Saliva and Dental Caries

M. Lenander-Lumikari*, V. Loimaranta

Department of Cariology and Turku Immunology Centre, Institute of Dentistry, University of Turku, Lemminkäisenkatu 2, FIN - 20520 Turku, Finland, *corresponding author, marlen@utu.fi

Adv Dent Res 14:40-47, December, 2000

Abstract – Caries is a unique multifactorial infectious disease. Our understanding of etiological factors, the progress of the disease, and the effectiveness of prophylactic procedures have led us to believe that we understand the disease. However, we still have too few answers to many questions: "Why can we not predict who will get the disease?" "Why do we not become immunized?" "How much saliva is enough?" or "Which salivary components are protective?" and "Which salivary components predispose for caries?" It is generally accepted, however, that saliva secretion and salivary components secreted in saliva are important for dental health. The final result, "caries to be or not to be", is a complex phenomenon involving internal defense factors, such as saliva, tooth surface morphology, general health, and nutritional and hormonal status, and a number of external factors-for example, diet, the microbial flora colonizing the teeth, oral hygiene, and fluoride availability. In this article, our aim is to focus on the effects of saliva and salivary constituents on cariogenic bacteria and the subsequent development of dental caries.

Human saliva not only lubricates the oral tissues, making oral functions such as speaking, eating, and swallowing possible, but also protects teeth and oral mucosal surfaces in different ways. The lubricating and antimicrobial functions of saliva are maintained mainly by resting saliva. Stimulation of saliva results in a flushing effect and the clearance of oral debris and noxious agents.

However, the protective functions of saliva are not limited to the above-mentioned functions. Recent studies have revealed a large number of functions, mediated by both the inorganic and organic components of saliva, that should be considered in assessments of the effects of human saliva on dental caries. Some of these studies have introduced a new approach to dental caries from being a bacterially induced multifactorial disease to a disease which may also be influenced by inherited salivary factors. Such genetically regulated salivary components may influence both the colonization and the clearance of micro-organisms from the oral cavity.

Caries–Who, When, and Where?

The notion that dental caries in animals is an infectious, transmissible disease was first demonstrated by Keyes (1960). Since then, a group of phenotypically similar bacteria, collectively known as mutans streptococci, has been implicated as the principal bacterial component responsible for the initiation and the development of dental caries (Loesche, 1986).

The tooth surface is unique among all body surfaces in two ways. First, it is a non-shedding hard surface, and, second, this surface is introduced into the human mouth during the first years of life. The earliest point at which the cariogenic mutans streptococci may become established is when the first teeth erupt. Solid surfaces are required for both streptococcal colonization and multiplication (Loesche, 1986).

The relationship between the establishment of mutans streptococci and the initiation of dental caries in young children has been extensively studied. Several studies have shown that children who experience colonization by mutans streptococci early in life are at greater risk of developing dental caries than those who are colonized later (Alaluusua and Renkonen, 1983; Caufield *et al.*, 1993). The extent of colonization of mutans streptococci and also, to some degree, subsequent caries activity experience are often correlated with the mother's salivary levels of mutans streptococci (Li and Caufield, 1995). Once mutans streptococci become established, they are considered difficult to eliminate, and the caries process is made possible.

The current concepts of dental caries focus on the fermentation of carbohydrates by cariogenic-bacteria-producing organic acids. Plaque bacteria produce a variety of end-products that may differ depending on the diet. When fermentable carbohydrates are present, the main organic acids produced are lactic, formic, and acetic acids (Geddes, 1975, 1981). These acids coincide with a pH drop in plaque, resulting in demineralization of the tooth (Loesche, 1986; Nyvad and Fejerskov, 1996) and creating an environment which is advantageous for further growth of *Streptococcus mutans* (Bradshaw *et al.*, 1989; Dashper and Reynolds, 2000). In addition to acid production, mutans streptococci express a wide range of virulence factors that are responsible for the cariogenicity of the dental plaque. However, saliva provides the main host defense systems against these virulence factors, and the balance between de- and remineralization is continuously affected by the interaction of bacterial virulence factors and host defense.

The final result, "caries to be or not to be", is a complex phenomenon (Fig. 1) involving internal defense factors, such as saliva, tooth surface morphology, general health, and nutritional and hormonal status, and a number of external factors-for example, diet, the microbial flora colonizing the teeth, oral hygiene, and fluoride availability. In this article, our aim is to focus on the effects of saliva and salivary constituents on cariogenic bacteria and the subsequent development of dental caries.

Salivary Flow Rate, Buffer Effect, and Dental Caries

Probably the most important caries-preventive functions of saliva are the flushing and neutralizing effects, commonly referred to as "salivary clearance" or "oral clearance capacity" (Lagerlöf and Oliveby, 1994). In general, the higher the flow rate, the faster the clearance (Miura *et al.*, 1991) and the higher the buffer capacity (Birkhed and Heintze, 1989).

Reduced salivary flow rate and the concomitant reduction of oral defense systems may cause severe caries and mucosal inflammations (Daniels *et al.*, 1975; Van der Reijden *et al.*, 1996). Dental caries is probably the most common consequence of hyposalivation (Brown *et al.*, 1978; Scully, 1986). Caries lesions develop rapidly and also on tooth surfaces that are

Key Words

Saliva, dental caries, buffer effect, adhesion, aggregation, antimicrobial agents.

Presented at the 16th International Conference on Oral Biology (ICOB), "Saliva in Health and Disease", held in Chantilly, Virginia, USA, April 9-12, 2000, sponsored by the International Association for Dental Research and supported by Unilever Dental Research

usually not susceptible to caries. Subjects with impaired saliva flow rate often show high caries incidence (Papas *et al.*, 1993; Spak *et al.*, 1994) or caries susceptibility (Heintze *et al.*, 1983). It must be emphasized, however, that no linear relationship exists among salivary secretion rate, caries activity, and DMFS/DMFT values (Birkhed and Heintze, 1989; Russell *et al.*, 1990). Only weak or no association between saliva secretion rates and caries incidence has been shown (Mandel, 1987, 1989; Russell *et al.*, 1991). Major and minor salivary gland secretion rates have also been assessed and correlated to the sensation and complaints of dry mouth (xerostomia), objectively reduced saliva secretion (hyposalivation), as well as to various oral health measures, and yet there is an unanswered question: How much saliva is enough? (Fox *et al.*, 1987; Ship *et al.*, 1991).

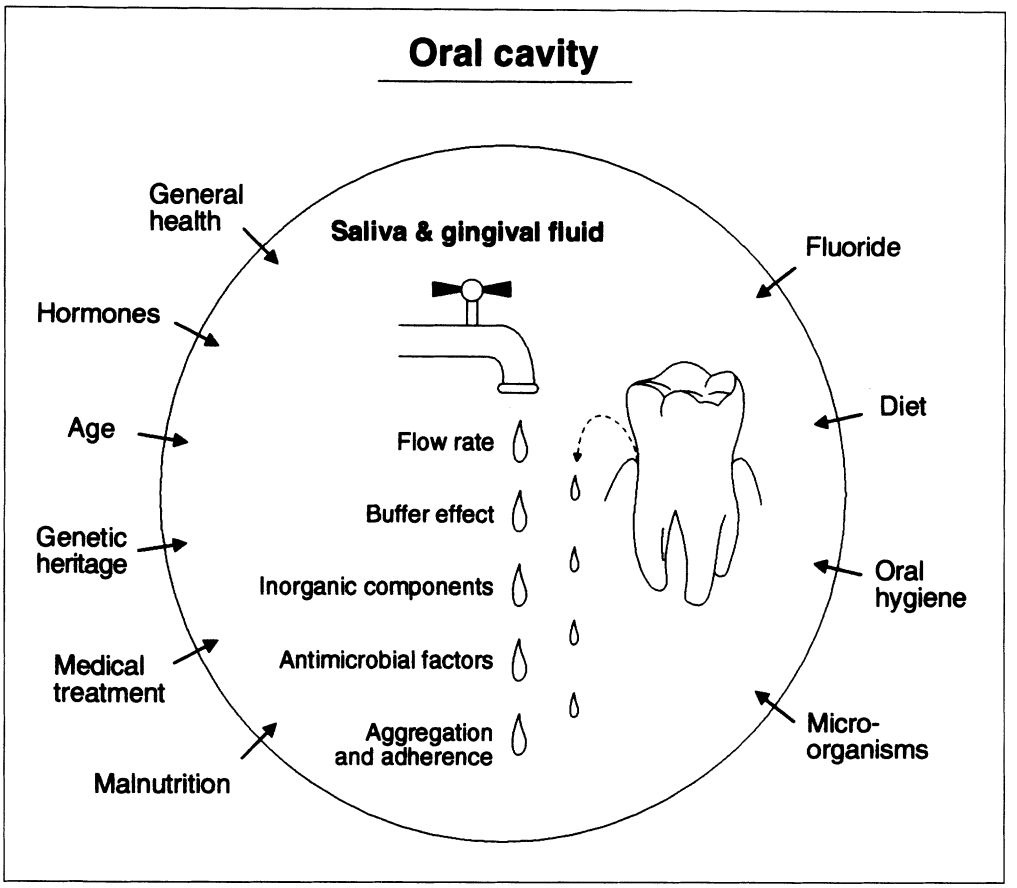

Fig. 1 — A schematic illustration of some of the factors affecting the development of dental caries.

The buffer capacity of both unstimulated and stimulated saliva involves three major buffer systems: the bicarbonate (HCO^-_3), the phosphate, and the protein buffer systems. These systems have different pH ranges of maximal buffer capacity (Bardow *et al.*, 2000), the bicarbonate and phosphate systems having p*K* values of 6.1-6.3 and 6.8-7.0, respectively. Since most of the salivary buffering capacity operative during food intake and mastication is due to the bicarbonate system (based on the equilibrium $HCO^-_3 + H^+ <=> CO_2 + H_2O$), sufficient saliva flow provides the oral cavity with the neutralizing components (Birkhed and Heintze, 1989). The phosphate and protein buffer systems make a minor contribution to the total salivary buffer capacity, relative to the bicarbonate system. The phosphate system is, in principle, analogous to the bicarbonate system but without the important phase-buffering capacity, and it is relatively independent of the salivary secretion rate.

A low flow rate combined with a low or moderate buffer effect clearly indicates poor salivary resistance against microbial attack (Lagerlöf and Oliveby, 1994). An inverse relationship between buffer capacity and caries experience is well-established according to Ericsson (1959), who evaluated 21 reports published up to 1956. On a population level, salivary flow rate and buffer effect show an inverse correlation (Heintze *et al.*, 1983) with caries susceptibility. Among the elderly, an inverse relationship of salivary buffer capacity in stimulated saliva has been established for both enamel (Guivante-Nabet *et al.*, 1998) and root caries (Ravald and Birkhed, 1991; Lundgren *et al.*, 1998). The salivary buffer effect in unstimulated saliva is sparsely documented. However, Larsen and co-workers (1999) have emphasized that the buffering capacity of unstimulated saliva varies so much that single measurements are not reliable for caries prediction.

The buffer effect of saliva is most obviously also affected by hormonal and metabolic changes, as well as by altered general health. It is generally accepted that the buffer effect is greater in men than in women (Heintze *et al.*, 1983). In women, the buffer effect decreases gradually, independent of flow rate, toward late pregnancy and promptly recovers after delivery (Laine *et al.*, 1988; Laine and Pienihäkkinen, 2000). The introduction of either hormone replacement therapy in menopausal women (Laine and Leimola-Virtanen, 1996) or low-dose oral contraceptives (Laine *et al.*, 1991) can slightly increase the buffer capacity.

Interestingly, although the secretion rate of stimulated saliva decreases as the degree of malnutrition increases, the buffer effect increases (Johansson *et al.*, 1992). The explanation for this phenomenon is still unclear, but a significant correlation between the degree of malnutrition and the severity of caries has been reported (Johansson *et al.*, 1992).

Carbonic anhydrases (CAs) participate in the maintenance of pH homeostasis in various tissues and biological fluids of the human body by catalyzing the reversible hydration of carbon dioxide, $CO_2 + H_2O <=> HCO^-_3 + H^+$. Eleven isoenzymes with CA activity have thus far been identified in mammals, and all of them are expressed in the alimentary tract. At least two isoenzymes are involved in salivary physiology (Kadoya *et al.*, 1987). CA II is a cytosolic, high-activity isoenzyme, expressed in the serous acinar cells of the parotid and submandibular glands. It is thought to produce bicarbonate in the saliva. CA VI is the only known secreted CA isoenzyme. It is expressed in the serous acinar cells of the parotid and submandibular glands, where it is secreted into the saliva (Kivelä *et al.*, 1999a).

The physiological role of salivary CA VI has been clarified during recent years (Kivelä *et al.*, 1999a). Low salivary concentrations of CA VI appear to be associated with increased prevalence of caries and acid-peptic diseases (Kivelä *et al.*, 1999a). Kivelä and co-workers (1999b) have shown that salivary CA VI correlates negatively with DMFT- values, especially in individuals with poor oral hygiene. In 1974, Szabó reported higher CA activity levels in caries-free children than in children with active caries. Since there is a positive correlation between CA VI concentration and salivary flow rate, and a negative correlation with the DMFT index, recent

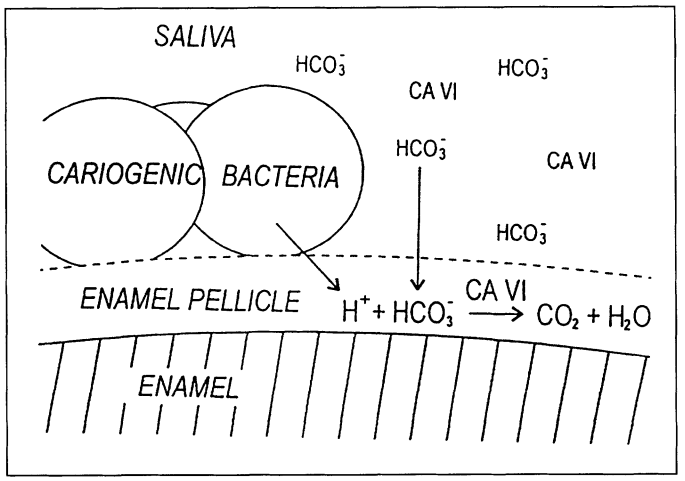

Fig. 2 — Suggested model for the function of CA VI on the dental surface. (Published courtesy of Kivelä *et al.*, 1999a)

research suggests that salivary CA VI plays a role in protecting the teeth from caries (Kivelä *et al.*, 1999a, b).

Contrary to earlier predictions, CA VI does not seem to be directly involved in the regulation of actual salivary pH or buffer capacity, and no correlation has been found between salivary CA VI concentration and mutans streptococci or lactobacilli levels (Kivelä *et al.*, 1999b). CA VI has been reported to bind to the enamel pellicle and retain its enzymatic activity on the tooth surface (Fig. 2; Leinonen *et al.*, 1999). In the enamel pellicle, CA VI may catalyze the conversion of salivary bicarbonate and microbe-delivered hydrogen ions to carbon dioxide and water.

Homeostasis of Inorganic Components

Human salivary secretions are supersaturated with respect to calcium and phosphate (Hay *et al.*, 1982; Lagerlöf, 1983), but spontaneous precipitation from saliva to dental enamel does not normally occur. This unexpected stability is mediated by a group of salivary proteins, namely, statherin, the acidic PRPs, cystatins, and histatins. These proteins differ from other salivary host defense proteins by having a specific function only for the oral environment, *i.e.*, the maintenance of the homeostasis of the supersaturated state of saliva. Interestingly, these proteins are multifunctional in that they are partly responsible for the remineralization capacity of saliva, but they also interact with some micro-organisms (Lamkin and Oppenheim, 1993).

Statherin is the only identified inhibitor of primary precipitation in saliva, and a very potent inhibitor of crystal growth. Statherin is a small, 43-amino-acid-containing protein with a highly negatively charged aminoterminal segment (Hay and Moreno, 1989). This negatively charged segment is likely to be the main inhibitory part of the molecule. According to Hay and Moreno (1989), statherin is present in stimulated saliva in concentrations sufficient to inhibit the precipitation of calcium and phosphate salts effectively. More recent studies have shown that statherin may contribute to the early colonization of the tooth surfaces by certain bacteria, such as *Actinomyces viscosus* (Gibbons and Hay, 1988).

The acidic proline-rich proteins (PRPs) account for 25-30% of all proteins in saliva, and they have high affinity for hydroxyapatite *in vitro* (Hay and Moreno, 1989). The acidic PRPs bind free calcium, adsorb to hydroxyapatite surfaces, inhibit enamel crystal growth, and regulate hydroxyapatite crystal structure (Hay and Moreno, 1989). The multifunctional properties of acidic PRPs, like statherins, are shown by their ability to promote the attachment of bacteria to apatitic surfaces (Gibbons and Hay, 1988, 1989; Gibbons *et al.*, 1991). Interestingly, the amount and quality of acidic PRPs, and agglutinins, are found to be different in caries-free and caries-active individuals (Rosan *et al.*, 1982; Stenudd, 1999).

Cystatins form a family of cystein-containing phosphoproteins, which may play a minor role in the regulation of calcium homeostasis in saliva (Johnsson *et al.*, 1991; Lamkin and Oppenheim, 1993). Phosphorylated and non-phosphorylated cystatins bind to hydroxyapatite, but the role of cystatins in the caries process is unclear.

There are very few reports on the possible correlation between the above-described proteins and dental caries. The fact that, for example, statherin, acidic PRPs, and cysteins play a key role in a protective and reparative system which is important for the integrity of the teeth is obvious. However, there are only two reports on the correlation between cystatin and caries prevalence (Table). Tabak and co-workers (1994) suggest that there is an inverse relationship between the levels of cystatin in resting whole saliva of children and their past and active caries experience, while the other study (Shomers *et al.*, 1982) found no association between cystatin concentration and caries.

TABLE — Numbers of Studies[a] on the Associations Between Salivary Components and Caries

Saliva Component	Pos. Corr.[b]	Neg. Corr.	NS
Cystatins	-	1	1
Statherin	-	-	-
Proline-rich proteins	-	-	1
α-Amylase	-	-	1
Lysozyme	-	1	10
Lactoferrin	-	-	7
Peroxidases	2	-	9
HOSCN/OSCN$^-$	1	2	4
Histatins	-	1	-
sIgA			
DMFT/DMFS	1	7	6
Active caries lesions	1	5	3
Mutans streptococci	1	5	3
IgG[c]	2	1	5

[a] List of references can be requested from the authors.
[b] The outcomes of the studies are marked as follows: pos. corr. = positive correlation; neg. corr. = negative correlation; NS = no significant association.
[c] Only studies with salivary IgG are included.

Salivary Adhesion and Bacteria-aggregating Proteins in Dental Caries

The acquired enamel pellicle is a thin film consisting mainly of salivary proteins selectively absorbed to the surface of the enamel. The pellicle protects the enamel from dissolution. Diffusion fluxes are reduced by 50% in the presence of pellicle (Zahradnik *et al.*, 1976), leading to a decreased demineralization potential of the acids secreted by bacteria (Zahradnik *et al.*, 1977). The pellicle is also a base to which the bacteria can adhere when they enter the oral cavity. The binding of bacteria is mediated by non-specific electrostatic and van der Waals forces, but also by specific interactions between bacteria and the proteins on the salivary pellicle. Thus, colonization of microbial flora on the tooth surface is strongly modified by salivary proteins (Gibbons, 1989). Several proteins-like parotid saliva agglutinins, α-amylase, statherins, mucins, acidic PRPs, and salivary immunoglobulins-are reported to bind with oral streptococci (Scannapieco, 1994). These proteins are also found in the salivary pellicle, and therefore, they are likely to mediate the specific adhesion of bacteria to tooth

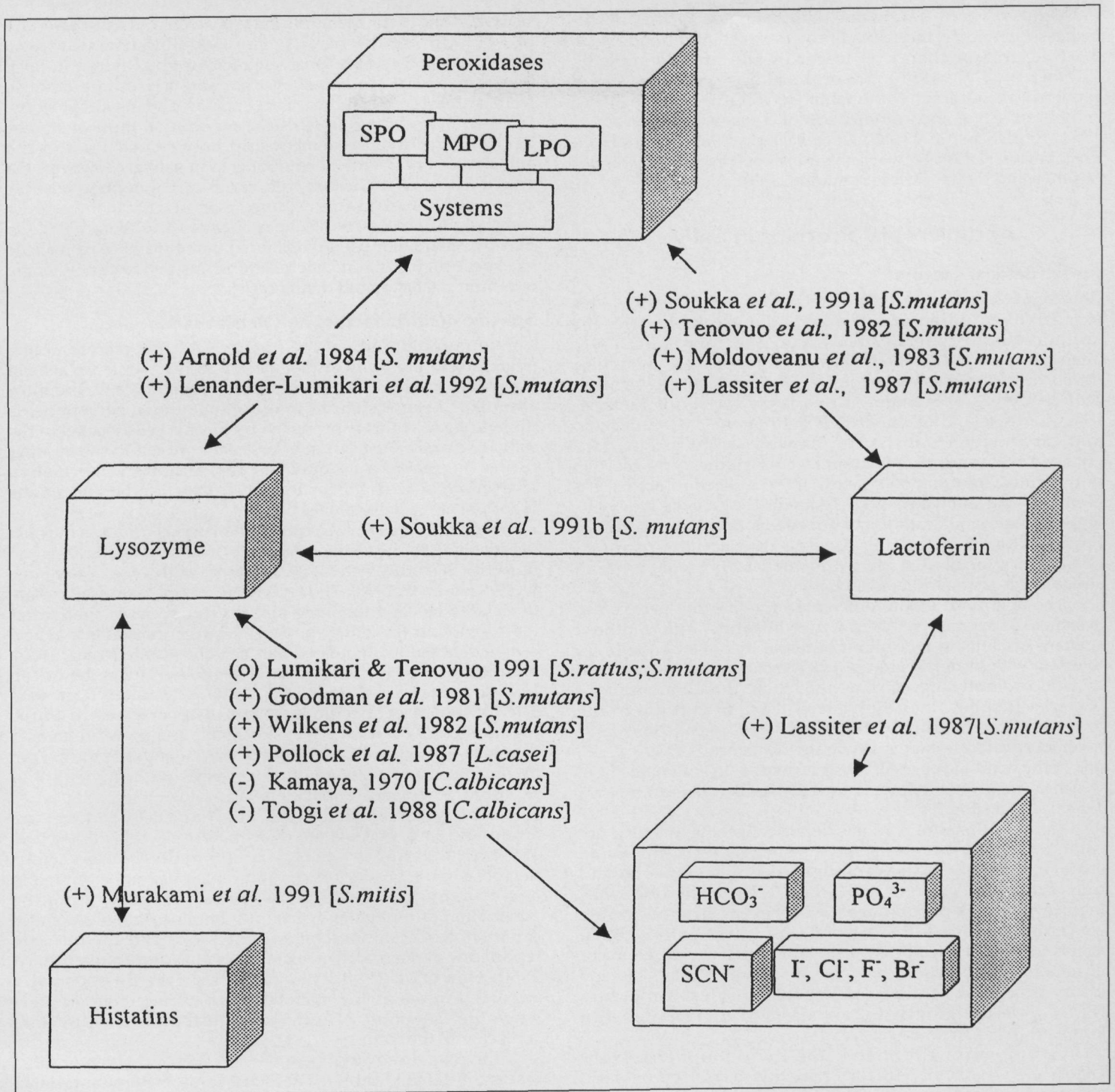

Fig. 3 — Interactions between innate host factors *in vitro*. The target organism studied is indicated in parenthesis. (+) = synergism or additive effect. (-) = inhibitory effect. (0) = no effect. (Published courtesy of Kivelä *et al.*, 1999a)

surfaces. It has been suggested that high-molecular-weight parotid saliva agglutinins, and similar proteins found in submandibular-sublingual saliva, are the most important salivary proteins in promoting the adhesion of *Streptococcus mutans* (Ericson and Rundegren, 1983; Kishimoto *et al.*, 1989; Carlén and Olsson, 1995).

On the other hand, when these same proteins exist in the liquid phase, they may promote bacterial aggregation and, hence, the clearance of bacteria from the oral cavity. The two most abundant agglutinins in saliva are high-molecular-weight agglutinin from parotid saliva and mucins. Of the mucins, the low-molecular-weight form, MG2, is more efficient in bacterial aggregation and clearance than the high-molecular-weight form, MG1 (Tabak, 1995). MG1 and MG2 proteins are products of different genes (Tabak, 1990, 1995), although it has recently been suggested that part of the low-molecular-weight mucins may be derived from high-molecular-weight mucins by the action of proteases in saliva (Slomiany *et al.*, 1996). This study, however, has not been further verified.

The ability of different salivas to promote aggregation or adhesion varies greatly among individuals. It has been speculated that the high aggregation and low adhesion activity of saliva against mutans streptococci could explain the differences in colonization susceptibility among individuals. Indeed, MG1 predominates in the saliva of caries-susceptible subjects, while the level of MG2 appears to be consistently higher in the saliva of caries-resistant

individuals. There is only one study suggesting that mucin protease activity in the saliva of caries-resistant individuals is 3.8-fold greater than that in caries-susceptible subjects (Slomiany *et al.*, 1996). Several studies, however, have reported an inverse relationship between the aggregating activity of saliva and colonization of *S. mutans* (Rosan *et al.*, 1982; Emilson *et al.*, 1989; Carlén *et al.*, 1996), and also a positive correlation between the adhesion-promoting activity of saliva and dental caries (Stenudd, 1999).

Antimicrobial Proteins in Saliva

Innate defense factors

The innate defense factors identified in saliva have been extensively studied *in vitro*, and they express different antimicrobial properties (Tenovuo and Lumikari, 1991; Tenovuo *et al.*, 1991). The modes of action of these molecules differ vastly, suggesting a long evolution during which the oral cavity has been exposed to a large variety of bacteria, fungi, viruses, and other noxious substances, *e.g.*, mutagenic and carcinogenic substances, as well as H_2O_2. The data obtained so far are mainly from *in vitro* studies, and there is only limited information on how these molecules act *in vivo* (Tenovuo and Lumikari, 1991; Tenovuo *et al.*, 1991). It is well-known that many antimicrobial proteins in saliva interact *in vitro* with each other (Fig. 2). The interactions result in additive, synergistic, or inhibitory effects on mutans streptococci, lactobacilli, or fungi.

The main oral innate defense factors are the peroxidase systems, lysozyme, lactoferrin, and histatins. *In vitro*, these proteins are known to (1) limit bacterial or fungal growth, (2) interfere with bacterial glucose uptake or glucose metabolism, and (3) promote aggregation and, thus, the elimination of bacteria. It should be emphasized that, in addition to the antimicrobial action of both salivary peroxidase and myeloperoxidase systems (Månsson-Rahemtulla *et al.*, 1987), one of the main purposes of these systems is to eliminate H_2O_2, which is highly toxic for mammalian cells (Hänström *et al.*, 1983; Tenovuo and Larjava, 1984).

Many of the antimicrobial defense systems in saliva are common to all exocrine secretions such as tears, milk, and seminal, vaginal, and gastrointestinal fluids (Tenovuo and Lumikari, 1991; Tenovuo *et al.*, 1991). Especially lysozyme, lactoferrin, and peroxidases are present in measurable concentrations in all these secretions. These antimicrobial agents are mainly synthesized in, and secreted via, the major or minor salivary glands, but a smaller amount enters the oral cavity from tissue fluid or polymorphonuclear leukocytes (PMNs) *via* the gingival crevicular fluid (Tenovuo and Lumikari, 1991; Tenovuo *et al.*, 1991).

During early childhood, the non-immune salivary factors–*e.g.*, lysozyme, salivary peroxidase, and peroxidase-generated hypothiocyanite ($HOSCN/OSCN^-$)–are present at levels similar to those in adults. However, lactoferrin, myeloperoxidase, and total protein are still significantly less abundant (Mandel *et al.*, 1983; Tenovuo *et al.*, 1987). All non-immune defense factors reach adult levels by the early teenage years (Kirstilä *et al.*, 1998) and remain at high concentrations even among elderly people with full dentition. If a considerable number of teeth are extracted, the components derived *via* gingival crevices are diminished (Närhi *et al.*, 1994).

Several attempts have been made to correlate salivary peroxidase activity, peroxidase-produced hypothiocyanite concentrations, lysozyme activity, lactoferrin or apo-lactoferrin concentrations, cystatin, histatin or proline-rich protein concentrations, and amylase activies to general, dental, gingival, or mucosal health (Table). The studies have been both cross-sectional and longitudinal. However, the literature presents controversial results. This may depend on inconsistency in study design, saliva collection methods, salivary analysis methods, statistical analysis, and the presentation of the results. The available literature was extensively and comprehensively reviewed by Rudney in 1995. Because several studies show that salivary innate defense factors affect cariogenic bacteria such as mutans streptococci, lactobacilli, and fungi *in vitro*, the expectation in most studies has been an inverse relationship between caries and the amounts of antimicrobial components in saliva. However, the only positive relationships with caries might be predicted for proteins that promote adhesion or maintain inorganic component homeostasis in the oral cavity (Rudney, 1995). On the other hand, it must be concluded that it may not be realistic to expect highly significant relationships between any single non-immune factor and dental caries.

Specific defense factors and dental caries

The immunoglobulins, IgG, IgM, IgA, and secretory IgA (sIgA), form the basis of the specific salivary defense against oral microbial flora, including mutans streptococci. The most abundant Ig in saliva, as in all other human secretions, is dimeric sIgA, which is produced by plasma cells located in the salivary glands. Two IgA subclasses are present in saliva; IgA1 forms the major component of Igs, although the relative amount of IgA2 is higher in saliva than in other secretions (Tappuni and Challacombe, 1994).

In human beings, IgG, mainly of maternal origin, is the only detectable Ig in the saliva of neonates. Salivary IgA is absent at birth but is readily detectable in infants at the age of only one week (Cole *et al.*, 1998). The IgG concentration decreases to non-detectable levels after some months but appears again after tooth eruption (Brandtzaeg, 1989). Low concentrations of IgG can be detected in stimulated parotid saliva (Brandtzaeg, 1989), but most of the IgG detected in whole saliva enters the mouth from the gingival crevicular fluid, thus originating from sera. The formation of specific IgAs in saliva correlates with the colonization of bacteria in the oral cavity. In most children over three years of age, salivary IgAs against mutans streptococci can be detected, and their amount increases with the length of exposure (Smith and Taubman, 1992).

Salivary Igs can bind to the salivary pellicle, and they are found also in dental plaque (Newman *et al.*, 1979; Fine *et al.*, 1984). In the oral cavity, Igs act by neutralizing various microbial virulence factors, limiting microbial adherence, and agglutinating the bacteria, as well as by preventing the penetration of foreign antigens into the mucosa. IgGs are also capable of opsonizing bacteria for phagocytes, which are reported to remain active in dental plaque and saliva (Scully, 1980; Newman, 1990). Phagocytosis may be especially important in modifying microbial flora during tooth eruption when high amounts of IgGs and neutrophils exist in close contact with the teeth.

The role of salivary Igs in dental caries formation is still a matter of debate (Table). There are some experimental data suggesting a protective role of the anti-streptococcal IgGs, mainly measured from serum, against caries and colonization of *S. mutans* in early childhood (Lehner *et al.*, 1978; Aaltonen *et al.*, 1987; Tenovuo *et al.*, 1987) and in adults (Challacombe *et al.*, 1984; Gregory *et al.*, 1990), but also contradictory results exist (Lehtonen *et al.*, 1984; Gråhn *et al.*, 1988; Camling *et al.*, 1991). Conflicting results are also reported for salivary IgA and dental caries, as extensively reviewed recently by Marcotte and Lavoie (1998). Comparison of different studies is complicated, however, since different samples are collected, and in some studies the Ig levels are correlated with DMFT/DMFS scores (that is, past experience of caries), whereas in other studies they are correlated with the presence of active caries (a situation which may take several months to develop), or with the levels of mutans streptococci in the mouth. It must also be noted that the presence of active caries lesions may induce the formation of specific IgGs (Challacombe, 1980; Kirstilä *et al.*, 1998), and that they may remain at a higher level for several weeks or months

after eradication of the lesions. Further, it has been postulated that part of the detected IgAs against mutans streptococci is generated by cross-reactivity with antigens from other bacteria.

Certain diseases, such as selective IgA immunodeficiency, should provide a unique model for the evaluation of the role of sIgA in the colonization of mutans streptococci and, more generally, in oral health. However, even these results are contradictory, and an increased, decreased, or lack of correlation between IgA deficiency and caries susceptibility has been reported (Tenovuo, 1998). Some studies, however, show increasing levels of other antimicrobial factors in the saliva of these patients (Tenovuo, 1998), thus supporting the previous conclusion about the clinical significance of the entire repertoire of antimicrobial components for oral health.

Concluding Remarks

The infectious nature of dental caries has already been known for decades. Ever since the recognition of *Streptococcus mutans* as the main microbial factor in the etiology of caries disease, a vast amount of work and effort has been devoted to the characterization of this bacterium. The number of published papers on mutans streptococci is enormous, exceeded only by the quantity of publications on *Escherichia coli*.

Today, we already have increased knowledge of the initiation, progression, and transmission of the disease. Still, we cannot fully explain what causes the disease in some persons but not in others, even though cariogenic microbes and other etiological factors are present. Also, the immunization methods against these bacteria are still lacking, even though promising results had already been obtained in animal studies in the 1960s.

The complexity of the oral biofilm and the microbial flora, the metabolic and adherence interactions between bacteria, *etc.*, obviously influence the outcome of the disease. However, phenotypic and genotypic differences inside a bacterial strain should also be recognized. Further, the skewed caries distribution observed nowadays in Western countries suggests that there might also be an important host-derived genetic background for the disease and, thus, the susceptibility for dental caries.

Acknowledgments

We thank Professor Jorma Tenovuo for many fruitful discussions. The late Professor Britta Månsson-Rahemtulla is acknowledged for introducing one of the authors (ML-L) to the fascinating world of saliva. The authors gratefully thank Timo Kattelus for preparing the figures.

References

Aaltonen AS, Tenovuo J, Lehtonen O-P (1987). Increased dental caries activity of preschool children with low baseline levels of serum IgG antibodies against the bacterium *Streptococcus mutans*. *Arch Oral Biol* 32:55-60.

Alaluusua S, Renkonen O-V (1983). *Streptococcus mutans* establishment and dental caries experience in children from 2 to 4 years. *Scand J Dent Res* 91:453-457.

Arnold RR, Russell JE, Devine SM, Adamson M, Pruitt KM (1984). Antimicrobial activity of the secretory innate defense factors lactoferrin, lactoperoxidase and lysozyme. In: Cariology today. Guggenheim B, editor. Basel, Switzerland: Karger, pp. 75-88.

Bardow A, Moe D, Nyvad B, Nauntofte B (2000). The buffer capacity and buffer systems of human whole saliva measured without loss of CO_2. *Arch Oral Biol* 45:1-12.

Birkhed D, Heintze U (1989). Salivary secretion rate, buffer capacity and pH. In: Human saliva: clinical chemistry and microbiology. Vol. I. Tenovuo J, editor. Boca Raton, FL: CRC Press, pp. 25-73.

Bradshaw DJ, McKee AS, Marsh PD (1989). Effects of carbohydrate pulses and pH on population shifts within oral microbial communities *in vitro*. *J Dent Res* 68:1298-1302.

Brandtzaeg P (1989). Salivary immunoglobulins. In: Human saliva: clinical chemistry and microbiology. Vol. II. Tenovuo J, editor. Boca Raton, FL: CRC Press, pp. 1-54.

Brown LR, Dreizen S, Daly TE, Drane JB, Handler S, Riggan LJ, *et al.* (1978). Interrelations of oral microorganisms, immunoglobulins, and dental caries following radiotherapy. *J Dent Res* 57:882-893.

Camling E, Gahnberg L, Krasse B, Wallman C (1991). Crevicular IgG antibodies and *Streptococcus mutans* on erupting human first permanent molars. *Arch Oral Biol* 36:703-708.

Carlén A, Olsson J (1995). Monoclonal antibodies against high molecular weight agglutinin block adherence to experimental pellicles on hydroxyapatite and aggregation of *Streptococcus mutans*. *J Dent Res* 74:1040-1047.

Carlén A, Olsson J, Ramberg P (1996). Saliva mediated adherence, aggregation and prevalence in dental plaque of *Streptococcus mutans*, *Streptococcus sanguis* and *Actinomyces* spp. in young and elderly humans. *Arch Oral Biol* 41:1133-1140.

Caufield PW, Cutter GR, Dasanayake AP (1993). Initial acquisition of mutans streptococci by infants: evidence for a discrete window of infectivity. *J Dent Res* 72:37-45.

Challacombe SJ (1980). Serum and salivary antibodies to *Streptococcus mutans* in relation to the development and treatment of human dental caries. *Arch Oral Biol* 25:495-502.

Challacombe SJ, Bergmeier LA, Rees AS (1984). Natural antibodies in man to a protein antigen from the bacterium *Streptococccus mutans* related to dental caries experience. *Arch Oral Biol* 29:1719-1784.

Cole MF, Bryan S, Evans MK, Pearce CL, Sheridan MJ, Sura PA, *et al.* (1998). Humoral immunity to commensal oral bacteria in human infants: salivary antibodies reactive with *Actinomyces naeslundii* genospecies 1 and 2 during colonization. *Infect Immun* 66:4283-4289.

Daniels TE, Silverman S Jr, Michalski JP, Greenspan JS, Path MCR, Sylvester RA, *et al.* (1975). The oral component of Sjögren's syndrome. *Oral Surg* 39:875-885.

Dashper SG, Reynolds EC (2000). Effects of organic acid anions on growth, glycolysis and intracellular pH of oral streptococci. *J Dent Res* 79:90-96.

Emilson CG, Ciardi JE, Olsson J, Bowen WH (1989). The influence of saliva on infection of the human mouth by mutans streptococci. *Arch Oral Biol* 34:335-340.

Ericson T, Rundegren J (1983). Characterization of salivary agglutinin reacting with a serotype c strain *Streptococcus mutans*. *Eur J Biochem* 133:255-261.

Ericsson Y (1959). Clinical investigations of the salivary buffering action. *Acta Odontol Scand* 17:131-165.

Fine DH, Wilton JMA, Caravana C (1984). *In vitro* sorption of albumin, immunoglobulin G and lysozyme to enamel and cementum from human teeth. *Infect Immun* 44:332-338.

Fox PC, Busch KA, Baum BJ (1987). Subjective reports of xerostomia and objective measures of salivary gland performance. *J Am Dent Assoc* 115:581-584.

Geddes DAM (1975). Acids produced by human dental plaque metabolism *in situ*. *Caries Res* 9:98-109.

Geddes DAM (1981). Studies on metabolism of dental plaque: diffusion and acid production in human dental plaque. *Front Oral Physiol* 3:78-87.

Gibbons RJ (1989). Bacterial adhesion to oral tissues: a model for infectious diseases. *J Dent Res* 68:750-760.

Gibbons RJ, Hay DI (1988). Human salivary acidic proline-rich proteins and statherin promote the attachment of *Actinomyces viscosus* LY7 to apatitic surfaces. *Infect Immun* 56:439-445.

Gibbons RJ, Hay DI (1989). Adsorbed salivary acidic-proline-rich

proteins contribute to the adhesion of *Streptococcus mutans* JBP to apatitic surfaces. *J Dent Res* 68:1303-1307.

Gibbons RJ, Hay DI, Schlesinger DH (1991). Delineation of a segment of adsorbed salivary acidic proline-rich proteins promotes adhesion of *Streptococcus gordonii* to apatitic surfaces. *Infect Immun* 59:2948-2954.

Goodman H, Pollock JJ, Katona LI, Iacono VJ, Cho M-I, Thomas E (1981). Lysis of *Streptococcus mutans* by hen egg white lysozyme and inorganic sodium salts. *J Bacteriol* 146:764-774.

Gråhn E, Tenovuo J, Lehtonen O-P, Eerola E, Vilja P (1988). Antimicrobial systems of human whole saliva in relation to dental caries, cariogenic bacteria and gingival inflammation in young adults. *Acta Odontol Scand* 46:67-74.

Gregory RL, Kindle JC, Hobbs LC, Filler SJ, Malmström HS (1990). Function of anti-Streptococcus antibodies: inhibition of virulence factors and enzyme neutralization. *Oral Microbiol Immunol* 5:181-188.

Guivante-Nabet C, Tavernier JC, Trevoux M, Berenholc C, Berdal A (1998). Active and inactive caries lesions in a selected elderly institutionalised French population. *Int Dent J* 48:111-122.

Hänström L, Johansson A, Carlsson J (1983). Lactoperoxidase and thiocyanate protect cultured mammalian cells against hydrogen peroxide toxicity. *Med Biol* 61:268-274.

Hay DI, Moreno EC (1989). Statherin and acidic proline-rich proteins. In: Human saliva: clinical chemistry and microbiology. Vol. I. Tenovuo J, editor. Boca Raton, FL: CRC Press, pp. 131-150.

Hay DI, Schluckebier SK, Moreno EC (1982). Equilibrium dialysis and ultra-filtration studies of calcium and phosphate binding by human salivary proteins. Implications for salivary supersaturation with respect to calcium phosphate salts. *Calcif Tissue Int* 34:531-538.

Heintze U, Birkhed D, Björn H (1983). Secretion rate and buffer effect of resting and stimulated whole saliva as a function of age and sex. *Swed Dent J* 7:227-238.

Johansson I, Saellström A-K, Rajan BP, Parameswaran A (1992). Salivary flow and dental caries in Indian children suffering from chronic malnutrition. *Caries Res* 26:38-43.

Johnsson M, Richardson CF, Bergey EJ, Levine MJ, Nancollas GH (1991). The effects of human salivary cystatins and statherin on hydroxyapatite crystallization. *Arch Oral Biol* 36:631-636.

Kadoya Y, Kuwahara H, Shimazaki M, Ogawa Y, Yagi T (1987). Isolation of a novel carbonic anhydrase from human saliva and immunohistochemical demonstration of its related isoenzymes in salivary gland. *Osaka City Med J* 33:99-109.

Kamaya T (1970). Lytic action on *Candida albicans*. *Mycopatholog Mycolog Appl* 42:197-207.

Keyes PH (1960). The infectious and transmissible nature of experimental dental caries. *Arch Oral Biol* 1:304-320.

Kirstilä V, Häkkinen P, Jentsch H, Vilja P, Tenovuo J (1998). Longitudinal analysis of the association of human salivary antimicrobial agents with caries increment and cariogenic micro-orgamisms: a two-year cohort study. *J Dent Res* 77:73-80.

Kishimoto E, Hay DI, Gibbons RJ (1989). A human salivary protein which promotes adhesion of *Streptococcus mutans* serotype c strains to hydroxyapatite. *Infect Immun* 57:3702-3707.

Kivelä J, Parkkila S, Parkkila A-K, Leinonen J, Rajaniemi H (1999a). Salivary carbonic anhydrase isoenzyme VI. *J Physiol* 520:315-320.

Kivelä J, Parkkila S, Parkkila A-K, Rajaniemi H (1999b). A low concentration of carbonic anhydrase isoenzyme VI in whole saliva is associated with caries prevalence. *Caries Res* 33:178-184.

Lagerlöf F (1983). Effects of flow rate and pH on calcium phosphate saturation in human parotid saliva. *Caries Res* 17:403-411.

Lagerlöf F, Oliveby A (1994). Caries-protective factors in saliva. *Adv Dent Res* 8:229-238.

Laine M, Leimola-Virtanen R (1996). Effect of hormone replacement therapy on salivary flow rate, buffer effect and pH in perimenopausal and postmenopausal women. *Arch Oral Biol* 41:91-96.

Laine M, Pienihäkkinen K (2000). Salivary buffer effect in relation to late pregnancy and postpartum. *Acta Odontol Scand* 58:8-10

Laine M, Tenovuo J, Lehtonen O-P, Ojanotko-Harri A, Vilja P, Tuohimaa P (1988). Pregnancy-related changes in human whole saliva. *Arch Oral Biol* 33:913-917.

Laine M, Pienihäkkinen K, Ojanotko-Harri A, Tenovuo J (1991). Effects of low-dose oral contraceptives on female saliva. *Arch Oral Biol* 36:549-552.

Lamkin MS, Oppenheim FG (1993). Structural features of salivary function. *Crit Rev Oral Biol Med* 4:251-259.

Larsen MJ, Jensen AF, Madsen DM, Pearce EI (1999). Individual variations of pH, buffer capacity, and concentrations of calcium and phosphate in unstimulated whole saliva. *Arch Oral Biol* 44:111-117.

Lassiter MO, Newsome AL, Sams LD, Arnold RR (1987). Characterization of lactoferrin interaction with *Streptococcus mutans*. *J Dent Res* 66:480-485.

Lehner T, Murray JJ, Winter GB, Caldwell J (1978). Antibodies to *Streptococcus mutans* serotypes in saliva for children ages to 3 to 7 years. *Arch Oral Biol* 23:1061-1067.

Lehtonen O-P, Gråhn EM, Ståhlberg TH, Laitinen LA (1984). Amount and avidity of salivary and serum antibodies against *Streptococcus mutans* in two groups of human subjects with different caries susceptibility. *Infect Immun* 43:308-313.

Leinonen J, Kivelä J, Parkkila S, Parkkila A-K, Rajaniemi H (1999). Salivary carbonic anhydrase isoenzyme VI is located in the human enamel pellicle. *Caries Res* 33:185-190.

Lenander-Lumikari M, Månsson-Rahemtulla B, Rahemtulla F (1992). Lysozyme enhances the inhibitory effects of the peroxidase system on glucose metabolism of *Streptococcus mutans*. *J Dent Res* 71: 484-490.

Li Y, Caufield PW (1995). The fidelity of initial acquisition of mutans streptococci by infants from their mothers. *J Dent Res* 74:681-685.

Loesche WJ (1986). Role of *Streptococcus mutans* in human dental decay. *Microbiol Rev* 50:353-380.

Lumikari M, Tenovuo J (1991). Effects of lysozyme-thiocyanate combinations on the viability and lactic acid production of *Streptococcus mutans* and *Streptococcus rattus*. *Acta Odontol Scand* 49:175-181.

Lundgren M, Emilsson CG, Osterberg T (1998). Root caries and some related factors in 88-years-old carriers and non-carriers of *Streptococcus sobrinus* in saliva. *Caries Res* 32:93-99.

Mandel ID (1987). The functions of saliva. *J Dent Res* 66(Spec Iss):623-627.

Mandel ID (1989). The role of saliva in maintaining oral homeostasis. *J Am Dent Assoc* 119:298-304.

Mandel ID, Turett H, Alvarez J (1983). Quantification of salivary defense factors in human infants (abstract). *J Dent Res* 62(Spec Iss):217.

Månsson-Rahemtulla B, Baldone DC, Pruitt KM, Rahemtulla F (1987). Effects of variations in pH and hypothiocyanite concentrations on *S. mutans* glucose metabolism. *J Dent Res* 66:486-491.

Marcotte H, Lavoie MC (1998). Oral microbial ecology and the role of salivary immunoglobulin A. *Microbiol Mol Biol Rev* 62:71-109.

Miura H, Isogai E, Hirose K, Wakizaka H, Ueda I, Ito N (1991). Application of sucrose indicator strip to evaluate salivary sucrose clearance. *J Dent* 19:189-191.

Moldoveanu Z, Tenovuo J, Pruitt KM, Månsson-Rahemtulla B, Mestecky J (1983). Antibacterial properties of milk: IgA-peroxidase-

lactoferrin interactions. *Ann NY Acad Sci* 409:848-850.

Murakami Y, Nagata H, Amano A, Takagaki M, Shizukuishi S, Tsunemitsu A, *et al.* (1991). Inhibitory effects of human salivary histatins and lysozyme on coaggregation between *Porphyromonas gingivalis* and *Streptococcus mitis. Infect Immun* 59:3284-3286.

Närhi TO, Tenovuo J, Ainamo A, Vilja P (1994). Antimicrobial factors, sialic acid, and protein concentration in whole saliva of the elderly. *Scand J Dent Res* 102:120-125.

Newman HN (1990). Neutrophils and IgG at the host-plaque interface on children's teeth. *J Periodontol* 51:642-651.

Newman HN, Seymour GJ, Challacombe SJ (1979). Immunoglobulins in human dental plaque. *J Periodont Res* 14:1-149.

Nyvad B, Fejerskov O (1994). Development, structure, and pH of dental plaque. In: Textbook of clinical cariology. Thylstrup A, Fejerskov O, editors. Copenhagen: Munksgaard, pp. 89-110.

Papas AS, Joshi A, MacDonald SL, Maravelis-Splagounias L, Pretara-Spanedda P, Curro FA (1993). Caries prevalence in xerostomic individuals. *J Can Dent Assoc* 59:171-179.

Pollock JJ, Lotardo S, Gavai R, Grossbard BL (1987). Lysozyme-protease-inorganic monovalent anion lysis of oral bacterial strains in buffers and stimulated whole saliva. *J Dent Res* 66:467-474.

Ravald N, Birkhed D (1991). Factors associated with active and inactive root caries in patients with periodontal disease. *Caries Res* 25:377-384.

Rosan B, Appelbaum B, Golub E, Malamud D, Mandel ID (1982). Enhanced saliva-mediated bacterial aggregation and decreased bacterial adhesion in caries-resistant versus caries-susceptible individuals. *Infect Immun* 38:1056-1059.

Rudney JD (1995). Does variability in salivary protein concentrations influence oral microbial ecology and oral health? *Crit Rev Oral Biol Med* 6:343-367.

Russell JI, MacFarlane TW, Aitchison TC, Stephen KW, Burchell CK (1990). Caries prevalence and microbiological and salivary caries activity tests in Scottish adolescents. *Community Dent Oral Epidemiol* 18:120-125.

Russell JI, MacFarlane TW, Aitchison TC, Stephen KW, Burchell CK (1991). Prediction of caries increment in Scottish adolescents. *Community Dent Oral Epidemiol* 19:74-77.

Scannapieco FA (1994). Saliva-bacterium interactions in oral microbial ecology. *Crit Rev Oral Biol Med* 5:203-248.

Scully C (1980). Comparative opsonic activity for *Streptococcus mutans* in oral fluids, and phagocytic activity of blood, crevicular fluid and salivary polymorphonuclear leukocytes in Rhesus monkeys. *Immunology* 39:101-107.

Scully C (1986). Sjögren's syndrome: clinical and laboratory features, immuno-pathogenesis, and management. *Oral Surg Oral Med Oral Pathol* 62:10-23.

Ship JA, Fox PC, Baum BJ (1991). How much saliva is enough? Normal function defined. *J Am Dent Assoc* 122:63-69.

Shomers JP, Tabak LA, Levine MJ, Mandel ID, Hay DI (1982). Properties of cysteine-containing phosphoproteins from human submandibular-sublingual saliva. *J Dent Res* 61:397-399.

Slomiany BL, Murty VLN, Piotrowski J, Slomiany A (1996). Salivary mucins in oral mucosal defence. *Gen Pharmac* 27:761-771.

Smith DJ, Taubman MA (1992). Ontogeny of immunity to oral microbiota in humans. *Crit Rev Oral Biol Med* 3:109-133.

Soukka T, Lumikari M, Tenovuo J (1991a). Combined inhibitory effect of lactoferrin and lactoperoxidase system on the viability of *Streptococcus mutans*, serotype c. *Scand J Dent Res* 99:390-396.

Soukka T, Lumikari M, Tenovuo J (1991b). Combined bactericidal effect of human lactoferrin and lysozyme against *Streptococcus mutans*, serotype c. *Microb Ecol Health Dis* 4:259-264.

Spak CJ, Johnson G, Ekstrand J (1994). Caries incidence, salivary flow rate and efficacy of fluoride gel treatment in irradiated patients. *Caries Res* 28:388-393.

Stenudd C (1999). Role of polymorphism of salivary molecules for bacterial adhesion in host susceptibility and resistance to dental caries (dissertation). Umeå, Sweden: Umeå University, pp. 4-24.

Szabó I (1974). Carbonic anhydrase activity in the saliva of children and its relation to caries activity. *Caries Res* 8:187-191.

Tabak LA (1990). Structure and function of human salivary mucins. *Oral Biol Med* 1:229-234.

Tabak LA (1995). In defence of the oral cavity: structure, biosynthesis, and function of salivary mucins. *Ann Rev Physiol* 57:547-564.

Tabak LA, Bowen WH, van Wuyckhuyse B, Frank D, Bessette J, Page DJ, *et al.* (1994). Levels of cystatin in whole saliva of children: association with oral health status (abstract). *Caries Res* 28:182.

Tappuni AR, Challacombe SJ (1994). A comparison of salivary immunoglobulin A (IgA) and IgA subclass concentrations in predentate and dentate children and adults. *Oral Microbiol Immunol* 9:142-145.

Tenovuo J (1998). Antimicrobial function of human saliva-how important is it for oral health? *Acta Odontol Scand* 56:250-256.

Tenovuo J, Larjava H (1984). The protective effect of peroxidase and thiocyanate against hydrogen peroxide toxicity assessed by the uptake of [^{3}H]-thymidine by human gingival fibroblasts cultured *in vitro*. *Arch Oral Biol* 29:445-451.

Tenovuo J, Lumikari M (1991). Organic factors in human saliva in relation to dental caries. In: Dental caries. Markers of high and low risk groups and individuals. Johnson NW, editor. Cambridge, UK: Cambridge University Press, pp. 382-399.

Tenovuo J, Moldoveanu Z, Mestecky J, Pruitt KM, Månsson-Rahemtulla B (1982). Interaction of specific and innate factors of immunity: IgA enhances the antimicrobial effect of the lactoperoxidase system against *Streptococcus mutans*. *J Immunol* 128:726-731.

Tenovuo J, Gråhn E, Lehtonen O-P, Hyyppä T, Karhuvaara L, Vilja P (1987). Antimicrobial factors in saliva: ontogeny and relation to oral health. *J Dent Res* 66:475-479.

Tenovuo J, Lumikari M, Soukka T (1991). Salivary lysozyme, lactoferrin and peroxidases: antibacterial effects against cariogenic bacteria and potential clinical applications in preventive dentistry. *Proc Finn Dent Soc* 87:197-208.

Tobgi RS, Samaranayake LP, MacFarlane TM (1988). *In vitro* susceptibility of *Candida* species to lysozyme. *Oral Microbiol Immunol* 3:35-39.

Van der Reijden WA, van der Kwaak JS, Veerman ECI, Nieuw Amerongen AV (1996). Analysis of the concentration and output of whole salivary constituents in patients with Sjögren's syndrome. *Eur J Oral Sci* 104:335-340.

Wilkens TJ, Goodman H, MacKay BJ, Iacono VJ, Pollock JJ (1982). Bacteriolysis of *Streptococcus mutans* GS5 by lysozyme, proteases and sodium thiocyanate. *Infect Immun* 38:1172-1180.

Zahradnik RT, Moreno EC, Burke EJ (1976). Effect of salivary pellicle on enamel subsurface demineralization in vitro. *J Dent Res* 55:664-670.

Zahradnik RT, Propas D, Moreno EC (1977). In vitro enamel demineralization by *Streptococcus mutans* in the presence of salivary pellicles. *J Dent Res* 56:1107-1110.