The variation of salivary peroxidase activities in persons of different oral health

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18 subjects (10 males, 8 females) with considerable differences in oral health conditions were examined regarding the peroxidase activity of oral fluid samples. Subjects with high caries prevalence and/or severe gingivitis displayed a significantly higher peroxidase activity than control subjects. Chromatographic experiments revealed that the increased activity was caused by salivary lactoperoxidase. This elevated activity does not, however, necessarily lead to better oral health because the antibacterial activity of lactoperoxidase also depends on the concentrations of thiocyanate ions and hydrogen peroxide. Hence the specific activity of lactoperoxidase, determined *in vitro*, is not as such an adequate measure of its antibacterial efficacy.

Key-words: Saliva; lactoperoxidase; oral health

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Only a few studies concerning the relationship between clinical conditions and salivary peroxidase activities in man have been performed (*Kraus et al.*, 1958; *Wolfe & Turner*, 1957; *Kato & Nagatsu*, 1967). Furthermore, the results obtained in these studies have been conflicting. *Kraus et al.* (1958) and *Wolfe & Turner* (1957) found that the average peroxidase values were significantly higher for the group of periodontal patients or those with extensive caries compared to normal subjects. On the other hand, *Kato & Nagatsu* (1967) found no remarkable differences in enzyme activity between the normal subjects and those with low-grade caries but patients with extensive caries, periodontosis or aphthous stomatitis displayed decreased salivary peroxidase values.

The connection between salivary peroxidases, especially lactoperoxidase, and the amount of dental plaque or the incidence of caries is still obscure. It has been observed that salivary lactoperoxidase can control the growth of lactobacilli (*Dogon*, 1962; *Zeldow*, 1963; *Iwamoto & Matsumura*, 1966) and some streptococci (*Jago & Morrison*, 1962; *Reiter et al.*, 1964; *Morrison & Steele*, 1968). *In vivo* experiments carried out by *Koch et al.* (1973), *Hoogendoorn & Moorer* (1973), *Hugoson et al.* (1974) and *Hoogendoorn* (1974) have shown that the amount of dental plaque could be controlled by using enzymes forming hydrogen peroxide and thus activating the salivary lactoperoxidase antibacterial system. These studies do not, however, clarify the significance of low or high salivary lactoperoxidase

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activities (secreted from the glands) on the clinical conditions, e.g. on the amount of dental plaque, in man.

MATERIALS AND METHODS

1. Collection and treatment of saliva samples

The experimental groups were formed according to the clinical conditions of the subjects (*cf.* the Results section). Group I consisted of 9 subjects, 4 males and 5 females, aged 19—25 years, and Group II consisted of 9 subjects, 6 males and 3 females, aged 17—42 years. Whole saliva samples (10 ml) were collected by paraffin stimulation after normal oral hygiene procedures between 8 and 11 a.m. The salivary flow rate was simultaneously determined. The samples were centrifuged immediately after collection with a Sorvall Superspeed RC-2 refrigerated centrifuge for 15 min at $23500 \times g$ in cold (+4°C). The supernatant fluids were stored in cold until enzyme and protein determinations were performed (within 1 week). The supernatants were then pooled to form two separate preparations representing Groups I and II and frozen (—20°C) until used for chromatographic analysis.

2. Chemical assays

Peroxidase activity was determined by the guaiacol method as suggested by *Chance & Maehly* (1964). Pertinent information about this method was also provided in other studies (*Mäkinen et al*, 1975). Proteins were determined by Lowry's method (*Lowry et al.*, 1951). Thiocyanate ions were determined using a colorimetric method presented by *Powell* (1945).

3. Chemicals

All chemicals were of analytical grade. Guaiacol was a product of BDH Chemicals Ltd. (Poole, England). Other chemicals were obtained, unless otherwise mentioned, from E. Merck AG (Darmstadt, Germany).

4. Chromatography and isoelectric focusing

Ion exchange chromatography was in principle performed according to *Peterson & Sober* (1962). For chromatography on DEAE-cellulose a special fraction (230—270 mesh) was sieved from the commercial preparation (Schleicher & Schüll, Dassel/Kr., Einbeck, Germany). The pooled saliva samples 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 20—25 ml. The concentrated samples were applied to DEAE-cellulose column.

The active fractions (Peak I, representing salivary lactoperoxidase according to *Mäkinen & Tenovuo*, 1976) from the two ion exchange chromatographies were pooled and focused in a pH gradient from 3.5 to 10 (Fig. 1). Other details are given in the Results section.

5. Methods of clinical examination

The methods used in this study for recording dental caries, gingival condition and the amount of dental plaque have been described earlier (*Tenovuo & Valtakoski*, 1976).

RESULTS

The subjects were divided into two groups according to the clinical conditions (Table I). The differences between the clinical

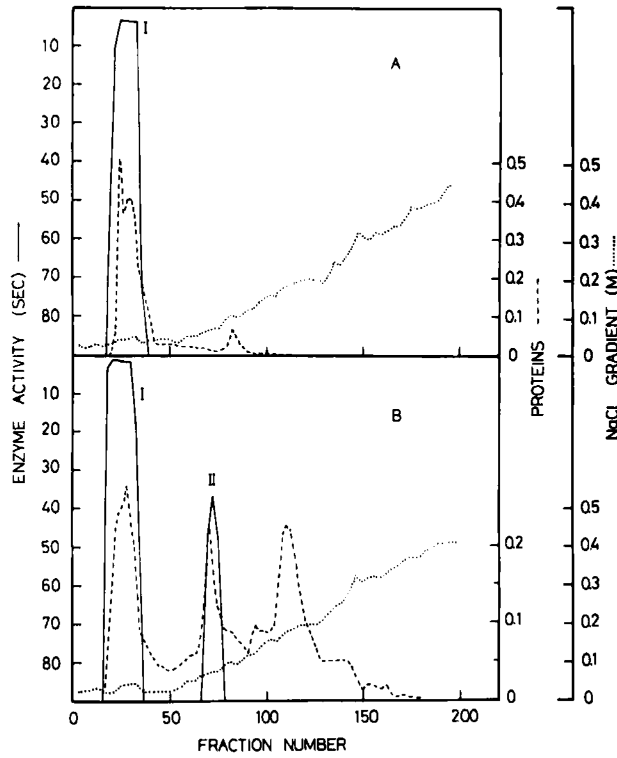

Fig. 1. DEAE cellulose chromatography of peroxidases of the pooled and concentrated human whole mouth saliva samples, determined according to the guaiacol method.
A. The sample (25 ml) representing Group I is described in the text. Column: 1.7×18 cm; Elution buffer: 0.01 M $\beta\beta$-dimethylglutarate 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 (20 ml) representing Group II is described in the text. Other details as for A. Fractions 15—36 (Peak I) and 66—78 (Peak II) were pooled.

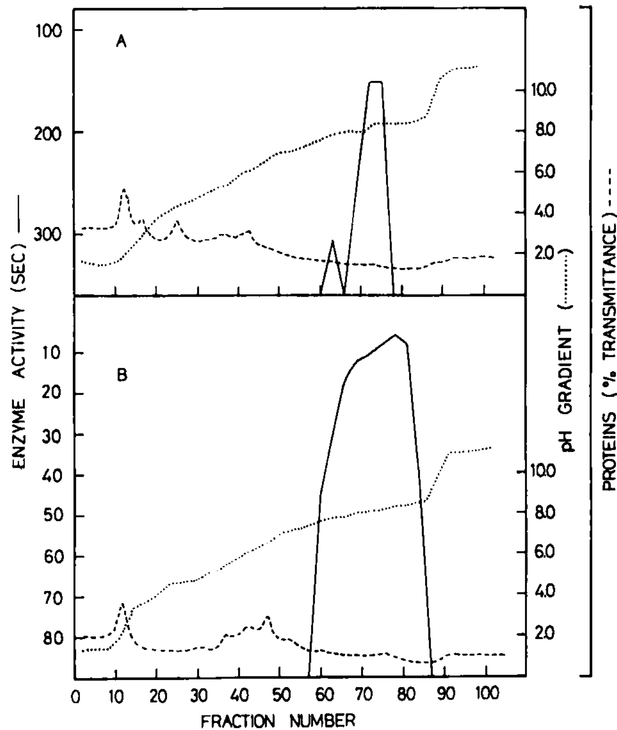

Fig. 2. Isoelectric focusing of concentrated and dialyzed pools from DEAE cellulose chromatography (see text). pH range from 3.5 to 10. Fraction volume: 1.1 ml; Voltage: 350 V; Current, <10 mA. A: Peak I of Fig. 1A. B: Peak I of Fig. 1B.

Table I. *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	1.40	0.30	++
GI	0.31	0.15	1.55	0.40	++
DS	7.6	3.8	20.0	15.1	+
DMFS	54.9	23.0	68.4	30.7	Φ

$P \leq 0.001$ ++ Φ = not significant
$P \leq 0.05$ + , Student's t-test

Table II. *The salivary peroxidase activity (in units per mg protein) of different samples of the test groups*

	Group I (n = 9)		Group II (n = 9)		
	$\bar{x}$	S.D.	$\bar{x}$	S.D.	
Individual samples	5.91	1.45	9.20	3.38	+
Pooled samples	16.1		19.5		
DEAE-Pool I	35.0		82.3		
DEAE-Pool II	—		1.7		

$P \leq 0.05$ +

recordings of these two groups were highly significant. The subjects of Group II had either extensive and active caries lesions or severe gingival inflammation or periodontosis. On the other hand, test persons of Group I were considered clinically healthy with only low caries frequency and mild gingivitis.

The saliva samples were comparable with regard to all procedures and assays performed. The results obtained show that the test persons with poor clinical conditions (Group II) displayed a much higher specific peroxidase activity of oral fluid samples than the control group (Group I). The differences between the individual samples of the two groups were statistically significant (Table II).

In order to clarify the origin of increased peroxidase activity a chromatographic separation of different oral fluid peroxidases according to *Mäkinen & Tenovuo* (1976) was performed (Fig. 1). The chromatographic experiments showed that the increased activity was due to a higher specific activity of salivary lactoperoxidase (DEAE-pool I and focusing Pool I). The isoelectric point (IP) of Pool I was the same (pH 8.1—8.2) as earlier presented for salivary lactoperoxidase (*Mäkinen & Tenovuo*, 1976), (Fig. 2).

Table III. *Salivary flow rate and the concentration of thiocyanate ions of the saliva samples of the test groups*

	Group I (n = 9)		Group II (n = 9)		
	$\bar{x}$	S.D.	$\bar{x}$	S.D.	
Salivary flow rate (ml/min)	2.34	0.73	2.12	0.84	Φ
SCN^- (mg/l)	90		115		

Φ = not significant.

There were no noticeable differences in salivary flow rate and thiocyanate concentrations between the test groups(Table III).

DISCUSSION

The results show that persons with poor clinical conditions (extensive caries and/or severe gingivitis) secrete a higher amount of salivary lactoperoxidase than subjects considered practically healthy. The increased activity cannot be a consequence of higher salivary flow rate (*Tenovuo & Valtakoski*, 1976) because there were no marked differences between the test

groups in this respect. This agrees with the results obtained by *Kraus et al.* (1958). They assumed that the increased peroxidase activity in the group of periodontal patients compared to normal subjects was due to certain homeostatic changes in the salivary glands rather than e.g. appearance of blood and leucocyte myeloperoxidase in saliva samples.

However, the increased salivary lactoperoxidase activity alone cannot control the poor clinical conditions enough although the increased activity may be a compensatory effect for reduced oral health. It has been observed with persons of lower caries activity and a low degree of gingivitis that the higher activity of lactoperoxidase antibacterial system in the oral cavity can significantly reduce the amount of dental plaque (*Koch et al.*, 1973). The xylitol-induced elevation of salivary lactoperoxidase activity could also be a factor in the reduction of dental plaque of xylitol-consuming persons (*Mäkinen & Scheinin*, 1975 a). As presented by many workers (*Wright & Tramer*, 1958; *Dogon et al.*, 1962; *Klebanoff & Luebke*, 1965; *Hoogendoorn*, 1974), lactoperoxidase alone is not antibacterial but the bacterial inhibition is a result of the combined working of the enzyme, thiocyanate ions and hydrogen peroxide. It is possible that adequate concentration of hydrogen peroxide is the most important factor in a good inhibition by the lactoperoxidase system (*Hoogendoorn*, 1974). In spite of increased enzyme activity the dental plaque reducing capacity of lactoperoxidase may be inadequate if there are no simultaneous and favourable changes in hydrogen peroxidase and/or thiocyanate concentrations. In this study no marked differences between the test groups in the concentration of thiocyanate ions were found. It was observed in Turku sugar studies that a xylitol diet increased considerably salivary lactoperoxidase activity and the increased activity correlated with low caries incidence (*Mäkinen et al.*, 1975; *Mäkinen & Scheinin*, 1975 a). However, there were a lot of simultaneous and favourable changes in dental plaque and oral flora (*Mäkinen & Scheinin*, 1975 a; *Larmas et al.*, 1975) and these changes may increase the antibacterial action of salivary lactoperoxidase system. As observed in the present study and by *Mäkinen & Scheinin* (1975 b), high peroxidase activity in saliva does not necessarily correlate with low caries incidence.

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