Microflora in Oral Ecosystems in Subjects with Hyposalivation due to Medicines or of Unknown Origin

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Purpose: To analyse the microbial flora in specific sites in 20 dentate (≥ 16 teeth) subjects with hyposalivation due to medicines or of unknown origin and in 20 controls matched according to age, sex and number of teeth.

Materials and Methods: The microbial samples were analysed for the following micro-organisms: mutans streptococci, *Lactobacillus* spp. and *Actinomyces* spp., associated with the development of caries, *Fusobacterium nucleatum* and *Prevotella intermedia/Prevotella nigrescens*, associated with plaque accumulation and gingivitis, *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*, frequently found in elevated numbers in periodontitis sites and *Candida albicans*, *Staphylococcus aureus*, enterics and enterococci, associated with mucosal infections. The total microbial count was also registered, together with the total number of streptococci, associated with good oral health.

Results: In the hyposalivation group, the mean age was 54 ± 8 years and the mean number of teeth 25 ± 3. The unstimulated secretion rate was 0.04 ± 0.04 mL/min and the stimulated secretion rate 0.98 ± 0.51 mL/min. Compared with the controls, the hyposalivation group tended to harbour a lower number and proportion of *F. nucleatum* on the tongue, and an increased number of enterococci in the vestibular region. In the supragingival plaque, the numbers of *Strepococcus mutans* and *Lactobacillus* spp. were significantly increased and the number of *C. albicans* tended to be increased.

Conclusion: The results indicate that subjects with hyposalivation due to medicines or of unknown origin have a supragingival plaque associated with the development of caries.

Key words: hyposalivation, microflora, mutans streptococci, *Lactobacillus* spp., *Fusobacterium nucleatum*, *Prevotella intermedia/Prevotella nigrescens*, *Candida albicans*, *Staphylococcus aureus*, enterics, enterococci

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Saliva has several important functions in the oral cavity. It moisturises the oral tissues and aids speaking and swallowing (Mandel, 1987). Saliva also rinses micro-organisms out of the mouth and contains buffering systems and antimicrobial components. Saliva is the major nutrient source for the micro-organisms, and microbial growth is supported by the slow degradation of salivary proteins and glycoproteins (Bradshaw et al, 1994). Saliva coats the oral mucosa and the teeth. Proteins in the saliva coating promotes the adherence of certain micro-organisms.

About 25% of the adult population experiences dry mouth – xerostomia (Nederfors et al, 1997; Sreebny and Valdini, 1988). In most of those subjects reduced salivary secretion rates can be determined. Hyposalivation is when the unstimulated secretion is ≤ 0.1 ml/min and/or the stimulated secretion rate is ≤ 0.7 ml/min. Common reasons

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for hyposalivation are radiation therapy in the head and neck region, inflammation in the salivary glands, hormonal changes and psychological disorders. Side-effects from medication is the most common reason for hyposalivation. It is not unusual, however, that the reason for hyposalivation cannot be determined. The majority of these subjects are women between 45 and 65 years old. In our previous study we found that subjects experiencing xerostomia with an unstimulated secretion rate ≤ 0.1 ml/min had an increase in *Lactobacillus* spp. in rinsing samples compared with subjects with normal salivary secretion rates (Almståhl and Wikström, 2003). The total number of anaerobically growing micro-organisms and the numbers of streptococci, mutans streptococci, *Fusobacterium nucleatum, Prevotella intermedia/Prevotella nigrescens*, *Staphylococcus aureus, Candida albicans* and enterics were comparable with the numbers found in controls with normal salivary secretion rates. In subjects with primary Sjögren's syndrome we found that samples taken from specific sites (tongue, mucosa, supragingival tooth surface and gingival crevice region) revealed further differences in microflora than the rinsing samples (Almstahl et al, 1999; Almståhl et al, 2001a). It is therefore likely that this is the case also for subjects with hyposalivation of other origins. Since this is a large group in which microbially induced oral health problems are common, knowledge about their oral microflora in specific sites is important.

For this purpose, microbial samples were collected and analysed from the dorsum of the tongue, the smooth mucosa, the supragingival tooth surfaces and the gingival crevice region in subjects with hyposalivation due to medicins or of unknown origin (not inflammation in the salivary glands or radiation therapy in the head and neck region) and from controls with normal salivary secretion rates. The samples were analysed for the following micro-organisms: mutans streptococci, *Lactobacillus* spp. and *Actinomyces* spp., associated with the development of caries (Van Houte, 1994), *F. nucleatum* and *P. intermedia/P. nigrescens*, associated with plaque accumulation and gingivitis (Moore et al, 1982), *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*, frequently found in elevated numbers in periodontitis sites (Renvert et al, 1990) and *C. albicans*, *S. aureus*, enterics and enterococci, associated with mucosal infections (Dahlen et al, 1992). The total microbial count was also registered, together with the total number of streptococci*, Streptococcus sanguis* and *Streptococcus salivarius*, associated with good oral health (Socransky and Manganiello, 1971).

MATERIALS AND METHODS

The study was approved by the Ethics Committee at Göteborg University.

The subjects with hyposalivation had experienced xerostomia for ≥ six month and had been referred to the Department of Oral Diagnosis, Faculty of Odontology, Sahlgrenska Academy, Göteborg, for lip biopsy. The criteria for being included in the hyposalivation group were an unstimulated secretion rate of ≤ 0.1 mL/min, ≥ 16 teeth and no antibiotics during the nearest two preceding months. They should also show no signs of inflammation in the salivary glands on biopsy or have a history of radiation therapy in the head and neck region. The 20 first subjects fulfilling the criteria were included in the study after giving their informed consent. Each subject was matched according to age, sex and number of teeth with a healthy control with normal salivary secretion rates. All participants were asked about their general health, medication, smoking and snuff habits, and subjective feeling of oral dryness. The subjects with hyposalivation were also asked about their use of saliva-stimulating agents and fluorides. At two appointments, 9 ± 7 days apart (median 7, range 2 – 30), the unstimulated and the stimulated secretion rates were measured and microbial samples taken. A clinical oral examination was performed at the first appointment. All measurements and examinations were performed between 8 and 11am by author AA.

The clinical variables registered were: status of the mucosal membranes, number of teeth, fillings and crowns, presence of plaque along the gingival margin after staining, bleeding following probing and periodontal pocket probing depths of ≥ 5 mm at four sites on each tooth. The unstimulated secretion rate was measured for 15 minutes. The subject leant forward and passively allowed the saliva to flow into a graduated test-tube. The stimulated secretion rate was measured using paraffin wax. The first saliva was swallowed, and then all saliva secreted during five minutes was collected in an ice-chilled test-tube. The pH and buffer capacity were measured in the stimulated saliva with a pH meter (Metrohm 632, Herisau, Switzerland) – with

the assay used "normal" pH range between pH 6.3 and 7.2 and "normal" buffer capacity between pH 5.0 and 7.0 (Ericsson, 1959).

Microbial sampling

Microbial sampling was performed after determination of the unstimulated secretion rate and before measuring of the stimulated secretion rate and the clinical examination. Before sampling the dorsum of the tongue, the buccal mucosa and the vestibulum, saliva was wiped off with sterile compresses. This was done to equalise the two groups. For sampling of the dorsum of the tongue, a plastic spatula with a circular hole (diameter 1.5 cm) was placed on the dorsal part of the tongue and the sample was taken with a sterile cotton pellet, which had been immersed in VMGA I (Möller, 1966). First, the pellet was swept along the margins of the hole and then back and forth over the area inside the hole. Sterile cotton sticks, which had been immersed in VMGA I, were used to collect the samples from the buccal mucosa and from the vestibulum in the molar region. The samples collected from the left and right buccal mucosa, centrally in the molar region, were pooled, as were the samples collected from the upper right and lower left vestibulum in the molar region. Supragingival plaque was collected with sterile toothpicks. The samples from between the upper right first and second molar and between the lower left first and second molar and buccally along the gingival margin on the upper right and the lower left first molar were pooled. Four gingival crevicular sites were sampled using the paper point technique (Renvert et al, 1990). The sites that were sampled and pooled were mesially on the upper right first molar, distally on the first premolar, mesially on the lower left molar and distally on the lower left premolar. Two paper points were used at each site. All the microbial samples were transported to the laboratory in bottles containing 3.3 mL of transport medium, VMGA III (Möller, 1966), and processed within four hours.

Cultivation media

Cultivation media used were Brucella agar (BBL Microbiological Systems, Cockeyville, MD, USA) with 50 ml/l of defibrinated horse blood, 20 ml/l of hemolysed human blood and 0.5 mg/l of menadione, Mitis-Salivarius (MS) agar (0298; Difco, Detroit, MI, USA), Mitis-Salivarius-Bacitracin (MSB) agar (Gold et al, 1973), Rogosa-SL agar (4080; Difco), CFAT agar plates (Zylber and Jordan, 1982), Staphylococcus agar (Staphylococcus medium 110: 0297; Difco), Drigalski agar (Vahlne, 1945), Enterococcus agar (Isenberg et al, 1971), Sabouraud-Dextrose agar (Difco) with 100 mg/l of tetrazoliumchloride (Sabouraud T) and Trypticase-Soy-serum-Bacitracin-Vancomycin (TSBV) agar (Slots, 1992).

Laboratory analysis

Dilutions of the samples were performed in VMGA I (Möller, 1966). For inoculation of TSBV agar plates, undiluted sample was used. All agar plates were inoculated from dilutions 10^{-1}. Plates with MSB agar, Rogosa agar and CFAT agar were also inoculated from dilution 10^{-3}. Plates with Brucella agar and MS agar were inoculated from dilutions 10^{-2}, 10^{-3} and 10^{-4}. Brucella agar plates were incubated using the hydrogen combustion technique (Möller and Möller, 1961) at 36°C for five to seven days. Plates with MS agar, MSB agar, Rogosa agar, CFAT agar and TSBV agar were incubated in an atmosphere of 90% CO_2 and 10% N_2 at 36°C for 3-5 days. Plates with Sabouraud T-, Staphylococcus-, Drigalski- and Enterococcus agar were incubated aerobically at 36°C for three to five days.

The detection limit was 100 colony-forming units (cfu)/mL for all species except *A. actinomycetemcomitans* where it was 10 cfu/mL. If possible, the number of the different micro-organisms in a sample was calculated from their number on a plate giving 30-300 colonies. The total number of bacteria growing anaerobically and the numbers of *P. intermedia/P. nigrescens, P. gingivalis* and *F. nucleatum* were calculated from their growth on Brucella agar plates. *P. intermedia/P. nigrescens* was identified as black, indole-positive colonies of Gram-negative coccoid rods showing dark-red fluorescence in long-wave (360 nm) UV light. *P. gingivalis* was identified as greenish-black colonies with Gram-negative coccoid rods not showing fluorescence. *F. nucleatum* was identified as grey colonies with a nacreous appearance of Gram-negative long and slender cells with tapering ends. Representative colonies were subjected to carbohydrate fermentation tests. On MS agar plates, *S. salivarius* was identified as large, light-blue colonies of Gram-pos-

itive cocci and colonies that were small, firm, and adherent were identified as *S. sanguis*. *S. mutans* and *S. sobrinus* were identified on MSB agar plates as Gram-positive cocci. *S. mutans* was identified as small, mucoid and irregular colonies and *S. sobrinus* as creamy marzipan-like colonies. *Lactobacillus* spp. were calculated from their growth on Rogosa agar plates and identified as Gram-positive rods. *Actinomyces* spp. were identified as small, yellow and high colonies of Gram-positive rods on CFAT agar plates. *S. aureus* was calculated from its growth on Staphylococcus agar plates. Colonies of *S. aureus* were distinguished from *Staphylococcus epidermidis* by their ability to degrade DNA on DNA agar plates (Difco). The number of *C. albicans* was calculated from its growth on Sabouraud T agar plates. It was identified as lustreless and creamy whitish-pink, or pink, colonies. Enterics were identified as large yellow or green colonies of Gram-negative rods on Drigalski agar plates and enterococci on Enterococcus agar plates as small brown colonies of Gram-negative cocci surrounded by a black zone. *A. actinomycetemcomitans* was identified on TSBV agar on the basis of a star-shaped colony morphology of coccoid rods and a positive catalase reaction. In doubtful cases, further identification was performed with API biochemical tests.

Statistical methods

To normalise the microbial data, the numbers were logarithmically transformed. Zero counts were treated as one colony-forming unit/mL. For the analysis of possible differences between the two groups, Student's two-sample (unpaired) *t* test was used. Due to the multiple influence aspect, single significances should be interpreted with care.

RESULTS

The hyposalivation group consisted of 18 women and two men with a mean age of 54 ± 8 years (median 54 years). The subjects had experienced xerostomia for 6 ± 8 years (median three years). In the hyposalivation group, 18 of the subjects used between one and five medicines. Most frequently used were: anti-depressants (five subjects), anti-rheumatics (four subjects), anti-inflammatory drugs (four subjects) and estrogen (three subjects). Six of the subjects were on medication with hyposalivation as a possible side-effect. Four of the controls used between one and three medicines; anti-hypertensives (three subjects), blood-thinning medicins (two subjects) and estrogen (two subjects). Five of the subjects in the hyposalivation group and one of the controls were smokers. None of the subjects in either group showed clinically visible signs of mucosal infection. Six of the subjects in the hyposalivation group and eight controls had sites with periodontal pocket probing depths of ≥ 5 mm (data not shown). For these subjects, the number of sites with pocket probing depths of ≥ 5 mm varied between one and eight sites. The mean pocket-probing depths of these sites were 5.3 ± 0.5 mm in the hyposalivation group and 5.7 ± 0.7 mm in the controls. In the hyposalivation group, three subjects used sodiumfluoride rinse daily, six subjects used chewing-gum and/or tablets with sodiumfluoride and two subjects used xylitol-sweetened chewing-gum daily.

The salivary secretion rates, pH and buffer capacities are shown in Table 1. One subject in the hyposalivation group had an undetectable unstimulated secretion rate. For 13 of the subjects with hyposalivation, the stimulated secretion rate was > 0.7 mL/min, varying between 0.8 and 2.0 mL/min. Seven hyposalivation subjects had a stimulated secretion rate varying between 0.3 and 0.7 mL/min. The pH in the stimulated saliva was normal for all subjects in both groups. Nine of the subjects in the hyposalivation group and four of the controls had a final pH ≤ 5.0, indicating a low buffering capactity, varying between 3.4 and 5.0 and between 3.8 and 4.8, respectively.

When the total microbial count per cm^2 in the sampled sites was calculated, 19 of the hyposalivation subjects and 19 controls displayed a higher microbial density on the tongue than on the buccal mucosa and in the vestibulum. In the hyposalivation group, the median total counts per cm^2 were 3×10^5 on the tongue, 3×10^3 on the buccal mucosa and 9×10^3 in the vestibulum. The corresponding numbers for the controls were 5×10^5, 3×10^5 and 7×10^3. Microbial species identified at the specific sites are shown in Figs 1-5.

Dorsum of the tongue

The total microbial count on the dorsum of the tongue was similar in the hyposalivation group and in the control group (Fig 1). The proportion of strep-

Table 1 Salivary secretion rates (mean of two measurements) and clinical features in the 20 subjects with hyposalivation (Hyposal.) and in the 20 controls. Molars and premolars with crown therapy were registered as 5 filled surfaces, and front teeth with crown therapy as 4 filled surfaces. N.S = not significant.

Subjects	Unstimulated secretion rate (ml/min)	Stimulated secretion rate (ml/min)	pH	Buffer capacity	Number of teeth	Number of crowned teeth	Number of filled surfaces	Surfaces with plaque along gingival margin (%)	Surfaces with bleeding on probing (%)
Mean ± SD									
Hyposal.	0.04 ± 0.04	0.98 ± 0.51	7.5 ± 0.3	5.2 ± 1.1	25 ± 3	4 ± 5	58 ± 23	48 ± 19	27 ± 16
Controls	0.31 ± 0.17	2.06 ± 0.68	7.6 ± 0.1	5.8 ± 0.9	26 ± 2	3 ± 4	51 ± 20	39 ± 25	25 ± 14
	p < 0.001	p < 0.001	N.S	N.S		N.S	N.S	N.S	N.S
Median									
Hyposal.	0.04	0.97	7.4	5.5	26	2	60	52	25
Controls	0.26	1.92	7.6	6.1	27	3	47	33	22
Rang									
Hyposal.	0.00 – 0.10	0.31 – 2.01	6.8 – 7.9	3.4 – 6.6	18 – 29	0 – 17	15 – 103	14 – 75	2 – 70
Controls	0.13 – 0.71	0.73 – 3.89	7.3 – 7.8	3.8 – 6.7	21 – 31	0 – 10	17 – 79	9 – 91	5 – 57

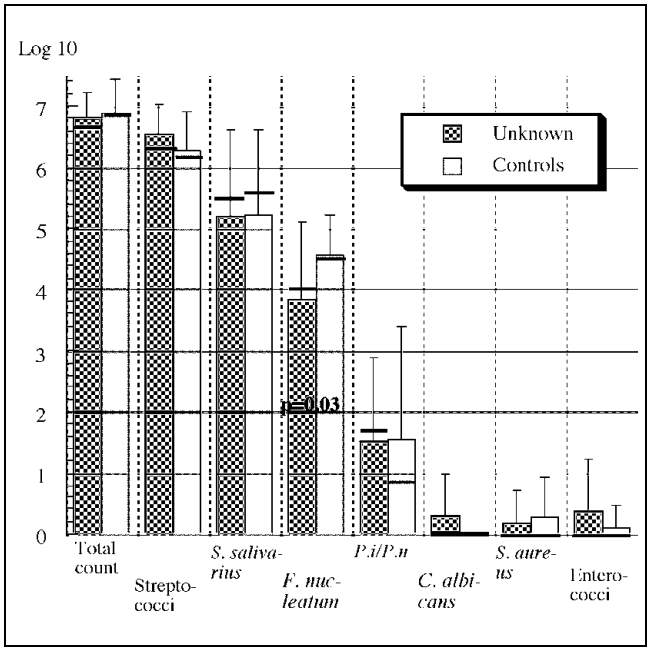

Fig 1 Numbers (mean of two samplings) of micro-organisms on the dorsum of the tongue (the sampled area was 1.8 cm^2) in the Unknown group (n = 20) and in the controls (n = 20). Mean (bars) + SD and median (lines) values are given. *P. i/P. n* stands for *P. intermedia/P. nigrescens*.

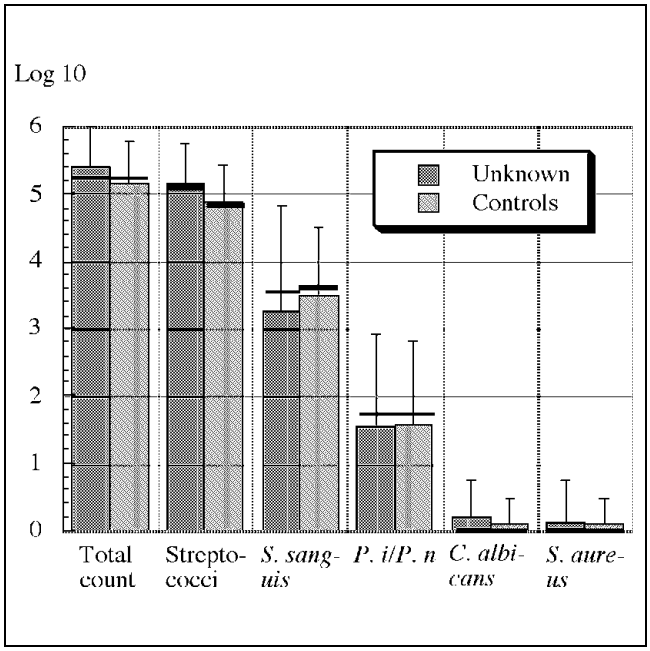

Fig 2 Numbers (mean of two samplings) of micro-organisms on the buccal mucosa (the sampled area was 6 cm^2) in the Unknown group (n = 20) and in the controls (n = 20). Mean (bars) + SD and median (lines) values are given. *P. i/P. n* stands for *P. intermedia/P. nigrescens*.

tococci of the total count, however, was about twice as high in the hyposalivation group as in the controls (mean ± SD: 61 ± 32 and median: 60% compared with 31 ± 25 and 26%). *F. nucleatum* was detected in 19 hyposalivation subjects and in all controls and *P. intermedia/P. nigrescens* in 12 hyposalivation sub-

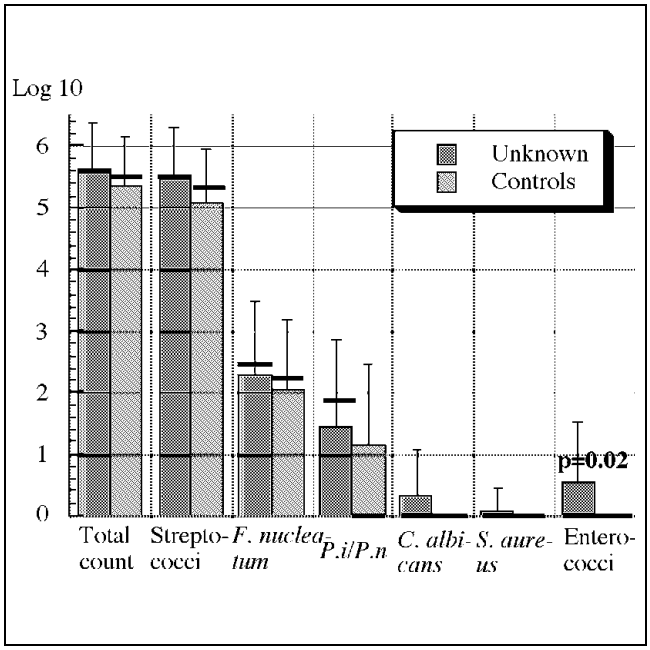

Fig 3 Numbers (mean of two samplings) of micro-organisms on the vestibulum in the molar region (the sampled area was 5 cm^2) in the Unknown group (n = 20) and in the controls (n = 20). Mean (bars) + SD and median (lines) values are given. *P. i/P. n* stands for *P. intermedia/P. nigrescens*.

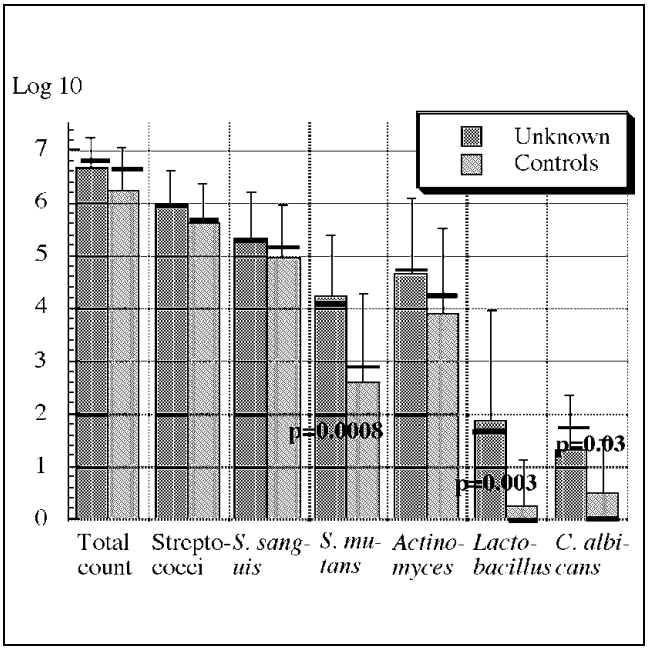

Fig 4 Numbers (mean of two samplings) of micro-organisms on the supragingival tooth surfaces, four sites sampled, in the Unknown group (n = 20) and in the controls (n = 20). Mean (bars) + SD and median (lines) values are given.

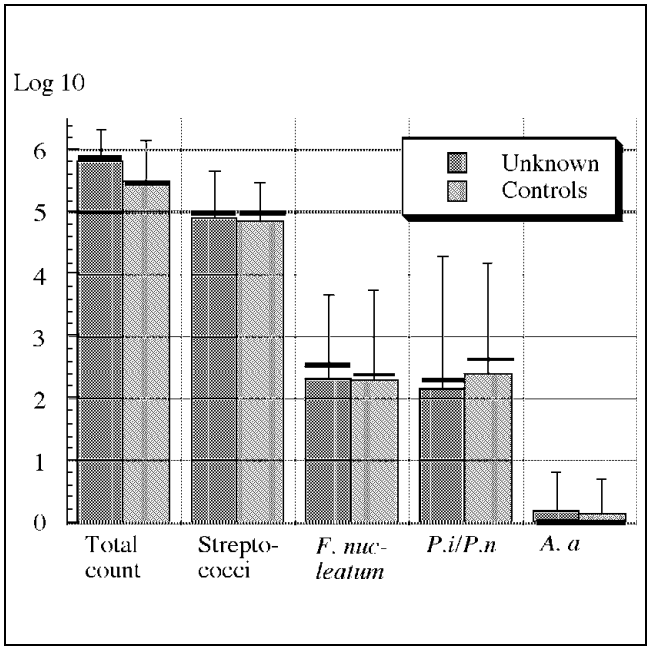

Fig 5 Numbers (mean of two samplings) of micro-organisms in the gingival crevice region, four sites sampled, in the Unknown group (n = 20) and in the controls (n = 20). Mean (bars) + SD and median (lines) values are given. *P. i/P. n* stands for *P. intermedia/P. nigrescens.*

jects and in nine controls. The number and proportion of *F. nucleatum* tended to be lower in the hyposalivation group than in the controls (p = 0.03 for both), while the number and proportion of *P. intermedia/P. nigrescens* was similar in the two groups. Six hyposalivation subjects and four controls harboured at least one of the following species: *C. albicans*, *S. aureus* and enterococci. Enterics were not detected in any of the subjects in neither group.

Buccal mucosa

No significant differences in the numbers (Fig 2) or in the proportions of the different micro-organisms analysed could be detected. *C. albicans* was detected in two hyposalivation subjects and in one control and *S. aureus* in one subject in each of the two groups.

Vestibulum

The number of enterococci tended to be higher in the hyposalivation group than in the controls (Fig 3).

The proportion of streptococci of the total count tended to be higher in the hyposalivation group than in the controls (mean ± SD: 71 ± 32% and median: 75% compared with 53 ± 30 and 38%). *F. nucleatum* was detected in 17 hyposalivation subjects and in 16 controls and *P. intermedia/P. nigrescens* in 11 hyposalivation subjects and in nine controls. Seven hyposalivation subjects, but none of the controls, harboured at least one of the following species: *C. albicans*, *S. aureus* and enterococci. *C. albicans* was found in three hyposalivation subjects, *S. aureus* in one subjects and enterococci in five subjects. Enterics were not detected in any subject in neither group.

Supragingival plaque

The numbers of *S. mutans* and *Lactobacillus* spp. were significantly higher and the number of *C. albicans* tended to be higher in the hyposalivation group than in the controls (Fig 4). There were no significant differences in the proportions of micro-organisms between the groups. *S. mutans* was detected in all subjects in the hyposalivation group and in 15 of the controls and *Actinomyces* spp. in 19 and 18 subjects respectively. *Lactobacillus* spp. was more frequently detected in the hyposalivation group, in 11 subjects compared with two controls. *S. sobrinus* was not detected in any subject in neither group, while *C. albicans* was detected in 11 subjects in the hyposalivation group and in four of the controls.

Gingival crevicular region

No significant differences in the numbers (Fig 5) or proportions of the micro-organisms analysed could be detected between the groups. *F. nucleatum* was detected in 16 subjects in each group and *P. intermedia/P. nigrescens* in 11 hyposalivation subjects and in 14 controls. *P. gingivalis* was not detected in any of the subjects and *A. actinomycetemcomitans* was detected in only two subjects in the hyposalivation group and in one of the controls.

Micro-organisms analysed in more than one site

Of the specific micro-organisms analysed in samples collected from more than one site, *F. nucleatum,* analysed in three sites, was most widespread. Nineteen subjects in each of the two groups harboured *F. nucleatum* in at least two of the three sites. *P. intermedia/P. nigrescens* was detected in at least three of the four sites analysed in nine subjects in each of the two groups. *C. albicans* was found in at least one of the four sites analysed in 11 of the hyposalivation subjects and in five of the controls. It should be noted that the supragingival plaque was the site in which *C. albicans* was most frequently detected, in 11 hyposalivation subjects. *S. aureus* was detected in at least one of the three sites analysed in four subjects in the hyposalivation group and in three controls. Enterococci were detected in both sites analysed in three subjects in the hyposalivation group.

Microflora in relation to other variables

In the hyposalivation group, 11 of the subjects had experienced xerostomia between 0.5 and three years (mean ± SD: 0.9 ± 0.6 years) and nine subjects longer than three years (mean ± SD: 10 ± 8 years, median: 10 years). The nine subjects who had experienced hyposalivation longer than three years had significantly higher numbers of filled surfaces ($p < 0.01$) compared with the controls. The only significant difference in oral microflora were found for the group who had experienced hyposalivation less than three years, they showed a higher proportion of streptococci of the total count on the tongue ($p < 0.01$).

Nine of the subjects in the hyposalivation group reported daily use of sorbitol-containing chewing-gum and/or tablets. We could not find any differences in the proportions of *S. sanguis*, mutans streptococci or *Lactobacillus* spp. in the supragingival plaque between the subjects that used sorbitol frequently and those that reported that they did not.

The hyposalivation group could also be divided according to fluoride use. Ten of the subjects used either fluoride-rinse or fluoride-containing chewing gum/tablets daily (fluoride-users) and 10 subjects reported that they only used fluoridated toothpaste. When these subgroups were compared with the controls, no significant differences in oral flora could be detected except for significantly higher numbers of mutans streptococci for both fluoride-users and the other subgroup ($p < 0.01$ for both groups).

For seven of the subjects the stimulated secretion rate was ≤ 0.7 ml/min. These subjects had experienced xerostomia a longer time, 12 ± 10 years, compared with the subjects that had a stimulated secretion rate > 0.7 ml/min, 3 ± 3 years. The subjects with stimulated secretion rates ≤ 0.7 ml/min tended to have a decreased number of *F. nucleatum* on the tongue and a higher total count in the supragingival plaque. The increase in mutans streptococci compared with the controls was seen for both groups.

DISCUSSION

In the present study, the oral microflora in different oral ecosystems was analysed in subjects with hyposalivation not caused by an inflammation in the salivary glands or due to radiation therapy in the head and neck region. The hyposalivation subjects had experienced xerostomia for ≥ six month and had been referred to the Department of Oral Diagnosis for investigation of a possible Sjögren's syndrome. The subjects' unstimulated secretion rate was measured and a lip biopsy taken. The subjects who had an unstimulated secretion rate < 0.1 ml/min and a lip biopsy not showing signs of inflammation were invited to participate in the study. For six of the 20 subjects in the hyposalivation group the decreased salivary secretion might have been due to their medication. For the 14 other subjects the reason for their hyposalivation is not known. Possible explanations are that they have secondary Sjögren's syndrome or are pre-Sjögrens syndrome patients. Their hyposalivation might also be due to depression or menopausal hormonal changes.

To the best of our knowledge, studies on the dental status in subjects with hyposalivation not caused by radiation therapy or Sjögren's syndrome are scarce. In the present study, clinically visible caries lesions were rare findings in the hyposalivation group, which is in accordance with results obtained in previous studies on subjects with primary Sjögren's syndrome (pSS) (Almståhl et al, 2001a; Ravald and List, 1998). Papas et al (1993) reported a higher number of decayed and filled surfaces in a group of subjects with hyposalivation due to either medicines or Sjögren's syndrome compared with healthy controls. This is in line with the pSS group in our previous study, which had significantly higher numbers of crowned teeth and filled surfaces compared with their controls (Almståhl et al, 2001a). In the present study, no significant differences in the number of crowned teeth or filled surfaces were found between the hyposalivation group and the controls. This might be explained by the fact that all hyposalivation subjects went to the dental clinic at least once a year. However, when the hyposalivation group was divided into two groups according to the time the subjects had suffered from xerostomia, it was found that the nine subjects who had experienced xerostomia longer than three years had significantly higher numbers of filled surfaces compared with the controls. These results suggest that the development of dental disorders is not inhibited by the preventive measures given, but the detoriation rate is fairly slow.

When rinsing samples from this group was analysed, the only significant difference in oral microflora compared with controls were an increase in *Lactobacillus* spp. (Almståhl and Wikström, 1999). In the present study, the hyposalivation group had significantly higher numbers of *S. mutans* and *Lactobacillus* spp. in the supragingival plaque compared with the controls. This is in congurence with the findings in the pSS group in our previous study (Almstahl et al, 2001a). In this study, the increase in *S. mutans* and *Lactobacillus* spp. were seen both in the nine subjects who had experienced xerostomia longer than three years and in the 11 subjects who had felt xerostomia less than three years. It could therefore be assumed that the increase in aciduric micro-organisms occur early after a decrease in the salivary secretion rate.

Nine of the subjects in the hyposalivation group reported daily intake of chewing gum and/or tablets sweetened with sorbitol. Plaque bacteria that can ferment sorbitol are mainly *Actinomyces* spp., *Lactobacillus* spp. and *Streptococcus* spp. (Edwardsson et al, 1977; Kalfas and Edwardsson, 1990; Mejare and Edwardsson, 1975). A clinical study has shown that the proportion of mutans streptococci increased while the proportion of *Lactobacillus* spp. remained unchanged in plaque after exposure to sorbitol 10-12 times/day in subjects with low salivary secretion rate of various origins (Kalfas et al, 1990). We could not find any differences in the proportions of mutans streptococci or *Lactobacillus* spp. between the subjects who used sorbitol frequently and those who did not. It is probable that the intake frequency of sorbitol in the study by

Kalfas et al (1990) was higher than in our hyposalivation group.

Factors that have been associated with mineral loss on root surfaces are medication intake, complaints of dry mouth, low unstimulated and stimulated saliva flow rates, low buffering capacity and high salivary *Lactobacillus* levels (Bardow et al, 2001). Of these factors, the unstimulated secretion rate was found to be the most important one. The increase in demineralisation was found not to be due to undersaturation of hydroxyapatite in saliva but to decreased outputs/min of calcium, phosphate, HCO_3- and proteins. In the present study, where the mean unstimulated secretion rate was 0.04 ml/min in the hyposalivation group, root caries was rarely found dispite the fact that the outputs/min of bicarbonate, sodium, potassium, calcium, phosphate, total protein, lactoferrin, amylase, albumin and mucin MUC5B were found to be decreased with between 40 and 70% compared with controls (Almståhl et al, 2001b; Almståhl and Wikström, 2003). Possible explanations to the divergent results might be that in the study by Bardow et al (2001) the subjects had experimental root surfaces inserted into their lower partial denture for 62 days and were instructed not to brush the root surfaces and to use non-fluoridated toothpaste for their oral hygiene. The subjects in our study all used fluoridated toothpaste daily and had good oral hygiene. Also, frequent use of fluoride containing products has been found to arrest root caries (Emilson et al, 1993).

It was shown in an *in vitro* study that, at a pH of 4.5, *P. intermedia* was killed while *Lactobacillus casei* and *Enterococcus faecalis* survived until the pH reached 2.5 (Svensäter et al, 1997). It was also shown in the same study that several species could adapt to a low pH, *Streptococcus gordonii, Streptococcus oralis, S. mutans, Streptococcus mitis* biovar 2, *S. salivarius*, *L. casei* and *E. faecalis*, but others could not, *P. intermedia*, *Actinomyces naeslundii*, *Streptococcus mitis* biovar 1, *Streptococcus oralis*, *S. sanguis* and *S. sobrinus*. A low buffering capacity was found for nine subjects in our hyposalivation group and *Lactobacillus* spp. were more frequently found in these subjects compared with the hyposalivation subjects with normal buffer capacity, supporting the adaptation theory. In this study, it is possible that the low buffering capacity in the hyposalivation subjects enhanced acid-tolerant species. Studies by Bradshaw et al (2002; 1989) showed that *in vitro* there is an increase in acid-tolerant species such as *Lactobacillus* spp. and *S. mutans* and a decrease in acid-sensitive species such as *Neisseria*, *S. sanguis*, *F. nucleatum* and *P. intermedia* when the pH is allowed to fall after a glucose challenge. However, the shifts in the microflora were not so pronounced when fluoride was present, which probably is the case in most of the hyposalivation subjects in our study. Xylitol, which was frequently used by many hyposalivation subjects in our study, has also been shown to decrease the pH fall after sugar intake and also to decrease the shift towards an aciduric and acidogenic microflora (Bradshaw and Marsh, 1994). This might explain why the changes in the microflora in the hyposalivation group in our study were not so large as could have been expected.

In accordance with our previous study on subjects with hyposalivation due to pSS (Almståhl et al, 2001a), the number and proportion of *F. nucleatum* on the tongue was lower in the hyposalivation group than in the controls. This result is in congruence with the clinical findings. We found the prevalence of sites with plaque, gingival bleeding and periodontal probing depths of ≥ 5 mm to be similar in the hyposalivation group and in the healthy control group. Also, Bradshaw et al (1989) found that *F. nucleatum* was more sensitive to an acidic environment than *P. intermedia*.

In the present study, the frequency and number of *Lactobacillus* spp. in the supragingival plaque were increased in the hyposalivation group. There are about 50 different species of *Lactobacillus*. The *Lactobacillus* spp. can be divided into homofermentative, heterofermentative and facultatively heterofermentative depending on their ability to ferment carbohydrates (Kandler and Weiss, 1986; Maiden et al, 1992). Preliminary results from studies on *Lactobacillus* spp. isolated from our groups with hyposalivation subjects have revealed that the ability to survive and produce acids at low pH varies between different strains.

In conclusion, subjects with hyposalivation have an aciduric supragingival plaque with high numbers of *S. mutans*, *Lactobacillus* spp. and *C. albicans*, which constitute a risk factor for caries. It is not known which *Lactobacillus* spp. that were present in the supragingival plaque. To examine the frequency, number and proportion of different *Lactobacillus* spp. in various oral sites will be the aim of future studies. Such knowledge will be used to for the development of oral health measures aiming at normalising their oral microflora.

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