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Continuous Monitoring of pH and Eh in Bacterial Plaque Grown on a Tooth in an Artificial Mouth

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Apparatus which enables the simultaneous continuous monitoring of pH and Eh of bacterial dental plaque as it develops on a tooth surface in an artificial mouth is described. Details of the electrodes used, monitoring equipment, and culture conditions are given. Preliminary results are given showing the Eh and pH values of plaque produced in vitro to be in close agreement with readings reported for plaque in vivo. The effect of the incorporation of 1% sucrose in the medium on these parameters is reported and a distinct inverse relationship between pH and Eh recorded.

Detailed study of the bacterial colonization of tooth surfaces and consequent plaque development poses two specific problems. Firstly, how to maintain controlled yet variable conditions thus facilitating evaluation of the influence of defined factors on plaque development; secondly, how to sample the plaque and monitor changes in its physico-chemical properties without interrupting its development and altering these very properties. To overcome the first of these problems, artificial mouth systems have been developed over the past 20 years (9, 10), mainly directed towards an understanding of the carious process. The system used in the present study is an adaptation of the apparatus developed by Wilson (13). The second problem, the measurement of parameters such as pH and Eh in developing plaque at the tooth surface without interrupting its development, necessitates the permanent positioning of electrodes in close contact with the tooth surface. The techniques used by many workers for measurement of pH (3, 11) and Eh (6), i.e., periodic insertion of electrodes into the developing plaque, has severe drawbacks. This procedure alters the structural integrity of the plaque and therefore its diffusion characteristics, and continuous recordings of pH and Eh are not possible. The development by Graf and Mühlemann (5) of a micro-glass pH electrode and by Kenney and Ash (6) of micro Eh electrodes made possible continuous monitoring of plaque properties as it developed in vivo. The artificial mouth system described in the present paper facilitates continuous recording of pH and Eh of uninterrupted plaque as it develops on a tooth and electrode surface under controlled defined conditions; some preliminary results obtained are reported.

MATERIALS AND METHODS

Artificial mouth apparatus. The artificial mouth apparatus shown in Fig. 1 consists essentially of a glass incubation chamber (A) which houses the tooth on which plaque is grown and a delivery chamber (B) which provides fluid to the tooth via a common delivery inlet. This system differs from that described by Wilson (13) in that the two lateral sockets fixed at opposite sides of the incubation chamber (A) are not off-set in vertical location, but positioned directly opposite one another. This enabled a tooth to be held in one socket and electrodes inserted through the other socket so as to contact the tooth as illustrated. A port in the incubation chamber (A) enabled samples to be taken of both the solid plaque developing on the tooth and of the fluid as it dropped from the tooth. Two small tubes were attached to the body of the apparatus to serve as gas inlets and to allow equalization of pressure within the apparatus (important during the sterilization procedure).

The teeth extracted were caries-free molars, cleaned by brushing followed by immersion overnight in H_2O_2 (3%). The pellicle was removed from the extracted teeth by immersion in N HCl for 5 min as described by Armstrong (1). The tooth was mounted in the widened end of a short length of thermo-setting, semi-rigid, polythene tube of suitable diameter, which was then inserted into the incubation chamber through a hollow glass stopper, cotton wool being used to fill the annular space.

Medium supply and inoculation method. Supplies of fluid (saliva, nutrient, and bacterial inoculum) were delivered via silicone rubber tubing to the upper delivery chamber (B) by means of a 10-track peristaltic pump (Watson and Marlow Ltd., Falmouth, England). The fluids, with the exception of the bacterial inoculum, then passed via a common delivery inlet to the tooth. The distance between the

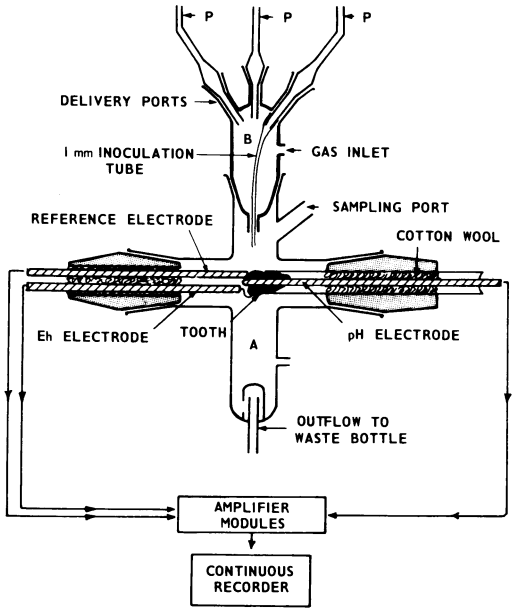

FIG. 1. *The artificial mouth apparatus: (A) incubation chamber with tooth and electrodes in situ; (B) delivery chamber; (P) peristaltic pump. Grey area represents glass stopper.*

delivery inlet and the tooth was arranged so as to minimize the physical washing effect of the medium without allowing back-contamination by direct contact. The bacterial inoculum passed via a 1-mm tube through the delivery inlet into the incubation chamber (A) and dropped onto the tooth. This arrangement served a dual function: first, it minimized bacterial contamination of the delivery chamber and hence blockage of the delivery inlet; second, by changing the length of the 1-mm tube that extended through the delivery inlet the distance that the fluid had to fall to make contact with the tooth could be varied, as this was found to influence the rate of plaque development. The drops of fluid wet the tooth and passed via the lip of the tooth mount to the base of the incubation chamber, where the effluent was periodically removed by the action of an internal self-sealing syphon.

Medium. The basal medium used (Table 1) was nutrient broth containing synthetic saliva (based on the report by Muhler and Swenson, 8). Sucrose and other agents were incorporated into the medium as supplements when required or solutions were delivered simultaneously to the delivery chamber via a separate port. The medium was supplied to the delivery chamber of the apparatus at a rate of 0.1 ml per min.

Inoculation. The tooth in the present series of experiments was inoculated with fresh whole human saliva. The saliva was allowed to fall onto the tooth continuously for 4 h from the beginning of the experiment at a rate of 0.05 ml per min.

Incubation. Incubation was carried out in a warm room at 35 C.

Sterilization. The fully assembled, dried apparatus, with tooth and electrodes in position, the plastic connection nipples at the end of the medium flow tubes wrapped in cellophane, and the gas inlets sealed with cotton wool, was put in a sterilization chamber and treated with ethylene oxide. The gas chamber was a converted portable autoclave which had a gas inlet, vacuum valve, and vacuum gauge fitted in the lid. A damp sponge was kept in the bottom of the sterilization chamber to maintain the high relative humidity essential for efficient sterilization by ethylene oxide and to cushion the glass apparatus. The lid was clamped tightly in place and the chamber was evacuated to 50 cm of Hg by a water pump. A mixture of 88% Freon and 12% ethylene oxide was then allowed into the chamber via the gas inlet until the pressure was equalized and allowed to act overnight. The sterilized apparatus was routinely tested by supplying sterile medium overnight before initiation of an experiment. All culture medium was autoclaved at 15 lb/in^2 for 15 min.

Electrodes. The system was designed to enable the use of readily obtainable commercial electrodes. The pH electrode was a tough membrane glass electrode with a 3.0-mm diameter bulb and a long slender 90-mm stem (Activion Glass Ltd., Fife, Scotland). This allowed it to be inserted up the center of the polythene tube, in which the tooth was mounted, and through a 4.0-mm-diameter hole drilled along the longitudinal axis of the tooth (Fig. 1). The electrode was positioned such that the pH-sensitive membrane of the bulb completely filled the hole in the crown of the tooth and was secured by means of autoclave tape, to the semirigid polythene tube.

A remote junction calomel reference electrode, with a 2-mm glass junction (Electronic Instruments Ltd. [E.I.L.], Richmond, England), was inserted through the side port of the apparatus facing that containing the tooth. The ceramic plug of the KCl bridge was placed in contact with the tooth surface (Fig. 1), thus completing the cell when medium was delivered over the tooth/electrode assembly.

The Eh was measured using a 6-mm square platinum plate electrode (E.I.L.) which was inserted alongside the reference remote junction and placed in contact with an area of the underside of the mounted tooth as illustrated.

Calibration of electrodes. The pH electrode was

TABLE 1. *Composition of basal medium*

Substance	Quantity (g/liter)
Lab lemco beef extract (Oxoid)	1.0
Yeast extract (Oxoid)	2.0
Peptone (Oxoid)	5.0
Sodium chloride	5.0
Mucin (crude porcine stomach)	2.0
Urea[a]	0.5
Sodium chloride	0.2
Potassium chloride	0.2
Calcium carbonate	0.3

[a] Sterile 40% urea solution was added aseptically to the sterilized medium (1.25 ml/liter).

calibrated at regular intervals with phosphate buffers pH 4.0 and 7.0 at 35 C. The Eh platinum electrode was calibrated with reference to the calomel electrode using the quinhydrone half-cell as a standard. The quinhydrone half-cell was recommended by Veibel (12) as one which, if prepared from day to day with a saturated solution, could serve in the standardization of hydrogen or calomel half-cells. Clark (2) reported that a saturated solution of quinhydrone in 0.1 N HCl had a potential (E) of 388 mV at 35 C with reference to the calomel electrode, and that the calomel electrode itself had a potential (E_{cal}) of 238 mV with reference to a hydrogen electrode. Thus, the potential of the quinhydrone electrode relative to a hydrogen electrode (Eh) is given by Eh = E + E cal, i.e., Eh = 388 + 238 = 626 mV. In practice, E cal may be incorporated by adjustment of the zero on the scale of the recorder so that the observed value directly indicates the Eh of any system.

Monitoring and recording equipment. The electrodes described above were connected outside the warm room via amplifiers to recording equipment and produced continuous recordings of the pH and Eh of the bacterial plaque as it developed.

The equipment consisted of: (i) a pH amplifier module (E.I.L. industrial system series 98) with scale set in the range pH 4.0 to 9.0; (ii) a redox amplifier module (E.I.L. series 98) with scales of +700 mV to -300 mV and 0 mV to +500 mV; (iii) a 3-channel continuous data recorder (Chessell Recorder Co., Worthing, England). Chart speed was fixed at 1 cm per h, allowing trends in the values to be easily observed; (iv) an electronic timer which operated a series of solenoid switches was developed, which allowed a single amplifier and channel of the recorder to be used to monitor the value of more than one continuous culture system. That is, the timer could be set to switch at 30-min intervals from an Eh electrode in one apparatus to another in a second apparatus and then back again, resulting in a semicontinuous recording of the Eh from the two plaques. The other two channels of the recorder were used to monitor changes in pH in each apparatus.

Bacteriological sampling. Samples of both the effluent as it fell from the inoculated tooth and of the plaque as it developed on the tooth were taken using a standard wire loop. These were examined both microscopically and culturally. A semiquantitative estimate of the composition of the plaque as it developed was obtained by sampling the effluent alone, thus allowing the plaque to develop without interference.

RESULTS

An experiment conducted to demonstrate the practical use of the artificial mouth apparatus and monitoring equipment is illustrated in Fig. 2, which shows the changes in pH and Eh that occurred when plaque developed in basal medium was changed to medium incorporating 1.0% sucrose. The pH fell rapidly from pH 8.0 to pH 4.6 in the first 4 h after change to the sucrose medium and remained at approximately this value; concurrently, the Eh of the plaque rose

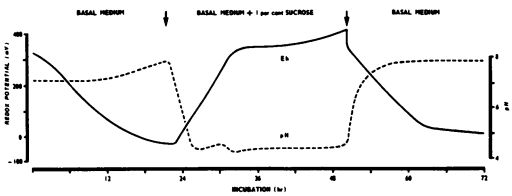

FIG. 2. *Eh and pH in plaque when the medium is changed.*

from below 0 to 200 mV in the same period and eventually leveled off at about 400 mV. Reversion to the absence of sucrose resulted in a steep rise in pH and fall in Eh (Fig. 2) to values similar to those found in plaque developed in the absence of sucrose from the beginning.

Smears of the effluent from plaque grown for 24 h in basal medium showed the presence of masses of neisseriae and streptococci; veillonellae, diphtheroids, and fusobacteria were isolated from effluent streaked onto selective media. In contrast, plaque produced after 24 h in the presence of 1.0% sucrose was predominantly streptococcal in composition with only low numbers of neisseriae and diphtheroids, whereas anaerobes were not recovered.

DISCUSSION

The results demonstrate the effectiveness of the artificial mouth system and monitoring equipment for studying changes in pH and Eh during plaque development and the effect of nutrition on these parameters. From Fig. 2 it is seen that plaque developed in basal medium produced Eh values below 0 mV within 24 h of incubation. These values are comparable to those reported by Kenney and Ash (6) for 3-day-old plaque in vivo. The absence of mechanical cleansing of the tooth in the artificial mouth allowed rapid colonization and development of plaque, with the consequent lowering of redox potential, in a shorter period of time than in vivo. Anaerobes were significantly absent from plaque developed in the presence of sucrose, because of the low pH and high Eh values resulting from acidogenic fermentation by streptococci. These recordings of pH agree with those reported by Mühlemann and de Boever (7) when sugar was applied to plaque in vivo. The rapid recovery in pH upon withdrawal of sucrose in the present experiment shows further the resemblance of the plaque produced in the artificial mouth to that in vivo. Of interest is the distinct inverse relationship clearly shown in Fig. 2 between pH and Eh, i.e., upon application of sucrose the Eh increased sharply as the pH fell, and when the sucrose was withdrawn the pH increased and the Eh fell. This inverse

relationship was reported by Eisenbrandt (4) for freshly collected saliva. It is of significance considering the importance of anaerobes and high pH values in peridontal disease and the low pH values associated with the carious process.

Results of more detailed studies involving both pH and Eh measurement and quantitative estimations of bacterial composition of plaque from pure and mixed culture plaques will be reported elsewhere.

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