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Lactoferrin, amylase and mucin MUC5B and their relation to the oral microflora in hyposalivation of different origins

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There are several reasons for hyposalivation, each affecting the salivary composition in different ways. The aim of this study was to analyze and compare lactoferrin, amylase and mucin MUC5B in stimulated whole saliva collected from subjects with hyposalivation of different origins and to relate the results to the presence of some microbial species associated with oral disorders. Albumin was determined as a marker of serum leakage. The characteristic feature for subjects with radiation-induced hyposalivation was a large increase in lactoferrin, probably due to leakage through inflamed mucosal tissues, while it was a high albumin content for the group with primary Sjögren's syndrome, probably due to disruption of the fragile mucosa. The saliva composition in subjects with hyposalivation of unknown origin or due to medicines was close to that in the healthy controls. All three hyposalivation groups tended to display a decrease in the concentrations of MUC5B and amylase. None of the microbial species analyzed (streptococci, mutans streptococci, *Lactobacillus* spp., *Fusobacterium nucleatum*, *Prevotella intermedia*/*Prevotella nigrescens*, *Candida albicans*, *Staphylococcus aureus* and enterics) correlated with concentration of MUC5B in saliva. The RT group, having the highest concentration of lactoferrin, had the lowest median number of *F. nucleatum* and was the only group in which median number of *P. intermedia*/*P. nigrescens* was zero.

Key words: hyposalivation; saliva; lactoferrin; amylase; mucin; MUC5B

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Saliva, consisting of 99% water and 1% organic and inorganic components, has several important functions in the oral cavity. One of them is to modulate the oral microflora by favoring the attachment and proliferation of certain microorganisms and promoting the clearance of others (36). Saliva also protects the oral tissues from desiccation and exogenous insult from acids and degradative enzymes (22, 25, 40).

Subjects with radiation-induced hyposalivation frequently have almost no salivary secretion due to the destruction of the salivary glands. In subjects with Sjögren's syndrome, there is an inflammation in the salivary glands, resulting in decreased salivary flow. In addition, several medicines, alone or in combination, affect the nerve signals to the salivary glands, also reducing the flow of saliva. Different reasons for hyposalivation will affect the salivary composition in different ways (14, 41, 44).

The parotid glands consist mostly of serous cells, whereas the minor glands consist of mucous cells. The submandibular and sublingual glands contain of both serous and mucous cells. The serous and the mucous cells are dissimilar in their secretion of water and saliva components (47). Lactoferrin is mainly secreted by the interductal cells of serous acini, amylase by serous cells (11, 34) and mucins by mucous cells (11).

Lactoferrin binds iron, which is an important nutrient factor for many microbial species (19). It also displays bacteriostatic and/or bactericidal activity towards several microbial species (5, 6, 31, 38). Lactoferrin has been found in increased concentrations in subjects with radiation-induced hyposalivation (42) and in subjects with Sjögren's syn-

drome (7, 20, 21, 39). A high lactoferrin concentration in saliva can result from damage to the salivary glands (42), gingival inflammation or leakage of serum through inflamed mucosal membranes (9, 17). Since saliva collected directly from the glands usually contains only trace amounts of albumin (32), it was therefore used as an indicator of serum leakage.

Amylase one of the most prevalent enzymes in the saliva, is generally considered to be a reliable marker of serous cell function. In addition to enzyme activity amylase coats the oral tissues, binds to streptococci, has antimicrobial activity and is involved in the selective clearance and adherence of microorganisms (11). The amylase concentration in subjects with radiation-induced hyposalivation has been found to be reduced (10, 23). In contrast, in patients with primary Sjögren's syndrome, the amylase concentration is comparable to that in healthy controls (24, 44).

Mucins are glycoproteins with low solubility and high viscosity, elasticity and adhesiveness (40) and have been shown to interact with several microorganisms (8, 18, 28, 46). When free in saliva, mucins agglutinate microorganisms. Their presence on the oral tissues, protect mucosal membranes and favour the attachment and proliferation of certain microorganisms. The carbohydrates, present in mucins, may also be a source of nutrients for some oral microorganisms (40). There are two types of mucin in saliva, MUC5B (formerly MG1), with a molecular weight of >1000 kDa, and MUC7 (formerly MG2), with a molecular weight of 130–150 kDa. The concentration of MUC5B in unstimulated saliva in subjects with primary Sjögren's syndrome has been found to be higher than that of healthy controls (35).

In our previous studies, we found differences in the oral microflora in subjects with hyposalivation of different origins (1, 2). These differences may be associated with altered salivary protein composition in these patients. The aim of this study was to compare lactoferrin, amylase and MUC5B concentrations in stimulated whole saliva collected from subjects with radiation-induced hyposalivation, subjects with pSS and subjects with hyposalivation of unknown origin or due to medicines. In addition, we analyzed the data in relation to the presence of selected microbial species that have been associated with oral disorders.

Material and methods

This study was approved by the ethics committee at Göteborg University. Three groups of subjects with hyposalivation and a healthy control group participated in the study. To be included, the subjects had to be between 35 and 70 years of age and had to have at least ten teeth, no removable prostheses or implants and an unstimulated secretion rate of ≤0.1 ml/min. The study groups comprised ten subjects who had completed radiation therapy in the head and neck region 6 months earlier (radiation therapy group), 20 subjects with primary Sjögren's syndrome, diagnosed according to the Copenhagen criteria (26) 7±5 years previously, 19 subjects with subjective feelings of hyposalivation for 6±8 years but who displayed no signs of salivary gland inflammation on biopsy (unknown group) and 26 healthy subjects with an unstimulated secretion rate of >0.1 ml/min.

The subjects were asked to refrain from eating, drinking, smoking and toothbrushing <2 hours before the appointment. Firstly, the unstimulated secretion rate was measured, after which a rinsing sample for microbial analysis was taken. Subsequently, the stimulated secretion rate was measured and finally the clinical examination took place.

Clinical examination

The clinical examination has been described previously (1, 2). In brief, the clinical parameters that were registered were the status of the mucosal membranes and the number of teeth, fillings, crowns and bridges, presence of plaque along the gingival margin after Diaplac® staining, bleeding following probing and periodontal pocket probing depths of >4 mm at 4 sites per tooth.

Secretion rate, pH and buffer capacity

The determinations of the salivary secretion rates and the measurement of pH and buffer capacity have been described previously (2). In brief, the unstimulated secretion rate was measured for 15 min and the paraffin-wax-stimulated secretion rate for 5–10 min. The stimulated saliva was collected in an ice-chilled test tube. The pH of stimulated saliva was measured using a pH meter (Metrohm 632, Herisau, Switzerland). For the determination of the buffer capacity, one part of the stimulated saliva was mixed with three parts of 0.005 M HCl and the final pH was measured. With the assay used here, "normal" pH ranges between pH 6.3 and 7.2 and "normal" buffer capacity between pH 5.0 and 7.0 (13).

Duplicate samples and determinations of the secretion rates, pH and buffer capacity were performed, 10±7 days apart, and a mean value was calculated. As ten the 50 subjects with hyposalivation had an unstimulated salivary flow rate below detectable levels, the stimulated saliva was used for the protein analysis in all subjects. For the protein analysis, the stimulated saliva was centrifuged at 11,600×*g* for 15 min in an Eppendorf centrifuge (Eppendorf M 5415) at room temperature. The supernatant was divided into aliquots and stored at −70°C pending analysis.

Microbial sampling and analysis

Sampling for microbial analysis was performed on the first appointment. For microbial sampling, the subject rinsed his or her mouth with 10 ml of sterile distilled water for 20 s before spitting into a sterile vessel. One ml of the fluid was transferred to a bottle with 5.7 ml of VMG IIS (29) anaerobically prepared. Dilution of the samples from 10^{-1} to 10^{-5} was performed in VMG I (29), and 0.1 ml was inoculated onto each agar plate. The cultivation and incubation procedures and the identification of microorganisms have been described previously (2). In brief, the total microbial count and the numbers of *Fusobacterium nucleatum* and *Prevotella intermedia*/*Prevotella nigrescens* were determined from their growth on Brucella agar, streptococci on mitis-salivarius agar, mutans streptococci on mitis-salivarius-bacitracin agar, *Lactobacillus* spp. on Rogosa-SL agar, *Staphylococcus aureus* on *Staphylococcus* agar, *Candida albicans* on Saboraud-T agar and enterics on Drigalski agar.

Protein analysis

The total protein concentration was determined using the BCA Protein Assay Reagent (Pierce, Rockford, IL) with bovine serum albumin (BSA) as a stan-

dard. The saliva supernatant was diluted 2- and 4- fold with distilled water and was analyzed in duplicate. Twenty µl of saliva or BSA in concentrations ranging from 0 to 1500 mg/ml and 180 µl of BCA reagent were added to wells in a microtitre plate (Greiner, Frickenhausen, Germany). The plate was incubated at 37°C for 30 min and was then read at 592 nm with an enzyme-linked immunosorbent assay (ELISA) reader (Molecular Devices, Sunnyvale, CA).

The concentration of lactoferrin was determined using the capture ELISA technique (43). Polysorb microtiter plates (Nunc, Roskilde, Denmark) were incubated at room temperature overnight with rabbit-anti-human lactoferrin (Dakopatts, Glostrup, Denmark) diluted in phosphate-buffered saline (PBS) to a concentration of 1 µl/ml. The plate was washed three times with PBS with 0.1% Tween 20 (PBS-T). The washing procedure was repeated after each step. A standard curve was prepared using human lactoferrin (Department of Oral Biochemistry, Amsterdam, the Netherlands) diluted in PBS with a start concentration of 250 ng/ml, which was then two-fold serially diluted. Duplicate wells were incubated with 200 µl of saliva supernatant diluted 20 times in PBS-T with 0.1% gelatin, which were then two-fold serially diluted in the plate. The plate was incubated for 2 h at room temperature. A 2000-fold dilution of goat-anti-human-lactoferrin conjugated with horseradish peroxidase (Dakopatts, Denmark) in PBS-T with 0.1% gelatin was then added, 100 µl/well, and the plate was incubated for 1 h at room temperature. After washing, 100 µl of TMB (3,3, 5,5,-tetramethylbenzidine) (ICN, Costa Mesa, CA) diluted in sodium acetate (pH 5.5) with a final concentration of 0.01% and with 0.003% H_2O_2 was added to the plate, 100 µl/well, and the plate was incubated at room temperature. The color reaction was stopped after 15 min by adding 2 M H_2SO_4, 50 µl/well, and the plate was read at 450 nm in the ELISA reader.

A competitive ELISA technique was used for the determination of the albumin concentration (45). Wells in a microtiter plate (Greiner) were coated with 0.1 mg/ml of human albumin diluted in 0.1 M Na_2CO_3 and the plate was incubated over night at 4°C. The washing procedure was the same as in the lactoferrin ELISA. After washing, 200 µl/well of a blocking solution, PBS-T with 1% gelatin, was added and the plate was incubated for 30 min at room temperature. The blocking solution was poured out and 160 µl of the saliva supernatant, diluted 10-fold in PBS-T with 1% gelatin, or 160 µl of human serum albumin with a start concentration of 50 µg/ml were added in duplicate wells in the microtiterplate. The solutions were 2-fold serially diluted in the plate. A mixture of a 1:1500 diluted rabbit anti-human albumin and a 1:3000 diluted goat-anti-rabbit immunoglobulin conjugated with horseradish peroxidase, in PBS-T with 1% gelatin, were added, 120 µl/well. The plate was incubated for 3 h at room temperature. After washing, 200 µl/well of the same color solution as in the lactoferrin ELISA was added. The color reaction was stopped after 5 min by adding 2 M H_2SO_4, 100 µl/well, and the plate was read at 450 nm in the ELISA reader.

The amylase concentration was determined using an amylase activity kit (Sigma Chemical Co., St Louis, MO). The saliva supernatant was diluted 80-fold with 0.85% NaCl and 10 µl was added to triplicate wells in a microtiterplate (Greiner). The multi-enzyme lintrol solution (Sigma Chemical) was used as a standard and was added, 10 µl/well, in concentrations ranging from 0 to 60%. Ninety µl of the amylase reagent was added to each well and the plate was incubated at 37°C. The plate was read after 2 and 4 min at 405 nm in the ELISA reader.

The concentration of MUC5B was determined using an ELISA method (47). The concentration of MUC5B as units/ml was determined relative to standard saliva (from one healthy person). Duplicate wells in a high-affinity microtiter plate (Greiner) were coated with 200 µl of saliva supernatant diluted 200-fold in 0.1 M Na_2CO_3, which were then 2-fold serially diluted in the plate. After incubation at room temperature overnight, the plate was washed 3 times as in the lactoferrin ELISA. The washing procedure was repeated after each step. A blocking solution of PBS-T with 2% BSA was then added in volumes of 150 µl to each well, and the plate was incubated at 36°C for 1 h. An antibody to human MUC5B (Department of Oral Biochemistry, Amsterdam, the Netherlands) was diluted 40-fold in PBS-T with 1% gelatin and was added in volumes of 100 µl/well to the plate. After incubation for 1 h at 36°C, a 1:2000 diluted rabbit-anti-mouse immunoglobulin (Dakopatts, Denmark) was added, 100 µl/well, and the plate was incubated again in the same conditions. One hundred µl of the same colour solution as in the lactoferrin ELISA was added and the plate was incubated at room temperature for 15 min, after which 50 µl/well of 2 M H_2SO_4 was added and the plate was read at 450 nm in the ELISA reader.

Statistical methods

The statistical methods were ANOVA and the Newman-Keul test, the latter at a significance level of 5%. Pearson's correlation was used to measure correlations between pairs of variables.

Results

Clinical recordings

In the radiation therapy group, 90% were men, while the primary Sjögren's syndrome group and the unknown group consisted of 95% and 89% women respectively. In the control group, the gender distribution was 23% men and 77% women. The mean age for all 75 subjects included in the study was 55±8 years with a median of 55 years. The mean unstimulated secretion rate in the hyposalivation groups was ≤0.04 ml/min and in the controls 0.33 ml/min. The mean stimulated secretion in the radiation therapy group was 0.39±0.41 ml/min, in the primary Sjögren's syndrome group 0.47±0.38 ml/min, in the unknown group 0.99±0.50 ml/min and in the controls 2.3±0.95ml/min. Compared with the controls, the pH and buffer capacity were lower in all three hyposalivation groups and lowest in the radiation therapy group. The three hyposalivation groups and the controls displayed similar proportions of surfaces with plaque along the gingival margin and surfaces showing bleeding on probing. Periodontal pocket probing depths of >4 mm were detected in 40% of the subjects in the radiation therapy group, 30% in the primary Sjögren's syndrome group, 37% in the unknown group and 38% of the controls. For subjects with periodontal probing depths of >4 mm, the number of pockets varied between 1 and 8. The mean probing depths of pockets of >4 mm were 5.8±0.3 mm in the radiation therapy group, 5.3±0.4 mm in the primary Sjögren's syndrome group, 5.0±1.2 mm in the unknown group and 5.6±0.6 mm in the controls. Clinically visible open caries lesion were rarely detected.

Table 1. Frequency (%) of microorganisms in subjects with radiation-induced hyposalivation (radiation therapy), subjects with primary Sjögren's syndrome, subjects with hyposalivation of unknown origin or due to medicines (unknown) and in healthy controls

Group	Number of subjects	Mutans streptococci	*Lactobacillus* spp.	*F. nucleatum*	*P. intermedia/ P. nigrescens*	*C. albicans*	*S. aureus*	Enterics
Radiation therapy	10	70	100	60	30	70	30	20
Primary Sjögren's syndrome	20	100	85	80	75	60	20	10
Unknown	19	100	79	74	80	53	21	5
Controls	26	81	35	81	50	19	27	4

Microbial results

The frequencies of microorganisms are presented in Table 1 and the mean±SD and median log 10 numbers in Fig. 1. No significant difference in total microbial count or in number of streptococci could be detected between the groups. Mutans streptococci were frequently detected in all four groups. There was a significant difference in the number of mutans streptococci between the groups (P=0.004), but the Newman-Keul test provided no further information about this difference. All three hyposalivation groups had more *Lactobacillus* spp. than the controls (P=0.0001). The number of *C. albicans* was significantly higher in the radiation therapy and primary Sjögren's syndrome group than in the unknown group and in the controls (P=0.0001). *F. nucleatum* was frequently found in all four groups. There were no significant differences in the numbers of *F. nucleatum* and *P. intermedia/P. nigrescens.* The lowest frequency of *P. intermedia/P. nigrescens* was found in the radiation therapy group, where it was detected in three of the 10 subjects, and the radiation therapy group was the only group in which the median number was zero. *S. aureus* and enterics were only detected in a few subjects in all four groups, and their median numbers were zero.

Protein concentrations

Table 2 presents the concentrations and outputs per min of total proteins, albumin, lactoferrin, amylase and MUC5B in the controls. Fig. 2 presents the concentrations of the salivary components per ml of stimulated whole saliva in the hyposalivation groups as proportions of the concentrations in the controls. There was a significant difference in the total protein concentration between the groups (P=0.0001). The total protein concentration in the radiation therapy group and the primary Sjögren's syndrome group was about twice as high as in the controls, while the concentration in the unknown group was comparable to that in the controls.

The albumin concentration also differ significantly between the groups (P=0.0001). Compared with the controls and the unknown group, the radiation therapy group and the primary Sjögren's syndrome group displayed an albumin concentration that was about five times higher.

The most pronounced difference between the groups was that in the lactoferrin concentration (P=0.0001). The lactoferrin concentration was about 24 times higher in the radiation therapy group. Only one subject in the radiation therapy group displayed a lactoferrin concentration comparable to that of the controls. This subject had a stimulated secretion rate within normal limits, 1.2 ml/min. In the primary Sjögren's syndrome group and unknown group, the lactoferrin concentration was about twice as high as in the controls.

The amylase concentration differed significantly between the groups (P=0.013). The radiation therapy group displayed the lowest amylase concentration, about 30% of the value in the controls (P<0.05). For the primary Sjögren's syndrome group, the amylase concentration was about 70% of that in the controls, while it was about 92% in the unknown group (not significant).

No significant difference in MUC5B concentration could be detected between the groups.

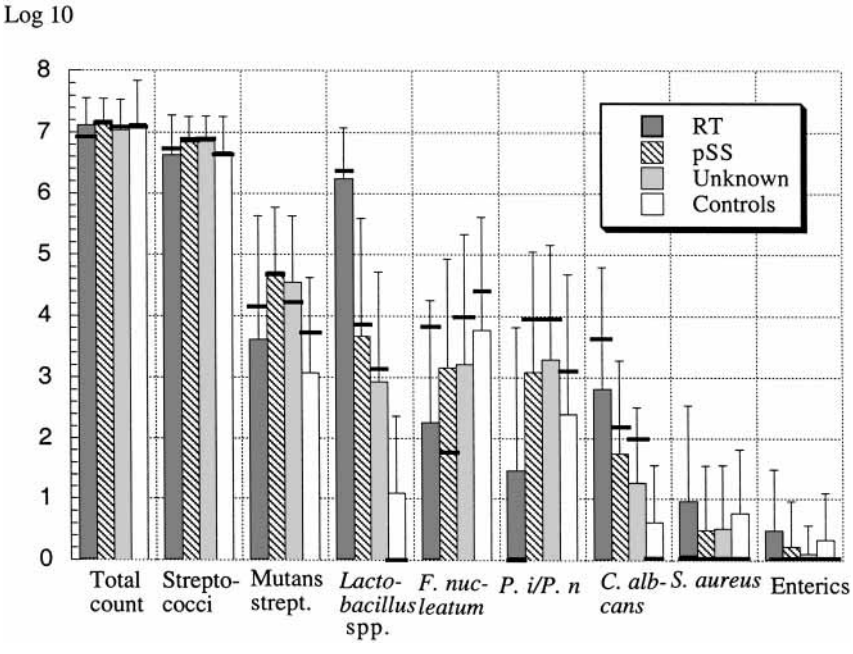

Fig. 1. Mean±SD and median log 10 numbers of microorganisms in the 10 subjects with radiation-induced hyposalivation (RT), the 20 subjects with primary Sjögren's syndrome (pSS), the 19 subjects with hyposalivation of unknown origin or due to medicines (unknown) and the 26 controls.

Proteins in relation to stimulated salivary secretion rate/min

Fig. 3 presents the proteins in relation to the stimulated salivary secretion rate per min (output per min) in the hypo-

salivation groups as proportions of the values in the controls. There were significant differences between the groups in the output per min of total protein, lactoferrin, amylase and MUC5B (P=0.0001 for all). Compared with the controls, all three hyposalivation groups displayed a lower output per min of total protein, amylase and MUC5B. In the radiation therapy group, the median output per min of lactoferrin was 3.5 times higher than in the controls. For albumin, the primary Sjögren's syndrome group showed a slightly higher output per min than the radiation therapy, unknown and control groups.

Correlations between salivary proteins and clinical parameters

Based on calculations for all 75 subjects, there was a weak positive correlation between the albumin concentration and the proportion of surfaces with bleeding on probing (P=0.02). The stimulated salivary secretion rate and the saliva pH were negatively correlated with the total protein and lactoferrin concentrations (P=0.0001 for all). No correlation between the buffer capacity and the proteins was detected.

Correlations between salivary proteins and microorganisms

The lactoferrin concentrations were positively correlated to the numbers of *Lactobacillus* spp. (P=0.0001) r=0.47 and *C. albicans* (P=0.0003) r=0.41. There was a weak negative correlation between amylase activity and the number of *Lactobacillus* spp. (P=0.035) r=−0.24. A weak negative correlation was also revealed between the lactoferrin concentration and the number of *F. nucleatum* (P=0.045) r=−0.24. The radiation therapy group, which had a very high lactoferrin concentration, was the only group in which the median number of *P. intermedia*/*P. nigrescens* was zero. None of the microbial species analyzed correlated with the concentration of MUC5B in saliva.

Discussion

In the healthy controls, the unstimulated and the stimulated salivary secretion rate, pH and buffer capacity as well as the total protein concentration were in accordance with what has previously been reported (16, 27, 33, 44).

The subjects in the radiation therapy

Table 2. Concentrations and outputs of proteins in the controls

	Concentration/ml Mean±SD (median)	Output per min
Total protein (mg)	0.9±0.2 (0.9)	2.0±0.9 (1.8)
Albumin (mg)	0.2±0.1 (0.2)	0.4±0.2 (0.4)
Lactoferrin (μg)	3.7±2.5 (2.7)	8.6±7.3 (5.7)
Namylase (U)	3257±1682 (3160)	7455±5537 (6220)
MUC5B (U)	2.4±1.7 (2.1)	5.4±4.9 (4.0)

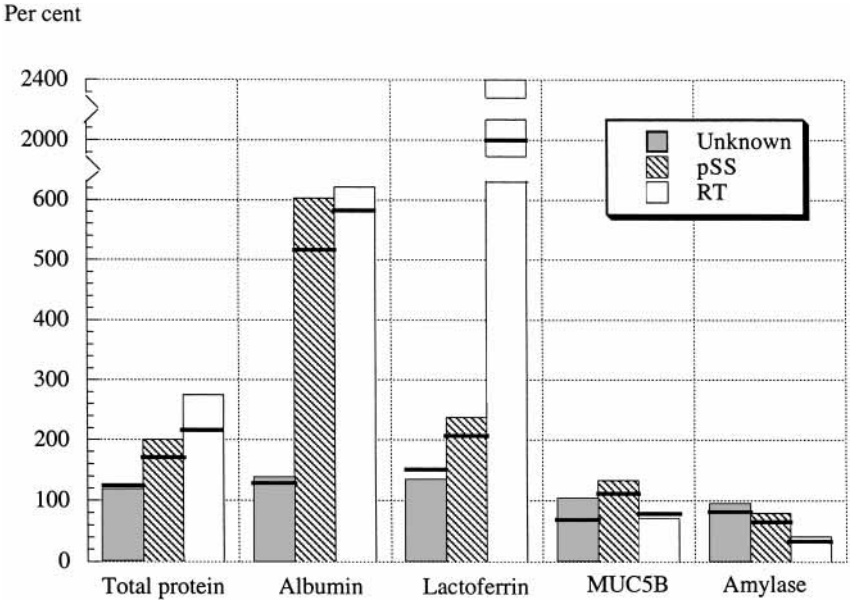

Fig. 2. Mean (bars) and median (lines) concentrations of total protein, albumin, lactoferrin, amylase and MUC5B in the three hyposalivation groups as proportions of the values in the controls. The concentrations in the controls are set at 100%.

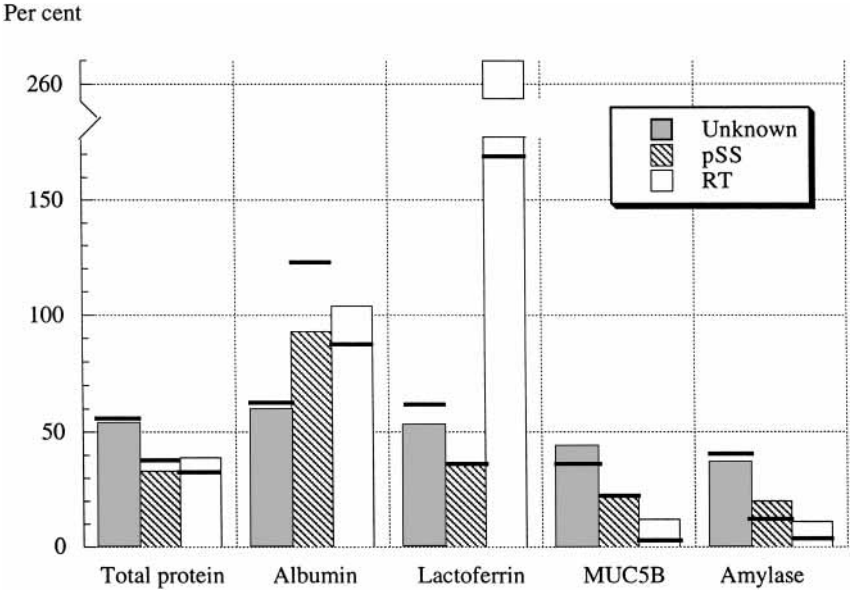

Fig. 3. Mean (bars) and median (lines) output per min of total protein, albumin, lactoferrin, amylase and MUC5B in the three hyposalivation groups as proportions of the values in the controls. The concentrations in the controls are set at 100%.

group had completed their radiation therapy 6 months prior to inclusion in the study. At this time, the clinically visible acute effect of the radiation therapy on the oral tissues was subsided. The subjects of the primary Sjögren's syndrome group and of the unknown group had had a decreased salivary secretion rate for several years. To avoid the effect of aging and of dental status on the salivary composition and the oral microflora, the subjects included in the groups were about the same age and had a similar number of teeth; the median number of the groups ranged from 26 to 28 teeth. The three hyposalivation groups and the controls displayed comparable proportions of surfaces tooth surfaces with plaque along the gingival margin and dental sites showing bleeding on probing, as well as number of periodontal pocket probing depths of >4 mm.

We chose to analyse protein concentrations in stimulated whole saliva in all subjects because several of the subjects in the hyposalivation groups displayed very low or no detectable unstimulated secretion. The results of the protein analysis in the hyposalivation groups are presented as proportions of the values in the controls.

While MUC7 readily interacts with microorganisms (8, 18, 28), the major function of MUC5B is assumed to be the coating and protection of the oral tissues (22, 25, 40). Only a few microorganisms have been identified as binding specifically to MUC5B (30). This could be the reason why no correlations were found between MUC5B and the microorganisms that were analyzed. Both the radiation therapy and the Sjögren's syndrome groups displayed a marked decrease in output per min of MUC5B. Their almost absent unstimulated salivary secretion rate and reduced concentration in stimulated salivary secretion may make the coating of MUC5B to the mucosal surfaces difficult. This could lead to a reduction in the protection of the oral tissues and increased susceptibility to mucosal damage.

We found a reduced amylase concentration in the radiation therapy group, a finding also made by others (10, 23). In primary Sjögren's syndrome subjects, Mandel & Baurmash (24) and Van der Reijden (44) reported that amylase concentration was comparable to that in healthy controls. This finding is supported by the fact that the mean amylase concentration in our primary Sjögren's syndrome group was similar to that in the controls. However, the primary Sjögren's syndrome group displayed a 30% lower median amylase concentration. The median amylase concentration and output per min in the hyposalivation groups suggests a loss of serous cell function that was most severe in the radiation therapy group.

In accordance with the results obtained by Valdez et al. (42), the radiation therapy group in our study displayed a 24 times higher concentration of lactoferrin than the controls. Since the radiation therapy group and the controls displayed a similar periodontal status, this factor is unlikely to explain the difference in lactoferrin concentration. Leakage of serum through the mucosal tissues infiltrated by acute-phase inflammatory cells due to radiation and low salivary secretion could be a more plausible reason. The high concentration of albumin found in the radiation therapy group (Fig. 2) supports this hypothesis. A high concentration of albumin in radiation therapy subjects has also been found by others (9, 23). Furthermore, the preliminary results of our ongoing longitudinal study indicate that the lactoferrin concentration in radiation therapy subjects decreases gradually over time.

The primary Sjögren's syndrome group displayed an albumin concentration close to that in the radiation therapy group (Fig. 2). Their output per min of albumin was even higher than in the radiation therapy group (Fig. 3). The primary Sjögren's syndrome group displayed a higher lactoferrin concentration than the controls. However, while the lactoferrin concentration in the radiation therapy group was 24 times higher than in the controls, it was twice as high in the primary Sjögren's syndrome group (Fig. 2). Konttinen et al. (21) found a similar increase in lactoferrin concentration in unstimulated whole saliva. An increase in the lactoferrin concentration in saliva from the parotid gland was found by Jezequel et al. (20) and by Konttinen et al. (21). However, there are conflicting results when it comes to the correlation between the lactoferrin concentration in stimulated parotid saliva and the degree of inflammation in the salivary glands (20, 21). We think it is reasonable to believe that part of the lactoferrin in the primary Sjögren's syndrome group comes from the inflamed salivary glands and the rest from leakage through disrupted fragile mucosal membranes due to a longstanding low salivary secretion rate.

The ELISA method used to determine lactoferrin in this study does not discriminate between iron-free lactoferrin, iron-saturated lactoferrin and fragments of lactoferrin. In contrast to iron-saturated lactoferrin, iron-free lactoferrin has bacteriostatic effects on streptococci, staphylococci, enterics and gram-negative bacteria for example (4, 12) and fungicidal effects on *C. albicans* and *Candida krusei* (4, 31). Iron-free lactoferrin also has a bactericidal effect on *Streptococcus mutans* (37), inhibits the adhesion of *S. mutans* to hydroxyapatite (49) and inhibits its glucose uptake and lactic acid synthesis (5). The degradation of lactoferrin reduces its antimicrobial function. Since the configuration of the lactoferrin molecule is of importance for its antimicrobial concentration, the lactoferrin in the different study groups will be analyzed further. The results of *in vitro* experiments suggest that the function of lactoferrin is also affected by pH, but in opposite ways on different microorganisms (6, 38).

The negative correlation between the lactoferrin concentration and the number of *F. nucleatum* might indicate an antimicrobial effect by lactoferrin (6). Previously, a negative correlation has been found between lactoferrin and the periodontal pathogen *Actinobacillus actinomycetemcomitans* in redundant diseased patients (15). The very low frequency and number of *P. intermedia*/*P. nigrescens* in the saliva in the radiation therapy group could be explained by both the iron dependence for growth for *P. intermedia*/*P. nigrescens* and an antimicrobial effect by lactoferrin. The pH and buffer capacity were lower in the hyposalivation group than in the controls and significantly lower in the radiation therapy and primary Sjögren's syndrome groups. The positive correlation between the lactoferrin and *C. albicans* could be due to a reduced antifungal function of lactoferrin at an acid pH (38). However, the fact that the acid milieu itself favours growth of both *Lactobacillus* spp. and *C. albicans* in the radiation therapy and Sjögren's syndrome groups is probably a stronger contributory factor (3, unpublished results).

It can be concluded that there are dif-

ferences in whole saliva protein composition in subjects with hyposalivation of different origins. The characteristic feature in the radiation therapy group was a large increase in lactoferrin, probably due to leakage through mucosal tissues, while in the primary Sjögren's syndrome group it was a high albumin content, probably due to the disruption of the fragile mucosa. The saliva composition in the unknown group was close to that in the controls. All three hyposalivation groups tended to have a decrease in the concentration of MUC5B. None of the microbial species analyzed here correlated with the concentration of MUC5B in saliva. The results suggest, however, that lactoferrin contributes to the differences in the oral microflora detected between the hyposalivation groups.

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