients with severely reduced salivary flow following head and neck irradiation had unstimulated flow measured by two techniques (Periotron® and spitting) and answered a standard xerostomia questionnaire. There was no correlation between the two sets of flow measurements. The Periotron® was able to detect differences in flow rates even in patients with minimal secretion. Whole salivary measurement (by spitting) over 5 minutes was not able to detect differences in flow rates in patients with minimal secretion. Only one question on the xerostomia questionnaire correlated at all well with flow (as measured by the Periotron®).

Salivary flow measurement methods do not seem to be inherently comparable between different methods. Spitting seems to be the most appropriate method for healthy adults while the Periotron® may be more appropriate to severely xerostomic patients. Conclusions drawn from studies measuring salivary flow with one technique, in normal or xerostomic patients, cannot be compared with studies using different techniques.
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Chapter 1. Review of the Literature

1.1 General Introduction

Saliva is a clear, usually alkaline, slightly viscid secretion from the three major paired salivary glands (parotid, submandibular and sublingual) and minor salivary glands. It consists primarily of water, but also contains enzymes, proteins, small organic molecules, electrolytes and constituents of a non-salivary origin. Saliva plays a critical role in the maintenance of oral health and the proximal portion of the gastrointestinal tract (Mandel 1987, Mandel 1989 Pedersen et al 2002). It performs several important functions; such as lubrication, hydration, buffering, mineralization, taste facilitation, tissue coating and antimicrobial activity. The protective functions can be due to dilution and/or cleansing effects. There are several families of salivary molecules, each with multiple members. It is now known that the majority, if not all, saliva molecules are multifunctional. Many molecules have overlapping functions, for example, mucins and amylase interact with viridans streptococci. Stetherin and proline-rich proteins play a role in mineralization. Nevertheless, some molecules are more effective with regard to a particular function (Levine 1993). Normal salivary function is vital to the protection of dental and oral health. Alterations in the quality and quantity of saliva has adverse effects on the oral cavity (Mason 1979, Pyykönen et al 1986, Mandel 1989, Tenovuo 1992) and influence patient quality of life (Fox et al 1985 Sreebny & Valdini 1987). Individuals with

Salivary flow rate of whole saliva and saliva from the parotid are most commonly used for different clinical purposes and for experimental studies. However, saliva from submandibular and sublingual glands is not widely used because it is more difficult to obtain due to poor accessibility.

Salivary flow rate can be measured after stimulation. It is generally stimulated using gustatory or mechanical stimuli, however it may also be collected without stimulation (resting or unstimulated). If unstimulated secretions are to be collected, subjects should be fasting for a minimum of 60 minutes prior to collection. During this period any oral stimulus should be avoided. On other hand saliva should be collected within a specified time of the day (Tenovuo & Lagerlöf 1994).

When the purpose of salivary collection is to analyse composition, individual gland secretion is superior to whole saliva, because the latter may contain some other elements such as crevicular fluid or food debris (Navazesh & Christensen 1982).

Variations in the rate of flow and composition of saliva have been studied for many years in an attempt to aid in the diagnosis of
salivary glands and systemic disease (Dawes 1987, Ship et al 1991, Pedersen 1999a). However since considerable variation exists in the normal range of salivary flow rates, it appears difficult to set standards to define what represents an abnormally low flow rate (Dawes 1987). However, how much saliva is needed to differentiate between a normal salivary flow and salivary hypofunction is debatable. Thus, the establishment of reference flow rates in various populations is considered important (Ship et al 1991, Mandel 1993a). However, the literature indicates contradicting results, which might be due to non-standardized collection techniques. Meanwhile, intra- and inter-individual variations may play an important role in the generation of such contradictory results. It is well known that secretion may greatly vary in an individual, and if repeated samples are taken at different time points varying flow rates will be obtained (Dawes 1987).

Saliva collection is a procedure that can provide sound clinical information about patients. It is a non-invasive means of assessing a variety of disease activities and the levels of certain drugs and hormones (Ferguson 1987, Tabak 2001). There is general agreement that saliva provides an easily obtainable, available, noninvasive, diagnostic medium for several dental and medical conditions and clinical situations (Mandel 1990, Tabak 2001). With the emergence of HIV and other blood-borne viral infections there has been a growing appreciation of the value of saliva as being a less hazardous specimen

Dry mouth is a common clinical problem, especially among elderly patients. However, it may also be found in younger individuals suffering from a variety of diseases (e.g. cystic fibrosis, AIDS, sarcoidosis) and in patients on various medications or who have had radiotherapy of the oral cavity and neck region. Studies report a weak correlation between subjective mouth dryness and objective sialometry (Ship et al 1991, Pedersen et al 1999a). This indicates that both quantity and quality of saliva are important for its surface-associated functions, such as lubrication and hydration (Pedersen et al 1999a).
1.2. Anatomy of the Salivary Glands

Saliva is secreted by three pairs of large glands; parotid, submandibular and sublingual and by numerous smaller glands located in the labial, buccal, palatal and lingual oral mucous membranes. All these sources contribute to the whole saliva (Berkovitz & Moxham 2002, Hand 2003). On the basis of size and distance from the oral mucous membrane, salivary glands are categorized into major or minor glands (Tandler & Philips 1998). The gross anatomy of the major glands is shown in Figures 1.1 and 1.2

1.2.1 Parotid Gland

The parotid is the largest salivary gland, is enclosed with a tough facial capsule, derived from the investing layer of deep cervical fascia. It is a serous gland and consists of two portions. Its superficial portion is located subcutaneously in front of the external ear, and its deeper portion lies between the ramus of the mandible and the mastoid process. The apex of the parotid gland is posterior to the angle of the mandible. Its duct (Stensen’s duct) runs forward from the anterior edge of the gland at the anterior border of the masseter muscle, pierces the buccinator and opens into the oral cavity at a papilla opposite to the maxillary second molar (Tandler 1987a, Berkovitz & Moxham 2002, Hand 2003, Avery & Chiego 2006). With in the gland lies the facial nerve and its branches, the
retromandibular vein and the external carotid artery. On the parotid sheath and within the gland are parotid lymph nodes.

It is supplied by branches of the external carotid artery with a richer vascular supply to the ductal system than the secretory system. Venous return is via the retromandibular vein. Sensory nerve fibres reach the gland through the great auricular nerve and auriculotemporal nerves, and postganglionic parasympathetic secretory fibres come from the otic ganglion through the auriculotemporal nerve. The preganglionic parasympathetic fibres reach the otic ganglia from the glossopharyngeal nerve. Sympathetic postganglionic fibres enter the gland from the plexus on the external carotid or middle meningeal arteries.

1.2.2. Submandibular Gland

The submandibular gland is a mixed (mucous and serous) salivary gland with the serous type predominating (Kerr 1961, Tandler 1987a, Cooper et al 1995, Hand 2003). It is situated in the posterior of the floor of the mouth, wrapping around the posterior border of the mylohyoid muscle. Thus the gland is divided into superficial and a deep part. Its duct (Wharton’s duct) runs forward above (deep to) the mylohyoid and opens into the mouth beneath the tongue at the sublingual caruncle (Hand 2003).
The arterial supply comes from several small branches of the facial and submental arteries and the venous drainage is to the common facial vein. The lymphatic vessels of the submandibular gland drain into the deep cervical lymph nodes. Secretory-motor fibres to the gland have their cell bodies in the submandibular ganglion or in small ganglionic masses on the surface of the gland itself. The preganglionic fibres pass from the cell bodies in the superior salivary nucleus in the pons and travel with the facial nerve. They leave the facial nerve in the chorda tympani. The sympathetic fibres from the superior cervical ganglion (Berkovitz & Moxham 2002).

1.2.3. Sublingual Gland

The sublingual gland is the smallest of the major salivary glands and is predominantly a mucous gland (Kerr 1961, Berkovitz & Moxham 2002). It is located in the anterior part of the floor of the mouth between the mucous membrane and the mylohyoid muscle. The glands from each side unite in the midline. Its secretions enter the oral cavity through a series of small ducts (ducts of Rivinus) opening along the sublingual fold and often through a larger duct (Bartholin’s duct), which opens with the submandibular duct at the sublingual caruncle (Berkovitz & Moxham 2002, Hand 2003).

The blood supply is from the sublingual branch of the lingual artery. Innervation is via branches of the lingual nerve, which contain
postganglionic parasympathetic fibres from the cells of the submandibular ganglion as well as postganglionic sympathetic fibres.

1.2.4. Minor Salivary Glands

The minor salivary glands occur as aggregations of glandular tissue in all areas of the oral cavity except for the anterior region of the hard palate and the gingiva. Some areas are richer in these glands (viz. sublingual, lingual, labial, buccal and palatal). The minor salivary glands are classified as follows: sublingual, lingual (Von Ebner glands), labial, buccal and palatal. These are predominantly mucous glands with few serous acini, with the exception of the lingual minor glands, which are considered to be serous (Dawes & Wood 1973a, Hand 2003). The Von Ebner seromucose glands are located intermuscularly at the stromal base of the circumvallate papillae (Berkovitz & Moxham 2002).

The blood supply is derived from adjacent vessels. The sympathetic innervation is derived from adjacent plexuses. The parasympathetic supply for buccal and lower labial minor glands is derived from the otic ganglion while those of upper labial and palatal glands are derived from the pterygopalatine ganglion while the lingual glands are supplied by the chorda tympani. Secretions from minor salivary glands are rich in mucins, which makes them highly viscous. Their purpose is protection and lubrication (Tabak et al 1982). Up to a half
of the secretory IgA in whole saliva is secreted by minor salivary glands (Crawford et al 1975).
Figure 1.1. Gross anatomy of the parotid gland (After Hand 2003).
Figure 1.2. Gross anatomy of the submandibular and sublingual glands (After Avery & Chiego 2006).
1.3. Histology of the Salivary Glands

Salivary glands consist of glandular secretory tissue and supporting connective tissue (stroma), which contain blood vessels, nerves and collecting ducts. The connective tissue forms a capsule, which surrounds the gland and passes septa, that subdivide the gland into lobes. These lobes are subdivided into lobules (Berkovitz et al 2002, Avery & Chiego 2006). Each lobe consists of clusters of secretory epithelial cells known as secretory end pieces. These secretory end pieces have several different shapes, depending on the gland and the nature of the secretory product. The most common configurations are globular (acini) or tubular (Tandler & Philips 1998, Avery & Chiego 2006). The acinus is lined by serous or mucus secretory cells, or both. However, when a gland is predominately mucous the serous cells are crescent in shape and are referred to as serous demilunes. The serous secretions are watery and protein rich, whereas mucus secretion are viscous and carbohydrate rich (Tandler 1987a, Avery & Chiego 2006). Acinar cells are highly polarized, surrounded by a plasma membrane with two general domains, basolateral and apical, which are separated by the tight junctions that link adjacent cells just below the luminal area (Young & Van Lennep 1978, Junqueira et al 1998, Berkovitz et al 2002). The acinar cells rest on a basement membrane, which separates them from the surrounding connective tissue. Ultrastructurally the serous cells are filled with secretory granules, rough endoplasmic reticulum, Golgi’s apparatus,
mitochondria and an oval nucleus. The mucous cells contain rough endoplasmic reticulum, a prominent Golgi's apparatus, flattened nucleus and large droplets of mucin (Chaudhry et al 1987, Berkovitz et al 2002, Avery & Chiego 2006).

Each secretory end piece empties into an intercalated duct, which has a small diameter and is considerably longer in the parotid than in the submandibular gland. These intercalated ducts are formed by cuboidal epithelial cells (Young & Van Lennep 1978, Tandler 1987a, Berkovitz et al 2002). Several acini drain into intercalated duct. It has been shown by histochemistry that lactoferrin staining is intense in these ducts, with lesser staining for lysozyme and weak staining for amylase. The intercalated ducts unite to form striated ducts. Ultrastructurally, these ducts are composed of cuboidal to columnar epithelial cells with marked infolded basilar plasma membrane, and are packed with mitochondria (Young & Van Lennep 1978, Tandler 1987a, Chaudhry et al 1987, Berkovitz et al 2002). Sodium resorption and potassium secretion occur in striated duct (Avery & Chiego 2006). On the basis of electron microscopy, fluorescent staining and immunohistochemistry for actin, it has been suggested that some basal cells in strated ducts of human salivary glands assume a myoepithelial phenotype (Dardick et al 1987). Both intercalated and striated ducts are part of the intralobular duct system, located inside the lobules. The later ducts empty into excretory ducts that open in the oral cavity. These ducts are embedded in the septal connective
tissue. The excretory duct cells are columnar with no obvious infolding at the base. As the duct enlarges and near its termination in the mouth, it is lined by pseudostratified epithelium. (Tandler 1987a, Berkovitz et al 2002, Hand 2003). The structures of the most common types of salivary gland secretory end-pieces are shown in Figure 1.3.
Figure 1.3. The secretory and ductal elements in a mixed salivary gland. Adapted from Berkovitz et al (2002).
Most secretory end-pieces are surrounded by contractile myoepithelial cells (basket cells) associated with their outer surface. These are stellate with a number of long, tapering processes that grasp the acini (Tandler 1987a, Berkovitz et al 2002, Avery & Chiego 2006). Contraction of these cells exerts pressure on the acini and forces preformed saliva into the mouth (Tandler 1987a, Cooper et al 1995). They have structural features of both epithelium and smooth muscle cells, and so are called myoepithelial cells (Palmer 1986). Myoepithelial cells can be identified by light microscopy through enzyme histochemistry and special stains and immunohistochemistry for their myofibrils, but the most reliable means of identifying myoepithelial cells is with a combination of histochemistry and electron microscopy. Ultrastructurally, myoepithelial cells contain myosin and a large number of actin microfilaments and also contain intermediate filaments of keratin. They lie beneath basal lamina on the surface of the acinar and intercalated duct cells and extend to the intra-and extralobular striated ducts (Dardick et al 1987, Chaudhry et al 1987). Monoclonal and polyclonal antibodies have been used to define salivary gland tissues, particularly with reference to the distribution of myoepithelial cells (Palmer 1986).

In a review article Garrett & Emmelin (1979) summarized myoepithelial activity as follows:

A - Speeds up the outflow of saliva.

B - Reduces luminal volume.
C - Contributes to the secretory pressure.

D - Supports the underlying parenchyma.

E - Helps salivary flow to overcome increases in peripheral resistance.
1.4. Physiology of Salivary Secretion

Salivary glands are composed of highly differentiated epithelial cells and are capable of secreting copious amounts of fluid and proteins. All secretions from human salivary glands occur in response to neurotransmitter stimulation (Baum 1987). This stimulation activates the secretory cells and leads to contraction of myoepithelial cells to support the secretory cells and to aid the flow of saliva (Emmelin 1987).

Salivary secretion is a reflex response. It occurs in response to neurotransmitter stimulation (Baum 1993), which is mediated through the autonomic nervous system (Garrett 1987). It can be stimulated by physical stimuli from the oral cavity and as a result of the presence of food in the mouth activating afferent neurons, which transmit to the superior and inferior salivatory nuclei in the medulla. While the superior salivatory nucleus controls the submandibular and sublingual glands the inferior nucleus controls the parotid glands. All glands receive sympathetic innervation from fibres, which have cell bodies arising in T-1 and T-2 that project to the superior cervical sympathetic ganglion. Post-ganglionic fibres pass through external carotid nerve plexus to all glands (Schubert & Izutsu 1987).

Salivary secretion can also be evoked by visual, olfactory, auditory stimuli and even by conscious, expectation and anticipation of food.
(Jenkins & Dawes 1966, Hector & Linden 1999). The types of receptors that are stimulated during eating have been classified as gustatory, masticatory, olfactory, psychic, visual and thermoreceptive (Hector & Linden 1999).

Both parasympathetic and sympathetic fibres innervate the acini and act collaboratively to activate the salivary glands. The main neurotransmitter for parasympathetic nerves is acetylcholine and that for sympathetic nerves is noradrenaline (Matsuo 1999). Parasympathetic stimulation has secretomotor and vasodilator effects and leads to increased salivary fluid secretion by all glands (Baum 1987, Herrera et al 1988, Edgar 1992). Parasympathetic stimulation results in a watery (serous) saliva due to the high contribution of the parotid gland to whole saliva.

Once in the glands the axons from each type of nerve intermingle and travel together in association with Schwann cells, forming Schwann-axon bundles. Two types of neuro-effector relationships exist with salivary parenchymal and myoepithelial cells: epilemmal (outside the parenchymal basement membrane) and hypolemmal (within the parenchymal basement membrane). Their relative frequencies with either type of nerve differ greatly between glands. Salivary blood vessels receive epilemmal innervations by both sympathetic and parasympathetic axons. A variety of non-conventional neuropeptide transmitters (such as vasoactive intestinal polypeptide, substance P) have also been found in salivary nerves by immunohistochemistry,
and they occur in large dense-cored vesicles. Prolonged high frequency stimulation has been found to cause depletion of large dense-cored vesicles from glandular nerves.

Stimulation of the sympathetic system (via noradrenaline) has vasoconstrictor, motor and trophic effects. Trophic effects cause a noticeable change in the composition of saliva, e.g. release of secretory proteins (Schubert & Izutsu 1987, Edgar 1992, Baum 1993). The saliva produced by sympathetic stimulation is thick and tacky (mucinous) due either to a weak stimulatory effect, or to the motor effect derived from the contractile activity of myoepithelial cells, or to both (Schubert & Izutsu 1987). The motor effect, however, is a complex reaction to a combination of parasympathetic and sympathetic stimulation (Emmelin 1979).

In general, parasympathetic stimuli may occur in isolation and may provide the principal reflex stimulation of salivary fluid formation. Meanwhile sympathetic stimuli do not occur in isolation and often produce synergistic effects when coupled with parasympathetic stimuli and tend to modulate the composition of saliva (Emmelin 1987, Schubert & Izutsu 1987).

Salivary secretion is a two-stage mechanism (Baum 1987, Baum 1993, Turner 1993, Poulsen 1998 Turner & Sugiya 2002). The first stage represents the formation of the initial secretion (primary secretion) by the secretory end-pieces, while the second stage
represents the modification of the initial secretion during its passage in the ductal system (Baum 1993, Baum 1989). (Figure 1.4).
Figure 1.4. Two stage saliva formation. The first stage takes place in the acinar region and is the generation of an isotonic primary fluid rich in exocrine proteins. The second stage occurs during passage of this fluid through the ductal region. This stage includes considerable electrolytes flux (reabsorption of most Na+ and Cl-; secretion of some K+ and HCO3-). A small amount of protein is also secreted by duct cells (After Baum 1993).
The initial secretion consists of interstitial fluids and the secretory products of acinar end-pieces. It is isotonic with plasma and is characterised by high concentrations of sodium and chloride ions and low concentration of potassium ions. It is believed that salivary secretion occurs by an osmotic mechanism. In particular sodium chloride is thought to be transported across the acinar cell into the lumen, thus creating an osmotic load, which draws water out of the cell, contributing to the initial secretion.

Based on studies of rabbit and rat salivary glands, three mechanisms have been suggested for primary fluid secretion by acini (Izutsu 1989, Turner 1993, Baum 1993). In mechanism (1), the fluid secretion is the result of action of four transport systems: (A) a Na⁺-K⁺-2Cl⁻-cotransporter (protein molecule) located in the basolateral of the acinar cell, (B) a basolateral Ca^{++} -activated K⁺ channel, (C) a Ca^{++} activated Cl⁻ and (D) the Na⁺/K⁺ ATPase. In this system sodium (Na⁺), potassium (K⁺), and two chloride (Cl⁻) ions are carried into the cell. In the unstimulated state K⁺ and Cl⁻ are concentrated in the acinar cell above electrochemical equilibrium. Salivary stimulation leads to a rise in intracellular calcium concentration, which results in the opening of the basolateral Ca^{++} -activated K⁺ channel and the apical Cl⁻ channel. These increases in K⁺ and Cl⁻ conductance allow KCl to flow out of the cell resulting in accumulation of Cl⁻ ions in the acinar
lumen. $\text{Na}^+$ then follows $\text{Cl}^-$. The resulting osmotic gradient for $\text{NaCl}$ causes a transepithelial movement of water to the lumen.

Mechanism (2) involves two ion exchanger systems: (A) a $\text{Na}^+ / \text{H}^+$ and (B) a $\text{Cl}^- / \text{HCO}_3^-$. Both are located in the basolateral membrane. The first system exchanges extracellular $\text{Na}^+$ for intracellular $\text{H}^+$, while the second exchanges extracellular $\text{Cl}^-$ for $\text{HCO}_3^-$.

Mechanism (3) involves bicarbonate secretions: $\text{CO}_2$ enters the acinar cell across the basolateral membrane and is converted to $\text{HCO}_3^-$ plus a proton ($\text{Cl}$) by intracellular carbonic anhydrase (Izutsu 1989, Turner 1993). The net result of these exchanges is that extracellular $\text{NaCl}$ is transported into the cell, while a carbonic acid molecule is transported out. Then $\text{Na}^+$ and $\text{Cl}^-$ exit the cell and enter the acinar lumen.

The initial secretion (now more properly called the lumenal fluid) is modified during its passage in the ductal system to the oral cavity. In the ducts, particularly the striated ducts which are lined by cuboidal cells rich in mitochondria, the sodium and chloride ions are reabsorbed into the cells while potassium and bicarbonate ions are secreted into the lumen (Figure 1.4). The lumenal fluid is changed into a hypotonic fluid with low sodium and chloride concentrations. The ionic exchange in the striated duct results in a hypotonic fluid since the duct cells do not absorb water under normal condition of flow. Further modification occurs in the excretory ducts by changes
in the electrolyte concentration and possibly by the addition of a mucoid component (Hand 2003). The excretory ducts can absorb sodium and excrete potassium.
1.5. Composition of Saliva

Many factors contribute to the composition of saliva. For example, stimulated whole saliva contains a higher proportion of fluid from the parotid glands than unstimulated saliva (Edgar 1992). Differences in salivary flow rate and in duration of stimulation of a gland have consequential effects on the composition of saliva (Dawes 1969, Dawes 1974, Edgar 1992). Salivary composition varies with the method of collection and stimulation (Lenander-Lumikari et al. 1995). Stimulus type appears to influence the protein content of parotid saliva even when the flow and duration of the stimulus are controlled (Dawes 1984a). It has been reported that parotid saliva secreted in response to a salty gustatory stimulus contains a higher protein concentration than saliva secreted in response to acid stimulus (Dawes & Jenkins 1964). Certain components of whole saliva may enter primarily by the gingival crevice rather than through the salivary glands. Thus, the concentration of these components may be affected by the number of teeth present and the degree of periodontal disease (Mandel 1980, Dawes 1993). Whole saliva is not sterile and contains a variety of proteolytic and other enzymes (Chauncey 1961). Thus organic components are susceptible to rapid catabolism unless the saliva is sterilised or the degradative enzymes are inactivated (Dawes 1993). Further changes in the composition may occur during collection and storage (Levine 1989a). The composition of stimulated and unstimulated saliva shows a circadian rhythm (Dawes 1969).
Factors influencing the composition of human whole saliva (after Dawes 1993):

1- Proportional contributions from different glandular sources

2- Contribution from blood and gingival crevicular fluid

3- Quality of oral hygiene

4- Plasma composition

5- Flow rate

6- Nature of the stimulus

7- Duration of stimulation

8- Time since previous stimulation

9- Circadian rhythm

10- Genetic polymorphisms

11- Antigenic stimulation

12- Exercise

13- Drugs

14- Various diseases

About 99% of saliva is water. The remaining 1% consists of a complex mixture of organic substances (proteins, glycoproteins, lipids, urea
and glucose), electrolytes; sodium, potassium, calcium, chloride, bicarbonate, phosphates, and small amounts of fluoride, iodide and magnesium (Mandel 1980, FDI 1992). Salivary constituents are listed in Table 1.1.
<table>
<thead>
<tr>
<th>Proteins</th>
<th>Small organic molecules</th>
<th>Electrolytes</th>
</tr>
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<tbody>
<tr>
<td>Albumin</td>
<td>Creatinine</td>
<td>Ammonia</td>
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<tr>
<td>Amylase</td>
<td>Glucose</td>
<td>Bicarbonate</td>
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<tr>
<td>β-glucuronidase</td>
<td>Lipids</td>
<td>Calcium</td>
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<td>Carbohydrases</td>
<td>Nitrogen</td>
<td>Chloride</td>
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<td>Cystatins</td>
<td>Sialic acid</td>
<td>Fluoride</td>
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<tr>
<td>Epidermal growth factor</td>
<td>Urea</td>
<td>Iodide</td>
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<tr>
<td>Esterases</td>
<td>Uric acid</td>
<td>Magnesium</td>
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<tr>
<td>Fibronectin</td>
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<td>Non-specific buffers</td>
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<td>Gustin</td>
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<td>Phosphates</td>
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<td>Histatins</td>
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<td>Potassium</td>
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<td>Immunoglobulin A</td>
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<td>Sodium</td>
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<td>Immunoglobulin G</td>
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<td>Sulphates</td>
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<td>Immunoglobulin M</td>
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<td>Thiocyanate</td>
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<td>Kallikrein</td>
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<td>Lactoferrin</td>
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<td>Lipase</td>
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<td>Lactic dehydrogenase</td>
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<td>Lysozyme</td>
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<td>Mucins</td>
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<td>Nerve growth factor</td>
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<td>Parotid aggregins</td>
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<td>Peptidases</td>
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<td>Phosphatases</td>
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<td>Proline-rich proteins</td>
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<td>Ribonucleases</td>
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<td>Salivary peroxidases</td>
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<td>Secretory component</td>
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<td>Secretory IgA</td>
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<tr>
<td>Serum proteins (trace)</td>
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<tr>
<td>Tyrosine-rich proteins</td>
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<td>Vitamin-binding proteins</td>
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Table 1.1. Salivary constituents (after FDI, 1992).
1.6. Functions of Saliva

The importance of salivary components and their function in maintaining oral health is well known (Table 1.2) (Sreebny 2000, Pedersen et al 2002). Saliva plays a major role in the health of the oral cavity (Mandel 1987, Valdez 1991). A reduction in salivary gland function may result in dental caries, difficulty in swallowing, speech and in denture wearing as well as alterations in taste and increased frequency of opportunistic infections (Fox et al 1985, Crow and Ship 1995, Mandel 1989, Diaz-Arnold & Marek 2002, Dawes 2004, Avery & Chiego 2006).

It is recognised that some salivary molecules are multifunctional (Dowd 1999, Niew Amerongen & Veerman 2002), some have amphifunctional properties and many molecules have overlapping functions (Levine 1993, Tenouvo 1998). This interact results in a functional compensation and may explain why large individual variations in the concentration of different components do not affect the overall protective qualities of saliva (Levine 1993; Van Nieuw Amerongen et al 2004 see Figure 1.5 below). Many interactions have been reported between salivary molecules (e.g. IgA and peroxidase, lactoferrin and lysozyme) (Tenovuo 1998).
Table 1.2. Functions of saliva (after Pedersen et al, 2002).
Figure 1.5. Salivary molecules are multifunctional (after Levine, 1993).
1.6.1. Digestion

The most abundant enzyme found in saliva is α-amylase (Mason & Chisholm 1975) and it is one of the most important salivary digestive component. It is synthesized within the serous acinar cells of the major salivary glands. Consequently, it is present in much higher concentrations in parotid saliva than submandibular saliva (Mason & Chisholm 1975, Noble 2000). It has been shown that amylase accounts for 40-50% of the total protein produced by salivary glands (Noble 2000). This enzyme digests cooked starch (Jenkins 1978). The end product of this digestion consists of approximately 80% maltose and 20% glucose. In addition to its well-known function as a digestive enzyme, amylase has been reported to act as antimicrobial enzyme. It has been found that salivary amylase inhibits the growth of *Legionella pneumophila* and *Neisseria gonorrhoeae* (Tenovuo 1989). On the other hand amylase adsorbs to tooth surfaces facilitating adherence of bacteria. This is an example of amphifunctional property of saliva (Levine 1993).

Amylase increases in stimulated saliva and decreases with increasing age. Thus, elderly people have a deficiency of starch digestion in the mouth and stomach (Meyer *et al* 1937). Age has also been found to affect amylase activity; its activity in the unstimulated and stimulated parotid is significantly lower in elderly healthy subjects (Ben-Aryeh *et al* 1986).
Other digestive enzymes present in saliva include proteases, lipase, gustin and nucleases (Fox 1989a, Diaz-Arnold & Marek 2002, Pedersen et al 2002).

1.6.2. Lubrication

Saliva keeps the mouth moist, facilitates speech, and lubricates food for chewing and swallowing (Rice 1984, Crow & Ship 1995, Humphrey et al 2001, Diaz-Arnold & Marek 2002, Pedersen et al 2002). It provides a tissue-coating film that allows food passage and provides smooth tissue surfaces, which exhibit minimal friction (Mandel 1987, Mandel 1989). The lubricant properties of saliva have been ascribed to the mucin and proline-rich proteins, which are the main proteins of saliva. Mucins are high molecular weight Glycoprotein which present in saliva in two forms MG1 and MG2. They provide a protective coating for both soft and hard tissues (Tabak et al 1982, Humphrey et al 2001). Mucins are at high concentration in minor salivary gland secretions, with some contribution from submandibular and sublingual glands secretions (Herrera et al 1988, Ferguson 1999). Saliva forms a mucoid coating on the mucous membrane, acting as a barrier to irritants and preventing desiccation (Tabak et al 1982, Rice 1984). Its flow helps to clear the mouth of food, cellular and bacterial debris, consequently retarding plaque formation. Certain salivary proteins act to reduce tooth wear by lubrication (Ben-Aryeh et al 1985, Herrera et al 1988),
to neutralise or dissolve weak acids or to modulate the oral microbial flora (Herrera et al 1988). Acquired pellicle, which consists of salivary proteins (Hensten-Pettersen 1975, Hall & Grigsby 1984), prevents diffusion of acids into the tooth surface, and calcium and phosphate out of the tooth (FDI 1992). It acts as a lubricant film, which aids in prevention of tooth wear. Salivary proteins interfere with bacterial colonization by binding to bacteria in the saliva solution and clear them from the mouth by aggregation. They can also promote colonization by providing receptor structures to which the bacteria adhere and become bound to a surface (Scannapieco 1994, Rudney 2000).

1.6.3. Regulation of Oral pH

Saliva has several buffering systems capable of regulating the pH of the oral cavity. The buffering capacity of human saliva is regulated by three systems (Bardow et al 2000).

1. The carbonic acid / bicarbonate system

2. The phosphate system.

3. The proteins

The carbonic acid / bicarbonate is based on the equilibrium

\[ H_2CO_3 \rightarrow HCO^-_3 + H^+ \]
When an acid is added, the bicarbonate releases weak carbonic acid. The carbonic acid is rapidly decomposed into water and CO$_2$, which leaves the solution, leading to a complete removal of acid. The bicarbonate concentration is strongly dependent on the salivary flow. As a consequence, most variables that may influence the secretion rate negatively will also affect the buffer capacity and pH.

The phosphate buffer system operates according to the following equilibrium

$$\text{H}_2\text{PO}_4 \longrightarrow \text{HP}^\text{+} + \text{H}^-$$

The tissue-coating properties of large molecular weight salivary mucins are also ideally suited to act as permeability and diffusion barriers and thereby maintain local pH.

Bicarbonate is the most important buffer, with phosphate playing a minor role (Humphrey et al 2001). Bacterial urease can convert salivary urea to ammonia, which neutralises acid (Mandel 1989, Humphrey et al 2001). There is an upward trend in the buffering power throughout the day until the evening when it usually tends to fall (Ericsson 1959). It increases about a quarter of an hour after meals but usually falls within 30-60 minute after. Meanwhile there is an inverse relationship between caries and the buffering capacity of saliva. Tooth wear has been speculated to be associated with buffering capacity, variation in calcium ion concentration and salivary flow rate (Dahl et al 1993, Johansson & Omar 1994,
Gudmundsson et al (1995). The tooth demineralisation caused by bacterial acids or acids in food can be inhibited by saliva bicarbonate that increases the pH and buffer capacity of saliva especially during stimulation (Bardow et al 2000).

1.6.4. Antimicrobial Function

Saliva has considerable antimicrobial capacity by virtue of its content of specific antibodies (secretory IgA) as well as non-specific antimicrobial factors such as histatin, calprotectin, lysozyme, lactoferrin, lactoperoxidase, hydrogen peroxidase and thiocyanate ion (SCN⁻) (Tenovuo et al 1982, Mandel 1987, Mandel 1989, Challacombe 1994, Tenovuo 1998, Salvolini et al 2000, Nieuw Amerongen & Veerman 2002). A glycoprotein, which is known as a secretory component, is produced by acinar cells of the parotid and submandibular glands. This protein binds two molecules of Ig A to stabilize and form secretory IgA (Rice 1984). Secretory IgA has the ability to agglutinate microorganisms, inhibit bacterial adherence and neutralize viruses (Mandel 1987, Tenovuo 1998, van Nieuw Amerongen et al 2004). In addition lysozyme can aggregate bacterial cells and inhibit their colonization on mucosal surfaces and teeth (Dowd 1999).

Secretory IgA concentration has been shown to be dependent on salivary flow rate (Streckfus et al 1991, Tenovuo 1992). Other
immunoglobulins such as IgG and IgM have been detected in saliva from parotid and minor glands (Rice 1984, Smith et al 1991). IgG and IgM salivary content, however, comes mostly from gingival crevicular fluid (Challacombe et al 1978, Parry 1993, van Nieuw Amerongen et al 2004).

Histatins are a family of salivary proteins, which are part of the non-immune host defence system in saliva. They have a potent and broad-spectrum antifungal activity and are also active against some bacterial species (Mackay et al 1984, Oppenheim et al 1988, Murakami et al 1991, Kavanagh & Dowd 2004), while non-toxic to human cells (Edgerton et al 1998). The mechanism by which histatins can kill or inhibit Candida remains unclear (Edgerton et al 1998, Xu et al 1999, Veerman et al 2004). However, several mechanisms of antifungal action of histamines against C. albicans have been suggested. One of these proposes that histamine may act against Candida through the binding to putative receptors on the candida cell membrane with subsequent interaction with the cell (Xu et al 1999). On the other hand (Helmerhorst et al 1999) reported that histatin is targeted to mitochondrial and results in a loss of transmembrane potential.

Lysozyme is an enzyme found in submandibular and parotid saliva but is produced mainly by the submandibular and sublingual glands (Mackay et al 1984, Noble 2000). It is able to split the cellular wall of some bacteria, especially S mutans and Veillonella, by hydrolysing.

Lactoferrin binds free iron, which is an essential element for bacteria and fungal multiplication (Challacombe 1994, van Nieuw Amerongen et al 2004). It has been reported that lactoferrin is a multifunctional protein having bacteriostatic, bactericidal, fungicidal, antiviral, antinflammatory and immunomodulatory properties (Nikawa et al 1993, Nieuw Amerongen & Veerman 2002).

Saliva contains sialin, a tetrapeptide, which is one of the glycolysis-stimulating factor or “pH-rise” factors (Edgar 1992). Sialin can induce a rise in the pH of dental plaque after sugar consumption. On the other hand, saliva contains proline-rich proteins that help to maintain the proper calcium phosphate environment required for tooth integrity by retarding demineralisation of tooth surfaces and inhibiting and excess mineral deposition (Bennick 1982).

It has been shown that saliva contains two peroxidase enzymes. Salivary Peroxidase, which is secreted by salivary glands and myeloperoxidase by oral polymorphonuclear leukocytes (Thomas et al 1994). Both salivary peroxidase and myeloperoxidase catalyse the oxidation of thiocyanate ions by hydrogen peroxide to form hypothiocyanite ions (Grisham & Ryan 1990, Humphrey et al 2001). Thomas et al (1981) found that accumulation of the antimicrobial agent hypothiocyanite in human saliva requires peroxidase activity, thiocyanate and production of hydrogen peroxide by oral bacteria.
Salivary peroxidase removes toxics hydrogen proxide produced by oral microorganisms and can reduce acid production in dental plaque (Mandel 1989, Dowd 1999).


They promote healing of ulcerations in the gastrointestinal tract. This could have a role in maintaining the integrity of this tract.

One recently discovered molecule called SLPI (secretory leukocyte protease inhibitor) is an antimicrobial protein found in saliva and having anti-HIV activity. McNeely et al (1995) reported that SLPI is present in whole and ductal saliva and this protein might block HIV infection by interacting with a cellular protein and thus blocking HIV entry.

1.6.5. Maintenance of the Integrity of Teeth

Theoretically, saliva can affect caries in four general ways: mechanical cleansing resulting in less accumulation of bacteria and debris on tooth surfaces, reducing solubility of enamel by providing a high level of calcium, phosphate and fluoride to the plaque, buffering and neutralising the acids produced within the plaque via the
carbonic acid-bicarbonate buffering system (ammonia and urea) and by anti-bacterial activity (Mandel 1987, Dowd 1999).

A reduction in the salivary flow rate has been followed by an increase in caries activity (Ericsson & Hardwick 1978, Lyons 1972, Saunders & Handelman 1992). The process of dental caries could be affected by the reduction in salivary flow by two major ways. It reduces its ability to clear sugar from the oral cavity after sugar intake (Dawes 1983, Dawes 1984b, Risheim et al. 1992) and minimise both the buffering activity of saliva and its degree of supersaturation with calcium, phosphate and hydroxyl ions (Dawes 1984b). This salivary supersaturation prevents demineralisation and enhances remineralisation. This action is enhanced by the increase in salivary flow rate through stimulation (Edgar 1998, Humphrey et al. 2001). While it is prevented when the plaque pH falls so that the hydroxyl and phosphate ion concentrations are reduced below a critical value. It has been shown that buffer capacity increases with increasing flow rate (Ericsson & Hardwick 1978, Bardow et al. 2001). Some salivary proteins contribute to remineralisation of enamel. Among these are statherin, the acidic proline-rich proteins and a number of phosphoproteins (Figure 1.5). These molecules have the capacity to bind calcium. With a drop in plaque pH, they release calcium and phosphate ions to the plaque, thus fostering remineralisation (FDI 1992). A summary of the possible roles of saliva in prevention of dental caries is given in Table 1.3.
1. Saliva aids enamel maturation

2. Saliva may inhibit plaque growth and metabolism

3. Saliva may reduce glycolysis in plaque

4. Saliva may buffer pH fall in plaque

5. Saliva may speed sugar clearance

6. Saliva may aid remineralisation by providing minerals

7. Saliva may help increase thickness of enamel pellicle

Table 1.3. The possible roles of saliva in prevention of dental caries
(modified from Levine 1989b)
1.6.6. Taste

Saliva is involved in maintenance of normal taste function and taste-bud integrity. The relationship of saliva to taste is well documented. Reduction of salivary flow may lead to alteration of taste (Lyons 1972, Tomita & Osaki 1990, Mott et al 1993). It has been proposed that saliva can affect taste through the following: its capability as a solvent, its role in transporting tastants to the taste receptors, provision of optimal ionic environment for taste transduction and provision of secretory proteins that may serve a carrier function (Mott et al 1993). Saliva can maintain oral acid-base balance, in addition to its immunoglobulin and antifungal contents, contributes to protection of taste pore integrity (Mott et al 1993, Hershkovich & Nagler 2004). On the other hand, low salivary flow has been found to be associated with loss of pleasure of tasting food (Ikebe et al 2002, Spielman 1990). Perceived low salivary flow has also been significantly associated with this loss of pleasure (Ikebe et al 2002).

In 1972, Henkin and co-workers reported that patients with dry mouths as a result of Sjögren’s syndrome showed significant decreases in taste acuity and that treatment of this dryness improved taste function. However, wearing a maxillary mouth guard for one hour resulted in a reduction in stimulated parotid saliva (Shannon et al 1970). The mean parotid saliva collected without the mouth guard was 0.78ml/min, which decreased to 0.64ml/min when the mouth guard was in place. This effect was attributed to reduced taste and
tactile sensation due to the palatal coverage with the mouth guard.

1.6.7. Saliva as a Diagnostic Fluid

With the emergence of HIV and other blood-borne viral infections there has been a growing appreciation of the value of saliva as a less hazardous specimen than blood for various Potential clinical diagnostic assays. In addition, collection of saliva is non-invasive, and several risks associated with drawing of blood and limitations with use of urine are reduced (Mandel 1993a, Lenander-Lumikari et al 1995). These make saliva a good diagnostic tool in epidemiological studies.

Potential advantages of saliva over serum specimens (after Parry 1993):-

1. More acceptable to patient
2. Non invasive
3. Painless
4. Fewer refusals
5. More convenient
6. Self-collected, cheap to collect
7. Rapid and simple
8. No sterile precautions

9. More versatile

10. Reaches difficult populations

1.6.8. Drug monitoring

In recent years saliva has attracted much attention, in particular among workers interested in the determination of drug concentrations, who suggest that saliva might be substituted for plasma in the areas of pharmacokinetic studies and drug monitoring (Mandle 1993, Streckfus & Bigler 2002). The traditional biological samples for the qualitative and quantitative measurement of most drugs are blood, plasma and urine. Many substances and their metabolites are present in different concentrations in these media. Blood or plasma provides an estimate of the current circulating concentration of the analyte of interest. Blood samples must be drawn by trained staff and particular care must be taken with samples from drug addicts given the high prevalence of hepatitis B, hepatitis C and human immunodeficiency virus (HIV) infections in this population. Urine permits measurement of the accumulated concentration of analytes since the last emptying of the bladder. Being readily accessible and collectable, saliva demonstrates many advantages over blood and urine. Pretreatment of saliva in analytical procedures is often easier and faster than pretreatment of blood.
Detection of drugs in urine is possible after 12 hour from taking the drug. In saliva low levels of drug may be detected within one hour after use, depending on the dose and kinetics of the drug. Therefore, saliva is more agreeable to pharmacokinetic interpretation, monitoring of drug abuse and therapeutic drug monitoring (Höld et al 1995 Tabak 2001). However, saliva also has its limitations (Haeckel 1993, Bailey et al 1997), some times there is poor correlation between saliva and plasma concentration for many substances (Siegel 1993).

Factors such as molecules size, lipid solubility and degree of ionization of the drug molecule as well as the effect of salivary PH and the degree of protein binding of the drug are important determinants of drug availability in saliva (Siegel 1993).

Never the less, a clear interpretation of the quantitative significance of saliva drug concentrations to explain the mechanisms of drug secretion into saliva has not been achieved. Therefore, it is important to use saliva concentrations in conjunction with concentrations recorded from paired plasma samples.

The increasing use of tricyclic antidepressants in the very young and in the elderly makes a non- invasive approach to the monitoring of adequacy of dosage and compliance highly desirable. Saliva is readily available and simple to collect utilizing non-invasive procedures (Tabak 2001). It may be possible to use saliva levels of the drug to monitor patients once their saliva / plasma ratio has been determined (Cooper et al 1981). Saliva sampling obviates the need for

In order to achieve an accurate screening for drugs of abuse, saliva should be collected under standardized condition. The following variables must be considered (Tabak 1993)

1. Time of day.


3. Analysis of mount (i.e. weight versus volume measurements).

4. Need for controlling proteolysis.

5. Method of collection.

6. Source of saliva (it is desirable to collect ductal secretions; parotid or submandibular-sublingual glands).
1.6.9. Systemic diseases

Saliva has been used to evaluate a variety of systemic conditions. Its changes may represent the most common oral manifestation of systemic disease. These characteristic changes may contribute to the diagnosis and early detection of these diseases.

Salivary gland dysfunction is a common clinical feature of Sjögren's syndrome (Sjögren 1933, Connolly 2001). The immune disorder involves a progressive lymphocytic infiltration of the affected glands with polyclonal B lymphocyte activation and autoantibody production. Reduced salivary flow rate has been used extensively as non-specific diagnostic criteria for Sjögren's syndrome. Various arbitrarily cut off values have been applied. However because lack of specificity and considerable overlapping with healthy individuals the use of sialometry in the diagnostic test set for Sjögren's syndrome has been questioned (Skopouli et al 1989). On the other hand sialometric and saliochemistry have been proposed as useful instruments in diagnosing Sjögren's syndrome (Vissink et al 1993, Kalk et al 2002). Vissink and colleagues (1993) demonstrated decrease in unstimulated salivary flow of the parotid and the submandibular/sublingual glands with change in composition in the early stage of the disease. In advanced stage parotid flow rates were markedly reduced with striking changes in salivary composition (increased Na⁺ and Cl⁻ and decreased total protein and amylase. They
concluded that analysis of the secretion and composition of parotid saliva is a sensitive tool in diagnosing advanced Sjögren’s syndrome.

In cystic fibrosis, there are dysfunctions of all major and minor salivary glands. Flow rates are decreased, which leads to an increase in \( \text{Ca} \), \( \text{PO} \) and protein (Kaufman & Lamster 2002).

1.6.10. Infectious diseases

Analysis of saliva for infectious diseases such as human immunodeficiency virus (HIV) is a good example of its usefulness in diagnosis of a number of such diseases. It has been demonstrated that the diagnosis of infection with (HIV) based on specific antibodies in saliva is equivalent to serum in accuracy and therefore applicable for both clinical use and epidemiological surveillance. Furthermore collection and analysis of saliva offer a simple, safe and accurate method for diagnosis of HIV infection (Kaufman & Lamster 2002). Clemmons et al (1993) has demonstrated that saliva screening may be useful to detect patient who carry hepatitis B surface antigen (87% sensitivity and 66.7% specificity) and those with anti- hepatitis B core antibodies (100% sensitivity and specificity). Saliva-based tests are also finding use in diagnostic and epidemiology studies of herps viruses (Zerr et al 2000, Spicher et al 2001) and Epstein-Barr virus (Ikuta et al 2000).
1.6.11. Monitoring of Hormone Levels

Many clinical assessments of endocrine function require the temporal monitoring of plasma steroid levels. Standard plasma sampling techniques or urinalysis do not necessarily provide the optimal sampling conditions required in these types of monitoring (Quissell 1993).

Saliva is becoming the specimen of choice for investigating hormones because of its ability to measure the free fraction as well as the other advantages associated with the case of obtaining multiple samples. It is actually superior to blood for evaluating these hormones because it accurately reflects the concentrations of free hormones in circulation (Ferguson 1987, Streckfus & Bigler 2002). Hormones in the bloodstream are mostly protein-bound, which makes them inactive. It is only the free or unbound hormone that is able to pass into the cells and exert activity (Quissell 1993). Hormones that can be measured from saliva include oestrogen, progesterone, testosterone, androstenedion, cortisol (Aardal & Holm 1995) and melatonin. Cortisol is a steroid hormone that is primarily involved in regulating metabolism and the body’s response to stress. A number of studies have revealed correlations between plasma and salivary levels of cortisol. The measurement of cortisol in saliva has become a reliable tool for studying adrenal cortical function. It has been suggested that saliva may be used for cortisol measurements in situations where
blood sampling is impractical and difficult to obtain (Aardal & Holm 1995). Due to the several advantages over blood cortisol salivary cortisol can be used as an alternative to serum cortisol (Aardal-Eriksson et al 1998). Saliva was also recommended to use in screening Cushing's syndrome (Castro et al 1999, Tabak 2001). Based on the ability of saliva assays to detect a cortisol increase, Duclos and co-workers (1998) suggested its use to study human hypo-thalamopituitary adrenal physiology.

Salivary diagnosis is an increasingly important field in forensic medicine, as saliva has proven to be a convenient source of host and microbial DNA (Tabak 2001). DNA typing of biological material has become one of the most powerful tools for personal identification in forensic medicine and in criminal investigation. Forensic studies have shown that DNA from saliva on stamp or cigarettes can be used in PCR analysis to identify suspects (Walsh et al 1992, Allen et al 1994).
1.7. Symptoms and Signs of Hyposalivation:

Dryness in the oral cavity is not only uncomfortable; it is also accompanied by an array of symptoms and signs (Nagler 2004). The most serious outcome, which is related to teeth, is dental caries and different types of tooth wear (Greenspan 1996, Young et al 2001, Lenander-Lumikari & Loimaranta 2000). Salivary hypofunction can alter the oral flora and contribute to plaque formation, thereby increasing the risk for opportunistic infections and the proliferation of cariogenic microorganisms (Papas et al 1993, Nagler 2004). On the other hand salivary deficiency may predispose to oesophageal injury by decreasing the clearance of acid and altering intraoesophageal pH (Korsten et al 1991). Certain signs and symptoms that could be associated with hyposalivation are listed below.

Symptoms

Thirst

Dysphagia

Dysguesia and dysosmia

Dysphonia
Difficulty in eating dry foods

Sore tongue and lips

Adherence of the tongue to the palate

Ulcerations

Difficulty with denture wearing and retention

Burning sensations (glossopyrosis)

Signs

Dental caries (rampant caries); increased prevalence; located at sites generally not susceptible to decay e.g. cervical margins of teeth

Loss of glistening of the oral mucous membrane

Pallor and atrophy of the oral mucous membranes

Fissuring and lobulation of the dorsum of the tongue

Epithelial atrophy

Candidosis; especially of the tongue and the palate

Angular cheilitis
Inflammation with consequent infection of salivary glands

Increased dental plaque accumulation and periodontal disease

Nutritional deficiencies because of eating difficulties.
1.8. Factors modifying salivary flow

Salivary flow is affected by many factors. It has been shown that factors such as chewing and swallowing during saliva collection influence saliva secretion rates (Kerr 1961). Mason et al (1966) studied the pattern of salivary flow (unstimulated & stimulated) from parotid and submandibular salivary glands with the aid of apparatus containing photoelectric detectors. They stated that there are many variables affecting flow rate: swallowing, chewing, sucking, the position of the stimulus in the mouth, the rate of application of the stimulus and the time interval following the previous stimulus. There are many other factors that may affect the salivary flow.

1.8.1. Degree of hydration

The degree of tissue hydration may have an essential effect on the salivary flow rate (Kerr 1961, Holmes 1964, Dawes 1987, Navazesh 1993). Dehydration occurs when the body loses more fluid than it takes in. This condition can result from illness, hot, dry climate, prolonged exposure to sun or high temperatures, not drinking enough water and over use of diuretics. For instance, Holmes (1964) reported a lack of salivary flow following an 8% reduction in body water content. On the other hand, Shannon & Chauncey (1967) demonstrated that water tissue overloading had a positive effect on
salivary flow rate. The latter finding was in contrast with previous observation of De Wardener & Herxheimer (1957) and Kerr (1961).

De Wardener & Herxheimer studied unstimulated salivary flow rate in two normal individuals. Each individual drank daily 10 litres of water for eleven days. The flow rate was found not be affected by this high water ingestion. Kerr (1961) has pointed out that this sample group was very small.

Salivary flow rate and composition are affected by seasonal and diurnal factors. Shannon (1966) found that parotid salivary flow rates were lower in summer than in winter. This may be attributed to the summer heat that leads to a dehydration of the body. On the other hand, Louridis et al (1970) found that unstimulated whole salivary flow rates increases during low temperatures and decrease during high temperatures when the whole saliva is collected from three healthy subjects every third or fourth day for one year in room temperature without a heater or an air condition. Accordingly, it reasonable to suggest that an interrelationship of heat, dehydration and reduction of salivary flow rate does exist. Ship & Fischer (1997) demonstrated reduction in parotid salivary flow rates in dehydrated young (20-40 years) and old (60-80 years) healthy subjects after they refrained from food and beverage intake for 24 hours.
1.8.2. Circadian rhythms

Salivary flow shows significant circadian trends (Dawes 1972, Ferguson et al 1973, Ferguson & Botchway 1980, Richardson & Feldman 1986). The amount of saliva in the mouth is not constant and varies within a person over time (Dawes 1972). Variations in individual flow rates can be as high as 50% over a twenty-four hour period due to circadian rhythms (Ferguson & Botchway 1979). In most individuals the volume of unstimulated and stimulated saliva is at a maximum in the evening (Shannon & Prigmore 1959, 1962, Dawes 1974, Ferguson & Botchway 1980, Edgar 1992, Dawes 1996). On the other hand, Palmai & Blackwell (1965) measured salivary flow by dental swabs left in the mouth at two-hourly intervals in 24 hours in normal controls and depressed patients. They found a diurnal rhythm in the salivary flow in the normal subjects with maximal secretion occurring in the morning and reaching its lowest level in the evening. During sleep, the flow rate is very low (Schneyer et al 1956, Edgar 1992), which may be due to a decrease in physical and psychic stimuli. Nederfors & Dahlöf (1992) noted that secretion rates for both unstimulated and stimulated saliva in healthy men were significantly lower in the morning than at lunch time. In an investigation of diurnal variations of salivary flow rate and composition, Dawes (1972) reported significant circadian rhythms in the flow rate and concentrations of sodium, chloride, protein and potassium, calcium, but not in phosphate or urea. Richardson &
Feldman (1986) noted considerable variations from day to day in unstimulated and, to a lesser extent, stimulated whole saliva. Ghezzi et al (2000) investigated the variability in stimulated parotid and submandibular flow rates over six hours in 36 healthy males and females (aged 20-77 years), and demonstrated that flow rates can fluctuate by as much as 45% with diurnal rhythms and could be considered normal salivary variation.

Flink et al (2005) investigated unstimulated whole saliva flow rate in 108 individuals, age 15-46 years. Saliva was collected at two time intervals (7:30 a.m. and 11:30 a.m.). The participants were allocated to one of three groups (very low< =0.1mL/min, low 0.1-0.2mL/min and normal >0.2mL/min) based on the unstimulated whole saliva flow rate at 7:30 a.m. The salivary flow rate was found to increase significantly at 11:30 am when compared to the one at 7:30 a.m. Subjects with a very low flow rate (< =0.1mL/min) in the early collecting time were found to exceed this limit at the second collecting time. According to this study, the time at which saliva is collected has a strong effect on the flow rate and therefore should be standardised. It was recommended that unstimulated whole saliva should be collected at a fixed time-point or in a limited time interval early in the morning.
1.8.3. Age

atrophy was observed in both acinar and ductal epithelia. With increasing age structural changes were also found in fibrous and elastic tissues as well as in the walls of arteries and veins (Scott 1977c). However, the high consumption of medications among elderly patients has been postulated as a cause of salivary flow reduction (Thorselius et al 1988, Sreebny et al 1989, Närhi et al 1992, Edgar & O'Mullane 1996). On the other hand, Ghezzi & Ship (2003) demonstrated that the salivary glands of older people are more susceptible to the adverse effects of medications compared with those of younger individuals.

It is difficult to obtain conclusive information by comparing the available data. The conflicting results in different studies may be due partially to sample selection, which in most cases was not randomised and included medicated individuals with difference in age between elderly subjects in different studies (Sreebny & Broich 1987, Percival et al 1994), or to the study design and the different collection methods (Bourdiol et al 2004, Nagler 2004). It is worth mentioning that study of parotid flow (Baum 1981a, Chauncey et al 1981, Heft & Baum 1984, Gandara et al. 1985, Percival et al 1994, Fischer & Ship 1999, Bourdiol et al 2004) and whole salivary flow in healthy individuals (Parvinen & Larmas 1982, Heintze et al 1983) as well as longitudinal studies (Österberg et al 1990, Ship & Baum 1990) did not demonstrate significant effects of age on salivary flow. Meanwhile Shern et al (1993a), examined 51 healthy subjects and
reported that age exerted no influence on unstimulated flow from buccal or lower labial glands but palatal unstimulated secretion showed a significant decrease with age. Nevertheless Fischer & Ship (1999) demonstrated that there is no increased age-related variability in parotid salivary flow rates over repeated measures; so parotid salivary flow rates remain stable over two hours in healthy, unmedicated subjects, and are similar in younger and older individuals.

It has been suggested that elderly people have no impairment in their ability to respond to sialogogues but unstimulated salivary flow rates are found to be lower in old individuals than in young ones (Percival et al 1994). Skopouli et al (1989) found that age influenced the rates of unstimulated whole salivary flow while it did not have any effect on the stimulated parotid flow rate. Flow rate of buccal minor salivary glands was found not to be influenced by age below 50 years, but decreased thereafter irrespective of the individual’s gender (Sivarajasingam & Drummond 1995).

In a review by Sreebny (2000) for numbers of studies that included only healthy, non medicated subjects, it was reported that in most of the studies the flow rate of resting whole saliva decreased with age whereas the flow rate of stimulated saliva provided mixed results because most organs when stimulated compensate for parenchymal loss. Furthermore, Vissink et al (1996) concluded that stimulated salivary flow rate in healthy adults did not demonstrate significant
age-related changes, which may be due to the high reserve capacity within the glands. On the other hand, Wu and Ship (1993) suggested that the submandibular gland may be more sensitive to physiological change than the parotid gland, which can maintain the output despite of the decrease in acinar cells in elderly people (Scott et al 1987). It has been speculated that this capacity may be due to the resilience nature of the oral cavity with ageing (Ship & Baum 1993). However, in the submandibular gland significant loss of secretory tissue occurs as a result of its replacement with fat and connective tissues (Scott 1977a).

Ship et al (1991) investigated unstimulated and stimulated submandibular and parotid salivary flow rate and oral health status in healthy individuals to determine if there is a discrete range of saliva production necessary to support normal oral function. They failed to define normal function and suggested that measurement of salivary production within an individual over time is necessary to determine any salivary gland hypofunction. They demonstrated that healthy individuals in the lowest 10th percentile of major salivary gland flow rates had oral health similar to that of those in the highest 10th percentile. However, the large amount of variability in parotid salivary flow rates in a healthy population could obscure important age-related changes in the output reported in cross-sectional investigations.
To conclude, the effect of age on the salivary flow rate as reported by several investigators shows a diversity among the studies. Table 1.4 shows several studies of the effect of age on the salivary flow rate.
<table>
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<th>Stimulated</th>
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table 1.4. Several studies of salivary flow rate: variations of flow rate with age
1.8.4. Sex

Lower secretion rates have been reported for unstimulated and stimulated salivary flow rates in women than in men (Bertram 1967, Heintze et al. 1983, Parvinen & Larmas, 1981, Parvinen & Larmas 1982, Parvinen et al. 1984, Thorselius et al. 1988, Percival et al. 1994, Lopez-Jornet & Bermejo-Fenoll 1994, Eliasson et al. 1996, Navazesh et al. 1996, Hargitai et al. 2005). Österberg et al. (1991) found men secreted more stimulated whole saliva than women (1.24 versus 0.92 ml/min). Nevertheless, these findings contradict the results reported by Heft & Baum (1984), Ben-Aryeh et al. (1984), Watanabe & Dawes (1988ab), Richardson & Feldman (1986) and Jones & Ship (1995) who did not find significant differences between women and men. These differences between the sexes have been explained on the basis of the male salivary glands being larger than those of females (Ericson 1970, Scott 1975). It is reasonable to suggest that this difference may be due to the differences in gland sizes. For instance, Ericson (1970) found that the maximum stimulated flow rate of parotid gland is directly proportional to its size (Ericson 1970) and that the size of the parotid gland is the best predictor of its secretory capacity (Ericson 1970, Ericson 1971, Dawes et al. 1978). This is, partially in accordance with the results reported by McCartan et al. (1987) who found correlation between parotid flow rate and parotid gland volume in young individuals with suspected salivary disorders (age range 20-29), but not in older individuals with a similar medical
condition. Recently Ono and co-workers (2006) investigated the relationships between salivary gland size and flow rate and salivary composition in 28 healthy young individuals. The three major salivary gland sizes were estimated using a magnetic resonance imaging technique. A positive correlation was observed between the unstimulated whole salivary flow rate and the size of the parotid (r = 0.50, P = 0.007) and submandibular glands (r = 0.44, P = 0.02), but not with the sublingual glands. The study also suggested that the larger the sizes of the parotid and the submandibular glands, the faster the un-stimulated whole salivary flow rates (Ono et al 2006).

However, other factor that might be expected to contribute to a decrease in flow rate is hormonal status (McCartan et al 1987). Women of post-menopausal age have been reported to have a decrease in salivary flow rate (Parvinen & Larmas 1982). Meanwhile, Patton et al (1990) did not demonstrate an effect of either menopause or hormonal replacement therapy on salivary flow rate.

1.8.5. Medications

Nederfors et al 2004). Medications can mimic or antagonize numerous regulatory aspects of salivation thus affecting both salivary flow rate and composition (Schubert & Izutsu 1987, Ship 2004). More than 400 medications have been reported to induce salivary gland hypofunction as a potential side effect (Sreebny & Schwartz 1986). Antidepressants, antipsychotics, antihistamines, antihypertensive, alpha receptor antagonists for treatment of urinary retention, amphetamines, anticholinergics, antimigraine agents, appetite suppressants, atropinics, hypnotics, opioids, drugs of abuse, bronchodilators, cytotoxins, diuretics, protease inhibitors, radioiodine, retinoids and skeletal muscle relaxants are the drugs that are most commonly associated with salivary gland hypofunction (Sreebny & Schwartz 1997 Scully 2003, Scully & Bagan 2004, Astor et al 1999). It has been shown that unstimulated and stimulated major salivary flow rates progressively diminish with increasing numbers of medications that a patient takes. (Thorselius et al 1988, Sreebny et al 1989, Handelman et al 1989, Närhi et al 1992, Nederfors et al 1997, Wu & Ship 1993, Bardow et al 2001). Subjective reports of mouth dryness have been positively correlated with the number of xerostomic medications taken (Österberg et al 1984, Locker 1993, Gilbert et al 1993). Furthermore the intake of drugs positively correlates with age (Sreebny et al 1989, Sreebny & Schwartz 1997). Saunders & Handelman (1992) found that amongst institutionalised adults aged 65 or older, 79% were taking at least one xerostomic medication. It has been reported that stimulated
whole saliva was significantly reduced in subjects over 30 years of age who were using certain xerostomic medications when compared with non-medicated subjects of the same age (Parvinen et al 1984). Mäkilä (1977) found that 73% of subjects, with a very low stimulated whole saliva (mean <0.30ml/min) were taking xerostomic medications. The effect of some cytotoxic medications on whole salivary flow has been studied by Main et al (1984). They found that the flow rate was significantly lower in non-hospitalised patients on cytotoxic therapy for more than three months than in a healthy control group. Some xerostomic medications can reduce salivary flow for several hours after ingestion (Persson et al 1991).

Xerostomic medication can reduce salivary secretion by several ways such as: altering fluid and electrolyte balance, altering acinar and ductal function, interfering with central salivatory centres and affecting postganglionic synaptic transmission (both adrenergic and cholinergic) (Grad et al 1985, Schubert & Izutsu 1987). This variety of mechanisms may explain why multiple drug therapy can give rise to a considerable decrease in salivary flow.

Typical xerostomic medications are shown in Table 1.5.
Drugs with anticholinergic effects
Atropine and analogs (antimuscarinics)
Tricyclic antidepressants
Serotonin reuptake inhibitors
Antihistamines
Antiemetics
Antipsychotics

Drugs with sympathomimetic actions
Decongestants
Bronchodilators
Appetite suppressants
Amphetamines

Other drugs
Lithium
Omeprazole
Oxybutynin
Disopyramide
Dideoxynosine
Didanosine
Diuretics
Protease inhibitors

Table 1.5. Medication groups causing hyposalivation (After Porter et al 2004).
1.8.6. Diseases


1.8.7. Irradiation of the Head and Neck

Salivary glands are sensitive to ionising radiation (Huber & Terezhalmy 2003). Treatment of head and neck cancer using ionising radiation usually leads to severe reduction in salivary flow rate as a result of acinar and vascular damage and salivary gland atrophy (Frank *et al* 1965, Glass *et al* 1984, Gornitsky *et al* 2004). The extent of degenerative changes depends on the dose and duration of the therapy (Glass *et al* 1984, Dreizen 1985, Schubert & Izutsu 1987, Cooper *et al* 1995) and on the position of the gland relative to the primary beam of radiation (Frank *et al* 1965, Al-Tikriti *et al* 1984, Valdez *et al* 1993, Atkinson & Wu 1994, Vélez *et al* 2004). Irradiation causes a great reduction 95% or more in salivary flow rate after a full course radiation therapy and this reduction is persistent (Dreizen *et al* 1977a, Shannon *et al* 1977). Both stimulated and unstimulated whole salivary flow rate are affected by radiation (Ben-Aryeh *et al* 1975, Shannon *et al* 1977, Dreizen *et al* 1977a). Since the acinar cells are the only fluid producing cells in the salivary glands (Turner & Sugiya 2002); then their loss in the gland will have profound consequences for the patient. The serous acini are the most radiosensitive (Frank *et al* 1965, Izutsu *et al* 1985, Tomita & Osaki
Minor salivary glands are less affected by irradiation, probably because they are predominantly mucous (Izutsu et al 1985, Niedermeier et al 1995, Huber & Terezhalmy 2003). The reduction of flow rate is usually associated with changes in salivary composition. For instance, an increase in sodium, chloride, calcium ions, and in nitrogen content and a decrease in bicarbonate were all previously reported to occur after radiation (Frank et al 1965, Dreizen et al 1976, Valdez 1991, Valdez et al 1991). Ionising radiation is a strong energy capable of removing an orbital electron from an atom and has the ability to damage the cell membrane permeability to ions, which affect the reabsorption ability, of the ductal system for example (Ben-Aryeh et al 1975, Vélez et al 2004). As a result of an increase in total salivary protein content and because saliva become more mucinious, salivary viscosity increases (Ben-Aryeh et al 1975, Kidd & Joyston-Bechal 1987, Tomita & Osaki 1990, FDI 1992).

Internal radiation, such as that from radioisotopes that are commonly used to treat thyroid tumours, can cause salivary hypofunction (Mason et al 1967, Maier & Bihl 1987, Laupa et al 1993, Malpani et al 1996). The isotope accumulates in the salivary glands and causes tissue damage (Wiesenfeld et al 1983, Schubert & Izutsu 1987). The radioiodine therapy produce significant effects on salivary gland function with the parotid glands more sensitive than the submandibular glands (Malpani et al 1995, 1996). However
Bohuslavizki et al (1998), reported that salivary glands lose their parenchymal function even with low doses of $^{131}$I. Meanwhile it has been suggested that hormonal and metabolic derangements are responsible for the glandular dysfunction rather than a direct effect of iodine on glands (Markitziu et al 1993).
1.9 Xerostomia and Hyposalivation

Xerostomia is defined as a subjective feeling of oral dryness (Sreebny & Valdini 1987, Nederfors 2000) while hyposalivation is a true demonstrable salivary flow reduction when the flow is measured as a result of pathological changes in the salivary glands. Dryness of the mouth (xerostomia) may be a result of reduction or lack of normal salivary secretion; increased speed of evaporation from the mouth and might occur without any apparent cause. The term xerostomia, however, has been used extensively to describe the symptoms of mouth dryness with or without objective measurement of salivary flow (Fox 1989b, Navazesh et al 1992a). Furthermore, the prevalence of xerostomia was said to increase with age (Sreebny & Valdini 1988, Närhi 1994).

The salivary flow rate (whole and individual glands) has a wide range and the value that differentiates a normal flow rate from an abnormal one has not yet been defined (Navazesh et al 1992a). As demonstrated by Ghezzi et al (2000), salivary flow rates are not constant and illustrate a moderate amount of variability between individuals, thus in order to set a standard flow rate, these variations should be considered and normal values should be individually determined (Ship et al 1991). However, hyposalivation has been estimated to be of a value less than 0.1 ml/min for whole unstimulated saliva and 0.5 to 0.7ml/min for stimulated whole saliva (Epstein & Scully 1992). Wang et al (1998) found that a symptom of mild dry mouth is
correlated with a decreased unstimulated whole saliva flow rate, while a greater severity of dry mouth is correlated with both decreased unstimulated and stimulated whole saliva flow rate.

The perception of dry mouth is not entirely understood. Some subjects with normal salivary flow rate may complain of mouth dryness, while others who have a low salivary flow rate may not (Gutman & Ben-Aryeh 1974, Österberg et al 1984, Dawes 1987, Fox et al 1987, Sreebny & Valdini 1988, Ben-Aryeh et al 1985).

There is a lack of correlation between the complaint of dry mouth and salivary gland hypofunction except for extreme levels of hypofunction (Sreebny & Broich 1987, Närhi 1994). Field and associates (1997) observed that only 53% of patients with xerostomia were found to have clinical evidence of a dry mouth. Nevertheless, the clinical assessments of oral dryness may correlate with the basal salivary flow rates (Field et al 1997). Thus, it is reasonable to suggest that the symptom of mouth dryness may be related to dehydration of areas of the mucous membranes or to the sensation caused by the friction between adjacent layers of dry mucous membranes (Dawes 1987).

Dawes (1987) suggested that the sensation of mouth dryness might occur when the sum of the rate of water absorption by oral mucous membranes, which evaporated into exhaled air, exceeds the amount of saliva secreted into the mouth. Dawes showed that only when the normal unstimulated salivary flow rate was reduced by 50% did the symptom of dry mouth appear regardless of the initial value. Dryness
of oral mucous membranes was found to be more prevalent in patients with xerostomia and with low salivary flow rates than in those who complained of xerostomia without a reduction in salivary flow rate (Ben-Aryeh et al 1985). It has been concluded that xerostomia seems to be strongly correlated with unstimulated saliva (Österberg et al 1984, Nederfors et al 1993) and it may be caused by change in the quality of the saliva rather than flow rate (Mandel & Wotman 1976, Ben-Aryeh et al 1988, Field et al 1997, Gilboe et al 2001) and/or to altered mucosal perception (Ben-Aryeh et al 1988). A strong positive correlation was found between submandibular and sublingual salivary gland hypofunction and dryness of the mouth (Fox et al 1987). This may be due to the fact that these two glands secrete mucins, which aid in lubrication (Fox et al 1987). It seems that although the feeling of mouth dryness is primarily associated with reduction of salivary flow rate, in some patients who complain of mouth dryness the salivary flow rate is within normal range (Spielman et al 1981, Österberg et al 1984, Fox et al 1985, Fox et al 1987, Dawes 1987, Sreebny & Valdini 1987, Sreebny & Valdini 1988).

The relationships between unstimulated whole saliva, xerostomia and changes in the oral soft and hard tissues have been reported by Bertram (1967) who associated xerostomia with unstimulated whole saliva based on a sample of 50 patients with symptoms of oral dryness, he associated xerostomia with unstimulated whole saliva
≤0.2ml/15 and hyposalivation with >0.2ml/15min. On the other hand, Wolff & Kleinberg (1998), demonstrated that when unstimulated whole saliva flow rate is equal to or less than 0.1ml/min, the thickness of the palatal film of saliva is less than 4 to 5 μm, but when stimulated whole salivary flow rate is normal (0.2-0.3ml/min), the thickness of the palatal film of saliva is between 14 and 18 μm. They concluded that the onset of the symptom of oral dryness corresponds to saliva thickness on the posterior hard palate of 10 μm or less. However; Lee et al (2002) stated that the function of minor salivary glands, especially the soft palatal glands, appeared to be well preserved, even in patients with severe dry mouth.
1.10. Sialometry

Accurate measures of salivary flow rate are required for a variety of clinical and experimental procedure. The flow rate is usually expressed in millilitres per minute (ml/min) and is obtained by measuring the volume of saliva collected over period of time. Salivary flow rates can be measured in resting (unstimulated) or stimulated glands using masticatory (paraffin wax or rubber band) or gustatory stimulation such as an acid (Enfors 1962, Birkhed & Heintze 1989, Mandel 1993a). Collection of saliva can be either as whole saliva or separately collected secretions from individual glands (parotid, submandibular, sublingual and minor); secretions from both submandibular and sublingual glands are collected together as these secretions enter the mouth by bilateral common ducts (Fox et al 1985, Navazesh 1993).

Collection of saliva is a painless and simple procedure. Several methods have been described for the collection of whole saliva and saliva from parotid, submandibular, sublingual and minor glands (Table 1.6).
<table>
<thead>
<tr>
<th>Method</th>
<th>Submandibular-sublingual</th>
<th>Submandibular</th>
<th>Parotid:</th>
<th>Whole saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suction</td>
<td>Micropipette</td>
<td>Suction</td>
<td>Polyethylene catheter</td>
<td>Spitting</td>
</tr>
<tr>
<td>Suction cup</td>
<td></td>
<td>Suction cup</td>
<td>Suction cup</td>
<td>Drainage</td>
</tr>
<tr>
<td>Combined catheter and</td>
<td></td>
<td>Suction cup</td>
<td>Combined catheter and</td>
<td>Suction rolls</td>
</tr>
<tr>
<td>suction cup</td>
<td></td>
<td></td>
<td>suction cup</td>
<td>Salivette®</td>
</tr>
<tr>
<td>SLURP collection cup</td>
<td></td>
<td></td>
<td>SLURP collection cup</td>
<td>Acid impregnated cotton</td>
</tr>
<tr>
<td>Material Quick Absorber (MQA) method</td>
<td></td>
<td></td>
<td></td>
<td>roll covered with polypropylene</td>
</tr>
</tbody>
</table>

Table 1.6. The main methods of saliva collection.
1.10.1. Whole Saliva

Whole saliva refers to saliva derived from the six major salivary glands and minor salivary glands (plus crevicular fluid). It is a colourless, clear, slightly foamy, viscous solution. Whole saliva is not inevitably the sum of individual gland secretions and may contain bacteria, bacterial products desquamated epithelial cells, serum, and blood cells, viruses and fungi, gingival fluid, food debris and expectorated bronchial secretion (Figure 1.6) (Mandel & Wotman 1976, Mandel 1980, Fox et al, 1985, Dawes 1987, Fox 1989a, Sreebny 1989, FDI 1992, Navazesh 1993, Kaufman & Lamster 2002), so individual gland secretions are superior to whole saliva for many compositional analysis (Navazesh 1993). On the other hand, whole saliva is easy to collect and gives a useful indication of the overall physiological status of salivary gland function (Sreebny 1989, Dawes 1987, Epstein & Scully 1992, Navazesh et al 1992a).
Figure 1.6. Components of whole saliva (after Kaufman and Lamster, 2002).

- Blood and blood derivatives: Intravital bleeding, gingival crevicular fluid
- Other fluids: Bronchial and nasal secretions
- Lining cells
- Extrinsic substances
- Microbiota: Oral bacteria (enzymes and bacterial products), Viruses, Fungi
- Salivary glands: Water, Proteins, Electrolytes and small organic molecules
There is lack of agreement in published literature as to the normal value of salivary flow rate. The unstimulated flow rate for the average whole saliva is about 0.19 to 0.72 ml/min with a wide range (Table 1.7). However average values of stimulated whole saliva range from 0.92 to 8.64 ml/min (Table 1.8). These wide ranges may be due to individual variations, to the many different methods used for collecting whole saliva that have been used and to the health status of subjects in the different studies (Kerr 1961). Skopouli et al (1989) assessed the unstimulated whole saliva flow and the stimulated parotid saliva flow in 188 healthy female subjects; their ages range from 20-85 years. They found that the stimulated parotid flow and unstimulated whole saliva flow rates presented a wide range of values. The values of the stimulated parotid flow ranged from 0.0 to 7.5ml/5min and from 0.2 to 6.8ml/min in unstimulated whole saliva flow. Salivary flow rates vary widely with time of day (Dawes 1972, Ferguson et al 1973, Richardson & Feldman 1986) and fluctuate with duration of collection time (Becks & Wainwright 1939, Dong & Dawes 1995). Such causes make it impossible to state a universal normal value. Salivary flow rates vary significantly among individuals and in the same individual under different conditions (Dawes 1987). There is ongoing debate in regard to the amount of saliva necessary to maintain oral homeostasis. Since there is a large variation in salivary output in healthy, unmedicated individuals, it is difficult to determine a cut off amount for normal salivary flow (Ship et al 1991, Dawes 1987).
Total salivary fluid produced during twenty-four hours is estimated to be about 0.5 to 1.5 litres (Jenkins 1978, Richardson & Feldman 1986, Watanabe & Dawes 1988b, Epstein & Scully 1992, Ganong 1997 Guyton & Hall 2000).

Most of the whole saliva is derived from the three pairs of major salivary glands with the submandibular-sublingual salivary flow rate exceeding parotid flow rate by about 50% (Nederfors & Dahlöf 1993). The proportional contribution of the parotid, submandibular and sublingual glands under resting condition has been estimated to be 26%, 69%, and 5% respectively while the minor salivary glands, under resting conditions, make no significant contribution (Schneyer 1956). At high levels of stimulation, parotid glands may contribute up to 49% of whole saliva (Shannon 1962). The relative contributions of the minor salivary glands have been estimated to be less than 10% of whole saliva (Dawes & Wood 1973b). The results reported by Speirs (1984) disagreed with those reported earlier by Dawes & Wood (1973b) who indicated that the percentage contribution made by the lower lip minor glands is the same when unstimulated and stimulated saliva is collected. Using 0.16 M ascorbic acid as a gustatory stimulus, the mean increase was three times for minor salivary glands of the lower lip and 10.7 times for parotid glands (Speirs 1984). The disparity between the two studies has been explained on the basis of the differences in the salivary glands under investigation and in the method used to collect saliva.
1.10.1.1. Unstimulated Saliva:

Unstimulated saliva is secreted in waking individuals when they are comfortable, at rest and when the glands are under minimal or no exogenous stimulation. However, it is obviously more or less impossible to obtain true unstimulated saliva as the major glands do not secrete spontaneously, so unstimulated saliva is largely or entirely stimulated (Schneyer et al 1956, Tenovuo & Lagerlöf 1994). Therefore the term unstimulated (resting) is not strictly accurate but is convenient and when used is to be taken as meaning the output from a gland in the waking subject in the absence of any known stimulation (Kerr 1961). Therefore saliva collected without any stimulation is less variable and is a more reliable indicator of reduced salivary flow rate than stimulated saliva (Navazesh & Christensen 1982, Tenovuo & Lagerlöf 1994, Mulligan et al 1995, Wang et al 1998, Longman et al 2000). Unstimulated whole saliva is mainly composed of submandibular saliva with significant contributions from the sublingual and minor salivary glands (Kerr 1961), while stimulated saliva is mainly composed of parotid and submandibular saliva (Dawes & Jenkins 1964).

Results of unstimulated whole salivary measurements are given in Table 1.7.
<table>
<thead>
<tr>
<th>Authors</th>
<th>No. of subjects</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Mean flow (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Becks &amp; Wainwright (1943)</td>
<td>661</td>
<td>Both</td>
<td>5-95</td>
<td>0.32</td>
</tr>
<tr>
<td>Gutman &amp; Ben-Aryeh (1974)</td>
<td>7</td>
<td>M</td>
<td>6-19</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>F</td>
<td>7-18</td>
<td>0.45</td>
</tr>
<tr>
<td>Gutman &amp; Ben-Aryeh (1974)</td>
<td>3</td>
<td>M</td>
<td>60-67</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>F</td>
<td>61-72</td>
<td>0.37</td>
</tr>
<tr>
<td>Ben-Aryeh et al (1981)</td>
<td>10</td>
<td>Both</td>
<td>39-68</td>
<td>0.39</td>
</tr>
<tr>
<td>Heintze et al (1983)</td>
<td>286</td>
<td>M</td>
<td>15-74</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>343</td>
<td>F</td>
<td>15-74</td>
<td>0.26</td>
</tr>
<tr>
<td>Österberg et al (1984)</td>
<td>23</td>
<td>M</td>
<td>70</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>F</td>
<td>70</td>
<td>0.19</td>
</tr>
<tr>
<td>Sreebny et al (1985)</td>
<td>20</td>
<td>Both</td>
<td>22-62</td>
<td>0.41</td>
</tr>
<tr>
<td>Gandara et al (1985)</td>
<td>12</td>
<td>Both</td>
<td>&lt;59</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>Both</td>
<td>≥59</td>
<td>0.52</td>
</tr>
<tr>
<td>Richardson &amp; Feldman (1986)</td>
<td>59</td>
<td>Both</td>
<td>20-52</td>
<td>0.62</td>
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<tr>
<td>Ben-Aryeh et al (1986)</td>
<td>39</td>
<td>Both</td>
<td>Mean 37</td>
<td>0.46</td>
</tr>
<tr>
<td>Ben-Aryeh et al (1986)</td>
<td>24</td>
<td>Both</td>
<td>Mean 66</td>
<td>0.39</td>
</tr>
<tr>
<td>Watanabe &amp; Dawes (1988b)</td>
<td>32</td>
<td>Both</td>
<td>Young adults</td>
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<tr>
<td>Shern et al (1990)</td>
<td>14</td>
<td>Both</td>
<td>Mean 37</td>
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</tr>
<tr>
<td>Dong &amp; Dawes (1995)</td>
<td>24</td>
<td>Both</td>
<td>13-73</td>
<td>0.32</td>
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<tr>
<td>Dodds &amp; Dodds (1997)</td>
<td>36</td>
<td>Both</td>
<td>Mean 55.2</td>
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<tr>
<td>Wang et al (1998)</td>
<td>23</td>
<td>Both</td>
<td>29-61</td>
<td>0.33</td>
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<tr>
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<td>Both</td>
<td>18-59</td>
<td>0.45</td>
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<td></td>
<td>52</td>
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<td>2-39</td>
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</tr>
<tr>
<td></td>
<td>40</td>
<td>Both</td>
<td>40-54</td>
<td>0.48</td>
</tr>
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<td>27</td>
<td>Both</td>
<td>55-69</td>
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<td></td>
<td>12</td>
<td>Both</td>
<td>≥70</td>
<td>0.51</td>
</tr>
<tr>
<td>Won et al (2001)</td>
<td>30</td>
<td>Both</td>
<td>Mean 25.2</td>
<td>0.43</td>
</tr>
<tr>
<td>Yurdukor et al (2001)</td>
<td>23</td>
<td>Both</td>
<td>Mean 59</td>
<td>0.26</td>
</tr>
<tr>
<td>Sánchez-Guerrero et al (2002)</td>
<td>73</td>
<td>Both</td>
<td>20-61</td>
<td>0.52</td>
</tr>
<tr>
<td>Lee et al (2002)</td>
<td>20</td>
<td>Both</td>
<td>24-63</td>
<td>0.43</td>
</tr>
<tr>
<td>Lin et al (2003)</td>
<td>50</td>
<td>M</td>
<td>Mean 32.2</td>
<td>0.48</td>
</tr>
<tr>
<td>Tenuta et al (2003)</td>
<td>13</td>
<td>Both</td>
<td>19-28</td>
<td>0.41</td>
</tr>
<tr>
<td>Hershkovitch &amp; Nagler (2004)</td>
<td>90</td>
<td>Both</td>
<td>20-86</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Table 1.7. Results from several studies of flow rate of unstimulated whole saliva.
1.10.1.2. Stimulated Saliva

Salivary flow is increased in response to exogenous stimulation. This type of stimulation includes gustatory (taste) and mechanical (chewing) stimuli (Kerr 1961, Bourdiol et al. 2004), even speaking can stimulate the minor salivary glands (Boros et al. 1999). The use of acid lemon drops or a few drops of citric acid are among the most potent of taste stimuli and will generally induce a maximum secretion (Jensen Kjeilen et al. 1987, Watanabe & Dawes 1988b, Vining and McGinley 1985, Edgar 1992). Chewing different types of food may act as both gustatory and mechanical stimuli (Watanabe & Dawes 1988a). However, results of the comparison between the taste and mechanical (chewing) stimuli on the salivary flow rate indicated that taste is a stronger stimulus (Watanabe & Dawes 1988a).

The effect of different concentrations of citric acid on whole saliva was investigated by Watanabe & Dawes (1988b) who reported that the highest flow rates were achieved when 5% citric acid was infused to stimulate saliva compared with those produced in response to infusion of 1% or 3% citric acid into the mouth, but Ericson (1971) showed that greater parotid saliva can be collected when 10% citric acid was used compared with 1% and 6%. Continuous gustatory stimulation, such as sucking on a sour lemon, produces stable whole salivary flow rates (Dawes & Macpherson 1993). Kerr (1961) reported no decline in the parotid or whole salivary flow rates in a single subject who sucked an acid drop sweet for the first of every five
minutes over a period of three hours. Recently, it has been demonstrated that even after ninety minutes of gum chewing, the salivary glands retained the capacity to respond with a marked increase in flow rate to the gustatory stimulus (Polland et al 2003, Dawes & Kubieniec 2004). Gustatory stimulants (e.g. citric acid) can result in changes in salivary pH and may cause precipitation of certain salivary proteins (Dawes 1987, Kaufman & Lamster 2002). Paton (1986) also mentioned the interference by citric acid in some immunoassays.

Mechanical stimulations such as chewing paraffin wax, rubber bands or chewing gum are also used. It has been shown that the salivary flow rate peaked in the initial minutes of chewing and then fell with time towards a relatively constant value greater than the unstimulated flow rate (Dawes & Macpherson 1992, Rosenhek et al 1993, Bourdiol et al 2004, Bots et al 2004, Dawes & Kubieniec 2004).

Jensen et al (1998) compared the effect of chewing gum and paraffin tablets on flow rate of whole and parotid saliva in eight healthy subjects (25-45 yrs). At the beginning of the collection with chewing gum there was significantly more salivary output compared with paraffin but in the final period of collection the paraffin chewing resulted in more salivary secretion than when chewing gum was used. Initially this high peak may be attributed to the synergistic effect between the mechanical and gustatory stimuli of the chewing gum (Chatoo et al 1993, Bots et al 2004). However, in the final period
and owing to the ability of paraffin to retain its hardness, weight and size throughout the chewing period more saliva was collected (Jensen et al 1998).

Mastication causes a reflex secretion of saliva from the parotid gland (Lashley 1916, Kerr 1961, Anderson & Hector 1987, Jensen Kjeilen et al 1987). The parotid secretion increases with increasing mechanical stimulation of the teeth. Kjeilen et al (1987) demonstrated that chewing movements without teeth contact as well as empty clenching did not induce an increase in parotid secretion. The reflex is associated with the stimulation of intraoral receptors, which may be mechanoreceptors in the periodontal ligament (Kerr 1961, Anderson & Hector 1987, Hector & Linden 1987), and in the oral mucosa (Scott et al 1998). Reduction in masticatory-salivary reflex was observed following anaesthesia of the teeth (Hector & Linden 1987). In general, the secretion rate increases with the size of the bolus and the pressure that is required to chew it (Kerr 1961, Jensen Kjeilen et al 1987). A study by Kerr (1961) showed that the flow of human parotid saliva increased when larger quantities of wax or rubber were chewed, and that the flow rate was proportional to the logarithm of sample weight. If chewing is unilateral, then the glands on the active side may secrete copiously while those on the inactive site secrete very little (Lashley 1916, Kerr 1961, Anderson & Hector 1987, Jensen Kjeilen et al 1987, Scott et al 1998). Mastication is important for the maintenance of salivary gland function. An increase in frequency of
chewing or a change of diet to harder foods leads to an increase in salivary flow rates (de Muñiz et al 1983, Dodds et al 1991). On the other hand, eating a liquid diet for a week or more results in a decreased flow of saliva in response to a stimulus (Hall et al 1967, Johansson et al 1984). While salivary gland function responds to increases and decreases in mastication, bite force appears to be responsive to it. It has been shown that a decrease in bite force is associated with a decrease in salivary flow rate (Yeh et al 2000). This decrease in magnitude of bite force is found to be due to loss of teeth, wearing of dentures, pain and aging. Furthermore, it is difficult to maintain a constant force (mastication) throughout the collection period (Navazesh 1993).

Mechanical stimulation is less effective in patients with dentures and is unsuitable in edentulous patients without dentures (Dawes 1987). Comparison of elderly and younger subjects using this method may be invalid because motor function is adversely affected by age (Baum 1981b). However due to lack of correlation between maximum flow in response to chewing and 5% citric acid, using both mechanical and gustatory stimuli was recommended in order to achieve a proper evaluation of salivary flow (Jensen Kjeilen et al 1987).

Secretion stimulating drugs, such as the parasympathomimetic drug pilocarpine, (Hensten-Pettersen 1975, Fox et al 1986) have sometimes been used orally, subcutaneously, or intravenously. However, in doses sufficient to produce very high flow rates,
parasympathomimetic drugs have undesirable side effects such as flushing, palpitations, abdominal colic, and an urgent desire to micturate. In addition, they appear to cause a transient increase in junctional permeability and thus result in the appearance in the saliva of compounds of higher molecular weight than would normally be expected. (Mazariegos et al 1984). Electrical stimulation can be used as a salivary stimulus, but it is not commonly used (Steller et al. 1988, Hargitai et al 2005).

Any physical or chemical stimulus used during the collection of saliva must not absorb or modify the compounds to be measured, nor must it introduce interfering factors into the assay procedure. In particular, Parafilm has been shown to absorb highly lipophilic molecules, leading to an apparent reduction in drug level (Chang & Chiou 1976, Taylor 1978). On the other hand, collection of saliva by plastic foam or cotton wool rolls for hormone analysis may possibly increase the non-specific adsorption (Vining & McGinley 1985).

Standardization of whole saliva collection plays an important role in the interpretation of the available literature. The following steps and instructions should be considered when saliva is collected (Tenovuo & Lagerlöf 1994)

- The patient should not eat or drink (except water) for one hour before collection
- Saliva should, if possible, be collected at the same time of day from the same subject.

- The patient should not smoke nor undergo heavy physical stress before collection.

- A pre-sampling period of one minute is recommended.

- A fixed collection time should be used (five minutes for stimulated saliva, 10 to 15 minutes for unstimulated saliva).

- The patient should sit in a relaxed position in an ordinary chair, not in a dental unit.

- Acute illnesses, chronic diseases and medication should be considered. If microbiological tests are planned, sample collection should be avoided within two weeks following antibiotic courses.

- Samples containing visible blood should be discarded if chemical analysis of saliva is planned.

Results of stimulated whole salivary measurements are given in Table 1.8.
Table 1.8. Results from several studies of flow rate of stimulated whole saliva

<table>
<thead>
<tr>
<th>Authors</th>
<th>No. of subjects</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Method of stimulation</th>
<th>Mean flow (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mäkilä (1977)</td>
<td>105 M 295 F</td>
<td>60-95 53-101</td>
<td>Paraffin wax</td>
<td>1.20 0.98</td>
<td></td>
</tr>
<tr>
<td>Crossner (1984)</td>
<td>57 M 58 F</td>
<td>15</td>
<td>Paraffin wax</td>
<td>2.25 1.83</td>
<td></td>
</tr>
<tr>
<td>Richardson &amp; Feldman(1986)</td>
<td>59 Both</td>
<td>20-52</td>
<td>Appetizing</td>
<td>2.78</td>
<td></td>
</tr>
<tr>
<td>Ben-Aryeh et al (1986)</td>
<td>39 Both</td>
<td>Mean 37</td>
<td>2% citric acid</td>
<td>1.53</td>
<td></td>
</tr>
<tr>
<td>Ben-Aryeh et al (1986)</td>
<td>24 Both</td>
<td>Mean 66</td>
<td>2% citric acid</td>
<td>1.28</td>
<td></td>
</tr>
<tr>
<td>Tenovuo et al (1986)</td>
<td>35 Both</td>
<td>17-61</td>
<td>Paraffin wax</td>
<td>1.62</td>
<td></td>
</tr>
<tr>
<td>Lassila (1987)</td>
<td>24 M 30 F</td>
<td>73-83 73-83</td>
<td>Paraffin wax</td>
<td>1.63 1.23</td>
<td></td>
</tr>
<tr>
<td>Watanabe &amp; Dawes (1988b)</td>
<td>32 Both</td>
<td>Young adult</td>
<td>5% citric acid</td>
<td>7.07</td>
<td></td>
</tr>
<tr>
<td>Shern et al (1990)</td>
<td>14 Both</td>
<td>Mean, 37</td>
<td>2% citric acid</td>
<td>1.49</td>
<td></td>
</tr>
<tr>
<td>Österberg et al (1991)</td>
<td>2600 M F</td>
<td>70-82</td>
<td>Paraffin wax</td>
<td>1.24 0.92</td>
<td></td>
</tr>
<tr>
<td>Shern et al (1993a)</td>
<td>9 M 8 F</td>
<td>&lt;41</td>
<td>2% citric acid</td>
<td>1.75 1.18</td>
<td></td>
</tr>
<tr>
<td>Shern et al (1993a)</td>
<td>8 M 8 F</td>
<td>41-60</td>
<td>2% citric acid</td>
<td>2.79 1.43</td>
<td></td>
</tr>
<tr>
<td>Shern et al (1993a)</td>
<td>8 M 8 F</td>
<td>41-60</td>
<td>2% citric acid</td>
<td>2.82 1.97</td>
<td></td>
</tr>
<tr>
<td>Wang et al (1998)</td>
<td>23 Both</td>
<td>29-61</td>
<td>Paraffin wax</td>
<td>1.89</td>
<td></td>
</tr>
<tr>
<td>Yurdukoru et al (2001)</td>
<td>23 Both</td>
<td>Mean 59</td>
<td>Paraffin wax</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>Siqueira &amp; Nicolau (2002)</td>
<td>18 Both</td>
<td>6-10</td>
<td>Paraffin wax</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Tenuta et al (2003)*</td>
<td>13 Not stated</td>
<td>19-28</td>
<td>Latex tube</td>
<td>1.54</td>
<td></td>
</tr>
</tbody>
</table>
1.10.1.3. Methods for Collecting Whole Saliva

There are several methods of collecting whole saliva, such as spitting, draining, suction and cotton wool rolls or swab method (Kerr 1961, Dawes 1987, Birkhed & Heintze 1989, Edgar & O'Mullane 1996, Navazesh 1993).

In the spitting method the subject spits out at specified intervals, e.g. one minute, into a receiving graduated tube or pre-weighed vessel, while in the draining (drooling) method the saliva is allowed to drip from the mouth into a graduated tube or pre-weighed container for a set period of time. In the suction method the saliva is continuously sucked with a suction tube and collected in a vessel, while in the swab method pre-weighed dry cotton wool rolls or absorbent swabs are inserted in the mouth and removed for weighing at the end of the collection period. The suction and the cotton wool rolls or swabs method result in some degree of stimulation and thus are not recommended for unstimulated whole saliva collection (Navazesh & Christensen 1982, Navazesh 1993). The spitting method is considered as a gold standard in measuring salivary flow rate. However, subject compliance is essential. The subjects should be able to expectorate the produced saliva and to clear their mouth and also not to swallow which might be difficult to achieve in elderly and young subjects (Jones et al 2000). The potential source of errors may be high when compared with other methods such as the suction
technique that can be more easily used in elderly subjects (Jones et al 2000).

Within the last few years, much research has been done to develop a method that solves some problems that are encountered when saliva is collected. May et al (1978) and Cooper et al (1981) were the first to use a dental cotton roll to collect saliva. They have used a dental cotton roll placed between the gum and cheek of the subject until saturated, for a maximum period of time (10 min). The cotton roll was placed in a vial, which was weighed with the roll before, and then reweighed after the collecting procedure. Over the years their method has undergone some improvements, and the dental cotton roll is nowadays available as the Salivette® (Haeckel & Bucklitsch 1987, Lenander-Lumikari et al 1995). Using this instrument, saliva is collected by absorption into a cotton roll either without or after stimulation. Unstimulated saliva is collected by placing the cotton roll under the resting tongue without chewing activity. In case of stimulated saliva, a cotton roll impregnated with, (e.g. 20 mg citric acid) is used. A mechanical stimulus (chewing on the roll) may also be used. After being soaked with saliva the cotton roll is placed in a container, which is closed with a plastic stopper. The container fits into a polystyrol tube that is then centrifuged for two minutes at about 1000 g. During centrifugation, the saliva passes from the cotton roll into the lower part of the tube. The container is then taken out of the tube and the clear saliva is poured out. Cellular particles
are retained at the bottom of the tube in a small sink compartment (Haeckel & Bucklitsch 1987). A disadvantage of the Salivette® is that the dental cotton roll interferes with several hormone and drug assays. However the cotton can be replaced by other absorbing materials. Meanwhile, the advantage of the Salivette® over many other sampling devices is that it reliably absorbs a relatively large volume of saliva (1.5 ml) in a short period and the sampling time of the Salivette® is very short (Höld et al 1995). Three different types of Salivette® kits are available (Lenander-Lumikai et al 1995), a non-covered cotton roll impregnated with citric acid, a neutral non-covered roll, and a neutral polyether roll covered with polypropylene.

Bacon and co-workers (1978) described a simple method for obtaining saliva from infants and young children. After stimulating the saliva, it is aspirated by means of a disposable mucus extractor from behind the lower lip and, if the child permits, from the buccal cavity and beneath the tongue. They found this method to be easy and non-invasive.

Kohler & Winter (1985) described a test which they called the Saxon test. In this test, they used a folded sterile gauze sponge, placed in a sterile plastic tube and the dry gauze and tube were weighed. Saliva was collected by having the subject forcefully chew on the gauze for exactly two minutes. After the gauze has been chewed, it is replaced in the same tube, and the amount of saliva produced in these two minutes determined by weighing. In their opinion, there is no need
for special items with the Saxon test, which in their opinion is a simple, reproducible and a low-cost method for measuring whole saliva (Kohler & Winter 1985, Mulligan et al 1995).

A method for whole saliva quantification has been used by López-Jornet & Bermejo-Fenoll (1994) and López-Jornet et al (1996). It consists of a piece of Whatman filter paper with a printed mm-ruler. The first millimetre is left with no print and the strip is introduced in a low-density polyethylene bag. The non-ruled portion of the strip of white paper is extracted from the bag and one of the extremes is folded at a 90° angle and introduced into the oral cavity, then held under the tongue with the lips closed. The saliva produced, accumulates in the lingual vallacula and slowly soaks the strip. The strip is carefully removed after five minutes and the humidified millimetres can be read. They described it as an inexpensive, hygienic, quick, requiring no special equipment and an easily run method (López-Jornet & Bermejo-Fenoll 1994, López-Jornet 1996).

1.10.2. Collection of Saliva from Individual Glands

Several methods of saliva collection from individual glands have been described. Polyethylene catheters are used for collection of saliva from the parotid and submandibular gland. The catheter is introduced into the duct through its orifice. This method is mainly used to collect a small sample of saliva from the parotid. However, it
has many disadvantages; it may be painful, the catheter is easily dislodged during use, or saliva may leak past the catheter. In addition, it is difficult to use in the submandibular duct, and tongue movements tend to displace it. Nevertheless, it is the only method that allows the collection of saliva from a single submandibular gland (Kerr 1961, Mason & Chisholm 1975).

A second method involves the use of suction cups. The basic principle of the suction cup method is that the stability of the collecting device on the orifices is dependent on vacuum pressure. There are suction cups suitable for either the parotid or the submandibular gland.

For collection of parotid gland saliva, a device described by Carlsson and Crittenden (1910) and by Lashley (1916) is used. It consists of two chambers (outer and inner) and two tubes. One of the tubes is connected to the outer chamber for application of vacuum. The inner chamber is placed over the parotid orifice and the cup is held in place by the vacuum in the outer chamber. The main disadvantages of this device are the inclusion of air bubbles in the collecting chamber, the need for supporting equipment and the need for good lighting conditions (Ericson & Nordlund 1993). Cup dislodgement by masticatory movement (Carlsson & Crittenden 1910) and occluding the tube with the buccal soft tissue are real problems (Mason & Chisholm 1975). Now, Carlsson-Crittenden cup is still used with slight modification.
Several modifications on the original Carlsson-Crittenden cup were described by Curby (1953), Shannon et al (1962), Terry & Shannon (1965) and Lewis et al (1993).

In an attempt to overcome the problem of catheter dislodgement that may occur in collection of parotid saliva, Kerr (1961) described a combined method. A suitable polyethylene cannula is inserted along into the tubing of a modified Lashley cup and ten mm beyond the fitting surface. The cannula is then inserted into the parotid duct, and when satisfactorily positioned, vacuum pressure is applied as with the standard Lashley cup. This has the advantage of stability of the device and avoidance of air inclusion. However, it may be painful and unsuitable for routine clinical use; it is also time consuming (Mason & Chisholm 1975).

Ericson & Nordlund (1993) described a SLURP cup. This consists of a cup with a collection chamber. When the device is introduced in the oral cavity against the parotid duct orifice, it is kept in place by the masseter, orbicularis oris and buccinator muscles. The capacity of the collection chamber is one ml. The device is used without supporting equipment and requires no trained personnel.

Results for unstimulated and stimulated individual glands are shown in Tables 1.9 to 1.12.
<table>
<thead>
<tr>
<th>Authors/ year</th>
<th>No. of subjects</th>
<th>Sex</th>
<th>Age</th>
<th>Mean flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shannon et al (1963)</td>
<td>513</td>
<td>M</td>
<td>Young adult</td>
<td>0.03</td>
</tr>
<tr>
<td>Shannon (1967)</td>
<td>4589</td>
<td>M</td>
<td>17-22</td>
<td>0.04</td>
</tr>
<tr>
<td>Shannon &amp; Chauncey (1967)</td>
<td>25</td>
<td>M</td>
<td>17-22</td>
<td>0.03</td>
</tr>
<tr>
<td>Epstein et al (1980b)</td>
<td>9</td>
<td>M</td>
<td>21-55</td>
<td>0.09</td>
</tr>
<tr>
<td>Ben-Aryeh et al (1986)</td>
<td>34</td>
<td>Both</td>
<td>Mean 37 yr</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Both</td>
<td>Mean 66 yr</td>
<td>0.07</td>
</tr>
<tr>
<td>Yeh et al (1988)</td>
<td>10</td>
<td>M</td>
<td>28-46 yr</td>
<td>0.05</td>
</tr>
<tr>
<td>Ship et al (1990)</td>
<td>35</td>
<td>Both</td>
<td>Mean 70 yr</td>
<td>0.10</td>
</tr>
<tr>
<td>Vissink et al (1993)</td>
<td>36</td>
<td>Both</td>
<td>19-75</td>
<td>0.05</td>
</tr>
<tr>
<td>Kalk et al (2001)</td>
<td>36</td>
<td>Both</td>
<td>23-58</td>
<td>0.05</td>
</tr>
<tr>
<td>Hargitai et al (2005)</td>
<td>22</td>
<td>Both</td>
<td>19-35</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 1.9. Results from several studies of flow rate of unstimulated saliva from parotid gland (ml/min).
<table>
<thead>
<tr>
<th>Authors</th>
<th>No. of subjects</th>
<th>Sex</th>
<th>Age</th>
<th>Method of stimulation</th>
<th>Mean Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Davis (1979)</td>
<td>15</td>
<td></td>
<td>9-28</td>
<td>citric acid</td>
<td>0.63</td>
</tr>
<tr>
<td>Epstein et al (1980b)</td>
<td>9</td>
<td></td>
<td>21-55</td>
<td>lemon lozenge</td>
<td>0.67</td>
</tr>
<tr>
<td>Gandara et al (1985)</td>
<td>12 Both</td>
<td></td>
<td>&lt;59</td>
<td>lemon drops</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>12 Both</td>
<td></td>
<td>≥59</td>
<td>lemon drops</td>
<td>0.71</td>
</tr>
<tr>
<td>Ben-Aryeh et al (1986)</td>
<td>39 Both</td>
<td>Mean 37 yr</td>
<td>2% citric acid</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Ben-Aryeh et al (1986)</td>
<td>24 Both</td>
<td>Mean 66 yr</td>
<td>2% citric acid</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Yeh et al 1988</td>
<td>10 M</td>
<td>28-46 yr</td>
<td>2% citrate</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Ship et al (1990)</td>
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<td>Mean 70 yr</td>
<td>citric acid</td>
<td>0.54</td>
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<td></td>
<td>13 Both</td>
<td>≥59</td>
<td>lemon juice</td>
<td></td>
<td>0.71</td>
</tr>
<tr>
<td>Streckfus et al (1991)</td>
<td>15 Both</td>
<td>≥65</td>
<td>citric acid</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>Vissink et al (1993)</td>
<td>36 Both</td>
<td>19-75</td>
<td>2% citric acid</td>
<td>0.52</td>
<td></td>
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<tr>
<td>Streckfus et al (1994)</td>
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<td>Mean 69.5 yr</td>
<td>citric acid</td>
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<td></td>
</tr>
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<td>Kho et al (1999)</td>
<td>22 Both</td>
<td>18-59</td>
<td>2% citric acid</td>
<td>1.11</td>
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<tr>
<td>Kalk et al (2001)</td>
<td>36 Both</td>
<td>23-58</td>
<td>2% citric acid</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>Lin et al (2003)</td>
<td>50 M</td>
<td>Mean 32.2</td>
<td>citric acid</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Hammi et al (2005)</td>
<td>20 Both</td>
<td>Mean 58 yr</td>
<td>2% citric acid</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>Hargitai et al (2005)</td>
<td>22 Both</td>
<td>19-35</td>
<td>transcutaneous electric nerve stimulation</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.10. Results from several studies of flow rate of stimulated saliva from parotid gland (ml/min)
A segregator appliance was described by Schneyer (1955) for the collection of separate submandibular and sublingual salivary glands saliva. This is constructed for each subject. It covers an area extending ventro-dorsal from the lingual surfaces of the incisors to the first molar region and the buccolingual from the lingual surfaces of the teeth under the tongue to cover the orifices of the submandibular ducts and the sublingual folds. In the lower surface there are three chambers; one central and two laterals. The central chamber overlies the submandibular duct while the lateral chambers cover the sublingual folds. The chambers are separated by acrylic rims and are connected to one of the three plastic tubes, which conduct the salivary secretion into collecting tubes. The main advantage of this method is that separate samples can be obtained from the submandibular and sublingual glands. The disadvantages include the need to construct a custom appliance for each subject; the need for a sufficient number of lower teeth; careful placement to avoid occluding the ducts (Mason & Chisholm 1975) and unsuitability for subjects with inflammatory or ulcerative soft-tissue disorders affecting the floor of the mouth (Navazesh 1993). This device has been further modified and used (Stephen et al 1978, Parr & Bustos-Valdes 1984).

A cup for collection of submandibular gland saliva has been described by Truelove et al (1967). It is a V-shaped appliance, consisting of three chamber (two outer and one inner), three tubes
and two rubber bulbs. The inner chamber, which is the collecting area, is located in the apex of the V and one collecting tube is attached to it. The two outer chambers, in the arms, stabilise the collector on the floor of the mouth at the submandibular gland orifices. A tube from each outer chamber is connected to a rubber bulb, which provides a vacuum, thus stabilising the device (Truelove et al 1967, Mason & Chisholm 1975, Francis & Hector 1995). This cup has four shortcomings: the device itself may act as a stimulant, the device does not permit collection of saliva from one submandibular gland; when intra-oral stimuli are being chewed or sucked movements of the device may occur; if the vacuum pressure is very great, the mucous membrane may be sucked into the suction tube occluding it (Mason & Chisholm 1975).

A method for collection of mixed submandibular and sublingual saliva was described by Fox et al (1985). After blocking the parotid ducts, the submandibular-sublingual ducts are isolated (submandibular and sublingual saliva frequently enter the mouth by a common duct) and saliva is collected from the floor of the mouth using a 100μl micropipette.

The use of a micropipette connected to a suction device overcomes the bulk difficulty associated with other methods. However, it still suffers several drawbacks. The collection procedure cannot possibly be carried out without interruption and the entire collected volume cannot be precisely measured since part of the saliva usually adheres
to the suction device. In addition, the use of a glass micropipette introduces a safety hazard (Wolff et al 1997).

Pedersen and colleagues (1985) used another device to collect saliva from submandibular glands. The device consists of a Drummond micropipette holder fitted with a 2 ml amber latex dropper bulb, which is used to apply suction. A length of a 50-microliter Van-Lab micropipette forms the central conduit through which the saliva enters the collection chamber.

A system for collection of submandibular/sublingual saliva has been described by Wolff et al (1997). This system consists of a collecting tube, a buffering chamber, a storing tube and a suction device. The main function of the buffering chamber is to avoid saliva being sucked into the suction device. The advantages of the this system as stated by Wolff et al (1997) are: adaptability to anatomic variation, small size, allowing for use for unstimulated collection, patient acceptance, ease to use, low cost, autoclave safety and reliability.

While parotid saliva is relatively easy to collect by established method (Carlson & Crittenden 1910), there is no universally accepted technique for the collection of submandibular/sublingual gland secretions.
<table>
<thead>
<tr>
<th>Authors/year</th>
<th>No. of subjects</th>
<th>Sex</th>
<th>Age</th>
<th>Mean flow (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enfors (1962)</td>
<td>54</td>
<td>M &amp; F</td>
<td>20-59</td>
<td>0.10</td>
</tr>
<tr>
<td>Epstein et al (1980b)</td>
<td>9</td>
<td>M</td>
<td>21-55</td>
<td>0.24</td>
</tr>
<tr>
<td>Pedersen et al (1985)</td>
<td>15</td>
<td>M</td>
<td>18-39</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>F</td>
<td>18-39</td>
<td>0.05</td>
</tr>
<tr>
<td>Pedersen et al (1985)</td>
<td>14</td>
<td>M</td>
<td>70-91</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>F</td>
<td>70-91</td>
<td>0.01</td>
</tr>
<tr>
<td>Tylenda et al (1988)</td>
<td>12</td>
<td>M</td>
<td>≤ 39</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>M</td>
<td>40-59 yrs</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>M</td>
<td>≥ 60</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>F</td>
<td>≤ 39</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>F</td>
<td>40-59 yrs</td>
<td>0.12</td>
</tr>
<tr>
<td>Yeh et al (1988)</td>
<td>10</td>
<td>M</td>
<td>28-46 yrs</td>
<td>0.10</td>
</tr>
<tr>
<td>Ship et al (1990)</td>
<td>35</td>
<td>Both</td>
<td>Mean 70 yr</td>
<td>0.09</td>
</tr>
<tr>
<td>Vissink et al (1993)</td>
<td>36</td>
<td>Both</td>
<td>19-75</td>
<td>0.12</td>
</tr>
<tr>
<td>Lin et al (2003)</td>
<td>50</td>
<td>M</td>
<td>Mean 32.2</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Table 1.11. Results from several studies of flow rate of unstimulated saliva from submandibular-sublingual glands (ml/min)
<table>
<thead>
<tr>
<th>Authors</th>
<th>No. of subjects</th>
<th>Sex</th>
<th>Age</th>
<th>Method of stimulation</th>
<th>Mean Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epstein <em>et al</em> (1980b)</td>
<td>9</td>
<td>M</td>
<td>21-55</td>
<td>lemon lozenge</td>
<td>0.78</td>
</tr>
<tr>
<td>Pedersen <em>et al</em> (1985)</td>
<td>15</td>
<td>M</td>
<td>18-39</td>
<td>lemon juice</td>
<td>0.28</td>
</tr>
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<td>15</td>
<td>F</td>
<td>18-39</td>
<td>lemon juice</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>M</td>
<td>70-91</td>
<td>lemon juice</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>F</td>
<td>70-91</td>
<td>lemon juice</td>
<td>0.09</td>
</tr>
<tr>
<td>Tylenda <em>et al</em> (1988)</td>
<td>12</td>
<td>M</td>
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<td>2% citric acid</td>
<td>0.47</td>
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<tr>
<td></td>
<td>19</td>
<td>M</td>
<td>40-59 yrs</td>
<td>2% citric acid</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>M</td>
<td>≥ 60</td>
<td>2% citric acid</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>F</td>
<td>≤ 39</td>
<td>2% citric acid</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>F</td>
<td>40-59</td>
<td>2% citric acid</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>F</td>
<td>≥ 60</td>
<td>2% citric acid</td>
<td>0.49</td>
</tr>
<tr>
<td>Yeh <em>et al</em> (1988)</td>
<td>10</td>
<td>M</td>
<td>28-46 yrs</td>
<td>2% citrate</td>
<td>0.45</td>
</tr>
<tr>
<td>Ship <em>et al</em> (1990)</td>
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<td>Bot h</td>
<td>Mean 70 yrs</td>
<td>citric acid</td>
<td>0.30</td>
</tr>
<tr>
<td>Vissink <em>et al</em> (1993)</td>
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<td>Bot h</td>
<td>19-75</td>
<td>2% citric acid</td>
<td>0.46</td>
</tr>
<tr>
<td>Kalk <em>et al</em> (2001)</td>
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<td>Bot h</td>
<td>23-58</td>
<td>2% citric acid</td>
<td>0.46</td>
</tr>
<tr>
<td>Lin <em>et al</em> (2003)</td>
<td>50</td>
<td>M</td>
<td>Mean 32.2</td>
<td>Citric acid</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Table 1.12. Results from several studies of flow rate of stimulated saliva from Submandibular-sublingual glands (ml/min)
1.10.3. Collection of Saliva from Minor Salivary Glands

Capillary tubes can be used with both non-suction and suction techniques. The non-suction technique depends on capillary action. Calibrated glass capillary tubes of 1, 5, & 10μl are placed over the duct of a labial gland in the everted lip (Kutscher et al 1967). This method was criticised by Dawes & Wood (1973a) because it is not possible to fill the capillary tube with the saliva secreted, and only a small percentage of the secretion can be collected in subjects with low flow rate. In both cases the actual volume of saliva is difficult to estimate.

The suction technique is a modification of the non-suction technique. It is not dependent on the capillary action of the tube but on suction applied through a rubber tube connected to the non-calibrated end of the tube. Dawes & Wood (1973a) argued that this method is more satisfactory.

Saliva from the labial minor salivary glands can be collected by absorbent materials in a blotting technique and then the volume of secretion is estimated by the increase in weight after the absorbent material has been in position for a certain period of time (Kaaber 1977, Speirs 1984, Smith et al 1992, Gaubenstock 1995, Ferguson 1996). It is also possible to calculate minor gland salivary flow by applying blotting paper over the lip. The paper is then chemically treated by dipping it into acid-Schiff's reagent. The droplets of saliva
appear as stained spots on the paper surface which help in the estimation of the studied salivary flow (Gaubenstock 1995).

Aspiration using small syringes has also been used for collection of saliva from minor glands (Hensten-Pettersen 1975).

The Periotron® is an electronic machine used for measuring volumes of biological fluids as little as 0.2 μL and can be used to measure fluid samples up to 3μL. The first generation of this machine was marketed as Periotron® 600. The Periotron® was originally designed for measurement of gingival crevicular fluids collected by Periopaper® strips (Suppipat & Suppipat 1977, Garnick et al 1979, Bickel & Cimasoni 1984), but later was extensively used to determine volumes of saliva from minor salivary glands and the residual saliva (Shern et al 1990, 1993a b, Sivarajasingam & Drummond 1995, DiSabato-Mordarski & Kleinberg 1996, Eliasson et al 1996, Wolff & Kleinberg 1998, Won et al 2001).

The Periotron® works by the principle of detecting the level of moisture in a filter strip by measuring its dielectric constant. It rapidly measures volumes of biological fluids by measuring the conductivity of the amount of fluid absorbed onto the filter paper strip of defined size. The essential part of the instrument is a set of jaws containing electrical sensors. The upper jaw can be raised and lowered. This permits insertion and removal of the filter strips, which are gripped firmly by the closed jaws. When the instrument is in operation, the sensor in one jaw possesses a positive charge and the
other a negative one. Electricity does not cross over because both sensors are anodised and kept apart, either by being open or, during the zeroing part of the calibration process, by separation using a dry filter strip, which acts as an electrical insulator. When a moistened paper is placed between the two sensors and the upper sensor is closed, the dielectric constant of the isolating material increases; permitting electrical current to flow. A digital meter of the machine gives a reading, which gradually increases over a few seconds until a stable maximum value is reached. The more moisture on the filter strip, the greater its electrical conductivity and, hence, the greater the meter reading (Suppipat & Suppipat 1977). The instrument can be used to measure fluid samples up to 3μl. The filter paper strips, which are known as Sialopaper® strips, are shaped like small frying-pan (see Figure 2.1) and are manipulated by the 'handle' with college tweezers.

The Periotron® 600 was later modified and replaced by new virgins such as the Periotron® 6000 I, 6000 II and 8000 in order to improve its performance. The Periotron® 8000 (Pro-Flow Inc., Amityville, New York, U.S.A.) obsoletes the older models (Periotron® 600 and 6000).

The Periotron® 8000 has a bimodal function. It can be used for measurement of gingival crevicular fluid or saliva by flicking the 'perio'/'sialo' switch that is located at the back of the machine. The machine was further updated in order to be used to measure a wider range of volumes. It could also be connected to a computer so the
volumes can be analysed directly (Ciantar & Caruana 1998, Chapple et al 1999).

The Periotron® provides an easy and efficient way of measuring small volumes of fluids regardless of their osmolarity and viscosity (Suppipat & Suppipat 1977, Shern et al 1990). It is said to be easy to use, requires minimum clinical time and more sensitive in measuring extremely small volumes of fluids such gingival crevicular fluids and saliva (Andors et al. 1982). However, several disadvantages are also present. For instance, the machine should be calibrated before its use and also, it gives a flow rate per unit area, not per gland (Shern et al 1990). The details of the Periotron® 8000 calibration technique are described in Chapter 2.

Calibration of the Periotron® is required to determine the relationship between volumes and the Periotron® readings, which will be employed to estimate the volumes of the biological fluid under investigation. Several fluids have been used as a calibrant in the calibration procedure. These include: deionised water, saline, saliva, serum, plasma and gingival crevicular fluid (Garnick et al 1979, Van Der Bijl et al 1986, Chappel et al 1995). Nevertheless, the calibrant fluid might have an effect on the calibration which should be considered when it is used. According to Bickel and Cimasoni (1984) the Periotron® 6000 readings seem to be affected by the ion concentration of the calibrants. Meanwhile, Chapple and colleagues (1999) used blood serum with different concentrations to study the effect of
protein concentration on the Periotron® 8000 output. They reported a minimum effect (<9%) of protein concentration when it was diluted by saline (1:5 dilution).

The true nature and shape of the calibration line and its validity for use in converting Periotron® readings into volumes is a controversial issue, while a number of groups (Bickel & Cimasoni 1984, Hinrichs et al 1984a, Chappel et al 1995, Medlicott et al 1995) have recommended the use of linear regression equation others (Van Der Bijl et al 1986, Griffiths et al 1988, Chappel et al 1995) advocated alternative methods. Bickel and Cimasoni (1984) recommended the use of linear regression equations to estimate the volume of the investigated fluid. They found that one unit of read out of the Periotron® corresponded to about 3.5 nl of fluid. However, this value was below that, which was stated by the manufacturer (3.5 nl/unit versus 5 nl/unit) (Bickel & Cimasoni 1984).

Chappie et al (1995) realised that the calibration line for serum has three sections: two linear and one quadratic. These sections are 0.0-0.1, 0.1-0.4 and 0.4-1.0μL respectively. So they recommended two separate regression equations for calibration of the Periotron® 6000. Nevertheless, it has been suggested that the calibration data is represented better with a quadratic regression equation than with a linear regression equation due to the errors that would be incurred in volume estimation when the latter equation was used rather than the earlier one (Preshaw et al 1996). They concluded, “The quadratic
regression equation can be used to determine 95% prediction intervals for estimating serum volume given by Periotron® value.

To my knowledge there is no study performed to compare between Periotron® 8000 machines when using Sialopaper® for the calibration. Nevertheless, Chapple and co-workers (1999) used the Periotron® 8000 and raised the following points that should be considered when the Periotron® is used.

1- The calibration of a machine using serum was not found to differ significantly when it is performed on different days.

2- When two different machines were calibrated using serum, a close correlation (r = 0.997) between runs performed on different days was found. These differences were attributed to the variations in thermal and humidity conditions within the laboratories, which might affect the degree of evaporation from the collecting paper (Chapple et al 1999). However, considerable differences between calibrations of different machines were obtained, therefore the calibration should not be crossed over between machines and each machine should use its own calibration to calculate the volume of the collected fluid. This was confirmed recently by Tözüm et al (2004) who reported a unique calibration data for each machine and attributed this to local conditions such as temperature and humidity of the room at which the machine is kept during the calibration procedure.
3- When the same machine was calibrated by a fluid of two different concentrations, both of the calibration lines were close to each other. This indicates an insignificant effect of varying protein concentration of samples for volumes greater than 0.1µL (Chapple et al 1999). However, this was not the case when deionised water was compared with the serum.

4- Deionised water is not a medium of choice to calibrate the machines to be used for GCF analysis due to its property differences.

5- Serum appears to be a suitable fluid for the calibration of the machine due to its similarity to GCF in its viscosity and composition. Thus it is recommended to use a fluid of as similar to fluid being studied as practical with respect to viscosity, pH, protein and electrolyte composition (Chapple et al 1995).

6- The calibration and the reading from the Periotron® 8000 appear not to be influenced by mild variations in serum concentration.

7- The Periotron® 8000 was found to be able to measure Periopaper® volumes up to 1.2 µL. However beyond this volume the calibration line began to level off and the volume greater than this point could not be accurately determined therefore resulting in an underestimation of the real volume (Chapple et al 1999). This was also reported to occur when the Periotron® 6000 was used (Van Der Bijl et al 1986, Griffiths et al 1988, Chapple et al 1995, Preshaw et al 1996).
8- When the Periopaper was completely saturated and placed between the jaws of the machine, a high digital score was observed which in a few seconds fell to settle down at a lower reading (Preshaw et al 1996). This may occur as a result of displacement of the calibration fluid towards and beyond the periphery of the electrodes. Preshaw and co-workers (1996) observed an increased scatter of the points on the calibration curve at higher volumes due to the saturation of the Periopaper and to the viscosity of the fluid.

9- When the machine is calibrated each fluid volume should be measured more than once in order to achieve a reliable curve. Preshaw et al (1996) suggested the use of a large range of fluid volumes for calibration and that three measurements should be taken at each test volume.

The use of the biological fluid, which is being investigated in the calibration procedure, is an ideal choice. However, collection of sufficient quantities of such fluid is considered difficult and impractical (Hinrichs et al 1984ab). As an alternative the calibration fluid should be of a similar composition and viscosity to the fluid intended to be collected and investigated. However, according to Shern and coworkers (1993b) water could be used as an acceptable medium for calibration. Their conclusion was based on an evaluation of spots on paper strips, which were obtained by saliva and a water-borne dye. Despite the fact that water stained a larger spot than did
an equal volume of saliva, the readouts of the Periotron® obtained by the two liquids were similar (Shern et al 1993b).

Another factor that should be considered is the time lapse between the fluid collection and its transfer and placement between the jaws of the machine. This time was found to be a detrimental effect on the readout value of the machine as a result of desiccation of the collecting paper (evaporation of the fluid) Shern et al (1993). Tözüm et al (2004) reported a lack of a major effect of time when the collected sample was transferred to the machine in five seconds, inversely a longer time (30 to 60 seconds) was found to cause a negative effect. This could lead to underestimation of the volume of the collected fluid. This later effect is a result of fluid evaporation. So an immediate transfer of the sample to the machine is of paramount important to eliminate or reduce this outcome.

Cleaning the jaws of the machine after each measurement is required to avoid the retained fluid on the volume measurement. This fluid retention was reported to occur if the machine was not cleaned, irrespective to fluid type and depending on the quantity and property of the fluid (Tözüm et al 2004).

To conclude, room temperature, humidity, location of filter paper between the jaws of the machine, fluid retention and reliability of calibration data should be monitored during the procedure in order to minimise their potential effect on the Periotron® readouts. Furthermore, the calibration fluid properties such as viscosity and
composition should be as close as possible to the fluid being investigated. The Periotron® 8000 was reported to be at least as reliable as the Periotron® 6000

Other techniques have been used for the collection and quantification of saliva from minor salivary glands. These techniques include; photographic method (Ferguson 1996) and an iodine-starch reaction (Shoji et al 2003, Ogami et al 2004).

In the photographic method a camera is used to photograph saliva droplets from the minor salivary glands of the lower lip (Ferguson 1996). Volume of saliva is then estimated from diameter of each droplet. One advantage of this method is that there is no contact with the area from which saliva is measured, thus unwanted salivary stimuli can be avoided. The method allows flow rates from individual glands and from defined areas of mucosal to be calculated. Furthermore, the fluid from other sources such as labial sulcus can be avoided. By this method a permanent documentation of the distribution and the numbers of functioning minor glands can be obtained. It has been claimed that the method permits an accurate measurement of the actual secreted saliva (Ferguson 1996). A high correlation between the flow rates as estimated from weighing filter paper and by the photographic method was reported (Ferguson 1996). The method gave a correlation coefficient of for 36 observations of 0.88, a highly significant value (p < 0.001) (Ferguson 1996).
In the iodine-starch reaction method, a strip of self adhesive tape is painted with a solution of iodine then by starch powder. The tape is placed against the mucosal surface of the lip. Discoloured spots of various sizes corresponding to individual glands could be visualised. After removal of the strip the total stained area could be calculated by computer. The number of stained spots represents the number of secreting glands during the application time. By this method, it is claimed that there is a close correlation between the known volume of saliva and the size of the stained spots by which the salivary flow rate of the minor glands could be estimated.

Results for various techniques for minor glands, both unstimulated and stimulated; are shown in Table 1.13.
<table>
<thead>
<tr>
<th>Authors</th>
<th>No. of subjects</th>
<th>Sex</th>
<th>Mean age (years)</th>
<th>Source of saliva</th>
<th>Method of stimulation</th>
<th>Mean flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiers (1984)</td>
<td>5</td>
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<td>----</td>
<td>lower lip</td>
<td>0.08 M ascorbic acid</td>
<td>3.4</td>
</tr>
<tr>
<td>Shem et al (1990)</td>
<td>14</td>
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<td>37</td>
<td>palate</td>
<td>2% citric acid</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>palate</td>
<td>Unstimulated</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>lower lip</td>
<td>2% citric acid</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>0.96</td>
</tr>
<tr>
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<td>13</td>
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<td>21-58</td>
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<td>2% citric acid</td>
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<td>Sivarajasingam &amp; Drummond (1995)</td>
<td>49</td>
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<td>Palate</td>
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<td></td>
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<td>Unstimulated</td>
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<tr>
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<td></td>
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<td>3.02</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>tongue</td>
<td>Unstimulated</td>
<td>3.10</td>
</tr>
<tr>
<td>Sivarajasingam &amp; Drummond (1995)</td>
<td>50</td>
<td>Female</td>
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<td>Unstimulated</td>
<td>0.53</td>
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<td>Lower lip</td>
<td>Unstimulated</td>
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<td>tongue</td>
<td>Unstimulated</td>
<td>2.96</td>
</tr>
<tr>
<td>Boros et al (1999)</td>
<td>38</td>
<td>Both</td>
<td>21-58</td>
<td>Lower lip</td>
<td>Unstimulated</td>
<td>1.09</td>
</tr>
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<td>Won et al (2001)</td>
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<td>25.2</td>
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<td>Unstimulated</td>
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<td></td>
<td></td>
<td></td>
<td>soft palate</td>
<td>Unstimulated</td>
<td>2.2</td>
</tr>
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<td>Lee et al (2002)</td>
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<td>Both</td>
<td>24-63</td>
<td>lower lip</td>
<td>Unstimulated</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>soft palate</td>
<td>Unstimulated</td>
<td>3.2</td>
</tr>
</tbody>
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Table 1.13. Results from several studies of flow rate from minor salivary glands (µl/min/cm²)
Other tests of salivary function, such as sialography and radioisotope scans, can provide a very crude measure of flow, in that they can demonstrate that flow has occurred but are not capable of estimating quantity. In sialography the contrast medium should clear from the gland within several minutes (aqueous contrast medium) or 45 minutes (lipid contrast medium) of stimulation. Failure of clearance demonstrates failure of flow. Similarly Tc$^{99}$ will be strongly concentrated within the glands but will disperse shortly after stimulation, again implying that salivary flow has occurred. Neither technique can give a quantitative measurement of flow, and both are inadequate for investigative purposes.

1.11. Correlations between Collecting Techniques:

A number of salivary collection devices and techniques have been developed due to the growing popularity of saliva as a substitute for other body fluids, in detection and/or determination of various disease indicators (Haeckel & Bucklitsch 1987). While numerous studies have collected saliva, a few of them have assessed the reliability, validity and correlation between different methods for measuring salivary flow.

In 1982 Navazesh and Christensen compared four methods for measuring whole salivary flow rate (suction, swab, spit and draining) in 17 healthy individuals. The suction method used a vacuum
suction device with an attached saliva ejector, which was placed under the subject's tongue to extract whole saliva from the mouth into a pre-weighed container. The swab method involved using pre-weighed cotton swabs placed in the floor of the mouth to collect saliva. The spit method required the subject to pool saliva in the mouth and then expectorate into a pre-weighed container at specific intervals. In the draining method, subjects were instructed to swallow prior to the collection procedure and were then asked to allow saliva to drain out between parted lips into a test tube fitted with a funnel, which was held near the mouth. At the end of the procedure, subjects were instructed to collect any remaining saliva and to expectorate it. The four methods did not significantly differ when mean flow rates were compared. The results of this investigation indicated the unreliability of the swab method. It also revealed that the suction method had the highest test / retest reliability; although it was found that the suction and swab methods introduced some degree of stimulation and thus are not recommended for unstimulated whole saliva collection. It is interesting to note that the authors concluded that the spitting and draining methods were more reliable and simple, and these make them the methods of choice for whole mouth salivary collections unstimulated and stimulated.

Mulligan et al (1995) investigated the reliability of three different collection methods (draining, chewing and saxon) for whole saliva in a sample of middle-aged and older adults with symptoms and signs of
diminished salivary flow and they compare the three tests with each other. This study showed that the draining and chewing stimulated methods are reliable and reproducible and also revealed a significantly high reliability for the saxon method. The study revealed no significant differences between the whole chewing-stimulated and the Saxon test but showed significant differences between the whole unstimulated draining and the other two collection methods.

Jones and co-workers (2000) compared salivary flow rate assessment methods in 16 elderly subjects aged 75 year and over who were taking various medications, which could possess a negative effect on the salivary flow. They used suction (an open and closed one) and spitting methods. In the open suction method saliva was aspirated from the floor of the mouth using a saliva ejector connected to a suction machine while the subject opened his/her mouth. However, in the closed method the saliva ejector was placed under the tongue and the subjects were asked to close their lips around the saliva ejector. In both of the suction methods saliva was collected for two minutes after which the tube was changed and the collection procedure was continued for an extra three minutes, thus a total of five minutes collection time was achieved. Meanwhile, the subjects in the spitting procedure were asked to expectorate into a pre-weighted test tube every 60 seconds. The saliva was collected for five minutes then the test tube was changed and the collection procedure was resumed for an additional five minute therefore; a total of 10-minute
collection time was achieved. They reported a strong correlation between the five minutes and ten minutes spitting methods (r=0.97, p<0.001). This suggested that five minutes is adequate collection period (Navazesh 1993, Jones et al 2000). However, the flow rates using the two minutes open suction method compared well with the 10-minute spitting method but not with the two minutes closed suction method (r=0.79 versus r=0.16). By using a test/retest with one-week period, both methods (open suction and spitting) demonstrated good comparable reliability. To conclude, the results of this preliminary study indicated that the two minutes open suction method is valid and reliable for measuring salivary flow. However, it needs lower levels of patient co-operation and is acceptable by elderly subjects. Therefore, this method can be used in frail elderly populations and large epidemiologic studies (Jones et al 2000).

Lenander-Lumikai et al (1995) investigated reliability and validity of two types of Salivette® collection kits (non-covered cotton roll and polypropylene covered polyether roll) relative to collection of saliva using paraffin wax. They found that the Salivette® method produces a significantly lower salivary volume (a range of 0.8 to 3.8 ml / 3 min versus 2.4 to 12 ml / 3 min). According to the authors the Salivette® method does not allow estimation of flow rate. They also did not observe any correlations between the flow rates obtained by chewing paraffin wax and the volume collected with either of the Salivette® rolls.
Lamey & Nolan (1994) investigated the recovery of human saliva by using two forms of Salivette® systems (plain cotton roll form and the new polyester insert form). They found that the polyester insert system allows a greater saliva recovery.

A comparison test by Whatman paper strip for the measurement of resting whole saliva with the draining and swab techniques was carried out by Lopez-Jornet et al (1996) in 159 healthy subjects. They found a significant correlation between the draining test and the swab one (r = 0.29 and P = 0.0006). On the other hand the correlation between whole saliva collected by the strip and the draining test was r = 0.3 and p = 0.0002 and the correlation between whole saliva test by the strip and the swab test was r = 0.24, p = 0.0031.

Speirs (1984) collected saliva from the parotid gland and the minor salivary glands in the lower lip. The volume of labial secretion was determined by weight. However by looking at the regression lines for every subject, they found that in many subjects there was a positive correlation between labial and parotid gland flow rates, but in only few subjects the correlation was statistically significant (P < 0.05).

Challacombe (1980) measured the parotid salivary flow in 44 healthy subjects (age range 18-24yrs) as collected in two different times. The authors reported a high significant correlation between the two measurements (r = 0.732, p<0.001).
In 1988b, Watanabe and Dawes carried out a study to investigate the effect of seven different commonly consumed food and 3 concentrations of citric acid on whole salivary flow rate. The study included 32 young adult healthy individuals. In all subjects, the highest flow rate was obtained when 5% citric acid was used as a salivary stimulant. In this investigation, although a significant correlation (P< 0.001) was observed between the unstimulated flow rate and the 5% citric acid stimulated flow, the correlation coefficient was weak (r = 0.56).

In fourteen healthy non medicated subjects, Shern et al (1990) assessed the flow rate of minor salivary glands, unstimulated and stimulated using the Periotron®, at various locations and unstimulated and stimulated whole saliva by spitting. They observed no association between the flow rates for whole and minor salivary glands saliva.

Ferguson (1996) used the photographic and gravimetric method to measure the unstimulated saliva from labial salivary glands. The study included 15 healthy non-medicated subjects aged 19 to 54 years. This study reported a high correlation between the methods. A correlation coefficient of 0.88 (p < 0.01) for 36 observations was reported. The slope of the regression line was very close to 45°, but the gravimetric method gave slightly lower values between 5-10% (Ferguson 1996).
Wang et al (1998) determined unstimulated and stimulated salivary whole saliva flow rates by spitting for twenty-three healthy subjects (11 males and 12 females). They found a high significant correlation between the unstimulated and stimulated whole saliva flow rates ($r=0.53$, $p<0.009$).

In one study Won et al (2001) measured the saliva secretion from the minor salivary glands at the soft palate and the lower lip, the residual saliva at six mucosal surfaces using the Periotron® 8000 and whole saliva with the spitting method. They found the mucosal wetness on the hard palate and buccal mucosa showed significant positive correlations with the unstimulated whole salivary flow rate, while the mucosal wetness on the soft palate was correlated positively with the minor salivary gland secretion rate.

To find if there is a positive correlation between salivary flow rate obtained by different methods, Ogami et al (2004) used the draining method to measure the resting whole saliva and strips that incorporated the iodo-starch reaction to collect saliva from minor saliva glands of the lower lip. Although a positive correlation was found between the salivary flow rates estimated by the two methods ($r=0.67$, $p = 0.01$). The age distribution of the subjects in this study was diverse (24-71 years of age), thus the correlation could not be implemented to estimate the resting whole saliva through the measurement of the resting saliva from the lower lip minor salivary glands.
Lee et al (2002) used a Periotron® 8000 to measure the minor salivary glands at the lower lip and the soft palatal mucosal and the spitting method to measure the unstimulated whole saliva flow rate in twenty healthy subjects (2 men, 18 women with age range 24-63 years). They demonstrated that the secretion rate from the lower labial mucosal was correlated with the unstimulated whole salivary flow rate ($r = 0.579$, $p < 0.01$).

Bourdiol et al (2004) reported a positive correlation between unstimulated and Parafilm stimulated whole saliva in elderly subjects but not in young ones. This correlation was also observed between unstimulated and Parafilm stimulated parotid salivary flow rate in the elderly age groups but was less significant in young groups.
1.12. Questionnaires for Assessment of Subjective Xerostomia

Questionnaires are the most commonly used method for screening xerostomia. Although symptomatic xerostomia is positively correlated with a decrease in salivary flow, it is highly individual-dependent. For example, while some individuals who complain of a dry mouth are found to have a reduction in salivary flow secretion others who do not complain are reported to pose a decrease in the flow (Uhlig et al 1999). Sreebny and Valdini (1988) found a statistically significant relationship between unstimulated whole saliva and the number of positive responses to ten questions about dry mouth when they compared a group of subjects with a complaint of dry mouth (< 0.1ml/min) with a wet mouth group (> 0.41 ml/min).

Fox and co-workers (1987) used questionnaire to study subjective xerostomic and its relationship with objective salivary gland performance. They found that while some questions were correlated with reduction in salivary flow rate other questions were not. Four of the questions which were correlated with a decrease in parotid and submandibular flow rates and which were considered to be highly predictive of salivary gland function are as follows:

Q 4- Do you have to sip liquids in order to swallow dry foods?

Q5- Does your mouth feel dry when eating?
Q 6- Do you have difficulty swallowing food?

Q 8- Is the amount of saliva in your mouth too little most of the time?

They also found that a positive answer to any of these questions was indicative of salivary dysfunction. Furthermore, they demonstrated that questions about dry-mouth symptoms helped to identify the patient who required additional salivary gland evaluation (Fox et al 1987).

Bretz et al (2000) reported that subjects who had at least one of Fox’s questionnaire question positively were found to have a reduction in unstimulated minor salivary flow when measured using Periotron®.

Visual Analogue Scales (VAS), which were originally used in assessment of pain, were also recommended for use in subjective assessment of salivary dysfunction. The subjects are instructed to mark their response to each item by placing a vertical line on a horizontal scale (Pai et al 2000, Navazesh 2003). Pai et al suggested that VAS may be helpful in the diagnosis of salivary dysfunction and for detecting changes in salivary flow rate over time.

Differentiation between subjects who have low salivary flow rates on the basis of a patient’s complaint is confusing because the feeling of a dry mouth is highly subjective. Some patients with a below normal salivary flow will not volunteer a subjective complaint whereas others may complain of a dry mouth when there is no relationship to a decreased salivary flow rate (Närhi 1994, Fox et al 1987 Sreebny
Poor agreement between xerostomia and reduced salivary flow was reported in a longitudinal study of an elderly population conducted by Thomson et al (1999). In this study each subject was considered to be xerostomic if he/she responded “Always” or “Frequently” to the following question: How often does your mouth feel dry? Individuals who had an unstimulated whole salivary flow rate less than 0.1 mL/min were categorized as salivary gland hypofunction patients. The results of this study reported that only 5.7% of contributors had both xerostomia and salivary gland hypofunction. It was suggested that subjective and objective measures of salivary function were not compatible and that the aetiology of xerostomia among the studied population may not solely depend on salivary gland hypofunction (Thomson et al 1999).

Navazesh et al (1992a) proposed a critical range of unstimulated whole saliva (0.12-0.16ml/min) that separated individuals with normal gland function from those with hypofunction, together with four clinical parameters that could be used successfully to identify subjects with salivary gland hypofunction. These parameters are dryness of lips, dryness of buccal mucous membrane, lack of saliva upon gland palpation and DMFT. Comprehensive medical and dental evaluations are required in order to diagnose the real salivary gland dysfunction despite that fact that there are no universal values of salivary flow rates that can be taken as a gold standard.

Xerostomia is most commonly reported by elderly individuals with a
prevalence rate of this subjective complaint ranged from 6% to 29% in the general population (Hay et al 1998, Dafni et al 1997). Locker (1993) investigated oral dryness as subjectively reported in a group aged 65 years and over without measuring salivary gland function. Almost 23% of the subjects reported mouth dryness.
1.13. Aims of the Present Study

Based on the above review of the literature, the aims of this study are:

To examine the reproducibility of methods of salivary flow measurements for minor salivary glands and for whole saliva, both stimulated and unstimulated subjects, in normal healthy adults.

To examine the relationships between different methods for the measurement of salivary flow, for minor salivary glands and for whole saliva, both stimulated and unstimulated subjects, in normal healthy adults.

To examine the relationships between salivary flow measurements for minor, parotid and whole saliva, both stimulated and unstimulated, in normal healthy adults.

To examine the relationships between different methods for unstimulated minor salivary gland flow in a cohort of patients with severely reduced salivary flow following head and neck radiotherapy.

To examine the relationship between unstimulated minor salivary gland flow and subjective xerostomia, as measured by a conventional questionnaire, in the same patient cohort.
Chapter 2. Reproducibility of Salivary flow Measurements

2.1. Introduction

Although great variation in salivary flow rate reported to occur, a relative repeatable flow rate could be achieved when the collection procedure is well standardised (Heintze et al 1983, Laine et al 1999). Variation in salivary flow rate can be due to a number of factors, which can be subdivided into (a) inter-individual variance that can be corrected for by taking standard samples and using a standard collecting procedure, and (b) intra-individual variance, which represents an inconsistency in the flow taken from the same individual. For instance, Crossner (1984) demonstrated that 84% of the variation in flow rate represented real differences between individuals in seven salivary samples over a 15-month period while the remaining 16% of the variation could be attributed to differences within individuals.

When paraffin stimulated saliva was collected from 41 children, on three successive days, Le Bell et al (1991) found that the flow rate of the first collection was significantly lower than that collected at the second and the third time. Several factors may attribute to this variation. For example, familiarity of the participant to the collecting procedure and condition could make the participant more relaxed. This may be an explanation to the increase in the flow rate obtained in the second collecting time when compared with the first one (Le Bell et al 1991, Laine et al 1999).
Laine et al (1999) collected paraffin-stimulated whole saliva for five times over a 7-week period in 12 women. The first, second and third collections were carried out on three successive days while the fourth and the fifth collections were made 6 and 7 weeks after the first collection. They observed a great variation between the collecting samples; the flow rates were significantly higher at the second and third collection than at the first collection. After six weeks the salivary flow rates were near first and the flow rate after seven weeks was higher than the first.

However, Heintze et al (1983) collected unstimulated and stimulated whole saliva by dripping, from 629 adults on two occasions within an interval of 1-2 weeks. They reported a highly significant correlation between the two measurements.

Crossner (1984) investigated a daily variation in salivary flow rate in 42 children. Three samples were collected on one day while two more samples were collected on the second and the third day. The result of the study demonstrated that no statistically significant difference in the flow rate between any of the five samples. According to this study neither the time of the day when the salivary sample was collected nor the timing in relation to the last meal were found to affect the salivary flow. Crossner in the same paper studied variations in the flow rate of salivary samples obtained at 10-week periods over 15 months in 115 children. The author did not observe any statistically significant variation between the obtained samples. In conclusion, the
salivary flow rate showed good reproducibility and stability when it was measured at different time of the same day or when calculated at different days over a longer period of time.

Periotron® 6000 II was found to be reproducible when saliva was collected from five healthy subjects on two successive days (Sivarajasingam & Drummond 1995).

When parotid salivary flow was obtained for double measurements from forty-four individuals, Challacombe (1980) observed a high significant correlation between the two measurements ($r = 0.732$, $p<0.001$).

Regarding the salivary flow of minor salivary glands, large intra- and inter-individual variations in the flow rate was observed by Eliasson et al (1996). The study included thirty-six randomly selected individuals. Unstimulated saliva was collected two times from buccal, labial and palatal minor glands and the flow rate was measured using the Periotron® 600 model 2. However the Periotron® readings were highly reproducible.
<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>No.</th>
<th>Collection method</th>
<th>Test-retest duration</th>
<th>Reliability (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enfors (1962)</td>
<td>16</td>
<td>Lashley cup (Parotid)</td>
<td>1 week</td>
<td>0.74</td>
</tr>
<tr>
<td>Palmai &amp; Blackwell (1965)</td>
<td>20</td>
<td>Unstimulated whole (Swab)</td>
<td>24</td>
<td>0.86</td>
</tr>
<tr>
<td>Ericson (1969)</td>
<td>92</td>
<td>Unstimulated Lashley cup (parotid)</td>
<td>1 week</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>92</td>
<td>Citric acid (1%)</td>
<td>1 week</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>92</td>
<td>Citric acid (6%)</td>
<td>1 week</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>88</td>
<td>Betacholy 0.2mg (cholinergic stimulus)</td>
<td>1 week</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>88</td>
<td>Betacholy 0.3mg (cholinergic stimulus)</td>
<td>1 week</td>
<td>0.76</td>
</tr>
<tr>
<td>Navazesh &amp; Christensen (1982)</td>
<td>17</td>
<td>Unstimulated whole (Draining)</td>
<td>1 day</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>Unstimulated whole (Spitting)</td>
<td></td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>Unstimulated whole (Suction)</td>
<td></td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>Unstimulated whole (Swab)</td>
<td></td>
<td>0.68</td>
</tr>
<tr>
<td>Navazesh et al (1992a)</td>
<td></td>
<td>Unstimulated whole saliva (Draining)</td>
<td></td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stimulated whole saliva (Draining)</td>
<td></td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right acid-stimulated parotid*</td>
<td></td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right candy-stimulated parotid*</td>
<td></td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left acid-stimulated parotid*</td>
<td></td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left candy-stimulated parotid*</td>
<td></td>
<td>0.66</td>
</tr>
</tbody>
</table>

* Modified Carlson-Crittendon.

Table 2.1 Test-retest
Reproducibility of measurement is important in any scientific method. Two possible problems were anticipated in consistency and reliability of salivary flow measurements. First, subjects would be measured on different days, with potential for unknown variations in conditions. Second, some of the methods appeared to be inherently difficult to control. For these reasons it was decided to undertake a reproducible study for each method.

Comparisons between pairs of measurements are often made using correlation analysis and the results shown as (Pearson’s) correlation coefficients (conventionally written as \( r \)). However, if one method of a pair, or if one set of measurements of a pair are, for example, consistently 20% greater than the second method or set of measurements, then the correlation will be perfect \( (r = 1) \) although there are major differences that would be unacceptable in experimentation. The same reasoning applies if one method, or if one set of measurements, are, for example, consistently greater than the other by a fixed amount. In such cases, the appropriate statistical treatment of the analysis of reproducibility is to use a paired t test (Bland & Altman 1986). To measure the reproducibility of salivary flow rates within the same subjects for each method it was decided to carry out a study of salivary flow in volunteer subjects on two separate morning sessions.
2.2. Materials and Methods

2.2.1. Subjects

Nine young adult volunteer dental nurses, all employees of the Dublin Dental Hospital, participated (only eight were used for whole salivary measurements). In each subject paired measurements were made at two time points for both unstimulated and stimulated salivary flow using four separate techniques: Sialopaper (Periotron®), weighing filter paper, collection of whole saliva by spitting and use of Salivette®s. For each technique, unstimulated flow was measured first followed immediately by stimulated measurements. Each technique was employed on a different day, in the morning. Subjects refrained from eating, drinking, smoking, for at least 1 hour before the test.

2.2.2. Collection techniques:

2.2.2.1 Periotron®

The Periotron® measure the moistness of filter strips by a dielectric method dependent on the differing resistances of dry and moist filter paper.

Special strips (Sialopaper, see Figure 2.1) are placed over the moisture source to be measured and then positioned between the jaws of the machine. When the jaws are closed (Figure 2.2) the
electric flow is measured and displayed on the digital read-out as units, which have no assigned value. The Periotron® 8000 is displayed in Figure 2.3.
A known volume of water for injection BP used for calibration procedure.

Figure 2.1. Sialopaper® strip with 3 spots of known volume of sodium chloride used for the calibration procedure recommended by the manufacturer.
Figure 2.2. Periotron® jaws closed on a piece of Sialopaper.

Figure 2.3. Periotron® 8000 showing the readout.
To enable conversion of these arbitrary units to volumes, the machine must be calibrated according to the manufacturer's instructions as follows.

a. The instrument is switched on 10 minutes before use to allow it to warm up. The 'perio'/'silao' switch is set to 'sialo'.

b. A new dry Sialopaper® strip is placed between the two jaws, using tweezers.

c. The jaws are closed, bringing them into contact with the dry strip

d. The zeroing dial on the face of the instrument is adjusted until the digital meter displays zero.

e. The jaws are then opened and the strip removed.

f. Using a Microliter® syringe (Hamilton), three drops of sterile sodium chloride were dispensed on the Sialopaper (Figure 2.1). Triplicate volumes of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4μl of sodium chloride were used

g. Again using tweezers, the strip was transferred to the Periotron® and the jaws were closed.

h. Each meter reading was recorded after it had stabilised.

i. The mean reading for each volume was plotted against the volume and, using curve-fitting software (JMP, SAS Corporation) a polynomial curve (Figure 2.4) was fitted.
Figure 2.4 Calibration curve for Periotron® measurements (Periotron readings/μl). The r value of the polynomial curve fit was 0.999, which is effectively an absolute correlation of +1.
With earlier version of the Periotron®, it was necessary to calibrate the equipment on each occasion. The model used for this experiment is stated to have a stable reading once calibrated. However, the calibration was repeated before each major experiment.

For flow measurement in subjects, unstimulated and stimulated saliva from labial gland/glands was collected from an area of about 4 mm from the outer border of the lower labial mucosa and a few millimetres from the mid line (to avoid the sparser distribution of glands near the midline). The lower lip was selected as a site to measure the salivary flow rate of minor salivary glands because it can be easily kept free of moisture and it allows easy retention of the strip. The following procedure was used:

a. A fresh, dry Sialopaper was placed in the Periotron® and the reading was zeroed.

b. The mucous membrane of the inner surface of the lower lip was dried with a sterile gauze and isolated with cotton wool rolls.

c. A fresh Sialopaper was placed with tweezers on the selected area and held in place with very light pressure from a gloved finger.

d. The Sialopaper was immediately placed with tweezers between the jaws of the Periotron® 8000®, so that the circular part of the strip was centralised on the lower sensor, and the jaws were closed.

e. The meter reading was noted and recorded after it had stabilised.
f. Salivary volumes were read in µl/min/cm^2 from the prepared calibration curve.

For stimulated flow the same procedure was carried out with the following addition. Before measurements were taken, one ml of 10% citric acid (pharmaceutical grade) was applied with a sterile disposable syringe to the dorsum of the tongue and labial sulcus and left for one minute. The subjects were instructed to rinse with clean water and the measurements made as above.

### 2.2.2.2 Weighing

The weighing technique relied on the increased weight of moist filter strips over dry strips. Strips (approximately 10mm x 2mm) were cut from Whatman filter paper and placed in Eppendorf tubes, which were then weighed. The weight of each tube was recorded on the tube in indelible marker. Unstimulated salivary flow was measured as follows:

a. The mucous membrane of the inner surface of the lower lip was dried with sterile gauze and isolated with cotton wool rolls.

b. Saliva from one minor labial gland was absorbed on to the tip of the strip for one minute.

c. The tube was reweighed and the weight of saliva calculated as the difference in the two weighings, before and after.
d. Assuming the specific gravity of saliva to be 1, the volume was calculated from the weight on the basis that \( 1 \text{gl} = 1 \text{ml} \). For stimulated flow measurements, the stimulation procedure was the same as for the Periotron\(^{5}\).

### 2.2.2.3 Whole Saliva (Spitting)

The measurement technique was the same for both unstimulated and stimulated collection; stimulation was carried out as described above. Subjects were seated in a dental chair and allowed to relax for several minutes. Subjects were instructed to make as few movements as possible, including swallowing, during the saliva collection procedure. Collection was initiated immediately after an initial swallow. Subjects were instructed to spit into a standard dental disposable plastic cup for 10 minutes. The saliva was then aspirated into a 10 ml sterile disposable syringe and the volume was read.

### 2.2.2.4. Salivette\(^{®}\)

The Salivette\(^{®}\) is a patent device that uses cotton wool rolls to collect saliva. The cotton wool roll is placed in the mouth and then transferred to a tube, which is pierced at the bottom. This, in turn, sits in a plastic centrifuge tube (Figure 2.5) so that when the entire device is spun, the saliva is forced out of the roll, through the hole in
the inner tube and down to the base of the centrifuge tube (Figure 2.6). The volume can then be measured by a variety of techniques. The Salivette® is used normally to collect saliva for experimentation rather than to measure volume.

In the present study, saliva was collected using the Salivette® (Sarstedt) with a polyester wool roll neutral in a polypropylene film (No/Ref. 51.1534.002).

As stated above, it is known that volume recovery from the Salivette® is incomplete as some liquid is retained by the polyester wool roll. This was confirmed in our laboratory, when different known volumes of water were absorbed by the polyester cotton rolls and centrifuged and the recovery volumes compared with the original volumes. The recovered volumes were always less than the original volumes.

Unlike several of the other methods, the Salivette has a limit to the amount of saliva that can be collected, as the cotton roll will rapidly become saturated with saliva in a normal subject. To enable accurate calculation of flow rates per minute, it is important to ensure that the rolls are left in for a short enough time so that they will not be saturated. To establish an appropriate time for this several rolls were saturated with water and the volume measured. From this, and from knowledge of standard whole salivary flow rates, it was determined that if rolls were left in the mouth for more than one minute, there
was a danger that they might become saturated before the end of the measurement time, thus giving falsely low values of flow per minute.
Figure 2.5. Salivette®. Top, assembled; bottom disassembled.
For both unstimulated and stimulated flow the following procedure was followed. Stimulated was carried out as described above.

1. The cotton roll was placed under the tongue for one minute.

2. The roll was then transferred to the tube assembly and sealed.

3. The assembly was centrifuged at 100 g for 2 minutes.

4. The extracted saliva was then aspirated in a 1 ml sterile disposable-use syringe and the volume read.

2.3 Results

2.3.1. Periotron®

The measurements are shown in Table 2.1.
<table>
<thead>
<tr>
<th>Case</th>
<th>Periotron® unstimulated 1ˢᵗ reading</th>
<th>Periotron® unstimulated 2ⁿᵈ reading</th>
<th>Periotron® stimulated 1ˢᵗ reading</th>
<th>Periotron® stimulated 2ⁿᵈ reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.65</td>
<td>1.08</td>
<td>3.00</td>
<td>2.08</td>
</tr>
<tr>
<td>2</td>
<td>2.00</td>
<td>1.60</td>
<td>4.15</td>
<td>3.25</td>
</tr>
<tr>
<td>3</td>
<td>1.65</td>
<td>2.83</td>
<td>3.00</td>
<td>3.45</td>
</tr>
<tr>
<td>4</td>
<td>0.26</td>
<td>0.81</td>
<td>0.84</td>
<td>3.05</td>
</tr>
<tr>
<td>5</td>
<td>0.70</td>
<td>0.30</td>
<td>1.80</td>
<td>2.55</td>
</tr>
<tr>
<td>6</td>
<td>1.85</td>
<td>1.71</td>
<td>3.23</td>
<td>2.28</td>
</tr>
<tr>
<td>7</td>
<td>2.28</td>
<td>1.53</td>
<td>4.76</td>
<td>3.05</td>
</tr>
<tr>
<td>8</td>
<td>1.58</td>
<td>1.71</td>
<td>2.80</td>
<td>2.03</td>
</tr>
<tr>
<td>9</td>
<td>2.08</td>
<td>2.28</td>
<td>2.73</td>
<td>3.60</td>
</tr>
</tbody>
</table>

Table 2.1. Duplicated Periotron® unstimulated and stimulated measurements (µl/min cm²)
The mean difference for unstimulated Periotron® flow measurements in nine subjects was 0.02, representing 1.44% of the mean paired measurements. Statistically, the two sets of measurements did not differ (p=0.92) (see Table 2.2).

The differences in the two sets of paired measurements are shown in Figure 2.7.
<table>
<thead>
<tr>
<th>Periotron® U2</th>
<th>1.54</th>
<th>t-Ratio</th>
<th>-0.11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periotron® U1</td>
<td>1.56</td>
<td>DF</td>
<td>8</td>
</tr>
<tr>
<td>Mean Difference</td>
<td>-0.02</td>
<td>Prob &gt;</td>
<td>0.92</td>
</tr>
<tr>
<td>Std Error</td>
<td>0.20</td>
<td>t</td>
<td>0.54</td>
</tr>
<tr>
<td>Upper 95%</td>
<td>0.45</td>
<td>Prob &lt;</td>
<td>0.46</td>
</tr>
<tr>
<td>Lower 95%</td>
<td>-0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation</td>
<td>0.64</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.2. Comparison of unstimulated Periotron® flow measurements (μl) in nine subjects.
Figure 2.7. Periotron® unstimulated flow measurements; means versus differences.
The mean difference for stimulated Periotron® flow measurements in nine subjects was 0.11, representing 3.76% of the mean paired measurements. Statistically, the two sets of measurements did not differ (p=0.80) (see Table 2.3).

The differences in the two sets of paired measurements are shown in Figure 2.8.
Table 2.3. Comparison of stimulated Periotron® flow measurements (µl) in nine subjects.
Figure 2.8. Periotron® stimulated flow measurements; means versus differences.
2.3.2 Weighing

The measurements are shown in Table 2.4

<table>
<thead>
<tr>
<th>Case</th>
<th>Weighing unstimulated 1st reading</th>
<th>Weighing unstimulated 2nd reading</th>
<th>Weighing stimulated 1st reading</th>
<th>Weighing stimulated 2nd reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0002</td>
<td>0.0006</td>
<td>0.0008</td>
<td>0.0007</td>
</tr>
<tr>
<td>2</td>
<td>0.0004</td>
<td>0.0007</td>
<td>0.0011</td>
<td>0.0014</td>
</tr>
<tr>
<td>3</td>
<td>0.0006</td>
<td>0.0009</td>
<td>0.0003</td>
<td>0.0011</td>
</tr>
<tr>
<td>4</td>
<td>0.0003</td>
<td>0.0002</td>
<td>0.0004</td>
<td>0.0003</td>
</tr>
<tr>
<td>5</td>
<td>0.0005</td>
<td>0.0013</td>
<td>0.0006</td>
<td>0.0007</td>
</tr>
<tr>
<td>6</td>
<td>0.0009</td>
<td>0.0003</td>
<td>0.0003</td>
<td>0.0006</td>
</tr>
<tr>
<td>7</td>
<td>0.0005</td>
<td>0.0007</td>
<td>0.0006</td>
<td>0.0008</td>
</tr>
<tr>
<td>8</td>
<td>0.0004</td>
<td>0.0008</td>
<td>0.0012</td>
<td>0.0012</td>
</tr>
<tr>
<td>9</td>
<td>0.0006</td>
<td>0.0003</td>
<td>0.0005</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

Table 2.4. Duplicated weighed unstimulated and stimulated specimens (mg/min).
The mean difference for weighed unstimulated flow measurements in nine subjects was 0.00016, representing 14.16% of the mean paired measurements. Statistically, the two sets of measurements did not differ (p=0.30) (see Table 2.5).

The differences in the two sets of paired measurements are shown in Figure 2.9.
<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Weigh U2</td>
<td>0.00</td>
<td>t-Ratio</td>
<td>1.11</td>
</tr>
<tr>
<td>Weigh U1</td>
<td>0.00</td>
<td>DF</td>
<td>8</td>
</tr>
<tr>
<td>Mean Difference</td>
<td>0.00</td>
<td>Prob &gt;</td>
<td>t</td>
</tr>
<tr>
<td>Std Error</td>
<td>0.00</td>
<td>Prob &gt; t</td>
<td>0.15</td>
</tr>
<tr>
<td>Upper 95%</td>
<td>0.00</td>
<td>Prob &lt; t</td>
<td>0.85</td>
</tr>
<tr>
<td>Lower 95%</td>
<td>-0.0002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation</td>
<td>-0.1165</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.5. Comparison of weighed unstimulated flow measurements (mg/min) in nine subjects.
Figure 2.9. Weighed unstimulated flow measurements (mg/min); means versus differences.
The mean difference for stimulated flow measurements in nine subjects was 0.00, representing 9.15% of the mean paired measurements. Statistically, the two sets of measurements did not differ (p=0.25) (see Table 2.6).

The differences in the two sets of paired measurements are shown in Figure 2.10.
Table 2.6. Comparison of weighed stimulated flow measurements in nine subjects.
Figure 2.10. Weighed stimulated flow measurements; means versus differences.
### 2.3.3. Spitting

The measurements are shown in Table 2.7.

<table>
<thead>
<tr>
<th>Case</th>
<th>Spitting unstimulated 1st reading</th>
<th>Spitting unstimulated 2nd reading</th>
<th>Spitting stimulated 1st reading</th>
<th>Spitting stimulated 2nd reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.20</td>
<td>1.50</td>
<td>4.20</td>
<td>5.80</td>
</tr>
<tr>
<td>2</td>
<td>1.10</td>
<td>1.90</td>
<td>8.70</td>
<td>8.00</td>
</tr>
<tr>
<td>3</td>
<td>0.90</td>
<td>1.00</td>
<td>1.30</td>
<td>1.50</td>
</tr>
<tr>
<td>4</td>
<td>0.80</td>
<td>1.00</td>
<td>1.80</td>
<td>2.00</td>
</tr>
<tr>
<td>5</td>
<td>6.20</td>
<td>4.20</td>
<td>9.30</td>
<td>9.00</td>
</tr>
<tr>
<td>6</td>
<td>4.20</td>
<td>6.00</td>
<td>9.40</td>
<td>12.00</td>
</tr>
<tr>
<td>7</td>
<td>1.00</td>
<td>1.00</td>
<td>5.70</td>
<td>4.00</td>
</tr>
<tr>
<td>8</td>
<td>1.60</td>
<td>2.00</td>
<td>7.80</td>
<td>6.00</td>
</tr>
</tbody>
</table>

Table 2.7. Duplicate spitting measurements, unstimulated and stimulated (ml/10min).
The mean difference for unstimulated spitting measurements in eight subjects was 0.02, representing 4.49% of the mean paired measurements. Statistically, the two sets of measurements did not differ ($p=0.61$) (see Table 2.8).

The differences in the two sets of paired measurements are shown in Figure 2.11.
Table 2.8. Comparison of unstimulated spitting measurements in eight subjects.

<table>
<thead>
<tr>
<th></th>
<th>Value 1</th>
<th>Value 2</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spit U2</td>
<td>2.33</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Spit U1</td>
<td>2.13</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Mean Difference</td>
<td>0.2</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>Std Error</td>
<td>0.37</td>
<td>0.31</td>
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</tr>
<tr>
<td>Upper95%</td>
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<td>0.7</td>
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</tr>
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<td>Lower95%</td>
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<td></td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation</td>
<td>0.85</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.11. Unstimulated spitting measurements; means versus differences.
The mean difference for stimulated spitting measurements in eight subjects was 0.0125, representing 0.21% of the mean paired measurements. Statistically, the two sets of measurements did not differ ($p=0.98$) (see Table 2.9).

The differences in the two sets of paired measurements are shown in Figure 2.12.
<table>
<thead>
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<th></th>
<th></th>
<th>t-Ratio</th>
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<tbody>
<tr>
<td>Spit S1</td>
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<td>DF</td>
<td>7</td>
</tr>
<tr>
<td>Mean Difference</td>
<td>0.01</td>
<td>Prob &gt;</td>
<td>0.98</td>
</tr>
<tr>
<td>Std Error</td>
<td>0.54</td>
<td>Prob &gt; t</td>
<td>0.49</td>
</tr>
<tr>
<td>Upper95%</td>
<td>1.28</td>
<td>Prob &lt; t</td>
<td>0.51</td>
</tr>
<tr>
<td>Lower95%</td>
<td>-1.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation</td>
<td>0.91</td>
<td></td>
<td></td>
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</tbody>
</table>

Table 2.9. Comparison of stimulated spitting measurements in eight subjects.
Figure 2.12. Stimulated spitting measurements; means versus differences.
### 2.3.4. Salivette®

The measurements are shown in Table 2.10.

<table>
<thead>
<tr>
<th>Case</th>
<th>Salivette® unstimulated (ml/min) 1&lt;sup&gt;st&lt;/sup&gt; reading</th>
<th>Salivette® unstimulated (ml/min) 2&lt;sup&gt;nd&lt;/sup&gt; reading</th>
<th>Salivette® stimulated (ml/30sec) 1&lt;sup&gt;st&lt;/sup&gt; reading</th>
<th>Salivette® stimulated (ml/30sec) 2&lt;sup&gt;nd&lt;/sup&gt; reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.40</td>
<td>0.40</td>
<td>1.90</td>
<td>1.80</td>
</tr>
<tr>
<td>2</td>
<td>0.40</td>
<td>0.20</td>
<td>1.60</td>
<td>1.80</td>
</tr>
<tr>
<td>3</td>
<td>0.30</td>
<td>0.60</td>
<td>1.50</td>
<td>1.80</td>
</tr>
<tr>
<td>4</td>
<td>0.40</td>
<td>0.20</td>
<td>1.60</td>
<td>1.80</td>
</tr>
<tr>
<td>5</td>
<td>1.20</td>
<td>0.80</td>
<td>1.90</td>
<td>2.00</td>
</tr>
<tr>
<td>6</td>
<td>0.20</td>
<td>1.00</td>
<td>0.60</td>
<td>1.90</td>
</tr>
<tr>
<td>7</td>
<td>0.30</td>
<td>0.10</td>
<td>2.00</td>
<td>1.10</td>
</tr>
<tr>
<td>8</td>
<td>0.50</td>
<td>0.20</td>
<td>2.00</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Table 2.10. Duplicate spitting measurements, unstimulated and stimulated.
The mean difference for unstimulated Salivette® measurements in nine subjects was 0.025, representing 5.56% of the mean paired measurements. Statistically, the two sets of measurements did not differ (p=0.86) (see Table 2.11).

The differences in the two sets of paired measurements are shown in Figure 2.13.
<table>
<thead>
<tr>
<th></th>
<th>U2</th>
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<th>t-Ratio</th>
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<tbody>
<tr>
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<td>DF</td>
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<td></td>
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<tr>
<td>Mean Difference</td>
<td>-0.03</td>
<td>Prob &gt;</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>Std Error</td>
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<td>Prob &gt; t</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Upper95%</td>
<td>0.31</td>
<td>Prob &lt; t</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Lower95%</td>
<td>-0.36</td>
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<tr>
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<td></td>
</tr>
<tr>
<td>Correlation</td>
<td>0.24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.11. Comparison of unstimulated Salivette® measurements in eight subjects.
Figure 2.13. Unstimulated Salivette® measurements; means versus differences.
The mean difference for stimulated Salivette® measurements in eight subjects was 0.0125, representing 0.21% of the mean paired measurements. Statistically, the two sets of measurements did not differ (p=0.98) (see Table 2.12).

The differences in the two sets of paired measurements are shown in Figure 2.14.
### Table 2.12. Comparison of stimulated Salivette® measurements in eight subjects.

| Salivette® | t-Ratio | DF | Mean Difference | Prob > |t| | Std Error | Prob > t | Upper95% | Prob < t | Lower95% |
|------------|---------|----|-----------------|--------|---|---------|---------|---------|---------|---------|
| S2         | 1.78    | 1.64 DF 7 | 0.14 | 0.14 | 0.21 | 0.21 | 0.21 | 0.64 | 0.64 | -0.37 |
| S1         | 0.65    | 0.54 | 7               | 0.73   | 0.73 | 8       | 0.27   | 0.27   | 0.73   | 0.73   |
| N          | 8       | 8   |                 | 8      | 8   | 8       | 8      | 8      | 8      | 8      |
| Correlation| -0.25   | -0.25 |                 | -0.25 | -0.25 | -0.25 | -0.25 | -0.25 | -0.25 | -0.25 |
Figure 2.14. Stimulated Salivette® measurements; means versus differences.
The mean differences (as a percentage of the means of the paired measurements) the methods varied from 0.2 to 14.16. Table 2.13 shows these percentage differences with the associated p values of the paired t-test. It can be seen that the poorest performing technique was weighing. Salivette®s also performed more poorly than the Periotron® and spitting. Of these latter, unstimulated flow was more reproducibly measured by the Periotron® while stimulated flow was more reproducibly measured by spitting.
<table>
<thead>
<tr>
<th>Measurement technique</th>
<th>Stimulation</th>
<th>Difference (per cent)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periotron® Unstimulated</td>
<td>1.44</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Stimulated</td>
<td>3.76</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>Weighing Unstimulated</td>
<td>14.16</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>Stimulated</td>
<td>9.15</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Spitting Unstimulated</td>
<td>4.49</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>Stimulated</td>
<td>0.20</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Salivette® Unstimulated</td>
<td>5.56</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>Stimulated</td>
<td>8.08</td>
<td>0.54</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.13. Mean differences in paired measurements, as a percentage of mean measurements, for four techniques both unstimulated and stimulated.
2.4. Conclusions

Each of the techniques investigated, Periotron®, weighing, spitting and Salivette® showed statistically reproducible measurements. The most reproducible result for unstimulated flow was given by the Periotron® and the least reproducible by weighing. The most reproducible result for stimulated flow was given by the spitting technique and the least reproducible by weighing. The weighing technique had, therefore the poorest reproducibility of any technique for both unstimulated and stimulated flow, but its reproducibility was still statistically acceptable.
Chapter 3. Comparison of unstimulated and stimulated salivary flow rates obtained by different collecting methods

3.1 Introduction

The reliability, validity and correlation between different methods for measuring the flow rate of whole saliva have been studied by various authors.

Kerr (1961) compared the volumes of resting saliva collected by spitting, draining and suction method in 3 subjects. He reported that inter-subject differences were larger than intra-subject differences with the three methods.

White (1977) compared the measurements of resting whole saliva flow rate with spitting and swab methods. He found a significant correlation between the swab and spitting methods. He compared chewing stimulated and resting whole saliva flow rates and found that they correlated significantly.

In 1982 Navazesh and Christensen compared four methods for measuring whole salivary flow rate (suction, swab, spit and draining) among 17 healthy individuals. The suction method used a vacuum suction device with an attached saliva ejector, which was placed under the subject's tongue to extract whole saliva from the mouth into a pre-weighed container. The swab method involved using pre-weighed cotton swabs placed in the floor of the mouth to collect saliva. The spit method required the subject to pool saliva in the
mouth and then expectorate into a pre-weighed container at specific intervals. In the draining method, subjects were instructed to swallow prior to the collection procedure and were then asked to allow saliva to drain out between parted lips into a test tube fitted with a funnel, which was held near the mouth. The four methods did not significantly differ when mean flow rates were compared. The results of this investigation indicated the unreliability of the swab method. It also revealed that the suction method had the highest test-retest reliability, but consistently produced more saliva volume than the draining or the spit. It was found that the suction and swab methods introduced some degree of stimulation and thus is not recommended for unstimulated whole saliva collection. Based on the results of the study the authors concluded that the spitting and draining methods were more reliable and simple, and these make them the methods of choice for whole mouth salivary collections unstimulated and stimulated.

Mulligan et al (1995) investigated the reliability of three different collection methods (draining, chewing and Saxon) for whole saliva in a sample of middle-aged and older adults with symptoms and signs of diminished salivary flow and they compare the three tests with each other. This study showed that the draining and chewing stimulated methods are reliable and reproducible and also revealed a significantly high reliability for the Saxon method. The study revealed no significant differences between the whole chewing-stimulated and
the Saxon test but showed significant differences between the whole unstimulated draining and the other two collection methods.

Jones et al (2000) compared salivary flow rate assessment methods in 16 elderly subjects aged 75 year and over. They used suction (open and closed) and spitting methods. In the open suction method saliva was aspirated from the floor of the mouth using a saliva ejector connected to a suction machine while the subject opened his/her mouth. However, in the closed method the saliva ejector was placed under the tongue and the subjects were asked to close their lips around the saliva ejector. In both of the suction methods saliva was collected for two minutes after which the tube was changed and the collection procedure was continued for an extra three minutes, thus a total of five minutes collection time was achieved. Meanwhile, the subjects in the spitting procedure were asked to expectorate into a pre-weighted test tube every 60 seconds. The saliva was collected for five minutes then the test tube was changed and the collection procedure was resumed for an additional five minute therefore; a total of 10-minute collection time was achieved. They reported a strong correlation between the 5-minute and 10-minute spitting methods (r=0.97, p<0.001). This suggested that five minutes is adequate collection period (Navazesh 1993, Jones et al 2000). However, the flow rates using the 2-minute open suction method compared well with the 10-minute spitting method but not with the 2-minute closed suction method (r=0.78 versus r=0.16). By using a
test/retest with a 1-week period, both methods (open suction and spitting) demonstrated good comparable reliability. In conclusion, the results of this preliminary study indicated that the 2-minute open suction method is valid and reliable for measuring salivary flow. Furthermore, it is acceptable by elderly subjects and needs lower levels of patient co-operation. Therefore, this method can be used in frail elderly populations and large epidemiologic studies (Jones et al 2000).

Lenander-Lumikai et al (1995) investigated reliability and validity of two types of Salivette® collection kits (non-covered cotton roll and polypropylene covered polyether roll) relative to collection of saliva using paraffin wax. They found that the Salivette® method produces a significantly lower salivary volume (a range of 0.8 to 3.8 ml / 3 min versus 2.4 to 12 ml / 3 min). According to the authors the Salivette® method does not allow estimation of flow rate. They also did not observe any correlations between the flow rates obtained by chewing paraffin wax and the volume collected with either of the Salivette® rolls.

Lamey & Nolan (1994) investigated the recovery of human saliva by using two forms of Salivette® system (plain cotton roll form and the new polyester insert form). They have found that the polyester insert system has allowed a greater saliva recovery.

Whatman paper strips for the measurement of resting whole saliva were compared with the draining and swab techniques in 159 healthy
subjects (Lopez-Jornet et al 1996). They found a significant correlation between the draining test and the swabs ($r = 0.29$, $P = 0.0006$). On the other hand, the correlation between whole saliva collected by the strip and the draining test was $r = 0.3$ and $p = 0.0002$ and the correlation between whole saliva test by the strip and the swab test was $r = 0.24$, $p = 0.0031$.

Speirs (1984) collected saliva from the parotid gland and the minor salivary glands in the lower lip. The volume of labial secretion was determined by weight. However by looking at the regression lines for every subject, they found that in many subjects there was a positive correlation between labial and parotid gland flow rates, but in only few subjects the correlation was statistically significant ($P < 0.05$).

Watanabe and Dawes (1988b) carried out a study to investigate the effect of seven different commonly consumed food and 3 concentrations of citric acid on whole salivary flow rate. The study included 32 young adult healthy individuals. In all subjects, the highest flow rate was obtained when 5% citric acid was used as a salivary stimulant. In this investigation, although a significant correlation ($p < 0.001$) was observed between the unstimulated flow rate and the 5% citric acid stimulated flow, the correlation coefficient was low ($r = 0.56$).

In fourteen healthy subjects, Shern et al (1990) assessed the flow rate of minor salivary glands, unstimulated and stimulated using the Periotron®, at various locations and unstimulated and stimulated
whole saliva by spitting. They observed no association between the flow rates for whole salivary flow and minor salivary glands saliva.

Ferguson (1996) used the photographic and gravimetric method to measure the unstimulated saliva from labial salivary glands. The study included 15 healthy non-medicated subjects aged 19 to 54 years. This study reported a high correlation between the methods. A correlation coefficient of 0.88 (p < 0.01) for 36 observations was reported. The slope of the regression line was very close to 45°, but the gravimetric method gave slightly lower values between 5-10%.

In one study Won et al (2001) measured the saliva secretion from the minor salivary glands in the soft palate and the lower lip, the residual saliva at six mucosal surfaces using the Periotron® 8000 and whole saliva with the spitting method. They found the mucosal wetness on the hard palate and buccal mucosa showed significant positive correlations with the unstimulated whole salivary flow rate, while the mucosal wetness on the soft palate was correlated positively with the minor salivary gland secretion rate.

Wang et al (1998) determined the unstimulated and stimulated salivary whole saliva flow rates by spitting for twenty-three healthy subjects (11 males and 12 females). They found a high significant correlation between the unstimulated and stimulated whole saliva flow rates (r 0.53, p< 0.009).
To find if there is a positive correlation between salivary flow rate obtained by different methods, Ogami et al. (2004) used the draining method to measure the resting whole saliva and strips that incorporated the iodo-starch reaction to collect saliva from minor saliva glands of the lower lip. Although a positive correlation was found between the salivary flow rates estimated by the two methods ($r=0.67$, $p = 0.01$). The age distribution of the subjects in this study was diverse (24-71 years of age), thus the correlation could not be implemented to estimate the resting whole saliva through the measurement of the resting saliva from the lower lip minor salivary glands.

Lee et al. (2002) used a Periotron® 8000® to measure the minor salivary glands at the lower lip and the soft palatal mucosal and the spitting method to measure the unstimulated whole saliva flow rate in twenty healthy subjects (2 men, 18 women with age rang 24- 63 years). They demonstrated that the secretion rate from the lower labial mucosal was correlated with the unstimulated whole salivary flow rate ($r = 0.579$, $p < 0.01$).

Bourdiol et al. (2004) reported a positive correlation between unstimulated and Parafilm stimulated whole saliva in elderly subjects but not in young ones. This correlation was also observed between unstimulated and Parafilm stimulated parotid salivary flow rate in the elderly age groups but was less significant in young groups.
This experiment was designed to examine the relationships between unstimulated and stimulated flow measurements for each of several techniques, and the relationships between the unstimulated and stimulated measurements made with different techniques.
3.2. Materials and Methods

3.2.1. Subjects

A group of dental students, ranging in age from 20-25 years, were randomly selected from lists of the 2nd, 3rd and 4th year in the Dublin Dental School & Hospital. Each selected student fulfilled the following criteria:

- willing to participate in the study
- healthy and not taking any medication
- the same subjects were tested with each method.

3.2.2. Collection techniques:

Using the materials and methods described in chapter (2) the salivary flow rates were determined.

Collections of unstimulated and stimulated saliva with different techniques were carried out in different sessions.

The stimulated specimen was collected immediately after collection of the unstimulated.

Collection of whole saliva was carried out with 2 methods: spitting and by using Salivette®. Saliva from minor salivary glands was collected with 2 methods: Sialopaper (Periotron®) and by filter paper.
The collected saliva was then analysed with Periotron® and weighted respectively. Stimulated parotid saliva was collected from the right side.

### 3.2.2.1 Periotron®

From 44 subjects, unstimulated and stimulated saliva from labial gland/glands was collected from an area of about 4 mm² from the outer border of the lower labial mucosa and a few millimetres from the mid line.

a. A fresh, dry Sialopaper was placed in the Periotron® and the reading was zeroed.

b. The mucous membrane of the inner surface of the lower lip was dried with sterile gauze and isolated with cotton wool rolls.

c. A fresh Sialopaper was placed with tweezers on the selected area and held in place with very light pressure from a gloved finger.

d. The Sialopaper was immediately placed with tweezers between the jaws of the Periotron® 8000®, so that the circular part of the strip was centralised on the lower sensor, and the jaws were closed.

e. The meter reading was noted and recorded after it had stabilised.

f. Salivary volumes were read in μl/min cm² from the prepared calibration curve as described in chapter 2.
For stimulated flow the same procedure was carried out with the following addition. Before measurements were taken, one ml of 10% citric acid (pharmaceutical grade) was applied with a sterile disposable syringe to the dorsum of the tongue and labial sulcus and left for one minute. The subjects were instructed to rinse with clean water and the measurements made as above.

3.2.2.2 Weighing method

With the same procedure described in chapter 2 the saliva of the lower labial minor salivary glands was collected from 35 subjects. Unstimulated salivary flow was measured as follows:

a. The mucous membrane of the inner surface of the lower lip was dried with sterile gauze and isolated with cotton wool rolls.

b. Saliva from one minor labial gland was absorbed on to the tip of the strip for one minute.

c. The tube was reweighed and the weight of saliva calculated as the difference in the two weightings, before and after.

d. Assuming the specific gravity of saliva to be 1, the volume was calculated from the weight on the basis that 1g = 1 ml.

For stimulated flow measurements, the stimulation procedure was the same as for the Periotron®.
3.2.2.3 Whole saliva (spitting technique)

The collection technique was described in the previous chapter. A brief description is presented here. All samples of unstimulated whole saliva were collected from 40 subjects during morning hours before noon.

At the start of each measurement, each subject was asked to rinse his/her mouth with tap water in order to remove any loose debris that might be present. Seated in the dental chair the subject relaxed for few minutes and then was instructed to make as little movement as possible, including swallowing, during the saliva collection procedure. The collection was initiated immediately after an initial swallow. The subjects were instructed to spit in to a plastic cup, for 10 minutes. The amount of collected saliva was calculated in a similar way used in chapter 2.

3.2.2.4 Salivette®:

Both unstimulated and stimulated salivary flow rates for 39 subjects were carried as follow

The cotton roll was placed under the tongue for one minute.

The roll was then transferred to the tube assembly and sealed.
The assembly was centrifuged at 100 g for 2 minutes.

The extracted saliva was then aspirated in a 1 ml sterile disposable-use syringe and the volume read.

Saliva stimulated by 1ml 10% citric acid.

3.2.2.5. Collection of parotid saliva

After failure of attempts to collect unstimulated parotid saliva, acid stimulated parotid saliva was collected from the right side with Carlessson-Crittenden cup. The 10% citric acid was applied to the dorsum of the tongue every minute for 5 minutes and saliva is collected in a graduated tube.

3.2.3. Statistical analysis:

One-way analysis of variance was used to test for significant differences in the salivary flow rates when the various methods were compared. Differences between means of collection methods were considered significant at a level of \( p < 0.05 \).

Correlation coefficients (r) were used to obtain a quantitative pattern of the interaction between flow rates obtained by different collection methods.
Figure 3.1. Interpretation of correlation coefficients (r). After Milton 1992.
3.3 Results

The overall results are shown in Table 3.2 and the correlations coefficients in Table 3.3.

<table>
<thead>
<tr>
<th>Number of subjects</th>
<th>Methods of collection</th>
<th>Duration of collection</th>
<th>Mean ± SD (ml)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>Spitting (Unstimulated)</td>
<td>10 min</td>
<td>4.34 ± 3.84</td>
<td>0.5 - 20</td>
</tr>
<tr>
<td>40</td>
<td>Spitting (Stimulated)</td>
<td>10 min</td>
<td>8.01 ± 4.21</td>
<td>3 - 22</td>
</tr>
<tr>
<td>39</td>
<td>Salivette® (Unstimulated)</td>
<td>1 min</td>
<td>0.22 ± 0.26</td>
<td>0 - 1.1</td>
</tr>
<tr>
<td>39</td>
<td>Salivette® (Stimulated)</td>
<td>30 sec</td>
<td>0.87 ± 0.63</td>
<td>0 - 2</td>
</tr>
<tr>
<td>44</td>
<td>Periotron® (Unstimulated)</td>
<td>1 min</td>
<td>1.51 ± 0.78</td>
<td>0.23 - 3.74</td>
</tr>
<tr>
<td>44</td>
<td>Periotron® (Stimulated)</td>
<td>1 min</td>
<td>2.87 ± 0.97</td>
<td>1.28 - 4.83</td>
</tr>
<tr>
<td>35</td>
<td>Weighing (Unstimulated)</td>
<td>1 min</td>
<td>0.0007 ± 0.0005</td>
<td>0 - 0.0024</td>
</tr>
<tr>
<td>35</td>
<td>Weighing (Stimulated)</td>
<td>1 min</td>
<td>0.0010 ± 0.0006</td>
<td>0.0002 - 0.0030</td>
</tr>
<tr>
<td>32</td>
<td>Parotid cup (Stimulated)</td>
<td>5 min</td>
<td>3.79 ± 1.91</td>
<td>1.3 - 9.9</td>
</tr>
</tbody>
</table>

Table 3.2. The number of subjects, methods and duration of collection and the mean and range of flow rates of stimulated and unstimulated whole saliva using four measurement techniques.
Table 3.3. Correlation coefficients between unstimulated and stimulated salivary flow rates from each of four methods. R values marked * are significant (p<0.05).

<table>
<thead>
<tr>
<th>Methods</th>
<th>SpittingU</th>
<th>Salivette®U</th>
<th>Periotron®U</th>
<th>WeighingU</th>
</tr>
</thead>
<tbody>
<tr>
<td>SpittingS</td>
<td>0.77*</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Salivette®S</td>
<td>-</td>
<td>0.55*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Periotron®S</td>
<td>-</td>
<td>-</td>
<td>0.46*</td>
<td>-</td>
</tr>
<tr>
<td>WeighingS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.00</td>
</tr>
</tbody>
</table>
3.3.1 Whole Saliva (Spitting)

The mean salivary flow rate of stimulated whole saliva measured by spitting was almost twice as great as the unstimulated one (8.01 versus 4.34) (Table 3.2). The correlation coefficients between unstimulated and stimulated flow rates was 0.77 (P<0.0001). The correlation coefficients is shown graphically in Figure 3.2.
Figure 3.2. Whole saliva (spitting), unstimulated versus stimulated
3.3.2. Salivette®

The mean salivary flow rate of stimulated whole saliva measured using Salivette®s was almost four times as great as the unstimulated one (0.22 versus 0.87) (Table 3.2). The correlation between unstimulated and stimulated flow rates was 0.55 (P<0.0005). The correlation is shown graphically in Figure 3.3.
Figure 3.3. Whole saliva (Salivette®), unstimulated versus stimulated
3.3.3. Periotron®

When the secreted saliva from labial salivary glands was collected and analysed with the Periotron®, the flow rate of stimulated minor salivary gland was a little under twice as great as the flow rate produced by unstimulated ones (2.87 versus 1.51) (Table 3.2). The correlation between unstimulated and stimulated flow rates was 0.46 (P<0.005). The correlation is shown graphically in Figure 3.4.
Figure 3.4. Minor salivary gland saliva (Periotron®), unstimulated versus stimulated
3.3.4. Weighing

When the secreted saliva from labial salivary glands was collected and analysed by weighing, the flow rate of stimulated minor salivary gland was approximately 50% greater than unstimulated flow (0.0007 versus 0.0010) (Table 3.2). The correlation between unstimulated and stimulated flow rates was 0.00 (p=0.81). The correlation is shown graphically in Figure 3.5.
Figure 3.5. Minor salivary gland saliva (weighing), unstimulated versus stimulated
3.3.5. Unstimulated techniques

The unstimulated measurements made with each of the four techniques were examined in pairs for correlations (six pairs in all). The correlation coefficients are shown in Table 3.4.
Table 3.4. Correlation coefficient between unstimulated salivary flow rates using different collection methods. R-values marked * are significant (p<0.05).
The correlation between the two whole salivary techniques measurements (spitting and Salivette®) was 0.36 (p<0.05). The correlation between whole saliva (spitting) and the minor salivary gland flow using the Periotron® measurements was 0.32 (p=0.05). The correlation between whole saliva (spitting) and the minor salivary gland flow using weighing was 0.00 (p=0.73). The correlation between whole saliva (Salivette®) and the minor salivary gland flow using the Periotron® was 0.1 (p=0.46). The correlation between whole saliva (Salivette®) and the minor salivary gland flow using weighing was 0.00 (p=0.89). The correlation between the two techniques of minor salivary gland flow (Periotron® and weighing) was 0.14 (p=0.45). All of these correlation coefficients are shown graphically in Figures 3.6 to 3.11.
Figure 3.6. Unstimulated flow, spitting versus Salivette®.

Figure 3.7. Unstimulated flow, spitting versus Periotron®.
Figure 3.8. Unstimulated flow, spitting versus weighing.

Figure 3.9. Unstimulated flow, Salivette® versus Periotron®.
Figure 3.10. Unstimulated flow, Salivette® versus weighing.

Figure 3.11. Unstimulated flow, Periotron® versus weighing.
3.3.6. Stimulated techniques

The stimulated measurements made with each of the five techniques were examined in pairs for correlations (ten pairs in all). The correlation coefficients are shown in Table 3.5.

<table>
<thead>
<tr>
<th>Methods</th>
<th>SpittingS</th>
<th>Salivette®S</th>
<th>Periotron®S</th>
<th>WeighingS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SpittingS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Salivette®S</td>
<td>0.14</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Periotron®S</td>
<td>0.33*</td>
<td>0.22</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WeighingS</td>
<td>0.00</td>
<td>0.47*</td>
<td>0.14</td>
<td>-</td>
</tr>
<tr>
<td>ParotidS</td>
<td>0.00</td>
<td>-0.26</td>
<td>-0.22</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 3.5. Correlation coefficient between stimulated salivary flow rates using different collection methods. R values marked * are significant (p<0.05).
The correlation between the two whole salivary techniques measurements (spitting and Salivette®) was 0.14 (p=0.40). The correlation between whole saliva (spitting) and the minor salivary gland flow using the Periotron® measurements was 0.33 (p<0.05). The correlation between whole saliva (spitting) and the minor salivary gland flow using weighing was 0.00 (p=0.87). The correlation between the whole salivary measurements (spitting) and parotid measurement (cup) was 0.00 (p=0.94). The correlation between whole saliva (Salivette®) and the minor salivary gland flow using the Periotron® was 0.22 (p=0.15). The correlation between whole saliva (Salivette®) and the minor salivary gland flow using weighing was 0.47 (p<0.005). The correlation between the whole salivary measurements (Salivette®) and parotid measurement (cup) was -0.26 (p=0.15). The correlation between the two techniques of minor salivary gland flow (Periotron® and weighing) was 0.14 (p=0.37). The correlation between minor salivary gland flow (Periotron®) and parotid measurement (cup) was -0.22 (p=0.24). The correlation between minor salivary gland flow (weighing) and parotid measurement (cup) was 0.00 (p=0.85). All of these correlations are shown graphically in Figures 3.12 to 3.21.
Figure 3.12. Stimulated flow, spitting versus Salivette®.

Figure 3.13. Stimulated flow, spitting versus Periotron®.
Figure 3.14. Stimulated flow, spitting versus weighing.

Figure 3.15. Stimulated flow, spitting versus parotid cup.
Figure 3.16. Stimulated flow, Salivette® versus Periotron®.

Figure 3.17. Stimulated flow, Salivette® versus weighing.
Figure 3.18. Stimulated flow, Salivette® versus parotid cup.

Figure 3.19. Stimulated flow, Periotron® versus weighing.
Figure 3.20. Stimulated flow, Periotron® versus parotid cup.

Figure 3.21. Stimulated flow, weighing versus parotid cup.
3.4. Conclusions

The present study was primarily concerned with the study of salivary flow rate with the aid of the most commonly used methods for salivary collection and evaluation in order to establish a range of values for each used method so these values could represent the normal flow rate in young healthy subjects. The intention of this study was also to find out whether there is a correlation between these different methods. Therefore, by using a single method one could predict salivary flow rate obtained from other sources. Although the various collection procedures were standardised and the subjects were cooperative and relaxed during the collection periods, almost all salivary flow rates did not appear to correlate. Only the flow rate from the spitting unstimulated whole saliva was found to be mildly correlated with its counter stimulated one. Meanwhile despite that all subjects were young and healthy, intra-individual variations were apparent and a wide range of salivary flow rate was observed. Accordingly, it is reasonable to suggest that the whole saliva as measured by a spitting technique (to a certain degree of accuracy) is more reliable in estimating the salivary flow rate. However, other collecting techniques should be used in very limited circumstances such as when the collection of the whole saliva is not obtainable. Saliva that is collected from individual glands such as parotid cannot be used to estimate the whole salivary flow rate as the coefficient correlation between them was found to approach zero.
Chapter 4. Salivary flow and subjective xerostomia in patients following radiotherapy for cancer of the head and neck.

4.1 Introduction

Radiation therapy is a common treatment for head and neck cancers and frequently has serious side effects to the oral cavity including the loss of salivary gland function and a persistent complaint of a dry mouth (Scully & Epstein 1996). The fields of radiotherapy to cancers of the head and neck often involve the major salivary glands. Salivary secretion decreases within a week of radiotherapy in almost all patients. It has been reported that after just five radiation treatment at a dose of 200cGy per day, salivary flow rate decreased by up to 57% (Dreizen et al 1977b). Al-Tikriti et al (1984) investigated the effects of irradiation on submandibular, sublingual and parotid unstimulated saliva in 22 patients with oral or circumoral carcinomas and 9 patients with laryngeal carcinomas. The results of this investigation reported differential effects on submandibular, sublingual and parotid glands, which reflect the beam position. They concluded that irradiation of laryngeal carcinoma would produce maximal effects in the submandibular and sublingual glands and peripheral effects on the parotid. In this study, although, all three major salivary glands in the two groups were affected by the radiation. The parotid salivary secretion in the laryngeal carcinoma patients returned almost to pretreatment flows while that in the oral or circumoral carcinoma group did not during the observation period.
(4 to 6 months). The different types of salivary gland cells react differently to the radiation. The mucous cells are less sensitive to radiation than the serous cells. Thus, the parotid glands, which are predominantly serous, are most likely to be affected with radiation when compared with the minor glands that are predominantly mucous (Niedermeier et al 1998).

Niedermeier et al (1998) demonstrated that after radiotherapy, the secretory performance of the parotid glands was found to decline rapidly and irreversibly when the unstimulated flow rate was collected during and up to 7 months after end of radiotherapy. During the course of the radiotherapy this decline in the flow rate could be observed immediately after commencement of the radiation and it was completely absent 10 days later.

On the other hand, salivary flow of palatal minor glands is not completely diminished after radiation and it returned to approximately 40% to 50% of the preradiation values. They reported that after an initial drop, the salivary flow of palatal minor glands increases, but to a lesser degree than the preradiation values, between 15th and the 35th days of radiation.

The histologic part of the study showed that irradiated tissue had a high degree of chronic inflammation, fibrosis and lipomatosis
4.2. Materials and Methods

4.2.1. Subjects

The study group consisted of 51 radiated patients (34 men and 17 women) with age ranged from 35 to 80 years. The group included 17 patients with carcinoma of the tongue, 14 had carcinoma of the tonsil, 9 had carcinoma of the floor of the mouth, 7 had carcinoma of oropharynx and 4 had carcinoma of the soft palate. The patients had received external beam radiotherapy ranging from 3500 cGy to 6500 cGy to the head and neck with the major salivary glands included in the radiated fields. All patients were seen for a routine follow up and reported having a feeling of dry mouth. On the other hand all of them were capable to perform the saliva collection by spitting.

Oral dryness was measured both subjectively, by questionnaire, and by salivary flow measurements.

4.2.2 Questionnaire

Patients were questioned regarding symptoms and severity of xerostomia by using a subjective questionnaire (Fox 1987). The detailed questionnaire is shown in Figure 4.1.
Please answer the following questions:

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Does your mouth feel dry at night or on awakening?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Does your mouth feel dry at other times of the day?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you keep a glass of water by your bed?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you sip liquids to aid in swallowing dry foods?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Does your mouth feel dry when eating a meal?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you have difficulties swallowing any foods?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you chew gum daily to relive oral dryness?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Does the amount of saliva in your mouth seem to be,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>too little, too much, or you don't notice it?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.1. Questionnaire to study subjective oral feelings and salivary flow (after Fox 1987).
4.2.3 Salivary flow

Unstimulated whole saliva was collected using the technique described in Chapter 2. A brief description is presented here. All samples of unstimulated whole saliva were collected from the patients during morning hours before noon.

At the start of each measurement, each patient was asked to rinse his/her mouth with tap water in order to remove any loose debris that might be present. Seated in the dental chair the subject relaxed for few minutes and then was instructed to make as little movement as possible, including swallowing, during the saliva collection procedure. The collection was initiated immediately after an initial swallow. The patients were instructed to spit in to a plastic cup, for 5 minutes. The amount of collected saliva was calculated in a similar way used in Chapter 2.

Labial minor salivary gland was collected using the Periotron® as described in Chapter 2. However, a brief description is presented here. The lower lip was selected as a site to measure the flow rate of minor salivary glands and the saliva was collected using Sialopaper for one minute.

The Sialopaper was then immediately placed between the jaws of the Periotron® 8000. The meter reading was noted and recorded after it had stabilised. Salivary volumes were read in μl/min/cm² from the prepared calibration curve.
4.3 Results

4.3.1 Whole saliva:

Whole salivary flow measurements are shown in Table 4.1. The frequency distribution of unstimulated whole salivary flow rate obtained from the radiated patients is shown in Figure 4.2. The flow rate presented a wide range of values. The values ranged from 0.0 to 4 ml/5min with a mean, standard deviation and a median of 0.44, 0.86 and 0.10 respectively. 22 patients had no saliva at all because their salivary glands did not produce any. The percentile values are shown in Table 4.2.
Figure 4.2. Distribution of unstimulated whole salivary flow rate in irradiated patients.
<table>
<thead>
<tr>
<th>Case</th>
<th>Periotron® unstimulated</th>
<th>Spitting unstimulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.43</td>
<td>0.40</td>
</tr>
<tr>
<td>2</td>
<td>0.81</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>1.03</td>
<td>0.09</td>
</tr>
<tr>
<td>4</td>
<td>0.33</td>
<td>1.20</td>
</tr>
<tr>
<td>5</td>
<td>1.08</td>
<td>0.02</td>
</tr>
<tr>
<td>6</td>
<td>1.43</td>
<td>1.20</td>
</tr>
<tr>
<td>7</td>
<td>0.23</td>
<td>1.00</td>
</tr>
<tr>
<td>8</td>
<td>1.08</td>
<td>0.00</td>
</tr>
<tr>
<td>9</td>
<td>1.14</td>
<td>0.93</td>
</tr>
<tr>
<td>10</td>
<td>0.40</td>
<td>2.80</td>
</tr>
<tr>
<td>11</td>
<td>0.33</td>
<td>0.00</td>
</tr>
<tr>
<td>12</td>
<td>0.21</td>
<td>0.00</td>
</tr>
<tr>
<td>13</td>
<td>1.12</td>
<td>0.20</td>
</tr>
<tr>
<td>14</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>15</td>
<td>0.32</td>
<td>0.50</td>
</tr>
<tr>
<td>16</td>
<td>0.33</td>
<td>0.00</td>
</tr>
<tr>
<td>17</td>
<td>1.06</td>
<td>0.00</td>
</tr>
<tr>
<td>18</td>
<td>0.10</td>
<td>0.00</td>
</tr>
<tr>
<td>19</td>
<td>0.08</td>
<td>0.00</td>
</tr>
<tr>
<td>20</td>
<td>0.56</td>
<td>0.00</td>
</tr>
<tr>
<td>21</td>
<td>2.04</td>
<td>0.80</td>
</tr>
<tr>
<td>22</td>
<td>0.65</td>
<td>0.00</td>
</tr>
<tr>
<td>23</td>
<td>1.84</td>
<td>4.00</td>
</tr>
<tr>
<td>24</td>
<td>0.56</td>
<td>0.20</td>
</tr>
<tr>
<td>25</td>
<td>0.49</td>
<td>0.90</td>
</tr>
<tr>
<td>26</td>
<td>0.71</td>
<td>0.00</td>
</tr>
<tr>
<td>27</td>
<td>0.49</td>
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Table 4.1. Salivary flow measurements in irradiated patients, derived from two measurement techniques.

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Table 4.2. Percentiles of whole salivary flow in irradiated patients

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<th>Median</th>
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<tbody>
<tr>
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4.3.2 Periotron®

Periotron® measurements are shown in Table 4.1. The frequency distribution of unstimulated minor salivary flow rate using the Periotron® is shown in Figure 4.3. As can be seen from Figure 4.3, the values were skewed markedly towards zero. The percentile values are shown in Table 4.3. Unlike whole saliva, the Periotron® was able to detect differences in flow rate even at minimal level.
Table 4.3. Percentiles of whole salivary flow in irradiated patients.

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<tr>
<th>25% ile</th>
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<tr>
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Figure 4.3. Distribution of unstimulated minor salivary flow rate.
4.3.3 Comparison of Flow Rates

The means of the salivary flow rates of whole unstimulated saliva were compared with those obtained by the Periotron® using the paired t-test. Regression analysis was used to investigate the possible relationship between salivary flow rates obtained by the two methods. The distribution differences between the flow rate of whole saliva and the Periotron® is displayed in Figure 4.4 below. The mean difference is 0.31 with a standard deviation of 0.99. There is a significant difference between the two methods ($p = 0.031$).
Figure 4.4. The distribution difference between flow rate of whole saliva and the Periotron®.
When the two sets of measurements were compared by correlation coefficients, there was a weak correlation ($r=0.1$). The resultant scatterplot is shown in Figure 4.5 and the analysis in Table 4.4.
Figure 4.5. Scatterplot of Periotron® versus spitting for radiotherapy cases.
Table 4.4. Pearson's correlation analysis of Periotron® measurements versus whole saliva in radiotherapy cases.

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4.3.4. Xerostomia questionnaire.

For analysis, each response was coded as 0 (No) or 1 (Yes) except for question 8 which was coded -1 (too little), 1(too much) or 0 (don’t notice). Detailed results by case are shown in Table 4.5.
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Table 4.5. Responses to Fox xerostomia questionnaire. See text for codings.
The responses were first analysed to look for redundancy, i.e. were any two questions show reasonable consistency of response suggesting that they were in fact measuring the same property or variable. Any such redundancy would effectively double the weight of that factor in the overall score.

To examine this, the correlations between pairs of questions were examined. The correlations are shown in Table 4.6. The highest correlation is between questions 1 and 2 but the value is low (0.51). This would suggest that there is little redundancy within the questionnaire but this conclusion is based on a relatively small sample size.
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<td>-0.1690</td>
<td>0.2390</td>
<td>1.0000</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Q7</td>
<td>0.0871</td>
<td>0.1720</td>
<td>-0.2246</td>
<td>-0.1213</td>
<td>-0.0381</td>
<td>0.0592</td>
<td>1.0000</td>
<td>--</td>
</tr>
<tr>
<td>Q8</td>
<td>0.3509</td>
<td>0.4055</td>
<td>0.1002</td>
<td>-0.1059</td>
<td>0.2396</td>
<td>-0.1647</td>
<td>0.1827</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

Table 4.6. Correlations of pairs of questions.
For completeness, non-parametric correlations (Spearman’s rho) were also calculated for each pair of question. Again the highest value was for question 1 versus question 2 (P = 0.51).

As the technique of minor gland flow as measured by the Periotron® seemed to be more sensitive to small variations in patients with severely diminished salivary flow, it was selected for comparison with the results of the Fox questionnaire.

On an initial, exploratory analysis, the score from each question was compared to minor gland unstimulated salivary flow. The results are shown in Figures 4.6 to 4.13 and the effects in Table 4.7. As can be seen, only question 4 showed any significant association with flow rates. Although the r value was low, 0.5, the probability was p<0.002. The weak correlation is probably to be expected in human experimentation of this type.
Figure 4.6. Bivariate fit of Periotron® by question 1

Figure 4.7. Bivariate fit of Periotron® by question 2
Figure 4.8. Bivariate fit of Periotron® by question 3

Figure 4.9. Bivariate fit of Periotron® by question 4
Figure 4.10. Bivariate fit of Periotron® by question 5

Figure 4.11. Bivariate fit of Periotron® by question 6
Figure 4.12. Bivariate fit of Periotron® by question 7

Figure 4.13. Bivariate fit of Periotron® by question 8
<table>
<thead>
<tr>
<th>Question</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.14</td>
<td>0.3236</td>
</tr>
<tr>
<td>2</td>
<td>-0.03</td>
<td>0.8195</td>
</tr>
<tr>
<td>3</td>
<td>-0.22</td>
<td>0.1247</td>
</tr>
<tr>
<td>4</td>
<td>-0.50</td>
<td>0.0002</td>
</tr>
<tr>
<td>5</td>
<td>0.05</td>
<td>0.6867</td>
</tr>
<tr>
<td>6</td>
<td>0.16</td>
<td>0.2586</td>
</tr>
<tr>
<td>7</td>
<td>0.25</td>
<td>0.0825</td>
</tr>
<tr>
<td>8</td>
<td>0.000</td>
<td>0.8796</td>
</tr>
</tbody>
</table>

Table 4.7. Correlation (r) and probabilities of individual questions of Fox’s questionnaire with Periotron® unstimulated minor salivary gland flow, only question 4 showed any significant association with flow rates.
Following this exploratory analysis, it was decided to construct a whole model incorporating salivary flow and all eight questions. The results are shown in Table 4.8. As had been found in the individual question analyses, only question 4 contributed significantly to the model (p<0.002).
| Term  | Estimate   | Std Error | t Ratio | Prob>|t| |
|-------|------------|-----------|---------|------|
| Intercept | 2.460111   | 0.602007  | 4.09    | 0.0002 |
| Q1    | -0.553861  | 0.519752  | -1.07   | 0.2927 |
| Q2    | -0.042786  | 0.303528  | -0.14   | 0.8886 |
| Q3    | 0.0235544  | 0.246156  | 0.10    | 0.9242 |
| Q4    | -1.32748   | 0.388361  | -3.42   | 0.0014 |
| Q5    | 0.0574899  | 0.204102  | 0.28    | 0.7796 |
| Q6    | 0.0307634  | 0.197789  | 0.16    | 0.8771 |
| Q7    | 0.3902615  | 0.243646  | 1.60    | 0.1167 |
| Q8    | -0.049656  | 0.138131  | -0.36   | 0.7210 |

Table 4.8. Whole model test incorporating all eight questions as predictors and Periotron® unstimulated minor salivary gland flow as outcome.
The effect is also shown graphically in Figure 4.14, which plots each effect with confidence intervals. As can be seen, question 4 shows a clear slope and the 95% confidence intervals of the ends of the slope do not overlap. This is not true of any of the other questions.

The second questionnaire property under investigation was whether all eight questions contributed to the predicted salivary flow level. To examine this, principal component analysis was carried out. The results are shown in Table 4.9. The first principal component contributed 81% of the variation. This was spread relatively evenly across seven of the questions, but question 7 explained only 0.07 of the variation. The second principal component explained only 7% of the variation and this was almost all accounted for by question 8 (0.84). The remaining six principal components explained less than 12% of the variation.
Figure 4.14. Plot of effects test of whole model incorporating all eight questions as predictors and Periotron® unstimulated minor salivary gland flow as outcome. Only question 4 showed any significant association with flow rates.
<table>
<thead>
<tr>
<th></th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>Q6</th>
<th>Q7</th>
<th>Q8</th>
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<tr>
<td>Q1</td>
<td>1.0000</td>
<td>0.5065</td>
<td>0.1715</td>
<td>-0.0505</td>
<td>0.2857</td>
<td>-0.1366</td>
<td>0.0871</td>
<td>0.3509</td>
</tr>
<tr>
<td>Q2</td>
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<td>1.0000</td>
<td>-0.0352</td>
<td>-0.0997</td>
<td>0.3223</td>
<td>-0.1469</td>
<td>0.1720</td>
<td>0.4055</td>
</tr>
<tr>
<td>Q3</td>
<td>0.1715</td>
<td>-0.0352</td>
<td>1.0000</td>
<td>0.3215</td>
<td>0.1091</td>
<td>0.0196</td>
<td>-0.2246</td>
<td>0.1002</td>
</tr>
<tr>
<td>Q4</td>
<td>-0.0505</td>
<td>-0.0997</td>
<td>0.3215</td>
<td>1.0000</td>
<td>-0.1768</td>
<td>-0.1690</td>
<td>-0.1213</td>
<td>-0.1059</td>
</tr>
<tr>
<td>Q5</td>
<td>0.2857</td>
<td>0.3223</td>
<td>0.1091</td>
<td>-0.1768</td>
<td>1.0000</td>
<td>0.2390</td>
<td>-0.0381</td>
<td>0.2396</td>
</tr>
<tr>
<td>Q6</td>
<td>-0.1366</td>
<td>-0.1469</td>
<td>0.0196</td>
<td>-0.1690</td>
<td>0.2390</td>
<td>1.0000</td>
<td>0.0592</td>
<td>-0.1647</td>
</tr>
<tr>
<td>Q7</td>
<td>0.0871</td>
<td>0.1720</td>
<td>-0.2246</td>
<td>-0.1213</td>
<td>-0.0381</td>
<td>0.0592</td>
<td>1.0000</td>
<td>0.1827</td>
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<tr>
<td>Q8</td>
<td>0.3509</td>
<td>0.4055</td>
<td>0.1002</td>
<td>-0.1059</td>
<td>0.2396</td>
<td>-0.1647</td>
<td>0.1827</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

Table 4.9. Principal component analysis of salivary flow measurement and Fox's questionnaire.
The prediction profile for the first and second principal components are shown in Figure 4.15.
Figure 4.15. Prediction profile for first and second principal components.
With three possible responses Question 8 it was difficult to analyse the direction of the (almost zero) correlation as the middle group ('don’t notice') was associated with the highest mean Periotron salivary flow. To simplify the analysis, the values were recoded successively in three different ways. First, all values of -1 (too little) were combined with 0 ('don’t notice'). When these scores were compared to Periotron unstimulated minor salivary gland flow, there was an almost complete lack of correlation (r=-0.066, p=0.64). Second, all values of 0 ('don’t notice') were combined with 1 ('too much'). When these scores were compared to Periotron unstimulated minor salivary gland flow, again there was very low level of correlation (r=0.026, p=0.86). Lastly, values of 0 ('don’t notice') were discarded leaving only -1 ('too little') and 1 ('too much'). Once again, when compared to Periotron unstimulated minor salivary gland flow, the correlation was weak (r=0.013, p=0.93). These results are tabulated in Table 4.16 and are shown graphically in Figures 4.16 to 4.18. The direction of the correlations is interesting. One would expect a positive correlation between an ordinal scale of {'too little'}, {'don’t notice'} {'too much'} and salivary flow level. However, the correlation was negative for the first of these recoded comparisons.
<table>
<thead>
<tr>
<th>Recoding employed</th>
<th>Correlation (r)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>{-1,0}, {1}</td>
<td>-0.066</td>
<td>0.64</td>
</tr>
<tr>
<td>{-1}, {0,1}</td>
<td>0.026</td>
<td>0.86</td>
</tr>
<tr>
<td>{-1}, {1}</td>
<td>0.013</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Table 4.16. Correlations of recoded values of question 8 with Periotron unstimulated minor salivary gland flow.
Figure 4.16. Bivariate fit of Periotron unstimulated minor salivary gland flow by recoded question 8 (-1,0),(1) (see text for detail).

Figure 4.17. Bivariate fit of Periotron unstimulated minor salivary gland flow by recoded question 8 (-1),(0,1) (see text for detail).
Figure 4.18. Bivariate fit of Periotron unstimulated minor salivary gland flow by recoded question 8 (-1), (1) (see text for detail).
The total score for the Fox questionnaire for each subject had been calculated (see Table 4.5). This overall score was also compared to Periotron unstimulated minor salivary gland flow. As with the majority of the Fox comparisons, the correlation was low ($r=0.043$, $p=0.76$). This is shown graphically in Figure 4.19.

However, this crude total ignores the 'direction' of each question i.e. would a yes response be expected to be associated with decreased or an increased salivary flow.

When analysed in this way, questions 1 to 7 would expect a negative correlation while question 8 would expect a positive correlation. The scores were re-totalled, therefore, with the score for question 8 being subtracted from the others rather than added. The resultant correlation with Periotron minor salivary gland flow is, as expected, negative, but is very weak and non-significant (0.029, $p=0.84$). This is shown graphically in Figure 4.20.
Figure 4.19. Bivariate fit of Periotron unstimulated minor salivary gland flow by total Fox questionnaire score.

Figure 4.20. Bivariate fit of Periotron unstimulated minor salivary gland flow by total Fox questionnaire score with question 8 scores adjusted (see text for details).
There is no correlation between Periotron® measurements of unstimulated minor salivary gland flow and whole salivary measurement in patients with severely reduced salivary flow due to radiation.

The Periotron® was able to detect differences in flow rates even in patients with minimal secretion.

Whole salivary measurement over 5 minutes was not able to detect differences in flow rates in patients with minimal secretion.

The Fox xerostomia questionnaire was a poor predictor of unstimulated minor salivary gland flow as measured by the Periotron®.

There appeared to be little redundancy in the eight questions of the Fox questionnaire, but this might be due to the small sample size as such analysis normally requires a larger sample size might.

There was no reason to consider removing any question other than Question 7 from the model used to predict salivary flow. Question 7, in fact, relates to the use of chewing gum, which would have cultural unacceptability for a large proportion of older Irish adults.

The pairwise correlation analysis and principal component analysis should provide a foundation for further studies of the value of the Fox xerostomia questionnaire. The standard minimum sample size for
such analysis is ten times the number of questions, a sample approximately twice that in this study.
Chapter 5. Discussion

5.1. Introduction

Collection of saliva, which is important for various clinical and experimental protocols, is a simple non-invasive procedure. Several factors are believed to influence the amount and composition of saliva that is produced from each of the major salivary glands as well as that produced by minor ones. For example, factors such as the intensity and type of stimulus, method of collection, emotional state and dehydration have been found to affect the flow rate. Furthermore, the production of saliva follows a circadian rhythm in which the flow rate rises during the day to an afternoon peak and decreases to almost zero during sleep (Dawes 1974). Since the type of collecting method has been found to influence the flow rate (Kerr 1961), and due to the complexity of endogenous control and the extreme susceptibility of salivary secretion to exogenous influences there is a high correspondence between flow rate and the degree of intra-oral stimulation produced by the collection method (Kerr 1961). Thus, standardization of salivary sampling is important in order to achieve reliable measurements in particular when comparison between the flow rates from the same individual are being made as well as comparison between different subjects (Crossner 1984, Le Bell et al 1991). It is important therefore, to select a technique that is well defined. It is also of a prime importance to describe the procedure and subjects involved in the measurements.
Assessment of salivary flow can be determined by measurement of both unstimulated and stimulated saliva production. The former indicates that no stimulus is used while in the later procedure a specific stimulus is applied just prior to the collection procedure.

It has been pointed out, that the results obtained from different sialometric methods are not comparable because individuals with a high secretory response to one stimulus do not necessarily have the same to another (Ericson 1969). So standardization of stimuli to a limited number of internationally acceptable methods would be of great scientific value (Ericson 1969).

Salivary flow rate has been extensively investigated in different age groups. However, there is lack of agreement in published literature as to the normal value of salivary flow rate. The results reported by several studied were inconclusive and no universal value could be taken as a gold standard. A wide range between individuals was found (Tables 1.5-1.11). This wide range may be due to individual variations, to the many different methods used for collecting whole saliva that have been used and to the health status of subjects in the different studies (Kerr 1961, Sreebny 2000).
5.2 Reproducibility

Heintze et al (1983) reported a highly significant correlation between test-retest (duplicate tests) of unstimulated whole saliva ($r=0.67$ for males and 0.72 for females) and even a higher correlation for stimulated 0.76 and 0.81 respectively at $P<0.001$ for all measurements. Heintze and coworkers studied unstimulated and stimulated whole saliva collected from 629 adults aged between 15 and 74 years. They suggested that the degree of correlation between salivary samples taken at different time intervals may indicate an individual characteristic of flow rate (Heintze et al 1983).

It has been found that biological inter-individual variations are greater than intra-individual variations (Ericson 1969). The correlation between two successive measurements taken at least one week apart for unstimulated parotid salivary flow rate was 0.83 (Ericson 1969). When 1% and 6% citric acid were used as stimulators the correlations between the test-retest measurements were 0.96 and 0.95 respectively (Ericson 1969).

Le Bell et al (1991) collected saliva from forty-one 9-year old children on three successive days. They studied the effect of repeated salivary sampling on paraffin stimulated salivary flow rates and found that the flow rates from the second collecting time were significantly greater than the first. Laine et al (1999) collected paraffin-stimulated saliva samples in a group of 12 menopausal women (eight of whom were taking hormone replacement therapy) in order to study the
effect of repeated sampling on the salivary flow rates. Saliva was collected for five times over a 7-week period. Collections 1, 2 and 3 were carried out on three successive days while the remaining two collections were made at 6 and 7 weeks after the first collection. The flow rates were significantly higher at the second and third collection than at the baseline collection (P=0.003 and P=0.005 respectively). However, the 6-week flow rate declined to approach the baseline flow rate, while after 7 weeks it increased to achieve a significantly higher value than the baseline rate (P=0.018).

Crossner (1984) collected stimulated saliva from 12-year old children five times and found no statically difference in the flow rate between any of the five sampling measurements. This study indicated that a relatively stable flow rate could be achieved during the daytime and from day to day as well as over a longer period of time when the collecting method was well standardised. A probable explanation of the contradiction between the two studies (Crossner 1984 and Le Bell et al 1991) may be the difference of statistical analyses used in the two studies.

Correlation between flow rates obtained for repeat measurements with a single salivary collection method has been shown to be higher than those obtained by different collection methods (Navazesh et al 1992a).

Shern et al (1990) observed that the mean flow rate of minor salivary glands as obtained by test-retest measurements using the Periotron®
were similar during the two measurements (Shern et al 1990) and flow rates were found to be similar across four different labial sites (Shern et al 1993a).

Nederfors and Dahlöf (1993) studied the reliability of stimulated submandibular/sublingual salivary flow rate as well as parotid flow rate. Salivary glands were stimulated by 3% citric acid. Twenty-nine healthy females mean age 36 years were involved. Saliva was collected in three consecutive days at two times each day; before and after breakfast. The two measurements were separated by about two and half hours. The salivary flow rate obtained after breakfast was significantly higher (P<0.05) than that obtained before breakfast, by about 13-14%. There was also a small (but statistically insignificant) decrease (P=0.35) in the flow rate between the first and the following two measurements.

In the current study the flow rate obtained in the second collection period was, in general, higher than that obtained from the first collection. This finding is in line with those reported by Heintze et al (1983), who found that the median flow rates of the second test were higher than those of the first. However, in the current study some individuals were found to secrete more saliva in the first collecting time. This could not be explained by the negative effect of stress on the flow rate (Bates & Adams 1968) and other causes would have a role to play.
To assess the spitting test-retest reliability (whole salivary flow rate), two measurements from eight subjects were obtained one day apart from each other. The correlations for the unstimulated and the stimulated salivary flow were 0.85 and 0.91 respectively, which are comparable favourably with those reported by White (1977) who used a different collecting method and a different stimulator. It is also comparable with those reported by Navazesh et al (1992a) who observed high correlations values for draining unstimulated and stimulated collection method (r= 0.84 and r= 0.87 respectively).

It is reasonable to suggest that there may be an individual range of flow rate values rather than an individual single flow rate and when an intra-individual variation is considered, it may be not possible to obtain the same flow rate value at every collecting time. Subjects may be more relaxed at the second collecting time as any unfavourable effects of stress may be diminished when the collecting periods are not widely separated (within a few days). In the present study, the subjects were dental nurses familiar with the collecting methods and some of them had their saliva previously collected; that may have had a positive effect on flow rates.
5.3 Comparison of unstimulated and stimulated salivary flow rates obtained by different collecting methods

In the present study, the group mean flow rate of spitting unstimulated whole saliva was 0.44 ml/min, which is almost equal to that reported by Navazesh and Christensen (1982) (0.47 ml/min). The value of the stimulated spitting method was almost doubled (0.8 ml/min). However, the group mean flow rate of unstimulated whole saliva obtained by the Salivette® method was almost half that obtained by the spitting method (0.2 ml/min versus 0.44 ml/min) which increased to about nine times when the stimulated whole saliva was considered (0.2 ml/min versus 1.74 ml/min). This difference between the two methods may be due to the lack of complete saliva recovery from the polyester cotton rolls used for the collection (Nolan et al 1991) and/or to the ability of the rolls to absorb saliva. This drawback was observed in the present study when different known volumes of water were absorbed by the polyester cotton rolls and centrifuged and the recovery volumes compared with the original volumes. The recovered volumes were always less than the original volumes.

This finding concurs with observations made earlier by Lenander-Lumikai and associates (1995) who investigated reliability and validity of two types of Salivette® collection kits (non-covered cotton roll and polypropylene covered polyether roll) used for collection of whole saliva stimulated by means of paraffin wax. They found that
the Salivette® method produces significantly lower salivary volumes and concluded that the Salivette® method does not measure real salivary flow rates. They also did not observe correlations between the flow rates obtained by the spitting collection method when paraffin wax was applied as a stimulator and the volume collected with either type of the Salivette rolls.

Nolan and colleagues (1991) investigated an in vitro ability of three Salivette® types to absorb and, subsequently, to release absorbed saliva during centrifugation. The polyester covered rolls were found to release 85% of the absorbed saliva sample, while the maximum release from plain cotton rolls was 48%. Volumes collected during 3-minute periods using a neutral non-covered cotton roll ranged from 1.2 to 3.8 ml, while volumes collected in the same period of time using polypropylene covered polyester rolls ranged from 0.8 to 2.7 ml. This inconsistency between the reported volumes could be attributed to the ability of the used rolls on absorption of the produced saliva and/or to the amount of saliva recovered from the rolls after being centrifuged.

Based on the available literature and on the result of the present study, it may be reasonable to conclude that the Salivette® method is unreliable and its use may lead to an underestimation of the flow rate. It is important to note that the Salivette® was not developed for sialometry.
The spitting and draining methods have been more commonly used in measuring whole salivary flow rate due to their simplicity and reliability (Navazesh et al 1982, Jones et al 2000). The spitting method is considered as a gold standard in measuring salivary flow rate. However, subject compliance is essential. The subject should be able to expectorate the produced saliva and to clear the mouth and also not to swallow, which might be difficult to achieve in older and younger subjects (Jones et al 2000). The potential for errors may be high when compared with other methods, such as the suction technique, that can be more easily used in elderly subjects (Jones et al 2000).

In the current study, spitting rather than draining was employed. The duration of collection was ten minutes, which might be considered lengthy and impractical especially for elderly subjects because it requires a high degree of co-operation from the participants. However, a 10-minute collecting period was chosen to as it was believed that the amount of secreted saliva is increased as the collection time increases and that fluctuations in the flow rate do occur with time (Becks & Wainwright 1939, Dong & Dawes 1995). Therefore, the amount of saliva obtained in a shorter collecting period may not be considered as an indicative of the real flow rate. Meanwhile, the 10-minute period may be required when a considerable amount of saliva is needed. Nevertheless, a strong correlation between 5-minute and 10-minute spitting methods
was reported by Jones and co-workers (2000). This suggested that the 10-minute collecting period may be safely reduced to 5 minutes in some situations without a major drawback (Jones et al 2000).

The present investigation used the spitting method and found a moderate correlation between the stimulated and unstimulated whole salivary flow \((r=0.77, P<0.0001)\). From this it may be reasonable to suggest that the unstimulated spitting method could be used to study the flow rate in order to avoid an unpleasant feeling of the stimulus such as citric acid. Navazesh et al (1992a) reported a moderate correlation between the flow rate for stimulated and unstimulated flow using the draining method (0.63), this value is comparable to the present study.

Although conventional methods used for assessment of salivary secretion are to some extent reliable, absolute differences do exist between methods (White 1977) thus extrapolation of the value obtained by a certain method cannot be made in order to predict the value obtained by other methods.

When the whole salivary flow rate was compared with minor salivary flow using the Periotron®, the correlation between unstimulated whole saliva (spitting) and unstimulated minor salivary gland flow (Periotron®) was 0.32 \((p=0.05)\) while that for stimulated was 0.33 \((p<0.05)\). This weak of correlation between the unstimulated and stimulated labial minor salivary gland measured by Periotron® and
the findings obtained by spitting is in accordance with those of Shern and colleagues (1990 and 1993b) who measured stimulated whole saliva using paraffin wax as a stimulator and saliva from the palatal minor salivary glands stimulated using 2% citric acid.

The use of high concentrations of citric acid is required in order to obtain enough saliva since mild gustatory stimulus was found to be unable to induce an increase in the minor gland salivary secretion (Shern et al 1990). In their investigation Shern et al (1990) failed to find any significant effect of a single application of 2% citric acid (which is considered a mild stimulus) on minor salivary gland secretion and this lack of effect was irrespective of the minor gland i.e. labial, palatal or buccal glands. This study may indicate that the minor glands have a threshold that must be exceeded in order to be stimulated. Shern’s study also indicated that the concentration threshold for the major glands is actually lower than that of the minor one, which should be considered. If this assumption is correct, the stimulated whole salivary flow using mild citric acid is actually representing the shared percentage of unstimulated minor glands to the total secreted saliva.

The use of Periotron® to measure salivary secretion of minor glands was reported to be reliable and consistent; however, it provides a flow rate per unit area of the covered mucous membrane and not per gland. Thus, the flow rate obtained by this method represents the saliva secreted by a number of glands present in that area. This
makes a comparison between several measurements problematic because anatomical variations in the distribution of glands in this area may have a role to play (Alsaade et al 2003). Furthermore, this speculation may partially explain the contradicting figures presented in the test-retest part of the current study because it was difficult to replace the Sialopaper® in the same position every time and thus each measurement represented the glands that were covered. Furthermore, lip elevation during the collection procedure and residual moisture on the lip may affect the amount of collected saliva and should also be considered. Comparison between saliva collected by papers with different sizes, which would cover a different number of glands, should also be considered. In the present study saliva was collected from a region about four millimetres from the outer border of the lower labial mucosa and a few millimetres from the midline in order to avoid the sparser distribution of glands near the midline (Alsaade et al 2003).

A good correlation was found between the right and left parotid glands (Ericson 1969), ranging from 0.78 to 0.97. This suggested that the parotid salivary flow rates are of the same magnitude and for both glands. However, intra-individual differences may exist because the data were presented as a mean obtained from the involved group and this should be considered when interpretation of results is made. In the present study, the right side parotid gland was used and the obtained flow rate represented a stimulated one. In the present study
no correlation was found between the whole salivary flow rate (using the spitting or the Salivette® method) and that of the parotid with correlations of 0.0 and 0.26 respectively.

Heintze et al (1983) suggested that the flow rate of unstimulated saliva is highly correlated to that of stimulated saliva. Unstimulated whole salivary flow rates have been found to correlate better with the stimulated counterpart ones (r=0.63) than with acid stimulated parotid and candy stimulated parotid (r=0.57 and r=0.44 respectively) (Navazesh et al 1992a). Nederfors and Dahlof (1993) found a strong correlation (P<0.0001) between the pre-and post-breakfast values when the submandibular/sublingual and parotid flow rate were considered (r=0.83 and r=0.81, respectively). However, no correlation was observed between the submandibular/sublingual and the parotid at the pre-breakfast collection time, while a weak correlation was found at the post-breakfast time (r=0.17 and r=0.27 respectively) when the flow rates obtained from the submandibular/sublingual glands were compared with that obtained from the parotid gland (Nederfors and Dahlof (1993).

5.4 Xerostomic patients

In the present study 22 subjects (44%) were found to produce no saliva in collection period of five minutes using the spitting method while only one subject had a lack of labial minor salivary secretion
after one minute of collection measured by Periotron®. This may indicate that the minor glands were not in the radiation field when the radiation treatment was being carried out while the major glands were. However, if we consider that the major glands as well as the minor glands were all in the radiation field, this may be explained on the basis of the effect of radiation on the mucous and serous acini. Histologically, it is believed that there is loss of salivary epithelial cells, predominantly acinar cells with fibrosis of remaining tissue (Cooper et al 1995). This finding is in accordance with the literature, which indicates that the major salivary glands are collectively dominated by serous acini, which are believed to be more negatively affected by radiation of the head and neck than the mucous acini (Izutsu et al 1985, Niedermeier et al 1995, Huber & Terezhalmy 2003). The minor salivary glands are predominantly mucous acini, thus they are affected by radiation but to a lower extent than the serous acini.

It was speculated that salivary secretion may be radiation dose-dependent and also dependent on the volume of salivary glands contained in the field of radiation. Variations in salivary gland recovery from the radiation effect may also explain the difference in the obtained salivary flow, since some individuals had a reasonable flow rate while some other individuals had no saliva at all.

In the present study the unstimulated whole saliva was chosen to evaluate the flow rate in the sense that it is more reliable to estimate
the real flow rate than the stimulated one. Unstimulated whole saliva was recommended as the test of choice for the diagnosis of hyposalivation because it may reflect a real reduction in the salivary flow even if the stimulated whole saliva is unaffected (Becks & Wainwright 1939, Sreebny 1989). However we found that the Periotron® was able to detect differences in flow rate even at minimal level, unlike whole saliva. The effect of radiation on the major glands may be present even after ten years, as it was found that some subjects in the present study still have a lack of saliva even 10 years after radiation of the head and neck.

After radiotherapy, the secretory performance of the parotid glands were found to decline rapidly and irreversibly when the unstimulated flow rate was collected during and up to 7 months after end of radiotherapy. During the course of the radiotherapy this decline in the flow rate could be observed immediately after commencement of the radiation and it was completely absent after 10 days (Nederfors and Dahlöf 1993). On the other hand, salivary flow of palatal minor glands is not completely diminished after radiation and it returned to approximately 40% to 50% of the preradiation values. After an initial drop, the salivary flow of palatal minor glands increases, but to less than the preradiation values, between 15th and the 35th days of radiation (Nederfors & Dahlöf 1993). In the histological part of the study, Nederfors and Dahlöf (1993) demonstrated that irradiated
tissue had a high degree of chronic inflammation, fibrosis and lipomatosis.

Subjective assessments of salivary flow reduction might aid in detection of individuals with salivary gland hypofunction. However, it is not accurate enough to be used for diagnosis and therapy. Many complaints of oral dryness may not indicate a real salivary reduction but may reflect changes in oral perception or alteration in mucosal tissue to degree of dryness (Field et al 1997, Longman et al 2000). However, normal flow is an individual feature and a subjective sensation of oral dryness is only poorly correlated with objective measures of salivary glands function in the general population (Hay et al 1998).

Questionnaires have been used in identifying individuals with xerostomia for further evaluation of salivary flow rate of those individuals. Each questionnaire consists of a series of questions concerning subjective oral feelings and oral functions. The response of each individual to these questions are then analysed. Fox et al (1987) have demonstrated that specific questions concerning symptoms of oral dryness can be helpful in identifying patients with hyposalivation. Four of the questions which were correlated with a decrease in parotid and submandibular flow rates and were considered to be highly predictive of salivary gland function.

Q.4 Do you have to sip liquids in order to swallow dry foods?
Q5. Does your mouth feel dry when eating?

Q6. Do you have difficulty swallowing food?

Q8. Is the amount of saliva in your mouth too little most of the time?

Subjective oral dryness (xerostomia) that persists during meals, and that complicates swallowing and leads to an impression of deficiency of saliva is most likely to reflect the actual decrease in salivary secretion and could be used to predict individuals with this reduction who might need further investigation (Fox et al. 1987). They reported a strong correlation between reduction in submandibular/sublingual salivary flow and subjective oral dryness. This may be due to the nature and properties of saliva secreted by these glands. The submandibular/sublingual glands consist predominantly of mucous acini, which produce mucous that has lubricatory properties and aids to improve sense of wetness and comfort during swallowing. In the current study, only question 4 (do you have to sip liquids in order to swallow dry foods?) was showed any significant association with flow rates of labial minor salivary glands.

Bretz et al. (2000) found that reduction in minor saliva gland secretion was associated with subjective complaints of dryness. He also found that a positive answer to at least one of questions of the Fox’s questionnaire was an indication of reduced salivary flow. The conflicting results obtained by different studies may be due to the variation in methods used and in the experimental conditions and
also to the fact that the secretion of saliva is affected by a number of psychological and environmental factors.

5.5 Conclusions

Salivary flow measurements with four measurement techniques, spitting, Salivette®, Periotron® and weighing of filter paper, in the same physiological state, unstimulated or stimulated, appear to be reproducible.

Measurements using three measurement techniques show correlations between unstimulated and stimulated flow rates for each method: spitting, Salivette® and Periotron®. No such correlation was shown with weighing of filter papers.

With one exception, there are no correlations between unstimulated flow, measured with four different techniques: spitting, Salivette®, Periotron® and weighing of filter paper. The exception was a correlation between unstimulated flow measured using spitting and Salivettes®.

With two exceptions, there are no correlations between stimulated flow, measured with five different techniques: spitting, Salivette®, Periotron®, Carlsson-Crittenden cups and weighing of filter paper. The exceptions are a correlation between stimulated flow measured using spitting and Periotron® and a correlation between weighing and Salivettes®.
In severely xerostomic subjects, there is no correlation between unstimulated salivary flow as measured by spitting and by the Periotron®. The Periotron is a better method for the detection of flow variations at low flow rates.

In severely xerostomic subjects, the Fox questionnaire is a poor predictor of unstimulated minor gland flow rates. The question “Do you have to sip liquids in order to swallow dry foods?” is a good predictor of unstimulated minor gland flow rates in these subjects.

Further studies are required to examine the possibility of internal redundancy in the Fox questionnaire.

Studies that have relied on single salivary flow techniques must be interpreted with caution as the results may not reflect the true salivary flow status of the subjects and the conclusions may therefore rest on inadequate evidence.
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