Oxidation Reduction Potential of Developing Plaque, Periodontal Pockets and Gingival Sulci

by

E. BARRIE KENNEY, B.D.SC., D.D.S., M.S.
MAJOR M. ASH, JR., D.D.S., M.S.

THIS STUDY WAS UNDERTAKEN to measure the oxidation reduction potential of developing dental plaque and of the gingival sulcus in order to obtain data which would add to the knowledge of the ecology of the gingival environment. This data would also indicate the level of oxidation reduction potential (Eh) required in vitro to isolate gingival organisms.

Bacterial metabolism requires hydrogen acceptors and results in the formation of "free" electrons which cause a fall in the Eh of the bacterial milleu. Therefore the level of Eh will change as bacteria in dental plaque proliferate and such changes may affect the type of organisms present at various stages of plaque maturation.

The role of microbial flora in the initiation of periodontal disease is not completely understood, but there is strong supportive evidence that bacterial plaque is an important etiologic factor in human periodontal disease. Changes in the ecology of bacterial plaque have been related to the initiation of inflammatory changes in the gingival tissues by Löe, et al[1] who showed that the appearance in developing plaque of two anaerobic organisms was accompanied by the first clinical evidence of inflammation in previously normal gingiva. Ritz[2] reported similar population shifts in developing human dental plaque with the initial predominant organisms being aerobic, while after seven days the predominant organisms were anaerobic. Further he suggested that these changes in plaque ecology were related at least in part to a lowering of the oxidation reduction potential of the environment.[2]

Strict anaerobic organisms have been identified from dental plaque and periodontal pockets,[3-8] and recent studies have shown that the isolation of many of these organisms in vitro requires a very low oxidation reduction potential.[9, 10] Although there have been studies of the oxidation reduction potential of saliva[11, 12] and of the gradient of electric potential from the inner to the outer surface of the dental plaque,[13] there is no data on the changes of oxidation reduction potential during the development of dental plaque on previously cleaned surfaces, nor is there any measurement of the oxidation reduction potential of the ginigval crevice and the periodontal pocket.

The University of Michigan, School of Dentistry, Ann Arbor, Michigan.

MATERIALS AND METHODS

In order to find the Eh, the potential difference was recorded between a stable reference electrode, and a variable measuring electrode which changed its potential according to the amount of oxidation or reduction in the environment. The base potential of the reference electrode was related to the standard hydrogen electrode to give absolute readings of the oxidation reduction potential.

The reference electrodes used were of two types (Fig. 1): (1) a miniature glass salt bridge electrode (1½ mm × 5 mm) with a silver-silver chloride internal element bathed in a solution of saline and connected to the outside environment by an asbestos wick; and (2) a pellet silver-silver electrode (1½ mm × 4 mm). The latter electrode was used as a reference in the study of the changes of oxidation reduction potential on the developing plaque. The pellet electrode is sturdier than the glass type and does not require a reservoir of chloride ions solution around it, but uses the chloride ions in the plaque environment. This electrode, described earlier,[14] is composed of a core of silver-silver chloride powder mixed with betonitic clay and epoxy resin to form a porous electrode element. It is covered with an adhesive resin in order to protect the junction of the electrode element and the wire lead from moisture contamination (Fig. 2).

The measuring electrodes were constructed of 26 gauge platinum wire that was covered with teflon which was sealed to the wire with epoxy resin. One projection of the wire acted as the measuring electrode while the other end of the wire was connected to an electrical lead. The potential difference between reference and platinum electrodes was measured with a Beckman Zero Matic pH Meter set to measure ± 700 millivolts.

The experimental electrodes were tested to ensure their accuracy by comparing them with the larger and more conventional electrode assemblies of a calomel electrode (Beckman #41239 fiber junction calomel) and a commercially available platinum oxidation reduction potential electrode (Beckman type #39271).

In the initial part of the study two subjects were used to measure the changes in oxidation reduction potential associated with changes in plaque ecology. In order to make the measurements pellet silver-silver chloride electrodes and platinum measuring electrodes were attached to veneers of sterilized human enamel from extracted teeth. This electrode assembly was then mounted to a fixed bridge pontic using acrylic resin, and the pontic

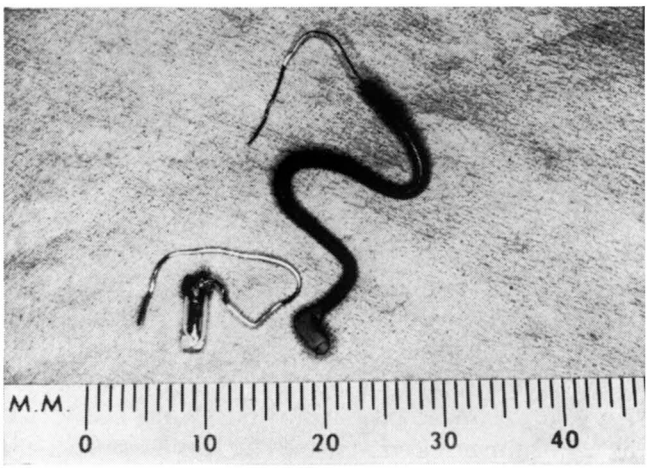

FIGURE 1. *Miniaturized silver-silver chloride electrodes. Glass salt bridge electrode on left, pellet electrode on right.*

plus electrode was seated in the patient's mouth (Fig. 3). Readings of the potential difference were recorded at the time of insertion of the electrodes and at varying intervals of time thereafter up to 7 days. At each recording the potential of the pellet electrode was measured against a miniaturized glass silver-silver chloride electrode placed next to the pellet—this was done in order to compensate for changes in the chloride ion concentration of the plaque milleu.

Bacterial smears were taken at the time of each recording and Gram and Tunnicliffes stain were used to identify plaque organisms. The patients were instructed not to brush or cleanse the area of the electrodes over the period of the study. After each recording the electrode leads were tucked under the pontic and covered with a neutral periodontal dressing.

In another group of 10 patients having periodontal disease, oxidation reduction potentials were recorded in

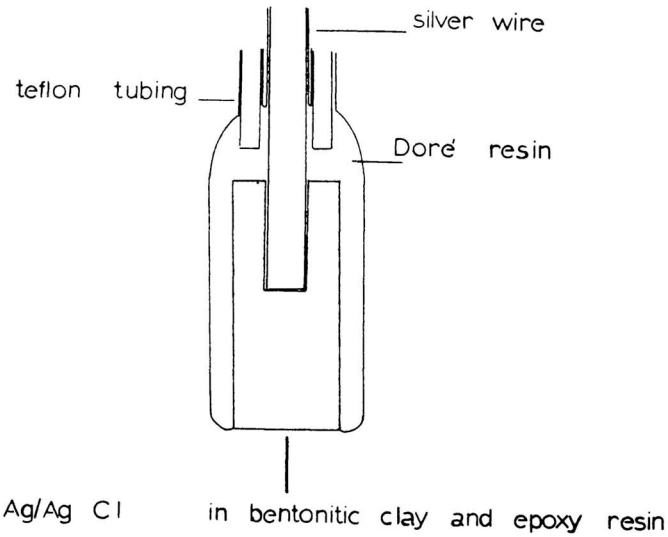

FIGURE 2. *Diagram of pellet silver-silver chloride electrode.*

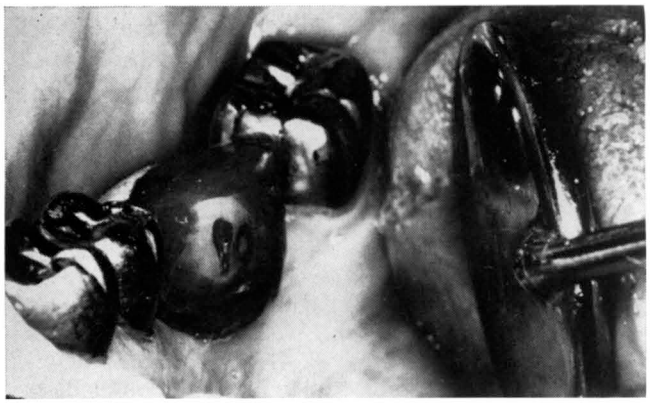

FIGURE 3. *Pellet silver-silver chloride electrode and platinum electrode mounted on removable bridge pontic in subject's mouth.*

periodontal pockets having gross subgingival calculus. The platinum electrode was inserted into the pockets and a glass miniaturized silver-silver chloride electrode was placed at the opening of the pocket. After the potentials were recorded the pocket depth was measured. Also in all the subjects Eh was measured in a gingival crevice of less than 3 mm depth with no calculus and minimal inflammation.

TABLE 1
Oxidation Reduction Potential of Developing Plaque

Subject 1 *Days After Insertion of Electrodes*	*Oxidation Reduction Potential (Millivolts)*	*Bacterial Smear*
0	+244	
1	+ 63	Gram +ve Cocci Gram −ve Cocci
3	− 31	Gram +ve Cocci Gram −ve Cocci Gram +ve Rods Gram −ve Slender Rods
4	−112	Gram +ve Cocci Gram −ve Cocci Gram +ve Rods in Chains Gram −ve Slender Rods
Subject 2		
0	+294	
2	− 30	Gram +ve Cocci Gram +ve Rods in Short Chains
3	− 67	Gram +ve Cocci Gram −ve Cocci Gram +ve Rods
7	−141	Gram +ve Cocci Gram −ve Cocci Gram +ve Rods in Chains Gram −ve Short Rods A Few Unbranched Gram −ve Filaments

Ten patients with no pocket formation and clinically healthy gingiva served as controls. The Eh was measured in gingival crevices which were less than 3 mm in depth.

RESULTS

The results from the initial study of two subjects with the electrodes in place for several days showed a fall in oxidation reduction potential as plaque bacteria accumulated. (Table 1).

In the group of subjects having periodontal disease there was a statistically significant difference between the mean Eh for periodontal pockets and the mean value for gingival sulci ($p < .0.001$). There was also a statistically significant difference between the mean Eh of gingival sulci from the control group and the mean Eh of periodontal pockets in the periodontal disease group ($p < .001$). The difference of the means of the Eh from the gingival sulci of the control and the experimental group was not statistically significant. (Table 2).

TABLE 2
Oxidation Reduction Potential in Millivolts

Subjects with Periodontal Disease		*Control Group*
Periodontal Pockets	*Gingival Sulci*	*Gingival Sulci*
+ 14	+113	+ 33
− 94	+ 53	+ 64
− 61	+ 10	+ 51
−157	+ 15	+ 73
+ 12	+102	+103
− 28	+103	+ 63
− 6	+ 47	+ 93
− 32	+ 67	+ 86
− 57	+103	+ 88
− 67	+113	+ 90
Mean −47.6; SE ± 15.6	+ 72.6; SE ± 12.0	+ 74.4 SE ± 6.5

DISCUSSION

Measurement of Eh in biologic material has limitations and results must be viewed with this in mind. The basic theoretical principle of the potentiometric readings is that the systems being measured are reversible. While such conditions may be satisfied by inorganic reactions they do not hold true for living tissue where there are many irreversible organic systems.

Silver-silver chloride electrodes are 3 phase electrodes of the second kind and as such their base potential is affected by changes in the concentration of the chloride ion as described by the Nernst equation.[15, 16] However, in the range of 10 to 200 milliequivalents of sodium chloride per litre the potential of the miniature glass silver-silver chloride electrode varied only 9 millivolts.

Since changes in pH will affect the oxidation reduction potential by changing the ionic equilibria,[17] the basic Eh data obtained would be enhanced by future studies of pH levels. Temperature changes of up to 12° did not cause more than 1 millivolt difference in the base potential of the reference electrodes in vitro. Platinum electrodes are relatively inert but they may be affected by thiol and other chemical groups found in biological material.[18] For this reason these electrodes were polished between each recording. Cater, et al[19] have suggested that hydrogen gas affects platinum electrodes to a far greater extent than gold electrodes and so may cause inaccurate readings of Eh. It was found that gold electrodes gave approximately the same reading as platinum electrodes but the readings from the gold electrodes were not as stable as those from the platinum electrodes.

The results of this study have significance from the point of view of relative measure of the amount of reduction occurring in each environment, and as such can be useful in predicting what type of environment is required to isolate gingival organisms in vitro.

The initial levels of Eh from the two patients with the electrodes in the bridge pontics are in effect measurements of saliva and as such are in agreement with previous reports[11, 12] obtained from saliva.

The fall in oxidation reduction potential which occurred with time may be the result of bacterial metabolism within the plaque, lowering the oxidation reduction potential as the organisms proliferate. This phenomena has been demonstrated in vitro using gingival organisms, by Socransky, et al.[20]

The oxidation reduction potential of mouse cecum has been reported to be at least as low as −339 millivolts and may even approach the theoretical limit of −420 millivolts which is the lowest limit of metabolic enzyme activity of DPN and FAD.[21] The cecum has a large number of anaerobic organisms in its flora, many of which are also found in the gingival flora. The most reduced environment found in the present study was −157 millivolts in a deep periodontal pocket. The placement of electrodes into periodontal pockets will inevitably introduce oxygen at the same time and so initial readings of Eh from pockets will tend to be higher than the actual level of oxidation reduction potential.

The levels of Eh obtained from all pockets are considerably lower than +200Mv which has been suggested as the level of aerobically metabolizing tissue.[19]

The measurements of the oxidation reduction potentials from normal healthy crevices and from deep periodontal pockets show that the environment of a pathologic pocket is much more reduced than is the gingival crevice. Anaerobic organisms would be favored by this low Eh in the periodontal pockets and these strict an-

aerobic organisms may play an important role in periodontal disease.

Methylene blue has been widely used as a redox potential indicator when anaerobic organisms are cultured in vitro. This dye discolorizes at +11 millivolts at Ph 7.[22] The findings of the present study suggest that such a level of reduction would be inadequate for the isolation of the most fastidious strict gingival anaerobes and supports previous findings of increased recovery of anaerobes when a technique which provides oxidation reduction potential as low as −290 millivolts through the incubation and plating of gingival bacteria was compared with conventional techniques which expose the organisms to atmospheric oxygen.[9]

Strict anaerobes survive and proliferate in the environment of the periodontal pocket but there does not appear to be a resident bacterial flora in the gingival tissues lining the periodontal pockets. It may be that the Eh of these tissues is not compatible with strict anaerobic organisms.

Previous studies have reported minimal[23] or no[24] pH changes between shallow and deep periodontal pockets; however, future pH measurements should take into account Eh changes particularly if antimony pH electrodes are used.

CONCLUSIONS

Periodontal pockets have a much lower Eh than healthy gingival sulci and this difference in one physical property of the environment can be expected to affect the ecology of the resident bacterial population. The level of the Eh in the periodontal pockets will favor the proliferation of facultative and strict anaerobic organisms.

The changes in the bacterial population that occurs as plaque develops on a fresh surface can be explained by the fall in Eh that occurs as plaque matures so that initial aerobic organisms are supplemented by facultative and strict anaerobes coming into the plaque after the first few days.

In vitro cultivation of gingival anaerobic organisms should be carried out completely in an environment that provides a level of Eh of approximately −150 Mv if the in vivo conditions of the periodontal pocket are to be duplicated.

REFERENCES

1. Löe, H., Theilade, E. and Jensen, S.B.: Experimental Gingivitis in Man. J. Periodont., 36:177, 1965.

2. Ritz, H. L.: Microbial Population Shifts in Developing Human Dental Plaque. Arch. Oral Biol., 12:1561, 1967.

3. McDonald, J. B. and Gibbons, R. J.: The Relationship of Indigenous Bacteria to Periodontal Disease. J. Dent. Res., 41:320, 1962.

4. Rosebury, J., McDonald, J. B. and Clark, A. R.: A Bacteriological Survey of Gingival Scrapings from Periodontal Infections by Direct Examination, Guinea Pig Innoculation and Anaerobic Cultivation. J. Dent. Res., 29:718, 1950.

5. Schultz-Haudt, S., Bruce, M. A. and Bibby, B. G.: Bacterial Factors in Non-specific Gingivitis. J. Dent. Res., 33:454, 1954.

6. Courant, P. R., Saxe, S. R., Nash, L. and Roddy, S.: Sulcular Bacteria in the Beagle Dog. J. Periodont., 6:250, 1968.

7. Dick, D. S., Shaw, J. H. and Socransky, S. S.: Further Studies on the Microbial Agent or Agents Responsible for the Periodontal Syndrome in the Rice Rat. Arch. Oral Biol., 13:215, 1968.

8. Dwyer, D. M. and Socransky, S. S.: Predominant Cultivable Microorganisms Inhibiting Periodontal Pockets. Brit. Dent. J., 124:560, 1968.

9. Aranki, A., Syed, S. A., Kenney, E. B. and Freter R.: Isolation of Anaerobic Bacteria from Human Gingiva and Mouse Cecum by Means of a Simplified Glove Box Procedure. App. Microbiol., 17:568, 1969.

10. Stutman, M. and Gordon, D. F., Jr.: Improved Isolation of Anaerobic Bacteria from the Human Gingival Crevice. I.A.D.R., 1969 (abst. 181).

11. Eisenbrandt, L. L.: Initial Oxidation Reduction Potentials of Saliva. J. Dent. Res., 22:293, 1943.

12. Eggers, Lura, H.: Investigations into the Oxidation Reduction Potentials of Saliva from Caries Resistant and Caries Susceptible Persons. J. Dent. Res., 1955 (abst. T 12).

13. Parker, R. B. and Snyder, M.: Electric Potentials of the Dental Plaque. Life Science, 3:1275, 1964.

14. Bond, H. W. and Ho, P.: Solid Miniature Silver-Silver Chloride Electrodes for Chronic Implantation. J. Electroenceph. and Clin. Neurophysiol. (to be published).

15. Leonard, J. E.: Electrochemistry of Bioelectrodes. Paper presented at Annual Conference on Engineering in Medicine and Biology, Cleveland, Ohio, 1964.

16. Janz, S. J. and Ives, D. J. G.: Silver-Silver Chloride Electrodes. Ann. N. Y. Acad. Sci., 148:210, 1968.

17. Hewitt, L. F.: Oxidation Reduction Potentials in Bacteriology and Biochemistry, ed. 6. Edinburgh, E. and S. Livingstone Ltd., 1950, p. 8-9.

18. Ives, D. J. G. and Jantz, S. J.: Reference Electrodes Theory and Practice. New York, Academic Press, 1961, p. 484.

19. Cater, D. B., Phillips, A. F. and Silver, I. A.: The Measurement of Oxidation Reduction Potentials, pH and Oxygen Tension in Tumors. Proc. of the Royal Soc., 146: 382, 1957.

20. Socransky, S. S., Loesche, W. J., Hubersak, C. and McDonald, J. B.: Dependency of Treponema Microdentium on Other Oral Organisms for Isobutyrate, Polyamines and a Controlled Oxidation Reduction Potential. J. Bact., 88: 200, 1964.

21. Wostman, B. S. and Bruckner-Kardoss, Edith: Oxidation Reduction Potentials of Germ Free and Conventional Rats. Proc. Soc. Exptl. Biol. and Med., 121:1111, 1966.

22. Clark, W. M.: The Determination of Hydrogen Ions, ed. 3. Baltimore, Williams and Wilkins Co., 1928, p. 683.

23. Kleinberg, I. and Hall, G.: pH and Depth of Gingival Crevices in Different Areas of the Mouths of Fasting Humans. J. Periodont. Res., 4:109, 1969.

24. Forscher, B. K., Paulsen, A. G. and Hess, W. C.: The pH of the Periodontal Pocket and the Glycogen Content of the Adjacent Tissue. J. Dent. Res., 33:444, 1954.