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Whole, Submandibular, and Parotid Saliva-Mediated Aggregation of *Pseudomonas aeruginosa* in Cystic Fibrosis

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The aggregation of mucoid and nonmucoid *Pseudomonas aeruginosa* by submandibular, parotid, and whole saliva from patients with cystic fibrosis (CF) and non-CF subjects was investigated. There were significant differences ($P < 0.01$) in aggregation of mucoid and nonmucoid variants of *P. aeruginosa* by submandibular and whole saliva from CF patients and non-CF subjects. However, the differences in the parotid secretion were not as pronounced. Patients with CF who were colonized with *P. aeruginosa* demonstrated a significantly higher ($P < 0.05$) percent aggregation of the mucoid variants by the submandibular secretion and of both mucoid and nonmucoid variants by whole saliva, compared with corresponding secretions from patients with CF not colonized with this pathogen. The parotid saliva aggregation activity was not markedly different for the two groups with CF. From patients with CF, whole saliva demonstrated a higher percent *P. aeruginosa* aggregation than did the submandibular saliva. In non-CF subjects, however, the percent aggregation of *P. aeruginosa* by submandibular saliva was higher than that by whole saliva. Our results indicate that the sero-mucous products of the submandibular gland have a more significant role in *P. aeruginosa* aggregation than the serous secreting parotid cells and that the submandibular secretion is possibly responsible for the differences in oral colonization by this pathogen in subjects with and without CF.

Cystic fibrosis (CF) is an autosomal recessive disorder which is characterized by chronic and recurrent pulmonary infections (15, 27, 31, 38). *Pseudomonas aeruginosa*, particularly the mucoid variant, has been identified as the major pathogen in patients with this disease (6, 14, 15, 22, 27, 31, 38). Although a great deal of research has attempted to identify the exact mechanism of colonization by *P. aeruginosa* in patients with CF, it still remains unknown.

Human saliva induces aggregation of some bacteria in the oral cavity (7, 8, 12, 13, 16, 24, 30). Several salivary molecules, such as lysozyme, secretory immunoglobulin A, and high-molecular-weight mucinous glycoproteins, have been known to cause aggregation (3, 7, 9, 23, 24, 32). It has been suggested that the saliva-mediated aggregation of bacteria may have two distinct functions in the oral cavity. First, saliva may promote the clearance of bacteria by aggregating them into masses more easily removed from the oral cavity by swallowing or flushing actions (10, 11, 23). Second, saliva-mediated aggregation may function to cause adherence of bacteria to oral tissues (7, 10, 18, 33, 34, 36, 37). Large numbers of bacterial cells would need to be present in saliva for attachment to be statistically probable. Therefore, saliva-mediated aggregation may provide the necessary numbers of cells for adherence and subsequent colonization (10). When studying mechanisms by which bacteria colonize or are cleared from the oral cavity, it is important to study bacterial interactions with salivary molecules.

Recently, we reported a high recovery rate of both mucoid and nonmucoid variants of *P. aeruginosa* from the oral cavities of patients with CF and suggested that this pathogen may initially colonize in the oral cavity prior to its colonization in the lower respiratory tract (19). More recently, we observed (20) that whole saliva of patients with CF (CF whole saliva) showed higher *P. aeruginosa*-aggregating activities than did whole saliva from non-CF subjects (non-CF whole saliva). In the same study, we have shown that it is the sialic acid content of whole saliva which acts as the salivary receptor for mucoid and nonmucoid *P. aeruginosa*. Therefore, this molecule may play an important part in the aggregation of this pathogen in the oral cavities of patients with CF. These studies raise the interesting possibility that intraoral colonization of *P. aeruginosa*, mediated by salivary aggregating activity, may adversely affect patients with CF in two ways: (i) inhalation of *P. aeruginosa* clumps, produced by salivary aggregating activity, which may result in colonization of the lower respiratory tract by this pathogen; and (ii) provision of a constant source of *P. aeruginosa*, allowing adherence to the various oral tissues.

Although we observed saliva-mediated aggregation of *P. aeruginosa* using whole saliva, this may not accurately reflect the whole answer regarding saliva-mediated aggregation of this pathogen in patients with CF. Human saliva is secreted by parotid, submandibular, sublingual, and minor salivary glands, and each of these glands produces a distinct pattern of macromolecules. It has been reported that whole, parotid, submandibular, and sublingual saliva all show varied bacterium-aggregating activities (33), with submandibular saliva exhibiting the highest activity in saliva-mediated aggregation of indigenous oral bacteria (7, 33). However, it is not clear what effect these differences have on the saliva-mediated aggregation of *P. aeruginosa* isolated from the oral cavities of patients with CF. Therefore, the present study was carried out to determine if there were any differences in the aggregation of both mucoid and nonmucoid variants of *P. aeruginosa* by whole, parotid, and submandibular saliva.

MATERIALS AND METHODS

Subjects. A total of 10 patients with CF (5 female, 5 male) regularly attending the CF clinic at the University Hospital,

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TABLE 1. Comparison of CF and non-CF whole-saliva aggregation of mucoid and nonmucoid *P. aeruginosa*

Patient	% Aggregation of *P. aeruginosa* strain							
	Mucoid				Nonmucoid			
	KBB2	TYT1	CZT1	CPT1	TYT2	HST2	HSS1	TYT3
With CF								
A	56.4	35.4	34.0	41.2	24.7	35.6	28.1	28.8
B	37.3	26.5	41.0	31.9	27.9	28.9	65.6	21.4
C	44.8	33.6	35.9	37.2	32.6	36.3	22.6	26.5
D	44.1	31.8	36.6	39.1	40.5	35.8	22.1	29.9
E	37.2	28.3	30.6	35.3	27.5	29.6	20.0	21.7
F	27.7	16.6	17.9	16.2	26.0	23.6	22.3	26.8
G	24.1	6.8	10.1	11.9	19.1	14.7	21.2	7.6
H	32.7	28.4	13.5	26.2	20.6	18.6	14.2	11.9
I	15.4	28.1	28.4	31.8	26.1	30.9	41.8	25.0
J	42.6	26.1	29.2	43.1	27.9	38.8	19.6	20.9
Without CF								
K	4.72	9.78	9.54	0.00	5.16	0.00	0.00	0.00
L	0.68	5.38	0.09	0.00	7.13	0.00	0.00	0.76
M	0.00	0.00	1.10	0.71	1.94	5.07	1.87	0.00
N	4.24	0.00	1.94	2.58	7.55	3.98	0.00	1.97
O	0.00	2.55	0.00	3.41	3.60	6.54	1.13	0.00
P	1.38	6.50	6.34	7.95	2.89	0.17	3.01	0.79
Q	5.82	5.11	5.30	0.57	0.00	5.96	5.66	0.00
R	0.00	2.60	0.00	2.98	0.90	6.66	1.32	0.00
S	0.00	8.39	10.20	7.60	8.47	3.29	5.76	2.37
T	0.00	4.18	6.21	0.00	0.00	10.30	9.83	0.00
P value (CF vs non-CF)	<0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Saskatoon, Saskatchewan, Canada, were examined. The patients were in generally good health at the time of sampling and were not undergoing drug therapy. The average age of the 10 patients was 13.1 years. Ten non-CF subjects served as a control group. This investigation was approved by the University of Saskatchewan Advisory Committee on Ethics in Human Experimentation, and informed consent was obtained from the patients or their parents.

Bacterial strains and bacteriological procedures. A total of 8 strains (4 mucoid, 4 nonmucoid) of freshly isolated *P. aeruginosa* were studied. These strains were isolated from various oral ecological sites, such as the buccal mucosa, tongue dorsum, saliva, and dental plaque. The methods used to isolate and identify *P. aeruginosa* from the oral cavities of patients with CF were described previously (19). Identified *P. aeruginosa* strains were transferred to several vials containing serum-coated glass beads and were immediately frozen at −70°C. These beads were used for subculturing. The *P. aeruginosa* strains were grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) at 37°C for 24 h. The cells required for the aggregation assay were harvested by centrifugation at 10,000 × *g* for 10 min and were subsequently washed three times with phosphate-buffered saline (pH 7.2). The cells were suspended in the same buffer and were dispersed with a 1-cm^3 (25-gauge, five-eighths-in. [1 in. = 2.54 cm] needle) syringe to an A_{700} of 1.5 (10^{10} cells per ml).

Saliva. Submandibular saliva was collected into ice-chilled vials, using the apparatus described by Truelove et al. (35). Parotid saliva was collected by a Carlsson and Crittenden-type apparatus as modified by Mason et al. (29). Salivary flow from the individual glands was stimulated by sucking on lemon-flavored candy. It was found that once the apparatus was suctioned over the individual gland, the secretion was free from saliva secreted by other glands. This was determined by placing the apparatus over the gland, getting suction, and then adding water that was colored by food coloring into the mouth of the subject. If the secretion from the saliva collection apparatus was not colored then, the collection of a pure secretion from the individual salivary gland was achieved. Whole saliva was collected by paraffin stimulation into ice-chilled vials.

The whole, submandibular, and parotid saliva samples were clarified by centrifugation at 10,000 × *g* for 10 min at 4°C. Clarified saliva was heated at 56°C for 30 min to destroy enzymes which may inactivate the aggregating factor. If saliva was not used immediately it was stored at −20°C until needed. Freezing and thawing of the saliva samples did not affect aggregation activity.

Aggregation assay. The saliva-mediated aggregation was assessed by the turbidimetric assay as described by Ericson et al. (8). Portions (0.5 ml) of the saliva were pipetted into small tubes and were warmed for 5 min at 37°C. At the same time, but in separate tubes, the bacterial suspension (A_{700} of 1.5) was heated at 37°C. Into the tube which contained the saliva, 0.5 ml of the bacterial suspension was added, as well as 0.5 ml of phosphate-buffered saline. The reaction mixture was stirred on a vortex mixer for 10 s. The contents were transferred immediately to a cuvette, and the A_{700} at zero time was measured. Phosphate-buffered saline was used as the reference blank. Cuvettes were maintained at 37°C for 90 min, at which time a final reading was taken. The results of the aggregation assay were expressed as percent aggregation after subtraction of a blank, obtained by incubating bacteria with phosphate-buffered saline alone. The aggregation assay was performed at least twice.

RESULTS

The *P. aeruginosa* aggregation abilities of whole, submandibular, and parotid saliva from 10 patients with CF and 10

TABLE 2. Comparison of CF and non-CF submandibular-saliva aggregation of mucoid and nonmucoid *P. aeruginosa*

Patient	% Aggregation of *P. aeruginosa* strain							
	Mucoid				Nonmucoid			
	KBB2	TYT1	CZT1	CPT1	TYT2	HST2	HSS1	TYT3
With CF								
A	42.2	29.7	28.9	37.9	15.4	24.9	21.8	20.5
B	26.8	22.2	32.5	26.9	19.1	19.9	46.8	15.4
C	37.8	24.2	28.9	27.8	24.1	27.3	20.0	23.0
D	40.7	28.2	33.6	34.9	37.2	33.1	20.0	26.7
E	28.5	20.8	23.2	29.0	22.1	21.7	15.5	15.9
F	22.9	12.7	12.5	13.0	20.1	15.8	16.5	20.2
G	19.7	4.97	6.75	7.74	14.9	11.7	16.0	5.38
H	27.2	22.0	9.68	20.4	17.9	16.5	11.8	8.72
I	13.1	24.2	22.4	28.4	23.5	26.5	37.1	21.0
J	37.6	22.6	26.7	36.2	24.8	35.9	17.3	18.8
Without CF								
K	14.10	11.70	16.80	7.06	11.80	10.50	3.73	0.23
L	7.29	14.30	9.59	5.68	10.40	0.95	0.31	1.88
M	14.20	10.00	17.30	10.70	8.09	6.70	0.55	4.17
N	15.00	6.12	13.50	9.62	6.26	4.15	4.15	4.31
O	14.60	8.04	7.15	13.70	8.15	6.14	6.14	3.92
P	14.00	19.90	20.70	8.82	4.39	12.10	13.60	0.00
Q	11.40	11.30	4.09	2.67	0.13	4.44	8.93	0.00
R	8.04	17.70	0.00	8.77	5.44	6.73	4.27	3.20
S	15.40	13.80	8.77	11.00	6.83	14.00	10.30	2.12
T	11.80	12.70	11.20	5.33	3.00	15.30	14.20	6.31
P value (CF vs non-CF)	<0.001	0.01	0.01	0.001	0.001	0.001	0.001	0.001

non-CF subjects were surveyed. In Table 1, the percent aggregation of *P. aeruginosa* mucoid and nonmucoid strains by CF and non-CF whole saliva is shown. Since the sample sizes in our study were small, the comparison of the percent aggregation scores of the individual strains of mucoid and nonmucoid *P. aeruginosa* by the CF and non-CF groups were analyzed by using nonparametric, Wilcoxon rank-sum statistics. Significant differences were found between CF and non-CF whole-saliva aggregation of the individual mucoid and nonmucoid strains of *P. aeruginosa* (*P* values listed in Table 1). Almost all CF saliva, except saliva from patient G, showed *P. aeruginosa* aggregation of greater than 10%. Alternatively, non-CF whole saliva showed less than 10% aggregation in almost all cases and often there was no recordable aggregation.

The CF and non-CF secretions from the submandibular and parotid glands were studied to determine if there were any significant differences in their contribution to *P. aeruginosa*-aggregating factors. Table 2 shows the percent aggregation of mucoid and nonmucoid strains of *P. aeruginosa* by CF and non-CF submandibular saliva. There were significant differences between CF and non-CF submandibular saliva aggregation of the four mucoid strains ($P < 0.01$) and of the four nonmucoid strains ($P < 0.001$). Therefore, like the whole saliva, submandibular saliva from CF patients and non-CF subjects also demonstrated significant differences in ability to aggregate *P. aeruginosa*.

Table 3 shows the percent aggregation of mucoid and nonmucoid strains of *P. aeruginosa* by CF and non-CF parotid saliva. There were no significant differences between CF and non-CF parotid saliva in aggregation of three of the four mucoid variants or of the four nonmucoid variants of *P. aeruginosa*. The parotid saliva from both the CF and non-CF groups showed lower overall percent aggregation of mucoid and nonmucoid *P. aeruginosa* strains compared with the submandibular- and whole-saliva aggregating activities.

A study was done to determine whether there were any differences in the aggregation of *P. aeruginosa* between the patients with CF colonized with *P. aeruginosa* in their oral cavities or sputum or both and the patients with CF from whom this pathogen was not isolated. Patients with CF from whom *P. aeruginosa* was isolated showed significantly higher ($P < 0.05$) aggregation by whole saliva of both mucoid and nonmucoid *P. aeruginosa* strains, compared with that of patients with CF from whom this pathogen was not isolated (Table 4). The submandibular saliva from patients with CF from whom *P. aeruginosa* was isolated also showed significantly higher aggregation of the mucoid variants ($P < 0.05$), compared with that of saliva from patients with CF from whom *P. aeruginosa* was not isolated. However, these two groups demonstrated no significant differences in the aggregation of nonmucoid variants by the submandibular saliva. There were also no significant differences between the two groups of CF patients in the parotid saliva aggregation of mucoid or nonmucoid strains of *P. aeruginosa*.

Table 5 shows *t*-test comparisons of the mean percent aggregation of *P. aeruginosa* by CF and non-CF whole, submandibular, and parotid saliva. The means of the aggregating activities (the average percent aggregation of the reactions between all the mucoid and nonmucoid *P. aeruginosa* and saliva) showed differences between CF and non-CF saliva similar to those of the individual saliva-strain aggregating activity comparisons (Tables 1, 2, and 3). That is, CF whole and submandibular saliva samples were significantly different from the corresponding non-CF secretions ($P < 0.0001$) in the aggregation of mucoid and nonmucoid strains. The parotid saliva from CF and non-CF groups were significantly different in the aggregation of mucoid variants ($P < 0.0001$), but no such significant difference was found in the aggregation of the nonmucoid variants.

General observations about the differences between aggregation of *P. aeruginosa* by CF whole, submandibular, and

TABLE 3. Comparison of CF and non-CF parotid saliva aggregation of mucoid and nonmucoid *P. aeruginosa*

Patient	% Aggregation of *P. aeruginosa* strain							
	Mucoid				Nonmucoid			
	KBB2	TYT1	CZT1	CPT1	TYT2	HST2	HSS1	TYT3
With CF								
A	13.10	5.33	5.46	3.35	3.66	8.08	4.40	5.49
B	5.05	2.29	3.06	4.70	3.94	4.11	12.60	3.89
C	4.67	3.09	4.41	6.24	8.20	4.53	0.00	4.13
D	3.33	4.05	1.71	4.16	2.35	0.00	0.00	0.85
E	4.19	3.40	5.57	4.67	4.82	6.23	0.00	4.78
F	2.15	2.59	0.66	1.95	3.75	0.71	1.93	5.94
G	3.56	0.00	0.96	0.44	2.46	3.28	2.54	0.13
H	4.26	4.42	1.33	4.84	0.00	0.00	0.54	0.00
I	0.00	5.61	3.19	3.27	2.42	0.00	3.50	3.71
J	4.24	2.21	3.18	2.77	0.82	0.00	0.65	0.00
Without CF								
K	0.00	0.60	3.15	0.00	0.91	0.00	0.00	0.00
L	0.81	0.53	1.58	0.00	5.23	0.00	0.00	2.98
M	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00
N	0.00	0.00	3.95	0.00	6.16	5.56	0.00	2.51
O	0.00	4.63	0.00	2.40	0.00	0.00	6.19	0.00
P	5.92	0.00	0.71	5.60	0.00	0.00	0.312	1.09
Q	5.15	1.07	6.82	0.00	0.34	3.21	6.11	1.88
R	0.00	5.20	0.00	0.00	1.00	5.61	0.00	3.79
S	3.27	6.99	4.33	5.92	4.19	1.13	6.01	6.96
T	4.72	1.59	1.80	0.00	0.75	5.72	7.04	4.67
P value (CF vs non-CF)	NS[a]	NS	NS	0.05	NS	NS	NS	NS

[a] NS, Not significant.

parotid saliva can be made by comparing the mean percent aggregation activity of these secretions. Similar observations may be made about any differences within the non-CF salivary groups. Table 6 shows the results of *t*-test comparisons between the individual salivary secretion aggregating activities within the CF group and within the non-CF group. Interestingly, non-CF submandibular saliva showed significantly higher aggregation than did the non-CF whole saliva ($P < 0.001$). However, the percent aggregation of *P. aeruginosa* by CF whole saliva was significantly higher than that of the submandibular saliva ($P < 0.001$). The non-CF whole-saliva aggregating activity is not significantly different from the non-CF parotid saliva aggregating activity. Yet the aggregating activities of the CF whole and parotid saliva are significantly different ($P < 0.0001$). CF and non-CF submandibular saliva demonstrated significantly higher aggregating activities than did the corresponding parotid secretions.

TABLE 4. Mean percent aggregation comparison of patients with CF colonized or not colonized with *P. aeruginosa*

Saliva source and *P. aeruginosa* variant	Mean % aggregation by saliva from CF patients		*P* value
	Colonized[a]	Not colonized[b]	
Whole			
Mucoid	37.9 ± 6.83	25.3 ± 9.89	<0.05
Nonmucoid	31.7 ± 10.60	23.2 ± 7.71	<0.05
Submandibular			
Mucoid	31.5 ± 5.89	20.5 ± 8.86	<0.05
Nonmucoid	24.7 ± 8.27	19.0 ± 7.28	NS[c]
Parotid			
Mucoid	4.64 ± 2.56	2.89 ± 1.70	NS
Nonmucoid	4.14 ± 3.38	2.01 ± 2.07	NS

[a] Patients A, B, C, and D.
[b] Patients E, F, G, H, I, and J.
[c] NS, Not significant.

TABLE 5. *t*-Test comparisons of the mean percent aggregation of *P. aeruginosa* by CF and non-CF saliva

Saliva source and *P. aeruginosa* variant	Mean % aggregation by saliva from patients		*P* value (CF vs non-CF)
	With CF	Without CF	
Whole			
Mucoid	30.4 ± 10.60	3.2 ± 3.2	<0.0001
Nonmucoid	26.6 ± 9.70	2.9 ± 3.1	<0.0001
Both	28.5 ± 10.30	3.0 ± 3.2	<0.0001
Submandibular			
Mucoid	24.9 ± 9.30	11.1 ± 4.5	<0.0001
Nonmucoid	21.3 ± 8.00	5.9 ± 4.3	<0.0001
Both	23.1 ± 8.90	8.5 ± 5.1	<0.0001
Parotid			
Mucoid	3.60 ± 2.20	1.9 ± 2.3	<0.01
Nonmucoid	2.90 ± 2.80	2.4 ± 2.5	NS[a]
Both	3.20 ± 2.50	2.2 ± 2.4	<0.001

[a] NS, Not significant.

DISCUSSION

Our present study showed that aggregation of *P. aeruginosa* by CF heat-treated whole saliva is higher than that by non-CF whole saliva (Table 1). This result is consistent with our previous study which used macroscopic aggregation

TABLE 6. *t*-Test comparison of *P. aeruginosa*-aggregating activities of the different CF and non-CF saliva

Saliva[a]	Mean % aggregation of *P. aeruginosa*		
	Mucoid and nonmucoid	Mucoid	Nonmucoid
CF			
Whole vs submandibular	28.50 ± 10.30	30.40 ± 10.60	26.60 ± 9.70
	23.10 ± 8.90	24.90 ± 9.30	21.30 ± 8.00
	<0.001	<0.05	<0.01
Whole vs parotid	28.50 ± 10.30	30.40 ± 10.60	26.60 ± 9.70
	3.20 ± 2.50	3.60 ± 2.20	2.90 ± 2.80
	<0.0001	<0.0001	<0.0001
Submandibular vs parotid	23.10 ± 8.90	24.90 ± 9.30	21.30 ± 8.00
	3.20 ± 2.50	3.60 ± 2.20	2.90 ± 2.80
	<0.0001	<0.0001	<0.0001
Non-CF			
Whole vs submandibular	3.00 ± 3.20	3.20 ± 3.20	2.90 ± 3.10
	8.50 ± 5.10	11.10 ± 4.50	5.90 ± 4.30
	<0.0001	<0.0001	<0.001
Whole vs parotid	3.00 ± 3.20	3.20 ± 3.20	2.90 ± 3.10
	2.20 ± 2.40	1.90 ± 2.30	2.40 ± 2.50
	NS[b]	NS	NS
Submandibular vs parotid	8.50 ± 2.20	11.10 ± 4.50	5.90 ± 4.30
	2.20 ± 2.40	1.90 ± 2.30	2.40 ± 2.50
	<0.0001	<0.0001	<0.001

[a] *P* value comparisons.
[b] NS, Not significant.

(20). The CF saliva from the submandibular gland also demonstrated overall higher aggregation activity than did the corresponding non-CF secretion (Table 2). The difference in *P. aeruginosa* aggregation by CF and non-CF parotid saliva was not significant (Table 3).

In this investigation, 4 of the 10 patients with CF were colonized with *P. aeruginosa* in their oral cavities or sputum or both, whereas 6 patients with CF were not colonized by this pathogen. Whole, submandibular, and parotid saliva from all patients with CF demonstrated high *P. aeruginosa*-aggregating activities. However, some differences were found between these two groups in the percent aggregation of the mucoid and nonmucoid strains of *P. aeruginosa* by the individual secretions (Table 4). There were significant differences ($P < 0.05$) between the percent aggregation of mucoid and nonmucoid strains of *P. aeruginosa* by whole saliva from colonized and noncolonized patients. Significant differences ($P > 0.05$) were also found between these two groups in submandibular-saliva aggregation of the mucoid variant. However, no significant differences were found in the aggregation by submandibular saliva of nonmucoid variants or in aggregation of either the mucoid or nonmucoid strains by parotid saliva. Although CF saliva-mediated *P. aeruginosa* aggregation activity was not dependent on colonization of the patient with *P. aeruginosa*, the degree to which the mucoid and nonmucoid variants were aggregated by the different saliva did vary.

The contribution to whole saliva from individual glands may vary, but it generally consists of an ~45% contribution from both the submandibular and parotid glands and an ~5% contribution from both the sublingual and minor salivary glands (26). The non-CF submandibular saliva demonstrated higher aggregating activity than did non-CF whole saliva ($P < 0.0001$), thereby indicating that an apparent dilution of the salivary *P. aeruginosa*-aggregating factors occurs in non-CF whole saliva. However, our results (Tables 5 and 6) indicate that in CF, such a dilution of the salivary aggregating factors does not occur, as whole saliva demonstrated a higher percent aggregation of *P. aeruginosa* than did the CF submandibular saliva ($P < 0.0001$). Furthermore, CF whole saliva showed a higher percent *P. aeruginosa* aggregation compared with the corresponding parotid secretion, yet no significant differences were found between the non-CF secretions. Therefore, the relative contribution of *P. aeruginosa*-aggregating factors to whole saliva from the individual salivary glands in patients with CF is not the same as in the non-CF saliva.

There is some conflicting information about the parotid gland secretory rates. A recent study by Kollberg et al. (17) found that there is a reduced volume of saliva from the parotid glands in patients with CF, even with strong stimulation. However, Marmar et al. (28) demonstrated an increased secretory rate by these glands in patients with CF, whereas Kutscher et al. (21) found no differences between the glands of CF patients and non-CF subjects in either unstimulated or stimulated secretion rates. The secretory rate of CF submandibular saliva has been found to be slightly lower than (4, 25) or no different from (2) the secretions from this gland in non-CF subjects. Since there is no evidence of increased secretory flow from the submandibular gland, a possible explanation of our results is that the parotid secretion rate is indeed reduced. If this is the case, then the submandibular saliva, which our study has shown to contain the majority of the aggregating factors, may be implicated as being responsible for the differences in *P. aeruginosa*-aggregating activities in the oral cavities of CF patients and non-CF subjects.

The submandibular gland saliva has been identified as having a significant role in the aggregation of some indigenous oral bacteria (7, 33). Our results confirm the observations of other studies (11, 33) that the sero-mucous products of the submandibular gland are more significant in their aggregating activities than are those of the serous fluid-secreting parotid cells. In patients with CF, the submandibular gland has been shown to be enlarged (1, 4), but such pathologic abnormalities have not been noted for the parotid

gland (1, 3, 5). The submandibular gland sero-mucoid secretion is similar to a secretion from the tracheobronchial tract, which in CF is pathologically altered, resulting in obstruction of the ducts (3). Therefore, examination of the submandibular saliva represents a novel method by which the effects of the sero-mucoid secretion may be studied and extended to examination of the tracheobronchial tract. Furthermore, on a more basic level, the present study indicates that salivary molecules from the submandibular gland may be directly involved in the mechanisms by which *P. aeruginosa* colonizes or is cleared from the oral cavities of patients with CF.

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