BIOCHEMICAL MEDICINE AND METABOLIC BIOLOGY 36, 260–265 (1986)

The Salivary Flow Rate and Composition of Whole and Parotid Resting and Stimulated Saliva in Young and Old Healthy Subjects

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Received October 16, 1985

Numerous studies have been published on the effect of age on salivary secretion (1,9). In many of the former studies young, healthy subjects were compared to institutionalized elderly subjects with systemic diseases and on various medications. As various diseases and drugs influence the salivary flow rate and composition, the picture that has evolved of extensive dysfunction of the salivary glands in the elderly appears to be overemphasized.

Recently several studies have been published on the salivary function in healthy, unmedicated elderly subjects in whom stimulated parotid saliva was analyzed. Heft and Baum (6) reported that parotid flow rate, both resting and stimulated, did not change significantly with age. Chauncey *et al.* (5) found that pH, sodium, chloride, calcium, and total protein decreased significantly with age.

In the present study, we examined young and old healthy volunteers to try to evaluate the effect of aging on the salivary glands by comparing resting and stimulated whole and parotid saliva.

MATERIALS AND METHODS

The population studied consisted of 63 volunteers residing in the community, 31 females and 32 males. They were divided by age into two groups: 39 "young" subjects 37 ± 10.5 years of age and 24 "old" subjects 66 ± 3.3 years of age. Only healthy, unmedicated subjects were included in the study. The choice was made by their physicians, relying on their medical files and a questionnaire.

The salivary samples were collected between 9 AM and 12 noon, at least 1 hr after a meal. Whole saliva was collected by the spitting method and parotid saliva by a Curby cup. Stimulation was performed by applying 2% citric acid every 30 sec to the sides of the tongue (3a). The sequence of collection was

[1] Based on a thesis submitted to the Faculty of Medicine, Technion–Israel Institute of Technology, in partial fulfillment of the requirements for the M.D. degree.

0885-4505/86 $3.00

resting whole, stimulated whole, stimulated parotid, taking down the cup, and resting parotid saliva. Each sample was collected for 10 min.

Sodium and potassium concentrations were measured by flame photometry and those of calcium and magnesium by atomic absorption.[2] Total protein was measured by the method of Lowry *et al.* (8) and amylase by the Phadebas amylase test.[3]

Statistical analysis was performed by SAS on an IBM 3081D computer. Equality of variance was examined by the F distribution test; a standard t test was used for equal variances, and a modified t test for unequal variances. The Wilcoxon two-sample test was also used. To evaluate the overall effect of age, a MANOVA test was performed for each parameter separately and for the "vector" of salivation.

RESULTS

No significant differences in any of the parameters examined were found between males and females, using the Wilcoxon two-sample test ($P < 0.01$). Therefore, the data for males and females were pooled.

Mean salivary flow rates of young and old are given in Table 1. Although the resting and stimulated whole saliva and the stimulated parotid flow rates were lower in the old, the differences were not statistically significant.

Salivary amylase activity and total protein concentration are given in Table 2. No significant difference in total protein in any of the salivary types was found between the young and the old. Salivary amylase activity was significantly lower in the old in resting and stimulated parotid saliva. In whole saliva the amylase activity was lower in the old; however, the difference was not significant.

Salivary sodium and potassium concentrations are given in Table 3. No significant differences were found between young and old in parotid saliva. The stimulated whole saliva sodium concentration was lower, and resting whole saliva potassium concentration was higher in the old.

Salivary calcium and magnesium concentrations did not differ significantly between young and old in whole saliva (Table 4). Stimulated parotid calcium concentration was lower in the old ($P = 0.07$). Resting parotid magnesium concentration was also lower in the old ($P = 0.04$).

By MANOVA analysis of resting whole saliva of the entire population, an overall age effect on salivary function was observed, but no significant change could be found in any of the parameters separately. In stimulated whole saliva an overall age effect, with a significant effect on calcium concentration (Ca concentration = 7.44–0.047 age), was detected.

DISCUSSION

In the present study we compared resting and stimulated whole and parotid salivary rates of secretion and composition in young and old healthy, unmedicated volunteers.

No significant differences in resting or stimulated parotid flow rates (Table 1)

[2] Model 107., Perkin–Elmer.

[3] Pharmacia Diagnostics, Uppsala, Sweden.

TABLE 1
Salivary Flow Rate in the Population Studied[a]

Type of saliva	Young[b] (ml/min)	Old[c] (ml/min)
Whole, unstimulated	0.460 ± 0.310 (39)[d]	0.390 ± 0.190 (24)
Whole, stimulated	1.530 ± 0.810 (39)	1.280 ± 0.500 (24)
Parotid, unstimulated	0.049 ± 0.039 (34)	0.066 ± 0.047 (18)
Parotid, stimulated	0.290 ± 0.198 (39)	0.223 ± 0.172 (24)

[a] Data given as means ± SD.
[b] Age of young group = 37.0 ± 10.5 years.
[c] Age of old group = 66.0 ± 3.3 years.
[d] Figures in parentheses indicate numbers of individuals. In 6 "old" volunteers out of 24 and in 5 "young" out of 39, resting parotid saliva could not be collected, in spite of prolonged endeavors.

TABLE 2
Salivary Amylase and Protein in the Population Studied[a]

	Amylase (IU/l × 10^3)		Protein (mg/100 ml)	
Type of saliva	Young	Old	Young	Old
Whole, unstimulated	516 ± 414 (35)[b]	454 ± 256 (21)	126 ± 49 (31)	142 ± 90 (22)
Whole, stimulated	580 ± 371 (34)	422 ± 283 (19)	97 ± 34 (34)	111 ± 44 (22)
Parotid, unstimulated	1043 ± 413 (31)[c]	639 ± 381 (14)[c]	190 ± 67 (12)	170 ± 91 (13)
Parotid, stimulated	834 ± 397 (34)[d]	505 ± 300 (19)[d]	158 ± 88 (27)	135 ± 53 (11)

[a] Data given as means ± SD.
[b] Figures in parentheses indicate numbers of individuals.
[c] $P < 0.005$, t test between young and old; $P = 0.0043$, Wilcoxon scores.
[d] $P < 0.005$, t test between young and old; $P = 0.0041$, Wilcoxon scores.

TABLE 3
Salivary Sodium and Potassium in the Population Studied[a]

	Sodium (meq/l)		Potassium (meq/l)	
Type of saliva	Young	Old	Young	Old
Whole, unstimulated	5.36 ± 2.17 (31)[b]	5.73 ± 4.39 (22)	19.33 ± 4.08 (31)[c]	21.48 ± 4.68 (24)[c]
Whole, stimulated	10.08 ± 5.64 (33)[d]	7.27 ± 3.59 (23)[d]	18.00 ± 3.15 (33)	18.39 ± 3.55 (23)
Parotid, unstimulated	2.13 ± 1.91 (19)	2.27 ± 1.14 (12)	26.84 ± 10.75 (21)	23.07 ± 4.15 (17)
Parotid, stimulated	4.42 ± 3.37 (30)	3.44 ± 3.09 (21)	23.08 ± 4.23 (30)	22.65 ± 4.19 (24)

[a] Data given as means ± SD.
[b] Figures in parentheses indicate numbers of individuals.
[c] $P = 0.0676$, Wilcoxon scores.
[d] $P = 0.0329$, Wilcoxon scores; $P = 0.027$, t test for unequal variance.

TABLE 4
Salivary Calcium and Magnesium in the Population Studied[a]

Type of saliva	Calcium (meq/l)		Magnesium (meq/l)	
	Young	Old	Young	Old
Whole, unstimulated	2.39 ± 1.18 (36)[b]	1.93 ± 0.71 (22)	0.58 ± 0.27 (32)	0.63 ± 0.26 (22)
Whole, stimulated	2.35 ± 0.99 (36)	2.17 ± 0.77 (22)	0.56 ± 0.24 (31)	0.53 ± 0.25 (22)
Parotid, unstimulated	2.31 ± 1.73 (15)	1.60 ± 0.98 (12)	0.88 ± 0.66 (9)[c]	0.41 ± 0.25 (12)[c]
Parotid, stimulated	2.05 ± 1.55 (32)[d]	1.43 ± 0.52 (19)[d]	0.53 ± 0.45 (28)	0.41 ± 0.22 (20)

[a] Data given as means ± SD.
[b] Figures in parentheses indicate numbers of individuals.
[c] $P = 0.07$, t test for unequal variance.
[d] $P = 0.04$, t test for unequal variance.

were found, in agreement with the results of Heft and Baum (6). No significant difference in stimulated whole salivary flow rate was observed, similar to our previous results (3a) and to those of Parvinen and Larmas (11), while resting salivary flow rate, although lower in the old, did not reach a level of significance. The present results fail to confirm our earlier observations (3a) and those of others (4,12) of significantly lower resting secretion in the old. Only a nonsignificant trend was observed here, indicating considerable sample variability. This variability might be due to such factors as the extent of cooperation or the quality of oral–motor coordination required for evaluating flow rate by the spitting method. Further, various environmental factors such as temperature and humidity could be involved.

No significant difference was found between young and old in protein concentration and amylase activity in resting or stimulated whole saliva (Table 2). Meyer *et al.* (10) reported on a pronounced deficiency in amylase activity in old people in both resting and stimulated whole saliva. However, they examined a far older population from a home for the aged, without excluding patients with various systemic diseases or treatments. Furthermore, their method of stimulation was by chewing, which is less efficient in the old.

The total protein concentration in the parotid saliva, both resting and stimulated, was lower in the old; however, the difference was not significant (Table 2). The amylase activity was significantly lower in the old when calculated per milliliter of parotid saliva and also when calculated per milligram of protein. Our data differ from those of Helfman and Price (7), who found that amylase activity in stimulated parotid saliva did not decrease with age. They examined parotid saliva collected in the afternoon and stimulated by acetic acid; the flow rates are not given.

Our data on nonsignificantly altered protein concentration and significantly lower amylase activity in parotid saliva of the old differ from those of Chauncey

et al. (5), who found that total protein concentration significantly decreased with age; however, the decrease in amylase activity was not significant. The dissimilarity to our results might be due to the differing method and extent of stimulation. Perhaps the quantitative relationship between total protein and amylase changes at varying levels of stimulation, as amylase secretion might increase after stimulation and that of various other proteins might decrease.

The discrepancy between our present results and those of others probably reflects the difference in the methodology used in stimulating salivary secretion as well as in selecting the volunteers. Also, possibly our method of sequential collection of the different salivary samples during a prolonged period of 40 min caused a depletion in the parotid amylase, revealing a difference between young and old. Indeed, Baum *et al.* (3) have reported that the parotid content of amylase was reduced about 50% in aged rats. No such study was performed in human parotid glands.

Only a few significant differences between young and old were found in the salivary electrolyte concentrations (Tables 3 and 4). The calcium in stimulated parotid, the magnesium in resting parotid, and the sodium concentration in stimulated whole saliva were lower in the elderly. Thus, a trend of lowered electrolyte concentrations in the saliva of old people was observed. Chauncey *et al.* (5) found significantly lower pH, sodium, chloride, and calcium concentrations in the old in vigorously stimulated parotid saliva. The dissimilarity to our results perhaps could be due to the difference in intensity of stimulation: while we worked at mean flow rates of 0.2 ml/min, Chauncey *et al.* (5) obtained mean flow rates of over 1 ml/min. Perhaps the parotid glands of the elderly at low flow rates secrete electrolytes similar to the young, while at a strong, continuous challenge of stimulation the difference between the young and old is demonstrated. Baum *et al.* (2) reported on age-related alterations in sodium handling, which seems to be more pronounced at high flow rates.

By multivariate analysis, an overall age effect on salivary function was detected; however, no significant effect could be found in any single parameter of resting whole saliva in the population examined. In stimulated whole saliva, an overall age effect with a significant change in calcium concentration was detected. No significant relationship to age was found by multivariate analysis for resting or stimulated parotid saliva.

In summary, it appears that the differences between young and old in most of the parameters examined in resting and stimulated whole and parotid saliva did not reach a level of significance. Thus, in spite of the extensive morphological changes reported in the salivary glands, the functional alterations appear to be very mild. These are revealed by special approaches such as extensive stimulation or prolonged follow-up (Chauncey *et al.*, personal communication) or by sequential collection (Table 2), probably fatiguing the glands.

Furthermore, the choice of volunteers is important. Perhaps one should examine an older population above the age of 70, which is the average life expectancy in our country. Also, as the effects of aging are mediated via circulatory or nervous pathways which regulate salivation, by choosing extremely healthy volunteers we artifically segregate a group of chronologically old but biologically young indviduals, obtaining a picture of intact salivary function.

SUMMARY

Resting and stimulated whole and parotid salivary composition and flow rate were examined in 63 healthy volunteers. No significant differences were found between the young and old in secretion rates and salivary concentrations of sodium, potassium, calcium, magnesium, and total protein. The activity of amylase in the resting and stimulated parotid saliva was significantly lower in the old.

ACKNOWLEDGMENT

The authors thank Miss Ruth Singer for excellent secretarial help.

REFERENCES

1. Baum, B. J., *Spec. Care Dent.* **1,** (1981).
2. Baum, J. B., Costa, P. T., and Izutsu, T. K., *Amer. J. Physiol.* **246,** R35 (1984).
3. Baum, J. B., Levine, R. L., Kuyatt, B. L., and Sogin, D. B., *Mech. Ageing Dev.* **19,** 27 (1982).
3a. Ben-Aryeh, H., Miron, D., Szargel, R., and Gutman, D., *J. Dent. Res.* **63,** 1147 (1984).
4. Bertram, V. *Acta Odontol. Scand. Suppl.* **49,** 13 (1967).
5. Chauncey, H. H., Borkan, G. A., Wayler, A. H., Feller, R. P., and Kapur, K. K., *Adv. Physiol. Sci.* **28,** 323 (1981).
6. Heft, M. W., and Baum, B. J., *J. Dent. Res.* **63,** 1182 (1984).
7. Helfman, P. M., and Price, P. A., *Exp. Gerontol.* **9,** 209 (1974).
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.* **193,** 265 (1951).
9. Mandel, I. D., *Gerodontology* **3,** 47 (1984).
10. Meyer, J., Golden, J. S., Steiner, N., and Necheles, H., *Amer. J. Physiol.* **119,** 600 (1937).
11. Parvinen, T., and Larmas, M., *J. Dent. Res.* **61,** 1052 (1982).
12. Yaegaki, K., Ogura, R., Kameyama, T., and Sujaku, C., *Int. J. Oral. Surg.* **14,** 47 (1985).