The correlation between salivary peroxidase activity, salivary flow rate, and the oxidation-reduction potentials of human saliva and dental plaque suspensions

JORMA TENOVUO & JUHA VALTAKOSKI

Institute of Dentistry, University of Turku, Finland

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18 subjects, 9 males and 9 females, were examined regarding salivary oxidation-reduction potential, salivary flow rate, salivary peroxidase activity, oxidation-reduction potential of dental plaque samples, and dental health. Both the peroxidase activity, expressed as the salivary lactoperoxidase, and the salivary oxidation-reduction potential increased with increasing salivary flow rate. The variation of these variables was obviously due to changes in salivary flow rate during the day. The remarkably slight differences in peroxidase activities, oxidation-reduction potentials and salivary flow rate in this study did not have any marked correlation with the clinical recordings of the test groups.

Key-words: Saliva; oxidation-reduction potential; lactoperoxidase; salivary flow rate

Jorma Tenovuo, Institute of Dentistry, University of Turku, Lemminkäisenkatu 2, SF-20520 Turku 52, Finland

The significance of the oxidation-reduction potential of human saliva for oral health is still obscure. *Eggers-Lura* (1955) has observed significant differences between the potentials in caries-resistant and caries-active persons; the potentials were more oxidizing with caries-resistant persons. On the other hand, a high oxidizing oxidation-reduction potential favours the growth of aerobic micro-organisms while some facultative and obligatory anaerobic bacteria need a negative, reducing potential. A typical aerobic culture broth may have an oxidation-reduction potential (E_h) of +300 mV, while anaerobic bacteria, e.g. oral lactobacilli have an E_h of —130 to —240 mV (*Burnett & Scherp*, 1968).

Studies of unstimulated or stimulated human saliva have shown that the mean values of E_h are about +250 to +300 mV, the range being about +150 to +500 mV (*Eisenbrandt*, 1943; *Turner et al.*, 1954; *Eggers-Lura*, 1955; *Scheinin & Mäkinen*, 1971; *Mäkinen, Tenovuo & Scheinin*, 1975). *Scheinin & Mäkinen* (1971) have measured a value of +401 ± 40 mV for human plaque suspensions (plaque collected *in situ* after four days neglect in oral hygiene).

Besides bacteria, there seem to be many other substances present in the saliva which can influence its reducing capacity such as glutathione, ascorbic acid, some enzymes and inorganic ions whose concentrations fluctuate from time

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to time. According to *Mäkinen et al.* (1975) the diurnal variation of E_h also seems to be considerable.

The purpose of this study was to examine the relationship between the activity of salivary oxidizing enzymes — in this case salivary lactoperoxidase — the E_h of dental plaque suspensions, the clinical conditions of patients, and the E_h of oral fluid samples. Further studies were made to determine the effect of sex, time of day, salivary flow rate and sample size on the potentials measured.

MATERIALS AND METHODS

1. Collection and treatment of samples

The subjects, 9 males and 9 females, aged 19—26 years, were dental students. Whole saliva samples of 6 ml were collected after normal oral hygiene procedures by paraffin stimulation at three hour intervals from 8 a.m. to 8 p.m. The samples were immediately chilled to + 4°C in an iced water bath. The flow rate of saliva was simultaneously determined. The subjects were asked not to eat or drink anything for half an hour before collection.

The plaque samples were collected *in situ* from all available surfaces during a three minute period. The collection was performed between 8 and 10 a.m. and all subjects were requested to refrain from all oral hygiene procedures for 24 hours before sample collection. The plaque samples were immediately suspended in 5 ml of cold 0.154 M NaCl solution (0.9 %).

2. Determination of redox potential (Eh), pH and rH

Redox potential was measured with a Radiometer pH Meter (Type 28) using a platinum and a calomel electrode according to the manuals of the supplier (Radiometer A/S, Copenhagen). The pH of the samples was measured immediately after collection with a glass and calomel electrode. The E_h and pH were determined at +22°C. In line with earlier studies carried out in this laboratory (*Scheinin & Mäkinen*, 1971; *Mäkinen et al.*, 1975) the redox potential was measured in open semicone-shaped plastic vessels (25 ml) immediately after collection of saliva and suspending plaque. The surface area and the volume of the samples in the vessels were approximately the same throughout the study. The potentials were recorded 5 seconds and 1 minute after the formation of the contact between the solution and the electrodes.

The measured potential difference, E, was used as an indication of the redox potential, E_h. rH (or pH_2, hydrogen pressure above the solution) was calculated from $rH = 2\ pE + 2\ pH$, where pE is the redox exponent defined as $pE = E_h/0.1984 \cdot T$, T being the absolute temperature and E_h being measured in mV.

3. Enzyme and protein assays

The peroxidase activity was determined by the guaiacol method as suggested by *Chance & Maehly* (1964). The specific activity was calculated as described earlier (*Mäkinen et al.*, 1975). Enzyme assays were performed with a Hitachi Perkin Elmer UV-VIS Spectrophotometer. Enzyme activity was determined from the saliva samples collected at 8 a.m.

Proteins were determined according to Lowry's method (*Lowry et al.*, 1951) by using bovine serum albumin as a standard (Sigma Chemical Company, Los Angeles, Cal., USA).

4. Chemicals

All chemicals were of analytical grade.

Guaiacol was a product of BDH Chemicals Ltd. (Poole, England). Unless otherwise indicated, all other chemicals were obtained from E. Merck AG (Darmstadt, Germany).

5. Chromatography

Ion exchange chromatography was in principle performed according to *Peterson & Sober* (1962). For ion exchange chromatography on DEAE-cellulose a special fraction (230—270 mesh) was sieved from the commercial preparation (Schleicher & Schüll, Dassel/Kr., Einbeck, Germany). The saliva samples for DEAE-cellulose chromatography were treated as follows. The samples were centrifuged for 10 min at 23500 $\times g$ in cold (+ 4°C) using a Sorvall RC-2B refrigerated centrifuge. The individual samples were pooled after analysis and stored frozen at —20°C. After approximately two weeks the pools were thawed and used for chromatographic steps. The chromatographic separation of peroxidases was carried out principally as described by *Mäkinen & Tenovuo* (1976). The pools were passed through a Sephadex G-25 (Coarse) column (5.5 × 32 cm) with the aid of 0.01 M $\beta\beta$-dimethylglutarate buffer, pH 7.2. The desalted solutions were then concentrated in an Amicon Ultrafiltration System TCF-10 (Membrane UM20E) to 23—25 ml. The concentrated pools were applied to DEAE-cellulose column. Other details are given in the Results section.

6. Methods of clinical examination

A. Recording of dental caries. The condition of the teeth was determined by means of the DMFS index (*Wessels & Cheyne*, 1947). The teeth were dried using compressed air during examination. Carious surfaces were demonstrated by visual examination and a sharp probe. Missing teeth were scored as extracted for caries, according to *Wessels & Cheyne* (1947) and weie considered to have been decayed on four surfaces.

Radiographical examination was performed by taking two or four bitewing radiographs (Kodak Ultra-Speed). The films were developed under standardized conditions and read in adjustable light. Both the clinical and radiographic examinations were performed by one and the same examiner who was not aware of the group the individual participant belonged to. The groups were formed according to the redox potentials of test subjects (see Results section).

B. Recording of gingival condition and the amount of dental plaque. The degree of gingival inflammation of the subjects was assessed by the Gingival Index (GI) of *Löe & Silness* (1963). The GI was determined by examining the four surfaces of all fully erupted teeth.

The amount of dental plaque was recorded from all four surfaces of teeth by using the Plaque Index (PI) presented by *Silness & Löe* (1964). The PI was determined for the following six teeth: 16, 21, 24, 36, 41, 44. These recordings were carried out by the same examiner as for dental caries.

RESULTS

The subjects were divided into two groups according to the redox potentials measured (Table I). The difference between the potentials of Groups I and II was statistically significant. Table I shows that in Group I where the E_h was higher (more positive) the peroxidase activity of both individual and pooled samples was also clearly higher than in Group II. The

Table I. *The means and standard deviations of salivary peroxidase activity, redox potential and pH in the test groups*

	E_h (mV)		pH		rH[1]		ΔE(mV/min)		Peroxidase activity (U/mg prot) Individual samples		Pooled samples	Peak I from DEAE-cellulose
	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.		
Group I (n = 9)	+189	24.1	7.39	0.28	13.9	0.3	—15.0	9.0	5.91	1.45	16.1	35.0
Group II (n = 9)	+152	11.9	7.46	0.29	12.7	0.1	—10.0	10.2	4.59	2.42	14.2	29.8
	++		Φ		++		Φ		+			

[1] Calculated from the formula rH = 2 pE + 2 pH, as described in the Materials and Methods section.

$P \leq 0.001$ ++ Φ = not significant.

$P \leq 0.2$ + , Student's t-test

Table II. *The correlation between salivary redox potentials and peroxidase activities in the test subjects*

E_h (mV)	+130 — +150 (n = 5) $\bar{x}$	S.D.	+150 — +180 (n = 8) $\bar{x}$	S.D.	+180 — +235 (n = 5) $\bar{x}$	S.D.
Peroxidase activity (U/mg prot)	3.68	2.76	5.72	1.76	6.14	1.54
		+		Φ		

$P \leq 0.2$ + Φ = not significant

Table III. *The means and standard deviations of redox potential and pH of dental plaque samples in the test groups*

	E_h (mV) $\bar{x}$	S.D.	pH $\bar{x}$	S.D.	rH $\bar{x}$	S.D.	ΔE(mV/min) $\bar{x}$	S.D.
Group I (n = 9)	+246	22.3	6.08	0.25	14.4	1.2	—6.7	7.8
Group II (n = 9)	+244	23.2	5.92	0.22	14.4	0.9	—8.8	6.7
	Φ		Φ		Φ		Φ	

Φ = not significant

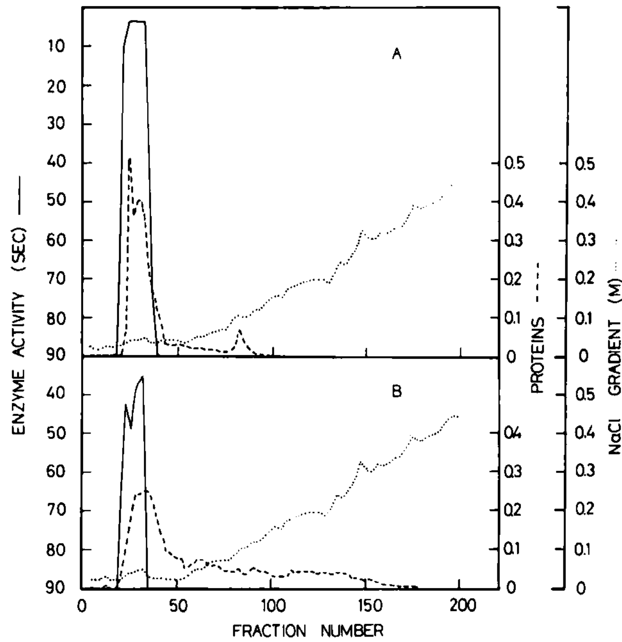

Fig. 1. DEAE cellulose chromatography of peroxidases of the pooled and concentrated human whole saliva samples, determined according to the guaiacol method. A. The sample (25 ml) representing Group I is described in the text. Column: 1.7 × 18 cm; Elution buffer: 0.01 M ββ-dimethylglutarate buffer, pH 7.2, containing a linear NaCl gradient from 0 M to 0.5 M (mixing volume 150 + 150 ml); Flow rate: 0.2 ml per min; Hydrostatic pressure in packing and elution: 120 cm; Fraction volume: 1.5 ml; Temperature: 4°C. Fractions 18—37 were pooled.
B. The sample (23 ml) representing Group II is described in the text. Other details as for A. Fractions 18—34 were pooled.

Table IV. *The means and standard deviations of redox potentials of saliva and plaque samples, peroxidase activity and salivary flow rate in male and female subjects*

	Saliva										Plaque suspension					
	Flow rate (ml/min)		E_h (mV)		pH		rH		Peroxidase activity (U/mg prot)		E_h (mV)		pH		rH	
	$\bar{x}$	S.D.	$\bar{x}$	S.D	$\bar{x}$	S.D	$\bar{x}$	S.D	$\bar{x}$	S.D	$\bar{x}$	S.D	$\bar{x}$	S.D	$\bar{x}$	S.D.
Males (n = 9)	2.1	1.1	+167	25.3	7.46	0.24	13.3	0.9	5.75	2.85	+240	21.7	5.99	0.25	14.3	1.2
Females (n = 9)	1.8	0.9	+176	27.1	7.37	0.26	13.4	0.9	4.91	0.86	+249	22.6	5.99	0.16	14.5	0.6
	Φ		Φ		Φ		Φ		Φ		Φ		Φ		Φ	
Total (n = 18)	1.9	0.9	171	25.4	7.43	0.25	13.3	0.9	5.29	2.04	245	22.0	5.93	0.22	14.4	1.0

Φ = not significant.

correlation between redox potential and peroxidase activity was confirmed by the results presented in Table II. It shows that the higher the E_h, the higher the peroxidase activity. The differences between the subgroups were significant.

DEAE-cellulose chromatography of whole saliva yielded two peroxidase peaks (Fig. 1). The specific activity of lactoperoxidase peaks are presented in Table I. The activity was somewhat higher in Group I than in Group II.

Table V. *The correlation between salivary flow rate, redox potential and peroxidase activity of the test subjects*

	n = 7		n = 7		n = 4		Group I		Group II	
	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
Salivary flow rate (ml/min)	0.7	1.5	1.6	3.0	3.1	4.5	2.34	0.73	1.49	1.40
		Φ		Φ				+		
E_h (mV)	+161	18.1	+177	24.5	+179	44.8	+189	24.1	+152	11.9
		+		Φ				++		
Peroxidase activity (U/mg prot)	4.24	2.19	5.87	1.93	6.27	1.41	5.91	1.45	4.59	2.42
		Φ		Φ				+		

$P \leq 0.001$ ++ Φ = not significant
$P \leq 0.2$ +

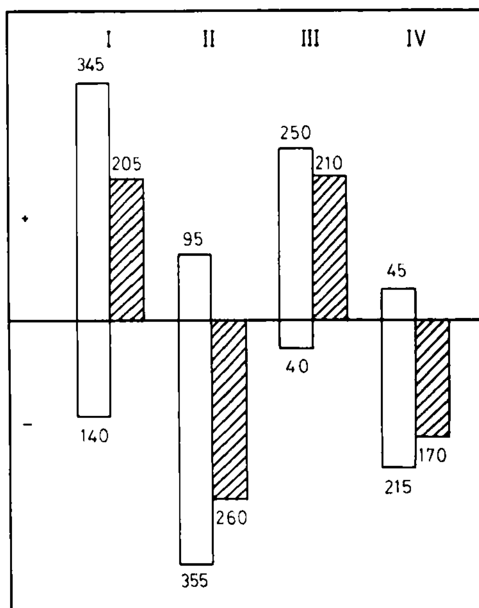

Fig. 2. Changes in oxidation-reduction potentials of human whole saliva samples. The sums of positive, negative and net changes are presented as a function of the time of day. The Roman numerals represent the following periods:

I 8 a.m. — 11 a.m.
II 11 a.m. — 2 p.m.
III 2 p.m. — 5 p.m.
IV 5 p.m. — 8 p.m.

The oxidation-reduction potentials of suspended dental plaque samples of the same test persons did not show any noticeable differences between the test groups (Table III). There were no remarkable differences between males and females with regard to salivary peroxidase activity or redox potentials and pH of saliva and plaque samples (Table IV).

The influence of salivary flow rate and saliva sample size on the potentials measured was also tested. Table V shows that increasing salivary flow rate led to more oxidizing and positive redox potentials. There was also a clear difference between the test groups with regard to salivary flow rate. On the other hand, there was no correlation between the sample size and the potentials measured. This was tested by first collecting a 4 ml saliva sample and then adding a 2 ml saliva sample at 1 min intervals to the test vessel. The addition did not alter the potentials in any direction.

The influence of the circadian rhythm was tested by measuring the individual increasing (oxidizing) or decreasing (reducing) redox potentials between different collection periods. The following periods were analyzed:

I 8 a.m. — 11 a.m.
II 11 a.m. — 2 p.m.
III 2 p.m. — 5 p.m.
IV 5 p.m. — 8 p.m.

Table VI. *The means and standard deviations of plaque index (PI), gingival index (GI), number of decayed surfaces (DS) and DMFS-index of the test groups*

	Group I (n = 9)		Group II (n = 9)		
	$\bar{x}$	S.D.	$\bar{x}$	S.D.	
PI	0.45	0.18	0.52	0.24	Φ
GI	0.31	0.15	0.26	0.15	Φ
DS	7.6	3.8	6.4	3.2	Φ
DMFS	54.9	23.0	52.8	22.8	Φ

Φ = not significant.

The results are shown in Fig. 2. The E_h of saliva samples was highest before noon, followed by a slight decrease and a new elevation in the afternoon. The potential was lowest at night. Table VI indicates that there were no remarkable differences between the test groups with regard to clinical conditions.

DISCUSSION

As mentioned earlier there are many factors which can influence the redox potentials of human saliva. The results obtained in this study indicate that at least two variables, the salivary lacto-peroxidase concentration and the salivary flow rate, have a marked effect on the oxidizing or reducing capacity of human saliva.

Kraus et al. (1958) have observed that the activity of salivary peroxidases was lowest on rising (7 a.m.). They assumed thatthis might be attributed to the lack of salivary secretion during sleep (*Schneyer et al.*, 1956). The difference between peroxidase values at 7 a.m. and 9 a.m. was highly significant. It seems likely that peroxidase activity of oral fluid samples depends on the salivary flow rate, which changes during the course of the day. The circadian variation of measured oxidation-reduction potentials seemed also to depend on the flow rate (Table V). The differences in peroxidase activity and redox potential between the test groups were probably due to different salivary flow rates in these groups. The higher per-oxidase activity itself should have a lowering effect on the redox potentials of the environment by decomposing hydrogen peroxide.

The redox potentials of dental plaque evidently do not correlate with the salivary potentials. According to *Kraus et al.* (1958) peroxidase values revealed no differences between male and female subjects. This agrees with the results of this study, the slight difference between the activities measured being most likely due to different salivary flow rates in males and females. The circadian variation in oxidation-reduction potentials evidently reflects the variations in the salivary flow rate during the day.

The differences between the two test groups found in this study in peroxidase activity, redox potential and salivary flow rate did not correlate to any notice-able extent with the clinical conditions of the test subjects. *Eggers-Lura* (1955) has observed that the mean E_h for a caries-active group, containing 50 persons, was significantly lower (more reducing) than for a caries-resistant group. The difference between the potentials in these two groups was about 70 mV, in this study 37 mV only. As presented earlier, this difference could be a secondary factor, the primary being the variation in salivary flow rate between the test groups. *Cushman et al.* (1940, 1941) and *Barany* (1947) have provided evidence that a low salivary flow rate leads to higher caries activity.

It is obvious that the differences in peroxidase activities and oxidation-reduc-

tion potentials, which are partly due to variations in salivary flow rate between different individuals, could be considered special indicators of oral health.

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