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Biochemical Composition and Electrolyte Balance of "Unstimulated" Whole Human Saliva

Nadja N. Rehak, Stacey A. Cecco and Gyorgy Csako

Clinical Chemistry Service, Clinical Pathology Department, Warren Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, Maryland, USA

The biochemical composition of "unstimulated" whole saliva was determined in healthy adult subjects. Based on their relative concentration, salivary analytes could be classified into three arbitrary categories: concentration lower than in serum (saliva/serum ratio < 0.5; 12 analytes), similar to serum (ratio = 0.5–1.5; five analytes), and higher than in serum (ratio > 1.5; five analytes). Consistent with local production, an elevated lactate dehydrogenase (LDH) activity in the saliva was associated with a non-serum like LDH isoenzyme pattern: LDH5 >> LDH4 > LDH3 >> LDH2 > LDH1. Compared with serum, the concentrations of hydrogen (as reflected in the pH), potassium and inorganic phosphorus were much higher (saliva/serum ratio $\geq$ 3), whereas that of sodium, total magnesium, chloride, and total carbon dioxide were lower (saliva/serum ratio $\leq$ 0.3). The concentration of ionized calcium was similar in saliva and serum (saliva/serum ratio = 0.8), while ionized magnesium was unmeasurable in saliva. The salivary ionized calcium fraction was higher (0.76) than previously suggested (0.51). The difference between the main salivary cations (potassium, sodium), and anions (phosphate, chloride) was similar to serum (anion gap: 4 *vs.* 11 meq/l). Highly significant ($p \leq 0.012$) correlations occured among salivary pH, dihydrophosphate, total calcium, and potassium. Our data suggest that calcium, potassium, chloride and phosphates are the major salivary complex-forming ions. The major compositional differences between serum and saliva show that saliva is not a passive "ultrafiltrate" of serum and salivary constituents may play a distinct physiological role.

Key words: Whole human saliva; Ionized calcium; Electrolyte balance; Anion gap; lactate dehydrogenase isoenzymes.

Abbreviations: AG, anion gap; bCa, bound calcium; fCa^{2+}, ionized calcium fraction; ISE, ion-selective electrodes; LDH, lactate dehydrogenase; P_{CALC}, total phosphate in meq/l assuming equal concentrations of mono- and dihydrophosphate; P_{HH}, total phosphate in meq/l as calculated from the Henderson-Hasselbalch equation (pK_2=7.2); Pi, inorganic phosphours; TCa, total calcium; TCO_2, total CO_2; TMg, total magnesium.

Introduction

The oral fluid of whole saliva is a complex mixture of salivary gland secretions, gingival crevicular fluid, cellular debris, and microorganisms, and provides the chemical milieu to the teeth and oral tissues (1, 2). Because saliva can be easily, non-invasively and inexpensively collected, it is increasingly used in diagnostic tests as a natural "ultrafiltrate" of plasma (3–6). However, in addition to being transferred from the plasma, salivary constituents may also originate from the salivary glands and, possibly, from other oral tissues, resulting in significant differences between the biochemical profiles of plasma and saliva (7).

Initially, we investigated saliva as a possible contaminant causing unexplained increases in serum potassium concentrations. Subsequently, we extended our investigations because we found that the existing information in the literature about the composition of saliva is limited for a number of reasons. On one hand, most of the studies were performed at the time when testing with high-precision modern automated instruments and high-quality specific methods were not yet available (8, 9). In fact, some presently used chemistry methods had not even been developed at that time (*e.g.*, ionized Ca (Ca^{2+}) (10) and ionized Mg (Mg^{2+}) (11)), whereas some others were not routinely available (*e.g.*, osmolality (12)) or were not satisfactory analytically (*e.g.*, lactate dehydrogenase (LDH) isoenzyme testing (13)). On the other hand, recent studies primarily focused on the use of saliva as a convenient sample for "free" (non-protein bound) drugs, "free" (non-protein bound) hormones, and toxicology testing (3–5, 14). Therefore, we re-examined the biochemical composition of "unstimulated" (resting) whole human saliva with special respect to previously unmeasured analytes and the electrolyte balance. We used whole ("mixed") saliva because it is more relevant to physiology and pathology of oral cavity than individual gland secretions (6, 7).

Materials and Methods

Specimens

Approximately 1.5 ml of "unstimulated" (*i.e.*, no specific gustatory or masticatory stimulation (7)) whole saliva was collected by the spitting method from apparently healthy adult subjects between 8 and 9 a. m. There were 43 females and 17 males aged 20 to 65 years (85% middle-aged (30–64 years old) as defined in reference 15) and all had a good oral hygiene. Only one volunteer was a smoker (male, 1 pipe per day). In the spitting method, the saliva first is collected with closed lips within the oral cavity, then it is expelled into a tube. To mini-

mize the contact of saliva with room air, the tube containing the saliva was immediately closed with a plastic stopper at the end of collection. After centrifugation (6 min at 3000 *g*), the supernatant (clarified saliva) was analyzed undiluted or, when analyte concentration was above the reportable range of the method, diluted with saline (NaCl = 145 mmol/l). There was only a single specimen that could not be analyzed due to extreme viscosity. Upon recollection on a different day, the new specimen was, however, amenable to analysis.

Measurement of pH, Ca2+ and Mg2+

The pH, Ca^{2+}, and Mg^{2+} were determined with Nova CRT (Nova Biomedical, Waltham, MA, USA) in undiluted saliva specimens. The Ca^{2+} and Mg^{2+} results were not normalized to pH=7.40. Theoretically, the ionic strength affects the response of an ion-selective electrode (ISE). Based on our observations with unbuffered $CaCl_2$ / saline solutions, the ionized Ca results indeed may be up to ~7% higher for solutions with $\mu \leq 0.075$, but this increase is close in magnitude to the between-run imprecision of the method (CV≤3%). Addition of small amounts of protein (*e.g.,* 350 mg/l albumin) to aqueous solutions had no effect on the measured Ca^{2+}. The within-run imprecision (CV) of pH (mean: 6.71 to 7.22) and Ca^{2+} (mean: 0.82 to 1.05 mmol/l) ranged from 1.2 to 3.0% and 0.3 to 1.0%, respectively (each of five different specimens analyzed 10 times). For comparison, the respective within-run imprecision of pH (mean: 7.28) and Ca^{2+} (mean: 0.98 mmol/l) was 0.8% and 0.3% for a human serum specimen analyzed 10 times.

Measurement of other analytes

Sodium (Na) and potassium (K) were measured in undiluted saliva specimens with flame photometer (IL 943, Instrumentation Laboratory, Lexington, MA, USA). Using diluted specimens, potassium was also measured with ion-selective electrodes (ISE1: Synchron Delta CX3, Beckman Instruments, Brea, CA, USA; ISE2: Lytening 2z Analyzer, AMDEV, Peabudy, MA, USA; ISE3: Nova CRT). Since the salivary concentrations of Na and Cl were below the reportable range of the ISE serum methods, their concentration on the Delta CX3 (ISE1) was determined with the respective urine methods. Total calcium (TCa) and total CO_2 (TCO_2) were measured with ISE on the Delta CX3. Total magnesium (TMg), inorganic phosphorus (Pi) and other common chemistry tests were measured with Hitachi 917 (Boehringer Mannheim, Indianapolis, IN, USA). For measurement of amylase, the specimens were diluted. Osmolality was measured with Multi-Osmette (Precision System, Natick, MA, USA). LDH isoenzymes were determined by the Rapid Electrophoresis System (REP; Helena Laboratories, Beaumont, TX, USA).

Data analysis

For all analytes, results below the reportable range but within the instrument low range were converted to the number nearest to the lower limit of the reportable range. Using this approach, 12 Cl results were converted from <15 to 14 mmol/l, 13 Na results from <10 to 9 mmol/l, 1 TMg result from <0.12 to 0.11 mmol/l, 5 TCO_2 results from <5 to 4 mmol/l, and 4 alanine aminotransferase results from <4 to 3 U/l. Results below the instrument low range were converted to zero: Cl (n=2), Na

Tab. 1 Biochemical composition of saliva compared with serum.[a]

	Saliva[b]				Mean
Analyte (units)	Mean	SD	Range	n	(Saliva/serum)
Glucose[H] (mmol/l)	<0.1	–	–	12	<0.03
Protein, total[H] (g/l)	<2	–	–	12	<0.03
Albumin[H] (g/l)	<2	–	–	12	<0.05
Creatinine[H] (mmol/l)	<9	–	–	12	<0.09
Magnesium, ionized[N] (mmol/l)	<0.1	–	–	29	<0.2
Alanine aminotransferase[H] (U/l)	3	3	0–11	12	0.1
Total carbon dioxide[D] (mmol/l)	4	3	0–11	34	0.1
Sodium[IL] (mmol/l)	9	6	3–29	60	0.1
Alkaline phosphatase[H] (U/l)	12	13	0–11	12	0.2
Chloride[D] (mmol/l)	16	6	0–27	34	0.2
Magnesium, total[H] (mmol/l)	0.22	0.11	0.08–0.56	29	0.3
Osmolality[M] (mmol/kg)	77	17	52–111	12	0.3
Uric acid[H] (µmol/l)	80	120	40–360	12	0.5
Calcium, total[D] (mmol/l)	1.3	0.27	0.88–2.05	34	0.6
Aspartate aminotransferase[H] (U/l)	16	12	4–43	12	0.7
Calcium, ionized[N] (mmol/l)	0.98	0.16	0.70–1.41	34	0.8
Urea nitrogen[H] (mmol/l)	4.5	1.5	2.9–6.8	12	0.9
Lactate dehydrogenase[H] (U/l)	314	154	113–609	12	1.9
pH[N]	7.01	0.26	6.42–7.41	34	
H^{+}[N] (nmol/l)	118	86	39–380	34	3.0
Phosphorus, inorganic[H] (mmol/l)	6.00	2.60	1.35–13.15	60	5.6
Potassium[IL] (mmol/l)	21.5	5.7	6.4–36.6	60	5.8
Amylase[H] (U/l)	73242	58402	11880–304720	26	1320.0

[a] Instrument codes: [N] Nova CRT, [D]Delta CX3, [H]Hitachi 917, [IL]IL 493, [M]Multi-Osmette;
[b] Conventionaly, reference interval defined as mean ± 2 SD

(n=13), and TCO_2 (n=20). The fraction of free Ca^{2+} (fCa^{2+}) was calculated as ratio of ionized to total Ca (fCa^{2+}=Ca^{2+}/TCa), whereas the bound Ca (bCa) was calculated as the difference between the total and ionized Ca (bCa= TCa-Ca^{2+}). For studying the relationship between salivary electrolytes the concentrations of cations and anions were expressed in terms of milliequivalents/l (meq/l). Since the mean salivary pH was closest to the dissociation constant of the mono- and dihydrogen phosphate anions (pK_2=7.2), for samples with a known pH (n=34) the concentration of each anionic form was calculated using Henderson-Hasselbalch equation ($pH_{measured}$ = 7.2 + log ($HPO_4^{2-}/H_2PO_4^-$)) and P_{HH} = HPO_4^{2-}+ $H_2PO_4^-$ (meq/l). For assessment of the electrolyte balance, the measured TCO_2 was assumed to be present as a HCO_3^- anion. Mean saliva/serum ratios were calculated by using previously determined in-house serum reference intervals for apparently healthy adults of both sexes. The analytical methods were the same for serum and saliva testing.

Results are expressed as mean±SD range. Additional statistical analyses included paired t-test, simple and debiased linear regressions and Spearman rank correlation coefficient (rho), as deemed appropriate. A $p<0.05$ was considered statistically significant.

Results

Biochemical composition of saliva

The biochemical composition of "unstimulated" whole saliva relative to serum concentrations of commonly measured analytes is summarized in Table 1. Under the conditions applied, the salivary glucose, total protein, albumin, creatinine and Mg^{2+} were unmeasurable and their concentrations are listed as below the reportable range of these methods. Other analytes with concentrations much lower in saliva than serum (saliva/serum ratio <0.5) included alanine aminotransferase, TCO_2, Na, alkaline phosphatase, Cl, TMg and osmolality. Analytes with similar concentrations in saliva and serum (0.5 ≥ saliva/serum ratio ≤1.5) included uric acid, total and ionized Ca, aspartate aminotransferase and urea nitrogen. Analytes that occurred with much higher concentration in saliva than serum (saliva/ serum ratio > 1.5) included LDH, pH (based on H^+ concentration), Pi, K, and amylase. The fCa^{2+} was higher in saliva (0.76±0.11) than in serum with a saliva/serum ratio of 1.6, whereas the bCa was lower in saliva (0.33±0.20 mmol/l) than in serum with a saliva/serum ratio of 0.25.

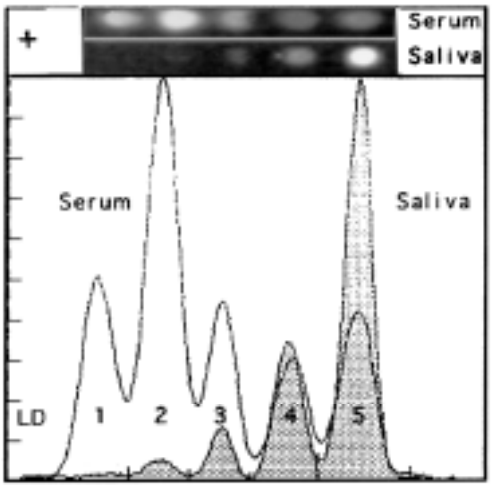

Fig. 1 Representative lactate dehydrogenase (LDH) isoenzyme patterns of "unstimulated" whole human saliva and normal human serum.

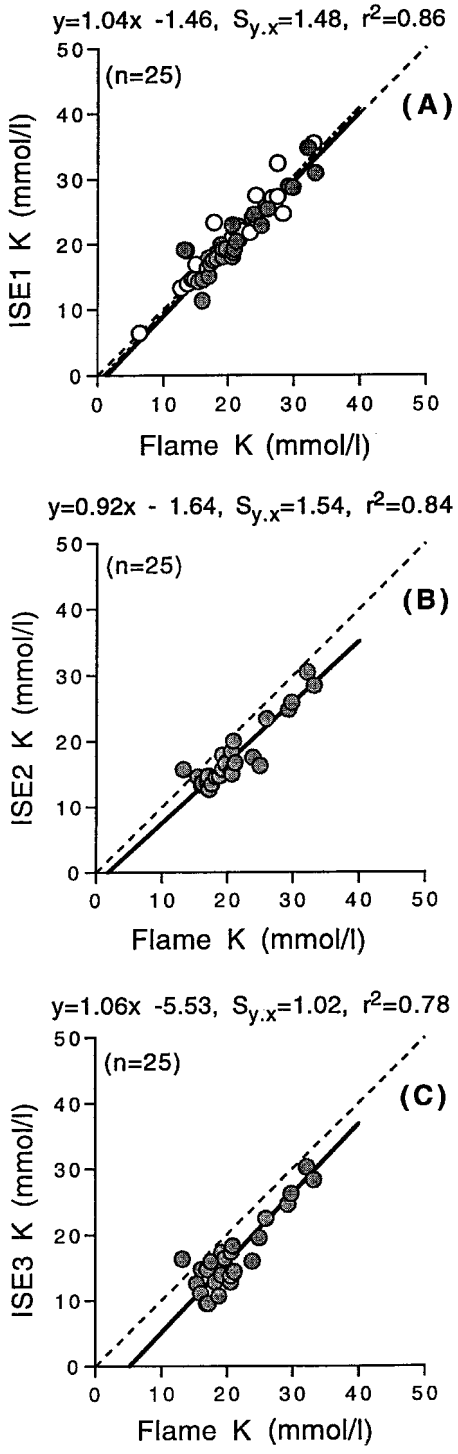

Fig. 2 Comparison of salivary K concentrations determined with flame photometry (undiluted specimens) to those determined with ion-selective electrodes (diluted specimens).
Inset (A): open symbol for n=41, y = 1.05x–1.02, Sy.x = 1.72, r^2 = 0.82.

All saliva samples analyzed (n=12) showed a similar LDH isoenzyme pattern. It was characterized by a virtual absence of the LDH1 fraction and a presence of strong LDH4 and LDH5 fractions: LDH5 (59.7±3.9%) >> LDH4 (24.0±1.8%) > LDH3 (9.4±2.9%) >> LDH2 (4.3±2.0%) > LDH1 (2.7±1.3%) (Figure 1).

The unusually low salivary Na obtained by flame photometry was also seen by various ISE methods (ISE1: 6±6, 0–26 mmol/l; IL: 8±6, 3–28 mmol/l; n=34). Figure 2 shows the relationship between the K results determined with flame photometry and those obtained with the ISE methods. While statistically significant differences (paired t-test $p<0.001$) were found between flame photometry and those measured by any of the three ISE methods, the intermethod correlations were relatively strong ($r^2>0.75$).

Electrolyte balance in saliva

The concentration of total phosphate anions calculated by the Henderson-Hasselbalch equation (P_{HH}) in a subset of saliva specimens (n = 34) was = 8.4±3.2 meq/l, range 2.1–16.4, with a mono- to dihydrophosphate anion ratio of 1.4±0.1, range 1.1–1.6. This ratio closely approximates an equimolar presence of the two phosphate anions in saliva, *i.e.* P_{CALC} (meq/l) ≃ 1.5 x Pi (mmol/l). Assuming equimolar presence of the two phosphate anions, the recalculation of total phosphate anion concentration in the same subset of saliva specimens gave a P_{CALC}=9.2±3.8 meq/l, range 2.0–19.8, and in the total of 60 saliva specimens gave a P_{CALC}=9.0±4.0 meq/l, range 2.0–19.8. The difference between the two calculated total phosphate concentrations was pH dependent: P_{CALC}–P_{HH} = 0.01 (H^+)–0.5. For saliva specimens with a pH of ≥ 6.8 (n=26) the difference was less than 1 meq/l, whereas for saliva specimens with a pH < 6.8 (n=8) the difference ranged from 1.1 to 3.5 meq/l. Further, the difference between P_{CALC} and P_{HH} was statistically significant (paired t-test $p<0.001$), but the results strongly correlated (P_{CALC} = 1.2 P_{HH}–0.9, r^2=0.94).

The summary of all statistically significant ($p<0.05$) correlations is shown in Figure 3. Figures 3and 4 show that significant correlations occurred between the salivary concentration of P_{HH} anion and several cation species such as Na, K, TCa and bCa, and between the Cl anion and K cation. The salivary concentration of P_{HH} and fCa^{2+} also significantly correlated even though the correlation between P_{HH} and Ca^{2+} was not significant ($p>0.8$). Figures 3 and 5 show that the concentrations of P_{HH}, K, Mg, TCa, bCa and Ca^{2+} all decreased with increasing pH, whereas the concentrations of Na and Cl were not affected by pH changes ($p>0.45$).

The calculated difference between measured equivalents of cations and anions (anion gap (AG)) in saliva (mean = 4.4 meq/l) was similar to that in serum (mean = 11 meq/l) only when P_{HH} was included in the calculation (Figure 6A). Assuming equimolar concentrations for the two phosphate anions had negligible effect on the calculated AG values (mean = 3.7 meq/l) but produced falsely low results for specimens with pH≤6.8. Inclusion of TCa and TCO_2 (as ≈HCO_3^-) in the formula barely affected the salivary AG (4.4 *vs.* 4.4 meq/l). Figure 6B shows that the salivary AG was only marginally dependent on the pH ($p > 0.74$).

Na	K	TMg	TCa	Ca^{2+}	fCa^{2+}	bCa	Cl	P_{HH}	$H_2PO_4^-$	HPO_4^{2-}	
	0.44	0.51	0.50	0.35		0.39			0.75		H^+
NS	**0.011**	**0.041**	**0.004**	**0.045**	NS	**0.026**	NS	NS	**<0.0001**	NS	
	-0.44	-0.51	-0.50	-0.35		-0.39			-0.75		pH
NS	**0.012**	**0.048**	**0.004**	**0.047**	NS	**0.027**	NS	NS	**<0.0001**	NS	
								-0.38			Na
	NS	NS	NS	NS	NS	NS	NS	**0.029**	NS	NS	
			0.53		-0.64	0.68	0.42	0.78	0.78	0.44	K
		NS	**0.002**	NS	**0.003**	**<0.0001**	**0.015**	**<0.0001**	**<0.0001**	**0.011**	
											TMg
			NS	NS	NS	NS	NS	NS	NS	NS	
				0.60	-0.60	0.77		0.45	0.58		TCa
				0.0006	**0.0005**	**<0.0001**	NS	**0.01**	**0.0009**	NS	
										-0.40	Ca^{2+}
					NS	NS	NS	NS	NS	**0.023**	
						-0.95		-0.66	-0.59	-0.46	fCa^{2+}
						<0.0001	NS	**0.0002**	**0.0008**	**0.009**	
								0.66	0.67	0.36	bCa
							NS	**0.0001**	**0.0001**	**0.037**	
											Cl
								NS	NS	NS	

Fig. 3 Significant correlations ($p<0.05$) among various analytes in "unstimulated" whole saliva specimens. Except for fCa^{2+} and pH, all analytes are expressed in meq/l. pH results also are shown in terms of H^+ concentration. All K results refer to those obtained with flame photometry. fCa^{2+}=Ca^{2+}/TCa, bCa= TCa-Ca^{2+}, P_{HH} = HPO_4^{2-}+ $H_2PO_4^-$. For number of specimens see Figures 4 and 5.
Top number, Spearman rank correlation coefficient (rho); bottom (bold) number, p-value of paired sign test; NS, not significant ($p\geq0.05$).

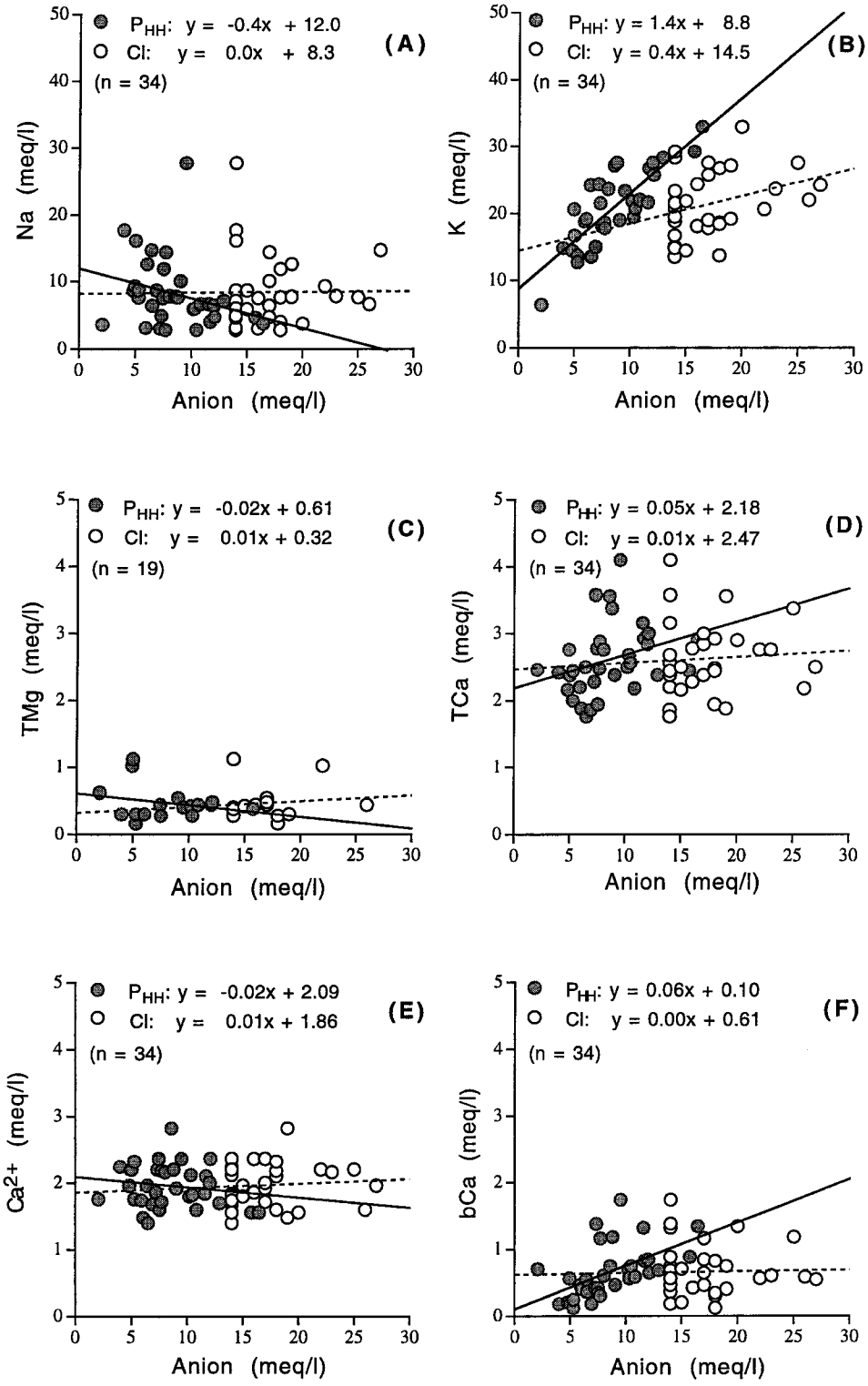

Fig. 4 Relationship between anions (x) and cations (y) in "unstimulated" whole saliva specimens.
All K results refer to those obtained with flame photometry.
Inset (B): for n=60, K = y, P_{CALC} = x; y =1.2 +10.6, r^2 = 0.69, rho = 0.79, p<0.0001.

Discussion

For most analytes (Na, K, Ca, Mg, Cl, TCO_2 (HCO_3^-), Pi and P_{HH}, urea, uric acid, total protein, albumin, and pH) in "unstimulated" whole saliva, we found concentrations similar to those reported earlier for stimulated and "unstimulated" whole or parotid salivary specimens (7,8, 15–22). The osmolality of saliva has been estimated to represent about half that of the plasma, but no direct measurements have been reported (1). According to our results, osmolality of "unstimulated" whole saliva amounts to only about one-third of that of

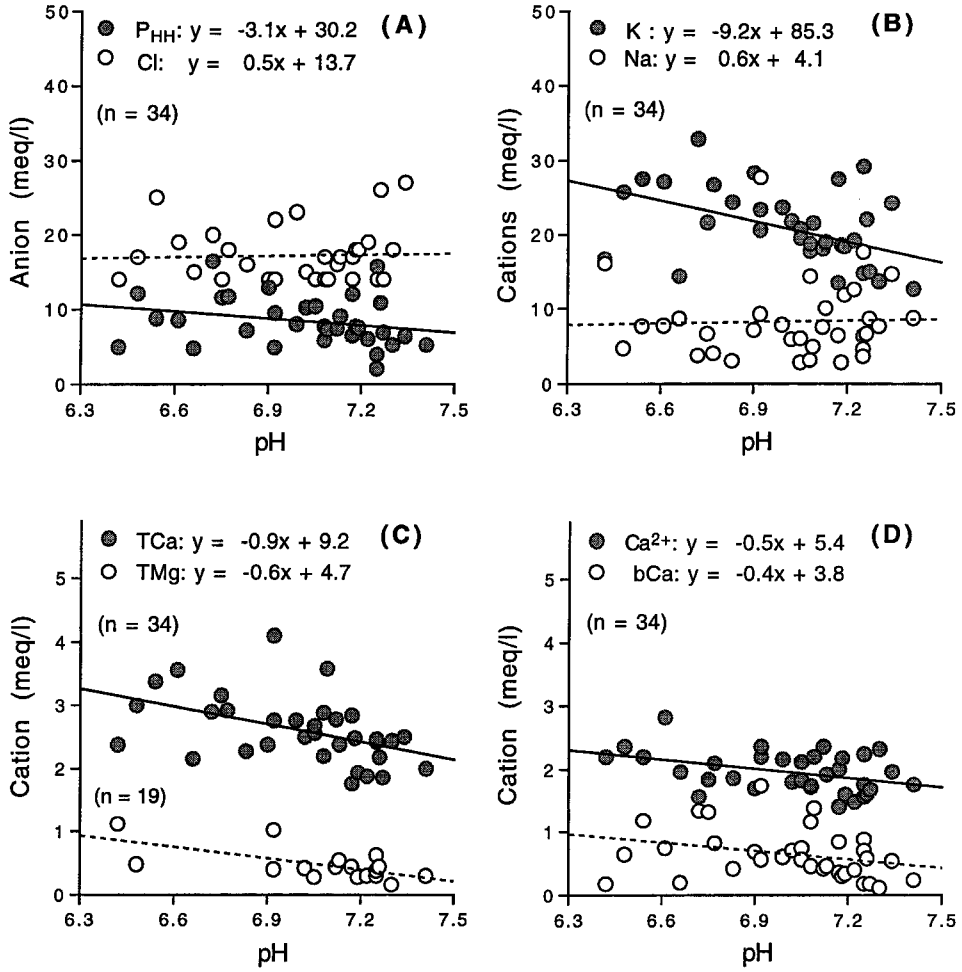

Fig. 5 Relationship between pH (x) and major ion species (y) in "unstimulated" whole saliva specimens. Inset (C): Note different (n) for the two regressions.

serum. α-Amylase is known to be the dominant salivary protein with a reported concentration of 0.6–1.2 g/l in the parotid saliva and 0.25 g/l in the submandibular saliva (1). Because chloride activates amylase, the chloride content of the solution used for sample dilution affects the amylase results (23). Using saline as a diluent and the Boehringer Mannheim defined-substrate α-amylase method, we observed an over 1000-fold excess of amylase activity in saliva compared with serum. Our finding of undetectable salivary creatinine and glucose concentrations is in apparent disagreement with previously reported reference ranges for creatinine 24.8–40.7 μmol/l (2.8–4.6 mg/l) and glucose 0.63–1.56 mmol/l (113–281 mg/l) in saliva (24). But we used an enzymatic creatinine method which is known to give up to 26.5 μmol/l (3 mg/l) lower results than the conventionally used Jaffe reaction. Further, our finding of undetectable glucose concentrations (<0.1 mmol/l (<20 mg/l)) is supported by recent observations of Weber (25) who reported similarly low salivary glucose concentrations (<0.15 mmol/l (<27 mg/l)) during intravenous glucose load in healthy men.

Besides confirming high salivary amylase levels, we detected the presence of the enzymes alkaline phosphatase, transaminases, and LDH in saliva. Reference ranges for salivary alkaline phosphatase and transaminases have been reported for infants and young children (26) but, because of analytical differences, they cannot be directly compared to our results. There are no published data about salivary LDH. Theoretically, the elevated LDH levels could have originated from the oral cavity (*i.e.*, contamination with bacterial flora and/or traces of blood). However, isoenzyme analysis revealed that all saliva specimens have virtually the same isoenzyme pattern (making contamination as a major source unlikely) and this pattern (LDH5 >> LDH4 > LDH3 >>LDH2 > LDH1) was very different from that of normal human serum (in-house reference interval: LDH2, 29–39% > LDH3, 15–29% ≥ LDH1, 13–25% > LDH4, 9–17% > LDH5, 6–16%). The predominance of LDH5 and LDH4, the consistent interspecimen isoenzyme pattern, and the higher-than-serum total enzyme activity all indicate local origin of salivary LDH.

Phosphate and K were the only inorganic components of saliva with a mean saliva to serum ratio of greater than five. The high salivary K results were method-independent: the results obtained with an "indirect" method that measures ion concentration (ISE1)

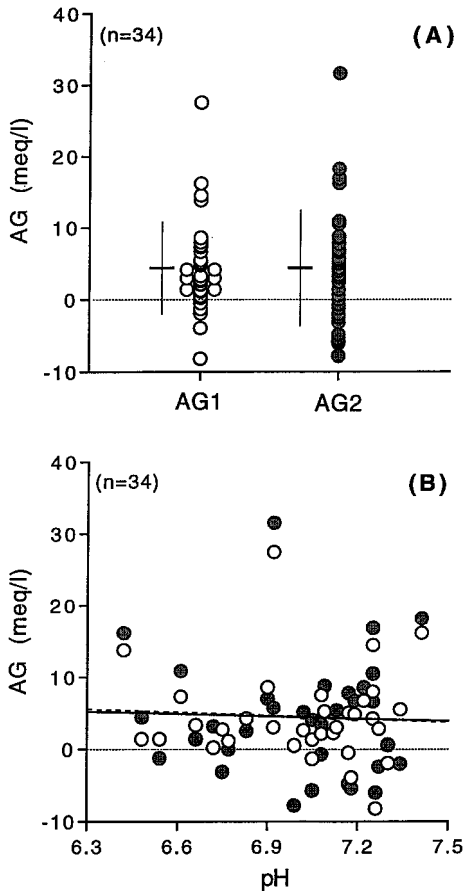

Fig. 6 Anion gap (AG) in "unstimulated" whole saliva specimens.
(A) AG calculated using two different formulas:
AG1 = (Na+K)-(P_{HH}+Cl), AG2 = (Na+K+TCa)-(P_{HH}+Cl+HCO_3^-).
(B) Relationship between AG and pH:
AG1 = –1.1pH + 12, r^2=0.002, AG2 = –1.5pH +15, r^2=0.002.

and "direct" methods that measure ion activity (ISE2 and ISE3) were comparable to those obtained with the reference method flame photometry. Because of the high Pi and K saliva/serum ratios, saliva should be considered as a potential source of preanalytical error for both analytes in serum. Comparatively small (as little as 2–5%) contamination of blood or other body fluids with saliva may cause not only clinically important erroneously high amylase results but also clinically important increases in K and, to a lesser extent, in Pi.

Similar to serum, Ca and Mg may occur in saliva as bound (either proteins or soluble complexes) or as a free ion (Ca^{2+}, Mg^{2+}); likely the most important fractions (7). Currently available information on the Ca^{2+} concentration in saliva is limited regarding the type of saliva analyzed, assessment of pH effect, and methodology. The first methods used for the measurement of salivary Ca^{2+} were based on murexide complexing in protein-free filtrate (27, 28) or gel filtration (29). Using ISEs, the concentration of Ca^{2+} in the stimulated parotid saliva was found to increase from 0.56 to 0.88 mmol/l as the salivary flow increased from 0.1 to 0.6 ml/min, but the Ca^{2+} fraction (fCa^{2+}=0.54±0.04) remained almost constant (30). With the same methodology, the concentration of Ca^{2+} was reported to be 0.53 ±0.07 mmol/l (estimated fCa^{2+}=0.51) in unstimulated whole saliva (31) and 0.66±0.15 mmol/l in stimulated whole saliva (32). We found no published data for salivary Mg^{2+}. With Nova ISEs, we observed Mg^{2+} concentrations below the usable range of the method (< 0.1 mmol/l) in all saliva specimens, resulting in a very low saliva/serum ratio (<0.2). In contrast, the salivary and serum Ca^{2+} concentrations were similar (saliva/serum ratio = 0.8) in our specimens. Since the TCa concentration in saliva is only about half of that in the serum (saliva/serum ratio = 0.6), the fCa^{2+} is much higher (saliva/serum ratio = 1.6) and the bCa is much lower (saliva/serum ratio = 0.2) in saliva than serum. Compared to a previous report (31), we found a higher fCa^{2+} in "unstimulated" whole saliva (mean: 0.76 *vs.* 0.51). In blood, albumin is the major binding protein of Caand Mg and the extent of binding is pH dependent as they compete with H^+ for binding sites. Because saliva contains only traces of albumin (20) and the H^+ concentration in most "unstimulated" whole salivary specimens is relatively high when compared to serum, the bound Ca and Mg ions in saliva are most likely present in the form of soluble complexes as previously suggested (33).

Salivary Ca and P_i are considered important in preventing dental caries by remineralising enamel surfaces of teeth via formation of hydroxyapatite at alkaline pH (1, 16). However, the relationship between salivary Ca and phosphate, as well as between the other major salivary cations and anions, has not been fully elaborated. Our data indicate that Na and K as cations and Cl and phosphates as anions account for most of the electrolyte balance in saliva, resulting in an AG (4±8 meq/l) that is similar to that of serum (11±2 meq/l). In "unstimulated" whole saliva specimens, TCa and TCO_2 appear to be minor constituents. Unmeasured anions may include thiocyanate (from dietary sources and smoking), fluoride (from fluorinated water and toothpaste), lactate, and possibly some other organic anionic species. The lack of correlation between the AG and pH is consistent with an exogenous origin of at least some of the unmeasured salivary anions.

The pH of "unstimulated" saliva has been reported to be acidic (<6.4) and to increase rapidly with increasing salivary flow rate (22). The flow rate is apparently much higher for "unstimulated" whole saliva (mean = 0.32, range: 0.1 to 0.5 ml/min) than for "unstimulated" parotid saliva (mean = 0.04, range 0.01 to 0.07 ml/min) (1). The difference in flow rates is reflected in different pH values and, consequently, different compositions of the two saliva types. At the pH range (6.42–7.41) of our "unstimulated" whole saliva specimens, the P_i is expected to be present as a mixture of mono- and dihydrophosphate anions (pKa ~ 7.2). The significant negative correlations of the pH with dihydrophosphate anion, TMg and K are consistent with the previously observed effect of flow rate on the composition of stimulated parotid saliva (22). However, in contrast to reported increases in Na and TCO_2 and, to a lesser extent,

Cl with increasing pH of stimulated parotid saliva (22), the correlations among these ions in "unstimulated" whole saliva were not significant. We also found negative correlations of the pH with TCa, Ca^{2+}, and bCa. A decrease in TCa with increasing salivary flow rate has been observed previously but only for the "resting" parotid saliva of children (16) and the salivary concentration of Ca^{2+} was reported to be unrelated to the pH (28). We observed that in "unstimulated" whole saliva the Ca^{2+} concentration decreased in direct proportion to the TCa concentration so that the fCa^{2+} was not affected by the pH value. Indeed, at any observed pH, like in a previous *in vitro* pH titration study (33), the fCa^{2+} ranged from about 0.3 to 0.9. However, the fCa^{2+}, as well as the TCa and bCa concentrations, decreased with decreasing concentrations of the phosphate anions. Since we found no relationship between Cl and any of the Ca forms ($p>0.3$), $H_2PO_4^-$ and, to a lesser degree, HPO_4^{2-}, can be considered important complex-forming anions of salivary Ca.

In conclusion, K, Na and Ca are the major cations, whereas Cl and different forms of phosphate are the major anions in "unstimulated" whole saliva. The main complex-forming ions are Ca, K, and phosphates. While our study addressed the normal (physiologic) status of "unstimulated" whole saliva, further studies with saliva specimens collected from patients with various diseases of the oral cavity are warranted to explore possible diagnostic utility of salivary Ca^{2+} and, possibly, Mg^{2+} measurements. We showed that these measurements are both feasible and practicable with currently available methodology.

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Corresponding author: Nadja N. Rehak, NIH, Bldg. 10, Rm. 2C-407, Bethesda, MD 20892-1508, USA
Fax: +301-402-1885, Email: nnrehak@nih.gov