SALIVARY FLOW AND COMPOSITION IN HEALTHY AND DISEASED ADULTS

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ABSTRACT

The aim of this study was to evaluate different aspects of salivary diagnosis. The specific aims were to investigate salivary flow rates and yeast counts in medicated and unmedicated adult patients, and to investigate one-day hourly pattern, correlations and within-subject variations of salivary viscosities and various salivary proteins in healthy adults. Further aims were to investigate salivary concentrations of cortisol and growth hormone (GH) in saliva and correlations with respective serum concentrations, and to analyze salivary albumin concentrations in patients in an acute geriatric ward and correlate the findings to the patients' oral health parameters and systemic condition.

The results of this study underline the need to take the patients' gender and systemic medication into account in all salivary diagnoses. The observed within-subject variations in the viscosity of unstimulated saliva, salivary IgA, albumin, amylase and total protein concentrations suggest that these variables are subject to short-term variation. The results also underline the need to accurately specify the time of saliva sampling. Salivary GH concentrations were 1900-fold lower than the values in serum but a positive correlation was found between salivary and serum GH levels. Further, there were significantly higher salivary albumin concentrations and albumin outputs in the frail elderly.

ABBREVIATIONS:

BALT	Bronchus-associated lymphoid tissue
Ca	Calcium
CBG	Cortisol binding protein
CI	Confidence interval
CO_2	Carbon dioxide
cP	CentiPoise (viscosity unit)
CV	Coefficient of variation
Da	Dalton (molecular weight unit)
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
GALT	Gut-associated lymphoid tissue
GH	Growth hormone
H^+	Hydrogen ion
HCO_3^-	Bicarbonate ion
H_2CO_3	Carbonic acid
HPO_4^{2-}	Secondary phosphate ion
IDDM	Insulin-dependent diabetes mellitus
IgA/G/E/D	Immunoglobulin A/G/E/D
IIC	Inter-item correlation
K	Potassium
Mab	Monoclonal antibody
MALT	Mucosa-associated lymphoid tissue
MANOVA	Multivariate analysis of variance
MG1	High-molecular- weight mucin
MG2	Low-molecular-weight mucin
MUC1/4/7	Mucin 1/4/7
MUC5b	Mucin 5b
mRNA	Messenger ribonucleic acid
Na	Sodium
N.S.	Not significant
OR	Odds ratio
PRPs	Proline-rich proteins
RIA	Radioimmunoassay
SS	Primary Sjögren's syndrome
SC	Secretory component
SD	Standard deviation
SE	Standard error
SIgA/M	Secretory immunoglobulin A/M
ξ	Viscosity
ν	Shear rate
ϑ	Shear stress

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Kuopio, November 2002   Panu Rantonen

LIST OF ORIGINAL PUBLICATIONS:

The papers are referred to in the text by these Roman numerals.

I. Meurman J.H., Rantonen P.: Salivary flow rate, buffering capacity, and yeast counts in 187 consecutive adult patients from Kuopio, Finland. Scand J Dent Res 102: 229-34, 1994.

II. Rantonen P.J.F, Meurman J.H.: Viscosity of whole saliva. Acta Odont Scand 56: 210-14, 1998.

III. Rantonen P.J.F., Meurman J.H.: Correlations between total protein, lysozyme, immunoglobulins, amylase, and albumin in stimulated whole saliva during daytime. Acta Odont Scand 58: 160-65, 2000.

IV. Rantonen P.J.F., Penttilä I., Meurman J.H, Savolainen K., Närvänen S., Helenius T.: Growth hormone and cortisol in serum and saliva. Acta Odont Scand 58: 299-303, 2000.

V. Meurman JH., Rantonen P., Pajukoski H., Sulkava R.: Salivary albumin and other constituents and their relation to oral and general health in the elderly. Oral Surg Oral Med Oral Pathol 94: 432-38, 2002.

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1. INTRODUCTION

A growing number of dental and medical doctors are finding that saliva provides an easily available, noninvasive diagnostic medium for a rapidly widening range of diseases and clinical situations (Mandel, 1990). Saliva is the principal defensive factor in the mouth, and a reduction in its flow rate affects orodental health. A reduced salivary flow may cause a variety of mostly unspecific symptoms and so the establishment of patients' saliva flow is of primary importance in oral medicine and dentistry. Salivary hypofunction is associated with oral and pharyngeal disorders and requires early diagnosis and intervention (Ghezzi et al., 2000).

It is important to establish reference flow rates in various populations (Mandel, 1993). Problems in clinical salivary diagnosis are mainly due to non-standardized collection procedures, further difficulties in interpretation caused by the great diurnal variation of salivary secretion, and individual differences in general. It is well-known that secretion may greatly vary in an individual, and if repeated samples are taken at different time points varying on flow rate results will be obtained (Dawes, 1987).

Moreover, clinical practice and recommendations for salivary diagnosis vary between centers. Unfortunately, saliva sampling has not yet become a routine in dental offices. It has also been claimed that no global consensus has been reached with regard to the terminology of the dry mouth, creating a substantial problem for research, education, diagnosis and therapy (Nederfors, 2000). International ICD-10 classification of diseases and "Application of the International Classification of Diseases to Dentistry and Stomatology" (ICD-DA) has considerably improved this situation (WHO, 1995).

The present series of studies were conducted in order to shed some more light on clinical salivary diagnosis. Both healthy subjects and diseased patients were investigated.

2. REVIEW OF LITERATURE

2.1. SALIVA AS A DIAGNOSTIC FLUID

2.1.1. The diagnostic uses of saliva

Salivary diagnosis is an increasingly important field in dentistry, physiology, internal medicine, endocrinology, pediatrics, immunology, clinical pathology, forensic medicine, psychology and sports medicine (Mandel, 1993). A growing number of drugs, hormones and antibodies can be reliably monitored in saliva, which is an easily obtainable, non-invasive diagnostic medium (Mandel, 1980; Mandel, 1990; Smith et al., 1991; Tabak, 2001). Thus, salivary diagnosis is anticipated to be particularly useful in cases where repeated samples of body fluid are needed but where drawing blood is impractical, unethical, or both. Salivary concentrations of drugs and hormones also represent the free fractions of serum in many instances, with good correlations with the respective total concentrations in serum (Westenberg et al., 1978; Cook et al., 1987; Read, 1989; Kirschbaum and Hellhammer, 1989; Hofman, 2001).

Assays of steroid hormones from saliva are widely used and well validated (Lac, 2001), providing an unstressful sampling instead of venipuncture. Multiple specimens of saliva for steroid hormone analysis can be easily collected by the patient, at home, to monitor fertility cycles, menopausal fluctuations, stress and other diurnal variations (Hofman, 2001). Also, some hormones other than steroids have been found to be reflective of their plasma levels and could be considered for salivary monitoring.

Salivary antibody levels can be determined to screen for infectious diseases. Anti-HIV antibody immunocapture assays have also been developed and tested for saliva, which could be useful in high-risk groups under field conditions in developing countries (Pasquier et al., 1997). Salivary assays have been used for monitoring of hepatitis A, B and C, measles, Epstain-Barr virus, rubella, parvovirus B 19, human herpesvirus 6, *Helicobacter pylori* and rotavirus infection (Mandel, 1990; Madar et al., 2002). In addition to measuring antibody, it is possible to identify a number of viral antigens in saliva, for example mumps and cytomegalo virus. Saliva has also proven to be a convenient source of host and microbial DNAs (Tabak, 2001).

There has been growing interest in the use of saliva in pharmacokinetic studies of drugs and in therapeutic drug monitoring in a variety of clinical situations. It has been suggested that drug levels in saliva reflect the free, non-protein-bound portion in plasma and hence may have a greater therapeutic implication than the total blood levels (Mandel, 1990). Lipid solubility is a determining

factor in saliva excretion of drugs, and the degree of acidity and basicity of a drug will determine its salivary/plasma ratio. The salivary flow rate, pH, sampling conditions, contamination and many other pathophysiological factors may influence the concentrations of drugs in saliva (Liu and Delgado, 1999). Drugs currently monitored in saliva include anticonvulsants, theophylline, salicylate, digoxin, anti-arrhythmic drugs, lithium, benzodiazepines, amitriptyline, chlorpromazine, methadone, ethanol, marijuana, cocaine and caffeine (Knott, 1989; Liu and Delgado, 1999).

It has become apparent that many systemic diseases affect salivary gland function and salivary composition. Studies of the effects of systemic diseases on salivary variables have been valuable in understanding the pathogenesis of the diseases, but their use as diagnostic markers has been limited. Primary Sjögren's syndrome (SS) is a common autoimmune disorder characterized by generalized desiccation, exocrine hypofunction and serologic abnormalities. More than 90% of the patients are women, and one of the main diagnostic procedures is biopsy of the minor salivary glands of the lip. It has been suggested that whole saliva flow rate (Sreebny and Zhu, 1996) and gland-specific sialometry and sialochemistry (Kalk et al., 2002) could be used to provisionally diagnose SS. In SS, salivary glands are indeed affected, resulting in a diminished salivary flow. It has been suggested that diminished output of salivary defense factors, rather than their absolute concentrations, may be related to the oral health problems seen in SS patients (van der Reijden et al., 1996).

Cystic fibrosis affects all of the exocrine glands to varying degrees (Ferguson, 1999). The most dramatic changes in the composition of saliva reported have been an elevation in calcium (Ca) and proteins, and this reduces the flow rate of minor salivary glands to virtually zero. Normally the flow rate of single labial gland is 0.1 µl/min (Ferguson, 1999). This phenomenon can be used as a diagnostic test by measuring the flow from labial glands of the lower lip (Mandel, 1990).

The sodium (Na) and potassium (K) concentrations of saliva are markedly affected by corticosteroids, especially aldosterone. The Na/K ratio of stimulated whole saliva can be used in diagnosing and monitoring Cushing's syndrome and Addison's disease. Investigators have also demonstrated the diagnostic value of Na/K ratio in primary aldosteronism (Wotman et al., 1969).

In several clinical situations salivary analysis has provided valuable information for both the clinician and the investigator. These situations include digitalis toxicity, affective disorders, stomatitis in chemotherapy, specific secretory IgA deficiency, smoking, ovulation time, relation of dietary factors to cancer and chronic pain syndromes (Mandel, 1990; Fischer et al., 1998).

Human saliva contains a large number of enzymes derived from the salivary glands, oral microorganisms, crevicular fluid, epithelial cells, and other sources. However, the use of whole saliva enzymes for diagnostic purposes has been more difficult than the use of serum enzymes. It

has been difficult to standardize saliva collection methods and enzyme analytical procedures so that direct comparisons between different laboratories would be possible (Mäkinen, 1989). Interpretation of results has also proved to be difficult. However, various studies have been made to find correlations between diseases or clinical situations and salivary enzyme levels.

Saliva is essential for alimentation, remineralization of teeth, and the protection and lubrication of oral mucosal tissues (Mandel, 1989). Measurement of the patient's saliva flow is of primary importance in oral medicine and dentistry (Ghezzi et al., 2000). For many years dental investigators have been exploring changes in salivary flow rate and composition as a means of diagnosing and monitoring a number of oral diseases. It has even been suggested that analysis of saliva may also offer a cost-effective approach to the assessment of periodontal diseases in populations (Kaufman and Lamster, 2000), even though no specific salivary marker of periodontal disease activity has been found so far.

A great number of studies with conflicting results have been published regarding various individual salivary agents and their possible association with oral health, particularly dental caries (Kirstilä et al., 1998). However, it appears that no single chemical agent is much more important than others. Many of the various defense factors show additive or even synergistic interactions against oral pathogens (Tenovuo, 1998).

2.1.2. Diagnostic tests for normal dental practice

Saliva is well adapted to protection against dental caries. The buffering capacity of saliva, the ability of saliva to wash the tooth surfaces and to control demineralization and mineralization, the antibacterial activity of saliva and perhaps other mechanisms all contribute to its essential role in the health of the teeth. Knowledge of the functional properties of saliva and of its separate components may permit a better assessment of dental caries susceptibility (Dowd, 1999).

Measurement of salivary flow is an invaluable diagnostic tool in determining the prognosis of alternative treatment plans (Strahl et al., 1990). In modern dental practice, diagnostic salivary measurements, at least salivary secretion rate and buffering capacity, should be used to supplement the anamnestic information and clinical findings with regard to prevention of dental caries. In order to gain reliable standardized results from the diagnostic tests, detailed instructions should be provided and followed by the dentist and patient (Tenovuo and Lagerlöf, 1994).

However, since caries lesions are the result of a multifactorial disease, assessment of a few salivary factors is not sufficient unless they are of overriding importance, which may occur in an individual patient (Birkhed and Heintze, 1989). Salivary bacterial counts, for example mutans streptococci and lactobacillus dip slide tests, are widely used in clinical practice in caries risk assessment. The current tests may be useful for estimating caries activity due to bad dietary habits, and establishing the

presence of infection and salivary yeasts for the determination of the patient's medical condition (Larmas, 1992). However, these tests may be limited in their applicability in the assessment of caries activity and in caries prediction (van Palenstein Helderman et al., 2001; Pinelli et al., 2001). However, they can be effective in a group of persons with high or low caries experience (Bowden, 1997).

Mutans streptococci are acidogenic and aciduric, and can produce extracellular glucans and adhere to tooth surfaces. Several methods are available to measure the levels of mutans streptococci in saliva and in plaque. The so-called ´Strip Mutans™ test´ is based on the ability of mutans streptococci to grow on hard surfaces, and it has been developed for chair-side use (Jensen and Bratthall, 1989). Other chair-side tests have also been developed (Bratthall and Ericsson, 1994).

Lactobacilli are associated with caries. They are more dependent on retentive sites being available in high numbers, and hence lactobacillus counts have been used to predict the increment of new caries lesions (Smith et al., 2001). The standard laboratory method of determining the number of lactobacilli includes the use of selective medium, Rogosa SL-agar. Chair-side methods for lactobacilli have also been developed, since the ´Dentocult™ LB´ method in 1975 (Larmas, 1975).

Yeasts, mainly *Candida albicans*, are commensals of the oral cavity in the majority of adult patients but many diseases may predispose to their dissemination (Odds, 1988). Mucosal surfaces are the primary oral reservoir for this microorganism, but dental plaque or prostheses can also harbor it (Cannon and Chaffin, 1999). Candidal colonization has been demonstrated in periodontal pockets, refractory periodontitis and failing dental implants (Pizzo et al., 2002). The emergence of *Candida albicans* may also occur as a result of the use of antibiotics or radiotherapy. In a review by Odds (1988) the prevalence of yeasts in saliva in healthy persons was stated to be 37%. In denture wearers yeasts may be cultivated from 85% of the subjects (Vandenbussche and Swinne, 1983).

In a study by Parvinen and Larmas (1981) on 1105 adults over 30 years of age the frequency of positive yeast counts was 59% in all subjects. From unmedicated subjects (n = 642) with full dentures (n = 104), 82% of women and 78% of men had salivary yeasts, whereas their incidence in subjects with complete dentition (n = 174) was 55% in women and 33% in men (Parvinen, 1984). In a study of the elderly by Närhi et al. (1992) the respective figure was 75% in all subjects. A chair-side method, `Oricult ®´, has been developed for detecting yeasts (Nicherson agar) in the mucous membranes and saliva.

It has recently been shown that low secretion rate of saliva and the high scores of lactobacilli and *Streptococcus mutans* have a significant influence on complications of fixed metal ceramic bridge prostheses and this should be taken into consideration in choosing patients for prosthetic treatment with fixed prosthodontics (Näpänkangas et al., 2002). Since salivary flow and its composition is essential in the protection and lubrication of oral mucosal tissues, salivary tests have also significant predictive

value in prosthodontic treatment planning. Successful management of complete and removable partial dentures is complicated by a reduction in salivary flow (Massad and Cagna, 2002). It has been suggested that salivary tests should be performed and analyzed before planning an extensive and expensive restorative therapy or orthodontic treatment (Birkhed and Heintze, 1989) and on a routine basis with geriatric patients (Strahl et al., 1990).

2.2. SALIVARY FLOW

2.2.1. Salivary glands

The major salivary glands are the parotid glands, submandibular glands and sublingual glands. Minor salivary glands are situated on the tongue, palate, and buccal and labial mucosa. They are small mucosal glands with primarily a mucous secretion (Ferguson, 1999).

The working part of the salivary gland tissue consists of the secretory end pieces (acini) and the branched ductal system. The fluid first passes through intercalated ducts which have low cuboidal epithelium and narrow lumen. Then the secretions enter the striated ducts which are lined by more columnal cells with many mitochondria. Finally the saliva passes through the excretory ducts where the cell type is cuboidal with stratified squamous epithelium (Whelton, 1996).

The acinar cells first secrete isotonic primary saliva and then the striated duct cells actively extract ions to render the saliva progressively more hypotonic as it passes down the ducts towards the mouth (Smith, 1996).

2.2.2. Flow rate

Diminished salivary output can have deleterious effects on oral and systemic health (Navazesh et al., 1992a; Atkinson and Wu, 1994). Unstimulated whole saliva is the mixture of secretions which enter the mouth in the absence of exogenous stimuli such as tastants or chewing. Several studies of unstimulated saliva flow rates in healthy individuals have found the average value for whole saliva to be about 0.3 ml/min. Values below 0.1 ml/min are considered as hyposalivation, and values between 0.1-0.25 ml/min low (Tenovuo and Lagerlöf, 1994). The normal range is very large and includes individuals with very low flow rates who do not complain of a dry mouth (Ship et al., 1991; Dawes, 1996). There is significant difference between genders in unstimulated flow rates (Dawes, 1996) . Xerostomia (dry mouth) is the subjective feeling of oral dryness. It is generally accompanied by salivary gland hypofunction and a severe reduction in the secretion of unstimulated whole saliva (Sreebny, 1989), but xerostomia is not necessarily reflected in the actually measured flow rates (Nederfors et al., 1997).

Table 1. Studies of unstimulated whole saliva flow rates (ml/min) in healthy individuals (Närhi et al., 1992; Dawes 1996).

Studies	Saliva	Sample number	Mean (ml/min)	SD
Andersson et al. (1974)	Whole	100	0.39	0.21
Becks and Wainwright (1943)	Whole	661	0.32	0.23
Heintze et al. (1983)	Whole	629	0.31	0.22
Närhi et al. (1992) (unmedicated elderly)	Whole	72	0.17	0.16

Unstimulated saliva is usually collected with the patient sitting quietly, with the head bent down and mouth open to allow the saliva to drip from the lower lip into a sampling tube (the so-called draining method). The other most commonly used techniques for measuring unstimulated saliva are the spitting method, suction method and swab method (Birkhed and Heintze, 1989).

The factors affecting unstimulated saliva flow rate are degree of hydration, body position, exposure to light, previous stimulation, circadian rhythms, circannual rhythms, and drugs. Less important factors are age, body weight, psychic effects, and functional stimulation (Dawes, 1987).

Widely accepted normal values for stimulated flow rates are 1.0 - 3.0 ml/min. Values below 0.7 ml/min are considered as hyposalivation, and values 0.7 – 1.0 ml/min low (Tenovuo and Lagerlöf, 1994).

Table 2. Studies of stimulated whole saliva flow rates in healthy subjects (Parvinen and Larmas, 1981; Närhi et al., 1992; Dawes, 1996).

Studies	Saliva	Stimulus	Sample	Mean (ml/min)	SD
Heintze et al. (1987) (healthy adults)	Whole	Paraffin wax	629	1.6	2.1
Närhi et al. (1992) (unmedicated elderly)	Whole	Paraffin wax	72	1.49	0.92
Parvinen and Larmas (1981) (unmedicated adults)	Whole	Paraffin wax	642	1.87	0.81
Shannon and Frome (1973) (healthy adults)	Whole	Chewing gum	200	1.7	0.6

Stimulated saliva is secreted in response to either masticatory or gustatory stimulation, or to other less common stimuli such as activation of the vomiting centre. A wide variation among individuals has been found (Ghezzi et al., 2000). Men have higher flow rates than women (Parvinen and Larmas, 1981). The factors affecting the flow of stimulated saliva are nature of stimulus, vomiting, smoking, gland size, gag reflex, olfaction, unilateral stimulation, and food intake (Dawes, 1996).

Reduced salivary flow may cause a variety of mostly unspecific symptoms to the patient, so the establishment of salivary flow rates is of primary importance in oral medicine and dentistry (Ghezzi et al., 2000). Saliva influences caries attacks mainly by its rate of flow and its fluoride content. The

salivary flow rate influences to a high degree the rate of oral and salivary clearance of bacterial substrates (Lagerlöf and Oliveby, 1994).

However, there are no uniformly accepted reference values for saliva, and clinical practice and recommendations for salivary diagnosis vary between centers. The amount of saliva in the mouth is not constant and varies within a person over time and between individuals (Ship et al., 1991). It has been shown that it is important to standardize the sampling intervals when salivary flow rates are studied (Laine et al., 1999). The standardized collection of saliva is most important to obtain reliable results (Tenovuo and Lagerlöf, 1994). Variation in individual flow rates can be as high as 50% over a 24-hour period due to circadian rhythms (Ferguson and Botchway, 1979), and have been reported to exceed 50% in cross-sectional healthy population studies (Ship et al., 1991). Normal variations have been shown to be age- and gender-independent (Fischer and Ship, 1999).

Several studies have been made to evaluate the role of aging in salivary flow. Basically, there seems to be no age-related decrease in salivary flow rates (Baum, 1981; Parvinen and Larmas, 1982; Thorselius et al., 1988), but medication is one of the main factors causing reduced salivary flow (Strahl at al., 1990; Persson et al., 1991), also in the elderly (Närhi et al., 1992). However, diminished resting salivary flow in unmedicated healthy elderly subjects has been found in one study (Percival et al., 1994).

Many investigators have attempted to establish normal ranges or "cut-off" values to distinguish normal from abnormal salivary function (Ghezzi et al., 2000). A value of 0.1 ml/min has been suggested as the lower limit of normal unstimulated whole saliva output (Sreebny and Valdini, 1988). On the other hand, it has been shown in one study that healthy persons in the lowest 10th percentile of major salivary gland flow rates had oral health similar to that of those in the highest 10th percentile (Ship et al., 1991). Single measurement of salivary flow rate may be insufficient to determine how much saliva is necessary to maintain oral health in particular individual (Dawes, 1987; Ship et al., 1991).

There are multiple causes of salivary hypofunction, including oral disorders, systemic diseases, prescription and non-prescription medications, chemotherapy, head and neck radiotherapy, psychogenic factors and decreased mastication (Sreebny 1989; Sreebny and Schwartz, 1997; Ship et al., 1999; Ghezzi et al., 2000). The most common cause of salivary gland hypofunction is the intake of medicaments, over 400 hundred of which possess the ability to diminish the flow of saliva. These have been identified and listed thoroughly in "Reference guide to drugs and dry mouth" (Sreebny and Schwartz, 1986; Sreebny and Schwartz, 1997). The feeling of dryness increases with the number of drugs taken per day, but drugs usually do not cause permanent damage to the structure of the salivary glands (Sreebny, 1989). Clinically, the most important classes of drugs that continuously diminish the

flow of saliva are antidepressants, anticholinergics, diuretics and antihypertensive agents, and psychofarmaca (Parvinen et al., 1984; Strahl et al., 1990).

2.2.3. Buffering capacity of saliva

Salivary buffering capacity is important in maintaining a pH level in saliva and plaque. The buffer capacity of unstimulated and stimulated whole saliva involves three major buffer systems (Bardow et al., 2000). The most important buffering system in saliva is the carbonic acid / bicarbonate system. The dynamics of this system is complicated by the fact that it involves the gas carbon dioxide dissolved in the saliva (Tenovuo and Lagerlöf, 1994). The complete simplified equilibrium is as follows:

$$CO_2 + H_2O \lozenge H_2CO_3 \lozenge HCO_3^- + H^+$$
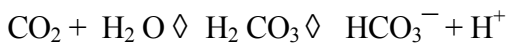
The increased carbonic acid concentration will cause more carbon dioxide to escape from the saliva. The saliva bicarbonate increases the pH and buffer capacity of saliva, especially during stimulation (Bardow et al., 2000).

The second buffering system is the phosphate system, which contributes to some extent to the buffer capacity at low flow rate. The mechanism for the buffering action of inorganic phosphate is due to the ability of the secondary phosphate ion, HPO_4^{2-} , to bind a hydrogen ion and form an $H_2PO_4^-$ -ion.

The third buffering system is the protein system. In the low range of pH the buffering capacity of saliva is due to the macromolecules (proteins) containing H-binding sites (Tenovuo and Lagerlöf, 1994).

The bicarbonate concentration is strongly dependent on secretion rate (Birkhed and Heitze, 1989). Since bicarbonate is the chief determinant of the buffer capacity, there is an interrelationship between pH, secretion rate and salivary buffering capacity.

Various methods have been used to measure the salivary buffer capacity, including titration under oil, titration while open to air and titration with CO2 (Bardow et al., 2000). Values obtained for buffer capacity in different studies are not comparable. However, final pHs under 3.5 for unstimulated saliva and 4.0 for stimulated saliva are considered low (Tenovuo and Lagerlöf, 1994) . From a practical point of view, the Dentobuff method has been developed to assess the buffering capacity in dental practice. Based on the color change of the indicator paper, the buffering capacity is assessed in comparison with a color chart. The Dentobuff method to assess the salivary buffering capacities has been shown to be valid (Ericson and Bratthall, 1989).

2.3. SALIVARY VISCOSITY

The lubricating action of saliva is important for oral health. It facilitates the movements of the tongue and the lips during swallowing and eating and is important for clearly articulated speech (Waterman et al., 1988). The efficacy of saliva as a lubricant depends on its viscosity and how it

changes with shear rate. The shear rate can obtain high values, e.g. 160 and 60 1/s during speaking and swallowing, respectively (Waterman et al., 1988).

Tribology is the science and practice of friction, lubrication, and wear applied to surfaces in relative motion (Schwarz, 1987). Rheology is the science associated with the deformation of materials subjected to stresses and forces. The rheological aspect includes viscosity and viscoelasticity (Aguirre et al., 1989). Saliva possesses specific rheological properties as a result of its chemical, physical and biological characteristics, these properties being essential for maintaining balanced conditions within the oral cavity.

A Newtonian viscous fluid is defined a fluid where the stress is linearly related to the rate-of-strain. However, there is a large group of materials (such as polymers, emulsions, and suspensions) and biomaterials, such as saliva, which cannot be described by simple elastic or viscous rheological models (Schwarz, 1987). It has been reported that the viscosity (ξ) (the ratio between shear rate ν and shear stress ϑ) of saliva depends on shear rate and on time, so that saliva can be classified as a non-Newtonian fluid (Roberts, 1982; Schwarz, 1987; Van der Reijden et al., 1993). The shear-thinning nature of saliva has several useful functions: for example, draining of the saliva from surfaces is reduced and saliva appears to be thin during manipulations.

It is assumed that the rheological properties of human saliva may be due to the salivary glycoproteins, mainly the high-molecular-weight mucins (MG1), which are secreted by the sublingual, submandibular and palatal glands. Differences in viscoelasticity between submandibular and sublingual salivas are not due to the differences in mucin concentrations in those secretions, but rather to mucin species (van der Reijden et al., 1993).

The significance of the viscosity of saliva in general has been the subject of many studies in odontology (Ericsson and Stjernström, 1951). It has been found that salivary viscosity is greatly influenced by pH and calcium (Nordbo et al., 1984). Increased salivary viscosity may also be associated with an increase in dental caries, although it is difficult to examine flow rate and viscosity independently from each other (Biesbrock et al., 1992). The apparent viscosity contributes to the rheological properties of saliva, and the elastic properties could be important as well (van der Reijden et al., 1993). Salivary viscosity is also suggested to contribute to denture retention. Retention of dentures is a dynamic issue dependent on the control of the flow of the interposed fluid and thus its viscosity and film thickness. In this, the most important concerns are good base adaptation and border seal of the prosthesis, so that full advantage is taken of the saliva flow-related effects (Darwell and Clark, 2000). Alterations in salivary composition appear to be reflected in its viscosity and in oral complaints (Chimenos-Kustner and Marques-Soares, 2002).

Until the 1970s most rheological measurements of saliva were made with the Ostwald-type U-type viscometer or its modifications (Waterman et al, 1988). Since then the Weissenberg rheogoniometer has been used, measuring the dynamic viscosity (ξ') and storage modulus (G') as functions of frequency (Schwarz, 1987). Oscillating rheometers and cone/plate microviscometers have also been used in viscosity measurements (Van der Reijden et al, 1993; Rantonen and Meurman, 1998). However, the phenomenon of a protein layer adsorbed at the air-liquid interface necessitated reinterpretation of the previous results (Levine et al., 1987a; Waterman et al, 1988; Mellema et al., 1992). The adsorbed layer at the saliva- air interface rises rapidly and after a definite time reaches thicknesses (100 nm) which are often larger than usual for protein solutions (<20 nm). Using the results of these studies as a framework for the development of saliva substitutes that mimic saliva rheologically, it is plausible to look for high molecular protein solutions that show rapid adsorption at the air-solution interface and form layers with complex shear moduli and flow behavior in the same range as determined for real saliva (Mellema et al., 1992). It has also been suggested that film-forming capacity of saliva substitutes is an important property to be considered in the exploration of clinically effective artificial salivas (Christersson et al., 2000).

2.3.1. Mucins

Human salivary mucins have a multifunctional role in the oral cavity in that they lubricate oral surfaces, provide a protective barrier between underlying hard and soft tissues and the external environment, and aid in mastication, speech and swallowing (Tabak, 1995). The high-molecular-weight mucin (MG1) and the low-molecular-weight mucin (MG2) have been isolated and characterized biochemically as glycoproteins (Levine et al., 1987b). Mucins have been intensively studied, and much has been learned about their biochemical properties and their interactions with oral micro-organisms and other salivary proteins (Offner and Troxler, 2000).

Mucins lack the precise folded structure possessed by many globular serum proteins. They tend to be asymmetrical molecules with an open randomly organized structure, consisting of a polypeptide backbone with carbohydrate side-chains. These molecules are hydrophilic and entrain much water (Hay and Bowen, 1996).

MG1 (predominantly mucins MUC5b and MUC4) and MG2 (product of a MUC7 gene) are the predominant mucins in human saliva, providing lubrication and antimicrobial protection for oral tissues (Baughan et al., 2000). MG1 is present in the mucous acini of submandibular, sublingual, labial and palatine salivary glands (Nielsen et al., 1996). The site of MG2 synthesis is controversial, in mucous acini in both submandibular and labial salivary glands (Cohen et al., 1991), and in serous acini in submandibular, sublingual, labial, and palatine salivary glands (Nielsen et al., 1996). In situ

hybridization studies have revealed a similar distribution of MUC7 mRNAs in mucous acinar cells of major and minor salivary glands (Khan et al., 1998).

These two major mucins create an enormous repertoire of potential binding sites for micro-organisms at one of the major portals where infectious organisms enter the body (Thomsson et al., 2002). MUC5b is one of four gel-forming mucins which exist as multimeric proteins with molecular weights greater than 20-40 billion Da. Membrane-bound mucins are another class of mucin molecules which exist both in secreted and membrane-bound forms (e.g. MUC1 and MUC4) (Offner and Troxler, 2000). Recently, capture ELISAs have been developed for measurement of MG1 and MG2 in whole saliva (Rayment et al., 2000).

2.3.2. Proline-rich proteins (PRPs)

Human salivary PRPs constitute a significant fraction of the total salivary protein and have important biological activities (Hay et al., 1994). PRPs are inhibitors of calcium phosphate crystal growth. Almost all crystal growth inhibition by PRPs is due to the first 30 residues at the negatively charged amino-terminal end of the molecule (Hay and Bowen, 1996). PRPs are present in the initially formed acquired pellicle, and have been reported to be present also in mature pellicles (Lamkin et al., 1996). It has also been shown that PRPs adsorbed onto hydroxyapatite are strong promoters of adhesion of many common bacteria (Gibbons and Hay, 1989; Douglas, 1994; Carlen et al., 1998; Li et al., 2001).

The molecular structures of the PRPs are highly asymmetrical. The PRP molecule is thought to bind to tooth surfaces via its amino-terminal segment. This leaves the carboxy-terminal region of the molecule, directed to the oral cavity, free to interact with oral bacteria.

Several salivary glycoproteins, including the proline-rich glycoproteins and mucins, have lubricatory roles in saliva,and the carbohydrate moieties of these molecules also affect their lubricating properties (Aguirre et al., 1989).

Salivary proline-rich proteins may act as defense against tannins by forming complexes with them and thereby preventing their interaction with other biological compounds and absorption from the intestinal track (Lu and Bennick, 1998).

2.4. SALIVARY ANTIMICROBIAL PROTEINS

2.4.1. Salivary immunoglobulins

Salivary secretory immunoglobulins (sIgA and sIgM) originate from immune cells which home to the salivary glands, and are produced as a host response to an antigenic stimulus (Brandtzaeg, 1989). The immunoglobulins may be directed at specific bacterial molecules, including cell surface molecules such as adhesins, or against enzymes. By binding to such molecules, adhesion of specific bacteria to oral surfaces may be blocked, so preventing colonisation by the affected species (Hay and Bowen, 1996, Zee et al., 2001). Several studies have confirmed that sIgA is mainly dimeric rather than monomeric, and it is associated with an epithelial glycoprotein called SC (secretory component) (Seidel et al, 2001). At least 95% of the IgA normally appearing in saliva is produced by the local gland-associated immunocytes rather than being derived from the serum (Brandtzaeg, 1989).

2.4.1.1. Induction of secretory immune responses

The main function of mucosa-associated lymphoid tissue (MALT) is generation and dissemination of stimulated B-cells. From these inductive sites they migrate as memory cells to exocrine tissues all over the body (Brandtzaeg et al., 1997). These B cells subsequently require some "second signals" for terminal differentiation in secretory tissues, for example salivary glands.

The lymphoepitelial structures include Peyer's patches and other gut-associated lymphoid tissue (GALT), bronchus-associated lymphoid tissue (BALT) (Escolar Castellon et al., 1992) and probably the tonsils (Brandtzaeg, 1989). Second signals inducing local B-cell differentiation are not fully known, but the importance of topical antigen for both local B-cell proliferation and differentiation has been shown (Figure 1).

Figure 1. Schematic representation of B-cell traffic in the secretory immune system (from Human Saliva: Clinical Chemistry and Microbiology, Vol II. Ed. Tenovuo JO. Figure 6. Boca Raton, Florida, 1989). Figure is used by kind permission of CRC Press.

2.4.1.2. Properties of salivary immunoglobulins

Most sIgA consists of an IgA dimer, one or two J chains, and one SC molecule. SIgA is remarkably stable and therefore well suited to function in protease-containing external secretions such as saliva. It is generally accepted that sIgA antibodies act in the first line of mucosal defense principally by simple binding to soluble and particulate antigens. As the first line of defense against microbial invasion, sIgA is the dominant immunoglobulin on all mucosal surfaces (Proctor and Carpenter, 2001). High levels of sIgA are found in saliva of newborn infants, indicating the existence of a competent oral mucosal immune system as early as within the first 10 days of life (Seidel et al., 2000). SIgA is capable of inhibiting various enzymes and retardate bacterial colonization on oral hard surfaces, also in the early stages (Zee et al., 2001). It has been found that chewing stimulates epithelial cell transcytosis of IgA and increases secretion of secretory IgA into saliva (Proctor and Carpenter, 2001). Salivary levels of IgA have been widely studied, in healthy and also in diseased patients. In a study with diabetic patients, significantly higher IgA concentrations in IDDM patients were reported (Ben-Aryeh et al., 1993). Salivary levels of IgA are elevated at pregnancy (Bratthall and Widerström, 1985). In HIV-infected patients the IgA levels were higher than in healthy controls (Mellanen et al., 2001). Salivary levels of IgA in triathletes have been reported to be reduced (Steerenberg et al., 1997).

Secretory IgM (sIgM) is not as resistant to proteolytic degradation as sIgA. SIgM levels have been shown to be increased in infancy and in selective IgA deficiency. It is probable, that sIgM may function like sIgA in the first line of mucosal defense (Brandtzaeg et al., 1997).

Salivary IgG reaches the oral cavity by leakage through various epithelia and is mainly added to whole saliva via crevicular fluid. It is mainly derived from serum, although a minor fraction of the crevicular IgG may originate in local plasma cells when the gingivae are inflamed. Salivary IgG can be expected to have the same functional properties as circulating IgG. Crevicular IgG antibodies have been shown to inhibit colonization of *Streptococcus mutans* and can protect significantly against tooth decay in monkeys.

Traces of IgD probably reach whole saliva by passive diffusion like IgG. IgD cannot be detected regularly in parotid fluid from normal adults but it appears in whole saliva when present in serum.

However, during the first 6 months after birth salivary IgD levels are often relatively high (Seidel et al., 2001). The functional significance of salivary IgD is not known.

Salivary IgE most likely reaches external secretions by passive diffusion, although a contribution from intraepithelial mast cells in atopic and allergic individuals is also a possibility (Vernejoux et al., 1994). The biological significance of the traces of IgE found in whole saliva is unknown.

2.4.2. Nonimmunoglobulin proteins

2.4.2.1. Lysozyme

Lysozyme represents the main enzyme of the nonspecific salivary immune defense (Meyer and Zechel, 2001), and it is secreted mainly by the submandibular and sublingual glands (Noble, 2000). Salivary lysozyme hydrolyses specific bonds in exposed bacterial cell walls, causing cell lysis and death. Many organisms, however, have cell capsules or other cell wall protective material, which confer resistance against lysozyme attack (Tenovuo, 1989). Lysozyme has been proposed as a lytic factor for bacteria which immunoglobulins have bound, mimicking in some respects the complement system in serum. Lysozyme aggregates cell suspensions of some bacterial species (Hay and Bowen, 1996). It is also known that lysozyme contributes to mucosal protection and modulates Candida populations in the oral cavity (Samaranayake et al., 2001).

2.4.2.2. Peroxidase systems

Peroxidases, salivary peroxidase and myeloperoxidase, catalyze a reaction involved in the inhibition of bacterial growth and metabolism, and the prevention of hydrogen peroxide accumulation, thus protecting proteins from the action of oxygen and reactive oxygen species (Salvolini et al., 2000; Battino et al., 2002). More precisely, salivary peroxidase catalyses the oxidation of thiocyanate ion (SCN^-) to generate oxidation products that inhibit the growth and metabolism of many micro-organisms (Battino et al., 2002). The primary oxidazer, hydrogen peroxide, is produced by the bacteria, and the production of the toxic agents is highly localized and occurs close to the bacterial target (Hay and Bowen, 1996).

2.4.2.3. Lactoferrin

Lactoferrin is present in plasma and in mucosal secretions (van der Strate et al., 1999). Salivary lactoferrin has antibacterial activity. Lactoferrin binds iron, making it unavailable for microbial use (Tenovuo, 1989). Lactoferrin, in its unbound state, also has a direct bactericidal effect on some micro-organisms including *Streptococcus mutans* strains.

2.4.2.4. Histatins

Histatins comprise a group of small histidine-rich proteins present in the saliva. The most significant function of histatins may be their anti-fungal activity against *Candida albicans* (Tsai and Bobek, 1998;

Koshlukova et al., 1999). Oral candidiasis may also modulate the levels of salivary histatin (Bercier et al., 1999; Jainkittivong et al., 1998). It has been suggested that histatins could be used as components of artificial saliva for patients with salivary dysfunction (Tsai and Bobek, 1998).

Histatins have been shown to be tannin-binding proteins in human saliva (Yan and Bennick, 1995; Wroblowski et al., 2001). Histatins also bind to enamel surfaces and hydroxyapatite in a complex manner (Lamkin et al., 1996).

2.4.2.5. Agglutinins

Salivary agglutinins are glycoproteins which have the capacity to interact with unattached bacteria, resulting in clumping of bacteria into large aggregates which are more easily flushed away by saliva and swallowed (Tenovuo and Lagerlöf, 1994). Bacterial binding to salivary proteins may in part account for individual differences in the colonization of tooth surfaces. Agglutinins induce the aggregation and clearance of streptococci from the oral cavity and are also important modulators of initial plaque formation (Carlen et al., 1998). On the other hand, it seems that salivary agglutinins may mediate the adherence of various bacterial species to the tooth surfaces (Lamont et al., 1991; Stenudd et al., 2001).

A number of salivary proteins with an agglutinating capacity have been identified: parotid saliva glycoproteins, mucins, sIgA, β2-microgolubin, fibronectin and lysozyme (Tenovuo, 1989).

2.4.3. Salivary antimicrobial factors and oral health

The relative concentrations of the organic salivary constituents are known to depend on salivary flow rate (Brandtzaeg, 1989). Some studies report temporal variations in salivary proteins (Oberg et al., 1982; Jenzano et al., 1987; McGurk et al., 1990). Little is known of the correlations of salivary proteins and possible changes in these correlations during daytime (Rudney and Smith, 1985). Salivary protein composition is also subject to variation in patients on different medications (Henskens et al., 1996).

Many investigators have attempted to relate differences in salivary levels of antibacterial proteins to differences in oral health. However, the results have been inconsistent and difficult to interpret. In almost all studies, single point measurements of salivary antimicrobial factors have been corre-lated to clinical indices, such as DMF scores, which represent cumulative disease experience (Tenovuo, 1989). Within-subject correlations among salivary antimicrobial protein levels over time can provide an indirect estimate of the extent to which analyses of these factors may influence the results of clinical investigations (Rudney et al., 1985). It is also important to remember that many salivary antimicrobial factors interact with each other (Tenovuo et al., 1987; Lenander-Lumikari et al., 1992).

It seems that no single salivary defensive factor (except flow rate) affects oral health to a significant degree (Kirstilä et al., 1998). This interaction may be one reason why it has been difficult to relate variations in salivary levels to oral ecology. It may be appropriate to consider salivary proteins as an integrated system (Rudney, 1989). The concerted action of all agents in whole saliva, both saliva- and serum-derived, provides a multifunctional protective network that collapses only if salivary flow rate is substantially reduced (Tenovuo, 1998).

2.5. AMYLASES

Amylase (α – amylase) is one of the most important salivary digestive enzymes. It consists of two families of isoenzymes, of which one set is glycosylated and the other contains no carbohydrate (Mäkinen, 1989). Salivary amylase is a calcium metalloenzyme which hydrolyses the alpha bonds of starches, such as amylose and amylopectin (Hay and Bowen, 1996). Maltose is the major end-product. It has been suggested that amylase accounts for 40 to 50% of the total salivary gland-produced protein, most of the enzyme being synthesized in the parotid gland (Noble, 2000). Human parotid saliva and submandibular saliva contain about 45 mg and 30 mg of amylase, respectively, per 100 mg of protein (Mäkinen, 1989). However, it has also been claimed that amylase makes up about 1/3 of the total protein content in parotid saliva, and the content in whole saliva would be lower (Pedersen et al., 2002). The concentration of amylase increases with the salivary flow rate, and it is generally considered to be a reliable marker of serous cell function (Almståhl et al., 2001).

In addition to its well-known function as a digestive enzyme, amylase has been reported to act as an antimicrobial enzyme. Amylase activity exists also in tears, nasal and bronchial secretions, milk, serum, urine and in the secretions of the urogenital tract (Tenovuo, 1989). Amylase also interacts specifically with certain oral bacteria and may play a role in modulating the adhesion of those species to teeth (Scannapieco et al., 1993). It has been found that salivary amylase inhibits the growth of *Legionella pneumophila* and *Neisseria gonorrhoeae* (Tenovuo, 1989). Amylase is also present in human acquired pellicle *in vivo* (Yao et al., 2001). Fasting has been found to decrease whole saliva amylase levels and activity (Mäkinen, 1989). The amylase concentrations in radiation-induced hyposalivation has been found to be reduced (Almståhl et al., 2001).

2.6. SALIVARY ALBUMIN

Albumin is the most abundant serum protein, accounting for more than 50% of all plasma proteins. Its molecular mass is 69 kDa and the normal serum reference limits are 40 - 52 mg/l. Albumin is synthesized exclusively in the liver at a rate of 100 - 200 mg/kg/day. Factors that regulate albumin

synthesis are nutrition, hormonal balance, and osmotic pressure. The half life of albumin is approximately 15 - 20 days. About 4% of albumin is degraded per day, but synthesis can be increased by as much as 100% by conditions that decrease serum albumin or lower intravascular osmotic pressure (Weisiger, 1996). Nephrotic syndrome is the best known example of systemic disorders with characteristic proteinuria and subsequent hypoalbuminaemia which lead to oedema (Appel, 1996).

In the oral cavity, albumin is regarded as a serum ultrafiltrate to the mouth (Oppenheim, 1970; von Meyer et al., 1973) and it may also diffuse into the mucosal secretions (Schenkels et al., 1995). Salivary albumin is selectively adsorbed by different materials in the oral cavity, which may enable the attachment of specific bacteria and thus alter the composition of dental plaque (Kohavi et al., 1997). Salivary albumin has been shown to increase in medically compromised patients whose general condition gets worse (Meurman et al., 2002). Immunosuppression, radiotherapy, and diabetes are examples of states where high concentrations of salivary albumin have been detected (Isutzu et al., 1981; Ben-Aryeh et al., 1993; Henskens et al., 1993; Meurman et al., 1994; Mellanen et al., 2001). There are no reference limits for salivary albumin, however.

Salivary albumin levels have been used as a marker for the degree of mucositis (Oppenheim, 1970; Izutsu et al., 1981; Makkonen et al., 1994) and inflammations in salivary glands (Lutz et al., 1991; Schiodt et al., 1992). Butler et al. (1990) found that albumin levels in whole saliva fluctuated in most of the elderly patients in their study. Cuida et al. (1997) found that albumin concentrations were higher in both parotid and whole saliva in patients with primary Sjögren's syndrome (SS) than in the control group. However, the output/min of albumin was lower in SS patients. Lenander-Lumikari et al. (2000) found that albumin concentrations were higher in patients with coeliac disease than in healthy controls.

It may be hypothesized that salivary albumin can be used to assess the integrity of mucosal function in the mouth (Meurman et al., 1997b). In periodontitis patients, significantly increased levels of salivary albumin have been reported (Henskens et al., 1993), and a significant correlation between salivary albumin and gingival index in diabetic patients has been found (Ben-Aryeh et al., 1993). On the other hand, Sweeney and coworkers (1994) did not find any difference in serum albumin concentrations in elderly patients with mucosal pathology in the mouth when compared with those with healthy mouths. In a recent study Yoshihara et al. (2003) found that there is a relationship between root caries and serum albumin concentrations in elderly subjects. Terrapon et al. (1996) found that the low salivary albumin of old edentulous people was similar to that in a group of younger individuals with a healthy periodontium.

2.7. HORMONES IN SALIVA

2.7.1. Transportation of hormones into saliva

In general, the presence of a molecule in saliva can occur through four mechanisms (Read, 1989):

1. Passage through the tight junctions of the cells of the salivary glands. This passage is dependent on molecular weight and will be relatively unimportant for compounds of 200 Da or more.

2. Passage through the lipid-rich cell membranes of the acinar cells of the salivary glands. This route is available to lipophilic molecules, such as progesterone and cortisol. It is not available to changed molecules such as steroid conjugates. Concentration reflects the free fraction in plasma.

3. Active secretion. This means exocrine gland secretion. Since salivary glands can secrete amylase and several proteases, for example, there is no reason why a protein hormone should not be secreted. Such secretion is energy dependent, and possible metabolism in the salivary gland may have an effect on the concentration. Hence, the correlation of saliva concentration with plasma concentration may be unsatisfactory.

4. Contamination. The blood and serum constituents may arise from minor abrasions and leakage from the gingivae. If oral health is poor, or the patient is malnourished, gingival leakage is even more significant.

In addition, pH and metabolism to the analyte in the salivary gland may have an effect on the concentration of hormone in saliva. Thus the saliva level of a hormone is more likely to reflect the plasma level only if the molecule is lipophilic. However, several peptide hormones have been found in saliva.

2.7.2. Steroid hormones

Interest in measuring hormones in saliva, particularly neutral steroids, has risen considerably in past decades. This interest has been stimulated by the noninvasive nature of salivary sampling (Lac, 2001).

Steroid hormones circulate in plasma either as free hormones or as hormone bound to proteins and this binding may be either "nonspecific" or "specific". Nonspecific binding is predominantly to albumin, and specific binding to such proteins as sex hormone binding globulin and cortisol binding

protein (CBG). Hence, steroid hormone in plasma is distributed in three different compartments: free, bound to albumin, and bound to specific binding protein.

The main advantage of saliva sampling for steroids in saliva is that concentrations of hormones in saliva reflect the free fraction in plasma (Lac, 2001; Hofman, 2001). Practically all steroids can be detected in saliva with good correlations with respective serum values (Read, 1989; Groschl et al., 2001; Hofman, 2001; Raff et al., 2002).

2.7.2.1. Cortisol

Salivary cortisol has been measured since 1978 (Walker et al., 1978). Studies suggest that salivary cortisol levels may be lower than the plasma free level by as much as 20% (Read, 1989; Raff et al., 2002). Good correlation coefficients have been reported between salivary cortisol and plasma free cortisol concentrations, with correlations of r =0.80 or even r = 0.90 (Read, 1989). Since the salivary cortisol assay is technically much simpler than any procedure for determining the free level, this makes the salivary assay useful in any situation in which cortisol binding is disturbed.
 Some investigators have found that salivary cortisol is a better measure of adrenal cortical function than serum cortisol and is particularly useful in studies with children (Mandel, 1990). Salivary determinations may also be used to elucidate the role of cortisol in stress. The saliva samples may also be used to follow the response to the ACTH (adrenocorticotropin hormone) test.
Cortisol has also been detected from gingival crevicular fluid (Axtelius et al., 1998).

2.7.3. Peptide hormones in saliva

It has also been suggested that proteins and even quite small peptides could occur in saliva only as a result of contamination by gingival fluid or plasma exudate. However, this cannot be the whole truth, since saliva also contains different proteins that have a physiological role and which are presumably secreted in the same fashion as are the proteins secreted by any exocrine gland. Such secretion is energy dependent, and it is not clear that the salivary concentration of any protein secreted by such a mechanism would bear any direct relationship to the circulating plasma concentration over short periods of time (Read, 1989). However, there are published data on many peptide hormones in saliva: human choriongonadotrophin, carcinoembryonic antigen , gonadotropins, prolactin, thyroxine, melatonin, insulin (Elson et al., 1983; Wright and Lack, 2001; Lac, 2001), and gastrin (Ingenito et al., 1986).

2.7.3.1. Growth hormone

Growth hormone (GH, somatotropin) contains 191 amino acids and it is secreted in pulses from the anterior part of the pituitary gland (Becker et al., 1990). Its function in the oral cavity is not known. As only minor amounts of GH have been detected in human saliva (Rantonen et al., 2000), it can be

supposed that it may not be actively excreted to saliva but that salivary GH rather represents serum ultrafiltrate and is probably part of the free fraction of plasma, devoid of any binding globulin. However, GH is a large molecule (molecular weight 21,500) and it is not probable that it can diffuse freely from serum to saliva as can steroid hormones, which are much smaller (Read, 1989). There are only a few published reports on GH and saliva. Alexander et al. (1989) found that transgenic mice began to secrete GH into saliva at puberty. On the other hand, it is known that saliva contains insulin-like growth factor (IGF-1), and salivary IGF-1 levels reflect the GH status of the donor (Costigan et al., 1988; Halimi et al., 1994). Baum et al. (1999) found that GH was secreted primarily in an exocrine manner into saliva after parasympathomimetic secretory stimulus in rat. It has also been found that hydroxychloroquine enhances the secretion of adenovirus-directed GH from rat submandibular glands into the blood stream *in vivo* (Hoque et al., 2001).

2.8. SALIVARY UREA

Urea is metabolized by the oral bacteria to ammonia and CO, resulting in an increase in the pH of the environment (Carlsson and Hamilton, 1994; Dibdin and Dawes, 1998). The influence of normal salivary urea levels on the pH of fasted plaque and on the depth and duration of a Stephan curve is uncertain. It has been suggested that plaque cariogenicity may be inversely related to salivary urea concentrations, not only when the latter are elevated as a result of some disease, but even when they are in the normal range (Dibdin and Dawes, 1998). pH gradients in dental plaque induced by urea metabolism have also been studied in "artificial mouths", and it has been found that urea-induced pH gradients may form, and can last many hours (Sissons et al., 1994). Little is known about whether the addition of a small amount of urea to chewing gum influences calculus formation or Stephan curve (Fure et al., 1998; Dawes and Dibdin, 2001).

It has been suggested that in certain metabolic conditions, mainly patients with renal dysfunction, urea is cumulated and it could be easily monitored in saliva (Khramov et al., 1994; Al Nowaiser et al., 2003).

3. AIMS AND HYPOTHESES OF THE STUDY

The overall aim of these series of studies was to investigate various aspects of salivary diagnosis in healthy and diseased subjects. The aim was also to determine whether saliva as a diagnostic fluid could be used to investigate growth hormone.

More specifically, the purposes were:

1. to investigate salivary flow rate, buffering capacity and yeast counts in medicated and unmedicated adult patients (I);

2. to investigate one-day hourly patterns of and within-subject variations in salivary viscosities and flow rates in a test panel of healthy adults (II);

3. to investigate the one-day hourly pattern and correlations between lysozyme, IgA, IgG, IgM, albumin, amylase and total protein concentrations in stimulated whole saliva in healthy adults over a 12-hour period. In addition, we wanted to study the within-subject variations in these variables (III);

4. to investigate salivary concentrations of cortisol and growth hormone (GH, somatotropin) in a healthy homogenous study group (IV); and

5. to analyze salivary albumin concentrations in patients on an acute geriatric ward and to correlate the findings to the patients´ oral health parameters and systemic condition (V).

One hypothesis was that differences exist in salivary flow rates and salivary composition, and between the different groups. Medication was expected to affect salivary flow rates and other variables. Other study hypotheses were that the time of saliva collection may affect its flow rate and composition, and that certain correlations may exist between these variables. Further, the salivary concentrations of GH were expected to correlate with their respective serum concentrations, if GH in general were detected in saliva. The salivary albumin concentrations were expected to be higher in medically compromised patients.

4. SUBJECTS, MATERIALS AND METHODS

Detailed description of the materials, subjects and methods are given in the original publications of this thesis (I-V). The following chapters briefly summarize the essential materials and methods.

4.1. ETHICAL CONSIDERATION

In all of the studies the study protocol had been approved by the joint Ethical Committee of Kuopio University Hospital and the University of Kuopio. The ethical guidelines of the Declaration of Helsinki (1975) were followed.

4.2. SUBJECT GROUPS

Altogether 651 subjects were included. Four different subject or patient groups (A, B, C and D) were studied:

4.2.1. Patient group A (I)
A total of 187 ambulatory adult patients (101 men, 86 women) of the University Dental Clinic in Kuopio were recruited. Most of the patients were enrolled in the study on their first visit to the Clinic.

Subgroup I
Unmedicated patients (n = 84).

Subgroup II
Medicated patients (n = 103).

The study group was divided into three different age groups: 20 - 40 years, n = 36; 40 - 60 years, n = 88; and over 60 years, n = 63 (Table 3).

Information about patients' condition and daily medicines was obtained via an interview before collecting salivary samples. Smoking was not recorded.

Table 3. Basic data of study group A.

	Men n	Women n	All n
Age			
20-40 yrs	15	21	36
41-60 yrs	51	37	88
>60 yrs	35	28	63
Medication			
Unmedicated	45	39	84
1-2 medicines	41	30	71
3-4 medicines	12	11	23
>5 medicines	3	6	9
All	101	86	187

4.2.2. Subject group B (II-III)

This consisted of 30 Kuopio University students, 16 male (mean age 24.2 ± 2.9 years) and 14 female (mean age 21.7 ± 1.5 years). All subjects were accepted on a voluntary basis, and only healthy, unmedicated and non-smoking subjects were recruited.

4.2.3. Subject group C (IV)

This consisted of 51 healthy, unmedicated and non-smoking subjects, 23 men, 28 women (mean age 23.7 ∂ 2.5 years). All subjects were accepted on a voluntary basis.

4.2.4. Patient group D (V)

This consisted of 383 patients. The study group was divided into two subgroups:

Subgroup I

Hospitalized patients (n = 131). The patients were selected from 199 consecutive patients referred, because of a sudden worsening of their general condition, to the Harjula geriatric hospital, Kuopio City Health Centre, in Kuopio, Finland. The patients' medical and dental history and status were recorded during the first week of hospitalization by a team of physicians, dentists and nurses, and blood and saliva samples were taken. The patients' subjective symptoms of the mouth, such as sensation of dry mouth, were recorded using structured forms. Smoking was not recorded. A detailed description and the inclusion and exclusion criteria of the original study have been given elsewhere (Meurman et al., 1997a; Pajukoski et al., 1999).

During two-year follow-up, 47 patients died. The death records were obtained from Statistics Finland. The cause of death was not recorded.

Subgroup II

Outpatients (n = 252). Subjects aged over 75-years from the same population as the patients were offered the opportunity to have a dental, salivary and x-ray examination at the Department of Oral and Dental Diseases, University of Kuopio, via an advertisement in the leading local newspaper. Altogether 260 subjects responded and all were examined using the same WHO criteria as with the hospitalized patients (World Health Organization, 1980; 1987) (Table 4). Similarly, the subjects' symptoms of the mouth were recorded. Panoramic x-rays were taken of the subjects' jaws, and cubital venous blood was drawn to analyze blood erythrocyte sedimentation rate, white blood count, and C-reactive protein values. These will be reported elsewhere. The subjects' medical history and drugs used daily were obtained via an interview. Smoking was not recorded. Drugs were classified according to a national manual for doctors (Pharmaca Fennica, 2000).

Table 4. Basic characteristics of study group D.

	Hospitalized patients		Outpatients
	Survived	Died	
N	84	47	252
Gender	23 men, 61 women	15 men, 32 women	71 men, 181 women
Age, mean with SD and range (years)			
Men	81.8 ∂ 6.4 (71 - 96)	81.8 ∂ 6.8 (70 - 94)	75.9 ∂ 5.7 (70 - 95)
Women	81.3 ∂ 5.5 (70 - 95)	83.2 ∂ 5.1 (72 - 92)	77.3 ∂ 5.5 (70 - 95)
All	81.4 ∂ 5.8 (70 - 96)	82.7 ∂5.7 (70 - 94)	76.9 ∂5.6 (70 - 95)
Mean number of concomitant systemic diseases	2.0 ∂ 1.6	2.4 ∂ 1.6	3.6 ∂ 1.9
Mean number of drugs used daily	6.0 ∂ 3.1	6.8 ∂ 3.0	4.1 ∂ 2.8***

*** $p < 0.001$, between hospitalized patients and outpatients. Differences between patients who died and those who survived were not statistically significant.

4.3. STUDY PLAN

Group	Unstimulated whole saliva	Stimulated whole saliva	Blood
Group A (I) Adult patients (n = 187): unmedicated and medicated 20-40 yrs n = 36 41-60 yrs n = 88 over 60 yrs n = 63	Flow rate	Flow rate Buffering capacity Yeast counts	
Group B (II, III) Healthy, unmedicated students (n = 30) Mean age 22.7 yrs	5 repeated samplings Flow rate Viscosity	5 repeated samplings Flow rate Viscosity IgA, IgG, IgM Lysozyme Albumin Amylase Total protein	
Group C (IV) Healthy , unmedicated students (n = 51) Mean age 23.7 yrs	Flow rate GH	Flow rate IgA, IgG, IgM Albumin Amylase Urea Cortisol	Cortisol GH
Group D (V) Elderly patients (n = 383): hospitalized (n = 131), mean age 81.9 yrs and outpatients (n = 252), mean age 76.9 yrs		Flow rate Yeast counts Total protein Albumin Urea	

+ medical and dental examination

Figure 2. Study plan.

4.4. SALIVARY SAMPLES

The subjects were given written instructions beforehand regarding the saliva collections. Subjects and patients were told not to eat, drink or smoke for 1 h before each sampling. The saliva samples were always collected in restful and quiet circumstances. Unstimulated whole saliva was collected for 3 minutes (group A) by the subject leaning forward and spitting saliva into the test tube once a minute, or for 5 minutes (group B and C) by the subject leaning forward and letting the saliva drain into a graded sampling tube.

Whole saliva flow was stimulated by chewing a piece of paraffin wax (Orion Diagnostica, Espoo, Finland) at a constant rate. Stimulated whole saliva was collected over a 3 min period (group A, B and C) or 5 minutes (group D), and the saliva secreted during the first 30 seconds (group A, B and C) or 60 seconds (group D) was discarded. The flow rates were evaluated visually from graded test tubes (as ml/min).

The collection procedure was practiced several times with the researcher before the collections in order to reduce the bias in repeated measurements (group B). In group B, the saliva samples were collected five times daily at 8:00 h, 11:00 h, 14:00 h, 17:00 h and 20:00 h. In group A, saliva samples were taken between 9:00 h and 12:00 h. In group C, saliva samples were collected at 8:00 h after 12 hours fasting, and in group D between 13:00 h and 15:00 h.

For salivary growth hormone analysis (group C), however, each participant gave stimulated saliva samples directly into albumin-coated test tubes that were kept in crushed ice. Immediately after collection, the samples were deep-frozen in dry ice and stored at -70° C until analyzed.

Salivary samples in groups A, B and C were collected by the same researcher.

4.5. BLOOD SAMPLES

Cubital venous blood was drawn into 5 ml Vacutainer tubes without anticoagulants. Coagulated blood was centrifuged (10 min, 720 x g), serum was taken and deep-frozen (-70° C) for analyses (group C).

4.6. BUFFERING CAPACITY

Buffering capacity was assessed using the Dentobuff-strip chair-side method (Orion Diagnostica, Espoo, Finland).

4.7. YEAST COUNTS

Yeast cultures were assessed with Oricult-N dip slides (Orion Diagnostica, Espoo, Finland) (groups A and D). The slides were incubated at 37° C for two (group A) or four (group D) days until read for colonies of yeasts, mainly Candida spp.

4.8. VISCOSITY MEASUREMENTS

A Wells Brookfield digital cone-plate viscometer, model LVTDV CP - II (Brookfield Engineering Laboratories, Stoughton, Mass., USA), was used. A cone of very shallow angle (0.8∀ spindle CP - 40) is allowed to rotate freely inside the testing cup. When the liquid (saliva) to be tested is introduced between the cone and plate, a resistance to the rotation of the cone is produced. A reading of the dial of the instrument gives a measure of this resistance, or shear stress (ϑ), of the fluid. The apparent viscosity of the fluid can be calculated from the ratio of the shear stress (ϑ) to the rate of shear (ν) applied. Apparent viscosity ξ was measured immediately after collection from the fresh saliva samples (1 ml, T= 37 ± 0.1∀C, shear rate 90 s^{-1}) (group B).

4.9. SALIVARY LABORATORY ANALYSIS

The sampling tubes were placed in crushed ice right after collection. Stimulated saliva samples were used for analysis. Lysozyme was assessed immediately using a modification of the lysoplate method (Rudney and Smith, 1985), which is based on the capacity of lysozyme to lyse cell walls of *Micrococcus lysodeikticus*. Saliva was pipetted on the agarose gel plates before the sample was centrifuged. The diameters of clearance zones were measured after 24 hours (group B). The rest of the saliva was centrifuged (2000 x g, 10 min) and the samples were stored at -75∀C until further analyzed. The total salivary immunoglobulin concentrations were then analyzed from thawed samples using an enzyme immunoassay (ELISA) according to Lehtonen et al. (1984) (group B and C). Albumin was assessed by a spectrophotometric method, and human serum albumin standards were used (Webster, 1977). Total protein was measured with the colorimetric Lowry method (Lowry et al., 1951) (group B, C and D). Bovine protein standards were used.

Amylase was analyzed using an enzymatic colorimetric test kit (ζ -amylase EPS, Boehringer Mannheim, Mannheim, Germany) (group B, C). Precinorm E was used as a control.

Urea was analyzed using the Boehringer kinetic method (MPR 2, Boehringer Mannheim, Germany) (group C and D). Precimat Urea was used as a standard.

4.9.1. Analyses of serum and salivary cortisol concentrations

To analyze serum cortisol (group C) an Orion Diagnostica RIA kit was used (Orion Diagnostica, Espoo, Finland). Detailed description of the laboratory procedures is given in paper IV. Radioactivities were assessed in a Gamma Master 1277 gammacounter (Wallac Oy, Turku, Finland) during 1 min. All the analyses were made in duplicate.

Salivary cortisol was assessed in the same way as from blood samples except that the standards were prepared from a 2000 nmol/l solution which was diluted in the 0.1 M Tris buffer (pH 7.4) to give the final concentrations of 0 - 100 nmol/l. Antiserum and assay buffer for the NSB tubes were diluted 1:5 in the Tris buffer. The salivary analyses were also made in duplicate. Concentrations were determined as nmol/l.

4.9.2. Assay for human growth hormone in saliva and in serum, and its validation

The concentrations of GH in saliva and in serum (group C) were determined with a highly sensitive immunoradiometric assay, which originally was introduced for urine samples (Helenius et al, 1989). Two monoclonal antibodies were used, both from Medix Biochemica (Kauniainen, Finland). Detailed description of the laboratory procedures is given in paper IV. According to Medix Biochemica both monoclonal antibodies recognize specifically human GH with molecular weight of 22 kDa (cross-reactivity 100 %). Neither of the monoclonal antibodies recognizes the molecular form of 20 kDa (cross-reactivities below 0.2 %). Cross-reactivity with human prolactin is 1 % with both antibodies. With human placental lactogen, the Mab 5801 has a cross -reactivity of 0.2 % and Mab 5802 below 0.2 %. The detection limit of the assay, estimated from the interpolated response at two standard deviations above zero dose, is 1.0 ng/l when fresh labeled antibody is used. Concentrations were determined as mU/l in serum and μU/l in saliva.

The possible blood contamination of the salivary samples was tested with semiquantitative strip-test (Redia-test, Boehringer Mannheim, Germany).

4.9.2.1. Radioimmunological method

Serum GH concentrations were also analyzed using the radioimmunological method of Orion Diagnostica Human Growth Hormone RIA kit (Espoo, Finland). Detailed description of the laboratory procedures is given in paper IV. Radioactivity was assessed in a Gamma Master 1277 gammacounter during 1 min. All the analyses were made in duplicate.

4.10. STATISTICAL ANALYSIS

Kolmogorov-Smirnov's test was used to test the normality of distributions. The SPSS for Windows program was used (Statistical Package for Social Sciences, SPSS Inc., Chicago, U.S.A.) in all statistical analyses.

Group A

Results were expressed as mean values ∂ SD. Frequencies were also used in the statistics when applicable. The results between the three age groups and between medicated and unmedicated groups were analyzed statistically. The statistical significance of the differences between the means were tested using Student's t test.

Group B

A repeated measures design was used in MANOVA. These statistical tests allow that data from repeated samplings correlate with each other, which was tested by correlation plotting. Initial results were computed on the basis of observed concentrations. The viscosity of saliva can be subject to variation due to differences in flow rates between subjects. We used MANOVA with covariates (flow rate) to test the within-subject variation in the repeated samplings.

Concentrations of salivary proteins can be subject to variation due to differences between subjects in flow rates and total protein output (Rudney et al., 1985). Since total protein concentration and flow rate seemed to correlate with the other variables in our data, we used MANOVA with covariates (flow rate and total protein) to test the within-subject variation of the repeated samplings.

To study the relationships between the variables at different time points we used partial correlations (Pearson). Flow rates were controlled for.

Correlations within subjects across samples were expressed as inter-item correlations (IIC). These correlations indicate how persons who show low or high values for a variable remain at the low or high end of the range for each time point (Rudney et al., 1985).

Group C

Mean concentrations and standard deviations of variables were calculated. Differences between the sexes were calculated by Student's t test for independent samples. To remove skewness in these values, concentrations were re-expressed as base 10 logs. Since total protein concentration seemed to correlate with other parameters also in our data, we used Pearson's partial correlation controlling for salivary total protein to calculate associations between variables.

Group D

Results were expressed as mean values ∂ SD. Median values were also used in the statistics when applicable. Differences were analyzed between men and women and between the hospitalized

patients and outpatients. Albumin values were studied with respect to the patients' age, and to a variety of other background variables, such as dental parameters and treatment need, mouth mucosal pathology, salivary yeast counts, number of the patients' concomitant systemic diagnoses, various disease groups, and drugs used daily. The differences between the groups were assessed by means of Student's t test, rank-sum test, or chi-square test when applicable. Analysis of variance was used for continuous data when applicable, and logistic regression analyses were made to investigate the effect of various variables on albumin values below or above the median, separately in the hospitalized patients and outpatients. Cox survival regression analysis was used to study risk factors regarding patients who died and those who survived.

5. RESULTS

5.1. SALIVARY FLOW, BUFFERING CAPACITIES AND YEAST COUNTS (STUDY I)

5.1.1. Patients' medication

Information about patients' health and daily medicines was obtained via an interview before collecting salivary samples. Altogether 103 patients were medicated and 84 were unmedicated (Table 3). The medicines used are listed in Table 5.

Table 5. Group A. Use of medicines (n = 187) . Each patient may concomitantly use several drugs, and altogether 103 patients were medicated.

Medication	Men (n = 101)		Women (n = 86)	
	n	%	n	%
Antidepressant drugs, tranquilizers, sedatives and antipsychotic agents	4	4.0	6	7.0
Diuretics, antihypertensive agents, nitrates, digitalis and antiarrythmic agents	33	32.7	23	26.7
Analgesics and antipyretics	5	5.0	9	10.5
Respiratory drugs	5	5.0	5	5.8
Drugs for metabolic diseases	2	2.0	7	8.1
Drugs for gastro-intestinal diseases	30	29.7	12	14.0
Other (hormones, antibiotics etc.)	4	4.0	11	12.8

5.1.2. Unstimulated saliva

The mean unstimulated whole saliva flow rate was found to be 0.5 ml/min (SD 0.4 ml/min) (Table 6). Women had a lower mean flow than men, and the differences between the sexes were statistically significant in all age groups in medicated and unmedicated patients (Tables 6 and 7).

The medicated patients had a slightly lower mean flow rates than unmedicated ones, the difference being statistically significant in patients aged over 60 years ($p < 0.010$) and in men aged over 60 years ($p < 0.050$) (Table 7).

Table 6. Unstimulated saliva flow (ml/min) in all patients (group A, n = 187).

Age	n	Men (n = 101) Mean	SD	Women (n = 86) Mean	SD	All (n = 187) Mean	SD	Significance between genders
20-40 yrs	36	0.8#	0.5	0.4	0.3	0.6	0.4	<0.050
41-60 yrs	88	0.7	0.5	0.4	0.2	0.6#	0.4	<0.001
>60 yrs	63	0.5#	0.3	0.3	0.3	0.4#	0.3	<0.010
All	187	0.6	0.4	0.4	0.3	0.5	0.4	<0.001

# $p < 0.050$; significance between age groups.

Table 7. Unstimulated saliva flow (ml/min) in unmedicated and medicated patients (group A, n =187).

Age	n	Men (n = 101) Mean	SD	Women (n = 86) Mean	SD	All (n = 187) Mean	SD	Significance between genders
Unmedicated								
20-40 yrs	31	0.8	0.5	0.5	0.3	0.6	0.4	<0.050
41-60 yrs	40	0.7	0.4	0.4	0.2	0.5	0.4	<0.010
>60 yrs	13	0.7*	0.5	0.5	0.6	0.6**	0.5	N.S.
All	84	0.7	0.5	0.4	0.3	0.6	0.4	<0.010
Medicated								
20-40 yrs	6	0.7	0.6	0.2	0.0	0.5	0.5	N.S.
41-60 yrs	48	0.7#	0.5	0.4	0.3	0.6#	0.5	<0.050
>60 yrs	50	0.5#*	0.3	0.3	0.2	0.4#**	0.3	<0.050
All	103	0.6	0.4	0.3	0.3	0.5	0.4	<0.001

** $p < 0.010$; *$p < 0.050$; significance between medicated and unmedicated.

# $p < 0.050$; significance between age groups.

5.1.3. Stimulated saliva

The mean stimulated whole saliva flow rate was found to be 1.6 ml/min (SD 0.8 ml/min) (Table 8). Stimulated saliva flow was significantly higher in men than in women in all age groups (Table 8). The medicated subjects had lower mean flow rates than unmedicated ones, the difference being statistically significant in women (p < 0.010) and in the over 60s, and in all subjects (p < 0.050). No difference in this respect was found in men (Table 9).

Table 8. Stimulated saliva flow rate (ml/min) in all patients (group A, n = 187).

Age	n	Men (n = 101)		Women (n = 86)		All (n = 187)		Significance between genders
		Mean	SD	Mean	SD	Mean	SD	
20-40 yrs	36	2.0	0.8	1.5#	0.8	1.7	0.8	<0.050
41-60 yrs	88	1.8	0.9	1.3	0.8	1.6	0.9	<0.010
>60 yrs	63	1.8	0.8	1.2#	0.5	1.6	0.7	<0.001
All	187	1.8	0.9	1.3	0.7	1.6	0.8	<0.001

# p < 0.050; significance between age groups.

Table 9. Stimulated saliva flow (ml/min) in unmedicated and medicated patients (group A, n = 187).

Age	n	Men (n=101)		Women (n=86)		All (n=187)		Significance between genders
		Mean	SD	Mean	SD	Mean	SD	
Unmedicated								
20-40 yrs	31	1.9	0.8	1.6	0.8	1.7	0.8	N.S
41-60 yrs	40	1.9	1.0	1.5	0.7	1.7	0.9	N.S
>60 yrs	13	2.2	0.7	1.4	0.6	2.0*	0.8	<0.050
All	84	2.0	0.9	1.6**	0.7	1.8*	0.8	<0.050
Medicated								
20-40 yrs	6	2.3	0.8	1.0	0.9	1.7	1.0	N.S
41-60 yrs	48	1.7	0.8	1.2	0.8	1.5	0.9	<0.050
>60 yrs	50	1.7	0.8	1.2	0.5	1.4*	0.7	<0.010
All	103	1.7	0.8	1.2**	0.7	1.5*	0.9	<0.001

** p < 0.010; * p < 0.050; significance between medicated and unmedicated.

5.1.4. Saliva secretion in various medication groups

A statistically significant difference in stimulated saliva flow was found between unmedicated persons and those taking 1 - 2 or over 3 medicines daily (p < 0.050). No statistically significant

differences were found in resting saliva flow in this respect (Figure 3). The most often encountered medicines were for cardiovascular diseases and for gastro-intestinal disorders (Table 5).
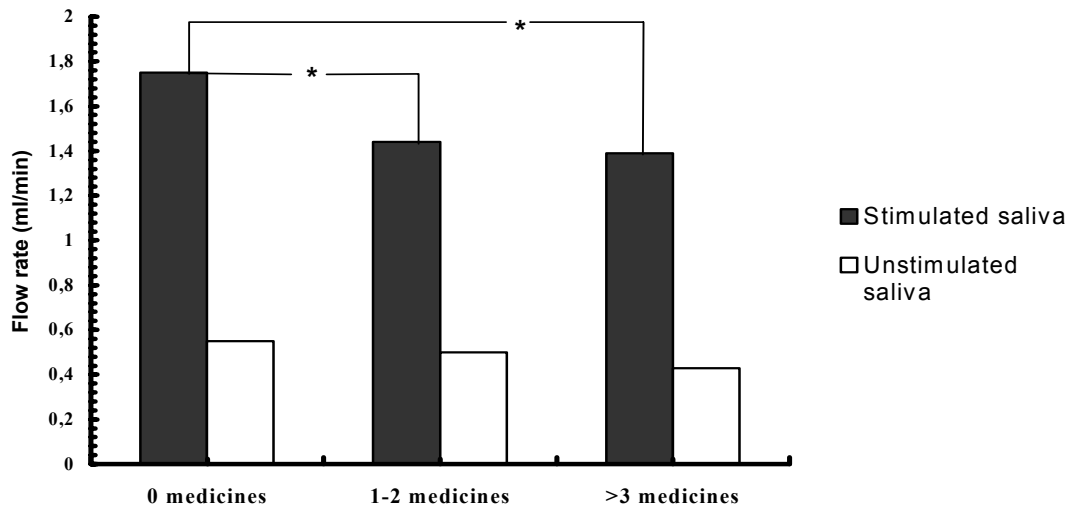
Figure 3. Number of medicines used daily versus saliva flow (group A, n = 187). Stimulated saliva flow was lower in patients taking 1-2 medications or over 3 medications than in unmedicated patients. * p < 0.050.

Antidepressants and other psychopharmaca known to reduce salivary secretion via an anticholinergic effect were used by 10 patients. Their mean flow rates were significantly lower than those of unmedicated patients: unstimulated flow 0.3 ± 0.3 ml/min, stimulated flow 1.2 ± 0.6 ml/min for the former and 0.6 ± 0.4 ml/min and 1.8 ± 0.8 ml/min for the latter (p < 0.050).

5.1.5. Buffering capacity

The frequencies of buffering capacities of all the patients are presented in Figure 4. Women had more low values (pH < 4.0) than men (p < 0.001). When the frequencies were compared between unmedicated and medicated patients, no statistically significant differences were found in men, whereas in women the differences were significant (Table 10). When both the sexes were observed together, the difference between unmedicated and medicated patients became statistically significant (p < 0.001).
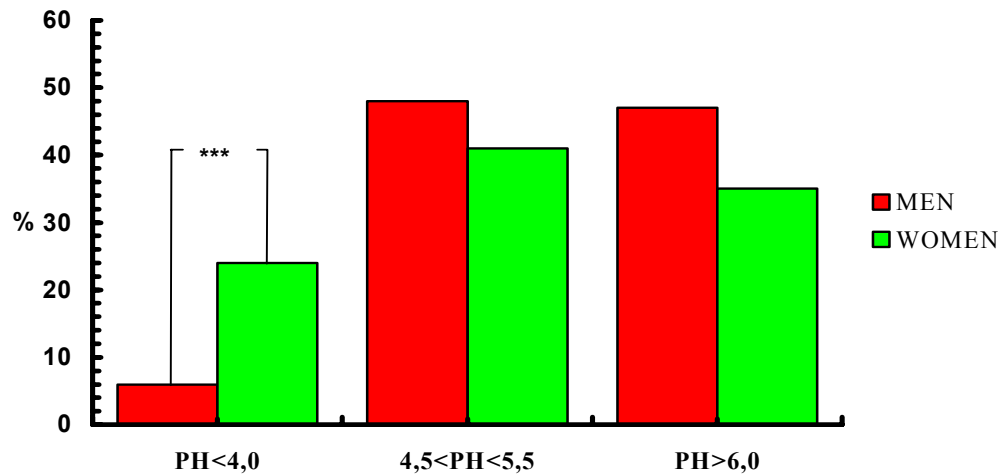
Figure 4. Buffering capacities in all subjects (group A, n = 187). Women had significantly more low scores than men (p < 0.001)

Table 10. Frequencies of buffering capacities in different subgroups (group A, n = 187).

	pH < 4.0 n	4.5 < pH < 5.5 n	pH > 6.0 n
Men (n = 101)			
Unmedicated (n = 45)	3 (6%)	17 (38%)	25 (56%)
Medicated (n = 56)	3 (5%)	31 (55%)	22 (39%)
Women (n = 86)			
Unmedicated (n = 39)	3 (8%)**	15 (38%)	21 (54%)***
Medicated (n = 47)	18 (38%)**	20 (43%)	9 (19%)***

** p < 0.010; *** p < 0.001; differences between
unmedicated and medicated.

5.1.6. Salivary yeasts

Of all the patients, 55% of men and 66% of women had positive salivary yeast counts. Altogether 63% of the medicated and 57 % of the unmedicated patients had positive yeast counts. The differences were not statistically significant between sexes or between medication groups . However, patients with positive yeast counts had statistically significantly lower flow rates, with respect to both unstimulated and stimulated saliva (p < 0.010).

5.2. SALIVARY VISCOSITY AND FLOW RATES OF REPEATED SAMPLES (STUDY II)

5.2.1 Observed data

The pilot study is presented in detail in Paper II. A fall in viscosity with higher shear rates was typical of a non-newtonian fluid exhibiting shear-thinning. We also measured viscosities of the fresh stimulated saliva samples and repeated this for the same samples 30 minutes after collection, and the results were almost the same (n =18, T = 37˚C).

The means of the observed flow rates and viscosities in different samplings are given in Figures 5 and 6. Initial results were calculated on the basis of observed data. The viscosity of stimulated saliva remained steady throughout the samplings. There were no statistically significant differences between genders in flow rates or viscosities.
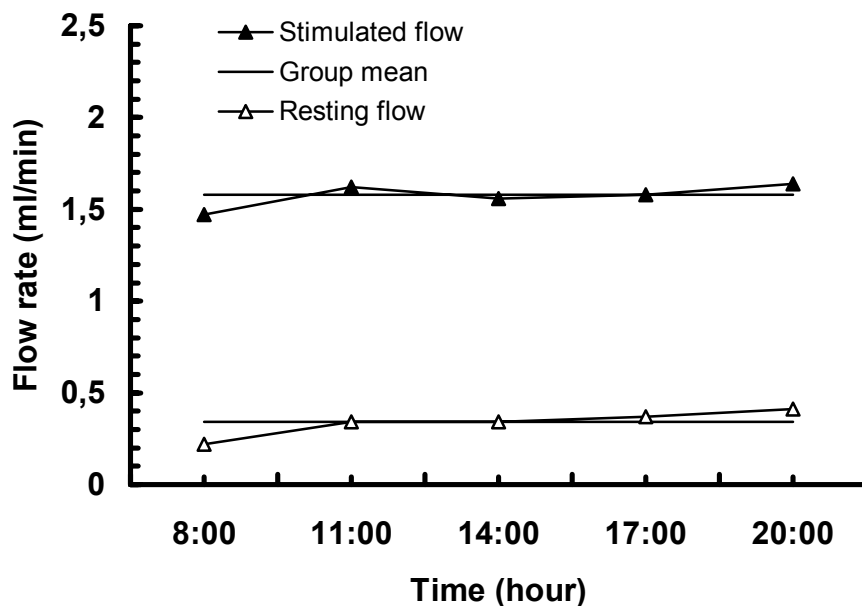
Figure 5. Daytime means of unstimulated and stimulated flow rates. Straight lines represent the respective mean flow rates (group B, n = 30).
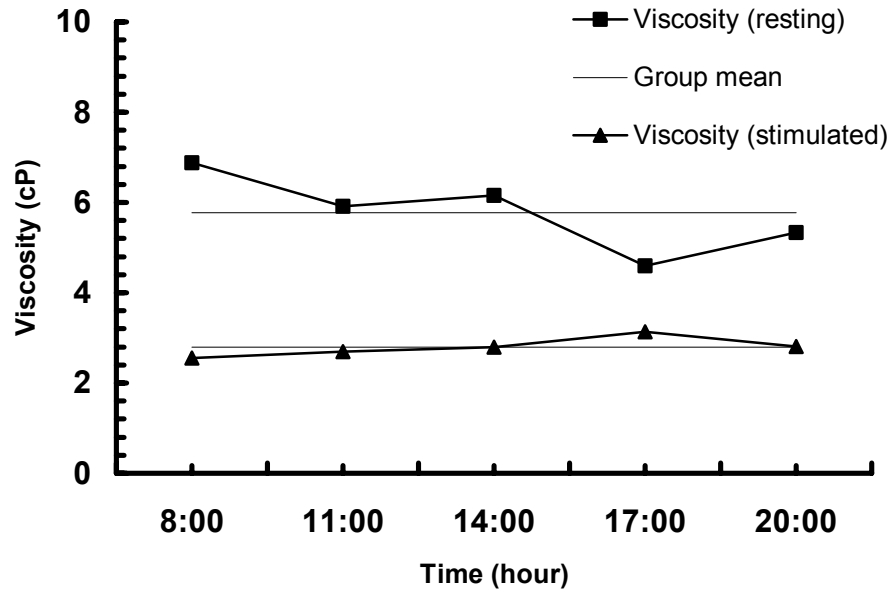
Figure 6. Daytime means of viscosities of unstimulated and stimulated salivas. Straight lines represent the respective mean viscosities (group B, n = 30).

5.2.2. Correlations of salivary viscosity and flow rate

When calculating the correlation coefficients between the variables at different sampling hours, it was observed that viscosities of unstimulated and stimulated salivas were correlated to each other at every sampling (r = 0.62, p < 0.001 at 17:00). Viscosities and respective flow rates were negatively and statistically significantly correlated to each other at every sampling hour (r = -0.43, p < 0.050 for unstimulated saliva and r = -0.53, p < 0.010 for stimulated saliva). Unstimulated and stimulated salivary flow rates were clearly correlated to each other at every sampling hour (r = 0.70, p < 0.001 at 17:00).

5.2.3. Within-subject variation and correlations across samples

The MANOVA analysis for salivary flow rates and viscosities with covariates is shown in Table 11. Flow rates were used as covariates and gender as a between-subject factor in analysis for viscosity. The viscosity of unstimulated saliva was found to have statistically significant within-subject variation (p < 0.001). However, the viscosity of stimulated saliva showed no significant within-subject variation across samplings. Unstimulated saliva flow rate showed statistically significant within-subject variation (p < 0.001).

The inter-item correlations were highly significant for both flow rates (p < 0.001). Inter-item correlation for the viscosity of unstimulated saliva was also significant (p < 0.050).

Table 11. MANOVA for repeated measures with covariates and inter-item correlation coefficients (IIC) (group B, n = 30, 5 repeated samples).

Variable	F-value	Significance	IIC
Unstimulated flow	11.8	$p < 0.001$	0.82***
Stimulated flow	1.9	N.S.	0.88***
Viscosity, unstimulated[†]	15.7	$p < 0.001$	0.54*
Viscosity, stimulated[‡]	0.8	N.S.	0.33

[†]Unstimulated and stimulated flow rates were used as covariates.

[‡]Stimulated flow rates were used as covariate. Gender was used as a between-subject factor in MANOVA.*** $p < 0.001$; *$p < 0.050$, significance of inter-item correlations.

5.3. PROTEINS IN REPEATED SAMPLES (STUDY III)

5.3.1. Observed data

Group means, standard deviations and skewness values of all samplings are given in Table 12. There were no statistical differences between genders.

Table 12. Group means, SD and skewness values for five repeated samplings in group B (n = 30).

Parameter	Mean	SD	Skewness
Unstimulated flow (ml/min)	0.3	0.2	1.5
Stimulated flow (ml/min)	1.6	0.7	0.6
Total protein (mg/ml)	1.2	0.4	0.8
Amylase (U/ml)	84.9	45.7	0.6
Albumin (µg/ml)	167.1	70.7	1.2
Lysozyme (mg/l)	103.4	77.5	1.4
IgA (mg/l)	37.4	16.8	0.9
IgM (mg/l)	4.5	3.5	1.2
IgG (mg/l)	11.4	9.2	1.8

The means of the observed concentrations at different time points and respective group means are given in Figures 7-10. The means of salivary flow rates at these time points are given in Figure 5. Salivary flow rates showed an increasing trend during the day.

The salivary IgA, IgG, IgM and albumin concentrations were at their highest in the morning, and they showed a decreasing trend during the day. Total protein, amylase and lysozyme concentrations showed an increasing trend from the morning to the evening samples.
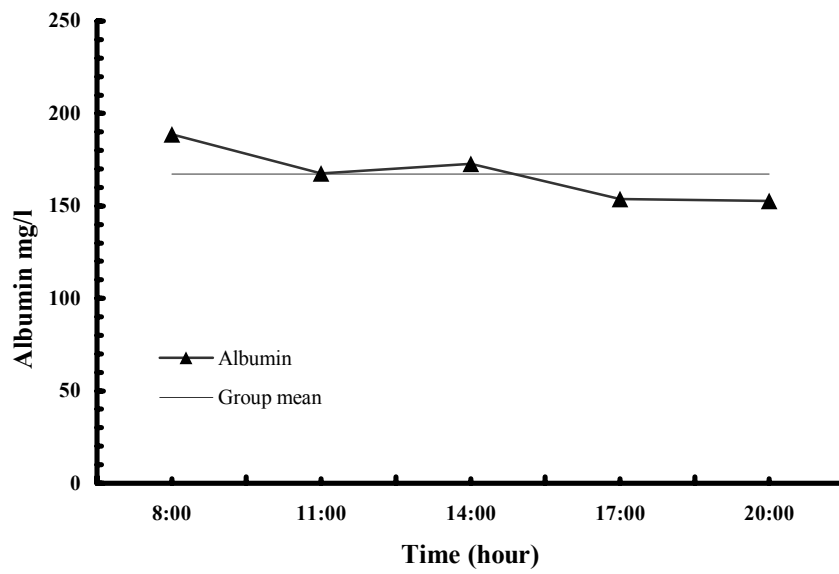
Figure 7. Albumin concentration over the 12-h period of sampling. The straight line represents the mean albumin concentration (group B, n = 30).
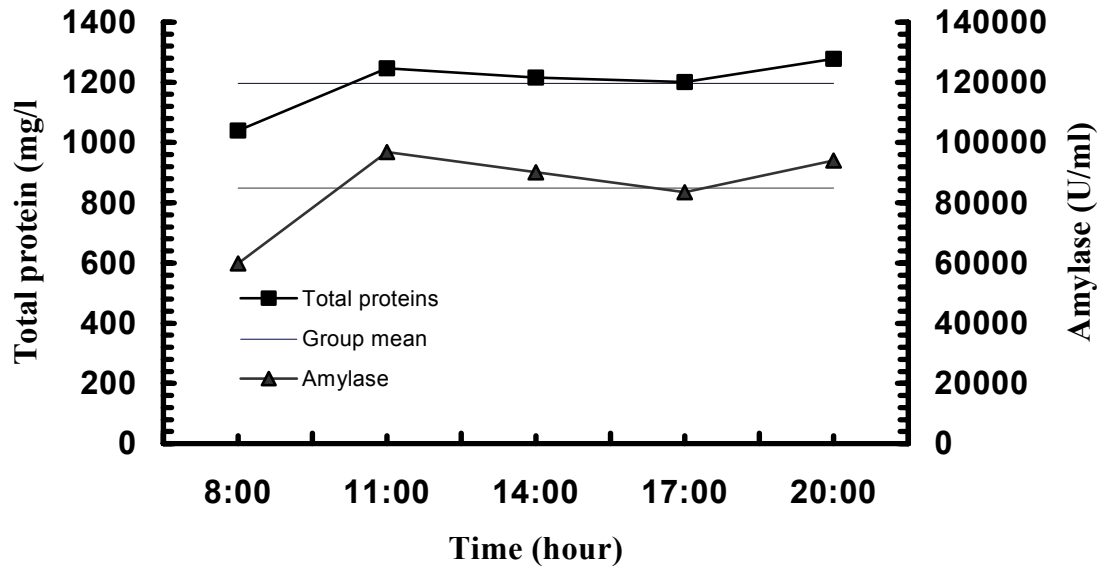
Figure 8. Salivary amylase and total protein concentrations over the 12-h period of sampling. Straight lines represent the mean of concentrations of variables (group B, n = 30).
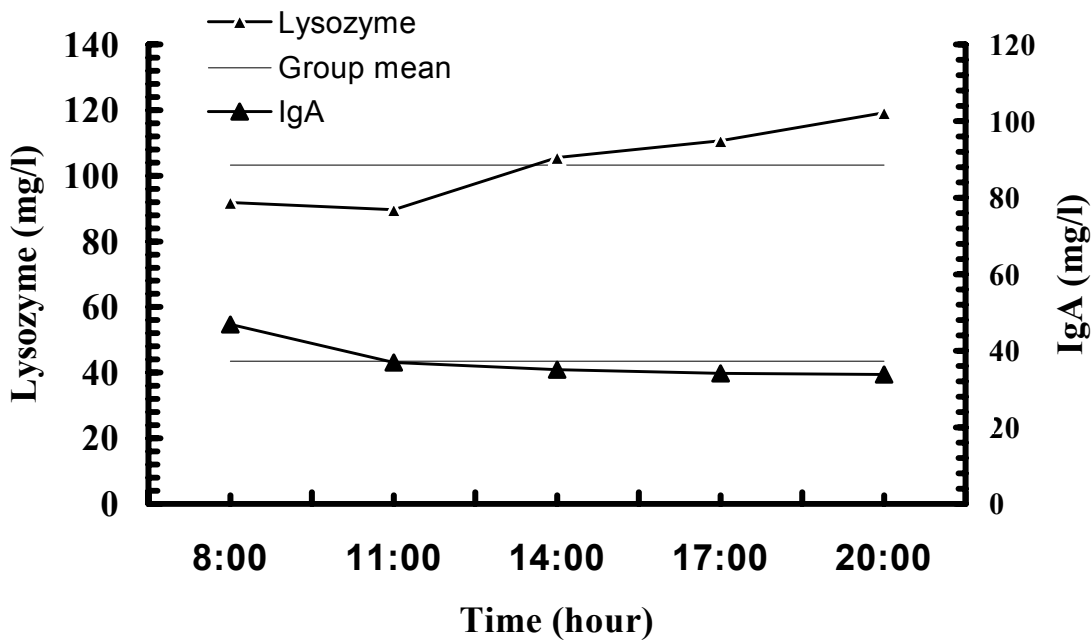
Figure 9. Salivary lysozyme and IgA concentrations over the 12-h period of sampling. Straight lines represent the mean concentrations of variables (group B, n = 30).
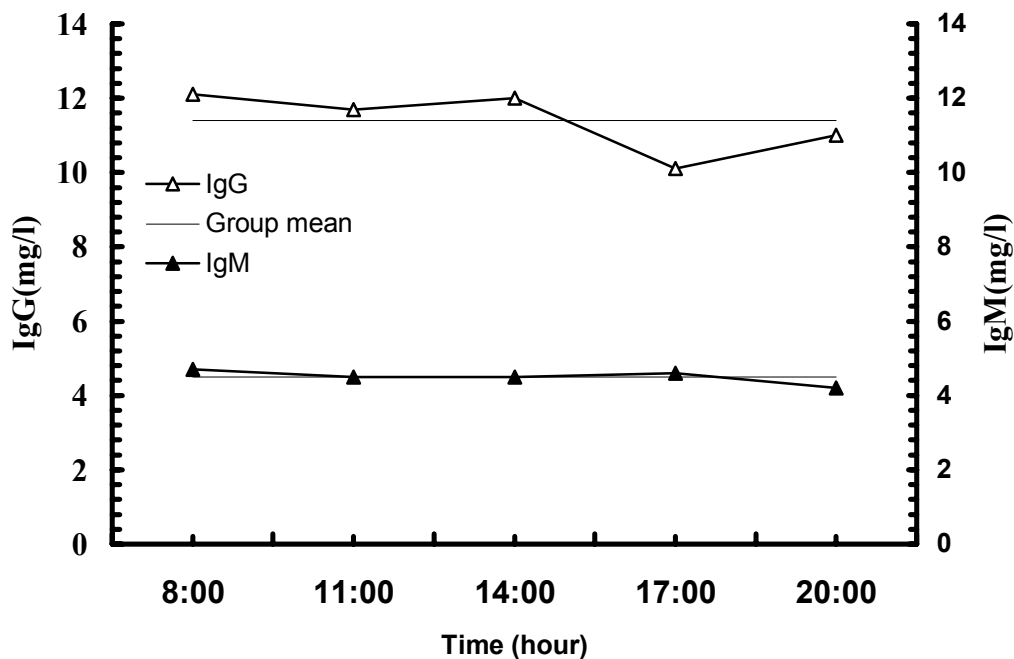
Figure 10. Salivary IgG and IgM concentrations over the 12-h period of sampling. Straight lines represent the mean concentrations of variables (group B, n = 30).

5.3.2. Within-subject variation and correlations across samples

The MANOVA analysis with covariates is presented in Table 13. Flow rate and total protein concentration were used as covariates and gender as a between-subject factor. After this adjustment salivary IgA ($p < 0.001$), albumin ($p < 0.010$), amylase ($p < 0.050$) and total protein ($p < 0.001$) concentrations still varied statistically significantly. Gender did not significantly affect the variation in any of these parameters.

Table 13. MANOVA for repeated measures with covariates and inter-item correlations (IIC) (n = 30, 5 repeated samplings).

Parameter	F	p-value	IIC
Total protein[a]	7.5	$p < 0.001$	0.80***
Amylase	3.0	$p < 0.050$	0.78***
Albumin	4.1	$p < 0.010$	0.79***
Lysozyme	1.6	N.S.	0.77***
IgA	6.7	$p < 0.001$	0.80***
IgM	0.5	N.S.	0.88***
IgG	0.9	N.S.	0.81***

Flow rate and total protein concentration were used as covariates and gender as a between-subject factor in MANOVA.

[a]adjusted for flow rate only

***$p < 0.001$

The inter-item correlations are also given in Table 13. There was a strong tendency for subjects to maintain a similar position relative to others across all samples, since IIC were significantly different from zero for all variables ($p < 0.001$).

The effect of covariates (flow rate and total protein) on the observed variation of repeated samplings was significant in amylase ($p < 0.001$).

5.3.3. Correlations between variables

Partial correlation coefficients and significances of the correlations between the variables in different samplings are presented in detail in Paper III. Flow rates and total protein concentrations were controlled for.

Total protein correlated significantly with amylase (r = 0.79, $p < 0.001$ at 14:00), albumin (r = 0.53, $p < 0.010$ at 14:00) and IgA (r = 0.39, $p < 0.050$ at 14:00) through different samplings. In addition, IgG correlated with albumin (r = 0.55, $p < 0.010$ at 14:00) and lysozyme (r = 0.58, $p < 0.010$ at 14:00) levels through one day.

5.4. SALIVARY CORTISOL AND GROWTH HORMONE (STUDY IV)

5.4.1. Observed data

Tables 14 and 15 show the basic characteristics of the salivary and serum samples. The salivary urea level was significantly higher in men than in women ($p < 0.050$). The salivary GH level was significantly higher in women than in men ($p < 0.010$). Serum cortisol and GH levels were also significantly higher in women than in men ($p < 0.001$).

Table 14. Group C. Basic data of salivary samplings after 12-hour fasting ($n = 51$).

Parameter	Women		Men		All	
	Mean	SD	Mean	SD	Mean	SD
Unstimulated flow (ml/min)	0.5	0.3	0.5	0.2	0.5	0.3
Stimulated flow (ml/min)	1.5	0.6	1.6	0.5	1.5	0.6
Protein (mg/ml)	0.8	0.3	0.8	0.2	0.8	0.8
Amylase (U/ml)#	34.9	25.1	45.3	22.0	39.6	24.1
Albumin (µg/ml)#	141.2	123.9	139.6	105.0	140.6	114.6
Urea (mmol/l)#	2.4	0.8	3.8	2.5	3.1*	1.9
IgA (mg/l)#	47.1	126.5	57.6	26.0	51.9	26.6
IgM (mg/l)#	5.1	4.0	3.6	2.2	4.4	3.4
IgG (mg/l)#	14.4	16.5	13.1	8.3	13.8	13.4

Difference between genders: *$p < 0.050$. # previously unpublished.

Table 15. Serum and saliva cortisol and GH samplings after 12-hour fasting (group C, n = 51).

	Women		Men		All	
Saliva	Mean	SD	Mean	SD	Mean	SD
Cortisol (nmol/l)						
Unstimulated	20.0	5.6	25.1	23.6	22.3	16.4
Stimulated	19.5	5.5	20.8	14.5	20.0	10.4
GH (µU/l)						
Unstimulated	12.8	13.5	3.4	2.3	8.6**	11.1
Serum						
Cortisol (nmol/l)	809.5	261.4	514.1	139.6	676.3***	259.8
GH (mU/l)	27.4	25.6	2.9	9.4	16.4***	23.3
Radioimmunological method:						
GH (µg/l)	13.7	9.3	1.5	2.3	8.2***	9.3

Difference between genders: ** $p < 0.010$; *** $p < 0.001$.

Distributions of cortisol and GH scores were substantially skewed, as is often the case with salivary data. To remove skewness in these values, concentrations were re-expressed as base 10 logs. This is explained in more detail in Paper IV.

5.4.2. Correlations between serum and saliva levels

Partial correlations were calculated between the serum and saliva levels of these hormones. Salivary cortisol was significantly associated with the serum cortisol level ($r = 0.47$, $p < 0.001$) (Figure 11). Correlations were also calculated separately for genders (women $r = 0.57$, $p < 0.050$; men $r = 0.36$, N.S.).

Salivary and serum GH levels also correlated significantly with each other (r = 0.59, p < 0.001) (Figure 12). The correlation was higher in men (women r = 0.45, p < 0.050; men r = 0.58, p < 0.010). The two different methods for analyzing GH serum concentrations correlated significantly with each other (r = 0.90, p < 0.001).

Partial correlations between salivary and serum cortisol and GH levels and other variables were also calculated. Salivary hormone levels did not correlate with other salivary

variables. Serum GH correlated negatively with salivary urea (r = -0.32, p < 0.050). Serum cortisol level correlated negatively with salivary lysozyme (r = -0.37, p < 0.010) and serum GH (r = 0.46, p < 0.001).
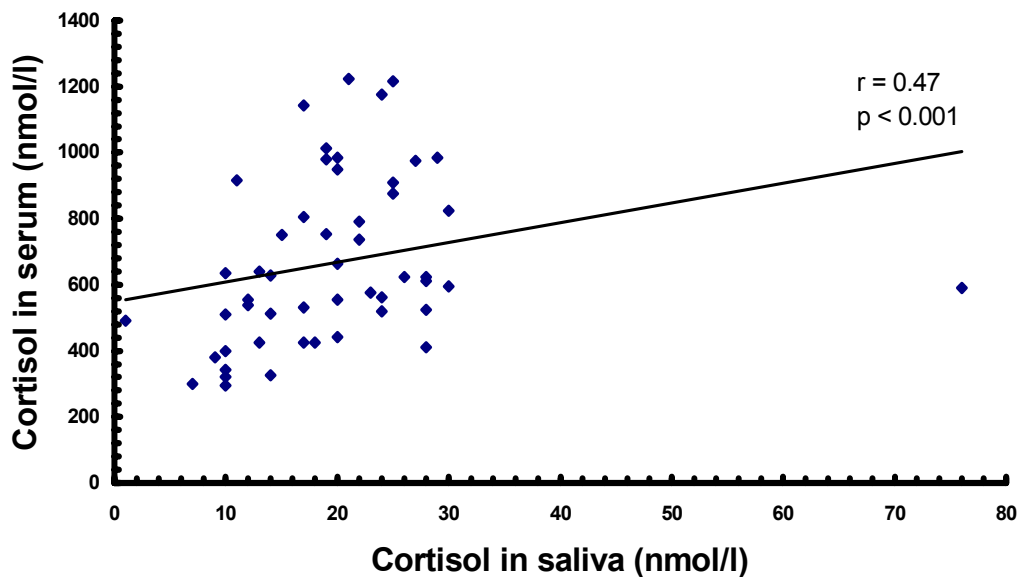
Figure 11. Correlation between serum total cortisol and stimulated saliva cortisol levels in group C (n = 51) after 12-h fasting.
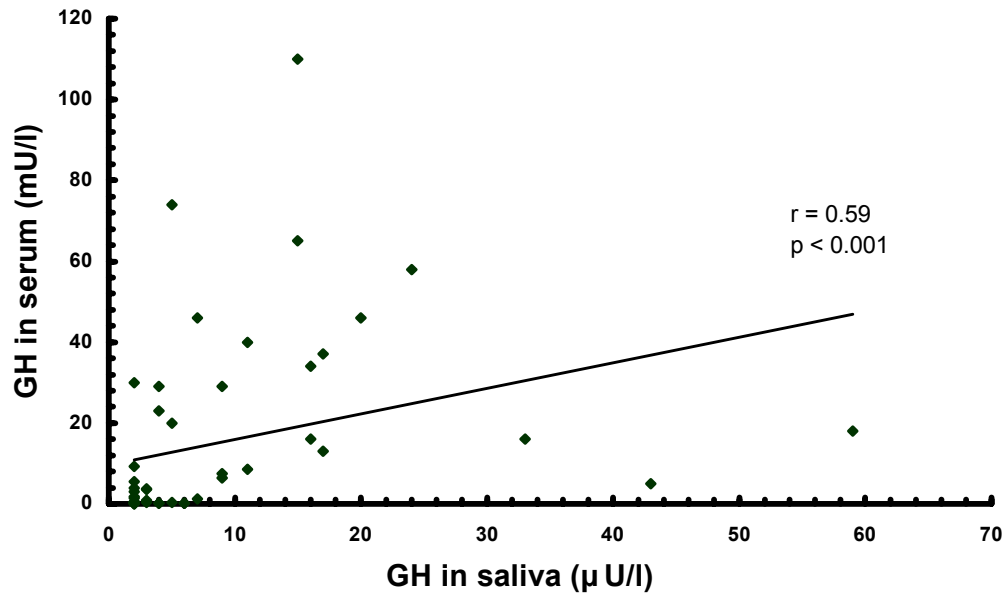
Figure 12. Correlation between serum GH and unstimulated saliva GH levels in group C (n = 51) after 12-h fasting.

5.5. SALIVARY ALBUMIN (STUDY V)

5.5.1. Patients' diseases and medications

The differences in the basic characteristics of group D, between hospitalized patients and outpatients and between those who died and survivors are given in Table 4.

Table 16. Percent distribution of use of drugs in group D. Each patient may concomitantly use several drugs.

	Hospitalized patients (n = 131)	Outpatients (n = 252)
Antimicrobial	19.8 %	6.3 %
Cardiovascular	77.9 %	65.5 %
Respiratory	13.0 %	6.7 %
Gastrointestinal	38.2 %	12.7 %
Sex hormones	1.5 %	2.4 %
Psychiatric	53.4 %	14.7 %
Neurological	6.1 %	7.1 %
Antihistamines	3.8 %	5.6 %
Endocrinological	35.9 %	19.4 %
Dermatological	0.8%	5.2 %
Analgesics	47.3 %	38.5 %
Electrolytes	9.2 %	4.8 %
Hematological	24.4 %	11.1%
Vitamins	10.7 %	2.4 %
Ophthalmological	8.4 %	6.7 %

Table 16 gives the medication groups of the hospitalized patients and outpatients. The higher percentages of systemic diseases among the outpatients are based on interview data. Both the hospitalized (39.7%) and outpatients (68.3%) had cardiovascular diseases. In the hospitalized patients, next in prevalence were endocrinologic (16.0%) and psychiatric (9.2%) diseases, whereas in outpatients musculoskeletal (56.3%), neurologic (43.7%) and urogenital (25.4%) were the most prevalent.

Table 17. Oro-dental findings of group D.

	Hospitalized patients (n = 131)	Outpatients (n = 252)
Percent edentulous	64.1	42.1***
Mean no of teeth with SD	11.0 ∂ 7.4	9.5 ∂ 9.9
Mean no of carious teeth	2.2 ∂ 2.8	0.6 ∂ 0.9**
CPI score (%)		
0	2.3	0
1	-	0.8
2	12.2	25.8
3	10.7	16.3
4	8.4	15.5**#
9	66.4	41.7
Percent with mucosal pathology	19.8	37.8 **

** p < 0.010, *** p < 0.001 between hospitalized patients and outpatients. # The significance in CPI values is calculated for total scores. Differences between patients who died and survivors were not statistically significant.

The main oro-dental findings of the hospitalized patients and outpatients are given in Table 17. The number of patients with mucosal pathology was greatest among outpatients. Among hospitalized, more mucosal changes had been recorded in patients who later died.

5.5.2. Salivary data

Mean salivary values are given in Table 18. The outpatients had significantly higher flow rates and less frequently positive yeast counts in saliva than patients taken to the hospital. Significantly higher yeast counts ($>10^4$ CFU/ml) were also observed in the hospitalized patients than in outpatients, but, contrary to our expectation, the yeast counts were more often positive in the survivors than among those who died ($p < 0.050$). Total protein and albumin concentrations were significantly higher in hospitalized patients than in outpatients ($p < 0.001$). Albumin output was also significantly higher in hospitalized patients than in outpatients ($p < 0.001$). In hospitalized patients, the salivary flow rate was negatively associated with higher than median concentrations of albumin ($r = -0.36$, $p < 0.001$) and urea ($r = -0.20$, $p < 0.010$).

The median age of the hospitalized patients was 82 years (survived 80, died 83 years), and that of the outpatients 76 years. The salivary albumin concentration was higher in all hospitalized patients and outpatients who were older than the median than in younger ones: 482.4 ∂ 333.8 μg/ml vs. 366.6 ∂ 264.6 μg/ml ($p < 0.050$) in hospitalized patients, 470.8 ∂ 320.9 μg/ml vs. 226.2 ∂ 174.7 μg/ml ($p < 0.010$) in outpatients. The albumin concentration increased statistically significantly in relation to the number of drugs used daily, both in hospitalized patients ($p < 0.050$) and in outpatients ($p < 0.050$).

Patients with the highest need for periodontal treatment (CPI score 2 - 4) did not have higher salivary albumin concentrations than those with less need for treatment. Salivary albumin did not correlate significantly with the number of concomitant systemic diseases of hospitalized patients and outpatients. Higher values were detected in patients suffering from cardiovascular and respiratory diseases than in other diagnosis groups.

The results of the logistic regression analysis relating to high salivary albumin concentration are given in Table 19. In outpatients, retaining own teeth and age were risk factors of clinical importance, while the use of analgesics was a risk factor in hospitalized patients.

In 68 % of those who died, salivary flow was below 0.7 ml/min, indicating hyposalivation. The respective figure among survivors was 47 % ($p < 0.010$). Altogether 69 % of the patients who died had suffered from dry mouth, according to the questionnaire results, compared with 59 % of survivors. The difference in xerostomia was not statistically significant, however. The strongest "saliva-associated risk factors for death" in the survival analysis were salivary flow rate (OR 2.7;

95 % CI 1.4-5.2; p < 0.010), the number of drugs used daily (OR 1.2; CI 1.0-1.3; p < 0.050) and high salivary urea concentration (OR 1.1; CI 1.1-1.2; p < 0.010).

Table 18. Salivary values of subject group D.

	Hospitalized patients (n=131)		Outpatients (n=252)
	Survived (n=84)	Died (n=47)	
Salivary flow rate (ml/min)	0.9 ± 0.6	0.8 ± 0.5	1.3 ± 0.7***
Yeast count (% positive)	84.8%	71.7% #	66.1%**
Total protein (mg/ml)	2.3 ± 1.0	2.6 ± 1.7	1.6 ± 0.8***
Albumin output (σg/min)	684.3 ∂ 396.8	700.0 ∂ 481.9	439.7 ∂ 432.8***
Albumin (µg/ml)	401.2 ± 247.3	501.1 ± 417.3	204.6 ± 179.5***
Urea (mmol/l)	6.4 ± 3.9	8.0 ± 4.8	4.9 ± 2.2* **

# p < 0.050 between survivors and hospitalized patients who died.

**p < 0.010 between hospitalized patients and outpatients.

***p < 0.001 between hospitalized patients and outpatients.

Table 19. Results of logistic regression analyses on the power of factors affecting higher than median salivary albumin values (group D).

Hospitalized patients
(n = 131)

Factor	Beta coefficient	SE	OR	95 % CI	Significance (p)
Use of analgesics	1.4	0.5	4.2	1.5 - 11.4	< 0.010
Use of vitamins	1.4	0.7	4.2	1.0 - 17.1	< 0.050
Neoplasia	2.5	1.2	12.3	1.2 - 129.0	< 0.050
Saliva flow rate	-1.3	0.5	0.3	0.1 - 0.7	< 0.010

Outpatients
(n = 252)

Factor	Beta coefficient	SE	OR	95 % CI	Significance (p)
Ophthalmological medicines	2.5	1.2	12.0	1.2 - 115.6	< 0.050
Age	0.1	0.04	1.2	1.1 - 1.3	< 0.001
Own teeth retained	1.5	0.4	4.3	1.9 - 9.3	< 0.010
High buffering capacity	-1.0	0.4	0.4	0.1 - 0.9	< 0.050

6. DISCUSSION

In the following sections the materials, methods and results of this thesis will be discussed and summarized.

6.1. SALIVARY FLOW-AND THE EFFECT OF MEDICATION ON IT (STUDIES I, II AND V)

One of the most important factors in salivary diagnosis is that the time of collection should be specified for diagnostic purposes. In the first study we did not specify the time of saliva collection. However, in that study samples were collected between 9 a.m. and 12 a.m. Our further studies with a repeated-measures-design (study II) showed that the within-subject variation of salivary flow rates during a day was significant, which is in agreement with earlier results (Ferguson and Fort, 1974). This emphasizes the need to specify the sampling time.

In the first study, the patients consisted of consecutive adult patients seeking dental treatment at the University Dental Clinic in Kuopio during the years 1992-1993. The Dental Clinic did not select the patients for the examinations, so that people from the area could freely come and participate in the study.

The salivary values obtained (study I) were in agreement with the mean values published by Parvinen and Larmas (1981) for an epidemiological sample of 1,105 adult Finns aged over 30 years: 1.79 ml/min for stimulated saliva. Parvinen and Larmas did not measure unstimulated saliva. Salivary values have been published for 629 patients from the University of Lund, Sweden, where the majority of the subjects had stimulated flow rates of 1.0 - 2.5 ml/min, and resting flow rates of 0.1 - 0.5 ml/min (Bratthall and Carlsson, 1986). The mean stimulated saliva flow rate value obtained also fall within the recommendations for reference values published in the Textbook of Cariology, representing the Nordic countries with the following value on average: 1.5 ml/min for stimulated flow (Ericson and Mäkinen, 1986). The unstimulated saliva flow rate in this study was somewhat higher than reference values for unstimulated flow, 0.25 – 0.35 ml/min (Ericson and Mäkinen, 1986). It is obviously practically impossible to obtain true unstimulated saliva, since saliva flow is always influenced by some kind of stimulation. Even slight movements of the tongue, cheeks, jaws or lips should be avoided (Tenovuo and Lagerlöf, 1994). In this study the patients spat the saliva into the test tube once a minute, which may have incluenced the flow rates of unstimulated saliva.

The range of normality is so broad that no reference values exist for the whole population (Ship et al., 1991). In order to reach the norm employed for decades in clinical chemistry and hematology, namely to give reference values based on means with two standard deviations, a lot more research must be done with saliva. The patients' subjective feelings of dry mouth are usually not associated with a particular level of saliva secretion capacity (Nederfors et al., 1997), but different results have also been published (Bergdahl, 2000), which complicates reaching an agreement on salivary reference values in clinical practice. However, information regarding salivary flow rate in adults is undoubtedly important in understanding and evaluating the relationships between salivary flow and various types of oral complaints in patients (Bergdahl, 2000).

Earlier studies, and these studies also, show that women have significantly lower flow rates than men. This difference has been suggested to be due to the size of the salivary glands (Dawes et al., 1978). In menopause, many women seem to suffer from xerostomia, which then ameliorates in older age (Parvinen and Larmas, 1982). In a study by Tarkkila et al. (2001) it was found that the occurrence of dry mouth in menopause-aged women was as high as 19.9% (n = 631) and occurrence of painful mouth 8.2% (n = 259).

In our repeated-measures-design study (study II) the correlations between unstimulated and stimulated flow rates were significant, a finding which is in agreement with earlier results. In a study by Navazesh and Christensen (1982), stimulation produced a fairly constant addition of saliva irrespective of low or high individual unstimulated flow levels. In the first sampling in the morning, the salivary flow rates were lowest, which is in agreement with other results. The collection procedure was practised several times beforehand in order to reduce the bias in repeated measurements, which is important in this kind of study. In a study by Laine et al. (1999) it was found that salivary flow rates showed significant variation between repeated samplings over a 7-week period. Also in that study the flow rate in the first sampling was the lowest, and the trend in the following samplings was increasing.

The salivary flow rates measured in the elderly (study V) were also not as low as we had expected. In our clinic, the lower reference limit for paraffin wax-stimulated salivary flow was 0.7 ml/min. Values below this indicate hyposalivation. Neither the hospitalized patients nor the outpatients had mean flow rates that low, and even patients who later died had mean flow rates above this reference limit. However, salivary flow rates in the elderly were lower than in healthy adult patients in general. Obvious explanatory factors for the lower flow rates were various diseases and use of drugs. The majority of elderly patients in eastern Finland are denture wearers, as was the case in this study. It has been found that retention of complete dentures is adversely influenced by the secretion rate of the minor salivary glands (Niedermeier and Kramer, 1992). It is probable that elderly patients will therefore have additional problems with dentures as salivary flow rates decrease.

It seems that old age as such does not cause diminished salivary flow (Shern et al., 1993; Närhi et al., 1999). However, some studies suggest that unstimulated salivary flow is related to age (Navazesh et al., 1992b, Percival et al., 1994; Yeh et al., 1998). It has also been suggested that there are some age-related alterations in salivary function (Yeh et al., 1998). In a study by Närhi et al. (1992) on very old home-dwelling people, medication was clearly the reason for reduced saliva flow. There are hundreds of different medicines which may reduce saliva flow through various mechanisms (Sreebny and Schwartz, 1997). Multiple systemic disorders and medications have been reported to cause xerostomia and/or salivary gland hypofunction (Navazesh et al., 1996). In an epidemiological study by Parvinen et al. (1984), the most frequently encountered medicines that were found to cause hyposalivation were diuretics, other drugs for cardiovascular diseases, and psychopharmaca. Medicines for cardiovascular diseases were the most frequently consumed drugs also among the elderly with reduced salivary flow in the study by Närhi et al. (1992). In 5002 dental outpatients surveyed in the universities of Kentucky and Texas, cardiovascular medicines were used daily by 23.1% of the 18- to 80- year-olds, representing the most common group of medication taken (Miller et al., 1992). Our patients in this study were over 70 years of age and suffered from a number of systemic diseases and took several drugs daily. The drugs were anticipated to cause dry mouth far more often than we observed. The xerostomia was reported by 69% of the patients who died, in comparison with 59% among the survivors. It is known from previous studies that xerostomia is not necessarily reflected in the actually measured flow rates, however (Nederfors et al., 1997).

In the first study, the results showing the significance of cardiovascular medicines in relation to salivary secretion were not unexpected in this respect (Tables 7 and 9). Clearly, the patient suffering from cardiovascular diseases is indeed a dental risk patient also regarding potential risk for reduced salivary flow due to his/her medication. However, many other medicines, in particular those with an anticholinergic effect, must also be taken into account. In the first study, the second most frequently used group of medicines found to cause reduced salivary flow were drugs for gastro-intestinal diseases; this group contains mainly H_2-receptor blocking agents but also anticholinergics, and hyposalivation is a well-known side effect of such drugs.

In the study V we also recorded subjects' medical history and daily used drugs (Table 16). Cardiovascular drugs, analgesics and endocrinological drugs were the most commonly used medications among outpatients. In the hospitalized patients, cardiovascular drugs, psychiatric drugs and analgesics were the most frequently used medications. Many patients did fall into the category of hyposalivation, and particularly those who died had had lower flow rates than the survivors or the outpatients at the start of the study. There were noticeable differences in the use of drugs, showing that the outpatients used more cardiovascular drugs and fewer phychiatric drugs. The

higher proportion of psychiatric illnesses in the hospitalized patients could be a factor simultaneously affecting salivary flow rate, too.

This study was not a two-year longitudinal investigation. Both the hospitalized patients and outpatients had been examined cross - sectionally at the beginning of the study and only mortality was followed up to two years. In retrospect it would have been interesting to take repeated saliva samples from the hospitalized patients but for practical reasons this was not possible. The examination was carried out in the acute geriatric ward and most patients were sent home, to nursing homes, or to other hospitals after their acute medical condition had been treated. The outpatients, on the other hand, were elderly patients who returned home the same day they had been examined. Unfortunately, the mean age of the outpatients was lower than that of the hospitalized patients, which is an evident bias in this study. The medical record of the hospitalized was based on the physician's examination at the time of hospitalization, while that of the outpatients was derived from their medical history and verbal report. Therefore, data regarding the number and distribution of systemic diseases and daily use of drugs are more reliable for the hospitalized patients than the outpatients. This is an evident source of error in this study, but it could not be avoided. Our principal aim was not to examine the medical status of the outpatients, but rather collect their saliva samples for comparison, because, as stated, there were no reference data on salivary albumin in the elderly. The community where the study was carried out was fairly small and the area is not densely populated. The population demographics did not allow the recruitment of enough age- and sex-matched material for this kind of study. It may therefore be anticipated that the patients represented the community, allowing us, according to our principal aim, to study the acutely hospitalized in comparison with those who were not in urgent need of medical attention.

6.2. SALIVARY BUFFERING CAPACITY (STUDY I)

Low buffering capacity of saliva is associated with a reduced salivary flow and increased risk for caries, in particular (Heintze et al., 1983). In a study by Heintze et al. (1983) it was found that low Dentobuff scores (final pH < 4.0) were found more often in women than in men. In the elderly, low Dentobuff scores were observed in 24% of over 75-year-olds, and high scores in 51% (Närhi et al., 1993). In people with diseases, such as diabetics and those suffering from malignant diseases, low buffering capacity of saliva seems to reflect systemic acidosis and may, consequently, be a sign of a worsening medical condition (Laine et al., 1992). In our study, low buffering capacity was more often found in patients with medication than in unmedicated ones, which supports the view that low

buffering capacity of saliva may indeed be associated with systemic diseases. However, the most important factor affecting salivary buffering capacity is salivary flow rate.

6.3. YEAST COUNTS (STUDIES I AND V)

The frequency of positive salivary yeast counts in these studies were somewhat higher than what has been reported in earlier studies with healthy adults. We found positive yeast counts in 63% of the medicated and in 57% of the unmedicated patients (study I), and in 66% of the outpatients and in 85% of the survived hospitalized patients (study V). In a review by Odds (1988) the prevalence of yeasts in saliva in healthy persons was found to be 37%. However, in denture wearers yeasts may be cultivated from 85% of the subjects (Vandenbussche and Swinne, 1983). In the study by Parvinen and Larmas (1981) the frequency of positive yeast counts was 59% in all subjects, and in the study of the elderly by Närhi et al. (1993) the respective figure was 75%.

In the first study we did not correlate the occurrence of yeasts with the patients' other orodental findings but the medicated subjects had more often positive yeast counts than the unmedicated ones. Yeasts are commensals of the oral cavity in the majority of adult patients but many diseases may predispose to their dissemination (Odds, 1988). Reduced salivary flow increases the risk for high yeast counts with subsequent infection, which is also supported by the results of these studies (Navazesh et al., 1995). Low buffering capacity has also been shown to be associated with high yeast counts (Närhi et al., 1993).

We found (study V) that yeast counts were more often positive in the hospitalized patients than outpatients but, astonishingly, patients who died had lower yeast counts than those who survived. We had anticipated the opposite to be true in this parameter since yeast infections are known to be prevalent in the frail elderly (Samaranayake, 1986; Stenderup, 1990; Lockhart et al., 1999). Patients who died also had elevated salivary urea levels, which is known to increase the pH of the environment. This could partly explain the lower yeast counts in these patients.

It has been found that smoking is strongly associated with the presence of yeasts and lactobacilli counts, independently of the oral status, oral hygiene or salivary factors (Sakki and Knuuttila, 1996). We did not investigate the patients' smoking habits, and this is an evident source of error in this study.

6.4. SALIVARY VISCOSITY (STUDY II)

In salivary diagnosis, viscosity has not been demonstrated as a clinically relevant variable. However, the apparent viscosity contributes to the rheological properties of saliva, and the elastic properties could be important as well (van der Reijden et al., 1993). The efficacy of saliva as a lubricant depends on its viscosity and how it changes with shear rate. This is thought to be especially important for patients using removable, partial or whole, dentures.

Many different kinds of viscometers have been used in previous salivary viscosity measurements. It has been reported that the apparent viscosity of saliva depends on the shear rate and time, so that saliva is a non-Newtonian fluid (Roberts, 1982; Schwarz, 1987). The viscometer used, a standard Brookfield digital cone/plate microviscometer, should be suitable for such systems. This apparatus gives the apparent viscosity in centipoises and shear stresses in dynes/cm^2. Such viscometers have also been used in salivary viscosity studies (Levine et al., 1987).

We studied salivary viscosity in a healthy, homogenous study group. Salivary viscosity should also be studied in medicated and diseased patients, so that we can get a better understanding of saliva and its function as a lubricant in different clinical situations. It has also been suggested that the quality of saliva could be as important as salivary flow rate to oral health and comfort (Chimenos-Kustner and Marques-Soares, 2002).

The within-subject variation of the viscosity of unstimulated saliva in repeated samplings was significant in our study. On the other hand, the viscosity of stimulated saliva seemed to remain stable through the repeated samplings. We did not find significant differences between genders in the viscosity of unstimulated or stimulated saliva. We used salivary flow rates as varying covariates in our analysis, since the viscosity of saliva can be subject to variation due to differences between the subjects' flow rates. Without the analysis of covariance, the within-subject variation in the viscosity of unstimulated saliva would not have been significant.

Our results suggest that the viscosity of unstimulated saliva is influenced by salivary flow rate, but there could be also another explanation for within-subject variation of viscosity. One possible reason for this could be changes in salivary composition in different samplings. It is assumed that the rheological properties of human saliva may be due to the salivary glycoproteins, mainly due to the high-molecular-weight mucin-glycoproteins (MG1), which are mainly secreted by the sublingual, submandibular and palatal glands. Differences in viscoelasticity between submandibular and sublingual salivas is not due to the differences in mucin concentrations in those secretions, but to the mucin species (van der Reijden et al., 1993). It seems that sublingual mucins are more elastic than submandibular and palatal mucins (van der Reijden et al., 1993).

It was also found that stimulated saliva remained viscosimetrically steady over a full day. The explanation for this could be the increased proportion of parotid secretion during masticatory stimulation and lower concentration of salivary glycoproteins in stimulated saliva. Salivary mucins, which are thought to have lubricating properties, are secreted mainly by sublingual, submandidular and palatal glands. This supports the theory of an association between salivary glycoproteins and salivary viscosity (van der Reijden et al., 1993).

The IIC for salivary flow rates and viscosity of unstimulated saliva were high, indicating that the rankings of subjects for these variables tended to be maintained across the samplings. The IIC for viscosity of stimulated saliva was not statistically significant, though. This means that the rankings of subjects for viscosity of stimulated saliva were not maintained across the samplings, but fluctuated randomly.

There were no statistically significant differences between genders in salivary viscosities, and gender did not affect the within-subject variation of salivary viscosities or flow rates. It needs to be remembered in this context that our study group comprised healthy, unmedicated students of the same age.

The results from the our pilot study indicated that stimulated saliva seems to be enzymatically steady viscosimetrically, as there were no differences in viscosimetric properties between fresh and 30 min old stimulated saliva samples stored at room temperature. This is a surprising result, since it has been generally recommended that viscosity should be determined from fresh saliva samples, but it has also been shown that the viscosity of unstimulated saliva under experimental conditions was relatively stable for the first 5-8 minutes after collection (Roberts, 1977). It would have been interesting to test this also in unstimulated saliva.

6.5. SALIVARY PROTEINS (STUDIES III, IV AND V)

It is established that salivary flow rate and composition of saliva vary rhythmically over 24 h periods (Dawes and Ong, 1973). Earlier investigations have mainly focused on inorganic constituents of saliva. The relative concentrations of the organic salivary constituents are known to depend on salivary flow rate (Rudney, 1989), but their fluctuation during a single day has not been investigated earlier. We used stimulated saliva as a test sample, since it is more easily collected, collection method is standardized and stimulated saliva is less adversely affected by storage than unstimulated saliva. Unstimulated saliva is also more difficult to collect enough for various

laboratory analysis. Hence, stimulated saliva is widely used for clinical studies and research purposes.

The repeated-measures-design experiments (studies II-III) were designed as a cross-sectional type of study, rather than a longitudinal type, in which the rhythms of single subjects are followed over several days (Ferguson et al., 1973; Ferguson and Fort, 1974). However, sampling procedure was practised several times before the saliva collections, to reduce the practise effect of repeated samplings. Another way of examining the temporal changes would have been the longitudinal method, in which the consistency of rhythms is studied in single subjects for a longer period. In most previous studies, saliva samples have been collected over a period of several days but only from a few subjects at a time (Ferguson et al., 1973). The subjects were instructed to eat breakfast at 6:30 a.m., and they were told not to eat, drink or smoke for 1 h before each sampling. However, we did not give instructions about other meal times during the day, which is an evident bias in this study. We used more time points and more subjects than most previous reports, and saliva samples were collected by the same researcher. In the light of these studies it seems that salivary sampling times between 11:00 and 14:00 are recommended, since most of the studied variables were closest to the daily mean at those time points.

There are only a few published reports on temporal variation of salivary proteins (Oberg et al., 1982; Jenzano et al., 1987; McGurk et al., 1990). Our results (study III) for these proteins are comparable with those previously reported. Our results are in agreement with the findings of Jenzano et al. (1987), who studied temporal variation of glandular kallikrein, amylase and total protein in stimulated human saliva in four repeated samplings. They also found significant differences for total protein and amylase levels within days, and the lowest values in the morning. Our observations of ranges for salivary IgA levels in stimulated whole saliva are in agreement with earlier findings in stimulated parotid fluid, although the pattern of hourly variation is different (Stutchell and Mandel, 1978). A negative correlation between stimulated flow rate and IgA concentrations has also been reported (Brandtzaeg, 1989). On the other hand, Bennet and Reade (1982) did not find any significant relationship between time of day and IgA concentration. Butler et al. (1990) found some fluctuations in IgA in whole saliva, but the levels were still more characteristic of the patient than the time of sampling. We did not find statistically significant variation in lysozyme concentrations in repeated samplings, though the trend was increasing from morning to the evening samples. However, Palenstein Helderman (1976) found variations in lysozyme in whole saliva as well as in saliva from the labial sulcus. On the whole, lysozyme concentrations of our subjects were somewhat higher than reported earlier for stimulated whole saliva (Tenovuo, 1989), though comparisons between different studies are difficult.

Our observations suggested that there was significant within-subject variation in salivary IgA, total protein, amylase and albumin levels. On the other hand, the hourly changes in lysozyme, IgM and IgG concentrations were statistically not significant.

Some relationships between salivary proteins could be seen in the results of the repeated measures experiments (study III). Amylase was negatively correlated with the other salivary proteins except total protein. Albumin, which is often used as a marker for the degree of mucositis or inflammation in the oral cavity (Isutzu et al., 1981), correlated well with IgG levels and partly with IgA levels. Lysozyme and IgG also correlated with each other. We did not find any significant relationship between lysozyme and IgA, which is in agreement with an earlier study by Rudney (1989). On the other hand, in one previous study lysozyme, lactoferrin and IgA correlated closely with each other (Rudney and Smith, 1985). On the whole, in our study the correlations remained rather steady during different samplings. However, some correlations, for example IgA and albumin, were not steady during a day, though the effect of salivary flow rate and total protein concentrations were controlled for.

It was also found that rankings of the subjects for total protein, amylase, albumin, lysozyme, IgA, IgM and IgG tended to be steady across samplings. This finding is also in agreement with that of Rudney et al. (1985), who found that subjects retain rankings for total protein, lysozyme, lactoferrin and salivary peroxidase even week-by-week.

In the repeated-measures study (studies II and III) salivary flow rates showed an increasing trend during the day. The salivary IgA, IgG, IgM and albumin concentrations were at their highest in the morning, and they showed a decreasing trend during the day. This can be partly due to the reduced flow rate in the morning. Another possible explanation could be the capacity for storage of IgA in salivary glands, so that the concentration would therefore be higher at the first sampling. On the other hand, total protein, amylase and lysozyme concentrations showed an increasing trend from the morning to the evening samples.

Variation in flow rates makes the interpretation of salivary concentrations difficult. It may be misleading to quantitate salivary protein levels without taking the salivary flow rate into consideration. Unfortunately, the reference protein for saliva has not been found, as the creatinine for urea. In this study amylase concentrations were strongly related to changes in flow rate and total protein concentration. Amylase is thought to be an indicator of acinar cell function in parotid glands (Almståhl et al., 2001), and its concentrations in this study during a day followed closely the increasing trend of salivary secretion rates and total protein (Fig. 8). It has also been found earlier that the concentration of amylase increases with the flow rate (Pedersen et al., 2002), and the profile of total salivary protein is largely influenced by the concentration of amylase (Brandtzaeg, 1989).

On the other hand, albumin showed a decreasing trend during a day. When taking salivary flow rate into consideration, the total amount of albumin (albumin output) secreted during a day is probably more stable.

In the growth hormone study (study IV), we also analyzed some basic salivary parameters in the samples collected at 8:00 after 12 hours fasting (Table 14). It may be assumed that the salivary variables were affected by fasting of twelve hours to some extent. Salivary amylase levels (39.6 U/ml ± 24.1 U/ml) were lower when compared with our results in study III (84.9 U/ml ± 45.7 U/ml; $p < 0.010$). A trend of lower total protein concentration (0.8 mg/ml ± 0.8 mg/ml) was also found (1.2 mg/ml ± 0.4 mg/ml; N.S), respectively.

When comparing the salivary values and composition of elderly patients (study V) with data from other patient materials, the observed values were not quite the same as those for adult patients representing different age groups previously reported from our laboratory (Meurman and Rantonen, 1994; Meurman et al., 1998; Rantonen and Meurman, 2000). We can compare the results of the elderly patients with the results of the repeated-measures-design (study III). When comparing total protein concentrations, we can see higher levels in hospitalized patients (survived mean 2.3 mg/ml ∂ 1.0 mg/ml, died 2.6 mg/ml ∂ 1.7 mg/ml) than in outpatients (mean 1.6 mg/ml ∂ 0.8 mg/ml; $p < 0.001$) or in healthy students (daily mean 1.2 mg/ml ± 0.4 mg/ml, $p < 0.001$). Hence, it seems that total protein levels also increase in the frail elderly population.

6.6. SALIVARY CORTISOL AND GROWTH HORMONE (STUDY IV)

These two hormones (cortisol and GH) were monitored from saliva because previous studies on the metabolism of fasting have shown that concentrations of these stress hormones in blood increase during fasting. GH stimulates lipolytic activity and ketogenesis during fasting (Keller et al., 1984).

In general, salivary diagnosis for analyzing the concentrations of these hormones would provide benefit over repeated blood samples. The diagnostic utility of single plasma cortisol concentration is limited by the episodic nature of cortisol secretion and its appropriate elevations during stress. In addition, only the unbound, free fraction of cortisol is thought to be biologically active (Tyrrell et al., 1994a). One possible advantage of salivary cortisol measurement is the hypothesis that it reflects the free fraction in plasma.

GH circulates mainly unbound in plasma and has a half-life of 20-50 minutes. Plasma GH values also fluctuate widely, and functional tests must be utilized for the diagnosis of GH deficiency or excess (Tyrrell et al., 1994b).

6.6.1. Cortisol

Cortisol, which is a glucocorticoid secreted by the adrenal cortex, is a well-known stress hormone with many functions, such as the regulation of cAMP (Newsholme and Leech, 1983). It has been found that normally approximately 70% of total plasma cortisol is bound to CBG, 20% to albumin, and 10 % is free (Tyrrell et al., 1994a).

The concentrations of stress hormones in blood increase during fasting. Cortisol secretion follows a diurnal rhythm with peak concentrations in the morning, and concentrations vary greatly during the day (Newsholme and Leech, 1983). Levels are increased in patients who are acutely ill, during surgery and following trauma. Total plasma cortisol concentrations may also be increased in severe anxiety, endogenous depression, starvation, anorexia nervosa and chronic renal failure (Tyrrell et al., 1994a).

Salivary cortisol concentrations have been stated to be about 20% lower than plasma free levels. Some of the salivary cortisol metabolism has been thought to take place within the salivary glands (Read, 1989). Its functions in the oral cavity, however, are not known. Salivary cortisol determinations have been used previously to elucidate its role in stress (Cook et al., 1987; Raff et al., 2002). The previously published correlation coefficients for serum free cortisol vs. salivary cortisol concentrations range from $r = 0.86$ to $r = 0.97$. Thus, our present material fell far behind the optimum in this respect ($r = 0.47$). We measured total serum cortisol concentration in this study, which partly explains the weak correlation. Another explanation could be the relatively small number of subjects in the present study; otherwise, our test group was a homogenous one, all members being healthy and unmedicated students.

In this study, the levels of total serum cortisol were higher than widely accepted reference values for morning samples (80 – 550 nmol/l) (Tyrrell et al., 1994a), especially in women. Women had significantly higher serum total cortisol values than men ($p < 0.001$). However, these samples were taken after 12-hours fasting, and single measurement of serum total cortisol may easily be elevated in stress. Salivary cortisol levels (20.0 nmol/l ± 10.4 nmol/l) were also somewhat higher than normal reference values in the morning (12.5 nmol/l ± 4.9 nmol/l; Diacor, 1992), but there was no difference between genders. This propably reflects an elevated free fraction in serum.

An important component of the host response in periodontal inflammation is gingival crevicular fluid, with constituents mainly derived from serum. Cortisol has also been measured in gingival crevicular fluid, and the total concentration of cortisol in gingival crevicular fluid might be below 10% of that in serum (Axtelius et al., 1998). How much of that flows into saliva is not known, but it may be assumed that the amount is not significant.

6.6.2. Growth hormone

This study was the first to assess growth hormone in human saliva. However, there are published reports on GH in saliva in mice and rats (Alexander et al., 1989; Baum et al., 1999). GH is a peptide hormone which contains 191 amino acids and it is secreted in pulses from the anterior part of the pituitary gland (Becker et al., 1990). Its function in the oral cavity is not known.

Routine methods used in clinical chemistry for the assessment of GH, such as the RIA method used in the present study for serum samples, were not directly applicable for the detection of the tiny amounts of GH in saliva. Therefore, we used an immunoradiometric assay originally developed for urine samples (Helenius et al., 1989). As it is important to use identical methods for both fluids, serum GH concentrations were also measured by immunoradiometric method.

Because only minor amounts of GH were detected in saliva, it can be assumed that it is not actively excreted saliva, and that salivary GH rather represents serum ultrafiltrate and is probably part of the free fraction of plasma, devoid of any binding globulin. Further, GH is a large molecule (molecular weight 21.500) and it is unlikely that it can diffuse freely from serum to saliva as steroid hormones, for example, whose size is much smaller (Read, 1989). The tiny amounts of GH detected in saliva also speak against any particular active transport system within the salivary glands and ducts. However, we cannot exclude the possibility that GH might still be excreted into the salivary gland secretions since we sampled whole saliva, or rather oral fluid, which is known to contain a variety of degradative enzymes and microbial products which may also degrade GH molecules. Salivary GH level was about 1900-fold less than in serum in all subjects. In men, the salivary GH level was about 850-fold less, and in women about 2100-fold less than in serum.

The early morning serum GH concentration in fasting adults is normally less than 5 μg/l (Tyrrell et al., 1994b). In this study, levels were somewhat higher (8.2 μg/l ± 9.3 μg/l; RIA method). It was also found that there was significant difference between the genders in salivary GH ($p < 0.001$), and also in serum GH with both methods used ($p < 0.001$). Women had significantly higher levels than men, which has not been detected earlier. This is probably due to the 12 hours fasting and the stressful sampling situation, which might have affected more female subjects. It has been found earlier that there is no difference between genders in serum GH concentrations (Tyrrell et al., 1994b).

Thus, further studies are needed to explain the secretion and eventual function of GH in saliva. However, we found a correlation between serum and salivary GH levels, which is a surprising result

considering the size of the GH molecule. The assay for salivary GH may offer interesting perspectives for salivary research.

6.7. SALIVARY ALBUMIN (STUDIES III, IV AND V)

The levels of salivary albumin during a day in healthy adult subjects had not been studied earlier. Our results (study III) suggest that there is a statistically significant hourly within-subject variation in salivary albumin levels even in healthy adults, which is in agreement with earlier results (Butler et al., 1990). In this study we did not examine the oral or dental status of the subjects, so we do not know the degree of mucositis or gingival inflammation in the study subjects. However, we have good reason to believe that the oral health status of our study subjects was above average, as the subjects were dental or medical students and very motivated to take care of their oral health. Hence, it is important to notice that salivary albumin is detecteable even in a study group of this kind.

In subject group C (study IV), the albumin levels (140 μg/ml ± 114.6 μg/ml) were somewhat lower than in the adults in study III (daily mean 167 σg/ml ± 70.7 μg/ml; N.S). However, in subject group C the samplings were made after twelve hours fasting, which may have affected the results. It is also possible to compare the albumin levels of healthy adults (study III) with the those of elderly patients (study V). As was expected, the albumin levels were indeed higher in the hospitalized patients (survivors, mean 401.2 ∂ 247 σg/ml; deceased 501.1 ± 417.3 μg/ml) than in outpatients (204.6 μg/ml ± 179.5 μg/ml; $p < 0.001$)) or than in healthy adults (daily mean 167 σg/ml ± 70.7 μg/ml; $p < 0.001$). This supports the earlier results of salivary albumin levels in medically compromised patients (Henskens et al., 1996; Mellanen et al., 2001). The analytical procedures used in these studies (studies III, IV and V) were the same in our laboratory.

Among the patients who died during the two-year follow-up, albumin concentrations were not significantly higher than in those who survived. Nevertheless, there was a highly significant difference in salivary albumin between the hospitalized patients and outpatients in general, particularly when comparing those who died with the outpatients. The same was observed in the other salivary biochemical parameters analyzed. It is possible that salivary flow rate partly explains the variation in composition. Salivary flow rates were lower in hospitalized patients than in outpatients, but there was no difference in flow rates between the survivors and deceased patients. However, salivary albumin output was also significantly higher in hospitalized patients than in outpatients. A previous study found that the prevalence of dentogenic infection among the hospitalized patients in this study group was high (Meurman et al., 1997a), and this might have an influence on albumin levels.

When looking at the results of the logistic regression analysis (Table 19), dentate status and high age were clinically apparent explanatory factors which could explain the high salivary albumin concentration of the outpatients. Old age as such probably affects mucosal integrity and function of salivary glands, and dentate patients are prone to periodontal disease, which was the case also in this group. All these factors may cause leakage of serum components into the oral cavity. Ophthalmologic medicines were also one factor affecting the higher than median salivary albumin values in this analysis. Use of ophthalmological medicines is not an obvious factor, but it may indicate problems with dry eyes and these patients may also suffer from hyposalivation. On the other hand, the number of patients using ophthalmological medicines was rather low and the variation was wide.

In the hospitalized patients, however, the strongest explanatory factors were the use of analgesic drugs and, surprisingly, vitamin supplements, while salivary flow rate had a negative effect. The use of analgesics may relate to serious diseases calling for such a therapy. The use of vitamins is not so obvious in this context, although significantly more hospitalized patients than outpatients used vitamins. This may indicate a malnourished general status at the time of hospitalization, which then led to the administration of vitamin preparations. The number of patients using vitamin supplements was low, however, which also explains the large variation. The same holds true to patients suffering from neoplasia.

Finally, there are very little data on the contribution and function of the minor salivary glands regarding old age and systemic disease (excluding Sjögren's syndrome) so that albumin, for example, has not been analyzed in the secretions of these glands (Ferguson, 1999). But for practical reasons it was not possible in the this study to investigate biochemical constituents collected from glandular secretions. There is an obvious need for such a study in the future.

6.8. SALIVARY UREA (STUDIES IV AND V)

In healthy adult patients (study IV) salivary urea levels were 3.1 mmol/l $\pm$ 1.9 mmol/l after 12 hours fasting. The urea levels in healthy adults were significantly lower than in elderly subjects (study V; deceased hospitalized patients mean 8.0 $\pm$ 4.8 mmol/l; outpatients mean 4.9 $\pm$ 2.2 mmol/l, $p < 0.001$). It has earlier been suggested that salivary urea levels reflect the progression of renal dysfunction and may even serve as a diagnostic criterion (Khramov et al., 1994; Al Nowaiser et al., 2003). Our results are in agreement with these earlier findings, since in the study V patients were medically compromised elderly people.

Salivary urea level was statistically significantly higher in men than in women after 12 hours fasting (Table 13, $p < 0.050$). To our knowledge, there are no earlier reports on this. However, these are preliminary findings of differences between genders in salivary urea levels. More studies are needed to find out salivary urea concentrations in different patient groups and in genders.

6.9. SUMMARY

To sum up, these studies confirmed that the patients' gender and systemic medication are the most important factors affecting salivary flow rates. Since other salivary parameters mainly depend on the secretion capacity, patients with reduced flow rate may suffer from inadequate buffering capacity and become liable to yeast infection, too.

Saliva has many diagnostic uses (Mandel, 1989), but there are a number of physiological and methodological factors affecting salivary diagnosis, which must always be taken into account. One of the most important is that the time of collection should be specified for diagnostic and research purposes. In the light of these studies it seems that salivary sampling times between 11:00 and 14:00 are recommended, since most of the studied variables were close to the daily mean at those time points. Our studies show that the within-subject variations of viscosity of unstimulated saliva, salivary IgA, albumin, amylase and total protein during a twelve-hour period were significant. The observed within-subject variations in these concentrations suggest that these proteins are subject to short-term variation. There were no differences in viscosimetric properties between fresh and 30 min old saliva samples stored at room temperature. Observed variation of viscosity of unstimulated saliva could be an indicator of changes in salivary glycoprotein secretion. In addition, rankings of subjects for total protein, amylase, albumin, lysozyme, IgA, IgM and IgG tended to be maintained across samplings.

GH was detected in saliva in tiny amounts, about 1900-fold less than in serum. However, there was a positive correlation between salivary and serum GH concentrations. Though this correlation was low and GH in plasma could not be inferred from salivary measurements. Further studies are called for to assess the role and function of GH in the oral cavity. Taking into account the very low concentration of this hormone detected in saliva, it is probable that salivary concentrations indeed reflect passive diffusion rather than active secretion to the mouth. Therefore, it can be assumed that aspects of oral health, such as the degree of periodontal and mucosal inflammation, may affect the concentration of GH detectable in saliva. Further studies are needed in this respect, too.

We found higher salivary albumin concentrations and albumin outputs in the frail elderly. More studies are also called for to find out how salivary diagnosis could be developed as an aid in clinical

decision making in future oral health assessments in the elderly. Salivary concentrations of serum proteins offer interesting perspectives in this regard.

7. CONCLUSIONS

1. The results emphasize the need for taking the patients' gender and systemic medication into account in all salivary diagnosis.

2. There was a significant within-subject variation in viscosity of unstimulated saliva. The results also emphasize the need to specify accurately the time of saliva collection.

3. Rankings of subjects for the salivary variables tended to be maintained across different samplings. The observed within-subject variations in salivary IgA, albumin, amylase and total protein concentrations suggest that these proteins are subject to short-term variation.

4. Salivary GH concentrations were 1900-fold lower than the respective values in serum but a positive correlation was found between salivary and serum GH levels.

5. There were significantly higher salivary albumin concentrations and albumin outputs in the frail elderly.

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