

On Minor Salivary Gland Secretion

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Göteborg 2006

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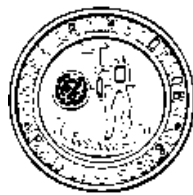
2006

ISBN 91-628-6947-7

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ABSTRACT

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The overall aim of this series of studies has been to examine palatal, buccal and labial minor salivary gland secretions in relation to age and gender and other factors and conditions that could have effects on the saliva. Further aims were to analyze minor salivary gland secretions in relation to feelings of oral dryness and dental plaque pH. The studies are based on the Periotron method, which measures minor fluid volumes collected in absorbent filter papers. The concentrations of proteins in the saliva samples, recovered from the filter papers, were also examined using ELISA techniques. The accuracy and variability of the Periotron measurements were evaluated (Paper I). Mucosal gland secretion rates were analyzed in relation to age and gender, common diseases and medications (Papers I and V), during oestrogen treatment (Paper II) and hyposalivation due to Sjögren's syndrome or head and neck radiation (Paper III). The secretion rates were also related to denture wearing (Papers I and V), pregnancy (Paper V) and tobacco use (Papers I and V). The salivary concentration of IgA was examined in relation to various factors in Paper V, while albumin and lactoferrin were examined together with IgA in relation to hyposalivation (Paper III). Acidogenic microorganisms and pH in dental plaque after a sugar challenge were determined in hyposalivation subjects and matched controls (Paper IV). The results showed high accuracy for the Periotron measurements but large inter- and intra-individual variations in minor gland secretions. The secretion rate per mucosal surface area was highest at buccal sites and lowest at palatal sites. In the palatal mucosa, the secretion rate was higher at medial sites than at lateral sites. Age was not correlated to the minor gland secretion rates but was positively correlated to the IgA concentration. Women displayed lower minor gland flow rates and lower buccal saliva IgA concentrations than men. The buccal mucosal secretion rate was reduced during the use of diuretics and anti-hypertensive medication, while elderly women had an increased labial salivary secretion rate during oestrogen treatment. Subjective feelings of oral dryness were reduced with increased labial salivary flow. Individuals with removable dentures had a significantly higher secretion rate in palatal glands compared with those without dentures. Lower secretion rates and higher protein concentrations were seen in minor gland saliva during hyposalivation and this applied especially to irradiated subjects. Compared with their respective matched controls, irradiated patients had a more acidic plaque after a sugar challenge than the Sjögren's syndrome patients. A low buccal gland secretion rate was correlated to increased plaque acidity in hyposalivation subjects. The number of acidogenic microorganisms had a more important effect on this acidity in the healthy controls. The present studies also showed that buccal gland secretion rates and IgA concentrations were positively correlated to whole saliva secretion rates and IgA.

Key words: age, dental plaque pH, dentures, gender, health, IgA, medication, minor salivary glands, oral dryness, saliva, salivary secretion rate.

ISBN 91-628-6947-7

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ORIGINAL PAPERS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

- I Eliasson L, Birkhed D, Heyden G, Strömberg N. Studies on human minor salivary gland secretions using the Periotron[®] method. Arch Oral Biol 1996;41:1179-1182.
- II Eliasson L, Carlén A, Laine M, Birkhed D. Minor gland and whole saliva in postmenopausal women using a low potency oestrogen (oestriol). Arch Oral Biol 2003;48:511-517.
- III Eliasson L, Almståhl A, Lingström P, Wikström M, Carlén A. Minor gland saliva flow rate and proteins in subjects with hyposalivation due to Sjögren's syndrome and radiation therapy. Arch Oral Biol 2005;50:293-299.
- IV Eliasson L, Carlén A, Almståhl A, Wikström M, Lingström P. Dental plaque pH and microorganisms during hyposalivation. J Dent Res 2006;85:334-338.
- V Eliasson L, Birkhed D, Österberg T, Carlén A. Minor salivary gland secretion rates and IgA in adults and elderly. Eur J Oral Sci 2006; accepted for publication.

INTRODUCTION

Saliva is rich in mechanisms and functions that are important not only for oral health and well-being but also for general health and well-being. It facilitates oral functions by lubricating the oral surfaces and it contains a number of protective mechanisms against microbial and chemical assaults. These functions are established by the salivary content of a number of inorganic and organic constituents (1). What we in daily speech refer to, as saliva is whole saliva. It is a mixture of secretions that is excreted into the oral cavity from a number of major and minor salivary glands. In all, they excrete between 0.6 and one liter of saliva per day (2).

Salivary glands

There are three major pairs of salivary glands. The parotid glands are the largest, and they are situated in front of the ear and behind the lower jaw. The glands deliver the saliva through ducts penetrating the buccal mucosa near the second upper molar. The submandibular glands are smaller and are situated in the posterior part of the floor of the mouth close to the inner aspect of the mandible. Their excretory ducts open underneath the tongue lateral to the lingual frenulum. The sublingual glands, which are also situated in the floor of the mouth, are the smallest of the three paired major salivary glands. Their secretions enter the oral cavity through a series of small ducts opening under the tongue. These three pairs of glands produce the largest part (>90%) of the total saliva volume (3). The remaining component of saliva originates from the mucosal, minor salivary glands (4). The minor salivary glands are located in the buccal, labial, palatal and lingual regions, including the base of the tongue (von Ebner's glands). They are estimated to occur in humans in numbers varying between 600 and 1,000 (3). The only regions of the mouth in which no glands have been found are the gingiva and the anterior part of the hard palate. The glands develop during embryonic life through the proliferation of a cord of cells from the epithelium into the underlying mesenchyma, followed by a branching process to form the grape-like arrangements of the secretory lobes (5). Unlike the major salivary glands, the minor salivary glands lack a branching

network of draining ducts. Instead, each salivary gland unit has its own simple duct (Fig.1).

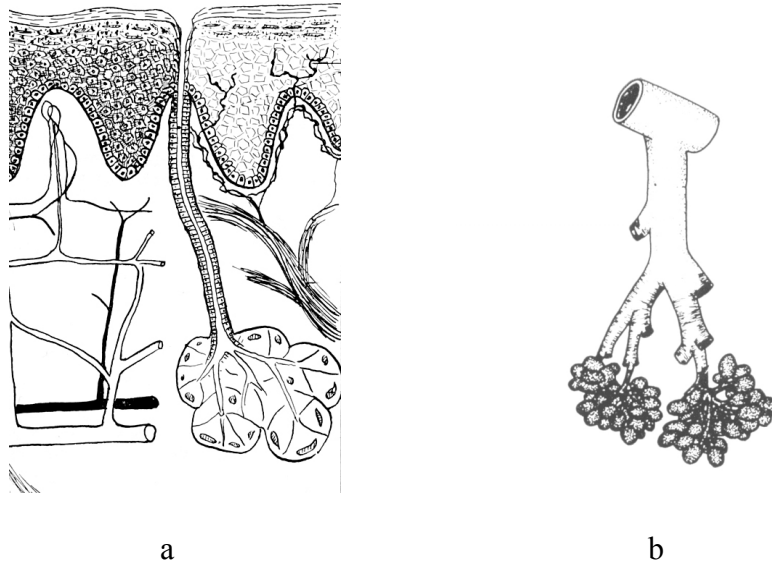


Fig. 1. Schematic pictures of secretory end pieces and excretory ducts from minor (a) and parotid (b) salivary glands (adapted from Orban: ORAL HISTOLOGY AND EMBRYOLOGY, 4th ed © 1957 Mosby, with the kind permission of Elsevier and Springer Science and Business Media).

Salivary secretion

The secretion of saliva is an energy consuming and active two-stage process. Salivary glands produce their secretions by transforming capillary blood into interstitial fluid, which is secreted by the acini as isotonic primary saliva in the terminal end pieces of the gland parenchyma. This saliva is finally modulated to a hypotonic secretion in the ductal systems (3). The secretion process of the major salivary glands is activated by both the sympathetic and the parasympathetic nervous systems. This mechanism regulate the secretory capacity in complicated interactions, varying between the separate glands (6). Since most of the minor glands have little or no sympathetic innervations (7), parasympathetic signals with cholinergic transmission (8) constitute the main_nervous drive to their salivary secretions. Also in contrast to the major glands, a small volume of saliva is continuously and spontaneously secreted from the minor glands.

The nature of saliva from different glands has traditionally been described as serous or mucous. Parotid secretion is referred to as serous whereas the submandibular/sublingual secretions are referred to as sero-mucous. Minor gland saliva is traditionally regarded as mucous in nature. Recent data from ultrastructural investigations have, however, shown that the separate minor glands differ in their secretions. Lingual glands are predominantly serous and the other minor glands, together with the mucous cells, possess a variable number of sero-mucous cells, with the relatively highest number in buccal glands (9). The protein content is also different in the minor gland secretions compared with that from the major glands (10, 11).

Saliva function

Major gland saliva has several functions that are of special importance for the teeth. The saliva is e.g. rich in buffering bicarbonate and in proteins that bind calcium and phosphate. The flow of the saliva solution after stimulation from eating facilitates oral clearance of food remnants and acids. These mechanisms allow for the maintenance of tooth mineral integrity. At reduced flow of saliva, the risk of oral mucosal lesions and caries are increased (1, 12). Reduced pH and higher numbers of acidogenic micro-organisms are also seen in hyposalivators (13-17).

In 1956, Schneyer (18) expressed the opinion that minor salivary glands make no significant contribution to the total unstimulated volume of saliva. It was subsequently reported that minor salivary gland secretion accounts for 7-10% of the total volume of whole saliva (4). The minor gland secretions are rich in high-molecular weight glycoproteins and secretory IgA (10, 19), which are important constituents of the protective layer covering the hard and soft oral tissues. This layer moistures the oral tissues and protects them from bacteria and mechanical wear and from chemical penetration of different substances. It also facilitates mastication, swallowing and speech by minimizing the friction between the teeth and the oral mucosa (20-22). In spite of the small volumes that are secreted, minor gland saliva may thus have a significant effect on salivary functions.

Considerable information on the secretion rate, composition and function of whole saliva and major gland saliva (parotid and submandibular/sublingual) is available in papers and textbooks. Fewer data have been published on minor gland function.

Methods for studying minor salivary gland secretions

Some investigations of minor salivary glands are based on histomorphologic studies (23-28). The difficulties involved in the collection of the small amounts of viscous saliva from these glands have hampered studies of their secretions. Micro-pipettes, capillary tubes, sponges, synthetic discs and filter papers have been used to collect minor salivary gland secretions for qualitative studies (22, 29-40). Weighing methods or semi-quantitative estimations as measurements of the secreted droplets (18, 27, 31, 33, 39, 41-49), as well as calculations based on measuring colored spots on chromatology papers (50, 51), have been applied in order to estimate minor gland flow rates.

The Periotron method

Following the introduction of an electronic device for measuring the oral mucosal flow of saliva (Periotron[®], Pro-Flow[™], Inc., Amityville, NY, USA), quantitative estimates of minor gland secretions have become fairly easy to perform. The method was originally designed for gingival fluid measurements (52), but it was subsequently modified for saliva research.

The introduction of this device has greatly improved precision and versatility when estimating minor gland secretion rates. Recent publications on mucosal salivary flow rates are based on this technique (53-65). The mean values for mucosal gland secretion rates presented in these publications are summarized in Table 1.

Table 1. Mean secretion rates ($\mu\text{l}/\text{cm}^2/\text{min}$) from oral mucosal glands in investigations using the Periotron method

	Nieder- meier & Huber (55)	Nieder- meier <i>et al.</i> (54)	Nieder- meier & Krämer (53)	Shern <i>et al.</i> (58)	Shern <i>et al.</i> (59)	Shern <i>et al.</i> (56)	Sivara- jasingam & Drum- mond (57)	Nieder- meier <i>et</i> <i>al.</i> (63)	Hedner <i>et al.</i> (64)	Won <i>et al.</i> (60)	Blom- gren <i>et al.</i> (65)	Lee <i>et</i> <i>al.</i> (61)	Sones- son <i>et</i> <i>al.</i> (62)	
Number of inves- tigated individuals	134	121	86	12	14	51	99	40	8	30	16 ^c	20 ^c	30 ^b	
Palatal glands	Males	1.36 ^a	1.06	-	0.31 ^a	0.68 ^a	0.58	0.59	0.6 ^a	2.5 ^c	-	3.2 ^{a, c}	-	
	Females		0.99	-			0.53	0.53		1.9 ^c	-		-	
	Denture wearers	^d	-	3.69 ^a	-	-	-	-	0.6 ^a	-	-	-	-	
Buccal glands	Males	-	-	-	-	2.60 ^a	2.36	3.02	-	-	-	-	12.7	
	Females	-	-	-	-		2.09	2.94	-	-	-	-	11.1	
Labial glands	Males	-	-	-	-	0.92 ^a	0.86	2.38	-	1.71 ^a	2.6	4.5 ^a	1.9 ^a	2.3
	Females	-	-	-	-		0.78	2.00	-		2.9			2.5

^a No gender difference shown; ^b Adults; ^c Values from reference/control group; ^d Increased secretion compared with non-denture wearers; ^e Soft palate.

Principles of the Periotron method

Moisture from the oral mucosa is harvested by applying a piece of blotting paper to the mucosa. The paper is placed between two plates on the measuring instrument and a voltage is applied across the plates. Due to the dielectric nature of saliva molecules, the instrument is capable of calculating the volume of moisture which has been absorbed by the blotting paper. In practice, the instrument is first zeroed to compensate for any moisture retained in the paper during storage. The harvesting of mucosal fluid is then performed and the paper is again placed in the instrument. The volume absorbed is calculated from a standard curve obtained with known volumes of water added to the papers. Due to the technique the saliva amount is usually expressed in $\mu\text{l}/\text{cm}^2/\text{min}$.

Factors affecting salivary secretions

Several factors that can affect secretion have been identified from studies of major glands and whole saliva. Our knowledge of the effects of these factors on minor gland saliva is, however, scarce or non-existent.

Age and gender

Conflicting data have been reported when it comes to the correlation between age and gender and major salivary gland and whole salivary secretion rates (66-71). Published studies of minor salivary glands have the same diversity, although several investigators have found an age-related lower salivary flow rate or structural, degenerative changes in labial glands, suggesting reduced function with increasing age (25, 26, 42). However, one study showed no age-related effect on labial salivary flow (56). In the case of palatal and buccal gland saliva, a decreasing or non-existent effect by age on the secretion rate was reported (27, 53-57). Apart from one study in which a higher palatal secretion rate was found in men compared with women (27), there are reports of no gender-related differences in minor gland secretion rates (43, 53-57). On the other hand, a study of the total sum of labial, buccal and palatal mucosal gland secretion rates revealed lower secretion rates for women than for men and reduced secretion with increasing age (72). This discussion is further complicated by data showing fewer secreting salivary glands per labial mucosal area in adults compared with children and a lower secretion

rate in buccal and labial mucosal glands in children compared with adults and adolescents (62). In this study of young adults, adolescents and children, no gender differences were found.

Stimulation

It has been reported that the minor glands display a poor response to stimulation (30, 31), in contrast to major glands (73). However, subsequent data report that the local application of physostigmine (64) and continuous speaking (48) could stimulate the labial mucosal gland secretion rate. The mechanical stimulation of a denture base plate on the palatal mucosa also appears to stimulate the underlying minor glands to increase their salivary secretion rate (53, 55).

Disease and medication

Medication, especially with drugs causing oral dryness symptoms, are known to reduce whole salivary secretion rates (74). An impaired palatal gland secretion rate among medicated persons has also been reported (53).

In longitudinal studies, medication with female sex hormones increased the flow rate of whole (75-77) and palatal (63) saliva, although this effect on whole saliva was not seen in cross-sectional investigations (78-80).

It is known that diseases, such as diabetes, hypertension (81), cystic fibrosis (41), and rheumatic diseases (82), regardless of treatment, can affect the salivary secretions. In Sjögren's syndrome, patients' salivary gland structures are infiltrated by lymphatic cells (23, 24). This interferes with glandular function and results in a lower salivary secretion rate (83). It is, however, believed that a residual function exists during the inflammation (84), which explains why stimulation of the function is possible (85).

Cancer patients are often treated with chemotherapy or irradiation. During chemotherapy, the labial minor salivary gland secretion rate but not the stimulated whole salivary secretion rate is hampered (65). Radiation therapy directed against the head and neck region causes permanent damage to the radiosensitive salivary gland structures. This leads to the depression of salivary flow (86), an increase in electrolyte and protein concentrations and a decrease in bicarbonate (87) and phosphate (88) concentrations. An

increase in whole saliva total protein, albumin and lactoferrin concentrations in both radiation therapy and Sjögren's syndrome patients is further reported (89). There are no reports of minor gland saliva in subjects with Sjögren's syndrome or irradiated patients.

Tobacco use

Tobacco habits are known to be hazardous to oral health. The relationship between tobacco use and a significantly lower number of remaining teeth (90, 91), a higher prevalence of periodontitis, the aggravation of the disease by increased pocket depth (92-94), a higher plaque index (95), lower salivary buffer effects, a higher number of lactobacilli and *Streptococcus mutans* (96) and an increase in caries incidence (97) among tobacco users has been reported. To some extent, these effects of tobacco could directly depend on the local or vaso-active effects in the oral cavity of noxious components from tobacco products (98), but effects on salivary glands with adverse effects on gland secretions must also be considered (99-101).

Oral dryness and salivary secretion rate

The prevalence of oral dryness is calculated to be 20-30% in an adult population, with a tendency to increase with increasing age (102, 103). This condition is more common among elderly women than among elderly men (104, 105) and is reported to be alleviated during hormone replacement therapy (106). A low secretion rate among persons complaining of oral dryness has been reported in some studies (107, 108), while others report a poor correlation between these oral discomfort symptoms and the whole salivary secretion rate (109, 110). During Sjögren's syndrome, a change in the viscosity of saliva, in addition to the reduced salivary secretion rate, could aggravate the feeling of oral dryness by increasing mucosal friction (83). Minor gland secretion in individuals with feelings of oral dryness has interested some authors, who report reduced palatal (54, 55) and labial (44) gland secretion rates among these persons. A correlation between oral dryness symptoms and reduced salivary output was also found when the sum of labial, buccal and palatal gland output was studied (72).

Co-variation between major and minor salivary gland secretion rates

A lack of co-variation between major and minor gland flow rates has been reported (54, 56, 58). On the other hand, a positive correlation was found between labial and resting whole salivary secretions in a study in which an iodo-starch reaction in paper strips was used to calculate the labial salivary secretion rate (51). A similar correlation was also reported for labial gland and parotid secretion rates (31).

Proteins in minor gland saliva

A few studies of proteins in minor gland saliva have been published. The major salivary immunoglobulin, IgA, is found in high concentrations in labial (29, 32-35, 43) and palatal (111) gland secretions. Amylase and lysozyme in labial saliva (112) and levels of amylase, cystatin and IgA in palatal saliva are also reported (111). The current knowledge of the influence of factors such as age, health status and medication on proteins in saliva is mainly restricted to whole saliva studies. In this saliva, lactoferrin (113) and IgA (71) concentrations were found to be elevated with increasing age. This correlation for age and whole saliva IgA was, however, not seen among persons aged 75 years and older (113). Albumin and lactoferrin concentrations are elevated in hyposalivating individuals (89) and IgA is reported to increase during pregnancy (114) and in rheumatic patients (82). No change in albumin concentrations was seen during hormone replacement therapy (76).

It would be true to say that the knowledge of minor gland secretions is limited. The studies in this thesis are inspired by the fact that minor salivary gland secretions may be more important than previously believed and that the new Periotron technique has enabled easy estimations of small fluid volumes.

AIMS

The main objective of these series of studies was to investigate some factors that could influence palatal, buccal and labial minor gland secretions. Whole saliva examinations were performed in parallel. The specific aims were:

- to evaluate the Periotron method and minor gland secretion rate variability (Paper I) and measure the salivary secretion rates from palatal, buccal and labial salivary glands (Papers I and V),
- to relate these secretion rates (Papers I and V) to age, gender and the effect of common diseases, medication, pregnancy and wearing removable dentures,
- to examine minor gland secretion rates and whole saliva buffer capacity and bacterial adherence and aggregation during oestrogen medication (Paper II),
- to examine IgA concentrations in minor gland and whole saliva and to relate these to various factors (Paper V),
- to investigate labial and buccal minor salivary gland secretion rates and concentrations of IgA, albumin and lactoferrin (Paper III) as well as plaque pH (Paper IV) in subjects with hyposalivation due to primary Sjögren's syndrome and head and neck radiation therapy, and
- to investigate minor gland saliva in relation to subjective oral dryness (Papers I and II).

MATERIALS AND METHODS

Test populations

The population included in Paper I consisted of 127 individuals, 61 females and 66 males (mean age 55 yr, range 22-89 yr). A total of 30 of these individuals were daily users of tobacco. Nine individuals were daily users of diuretics. Twenty individuals wore full upper dentures. Thirty-six individuals (20 females and 16 males) were randomly selected for duplicate measurements in order to calculate the variance of the Periotron method (Paper I). The two measurements at the different mucosal sites were performed with a 20-minute delay.

In the one-year study of oestriol treatment (Paper II), postmenopausal women were recruited through advertisements. Eighteen women with a mean age of 68 years (range 61-76 yr), with no medical problems or medication affecting salivary gland secretion during the last 3 months, were included in the study. Two women discontinued the medication after 6 months. Nine peri- and postmenopausal women (mean age 57 yr, range 53-61 yr) in good health and with no medication were included as a reference group.

In Papers III and IV, 10 subjects with primary Sjögren's syndrome and 10 subjects irradiated in the head and neck region and their age- and gender-matched healthy controls were investigated. All the subjects in the primary Sjögren's syndrome group were women with a mean age of 63 years (range 42-76 yr). In the irradiated group, four were women and the mean age in the group was 55 years (range 38-76 yr). Both patient groups were hyposalivators.

The test population in Paper V consisted of 96 women and 46 men, aged 18–82 years. Fifty-one individuals had no conditions affecting health, 35 suffered from circulatory diseases, 10 from diabetes mellitus and 12 from thyroid hypofunction. Twenty pregnant women and 25 daily smokers and their matched controls were included. Among the elderly individuals, 8 persons wore removable dentures.

Salivary secretion and plaque pH measurements

All saliva sampling in Papers I-V was performed between 8 am and 1 pm. Saliva sampling was performed at the same time of the day for each individual during the study period in Paper II. The individuals were instructed not to eat or drink and not to perform oral hygiene for at least 1 h prior to sampling. For the participants in the studies in Paper III and IV, where proximal plaque was measured and collected, the instructions were not to clean their teeth proximally for 3 days, not to brush their teeth the same day and not to eat and drink for at least 2 h prior to the test.

Minor salivary gland measurements

A Periotron 6000, model II (modified for minor salivary gland measurements) (Pro-Flow™, Inc.), and pre-cut pieces of filter paper (Munktell, Stora Kopparberg, Sweden) were used to measure unstimulated secretion rates. Prior to use, the reliability of the instrument was tested by checking the standard curve obtained with known volumes of water added to the filter papers. The wetted area of the paper was placed between the sensors of the device and the three-digit readings (0-200) were recorded according to the Periotron manual.

The values obtained when calculating the standard curve, together with an additional check-up one month after the study, showed both high reproducibility (SD=0.08 $\mu\text{l}/\text{cm}^2/\text{min}$) and stability in the readings of the instrument (Paper I).

The instrument was able to register harvested volumes of moisture of $<1.2 \mu\text{l}$. In order not to exceed the measuring range of the instrument, readings were made after collection times of 5 s from the buccal mucosa, 15 s from the labial mucosa and 30 s from the palatal mucosa in Papers I, II and V. In Paper III, 10 s was used for buccal and 30 s for labial measurements because of the low salivary flow. The filter paper was placed in the instrument for the adjustment of zero and, after drying the actual mucosal area with a cotton gauze pad, it was placed on the mucosa. The filter paper was handled using surgical gloves and was held in place with light finger pressure to ensure mucosal contact and block the paper from contact with moisture from the breathing air. Lower labial secretion was collected near the mid-line and 3 mm from the other border of the mucosa. Buccal secretion was sampled on the left side at the level of the parotid excretory duct

orifice, approximately 2 cm in front of it and ≥ 1 cm posterior to the angulus oris when the buccal mucosa was held and slightly stretched with a finger. Palatal secretions were measured bilaterally 5 mm from the first molars and medially at the border of the soft and hard palate (Paper I) and 5 mm medially from the first molar on the right hand side (Papers II and V). Four samples were collected from each mucosal area and the mean of the different readings was used for all calculations of minor gland secretion rates (Papers II and V). In Paper III, eight to twelve samples were collected from each mucosal site. No systematic variations were seen for the readings of secretion rates during the collections.

Whole salivary secretion rate and buffer pH measurements

Unstimulated whole saliva was collected for 5 min using the draining method according to Dawes (115). Paraffin-stimulated saliva was collected in ice-chilled tubes after one minute of pre-stimulation and during 5 min or until 8 ml had been collected. Determinations of secretion rates were performed immediately after the saliva collections and buffering capacity was determined according to Ericsson 1959 (116) within 15 min (Papers II and IV).

Tests of bacterial adherence and aggregation

Stimulated whole saliva from the test persons in Paper II was clarified by centrifugation (1200g; 7 min) and kept over night at 4°C for adherence and aggregation tests the following day.

Streptococcus mutans strain Ingbritt, *Streptococcus sanguis* strain ATCC 10556 and *Actinomyces naeslundii* strain ATCC 12104 were labeled by anaerobic growth over night in broth supplemented with ^{35}S -methionine. For the aggregation tests, bacteria were cultured over night in the broth.

Bacterial adherence and aggregation was tested at baseline and at 6 and 12 months. The adherence tests were performed in a tube with 40 mg of hydroxyapatite beads that were incubated for 60 min with 1.0 ml of saliva. After washing, the saliva-coated beads were incubated for 60 min with 1.0 ml of bacterial solution and the number of bound bacteria were determined by scintillation counting. Adherence was expressed as the

percentage of bound cells in relation to the number of cells added. For the aggregation test, aliquots of 0.6 ml of the saliva sample were mixed with 1.2 ml of the bacterial solution and the absorbance at 720 nm was registered every three minutes during one hour in a Shimadzu UV-160 spectrophotometer. The rate of aggregation was determined as the time in minutes required to reduce the absorbency by 0.2 units.

Feelings of oral dryness

In the study described in Paper I, the test persons were asked whether or not they had any subjective feelings of oral dryness. In Paper II, the participants were asked about their sensations of oral dryness at baseline using the following questions: do you experience feelings of oral dryness permanently or temporarily? If temporarily, do you experience them i) during the daytime? ii) during the night? After 3, 6 and 12 months of medication, the participants were asked if their sensations of oral dryness had changed.

Determination of protein concentration in saliva

The samples of stimulated whole saliva and filter papers used in the Periotron measurements in Papers III and V were kept frozen (-70°C). A method for recovering and measuring the proteins IgA, albumin and lactoferrin in the filter papers was evaluated. Pilot tests were performed using known volumes of whole saliva added to and recovered from the filter papers. It was shown that the majority of proteins could be recovered from samples of approximately 1 µl saliva using the applied method. For the IgA estimations, 20 µl of 2% SDS solution were added to the tube with minor gland saliva samples and, after heating the tube (90°C; 5 min), the solution was centrifuged into a larger, outer tube. The centrifugation was repeated after the addition of phosphate buffered saline (PBS) to the filter paper in the tube. The recovered saliva sample was diluted to 1/800 and further serially diluted in microtiter plates for ELISA analysis with specific antibodies. For the other protein tests, papers were eluted without heating using PBS supplemented with 0.1% Tween 20 and 1% gelatin for albumin and with 0.1% Tween 20 and 0.1% gelatin for lactoferrin. The volumes were adjusted to 160 µl (albumin) and 200 µl (lactoferrin) before further serial dilutions in the ELISA analyses.

Plaque-pH measurements

Plaque pH was measured using the microtouch method (117) (Paper IV). Measurements were performed at two proximal dental sites; in the front and in the premolar/molar region. With the exception of two individuals, both sites were in the upper jaw. No metal fillings were present at the sites of measurements. After registering the resting pH (baseline = 0 min), a mouth rinse was performed with 10 ml of 10% sucrose for 1 min. Plaque pH was recorded at 2, 5, 10, 15, 20, 30, 40, 50 and 60 min thereafter. During this period, the subjects were told to remain still and avoid talking.

Microorganisms in plaque

Directly after the pH measurements (Paper IV), plaque was collected from the same two sites using sterile toothpicks. After diluting the samples, 100 µl was inoculated on agar plates for the enumeration of the total number of bacteria, mutans streptococci, lactobacilli and *Candida* spp.

Statistical methods

Repeated calibration curves for the Periotron instrument showed direct proportionality between the test volumes of water and the instrument readings. The raw data from the Periotron display could therefore be used for the statistical calculations of minor gland saliva in all papers. The co-variations in fluid output between the different mucosal sites in Paper I were analyzed using simple, linear regression. Simple regression analyses were also used to test the correlation between the secretion rates and concentrations of proteins in separate types of saliva in Paper III. The correlation between fluid output and age, gender, upper dentures, use of diuretics and tobacco in Paper I and the possible influence of age and groups on the secretion rates in Paper II were estimated using multiple regression tests.

In Paper V, simple regression tests were initially used to examine whether any of the examined factors correlated with the saliva outputs and IgA concentrations. A correlation was seen for some drug categories and they were included as independent factors in further stepwise multiple regression analyses to test the effect of age, gender and the other factors on saliva.

A stepwise regression model was also used in Paper IV to test the importance of minor gland and stimulated and resting whole salivary secretion rates, stimulated whole saliva buffer capacity and the numbers of acidic microorganisms in relation to plaque acidogenicity.

Paired *t*-tests were applied for the intra-individual comparisons of minor gland secretion rates or protein concentrations in separate mucosal sites in Papers I, II, III and V.

The influence of mucosal fluid output on the subjective feeling of oral dryness in Paper I was tested with a one-tailed, unpaired *t*-test. Unpaired, two-tailed *t*-tests were used for comparisons at baseline between the hormone and reference groups in Paper II, for the comparisons of all test variables between the primary Sjögren's syndrome and the irradiated patients and their respective control groups in Paper III and for differences between pregnant women or smokers and their respective matched controls in Paper V. Individual means of plaque pH and the logarithmically transformed microbial data in Paper IV obtained from two dental sites were also compared between the test and their control groups using unpaired, two-tailed *t*-tests.

In Paper II, the data were subjected to two-way analysis of variance (ANOVA, repeated measures). When ANOVA rejected the multiple hypotheses of equal means, Fisher's PLSD *post hoc* test was applied for comparisons between baseline and the longitudinal measurements.

StatView (Abacus, Berkeley, CA) and SPSS 11.0 for Macintosh computer software were used. *p*-values below 0.05 were regarded as statistically significant.

RESULTS

Salivary secretion rates

The standard deviations for minor gland secretion rates that were calculated ($\mu\text{l}/\text{cm}^2/\text{min}$), including both intra-individual and method variations, were 0.6 (43%) for the palatal mucosa, 2.7 (17%) for the buccal mucosa and 0.9 (18%) for the labial mucosa. As the Periotron method was highly reproducible (see above), the intra-individual variations constituted the main parts of the values. Large inter-individual variations were also found in Papers I, II, III and V.

The ranges of mean mucosal outputs of fluid ($\mu\text{l}/\text{cm}^2/\text{min}$) found in Papers I, II, III and V were 11.6-16.0 for buccal glands, 2.3-4.8 for labial glands and 0.8-0.9 for palatal glands. The site-related differences were statistically significant in all studies.

The co-variation of fluid output from the various sites of the oral mucosa was calculated. A correlation was observed for the buccal and labial mucosa (Papers I and III). A strong co-variation was also observed for the output from three different sites of the palatal mucosa. However, medial sites displayed a higher secretion rate (about 45%) than lateral sites (Paper I).

The influence of age on the minor gland salivary flow rates was examined in Papers I and V. In both studies, a number of other factors, which could affect salivary secretion rates, were accounted for. Multiple (Paper I) or stepwise multiple (Paper V) regression tests did not reveal any age-related effect on palatal, buccal or labial minor gland secretion rates. In contrast, a lower stimulated (Paper II) and resting whole (Paper V) salivary secretion rate was seen with increasing age.

In Paper I, females were found to have significantly lower (10-20%) secretion rates in all minor glands compared with men. In Paper V, these differences were statistically verified for the buccal and labial secretions, but not for the palatal secretions. In this study, women also had a lower stimulated whole salivary secretion rate than men.

Diabetics had an increased buccal gland and a reduced resting whole salivary secretion rate. During pregnancy, the resting whole salivary secretion rate was lowered. In patients with Sjögren's syndrome or irradiated due to cancer of the head and neck region, we found significantly impaired whole and minor salivary gland secretion rates,

with one exception. The buccal mucosal gland salivary secretion rate was not significantly reduced in the Sjögren's syndrome group, compared with their controls (Paper III). Medication with diuretics significantly reduced the secretion rate of the buccal mucosa (about 15%) (Paper I). This was not seen for the study population in Paper V, where diuretics correlated to a lower stimulated whole salivary secretion rate. However, in this study, the buccal secretion was significantly reduced by anti-hypertensive medication, other than diuretics and beta-receptor blockers. Compared with a reference group, the labial but not the buccal or palatal secretion was increased by 3 months of medication in women taking a low dose of unopposed oestrogen preparation (Paper II). An increase in the stimulated whole saliva flow rate was seen in both groups after 3 months. The resting whole saliva flow rates did not vary significantly during the 12-month test period in any of the groups.

The use of tobacco (smoking and snuff) resulted in a significantly higher palatal fluid output (27%) (Paper I). A similar observation was not seen in Paper V, where only smoking was accounted for. The wearing of upper dentures resulted in a considerably elevated (300%) palatal gland secretion rate (Paper I). A statistically verified increase was also found in Paper V, in spite of the fact that there were fewer denture wearers in this study.

The multivariate statistics in Paper V showed a significant co-variation between buccal mucosal gland secretion rate and both resting and stimulated whole salivary secretion rates. An overview of the findings for the mean minor gland secretion rates, found for all the participants in the studies, and affecting factors is given in Table 2.

Feelings of oral dryness

A possible relationship between mucosal fluid output and subjective feelings of oral dryness was investigated. Of 127 individuals in Paper I, 14 experienced feelings of oral dryness. In these subjects, the labial fluid output was reduced by 21%, while the buccal and palatal outputs did not show any differences compared with others. Ten women in the hormone replacement group (Paper II) experienced permanent feelings of oral dryness, while 8 experienced temporary feelings of oral dryness at baseline. After 3 months of medication and throughout the test period, nine of the women with permanent dis-

comfort and three with temporary discomfort reported a noticeable relief of this symptom. In the reference group, there were no reports of permanent feelings of oral dryness and one woman reported temporary feelings of oral dryness at baseline and throughout the 1-year study period.

Protein concentrations in minor gland saliva

Our studies revealed that the buccal salivary IgA concentration was significantly higher than the labial concentration (Papers III and V), especially during hyposalivation (Paper III) (Table 2). In Paper V, even higher IgA concentrations were found in palatal secretions and the highest values were seen for the elderly (≥ 65 yr), both men and women. The concentration of IgA was statistically significantly elevated in buccal saliva in irradiated subjects and in stimulated whole saliva in both irradiated and Sjögren's syndrome patients, compared with their controls (Paper III).

The multivariate statistical tests in Paper V revealed that increasing age was correlated to increasing concentrations of IgA in all measured minor gland secretions, when possible interfering factors were accounted for (Fig. 2; Table 2). Additional observations were that the buccal saliva IgA was correlated to the stimulated whole saliva concentration of IgA and it was higher in men than in women (Table 2). When the pregnant women were compared with a matched control group, a significantly reduced IgA concentration was observed in buccal saliva. This observation was, however, not verified in the multivariate statistical test.

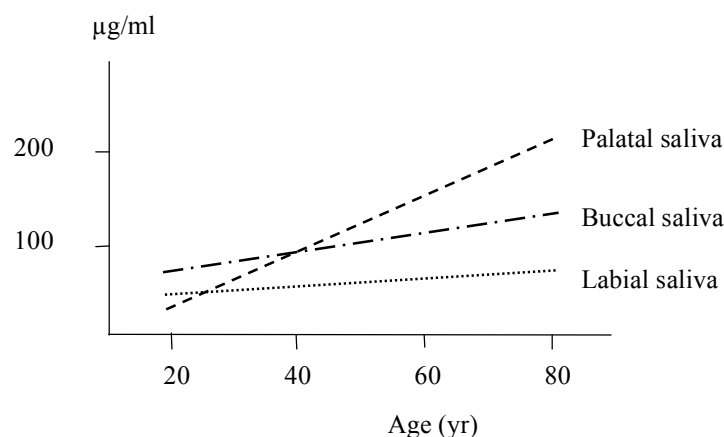


Fig. 2. Minor salivary gland saliva concentrations of IgA in relation to age. Linear regression lines calculated from scatter diagrams.

Albumin was significantly elevated in buccal secretions from the Sjögren's syndrome and irradiated subjects and in whole saliva from the latter group. Lactoferrin was significantly increased in both the minor and whole salivary secretions, but labial saliva was increased in the Sjögren's syndrome individuals. Statistical tests, including data from all the examined hyposalivation and control subjects, revealed higher concentrations of albumin and lactoferrin in buccal secretions than in labial secretions (Paper III) (Table 2).

Plaque pH

Compared with the healthy controls, the primary Sjögren's syndrome group displayed an almost identical plaque-pH response to a sugar challenge in proximal dental plaque. In the irradiated group, a lower minimum pH, lower final pH and larger area under the curve were seen (Fig. 3) (Paper IV).

Multivariate statistics revealed that the buccal minor gland secretion rate was negatively correlated to the plaque-pH response, expressed as both $AUC_{6.2}$ and $AUC_{5.7}$ (area under the curve; pH x min) in the hyposalivators (Fig. 4) (Paper IV).

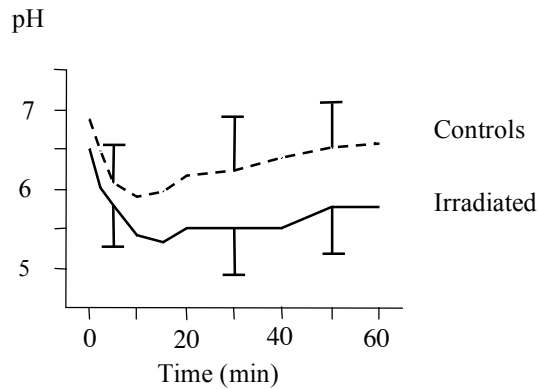


Fig. 3. Mean values of plaque-pH response to sugar challenge among head- and neck-irradiated subjects and their matched controls ($n = 10$ in each group). The estimations are based on the mean from two different sites in each individual. Bars at 5, 30 and 50 min represent SD

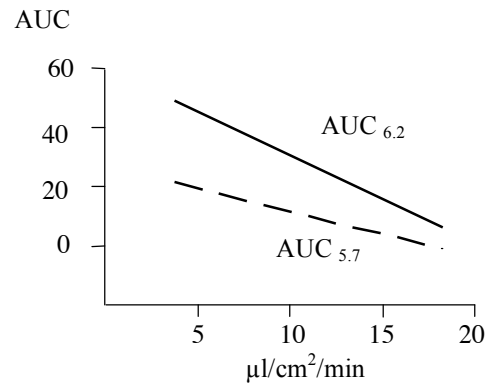


Fig. 4. Plaque acidogenicity (AUC; area under the curve; pH x min) in relation to buccal secretion rate among the hyposalivators. Linear regression lines calculated from scatter diagrams.

Microorganisms in plaque

In microbial analyses of the proximal dental plaque (Paper IV), the total number of microorganisms was of the same magnitude in the hyposalivation and control groups. Together with a more acidogenic plaque, a shift towards a higher number of acidogenic microorganisms was seen in the irradiated individuals. These patients had significantly higher numbers of lactobacilli and *Candida* spp. in their plaque compared with their controls (Fig. 5). In a stepwise regression model, it was revealed that the number of mutans streptococci was correlated to plaque pH in the controls.

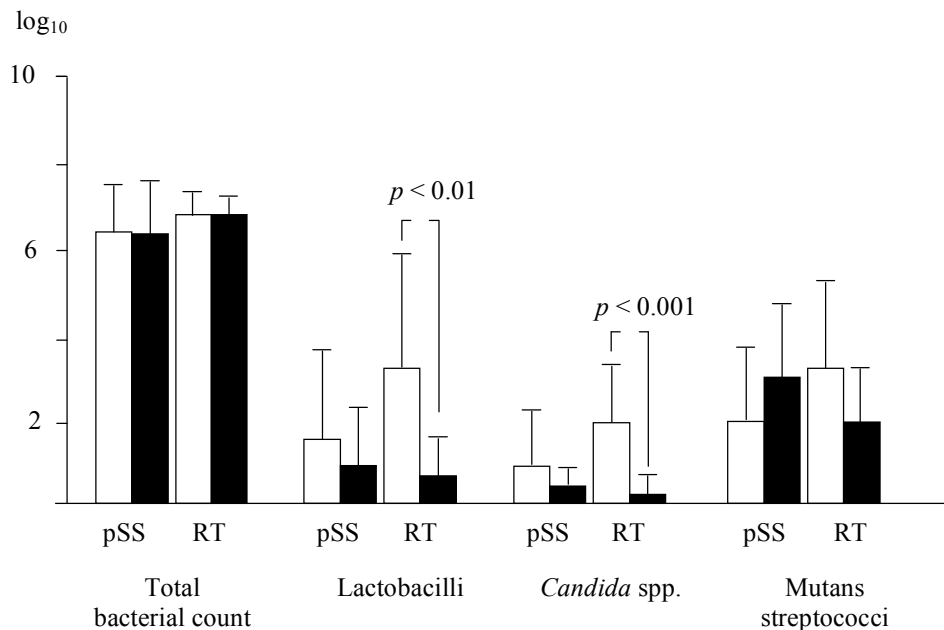


Fig. 5. Mean number of microorganisms in proximal dental plaque in patients with primary Sjögren's syndrome (pSS) and head- and neck-irradiated subjects (RT) (open bars) and their matched controls (filled bars) ($n = 10$ in each group). The estimations are based on the mean from two different sites in each individual. Error bars represent SD.

Stimulated whole saliva buffer capacity and bacterial adherence and aggregation during oestrogen treatment

The stimulated whole saliva buffer capacity increased parallel to the flow rate (Papers II and IV).

In the 12-month study during oestrogen therapy (Paper II), the ability of whole saliva to mediate the adhesion of *A. naeslundii* strain ATCC 12104 to hydroxyapatite was significantly reduced at 6 and 12 months compared with baseline in both the study and reference groups. The ability to aggregate the bacteria was only reduced in the hormone group. It was significantly reduced (25%) after 6 months and remained at this low level after 12 months. No changes were seen in the adhesion and aggregation of *S. mutans* and *S. sanguis*.

Table 2. Summary of findings in this thesis on minor salivary gland secretions

	Secretion rates ($\mu\text{l}/\text{cm}^2/\text{min}\pm\text{SD}$)			IgA ($\mu\text{g}/\text{ml}\pm\text{SD}$)			Albumin ($\text{mg}/\text{ml}\pm\text{SD}$)		Lactoferrin ($\mu\text{g}/\text{ml}\pm\text{SD}$)	
	Palatal	Buccal	Labial	Palatal	Buccal	Labial	Buccal	Labial	Buccal	Labial
Paper I	0.9 \pm 0.5	16.0 \pm 4.5	4.8 \pm 1.9							
Paper II	0.8 \pm 0.6	14.2 \pm 3.7	2.0 \pm 1.0							
Paper III		11.6 \pm 5.3	2.3 \pm 1.6		200.3 \pm 155.5	87.2 \pm 70.2	0.5 \pm 0.5	0.3 \pm 0.2	39.7 \pm 38.1	13.9 \pm 14.9
Paper V	0.9 \pm 0.5	14.8 \pm 4.0	3.0 \pm 1.5	149.8 \pm 185.8	114.6 \pm 113.8	50.3 \pm 34.6				
Intra-individual variations	43% ^a	17% ^a	18% ^a							
Correlating factors	Age			+ ^c	+ ^e	+ ^e				
	Gender	x ^a	x ^{a, c}		x ^c					
	Pregnancy				- ^e					
	Tobacco	+ ^a								
	Dentures	+ ^{a, c}				+ ^c				
	Oestrogen			+ ^b						
	Diuretics		- ^a							
	Anti-hypertensive medication		- ^c							
	Oral dryness			- ^{a, b}						
	Plaque acidity		- ^d							
	Sjögren's syndrome			- ^c					+ ^c	
	Radiation therapy		- ^c	- ^c		+ ^c		+ ^c	+ ^c	+ ^c
	Whole saliva secretion rate		+ ^e							
	Whole saliva IgA					+ ^e				
	Buccal secretion rate					- ^e				

+ Positive correlation; - Negative correlation; x Lower in women; ^a Paper I; ^b Paper II; ^c Paper III; ^d Paper IV; ^e Paper V.

DISCUSSION

The studies in this thesis were initiated by observations of small red dots in the somewhat pale palatal mucosa of some individuals. It was soon revealed that these dots were the expression of more or less intense inflammation around the excretory ducts of minor, mucosal salivary glands (118). Until this point, a few research reports had been published on these glands. These reports mainly focused on the labial glands and their excretions, primarily because this saliva could be harvested from small droplets, which are easily seen when the lower lip is pulled slightly outwards. In 1990, however, Shern *et al.* (59) published their report on a method using which mucosal secretions could be collected and measured fairly easily and from almost any mucosal site.

The Periotron method is a considerable improvement compared with older methods (as described in the introduction) for measuring the secretion rate in mucosal salivary glands. The simplicity and accuracy of the instrument are major advantages. When collecting saliva from mucosal surfaces, it is, however, always important to consider the risk of contamination of saliva from the major salivary glands. This is especially obvious for buccal measurements. The excretory duct of the parotid gland reaches the oral cavity via the lateral surface of the masseter muscle, turns dorsally at the medial edge of the muscle, pierces the buccinator and delivers the parotid saliva through the orifice at the level of the second molar (119). The measurements in this thesis of buccal minor gland saliva were performed well away from the parotid orifice and the very short collection period further eliminates the risk of contamination by parotid saliva. In addition, a fingertip with an operating glove was held over the measuring filter paper during the collection of saliva. Moreover, a study of minor gland secretions, collected in filter papers and measured using the Periotron method, and conventionally collected major gland saliva reported distinct protein patterns and differences in the ability to mediate bacterial adherence between the individual saliva collections (11). This further supports the opinion that mucosal secretions, collected in the same way as in this thesis, may not be contaminated to any significant extent by major gland saliva.

The lower secretion rates in the minor salivary glands in women compared with men and the finding that age did not appear to affect the secretion (Papers I and V) are not in

agreement with most of the previous studies using the Periotron method (Tables 1 and 2). One explanation for these deviating results could be that individuals with a different distribution of age groups were included in the studies. The number of individuals included in the studies could also vary and many of the studies shown in Table 1 are based on relatively few individuals compared with Paper I and V (Table 2). Another explanation could be that in other studies the possible effects of health variables and measuring variability were not considered. As has been reported for parotid saliva (120), the study in Paper I reports large intra-individual variations when measuring the secretion rates of the minor salivary glands. Repeated measurements are therefore preferable in population studies. In the studies in Papers II-V, the mean of at least four different readings from each site was used for the calculations.

In accordance with previously published investigations, we found a large variation in the secretion rates between different sites of the mucosa. Even within anatomically well-defined mucosal regions, there could be large variations. Paper I reveals that the secretion rate is higher in the medial part of the palate than in the lateral part. The mean values for the secretion rates in the buccal and labial areas of the mucosa, reported in the present studies, are higher than those in other investigations (Tables 1 and 2). This may be due to measuring in different parts of the mucosa where the number of glands can vary (45, 60, 121). There might be an additional explanation for the relatively high labial secretion values in Paper I. These subjects participated in a larger population study on the Koster islands, Strömstad and measurements were made directly after an extensive medical anamnesis with a lot of questions and discussions. Talking in this way may have stimulated the labial mucosal glands and resulted in relatively high secretion rate values, in accordance with the findings of Boros *et al.* (48). The labial secretion rates reported in Papers II–V were obtained during more resting conditions and these are not very different from the secretion rates reported by others (Table 1).

The palatal glands may also be mechanically stimulated (111). In 1994, when the study in Paper I was performed, the wearing of removable dentures was quite common in rural populations. The increased palatal gland flow rate in denture wearers reported in this and earlier studies (54) could be verified ten years later in Paper V, in spite of the more limited number of denture wearers that were included. It can therefore be stated

that the minor salivary glands could be mechanically stimulated and are not uniformly distributed throughout the mucosal membranes. In measurements of minor salivary gland function, it is therefore important to define the sites that are used and, as for all saliva sampling, standardize the overall examination procedure.

Medication is known to affect saliva and the most known oral side-effect is reduced salivation. Some medications, although few in number, appear to increase the whole salivary secretion rate or buffer capacity. They include physostigmine (64), pilocarpine (122) and female sex hormones (75, 76). The longitudinal findings in Paper II of whole salivary secretion rates and buffer pH during a 12-month period of oestrogen treatment appeared to support these earlier data. However, the whole resting and stimulated salivary secretion rate and buffer pH, as well as the minor palatal and buccal secretion rates, showed variations during the test period that were not significantly different from those seen for a reference group of unmedicated women. We therefore concluded that a low dose of oestrogen had no effect on secretion rate and buffer capacity of whole saliva. The different conclusions from our study, compared with the previous ones, may not only be explained by the lack of a control or reference group in the previous studies. Another explanation could be that the previous studies used more potent oestrogen preparations and in higher doses than in Paper II.

Our findings of decreased whole saliva aggregation of early colonizing *Actinomyces naeslundii* after 6 and 12 months of a low dose oestrogen treatment suggested that the oestrogen treatment affected the biological activity of saliva. Female hormones has been reported to modulate the whole saliva content of sialic acid (123). This carbohydrate structure is involved in the binding of oral streptococci and actinomyces to aggregating salivary glycoproteins (124). It remains to be examined if the observed changes in *A. naeslundii* aggregation resulted from changed expression of bacteria-binding receptor structures in saliva.

At baseline, the women taking oestrogen had a significantly lower secretion rate from their minor labial salivary glands than the reference group. The longitudinal data showed a significantly increased flow rate for this saliva after 3, 6 and 12 months of medication. This increase resulted in a labial salivary secretion rate of approximately the same magnitude as that in the reference group. The concomitant relief of feelings of

oral dryness, which were more common in the test group than in the reference group at baseline, was registered. The relief of oral dryness symptoms during hormone replacement therapy has also previously been reported (106). An observation of a correlation between subjective feelings of oral dryness and the labial mucosal salivary secretion rate was also made in Paper I. The explanation of these observations may be that saliva from minor mucosal glands is rich in mucins that bind water and have a high viscosity. Moreover, some mucins are bound to mucosal cells (125) and therefore remain for long periods on the oral mucosal surfaces. Reduced minor gland secretion rates (72) and increased friction on the labial mucosa (126) in individuals complaining of oral dryness have also been found in other studies. It could be summarized that reduced labial gland salivary secretion seems to be correlated to feelings of oral dryness.

Sjögren's syndrome patients and individuals who have undergone irradiation of the head and neck region for the treatment of cancer are known to suffer from oral dryness (87, 127). Accordingly, a reduced labial minor gland secretion rate was found among these patients, together with reduced resting and stimulated whole saliva (Paper III). These two groups of patients differed, however, in that buccal saliva was only significantly reduced in the irradiated group. Earlier research on Sjögren's syndrome patients has revealed reduced major gland salivary secretion rates and inflamed tissues in the labial glands (24). The data in Paper III suggest that the buccal glands are relatively less affected by sialoadenitis among these patients. In contrast, irradiation may cause the generalized destruction and impaired function of all salivary glands (88).

As mentioned in the introduction, there are a few reports in the literature on proteins in labial and palatal secretions. To my knowledge, there are none on protein concentrations in buccal secretions. In Paper III, proteins recovered from filter papers were used for the determination of IgA, albumin and lactoferrin concentrations in buccal and labial minor gland saliva. In our pretests, we found that the vast majority of these proteins could be recovered from the filter papers with samples of whole saliva. This is in accordance with a previous report of the successful elution and determination of proteins from filter papers (60). The minor gland and whole salivary secretions are, however, differently composed and mucins, for example, could retain complex bound proteins, such as IgA, in the filter papers (128). However, the aim of our investigations was not

to establish exact quantifications of minor gland proteins but to make comparisons between groups, which we think the method may permit. The protein concentrations reported in Papers III and V are also largely within the ranges of previous reports (Tables 2 and 3), although data from minor mucosal secretions are scarce.

The IgA concentration in labial minor and parotid gland saliva was examined by Crawford *et al.* (32) and Smith *et al.* (34, 35, 43), who found that the concentration was higher in labial saliva than in parotid saliva. In Papers III and V, the levels of labial IgA were found to be of the same magnitude as in these previous studies (Tables 2 and 3). High concentrations of IgA were also revealed in palatal and buccal salivary secretions. In view of the large area of the buccal mucosa, its secretion may be an important contributor to the whole saliva IgA. It should be mentioned that, as in the previous studies, the quantification of IgA in Papers III and V was performed with alpha-chain specific antibodies that recognize not only secretory IgA but also IgA from serum. Under normal conditions, the major part of IgA in secretions from the labial mucosa is secretory IgA (32). Serum IgA may, however, leak out from a damaged or fragile mucosa. The mucosal status was not recorded in the studies and the possibility that some of the IgA in the mucosal secretions emanated from the mucosal cells, especially in the hyposalivating subjects (Paper III), cannot therefore be excluded. Our findings and previous findings (89, 129-132) of high lactoferrin and albumin levels in minor gland and stimulated whole saliva during hyposalivation suggest that the mucosa or mucosal cells are damaged in these subjects.

Table 3. Mean protein concentrations ($\mu\text{g/ml}$) reported in whole, parotid and minor gland saliva

Saliva	n	IgA				Albumin	Lactoferrin	
		Whole	Parotid	Palatal	Labial	Whole	Whole	Parotid
Crawford <i>et al.</i> (32)	17		62		194			
Bennet & Reade (133)	10	30-260 ^c						
Wiederström & Bratthall (114)	15 ^a	48.3 ^d						
Izutsu <i>et al.</i> (33)								
Gandara <i>et al.</i> (46)	25 ^a	32.3 ^d				60.9 ^d		3
Smith <i>et al.</i> (34)	20		35.5 ^e		36.2 ^e			
Brandtzaeg (134)		194 ^c						
Smith <i>et al.</i> (35)	18		79	88	111			
Kugler <i>et al.</i> (135)	18 ^b	≈230						
Smith <i>et al.</i> (43)	264		32 – 35 ^e		52 – 145 ^e			
Sundh <i>et al.</i> (136)	12 ^a	237 ^c					22 ^c	
Henskens <i>et al.</i> (137)	19 ^a					80 ^c		
Henskens <i>et al.</i> (138)	25 ^a	164 ^c				123 ^c		
Närhi <i>et al.</i> (104)	71	53 ^d					13.2 ^d	
Veerman <i>et al.</i> (111)	5		26	85				
Leimola-Virtanen <i>et al.</i> (106)	27	41						
Almstähl <i>et al.</i> (89)	26 ^a					200 ^d	3.7 ^d	
Nagler & Hershkovich (71)	45	44.9						
Helenius <i>et al.</i> (82)	77 ^a	30.9 ^d						

^a Controls; ^b Adults; ^c Resting whole saliva; ^d Stimulated whole saliva; ^e Median values shown.

Saliva is important for the humoral protection of the oral cavity and the upper respiratory tract. The salivary concentration of immunoglobulin is essential in this respect and could be expected to increase with increasing age, due to challenges from resident or transient microorganisms. Studies of whole saliva in children reveal a rapid increase in IgA concentration soon after birth (139), which is followed by a slowly reduced increase during the first year of infancy (140). Data from childhood, adolescence (135) and in adults (71) also show this increase in whole saliva IgA with increasing age. Our data in Paper V did not show this correlation, when minor gland IgA was included as an independent factor in the statistical analyses. In contrast to an earlier finding of stimulated labial saliva (43), Paper V showed a clear positive correlation between age and the IgA concentrations in palatal, buccal and labial mucosal gland saliva in individuals aged from 18 to 83 years. Thus, our findings suggest that increased levels of whole saliva IgA with age in adults could be a reflection of increased minor gland IgA. The relationship between minor gland IgA and age in younger age groups of individuals remains to be examined.

It is known that the concentration of whole saliva IgA is negatively correlated to the secretion rate (113, 135) and this was also seen in Papers III and V. For the minor gland secretions, such a relationship was only found for buccal saliva.

In Paper IV, plaque pH during hyposalivation was examined in relation to acidogenic microorganisms in that plaque and to minor labial and buccal gland secretion rates. Relationships to whole saliva buffer pH and secretion rate were also examined. The oral microflora is reported to be differently affected by hyposalivation due to different conditions, i.e. Sjögren's syndrome or irradiation directed against the head and neck (16, 89). In Paper IV, the microbiota in proximal dental plaque was examined in these patients and matched controls. The numbers of mutans streptococci did not differ significantly between the hyposalivation subjects and their controls. However, a shift towards higher proportions of lactobacilli and *Candida albicans* was seen in the irradiated group but not in the Sjögren's syndrome patients. The irradiated patients also had a reduced whole saliva buffer capacity and higher acidogenic capacity in dental plaque, which were not seen among patients with Sjögren's syndrome. According to previous data (13, 141), it could be expected that a reduction in stimulated whole salivary secretion rate in

both hyposalivation groups (Paper III) would result in more acidogenic plaque in both groups, compared with their controls. There were, however, two striking differences in salivary secretion rates when comparing the Sjögren's syndrome and the irradiated groups. The irradiated patients had a comparably more pronounced reduction in the stimulated whole salivary secretion rate compared with the Sjögren's syndrome group. The buccal minor gland salivary secretion rate was only significantly reduced in the irradiated group. In the stepwise multiple regression tests, which were performed to test the influence of all measured saliva variables, it was revealed that the buccal salivary secretion rate was the only factor correlating with plaque acidogenicity after a sugar rinse in individuals with generally reduced salivation. In the controls, the numbers of mutans streptococci was the most important factor for plaque acidogenicity. These findings show that buccal secretion may be critical for caries protection during hyposalivation.

This series of studies demonstrated previously unknown functions of the minor salivary gland secretions. Interesting observations included the fact that buccal secretion may be of special importance during hyposalivation and for IgA in whole saliva. More studies of these glands in larger population groups are therefore of future interest.

MAIN CONCLUSIONS

The Periotron method enabled reliable and simple measurements of minor mucosal gland secretion rates. The secretion rates showed large intra- and inter-individual variations and differed between separate mucosal sites.

The minor mucosal gland secretion rates did not seem to be reduced with increasing age. The secretion rates were, however, affected by medication, diabetes and wearing of dentures, but not by pregnancy. Women had lower minor mucosal gland secretion rates than men.

During medication with a low dose oestrogen, the labial secretion rate increased and the whole saliva aggregation of a strain of *A. naeslundii* decreased.

The concentrations of IgA in minor gland saliva increased with increasing age. The buccal salivary secretion rate and IgA content were correlated to the secretion rate and the IgA content in whole saliva.

The minor gland secretion rates were reduced and the concentrations of IgA, albumin and lactoferrin in minor gland saliva were generally increased with hyposalivation, and especially in irradiated subjects. Especially the buccal saliva seems to be of importance for plaque pH in hyposalivated subjects.

The complaints of oral dryness were related to a low labial gland secretion rate.

ACKNOWLEDGEMENTS

First of all, I would like to thank my supervisors Anette Carlén and Downen Birkhed for their support and willing help.

I would also like to extend my thanks to Ann-Charlott Börjesson for her excellent technical assistance with methods and samples and her patience with laboratory matters.

I am also grateful to my co-authors, Annika Almståhl, Guy Heyden, Merja Laine, Peter Lingström, Nicklas Strömberg, Maude Wikström and Tor Österberg, for fruitful discussions and help during the work and in the preparation of the manuscripts.

I would also like to express my sincere gratitude to all my friends among the staff at the Departments of Cariology and Oral Microbiology, Institute of Odontology, University of Göteborg, who have contributed in different ways to the studies in this thesis.

Many thanks also to Henrik, for explaining the theories of dielectric measurements and for assistance with the photographs, and to Lilian and Jonas for their support and patience.

Göteborgs Tandläkaresällskap, The Institute of Odontology, Sahlgrenska academy at Göteborg University, Patentmedelsfonden för Odontologisk Profylaxforskning, Praktikertjänst AB, Sigge Persson och Alice Nybergs Stiftelse, Svenska Tandläkarnes Inköpsförenings Stiftelse för forskning och studier and the Swedish Dental Association provided research grants.

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