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Research Article

Parotid Gland Chemical Secretion under Identical and Diverse Forms of Stimulation: Major Physiologic Components

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Abstract

Context: In our days, the mechanism of salivary glands excretory and/or secretory functions are still a challenge.

Aims: To study the effects of varying parotid gland flow rates upon the levels of bicarbonate, calcium, pH, inorganic phosphate, potassium, sodium, total protein, and chloride in parotid fluid.

Methods and Material: Healthy individuals (N = 28; 17-22 yrs.) were participated in this study and each given parotid saliva samples (₦ = 53) under different stimulation conditions. The stimulated samples were categorized as the following: one rubber band (size 32), one-half stick of gum base, one and one-third sticks of sugared gum, one-half stick of gum base, and one rubber band (size 32). Using the chloridometer, the chloride measurement method was validated and compared with Sendroy titration.

Statistical analysis used: Data were expressed as mean and standard deviation. In addition, the correlations of the flow rate and the measured concentrations "between-subjects" and "within-subjects" were assessed based on 5-pairs of observations and the probability of significance for each coefficient was defined.

Results: Contrary to phosphate, the salivary flow rate, pH, chloride, and bicarbonate with rubber band (nonrepeat experiment) and sugared gum, were the lowest and the highest values. Opposing to total protein, calcium reached the highest and the lowest concentrations with repeated gum base and sugared gum, respectively. Potassium contents were lowest and highest with rubber band (nonrepeat experiment) and gum base, respectively. In within-subjects condition, the bicarbonate was positively correlated with calcium and pH and negatively with inorganic phosphate. However, in between-subjects, with totally insignificant correlations, the bicarbonate was negatively correlated with calcium contrary to pH and inorganic phosphate. Potassium in between-subjects was negatively correlated with sodium and chloride and insignificantly related to flow rate and total protein. Sodium was correlated with flow rate and chloride within subjects. Between-subjects, total protein was insignificantly related to flow rate, potassium, sodium, and chloride. "Between-subjects", chloride was negatively correlated to potassium, positively correlated to sodium, and insignificantly related to flow rate and total protein.

Conclusions: Most surprisingly, salivary flow rate is correlated significantly and negatively with inorganic phosphate and with no significant correlation with potassium.

Keywords: Bicarbonate; Rubber Band; Glandular Physiology; Duct Cells; Dietary Control.

Introduction

One of the most intriguing problems in glandular physiology is that concerned with the understanding of the excretory and/or secretory functions of the salivary glands [1-3]. The mechanisms by which certain chemical constituents enter and are released by the gland cells are not at all clear [4,5]. There is distinct disagreement as to whether or not there is active reabsorption by the duct cells and, conversely, whether or not certain duct cells actually elaborate chemical substances into the saliva [6,7]. For over a century, it has been known that the rate of flow from the gland exerts an influence on the concentrations of some of the chemical components of saliva [3], and many investigations have been carried out in flow rate-concentration relationships in efforts to clarify the mechanisms of glandular function [8]. Unfortunately, there has been a marked diversity in duration and methods of glandular stimulation [9], procedures for chemical determinations, time of day of collections, dietary control, age of subjects, and other variables under the control of the investigator [5,10].

It was our purpose in the present study to investigate, under controlled experimental conditions, the effects of varying parotid gland flow rates upon the levels of bicarbonate, calcium, pH, inorganic phosphate, potassium, sodium, total protein, and chloride in parotid fluid. The performances of three different methods for chloride determination were also evaluated. Of further concern was the determination of intercorrelations for findings other than flow rate and the development of a pattern of stimulation that would consistently provide a reproducible, well-spread flow rate pattern for this and future correlation studies.

Materials and Methods

This report includes the data from two separate flow rate experiments, each utilizing identical stimulatory procedures to elicit parotid flow. All subjects were young men between the ages of 17 and 22 years; each had recently undergone a physical examination and had been found fit for military service. Dietary factors, hours of waking and sleeping, physical exertion, and environmental exposure were identical within the experimental groups. Admitted recent bouts of upper respiratory infection, nausea, vomiting, diarrhea, constipation, or any significant systemic symptom disqualified a prospective subject.

Ten-minute parotid fluid test samples were collected by vacuum cap from the right parotid gland with the following sequence of stimulants: Sample 1. one rubber band (size 32); Sample 2, one-half stick of gum base; Sample 3, one and one-third sticks of sugared gum; Sample 4, one-half stick of gum base; Sample 5, one rubber band (size 32). This procedure represented an attempt to study flow rate-concentration relationships with significantly different rates of flow in both ascending and descending order. All samples were collected under mineral oil with the collection tube packed in ice. Prior to collection of each test specimen, a ten-minute accommodation (waste) sample was collected with the identical eliciting agent and discarded. The sugared gum bolus was renewed at the expiration of five minutes. The different quantities of gum base and sugared gum were employed since the hydration of one-half stick of gum base over a five-minute period produces a final bolus volume approximating that found for one and one-third sticks of sugared gum, the decrease in volume of the latter being due primarily to the rapid loss of sugar from the gum bolus.

In the first experiment, five test samples were collected from each of twenty-eight subjects, and levels were determined for rate of flow, bicarbonate, calcium, pH, and inorganic phosphate. In the next phase, twenty-five subjects were sampled and observations were recorded for a flow rate, potassium, sodium, total protein, and chloride. Volume was read directly from the graduated centrifuge tubes in which collections were made; pH was determined on a Beckman Zeromatic pH meter; bicarbonate was measured by the method of Van Slyke [11]; calcium and inorganic phosphate were determined on the Autoanalyzer by methods that we have previously reported [3,5]. Sodium and potassium analyses were performed on a Beckman DU flame spectrophotometer as previously described [12,13]. Total protein levels were ascertained by the biuret reaction and checked by HPLC [14]. Chloride concentrations were determined on the Cotlove chloridometer [15,16], and an evaluation of this method as compared to the Autoanalyzer method and an iodometric titration method [17] was included. Since several reports have been based upon the Van Slyke and Hiller modification of the method of Sendroy for chloride determination [18,19], and since we were aware of a discrepancy between chloride findings and actual concentration chloride at low levels, the Sendroy procedure [20] was the hand method chosen for comparison.

In the statistical analysis of the flow rate-concentration data, correlation coefficients were computed for each pair of variables of each subject, based upon the five pairs of observations. Then an average "within-subjects" correlation coefficient was calculated for each pair of variables. In addition, means and standard deviations of the five observations were computed for each variable and for each subject, and a "between-subjects" correlation coefficient was calculated for each pair of variables. The probability of significance was determined for each coefficient.

Results and Discussion

Flow rate experiment 1

In evaluating the three methods for the determination of parotid fluid chloride concentration, the Autoanalyzer portion was discontinued for two reasons: (1) A drift in the digital response to repeated running of standards made accurate estimation difficult and (2) when the untreated saliva was mixed with the sulfuric acid (H_2SO_4)-hyamine (benzethonium chloride) diluent, a turbidity was produced which led to the plating-out of a white precipitate upon the glass wall of the cactus-tree fitting in which the mixing took place.

Chloride method validation

A comparison of the iodometric titration chloride method to the Chloridometer method is shown in Figure 1, a graph which plots actual chloride concentrations of 5 to 50 mEq. per liter chloride standards against chloride concentration for these standards as calculated by titration and by the apparatus. Titration values were found to be higher; this effect being especially pronounced at the lower levels of concentration where a standard of actually 5.0 mEq. per liter chloride concentration gave a titration value of 10.1 mEq. per liter. Between 5.0 and 15.0 mEq. per liter, the titration results were linear, while between 15.0 and 50.0 mEq. per liter the results, although linear, were on a slightly different plane. From 60.0 to 100.0 mEq. per liter neither method varied more than 0.5 per cent from the actual standard value and no systematic pattern of difference was found. Throughout the range of from 5.0 to 100.0 mEq. per liter, the Chloridometer values were virtually identical to the actual chloride concentration. The difference in performance of the two methods on a series of parotid fluid samples was very similar to that found with standards.

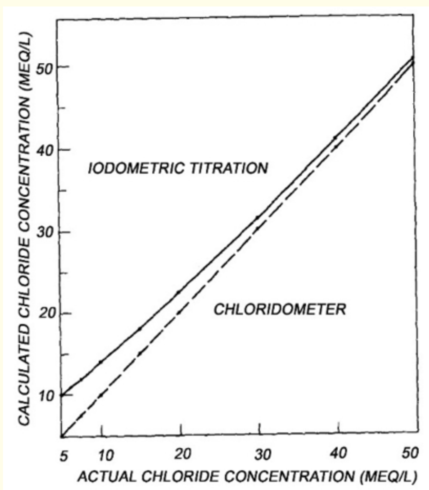

Figure 1: The chloridometer versus Sendroy titration in chloride determination.

Performance of flow rate stimulation procedure

Flow rate, as it was produced in the current experiments, was spread sufficiently to provide an adequate range for correlation studies. That the reproducibility of this method is of a high order is demonstrated when the results for the two experiments are compared (Figure 2).

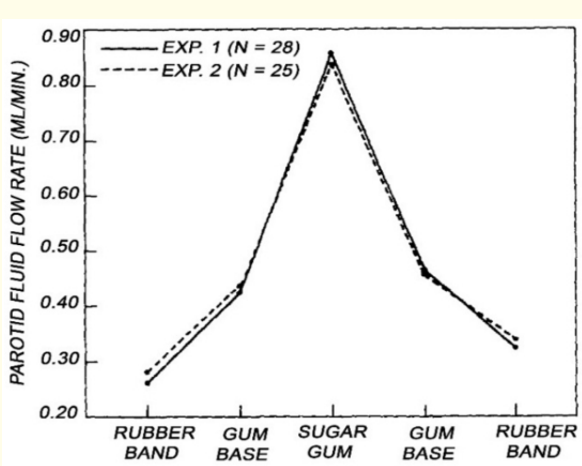

Figure 2: Reproducibility of the elicitation procedure.

In the first study, a rubber band flow rate mean of 0.261 ml. per minute (S.D., 0.107) increased to 0.425 (S.D., 0.139) with gum base and to 0.858 (S.D., 0.227) with sugared gum and fell to 0.465 (S.D., 0.165) and to 0.326 (S.D., 0.144) when gum base and rubber bands were repeated. Comparable figures for the second experiment were 0.279 ml. per minute (S.D., 0.121), 0.436 (S.D., 0.194), 0.838 (S.D., 0.284), 0.458 (S.D., 0.191), and 0.340 (S.D., 0.147), respectively.

On the basis of these findings, this elicitation procedure could be recommended as satisfactory with respect to simplicity, time required, flow rate range, and reproducibility. It is extremely difficult to interpret flow rate correlation studies with stimulation patterns providing fewer degrees of freedom for statistical analysis.

Bicarbonate

Within subjects, a significant ($P < 0.01$) positive correlation ($r = 0.98$) was found between flow rate and bicarbonate (Figure 3).

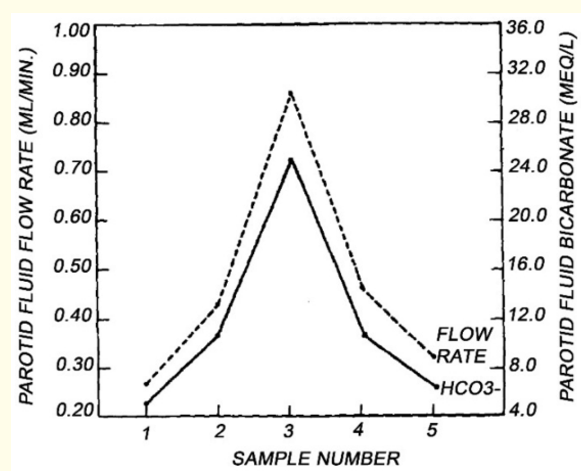

Figure 3: Correlation between flow rate and bicarbonate.

For the five samples, the bicarbonate mean values were 5.06 (S.D., 4.49), 10.65 (S.D., 5.94), 24.77 (S.D., 7.54), 10.52 (S.D., 5.30), and 6.23 (S.D., 4.38) mEq. per liter. Bicarbonate was found to be positively correlated with calcium and with pH and to be negatively correlated with inorganic phosphate (Table 1). When an entry in Table 1 was marked with an (a), the correlation between these two variables had been found to differ significantly from one subject to another, and therefore the average was not an estimate of the correlation for a given person.

The average correlations not marked with an (a) were estimates of the correlation coefficients for all subjects in the population. In other words, from the data in these experiments, there was no reason to suspect that the correlations not marked with an (a) differed from one subject to another.

	Bicarbonate	Calcium	pH	Inorganic phosphate
Flow rate	0.98 (< 0.01)	0.55 (< 0.01)[a]	0.93 (< 0.01)	-0.86 (< 0.01)
Bicarbonate		0.54 (< 0.01)[a]	0.93 (< 0.01)	-0.86 (< 0.01)
Calcium			0.27 (< .05)	-0.29 (< 0.05)[a]
pH				-0.87 (< 0.01)

[a]The correlation between these two variables differed significantly from one subject to another.

Table 1: The average "within-subjects" correlation coefficients for five parotid fluid samples from each of twenty-eight male subjects and the probability of coefficient significance.

In the "between-subjects" correlations, bicarbonate and flow rate demonstrated a significant positive correlation (r = 0.59), but the correlations of bicarbonate with calcium, pH, and phosphate were not significant (Table 2).

Calcium

The positive correlation coefficient for flow rate and calcium (r = 0.55), calculated within subjects, was significant at the 1 per cent level, but it differed significantly from one subject to another (Figure 4). The mean calcium values for the five specimens were 1.61 (S.D., 0.47), 1.45 (S.D., 0.36), 1.70 (S.D., 0.36), 1.39 (S.D., 0.30), and 1.50 (S.D., 0.47) mEq. per liter. Calcium was positively correlated, with bicarbonate (P < 0.01) and pH (P < 0.05) and negatively correlated with phosphate (P < 0.05) (Table 1).

	Bicarbonate	Calcium	pH	Inorganic phosphate
Flow rate	0.59 (< 0.01)	-0.36 (> 0.05)	0.45 (< 0.05)	-0.06 (> 0.05)
Bicarbonate		-0.28 (> 0.05)	0.32 (> 0.05)	0.23 (> 0.05)
Calcium			-0.60 (< 0.01)	-0.04 (> 0.05)
pH				0.29 (> 0.05)

Table 2: The "between-subjects" correlation coefficients and the probability of their significance.

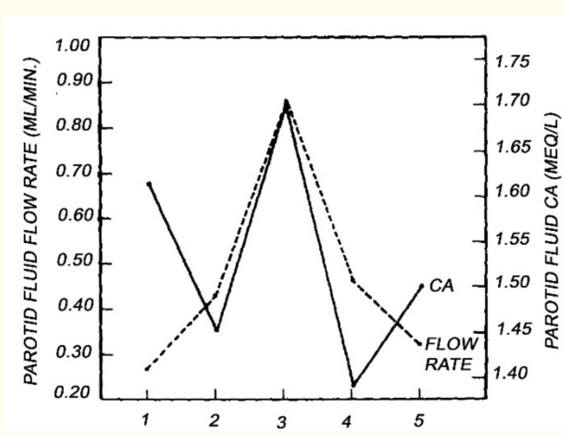

Figure 4: The correlation between flow rate and calcium.

In the "between-subjects" correlations, calcium was negatively correlated with flow rate (P < 0.0l) and negatively correlated with pH (P < 0.0l), while the correlations with bicarbonate and phosphate were not significant (Table 2).

pH

The positive correlation, within subjects, between pH and flow rate was significant at the 1 per cent level (Figure 5). Means for pH for the twenty-eight subjects were 6.77 (S.D., 0.37), 7.07 (S.D., 0.27), 7.38 (S.D., 0.17), 7.08 (S.D., 0.22), and 6.88 (S.D., 0.29). pH was found to be positively correlated with bicarbonate (P < 0.0l), positively correlated with calcium (P < 0.05), and negatively correlated with phosphate (P < 0.01) (Table 1). In the "between-subjects" analysis, pH was positively correlated with rate of flow (P < 0.0l) and negatively correlated with calcium (P < 0.0l). The correlations with bicarbonate and with phosphate were not significant (Table 2).

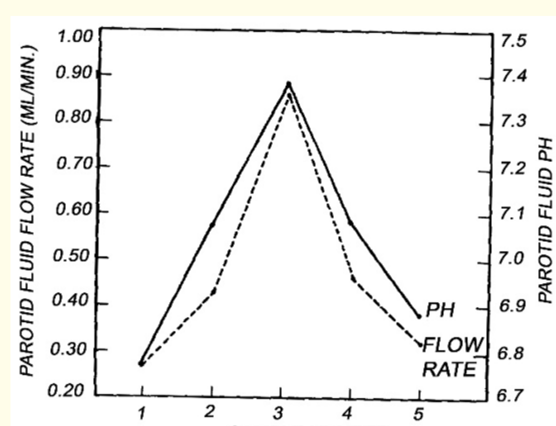

Figure 5: The correlation between flow rate and PH.

Inorganic phosphate

A strong negative correlation ($P < 0.01$) was noted within subjects between phosphate and flow rate (Figure 6). Means for phosphate were 13.58 (S.D., 2.40), 11.88 (S.D., 2.03), 9.77 (S.D., 1.96), 12.36 (S.D., 2.40), and 14.20 (S.D., 265) mg. per 100 ml. of parotid fluid.

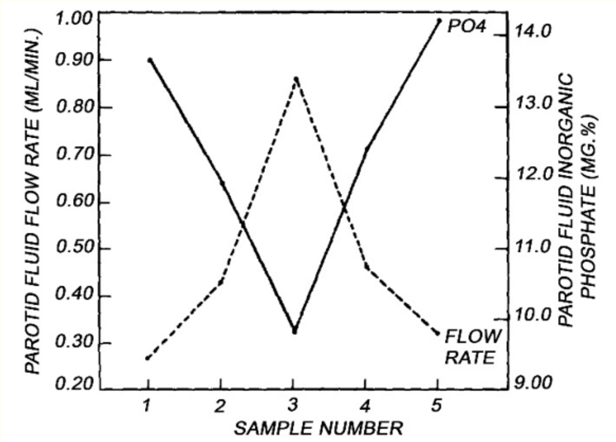

Figure 6: The correlation between flow rate and inorganic phosphate.

Phosphate was negatively correlated with bicarbonate ($P < 0.01$), calcium ($P < 0.05$), and pH ($P < 0.01$) (Table 1). "Between-subjects" phosphate was not significantly correlated with any of the four other variables under study (Table 2).

Flow rate experiment 2

Potassium

In the "within-subjects" analysis, no significant correlation could be found for potassium with flow rate (Figure 7), sodium, or chloride. A positive correlation of borderline significance ($P < 0.05$) was noted for potassium and total protein (Table 3).

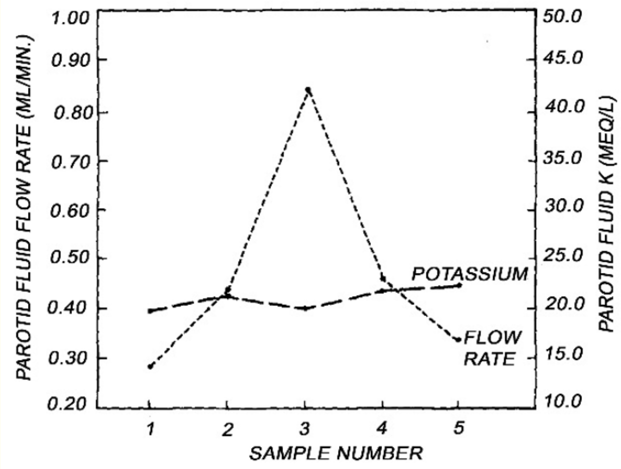

Figure 7: The correlation between flow rate and potassium.

For the twenty-five subjects, the potassium means for the five test samples were 19.80 (S.D., 4.30), 21.16 (S.D., 3.07), 20.12 (S.D., 4.44), 21.79 (S.D., 3.74), and 22.39 (S.D., 2.94) mEq. per liter. "Between-subjects" potassium (Table 4) was negatively correlated with sodium ($P < 0.01$) and chloride ($P < 0.01$) but insignificantly related to flow rate and to total protein.

Sodium

Sodium was found to be strongly correlated with flow rate (Figure 8), within subjects, in a positive fashion ($P < 0.01$). Significant ($P < 0.01$) correlations were also seen for sodium with total protein and for sodium and chloride. The sodium-potassium correlation was not significant (Table 3). As stimulation intensity was increased, the sodium mean rose from 9.95 (S.D., 14.56) to 20.22 (S.D., 20.27) to 49.88 (S.D., 28.43) mEq. per liter, and as intensity was decreased the mean fell to 20.20 (S.D., 17.61) and to 12.88 (S.D., 14.47) mEq. per liter.

	Potassium	Sodium	Total protein	Chloride
Flow rate	-0.12 (> 0.05)[b]	0.98 (< 0.01)	0.82 (< 0.01)	0.96 (< 0.01)
Potassium		-0.16 (> 0.05)[b]	0.27 (< 0.05)	-0.25 (> 0.05)
Sodium			0.85 (< 0.01)	0.95 (< 0.01)
Total protein				0.83 (< 0.01)
[b]The correlation between these two variables differed significantly from one subject to another.				

Table 3: The average "within-subjects" correlation coefficients for five parotid fluid samples from each of twenty-five male subjects and the probability of their significance.

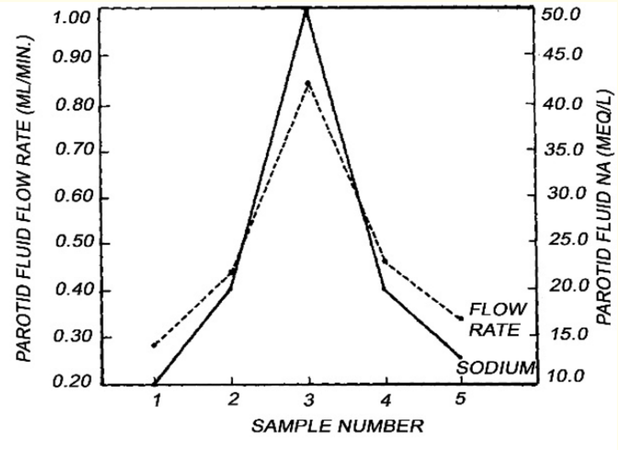

Figure 8: The correlation between flow rate and sodium.

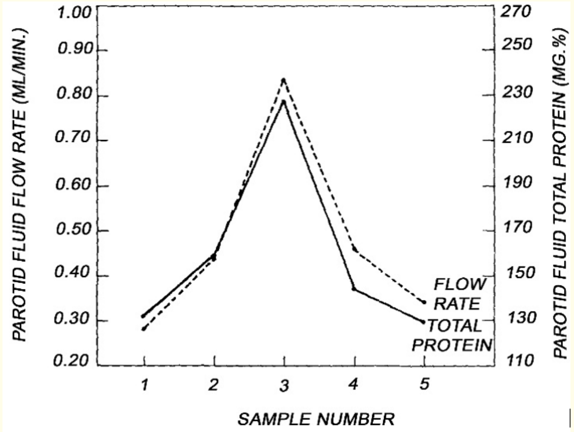

Figure 9: The correlation between flow rate and total protein.

"Between-subjects", sodium (Table 4) was negatively correlated with potassium ($P < 0.01$) and positively correlated with chloride ($P < 0.01$) but insignificantly related to flow rate and total protein.

Total protein

This variable was positively correlated with rate of flow (Figure 9), sodium, and chloride ($P < 0.01$) within subjects. The positive correlation between total protein and potassium (Table 3) was barely significant ($P < 0.05$). Total protein mean values for the five specimens were 132 (S.D., 56.8), 158 (S.D., 47.1), 227 (S.D., 65.2), 144 (S.D., 51.7), and 129 (S.D., 41.7) mg. per 100 ml. of parotid fluid. "Between-subjects" total protein (Table 4) was insignificantly related to flow rate, potassium, sodium, and chloride.

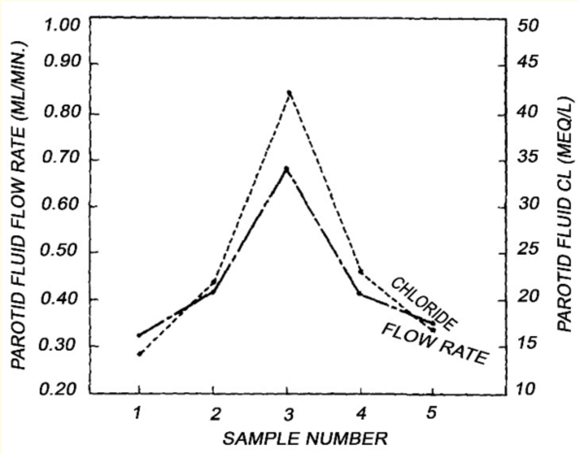

Figure 10: The correlation between flow rate and chloride.

	Potassium	Sodium	Total protein	Chloride
Flow rate	0.09 (> 0.05)	0.36 (> 0.05)	-0.25 (> 0.05)	0.35 (> 0.05)
Potassium		-0.71 (< 0.01)	0.25 (> 0.05)	-0.60 (< 0.01)
Sodium			-0.29 (> 0.05)	0.94 (< 0.01)
Total protein				-0.22 (> 0.05)

Table 4: The "between-subjects" correlation coefficients and the probability of their significance.

Chloride

Flow rate and chloride were positively correlated ($P < 0.01$) within subjects (Figure 10). Chloride was also positively related to sodium ($P < 0.01$) and to total protein ($P < 0.01$). The negative correlation with potassium was not significant (Table 3). As stimulus intensity was altered, the mean chloride value changed from 16.15 (S.D., 7.66) to 20.75 (S.D., 12.13), to 34.02 (S.D., 17.25), to 20.68 (S.D., 11.99), and to 17.57 (S.D., 9.01) mEq. per liter.

"Between-subjects" chloride (Table 4) was negatively correlated to potassium ($P < 0.01$), positively correlated to sodium ($P < 0.01$), and insignificantly related to flow rate and to total protein.

The findings had displayed significant positive correlations existing between flow rate and bicarbonate, calcium, pH, sodium, total protein, and chloride, and that inorganic phosphate was negatively correlated with rate of flow. In addition, potassium appeared to be independent of flow rate which were in agreement with the majority of published reports [21-25].

In a study of human parotid secretion [21], found a positive correlation between flow rate and sodium (0.50), calcium (0.38), bicarbonate (0.55) and pH (0.52). Potassium exhibited a negative correlation (-0.30). All of these coefficients were significant at less than 0.05. In this study, two 12.5 ml. samples of fluid were collected from each subject on each of two successive days under identical stimulation. The mean flow rates and chemical composition are not provided, but only the first sample mean flow differed significantly from the other samples. From a statistical point of view, flow rate-concentration correlations based upon three nondiffering samples and one sample of lower flow rate from each person would be most difficult to interpret, particularly when samples from each participant were collected on different days.

Blomfield., *et al.* [22] used glucose candy and dilute acetic acid as stimulants and found that human parotid fluid sodium, chloride, pH, and bicarbonate were positively correlated with flow and that potassium and phosphate concentrations were negatively correlated with flow up to a flow rate of 500 µl. per minute and thereafter independent of flow rate influences. Mureşsan and Mureşsan [26] in comparing the secretions of the pancreas and the parotid gland, found that sodium, bicarbonate, and chloride were positively correlated with parotid gland flow rates and that potassium and phosphate were independent of secretion rates.

Loy and colleagues [27], analyzed forty-seven human parotid fluid samples collected at secretory rates of from 0.20 to 4.26 ml. per minute and found a positive flow rate effect on sodium and chloride while potassium concentration was largely independent.

The positive correlation between human parotid flow rate and chloride concentration has been confirmed by Shannon., *et al.* [28] while Dawes [29] could find no clear relationship between parotid flow rate and chloride concentration in sheep. Hugoson and associates [21] found human parotid flow rate and potassium concentration to be negatively correlated, while in dogs, Langley and Smith [30] determined a negative correlation between these two variables and Tilve., *et al.* [31] concluded that potassium was secreted from the dog parotid gland in two distinct phases: (1) a transient high rate of potassium secretion immediately upon activation of the previously inactive gland followed by (2) the establishment of a steady state when potassium became independent of flow rate. A negative flow rate potassium relationship was also found for sheep parotid fluid. Prabhakar and co-worker [24] found human parotid fluid pH to vary directly with flow rates, but Langley and Smith [30] found no flow rate-pH correlation in dog parotid fluid collected under pilocarpine stimulation. The positive correlation between parotid flow rate and sodium has been confirmed in animal studies. Bicarbonate has been found to be positively correlated with parotid flow in experimental animals, while phosphate was found to decrease with increases in flow rate. Prabhakar., *et al.* [24] found protein nitrogen in human parotid fluid to increase progressively with increased secretion, but Hegde [25] found that, at rates of flow exceeding 100 µl. per minute, the protein concentration of human parotid secretion was not related to rate of flow.

Conclusion

Using different stimulatory agents at different stimulation intensities, the composition of the parotid secretion was varied not only from person to person, but, between the glands in the same individual. Besides, the possible interrelationships between the parotid saliva-biochemical indicators have been identified and the results of the biochemical modifications of human parotid saliva fluids confirmed the importance of the buffer role of the salivary bicarbonate and phosphate, in addition to the flow rate, pH, total protein, chloride, and essential alkaline and earth-alkaline ions within the oral media which may potentially serve as salivary bioindicators to such oral health risks and can be used for screening and early diagnosis of several local and systemic disorders.

Conflict of Interest

No conflict of interest exists.

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