CASE WESTERN RESERVE UNIVERSITY

GRADUATE STUDIES

We hereby approve the thesis of

Ira H Weiss

candidate for the _____

degree.

Signed: Donald H. Enlow
(Chairman)

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Date April 25 1983

THE MEASUREMENT OF CHANGES IN OXYGEN CONCENTRATION, REDOX POTENTIAL, pH, AND TEMPERATURE IN THE PERIODONTAL MEMBRANE DURING FORCE APPLICATION

by

IRA HAROLD WEISS

Submitted in partial fulfillment of the requirements for the Degree of Master of Science

Department of Orthodontics
School of Dentistry
CASE WESTERN RESERVE UNIVERSITY

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THE MEASUREMENT OF CHANGES IN OXYGEN CONCENTRATION, REDOX POTENTIAL, pH, AND TEMPERATURE IN THE PERIODONTAL MEMBRANE DURING FORCE APPLICATION

Abstract

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This study dealt with the biological responses of the tooth supporting apparatus to an application of force of sudden onset and short duration. Four parameters were selected, which were believed to be especially sensitive to significant changes in tissue respiration and energy dependent biochemical reactions.

The parameters selected for evaluation during force application were: oxygen tension; redox potential; pH; and temperature. All of these factors are extremely important in a consideration of any biochemical reaction or chain of reactions that either requires or liberates energy.

Electrodes and companion measuring systems were designed, tested, and applied to an in vivo model. Two animal systems were utilized. One was the anterior maxillary dental incisors of male hamsters. The periodontal membrane in these teeth was reasonably wide, and the bone sufficiently elastic to allow the insertion of extremely thin redox sensing electrodes.

The second animal model was the mandibular bicuspid teeth in a female dog. This model permitted the application of

calibrated forces and also the access to the periodontal membrane via two approaches: (1) an apicoectomy in the area of the distal root of the mandibular first bicuspid, (2) direct contact with the periodontal membrane through the pulp chamber of the distal root of the mandibular first bicuspid.

The important observations, findings, and conclusions from this study may be summarized:

1. The periodontium in the hamster incisor undergoes changes in its redox potential during sudden force application;

2. This alteration tends to drive the baseline value to increased negative potentials;

3. This increase in redox negativity shows a linear response in the force range of 1 to 4 ounces;

4. The negative increase per ounce of force is in the range of approximately 122 millivolts;

5. The recovery time after force removal--expressed as the time in seconds to restore the previous redox baseline value--tends to increase after 2 oz. of force;

6. The 50% recovery rate of the increased redox value is markedly increased with a 4 oz. load which shows evidence of having caused damage to the tooth supporting apparatus in the hamster's maxillary incisors;

7. This study developed the technique to conduct polarographic analyses in the periodontal membrane of the hamster incisor;

8. Using this analytical electrochemical analysis, an oxygen concentration fall was demonstrated in the hamster periodontium during force application;

9. This oxygen fall was immediate on force application, and a recovery of normal oxygen levels began as soon as force was released.

10. There seemed to be an indication that this fall in oxygen was proportional to the applied force in the 1 to 4 oz range. The relationship was not as definite as that seen in the redox change;

11. There appeared to be a slight increase in the acidity of the periodontium in the mandibular bicuspid region of a dog during force application;

12. The increase in acidity was very slight, but positive. An increase in acidity equivalent to 0.11 pH units occurred after an application of a 390 gram force for 6 minutes duration;

13. A decrease in temperature in the periapical region in the mandibular bicuspid area was demonstrated;

14. The temperature fall varied with the force applied. The application of 610 grams of force caused a reduction in temperature of .77°C after five minutes.

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DEDICATED

TO

My family, who gave me

the will to strive for

excellence

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INTRODUCTION

The science of orthodontics can be roughly categorized into two areas of knowledge. One is the mechanical basis for tooth movement, which accomplishes its goals by delivering forces to the periodontium through the banded teeth. The application of these forces changes the pre-existing biologic equilibrium, which results in tooth movement. This second area of knowledge, which deals with the underlying nature of the biologic response to forces, was recognized early as an essential part of the field of orthodontics. F. Noyes in 1942 stated: "It seems axiomatic... that mechanical forces applied to the teeth are not used to produce mechanical movement, but are used as biological stimuli to set up vital reactions in the tissues."[1]

The quest for knowledge of the biologic response continues. Some milestones are reviewed by Reitan.[2]

> "Sandstedt first observed hyalinized tissue in the periodontal membrane, and Schwarz rediscovered this interesting biologic phenomenon; in the field of transformation tissue, Sicher was the first to show that there is a constant rearrangement of periodontal fibers during tooth eruption, a finding which was later corroborated by Orban in studies on human material."

Parameters expressing tissue respiration and metabolism, namely redox potential, O_2 tension, pH, and temperature were studied in the present experiment to test a new electrophysiological, <u>in vivo</u> approach and a new quantitative technique for

analysis in the periodontal membrane subjected to orthodontic forces.

It is generally acknowledged that among the array of metabolic changes that occur upon application of force to a tooth, that there is a general collapse of the vascular bed on the pressure side of the periodontal membrane inhibiting blood flow to the area. The emphasis at this point is not to state that pressure causes resorption, and tension deposition, as this issue has been questioned by Baumrind in his article on a re-consideration of the propriety of the "pressure-tension" hypothesis,[3] and Enlow in his book on Facial Growth,[4] but to acknowledge that there is a decrease in blood flow to the area. This decrease in blood flow can be measured in the consideration of O_2 content in the area and in the subsequent change in pH, oxidation-reduction, and temperature.

An investigative study by Bien and Ayers[5] questioned the effects of blood flow in the periodontium with regard to forces acting on the maxillary incisor. Pressures were exerted on the periodontium which exceeded capillary blood pressure. Their findings were that in general the periodontal membrane is made up of a fine network of capillaries taking their origin from "an upward coursing vertical system of arteries, partly buried in bone and emptying into a downward coursing vertical system of veins interlaced through randomly oriented collagen fibers."

This makes up a complex hydrodynamic structure in which three independent fluid systems interact with each other. These systems are: "(1) The circulatory system of blood and lymph which course within vessel walls; (2) the visco-elastic system made up of the cells and fibers of the periodontium; and (3) the extra-circulatory system of interstitial fluids which fill the spaces between the cells, fibers, and vessels..." Their conclusions were that "...blood flow gives the periodontal membrane the characteristics of a hydraulic damper."[5] The study by Gianelly,[6] "Force-induced changes in the vascularity of the periodontal ligament," also concluded that "the type of resorption is related to the integrity of the periodontal membrane which in turn, is dependent on the vascularity of the membrane." He went on to theorize that forces which occlude the vascular network of the ligament led to the process of undermining resorption and forces not occluding the vascular network stimulate frontal resorption.

It is basic that an <u>in vivo</u> study undertake these metabolic parameters just outlined. Then the orthodontists of the future could begin to monitor the forces being delivered to such an optimum range,so that as little harm to the periodontium as possible, with the greatest amount of tooth movement, can occur.

As stated by Lehninger,[7] the "measurement of pH is one of the most common and useful analytical procedures in biochemistry, since the pH determines many important aspects of the

structure and activity of biological macromolecules and thus of the behavior of cells and organisms. There are two ways of deriving energy in the body. One is aerobic glycolysis which actively uses O_2 to generate energy. The other, anaerobic glycolysis, by its name means without oxygen. By-products of this anaerobic process are lactic acid and pyruvic acid, which accumulate in tissues where this form of glycolysis occurs. Monitoring acid, or, stated biochemically, hydrogen ion production gives some insight into the degree of this anaerobic process, particularly if the blood flow to the area is diminished and cannot carry away acid products formed, or exert its strong protective buffering action. Therefore hydrogen ion generation in tissue may be expected if there is a compromise of blood circulation which usually entails a slight diminution in O_2 availability. One might expect that a decrease in O_2 tension would have a tendency to increase anaerobic glycolysis as a compensatory mechanism.

Mast cells have been shown to respond immediately to the application of force, according to Yamasaki et al.[8] This response is expressed by an immediate discharge of products contained within the granules of the mast cells, such as muccopolysaccharides, amines, and other enzymes. These products are highly charged and may change the pH in the area for a short period of time.

Closely related are pH changes to the solubility of hydroxyapatite crystals which are the predominant crystals in organic components of enamel, dentin, cementum and bone--components which make up the hard structure architecture of the tooth supporting apparatus. In past research performed by this investigator to measure the rate of solubility of enamel surface to pH, it was noticed that hydroxyapatite concentrations are very sensitive to changes in pH. In that study, calcium ion activity was measured with a specific ion electrode on enamel surfaces exposed to buffers of various pH levels.

It is widely accepted that localized acidity in the living system favors bone resorption.

Oxidation reduction reactions as defined by Lehninger,[9] "are those in which there is transfer of electrons from an electron donor....to an electron acceptor." The standard oxidation-reduction (redox) potential, E_o' .....is defined by Lehninger[10] "as the electromotive force (emf) in volts given by a responsive electrode placed in a solution containing both the electron donor and its conjugate electron acceptor at 1.0 M concentration, at 25 C and pH 7.0. The electrode must be able to accept electrons from the electron donor and donate them to the conjugate electron acceptor. Such an electrode immersed in a mixture of the conjugate redox pair constitutes a half-cell." In many ways redox can serve as an essential parameter for the expression of

alterations in metabolic activity and therefore subtle changes in oxygen concentrations. It is a known fact in microbiology that anaerobic microorganisms can only be adequately cultured in media that are low in O_2 tension, and usually manifest very negative redox potentials. The pathways to energy consist of a series of steps in which the hydrogen atom possessing a -.421 volt redox potential is combined and reacts with oxygen, possessing a +.816 volt redox potential.[11] It is this change in electromotive pressure of 1.2 volts, which in the final analysis provides the energy to carry out all activities in living systems that require energy input.

Thus it is seen that metabolism, oxygen tension and hydrogen ion concentration are all intimately linked in the energetics of the living system. Therefore monitoring these fundamental expressions of living dynamics in the periodontium is most central to a fundamental, in-depth understanding of the mechanisms of tooth movement at a molecular level. While apparently no investigator to date has made measurements of the redox potential in the periodontal membrane during application of forces to the tooth and supporting structures, it is interesting to note that a recent study [12] addresses the subject of the redox status of growth plate cartilage in the rachitic chick. The findings were that "...chondrocytes in the rachitic growth plate are in a relatively reduced state. The least reduced zone is in

proliferating cartilage.....In addition, these analyses reveal that the total concentration of NADH and NAD in all zones of the growth plate is very low. It is concluded that Vitamin D modulates the metabolic activity of cells associated with the mineralization of the extracellular matrix." In other words, Vitamin D deficient animals have a decreased metabolic activity in association with the mineralization of the extracellular matrix.

The showering of mast cell products in an area of pressure, such as serotonin and histamine, both of which are highly charged particles, contribute to the change in redox potential. It is not inconceivable that the immediate expression of the mast cell degranulation can generate a local response or change in redox potential which could then be felt by biological transducers. As mentioned by Becker,[13] this could then make the appropriate response to initiate bone resorption or apposition.

As mentioned earlier in reference to the work of Bien and Ayers[5] and Gianelly,[6] the decrease in blood flow due to the occlusion of the vascular network caused by orthodontic forces most definitely affects the oxygen tension in the area and would probably be expressed as a decrease in oxygen tension. This decrease in oxygen tension must have a profound expression in the activity of the adjacent cells, and this has been in fact proposed by several investigators.

One study by Brighton and Friedenberg[14] measured the effects of electrical stimulation and oxygen tension. Some of their conclusions were: "....that the oxygen tension at a bone-cartilage front is low.....Several investigators have indicated that both epiphyseal plate cartilage cells and bone cells follow predominantly an anaerobic metabolic activity...." They went on to conclude that in zones of hypertrophy and especially at the bottom of the zone of hypertrophic cells, the oxygen tension is low and glycogen is almost entirely used up.

Another theory which seems possible is that the "mitochondria near the bottom of the zone of hypertrophic cells or at a newly forming bone front in the callus of healing fractures, because of oxygen and energy source deprivation, release calcium into the extracellular matrix.[15] This calcium may be the initiating factor for the extracellular matrix's calcification process. Another observation was that voltage of the range of 10-20μA at a potential less than 1 volt in the area of the cathode has two effects..."..namely the lowering of the local oxygen tension and a raising of the local pH. A decrease in oxygen tension is favorable to cartilage and bone formation...this could be due to....differentiation of multipotential cells into cartilage and bone-forming cells."[16] A further conclusion was that endochondrial bone formation, or cartilage calcification, is sensitive to an increase in pH.

The present investigator has been unable to find reference to *in vivo* measurement of the dissolved oxygen in the periodontal membrane under force application. One of the reasons for this may be that the electrode most used to measure physically dissolved oxygen (the Clark Electrode) is too large to fit into so minute a space.

One of the goals of this investigation was to apply force and then obtain a detailed profile of a selected number of the intermeshing parameters that yield insight into the active metabolic responses of cells located in the tooth supporting apparatus.

One of the selected parameters, temperature, is a frequently used biological monitor for sensing changes in the metabolic rate of a living system. Patient's temperatures are taken orally to monitor states of health. Temperature readings are taken in the leg to monitor alterations in blood flow during suspected cases of vascular changes in the lower limb (e.g. frost bite, arteriosclerosis, gangrene). One of the cardinal signs of inflammation is an increase in temperature in a localized area due to an active hyperemia in the initial states of the inflammatory response.

An interesting, unpublished pilot study by Batterman[17] deals with the use of infrared sensors to detect heat and spot cavities in the oral cavity. According to Batterman, the

detection of gum diseases and inflamed joints will also be possible. Thermograms for the detection of breast cancer are also now widely used.

Thus, in the present study, temperature changes that occur during the application of force to the tooth and supporting structures are monitored as one of the sensing parameters of important biological changes.

METHODS AND MATERIALS

This section is divided into four separate parts outlining the four different biological parameters tested.

Oxidation-Reduction Potentials

The term "redox potential" will be used interchangeably with "oxidation-reduction potential" for the sake of convenience or to conserve space in the labelling of charts.

Redox potentials were measured utilizing a No. 10 Kerr endodontic file as the redox sensing electrode. This file is extremely thin (100 microns at its thinnest)* and yet exceedingly flexible. It is made up of sharp filing flutes which permit one to work it into the thread-like curved canals often seen in many molar teeth. This probing electrode proved ideal for penetrating the periodontal membrane and insertion into the resting position seen in Plate VI. This was referenced against a silver/silver chloride electrode. During the course of this experiment, different reference electrodes were fabricated to best conform with the configuration of the particular area where the measurements were being conducted. The reference electrodes were all constructed the same way--starting with pure silver onto which a silver-chloride layer was electrically plated. This was done by

*The tip of a #10 K-Type file is 0.10 mm (100 microns), and it shows a gradual taper of 0.02 mm (20 microns) per mm in its 16 mm length.

connecting the silver wire to the anode (positive pole) of a simple direct current source derived from a direct current step-down transformer (Sharp Model AD-112 power supply, output DC 12 V, 1A). Two potentiometers were wired to the transformer in order to regulate the amount of voltage and current generated. By controlling the voltage and current densities, the rate at which silver chloride was formed on the electrode surface was also controlled. A standard metal paper clip was connected to the cathode (negative pole) to complete the circuit. An electrolytic NaCl solution was utilized in the plating procedure for the purpose of completing the circuit and to provide chloride ions for deposition on the silver electrode. The strength of the solution was adjusted to control the rate of silver-chloride deposition. Plate I illustrates the plating procedure.

After the plating procedure was completed, all the silver/silver chloride electrodes were carefully washed and then tested using a high resistance potentiometer (Plate II) against a commercial reference electrode for correct voltage and stability. A pure silver earing was plated, utilizing the procedure previously described. This proved very stable and was extremely useful for fixation to the cheek or palm, areas selected for anatomical reference points. The redox sensing electrode was cleaned of any surface impurities or oxides and tested against a reference electrode in an <u>in vitro</u> system before utilization in the animal. This redox electrode was then inserted into the

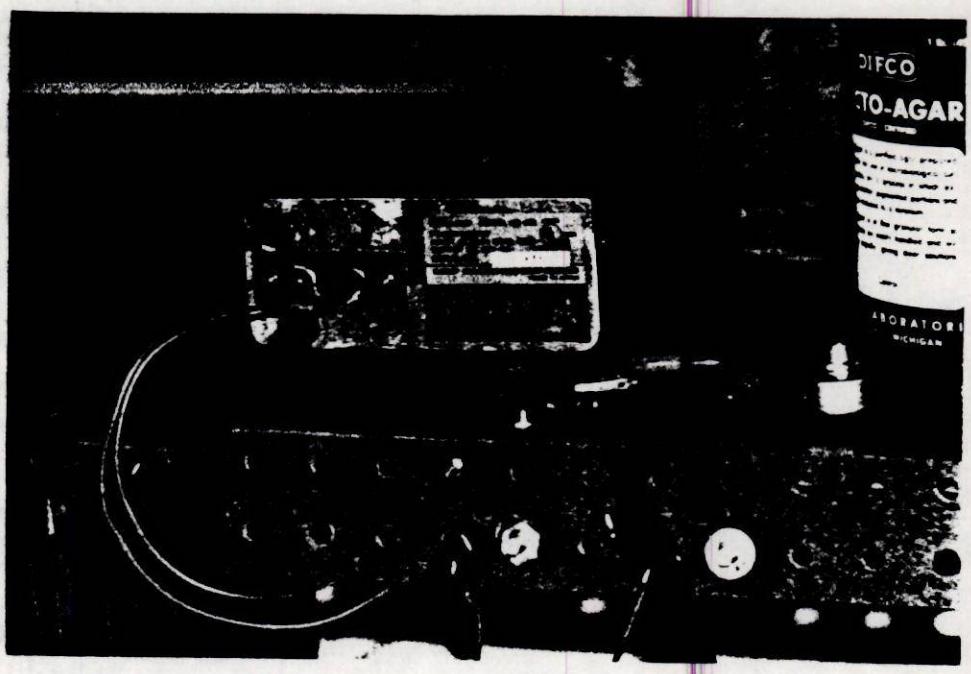
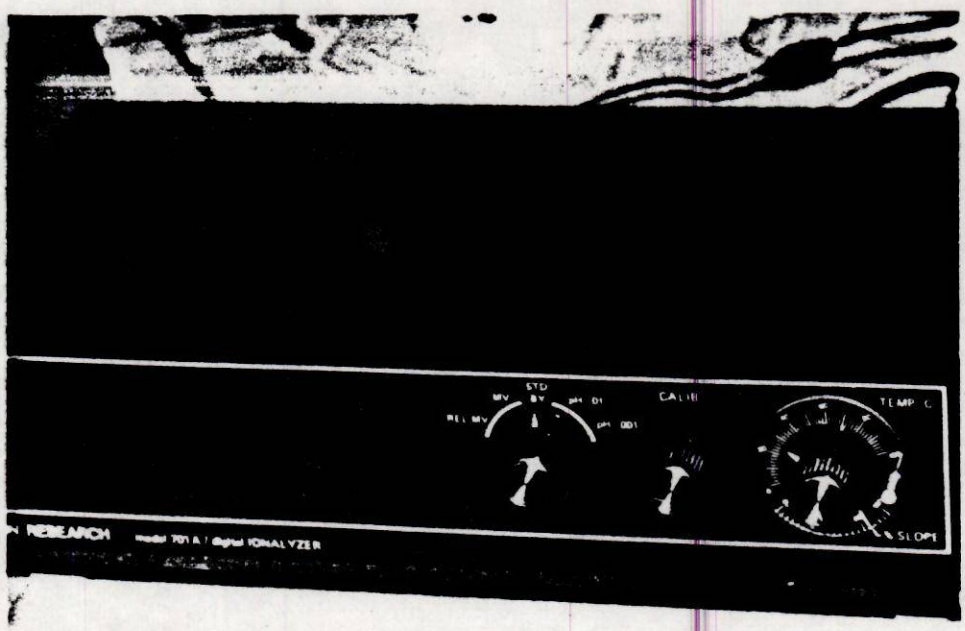

DIFCO
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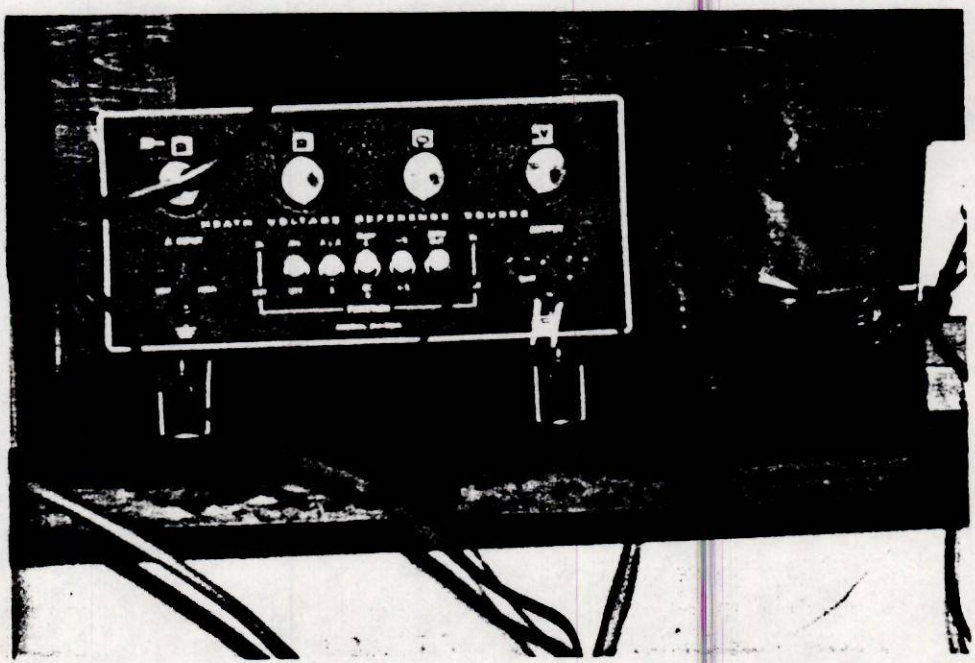


PLATE II

(UPPER) HIGH RESISTANCE POTENTIOMETER
(used in Redox and pH Measurements)

(LOWER) VOLTAGE REFERENCE SOURCE
(used to calibrate the measuring systems and the chart recorder)


STD
CALIB
TEMP C
RESEARCH



interproximal periodontal membrane of the upper incisor of an anesthetized male hamster, making sure to stay parallel to the root surface. Plates VI and VII show the relative placement of the Redox sensing electrode in the periodontal membrane. The anesthesia regimen consisted of 1 cc of Acepromazine (Ayers Co.) in 10 ml of Ketaset (ketamine HCl, Bristol Co.). Three-tenths of a ml of this mixture and 0.1 ml of Rompum (xylazine, Haver Lockhart Co.), were injected intramuscularly in the thigh area.

The anatomy of the maxillary anterior incisors and the relatively broad dimension of its periodontal membrane allowed good placement of the electrode. Plates IV, V, VIII, and IX show a histological section of the hamster maxillary anterior incisors, with and without placement of the electrode.

The reference electrode, as shown in Plate III, was clamped to the hamster's cheek. Both electrodes were connected by alligator clamps to a high resistance potentiometer (Orion Model 701A digital pH/mv meter). The purpose was to record the potential difference between the redox sensing stainless steel electrode and the reference electrode. This potential difference was fed to a potentiometric chart recorder (Linear Instruments Corp.), as seen in Plate X which was set at a chart speed of 10 cm/min. Forces ranging from 1-4 ounces were applied to the incisal edge of one of the maxillary central incisors. The other incisor was kept stationary by holding a rigid object against the distal surface of the tooth, and an attempt was made to maintain each of the forces for approximately 1/2 a minute.

PLATE III

MALE HAMSTER

A. Redox electrode

B. Reference electrode

(Pure silver earing plated with a layer of silver chloride)



PLATE IV

HISTOLOGICAL SECTION OF THE REGION BETWEEN THE TWO MAXILLARY INCISORS IN A MALE HAMSTER

(Magnification x 50)

A. Cementum

B. Periodontal Membrane

C. Alveolar Bone



PLATE V

A REGION IN THE PREVIOUS SECTION AT
INCREASED MAGNIFICATION
(Mag. x 140)

A - One of a linear chain of blood
vessels in the periodontium

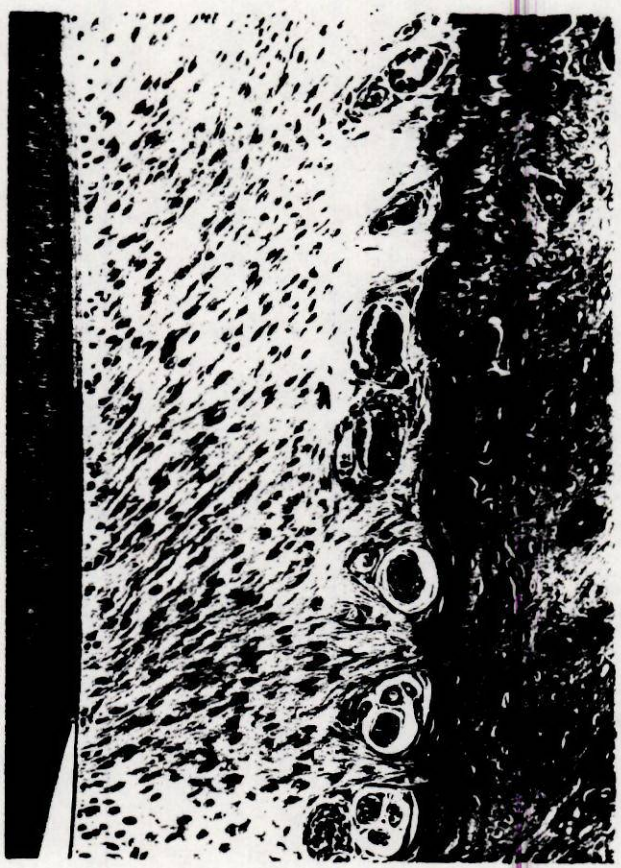


PLATE VI

LATERAL VIEW OF A DISSECTED BLOCK SECTION
OF THE ADULT HAMSTER'S MAXILLARY INCISOR
AND SUPPORTING ALVEOLAR BONE

(The relative position of the redox or
oxygen sensing electrode is shown)

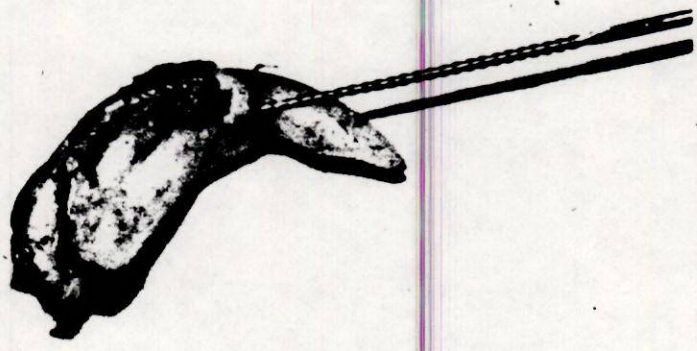


PLATE VII

LATERAL VIEW OF A RADIOGRAPHIC APPROXIMATION OF THE POSITION OF THE REDOX OR OXYGEN SENSING ELECTRODE IN AN ADULT MALE HAMSTER

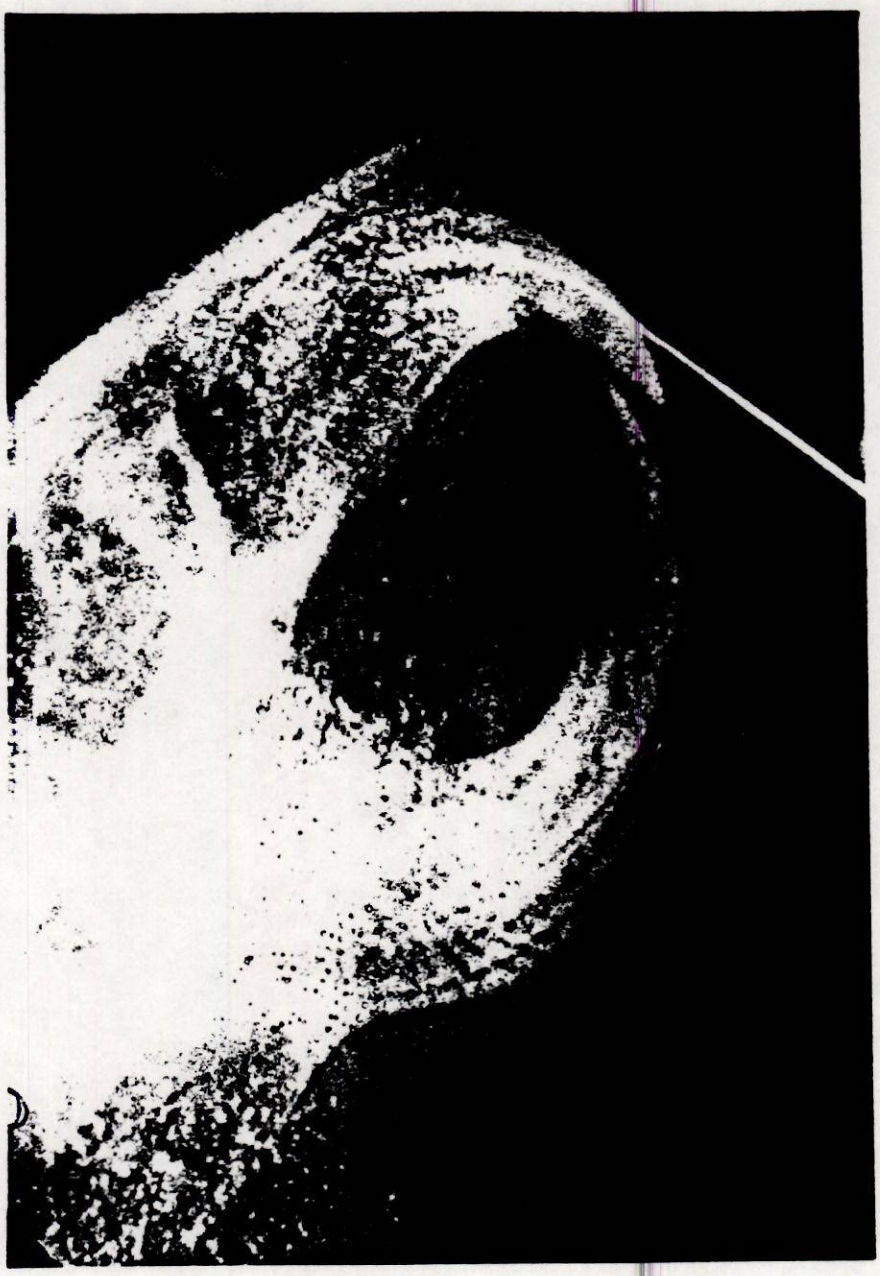


PLATE VIII

THE APPROXIMATE PLACEMENT
OF THE REDOX OR OXYGEN SENSING ELECTRODE
IN THE PERIODONTAL MEMBRANE OF AN
ADULT MALE HAMSTER'S MAXILLARY INCISOR
(Magn.- x38)

A. Redox or oxygen sensing electrode
(Tip diameter is 100 microns
before magnification)

B. Periodontal membrane

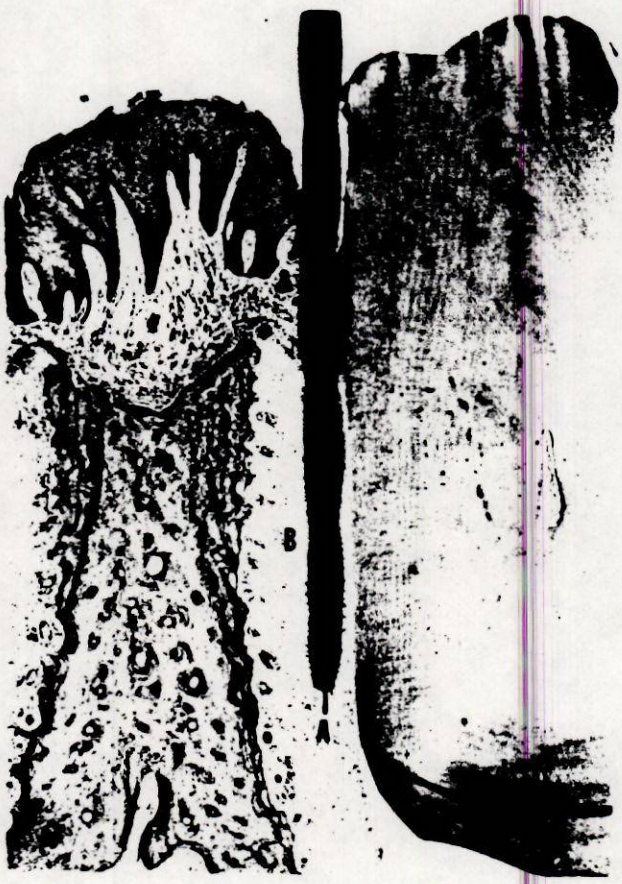

B

PLATE IX

AN ENLARGEMENT OF THE PREVIOUS SECTION SHOWING
THE APPROXIMATE PLACEMENT OF
THE REDOX OR OXYGEN SENSING ELECTRODE IN THE PERIODONTAL
MEMBRANE OF AN ADULT MALE HAMSTER'S MAXILLARY INCISOR

(Magn. x 70)

A. Redox sensing electrode
(Tip diameter is 100 microns before magnification)

B. Periodontal membrane

C. Approximate distance between blood vessels and electrode

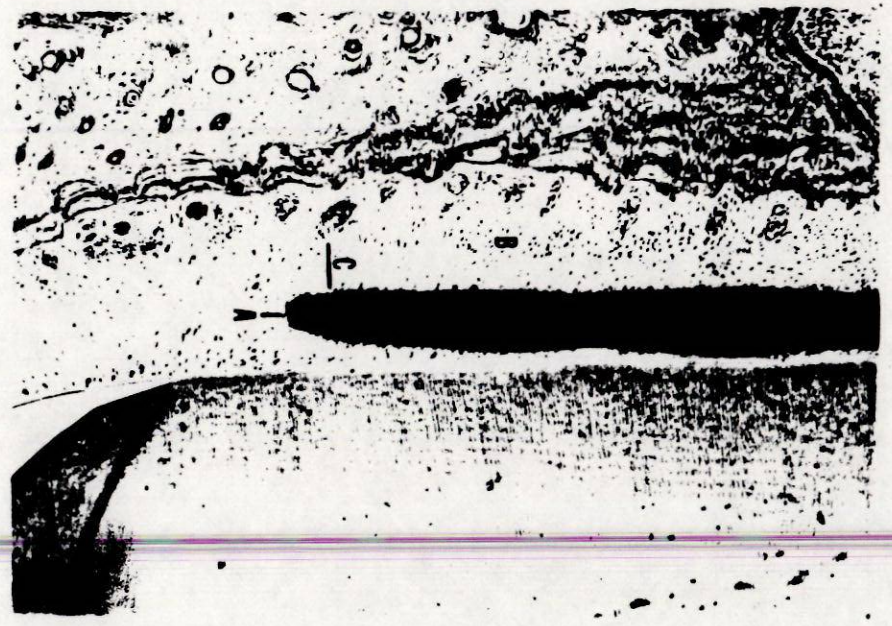
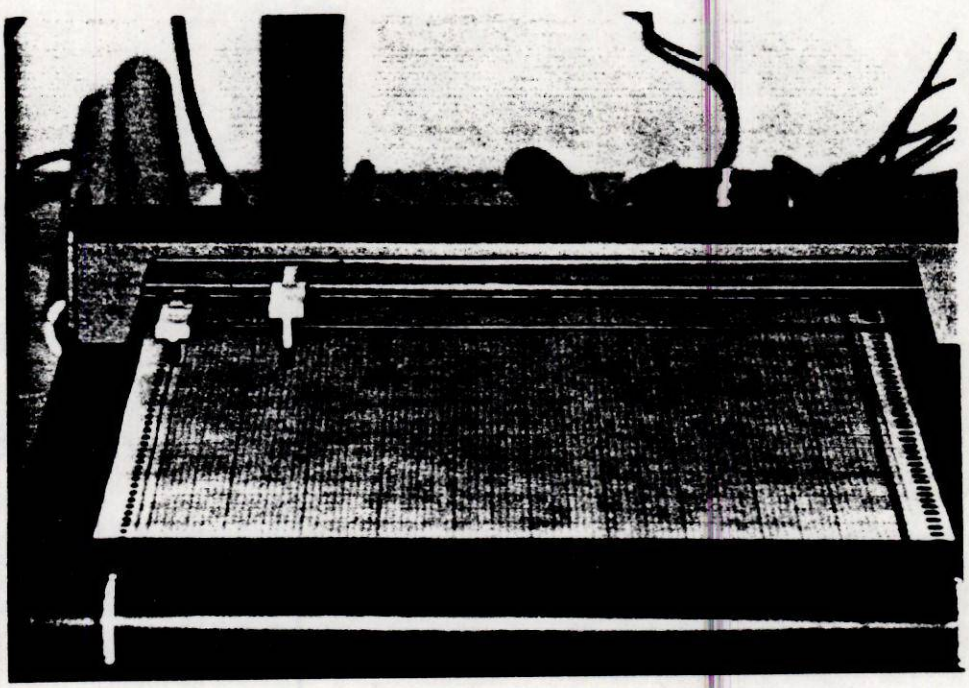


PLATE X

POTENTIOMETRIC CHART RECORDER



Oxygen Tension (Oxygen Concentration)*

Oxygen tension was measured by an oxygen electrode which was constructed from a No. 10 endodontic stainless steel file, charged with a potential of -700 mv and referenced to a silver/silver chloride electrode. The reason for the -700 mv charge will be discussed later, but it is at this negative potential that oxygen accepts electrons and this acceptance can be measured by a procedure called the polarographic analytical technique. This is the same analytical approach used to measure dissolved oxygen with the Clark electrode, which is widely used in industry today. In the polarographic technique, a voltage is superimposed on an electrode immersed in a solution. The voltage has sufficient electromotive potential to force an electron acceptance by the ion or molecule being analyzed in the solution. As the ions or molecules (in this case, the oxygen molecules) accept electrons, the degree of reduction occurring is measured by means of a recording of the increase in current flow. Thus, a polarograph analysis generates a current-voltage curve. By scanning slowly through a voltage range, and recording the current flow variations, one can analyze numerous substances and the energy and steps required for their oxidation or reduction.

The construction of the oxygen sensing electrode utilizes a No. 10 Kerr endodontic stainless steel file covered

*In this study, the term, "oxygen tension" will be used interchangeably with "oxygen concentration".

by a silver paint. This paint is used to repair printed circuits and dries to a reasonably hard finish. The surface is thus composed of almost pure silver with virtually no other metal contaminants. The coated surfaces were inspected under the dissecting microscope for gross imperfections and checked by observing their potential and stability against a silver/silver chloride electrode in an isotonic saline solution. In order to determine if this electrode was sensitive to dissolved oxygen, complete polarographic analyses were run, one at room temperature in isotonic saline, and the other in the periodontal membrane of the adult male hamster. (The identification of O_2 by this analysis will be discussed later after a few basic concepts have been defined.)

The placement of the O_2 sensing electrode, the reference electrode, and the anesthesia regimen were the same as in the redox experiment. In the experiments wherein oxygen concentration was monitored, two methods of force application were utilized. First forces were applied to the hamster's maxillary incisors by means of a hemostat pressing the incisors together. This force, while not calibrated, was of sufficient magnitude to ensure a maximal compression of the vasculature within the periodontal membrane. Once ascertaining a range for a severe compressional force, an attempt was then made to measure forces in the 1-4 oz range using a Dontrix Gauge.

A complete polarographic analysis refers to a slow scan of the voltage range from 0 to -1000 mv. The silver O_2 sensing electrode was made negative throughout the scan, and the reference electrode was connected to the positive terminal. The polarographic circuitry with which the scan was conducted is contained in the device seen in Plate XI. It consists of a precision motor which revolves at a slow rate of one revolution in six minutes. This motor drives a six turn potentiometer which generates a slowly increasing voltage from a 1.35 volt battery to the electrodes.

The current flow in the circuit is measured by feeding the potential drop of a resistor to a potentiometric chart recorder with a set constant speed. The voltage generated at the oxygen sensing electrode in the isotonic saline solution and in the periodontal membrane is read on a digital volt meter (Keithley) which is wired in series. Plate XI shows the set up for the complete polarographic analysis. Once the analysis was generated, it revealed the classical series of reductions seen in isotonic saline.

Figure 1 shows a steady state current for an open type oxygen cathode in unbuffered aerated 0.1M NaCl solution, as a function of polarization potential (adapted from Davies[18],), and this is almost identical to that obtained in the present study. From this it was possible to identify the region in which oxygen

PLATE XI

COMPLETE POLAROGRAPHIC ANALYSIS INSTRUMENTATION

LEFT: Polarographic circuitry RIGHT: Digital Volt Meter (Keithley)

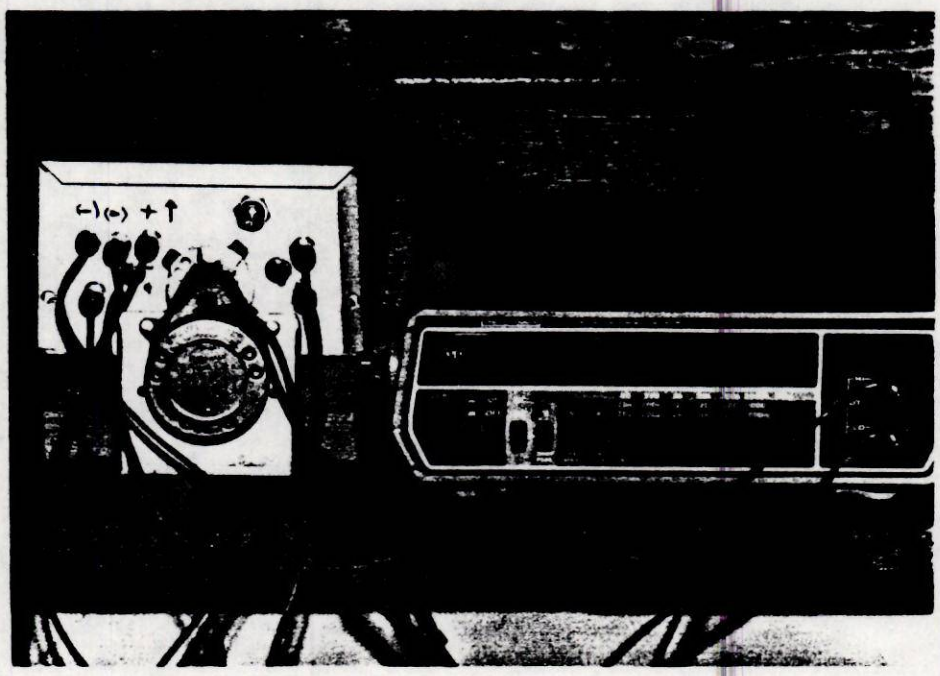


Figure 1

(From Davies, in Nastuck, 1962)[23]

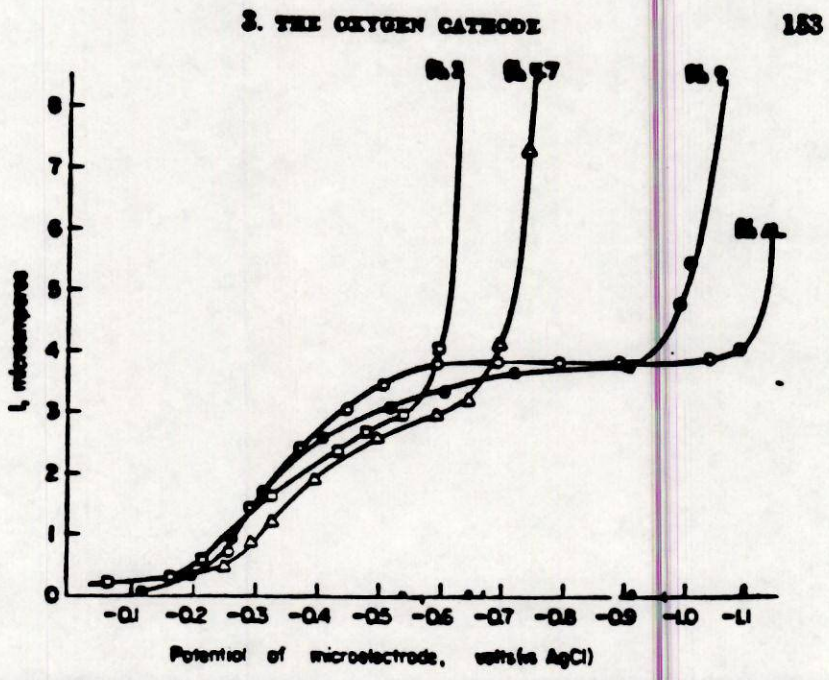


FIG. 8. Current-voltage curves for an open-type oxygen cathode in undisturbed aerated solutions at various levels of pH. The electrode was made from a 0.5 mm diameter Pt wire with 4 mm of its length exposed. Squares, phthalate buffer, pH 3.0. Triangles, acetate buffer, pH 4.7. Solid circles, borate buffer, pH 9.0. Open circles, 0.01 *M* NaOH in 0.1 *M* KCl, pH 12.0. (From Laitinen and Kolthoff, 1941.)

was being reduced and the voltage needed for its reduction. It was concluded that the most sensitive and stable potential for oxygen measurement is -700 mv, which was thereafter the voltage utilized. In order to ascertain that a small fall in oxygen could in fact be detected by a silver electrode at -700 mv, a hypodermic needle was modified to represent a miniature self-contained air tight oxygen sensing chamber, as seen in Plates XII and XIII. A scan of a 0 to -1 volt charge was run through the silver electrode and the silver/silver chloride electrode was connected to the positive pole to complete the circuit. An isotonic saline solution was aspirated and the analysis was run to generate a current-voltage curve. This was used as the control. Next, a fresh isotonic saline solution was brought to a vigorous boil. This brought molecules of O_2 to the surface of the solution, thus depleting the O_2 content of the solution, and the solution was then aspirated into the syringe and cooled to room temperature. It was noticed that the silver had been removed from the electrodes, so this experiment was repeated with new electrodes but two syringes were utilized: one to aspirate the boiling saline solution which was cooled to room temperature; and the second, the oxygen sensing syringe, inserted into the first syringe and the solution was aspirated. Then a new polarographic analysis was generated.

PLATE XII

HYPODERMIC NEEDLE
MODIFIED TO REPRESENT A MINIATURE SELF-CONTAINED, AIR-TIGHT
OXYGEN SENSING CHAMBER

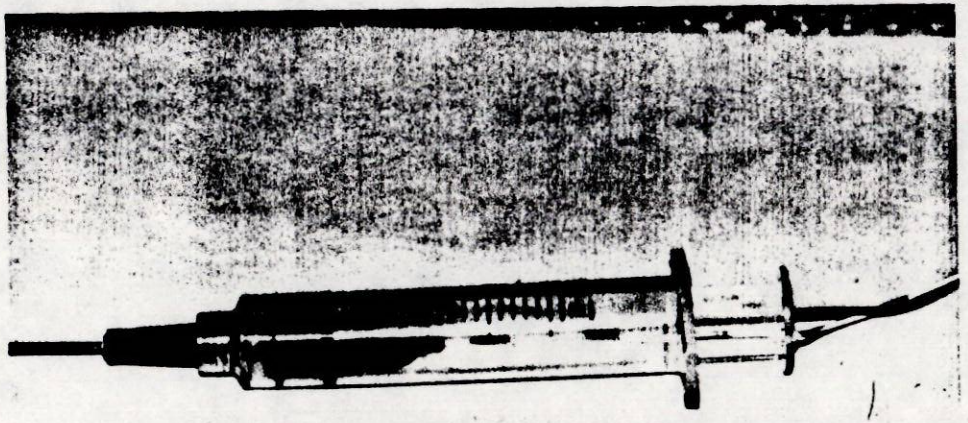
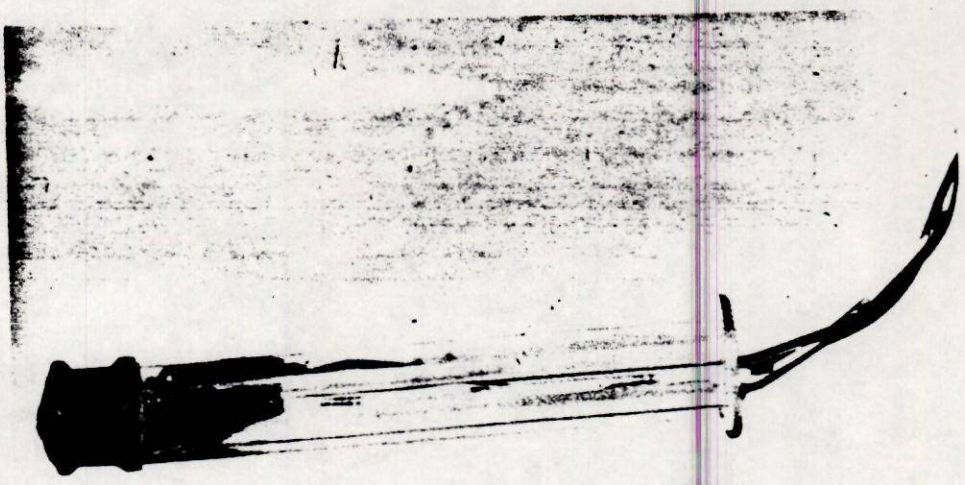
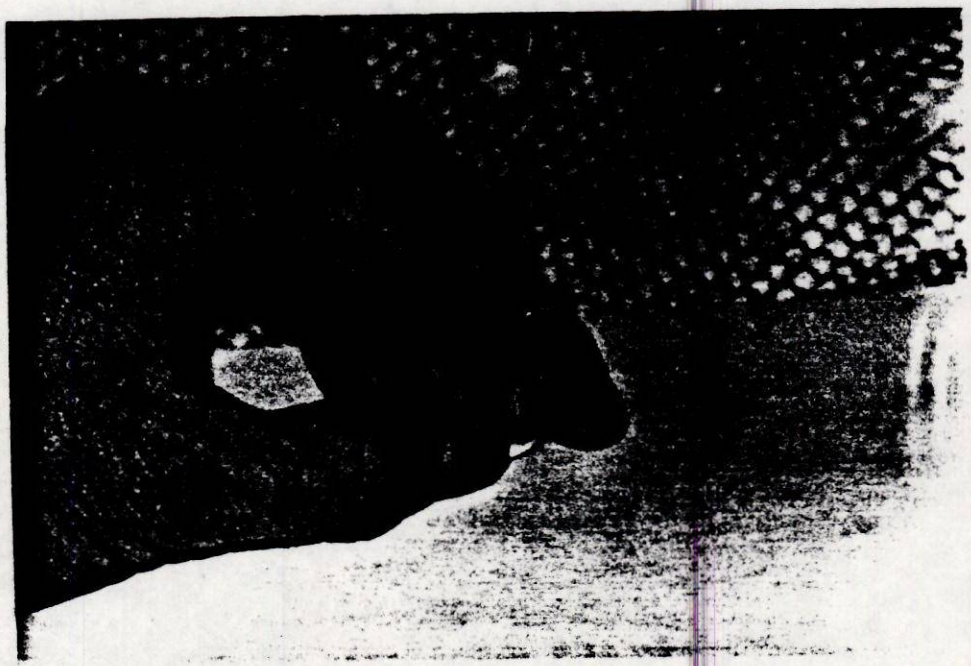


PLATE XIII

(UPPER) ELECTRICAL WIRING TO PLUNGER OF OXYGEN SENSING CHAMBER

(LOWER) O_2 SENSING ELECTRODE ON SURFACE OF RUBBER PLUNGER and below that a REFERENCE ELECTRODE ON SURFACE OF RUBBER PLUNGER





Hydrogen Ion Concentration (pH) Studies

In the series of studies to determine pH in the periodontium, a larger experimental animal was used because the sensing electrode was too large to insert into the periodontal membrane of the hamster model used in previous experiments. A one-year old female Labrador Retriever was anesthetized with 40 mg of morphine and 1 cc of atropine, injected subcutaneously, and 10 cc of Nembutol injected intravenously. She was then intubated ready for surgery. A surgical attempt was made to gain access to the periapical region of the lower first bicuspid with a semi-lunar flap. Once the flap was retracted, the approximate area of the apex surrounding the first bicuspid was located, and a circumscribed section of the buccal plate was removed. An endodontic file was gently manipulated to create a channel of communication with the surgical preparation and the periapical region. This allowed a sampling of the periapical fluids by the pH sensing electrode positioned in the surgically created hole. Another hole was created on the opposite side of the first bicuspid so that pH changes that occurred on both the pressure and tension sides could be determined during force application. Braces were put on the molar, second bicuspid, and first bicuspid by means of the Ormco one-step bonding system, as illustrated in Plate XIV. The entire surgical set-up is shown in Plates XV and XVI. However, this approach, while surgically successful, had to

PLATE XIV

BONDING PROCEDURE

(UPPER) ORMCO ONE-STEP BONDING SYSTEM

(LOWER) PLACEMENT OF EDGEWISE BRACKETS



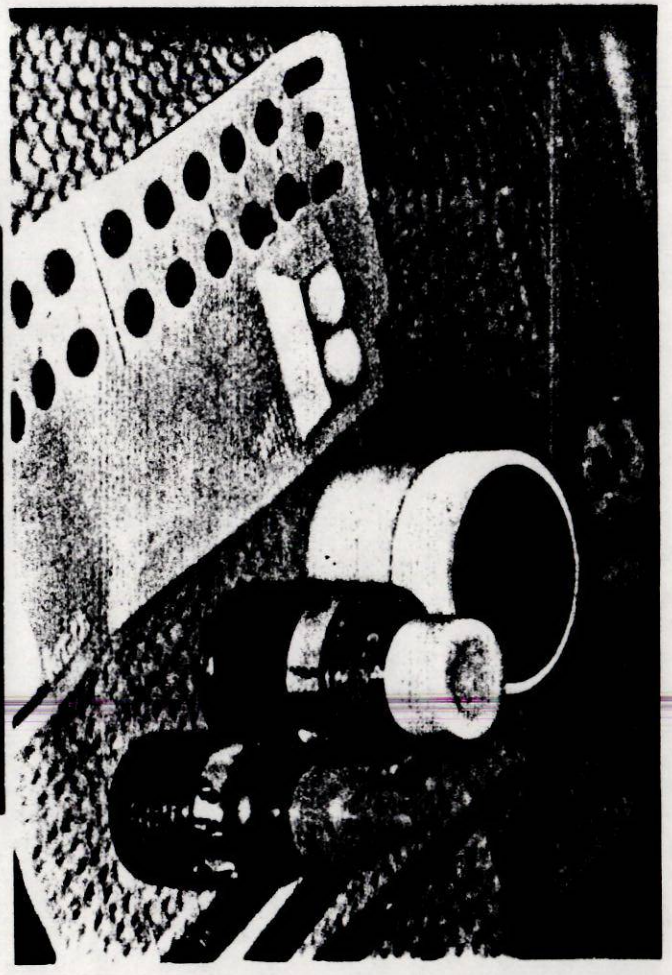


PLATE XV

(UPPER) SURGICAL SET-UP FOR pH AND TEMPERATURE STUDIES

(LOWER) SURGICAL OPENING TO THE PERIODONTIUM IN THE PERIAPICAL AREA OF THE MANDIBULAR BICUSPID IN THE FEMALE DOG

PLATE XVI

PLACEMENT OF pH ELECTRODE IN DISTAL APICAL PREPARATION



be abandoned because the glass electrode was very fragile and broke after a few initial runs upon insertion into the rigid cavity of bone.

Plate XVII shows a radiograph of the surgical area packed with amalgam to ascertain the position of the apex and its relation to the two surgical cavities. A new approach was then attempted.Close inspection of the radiograph of the first bicuspid revealed a straight canal extending to the apex. It seemed possible that this canal could be enlarged to a No. 80 file size (.8 mm). If a pH electrode could then be inserted into the canal to the approximate apex, this would permit contact with periodontal tissue in the area of the apex and thus permit monitoring of pH changes occurring during force application. A search of supply catalogues for the smallest glass electrode commercially obtainable with a protective sleeve was done, and the availability of an appropriate miniature electrode was determined. This electrode (Micro-electronics Inc.), consisted of a glass pH sensing electrode encased in a No. 28 gauge syringe stainless steel needle. The outer diameter of the needle was 0.8 mm and its length was 12 mm. The sensing glass was shielded by a beveled point. In addition to breakage resistance the shielding proved advantageous for another reason: a better resolution of pH was possible. This new electrode had the capability to ascertain changes to within a few hundreds of a pH unit. This was essential, as the pH changes during force

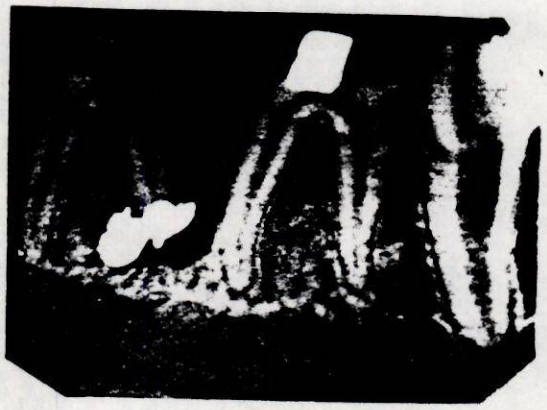
PLATE XVII

RADIOGRAPH OF THE MANDIBULAR LEFT POSTERIOR QUADRANT

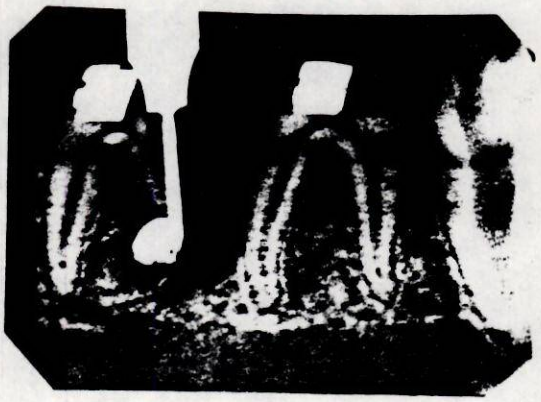
A. Bone markers placed to ascertain the relative position of the surgical opening to the mesial and distal aspects of the root apex

B. pH sensing needle type electrode positioned in the distal canal in the approximate area of the apex

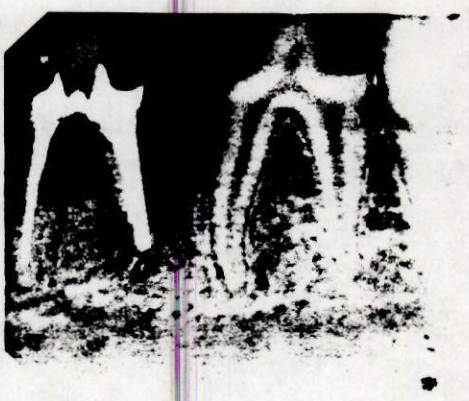
C. Both root canals filled with gutta percha and the entrance preparations with silver amalgam



A



B



C

application were expected to be small. The electrode was calibrated in a series of buffers (Markson standard buffer solution) in order to monitor accuracy, reproducibility, and response time, (Plate XVIII.) The pulp chamber was exposed in the lower first mandibular bicuspid with a #4 round bur. The canal was debrided of pulpal tissue and enlarged by a #90 Kerr endodontic stainless steel file, which is .9 mm in diameter. The electrode was then inserted to a distance of 12 mm, roughly approximating the area of the apex as seen in the radiograph (Plate XVII). A drop of isotonic saline was placed in the canal to establish liquid contact with the periapical fluid environment. Blood was present in the canal, which indicated that a communication had been made with the periapical area. A micro reference electrode (Silver/ Silver Chloride Reference Electrode Model No. 01973)** was placed in contact with the side of the tongue,as seen in Plate XIX. The two electrodes were connected to an Orion Model 701A pH meter and the range set at its most sensitive position (.01 pH sensitivity). The recorder used was the same as in the first two experiments. Forces were applied by placing Unitek elastic loop modules of varying sizes (two up to five) onto the molar and first bicuspid, producing progressive increases in elastic pull between the two bonded teeth. At one point of application the molar bond broke, so the elastic force was placed between the second and first bicuspid instead.

**Ionetics Inc.

PLATE XVIII

MARKSON STANDARD BUFFER SOLUTION pH 4 - 8

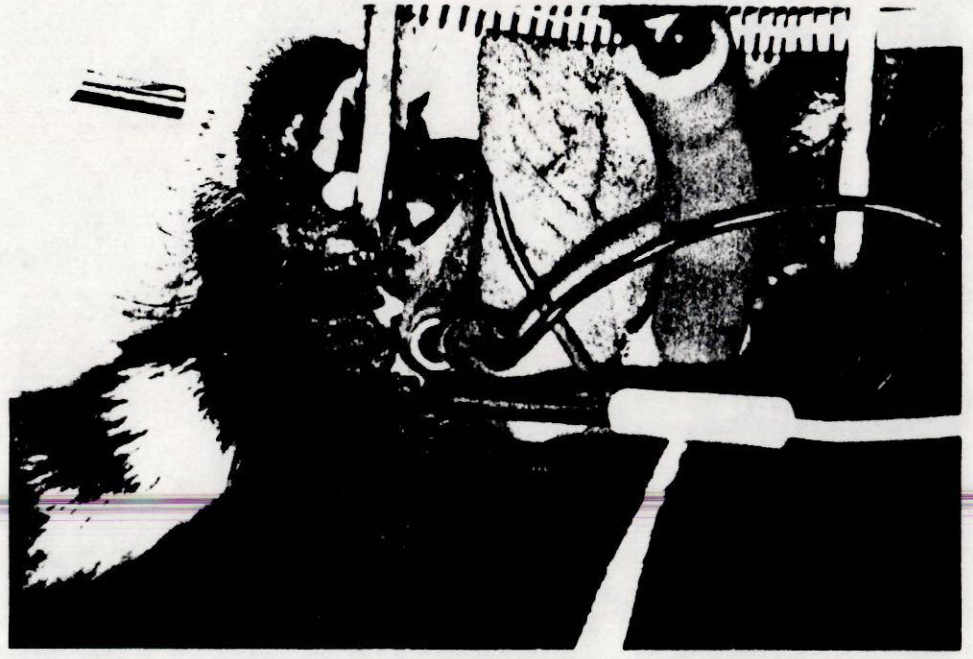
(Used to calibrate the pH electrode and to measure the pH sensitivity of the redox electrode)



PLATE XIX

pH ELECTRODE PLACED IN THE PULP CHAMBER OF THE DISTAL CANAL OF THE MANDIBULAR FIRST BICUSPID

SILVER/SILVER CHLORIDE REFERENCE ELECTRODE PLACED IN CONTACT WITH THE SIDE OF THE TONGUE



During the period of force application, the electrode recorded any subtle changes which occurred in pH. Plate XX shows the lower left first bicuspid after the gutta percha root canal filling and subsequent amalgam restoration. (While not central to the study, these were done so that the labrador retriever would not experience subsequent pain, and would have normal eating function.)

In order to determine the forces that were being applied by the different number of loops, the following system was devised. First, an impression via an acrylic tray, was taken of the lower left quadrant of the anesthetized dog. A stone cast was made and measurements were taken of the distance between the brackets to which the elastic modules were attached. Another set of similar brackets was purchased and one bracket was then cemented (Eastman 910 Superglue) to a Centimetric Triple Beam Balance at a position to one side of the fulcrum. A bracket was then glued to an aluminum rod which was positioned and fixed in such a way that both brackets were parallel to each other. The distance between the balance bracket and the rod bracket was identical to that measured on the cast. This set-up is shown in Plates XXI and XXII. The set-up permitted a stretching of the elastic modules at the same distance as that in the dog's mouth, and allowed measurements of the force at the same time. A glass beaker was placed in the weighing platform, and the

PLATE XX

MANDIBULAR LEFT FIRST BICUSPID FOLLOWING

ENDODONTIC ROOT CANAL AND AMALGAM BUILD-UP

PLATE XXI

(UPPER) STONE MODEL OF THE MANDIBULAR LEFT POSTERIOR QUADRANT OF THE FEMALE DOG WITH BRACKETS IN PLACE

CENTIMETRIC TRIPLE BEAM BALANCE

PLATE XXII

(UPPER) BRACKET PLACEMENT ON TRIPLE BEAM BALANCE AND ALUMINUM ROD AT THE DISTANCE ESTABLISHED FROM MEASUREMENTS TAKEN FROM THE CAST OF THE FEMALE DOG

(LOWER) SIDE VIEW SHOWING PARALLELISM OF SET-UP

balance was leveled to the midline by adding water to the beaker. Then elastic modules of different sizes, ranging from two to five, were placed onto the brackets. The subsequent amounts of weights needed to again register the balance to the midline were recorded in grams, thereby giving a precise measurement of force magnitude. The weight value equivalents of the various elastic modules deformed to specified distances are seen in Table I.

Temperature

Changes in temperature during force application were also measured in the female Labrador Retriever (for the same reasons given in the pH section previously discussed). The anesthesia regimen was also the same.

The temperature sensing electrode consisted of a Bailey Instrument Inc. micro right angle thermocouple, Type T Copper/ Constantan, Model No. MT-D. This fast response surface probe had the following specifications: probing surface point-3/8 of an inch in length; bend 1/8 of an inch long; diameter of needle portion of the probe-(.013 inches).

The constant temperature junction leads were connected to a Bailey Instruments Stable Oven Model OST-6, which holds a constant temperature at 44.98°C, within a fluctuation of less than .01° C. The second junction of the thermacouple (which senses varying temperatures) was connected to a Bailey Model TH-60 Thermalert Monitoring Thermometer, designed for the

TABLE I

FORCE EQUIVALENTS OF ELASTIC LOOP MODULES

DISTANCE (MOLAR TO 1ST BICUSPID) - 29 mm

FORCE	WEIGHT
5 loop elastic module	275 g
4 loop elastic module	440 g
3 loop elastic module	610 g

DISTANCE (2ND BICUSPID TO 1ST BICUSPID)- 19.5 mm

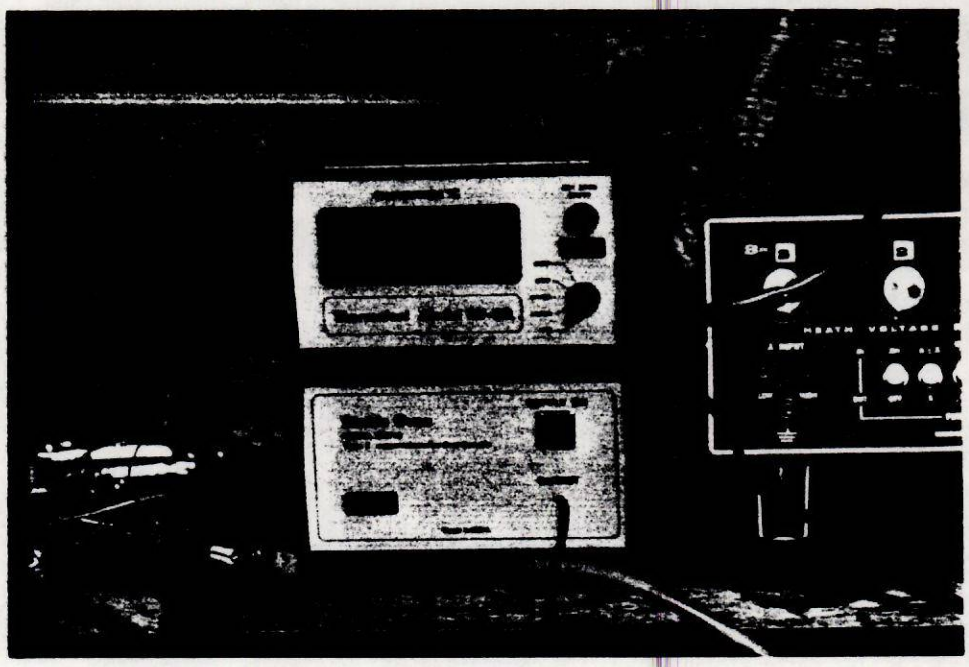
FORCE	WEIGHT
4 loop elastic module	110 g
3 loop elastic module	324.2 g
2 loop elastic module	390.0 g

A SERIES OF PHOTOGRAPHS OF

TEMPERATURE SENSING EQUIPMENT UTILIZED

IN THIS EXPERIMENT

FOLLOWS




Thermalert Model TH-60

temperature sensing electrode. Plates XXIII and XXIV show the set-up and the instruments utilized for these measurements. The readout meter was set at the differential mode, which compares the temperature of the experimental site to the constant temperature of the oven. It was possible during the experiment to occasionally switch to the non-differential mode, which gives direct readings in degrees centigrade. Also, the differential mode readings can easily be converted to degrees centigrade by simple arithmetic conversion of the millivolt readout. The thermocouple temperature sensing electrode was placed in the distal surgical preparation communicating with the periapical region of the tooth. Insulating rubber putty was placed over the temperature electrode, as seen in Plate XXV, to insulate the region being monitored from the dog's own breathing, room air currents, and also the heat generated from the lips and cheeks. Forces were applied, as previously specified, with elastic modules, and the temperature values were recorded manually before and after the force application. A plot was constructed, the results of which are discussed in the next section.

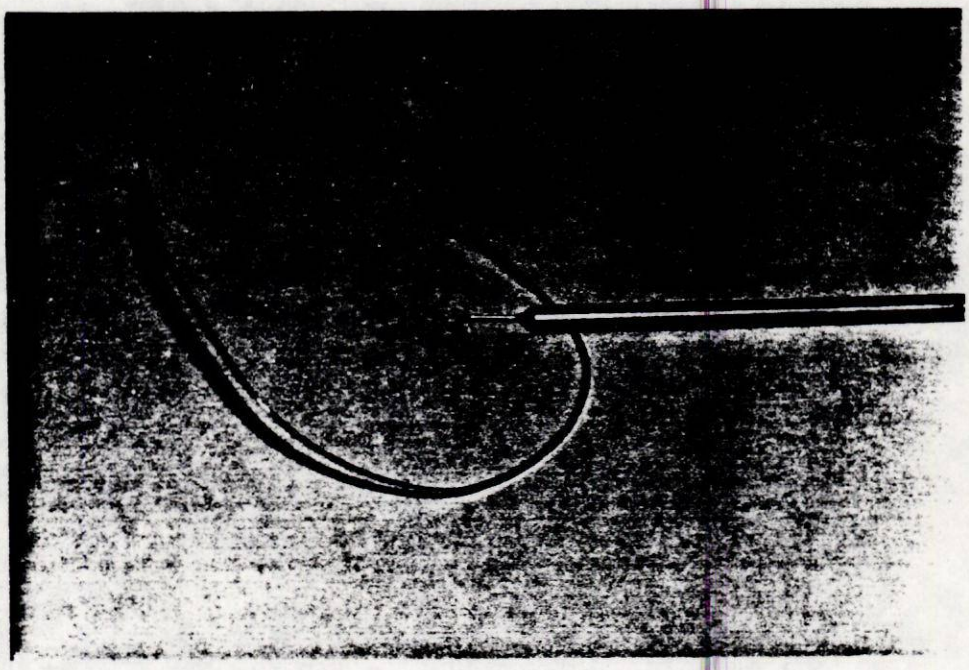


PLATE XXV

(UPPER) SURGICAL PREPARATION FOR THE TEMPERATURE MEASUREMENT AND APPLICATION OF THE ELASTIC LOOP MODULE

(LOWER) TEMPERATURE SENSING ELECTRODE INSERTED INTO THE SURGICAL PREPARATION AND COVERED WITH AN INSULATING RUBBER PUTTY





RESULTS

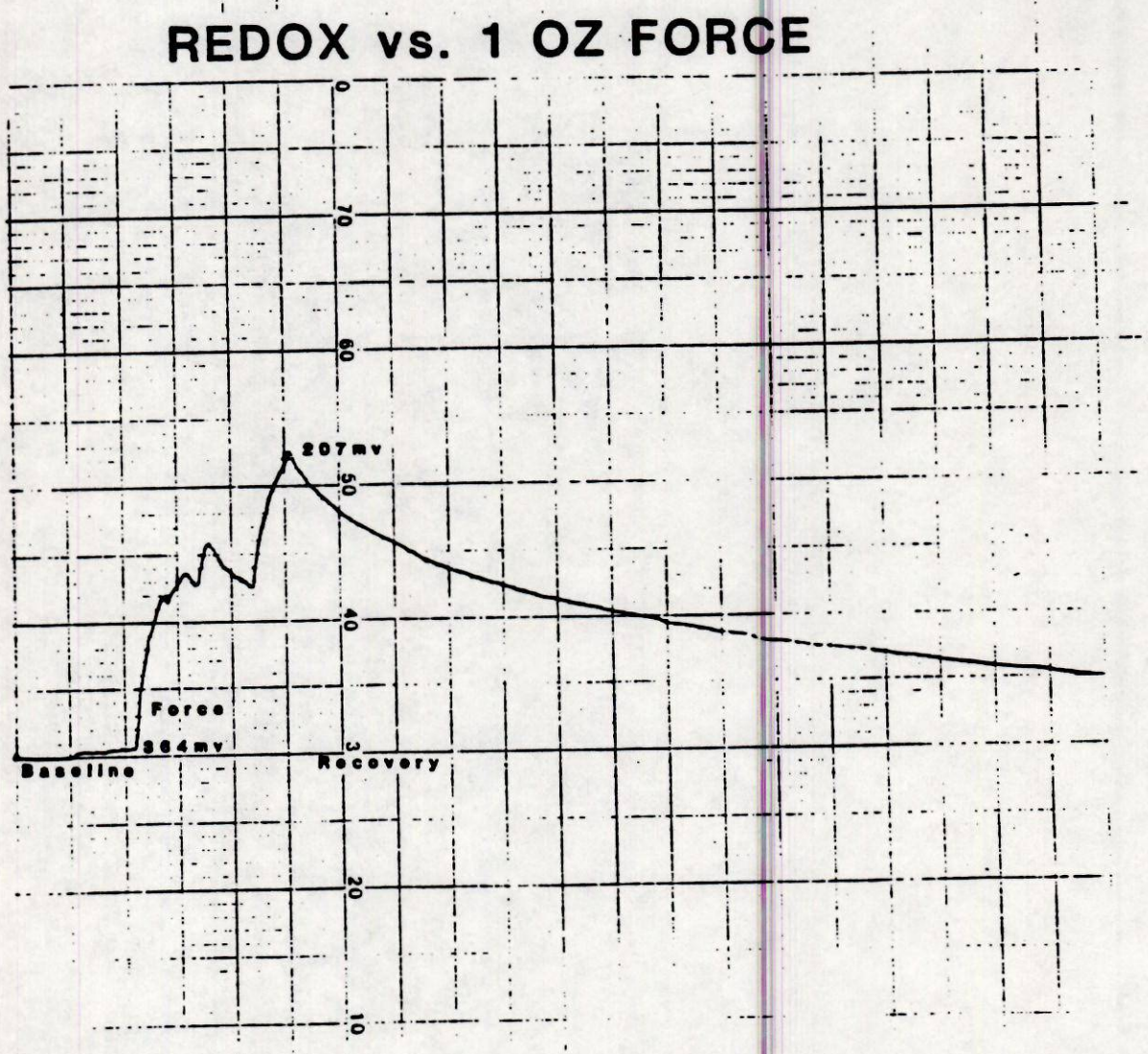
Inasmuch as this investigation studied the effects of applied force to the periodontium by monitoring four distinct variables, this section will discuss the findings for each variable separately.

Redox Results

Figures 2 - 7 depict the effect of graded forces on the redox potential in the periodontal membrane. In Figure 2, redox vs. 1 oz force, a starting pre-force baseline of +364 mv was noted. The application of a 1 oz force via the Dontrix Gauge caused an immediate change of redox potential in a negative direction, attaining a maximum value of +207 mv. This represents a change of -157 mv resulting from the applied force. The stepwise aspect of the rise to maximal negativity is not fully understood. It may represent an expression of a phasic stretching of fibers or vascular walls in the periodontium, or in part reflect an irregularity in the build up of force to the 1 oz level. This might be due in part to a slight slippage of the rod of the Dontrix Gauge.

After the force was released, the recovery towards the original baseline value proceeded at an exponential rate of

FIGURE 2


REDOX vs. 1 OZ FORCE
0
70
60
207mv
50
40
Force
364mv
Baseline
3
Recovery
20
10

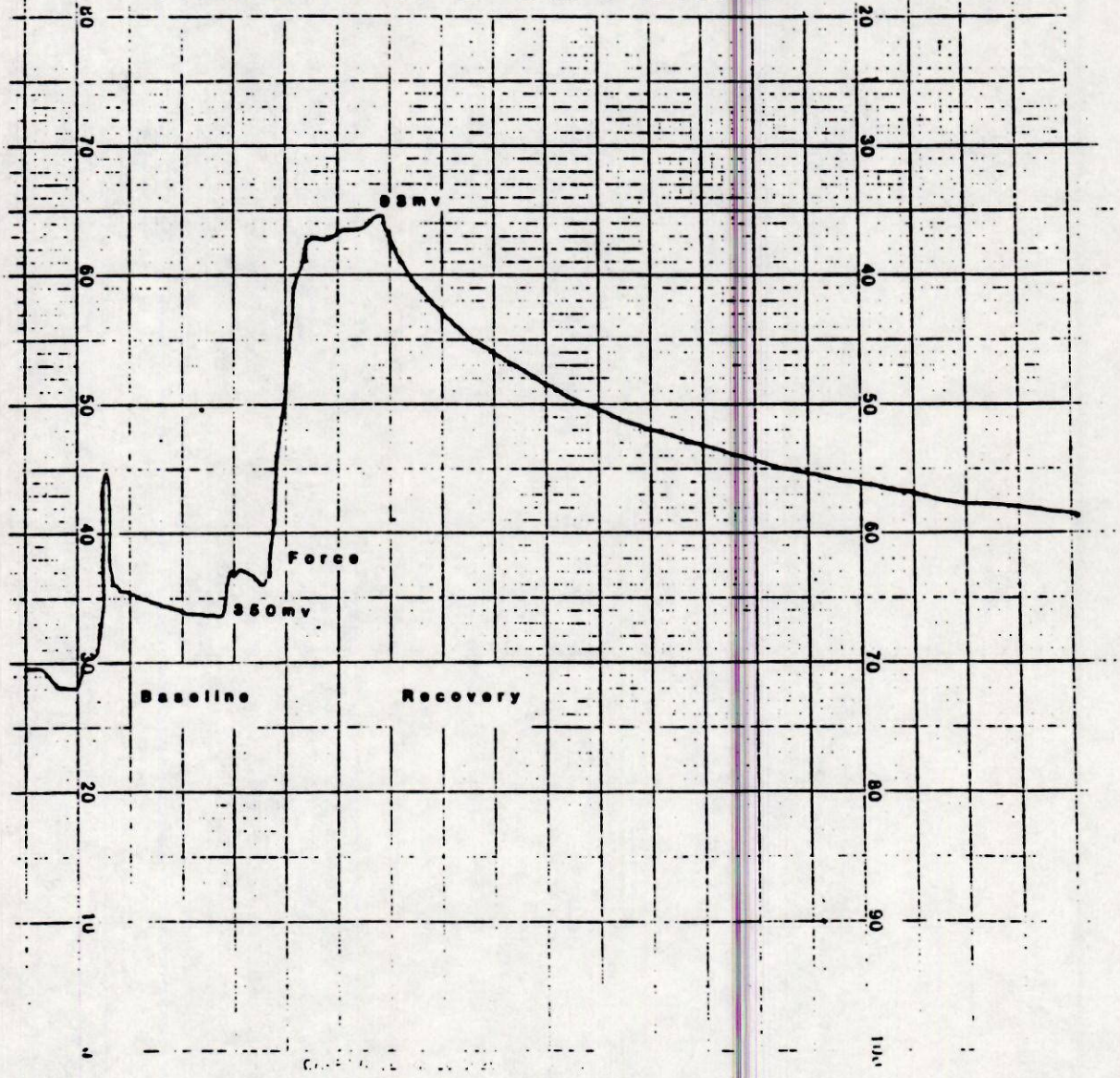
response. A value of 36 seconds for the 50% recovery rate was calculated from the curve. The 50% recovery rate represents the time after the force was removed from the periodontium to recovery of 50% of the changes induced by force application. This represents the most accurate method for evaluating a recovery rate, since the major dynamics of recovery occurred during the first half of the curve. In addition, this eliminates the problem, wherein a complete re-establishment of baseline is not attained. This 50% recovery rate is easily calculated on the curve since each centimeter division on the time scale is equal to 6 seconds. Since the rise after force reached 157 mv in a negative direction, 50% recovery calculates the time taken to reduce this potential to 78.5 mv.

In Figure 3, redox vs a 2 oz force, a starting pre-force baseline value of +350 mv was noted. The application of a 2 oz force with the Dontrix gauge caused an immediate change of redox potential in a negative direction, attaining a maximum value of +93 mv. This represents a change of -257 mv as a result of the applied force (93 mv - 350 mv). Again, the gradual exponential fashion of recovery was seen after the force was released. The 50% baseline recovery time was 36 seconds--a surprising agreement with that seen in the previous experiment.

Figure 4, redox vs a 3 oz force, shows a starting pre-force baseline value of +340 mv. The application of a 3 oz force with the Dontrix gauge caused an immediate change of redox

Figure 3

REDOX vs. 2 OZ FORCE



potential in a negative direction to a maximum value of +40 mv. The sharp drop after the attainment of this value, and the return to the level previously attained, was almost certainly due to the momentary slippage of the engaging rod of the Dontrix gauge. This rod was not fused to the enamel surface and at times did disengage during the establishment and maintenance of the force.

The maximal value caused by the 3 oz force represents a change of -300 mv. After the force was released the recovery towards the original baseline value began in the same gradual exponential fashion as seen in the previous runs. The 50% baseline recovery time was 51 seconds. This represents a 41.7% increase in the recovery rate when compared to the 1 oz and 2 oz forces.

Figure 5, redox vs a 4 oz force, shows a starting pre-force baseline value of +434 mv. The application of a 4 oz force with the Dontrix gauge caused an immediate change of redox potential in a negative direction to a maximum value of +29 mv. This represents a change of -405 mv as a result of the force applied. After the force was released the recovery towards the original baseline value began in a gradual exponential fashion. The 50% baseline recovery time was 72 sec., which represents a 41.2% increased time of recovery when compared to the 3 oz force, and a 100% increase in recovery time when compared to the 1 oz and 2 oz forces. What this tends to indicate is that

Figure 4

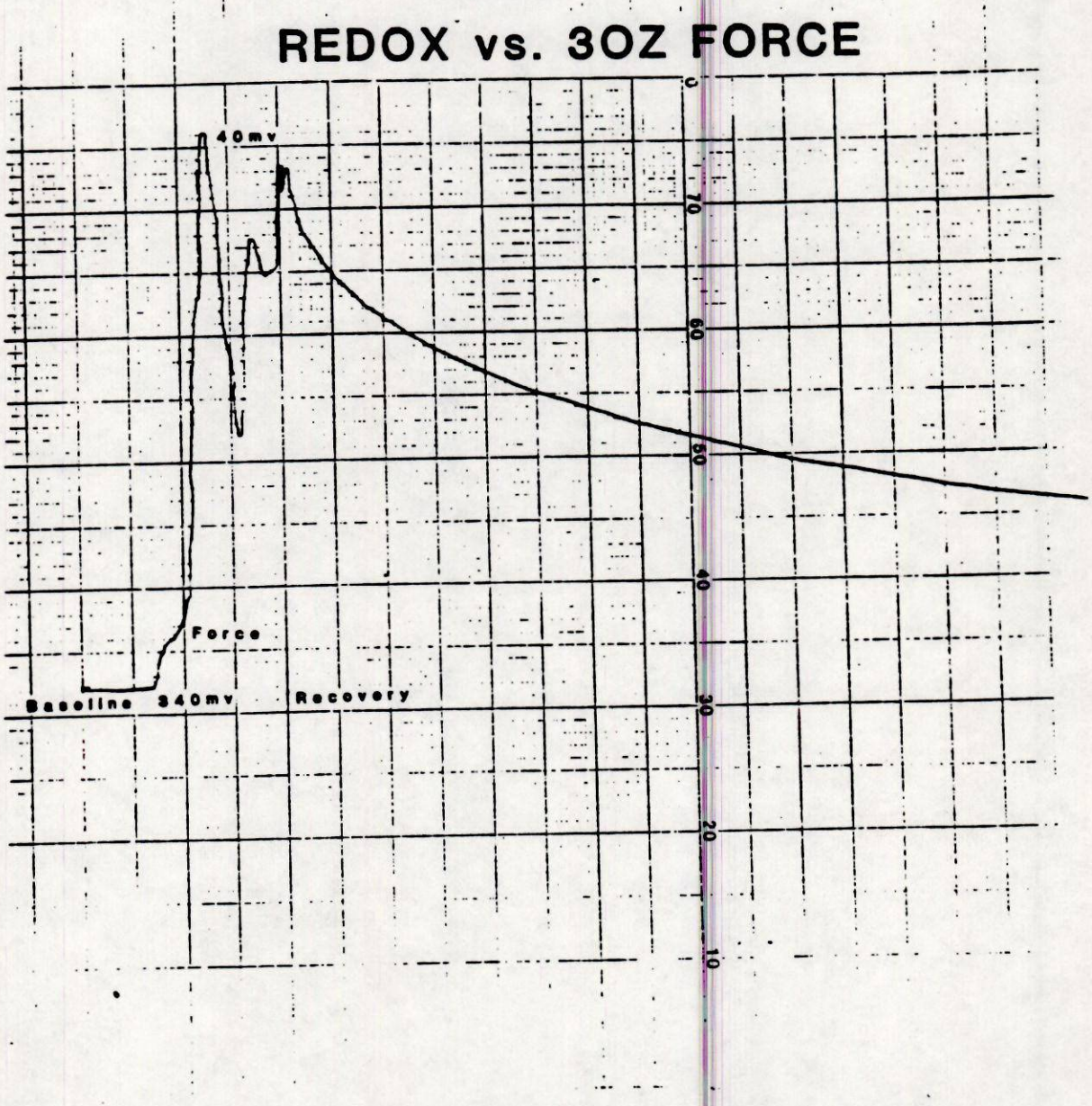
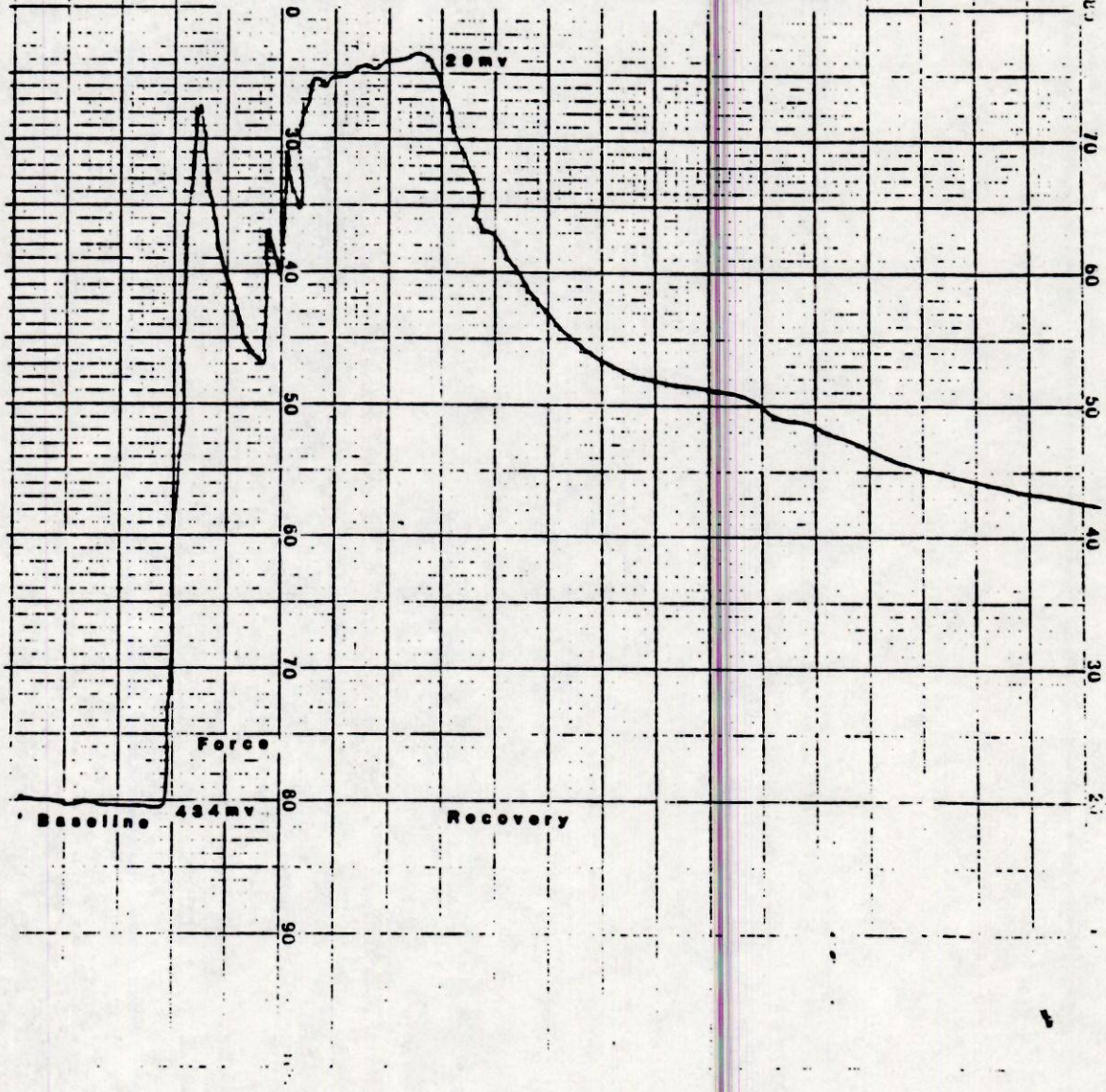

REDOX vs. 3OZ FORCE
40mv
Force
Baseline
340mv
Recovery
70
60
50
40
30
20
10

Figure 5

REDOX vs. 4 OZ FORCE



a 1 oz or 2 oz force in the incisor area of a maxillary hamster incisor causes a physical displacement of tissue, or a change in vascular perfusion, or tissue chemistry that takes 36 seconds to reverse after the force removal. The 3 oz force showed greater reaction and perhaps the beginning of over-loading. The 4 oz force may be pushing the extreme of tissue tolerance in this region. In order to test this, a second trial with 4 ounces was undertaken, and the results are summarized in Figure 6 and Fig. 6 -Cont,redox vs a 4 oz force (second trial). These curves depict a second attempt to apply a 4 oz force to the same tooth. It can be seen from an observation of the data that there was an altered response in this second application of force. The change in the negative direction of the redox potential was not as great as that exhibited during the first 4 oz application. More importantly, a drastic change in the recovery aspects of the curve was immediately noted. Instead of the usual gradual exponential recovery rate, a prolonged recovery time approximately three-fold greater was seen. Also, the recovery was marked by a series of step-like progressions towards the baseline. What is extremely interesting is that the recovery time took approximately 220 seconds. This value agrees closely with the loading experiment by Saul Bien et al previously cited.[5] They found that it took approximately 240 sec. for a maxillary incisor tooth of a freshly killed rodent

Figure 6

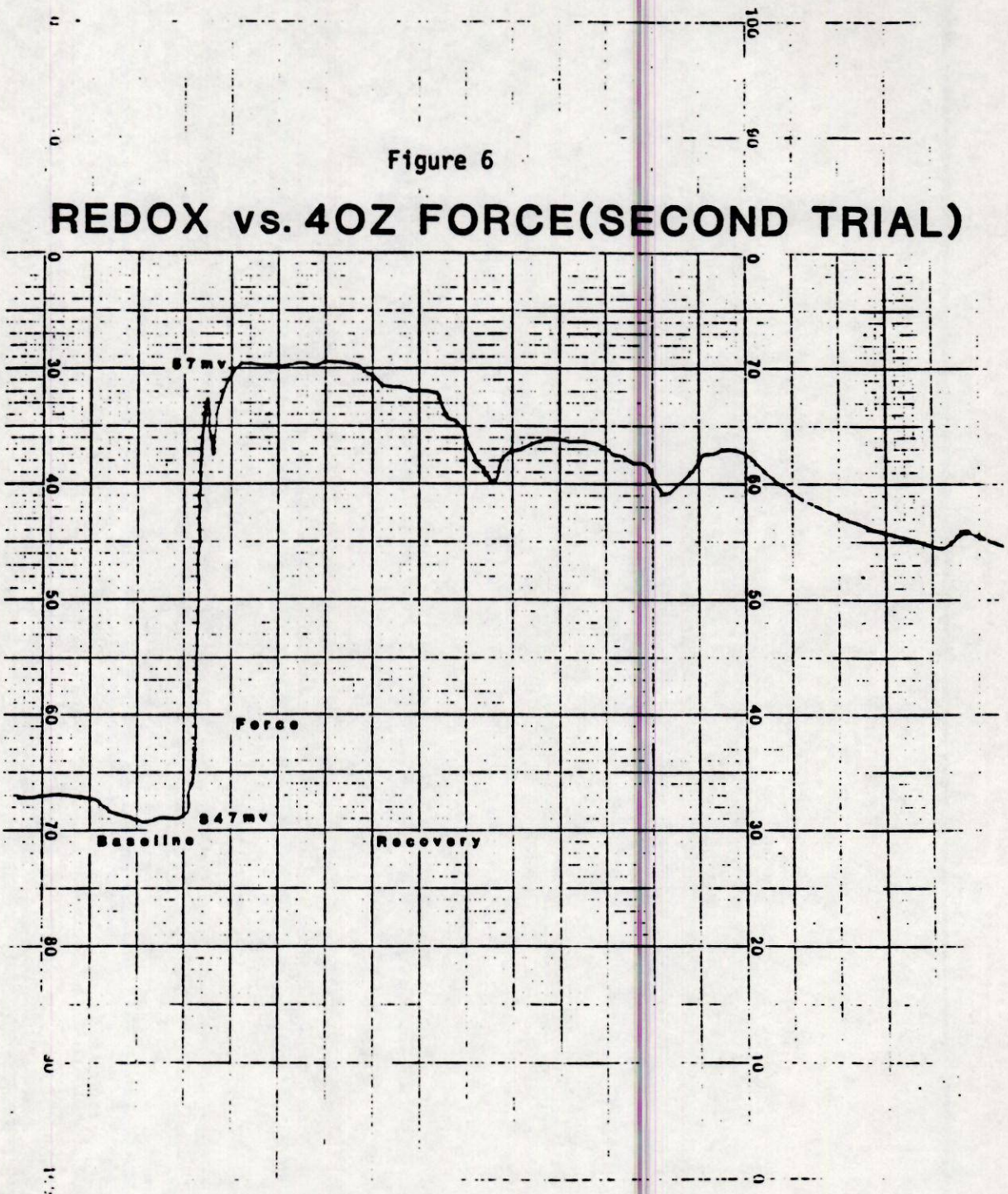
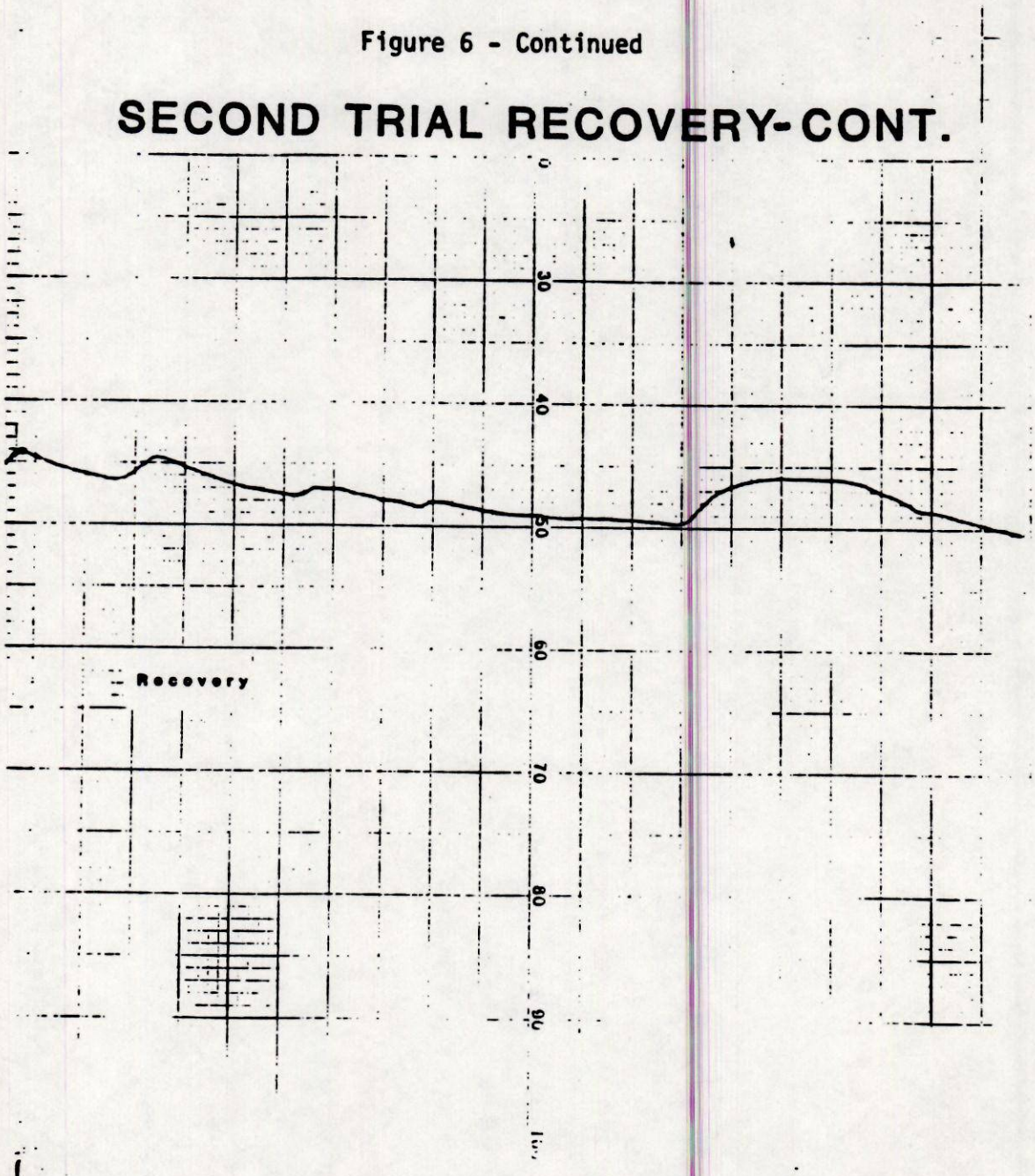

REDOX vs. 4OZ FORCE(SECOND TRIAL)
87mv
Force
847mv
Baseline
Recovery

Figure 6 - Continued



to return to a level near the zero level when loaded incisally with weights. In the deceased animals, some of the intruded teeth never reached the zero level.

This strongly suggests that the second four oz loading injured the periodontal membrane severely, as seen by a recovery rate similar to that reported by Bien et al. In light of this, only the value for 4 oz in the first trial was utilized in the plot of redox change vs. force, since the second 4 oz trial must have taken place in a disrupted and severely damaged periodontium.

Figure 7 shows the relationship between the applied force and the increase in negativity of the periodontium, the result of which is a strong linear relationship, with a correlation coefficient of 0.9. The change of negativity per ounce is approximately 122 mv.

Figure 8 shows the relationship between the applied force and the 50% recovery time of the periodontium baseline potential. One can note the increased time of recovery rate for a 3 oz force, and even greater increased time for a 4 oz force. Finally when the periodontium was overloaded, the three-fold increase in recovery time ensued.

Following the redox vs. force study, an experiment was undertaken to determine the sensitivity of our redox sensing electrode to changes in hydrogen ion concentration.

Figure 7

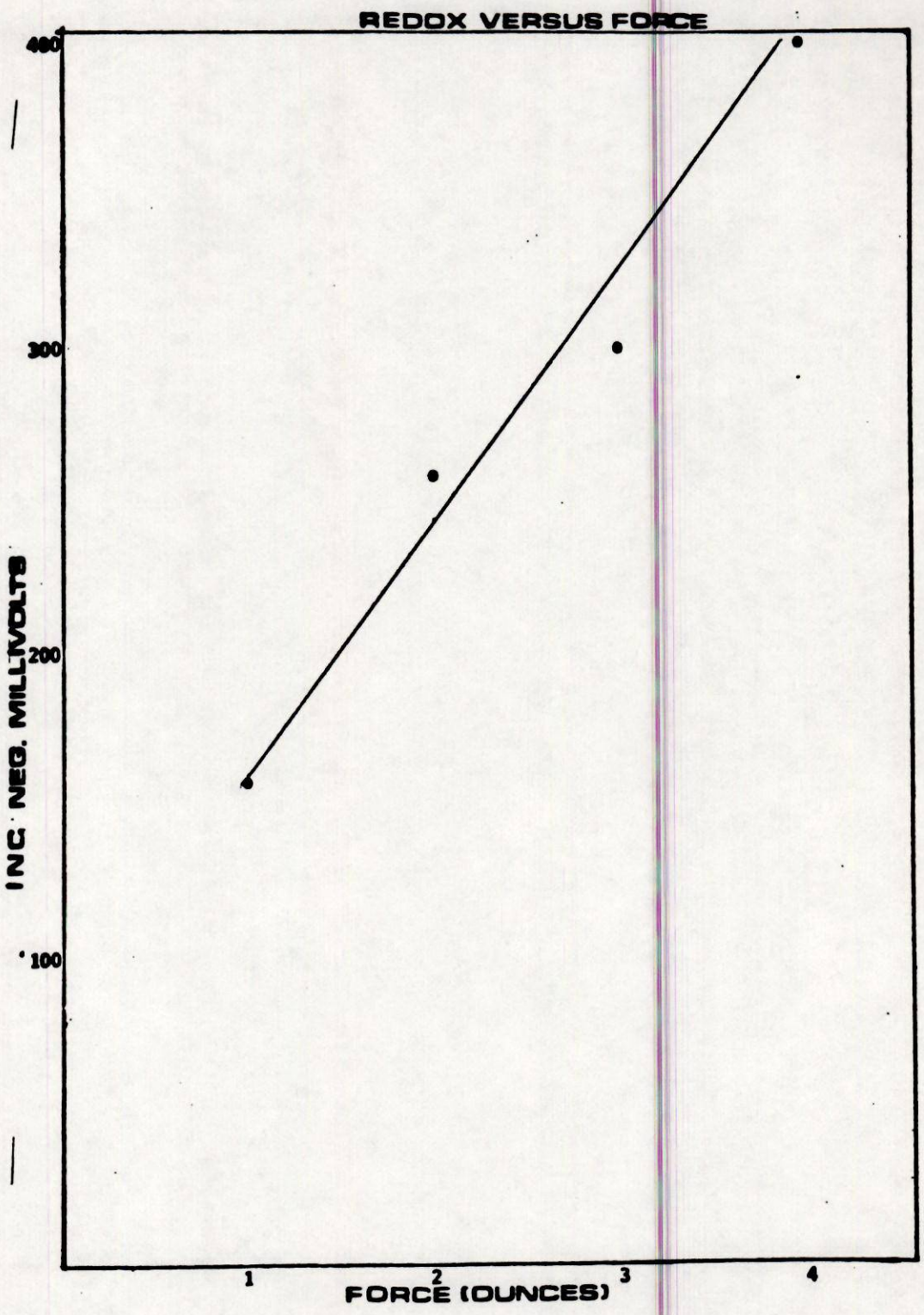
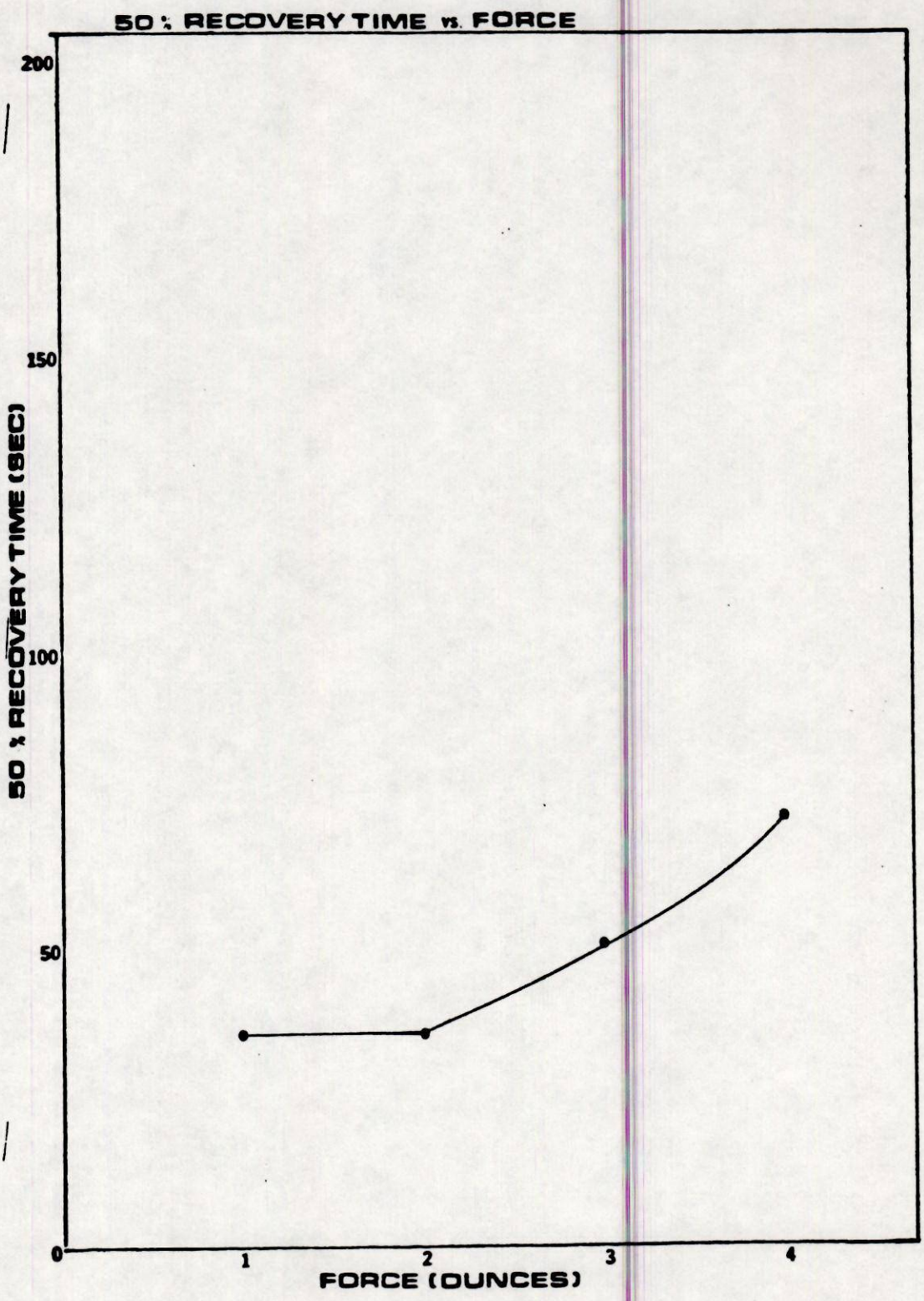

REDOX VERSUS FORCE
400
300
200
100
INC. NEG. MILLIVOLTS
1
2
3
4
FORCE (OUNCES)

Figure 8


50 % RECOVERY TIME vs. FORCE
200
150
100
50
0
50 % RECOVERY TIME (SEC)
1
2
3
4
FORCE (OUNCES)

This was done to answer the question as to whether changes in hydrogen ion concentration contributed to the potentials. In order to test this, a series of standard buffers of known pH values were arranged in a systematic order as seen in Plate XVIII. A glass electrode with an internal reference was then used to confirm the pH value of these buffers. The same endodontic file used in the redox experiment was placed in the series of buffers and the redox values then recorded. The values of these recordings are seen in Table II.

The silver chloride reference electrode in the combination glass electrode was also used as the companion reference electrode for the endodontic file. Figure 9 shows the plot of pH vs. redox potentials generated by the endodontic file. It is evident that our redox sensor is not a good pH electrode in the range of pH changes seen in biological systems. Actually, it was not expected to show high sensitivity to pH changes.

What the experiment did indicate is that the redox changes as seen by the probing electrode were most probably due to biochemical factors other than hydrogen ion changes, or in addition to them. Also, to measure pH changes during force application, an electrode specific for hydrogen ion analysis would have to be utilized.

Results of Oxygen Series Experiments

Figure 10 shows a polarographic recording generated in a 0.9% sodium chloride (isotonic) solution. The purpose of this

TABLE II

pH CALIBRATION OF REDOX ELECTRODE

(in millivolts)

TYPE OF ELECTRODE	pH of BUFFER				
	pH 4	pH 5	pH 6	pH 7	pH 8
Glass Electrode	165.5	111.5	46.4	-7.9	-64.6
Stainless Steel (Redox Sensing) Electrode	88.6	47.5	56.5	15.5	-110.9

Figure 9
pH vs. Millivolts

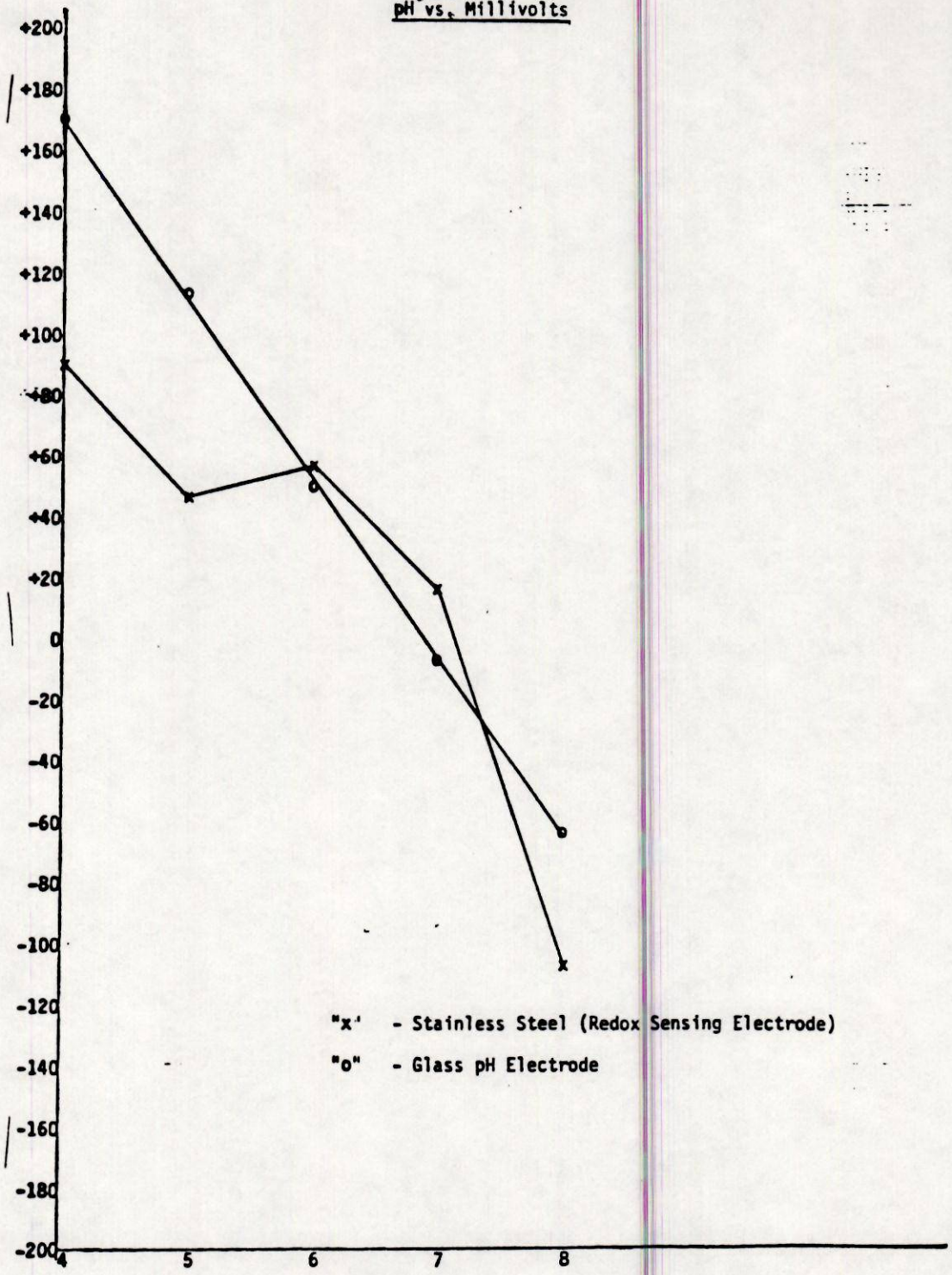
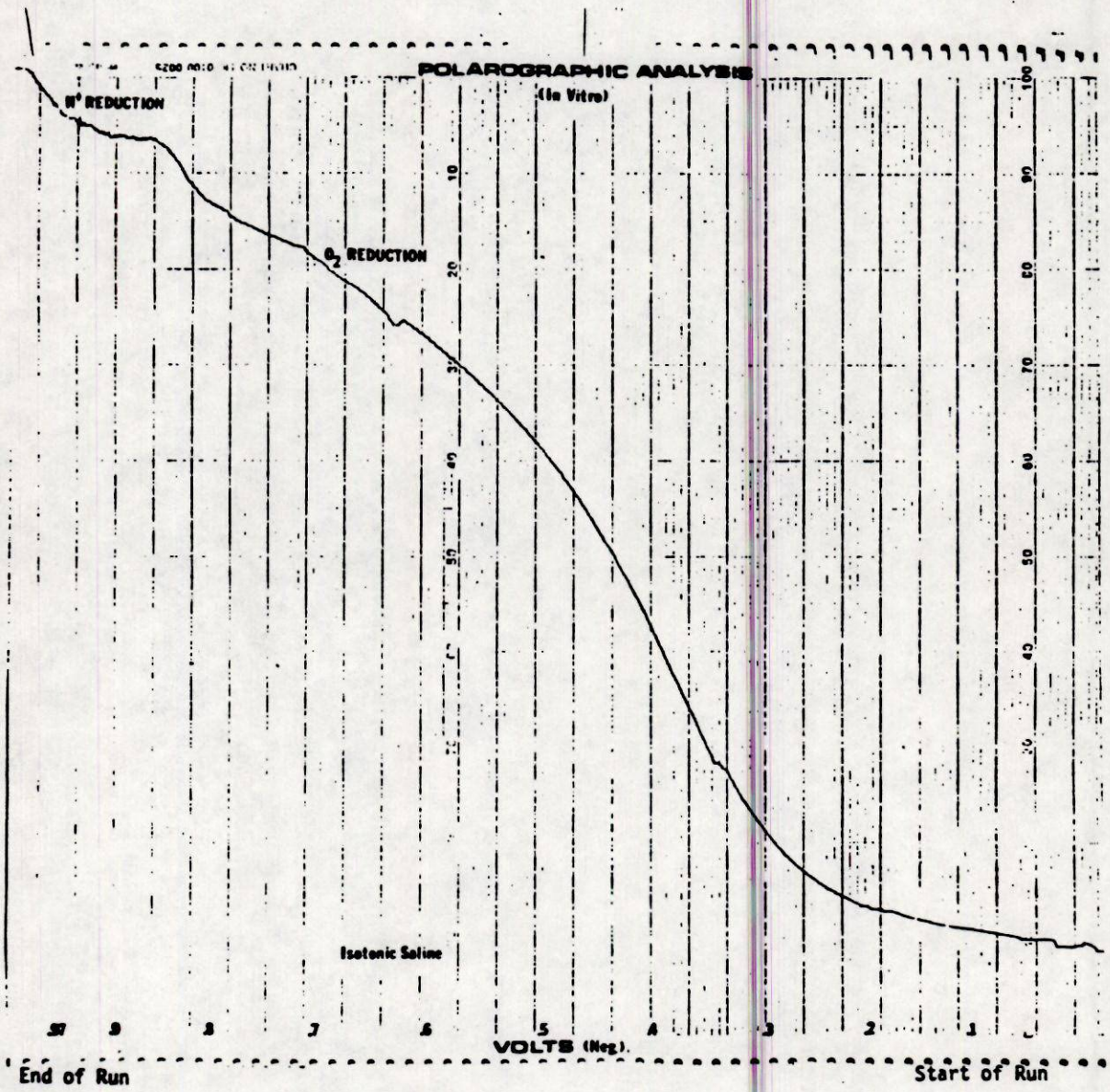


FIGURE 10


POLAROGRAPHIC ANALYSIS
(In Vitro)
H⁺ REDUCTION
O2 REDUCTION
Isotonic Saline
VOLTS (Neg).
End of Run
Start of Run

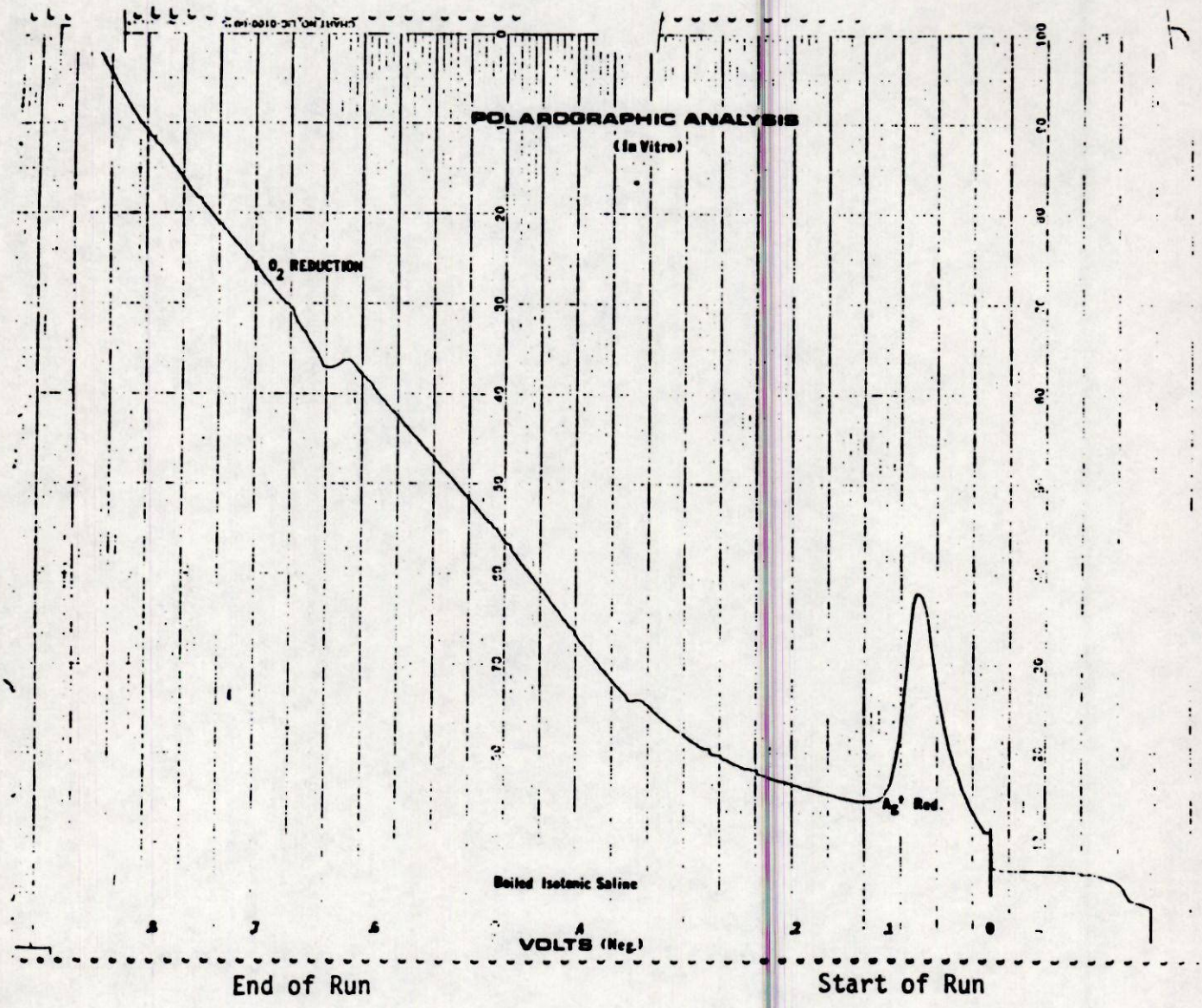
run was to attempt to duplicate the classical curve as described by Davies[18] and others, in a similar electrolyte. This would serve to establish the validity and reliability of the silver coated endodontic file electrode used in our animal experiments to penetrate the periodontium. The silver electrode and a fine silver chloride electrode (constructed from a #10 silver point, and plated with AgCl) were mounted in the barrel of a syringe, as illustrated in Plate XIII. This permitted aspirating the isotonic saline. Another advantage of this system is that it permitted a polarographic analysis in a minute amount of electrolyte and in a confined region, which tended to mimic that of the periodontal membrane.

Note the slight Ohm's Law-governed type of early rise and then the classical oxygen reduction wave. The two-step reduction of oxygen often mentioned in the general literature is well defined. Hydrogen ion reduction begins a -.94 volts.

These experimental results confirm the reliability of our oxygen sensing system used in the animal experiments, since all waves of reduction described in classical polarography literature are seen, and the potentials at which reduction begins are duplicated.

In this experiment, as summarized in Figure 11, the isotonic saline was boiled in order to drive off some of the oxygen. Then it was aspirated into the syringe and the

FIGURE 11


POLAROGRAPHIC ANALYSIS
(In Vitro)
O_2 REDUCTION
A_g^+ Red.
Boiled Isotonic Saline
VOLTS (Neg.)
End of Run
Start of Run

polarographic run repeated. The purpose was to ascertain if the oxygen value at -0.7 would drop, indicating a slight loss of oxygen due to the boiling. No large drop was anticipated since atmospheric oxygen equilibrates with the boiled solution very rapidly, and our transfer system may not have been totally air-tight. However, any lower value would be significant, since cleaning the electrodes between the experiments and the increased temperature of the second experiment are two factors that favor increased current flow, and therefore increased elevation of the oxygen curve.

The interesting point is the immediate appearance at the beginning of voltage application (below 0.1 volt) of a new wave. Examination of the electrodes with a dissecting microscope--after the run had been completed--showed a partial disintegration of the silver chloride coating of the reference electrode. The nature of this new wave is apparent. In the extremely hot solution, the silver-chloride coating of the reference electrode (a relatively insoluble compound at room or body temperature) began to dissolve into free silver and chloride ions. The silver ion has a great tendency to accept electrons and be reduced at a very low negative potential. Thus what was seen was the silver reduction wave. This observation, while not anticipated in the experimental design, did serve to prove the sensitivity and reliability of the analytic system. It also added confidence for the use of this approach in <u>in vivo</u> studies.

The -0.7 volt current value did in fact drop approximately 7 units, again adding confirmation to the validity of this analytic set-up.

However, due to the breakdown of the Ag/AgCl reference electrode in this experiment, the experiment was repeated utilizing a room temperature isotonic saline, compared against a boiled isotonic saline cooled to a temperature approaching room temperature. As was mentioned in Methods and Materials, a second syringewas used to aspirate the boiled isotonic saline. After a period of cooling, the solution was transferred, by a reasonably air-tight method, to the electrode-containing syringe. This solved the silver chloride solubility problem, but probably introduced some oxygen since the transfer may not have been completely air tight.

Figure 12 shows the first run in room temperature isotonic saline. Figure 13 shows the second run after the cooling of the boiled isotonic saline solution. Again, as in the previous runs, the -0.7 value, which represents the selected voltage point to evaluate the oxygen level, is 8 units lower. This indicates an oxygen loss due to boiling, and agrees with the results seen in the previous run.

Oxygen Studies in the Hamster Periodontium (in vivo series)

The results of these studies are best presented by an analysis of the polarographic charts. Figure 14 is a recording

FIGURE 12

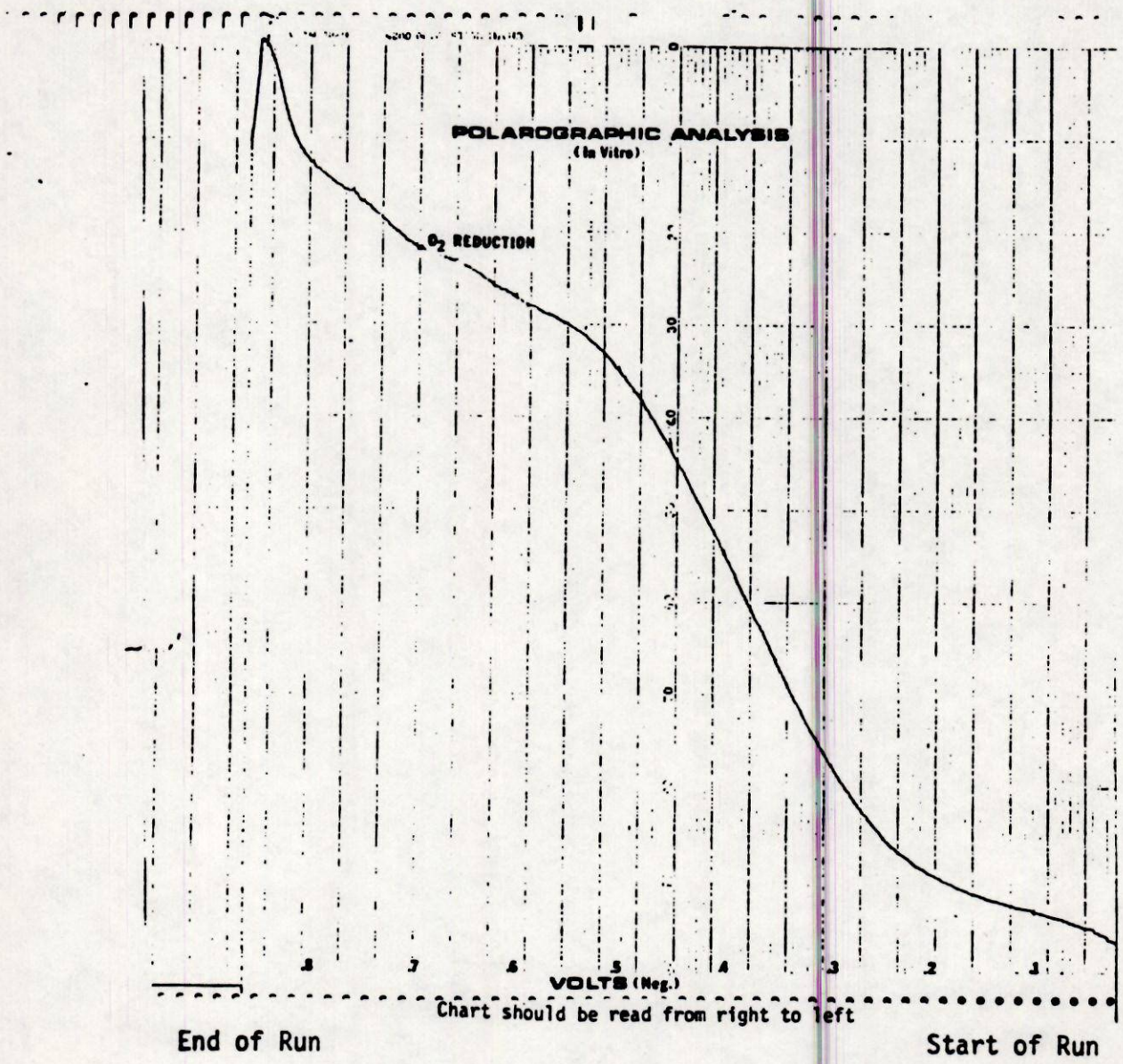
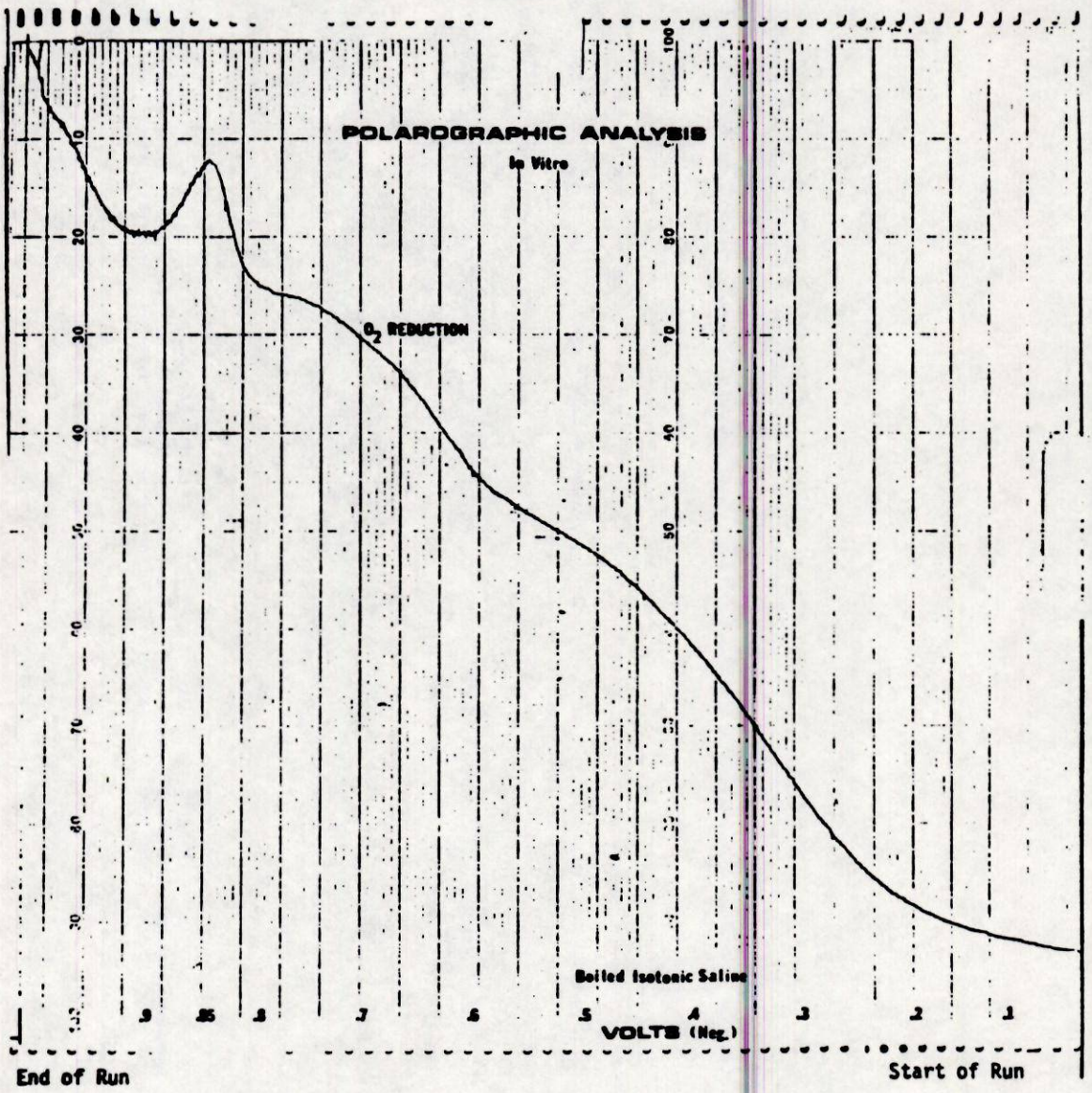

POLAROGRAPHIC ANALYSIS
(In Vitro)
O_2 REDUCTION
.8
.7
.6
.5
.4
.3
.2
.1
VOLTS (Neg.)
Chart should be read from right to left
End of Run
Start of Run

FIGURE 13


POLAROGRAPHIC ANALYSIS
In Vitro
O_2 REDUCTION
Boiled Isotonic Saline
.9
.85
.8
.7
.6
.5
.4
.3
.2
.1
VOLTS (Neg.)
End of Run
Start of Run

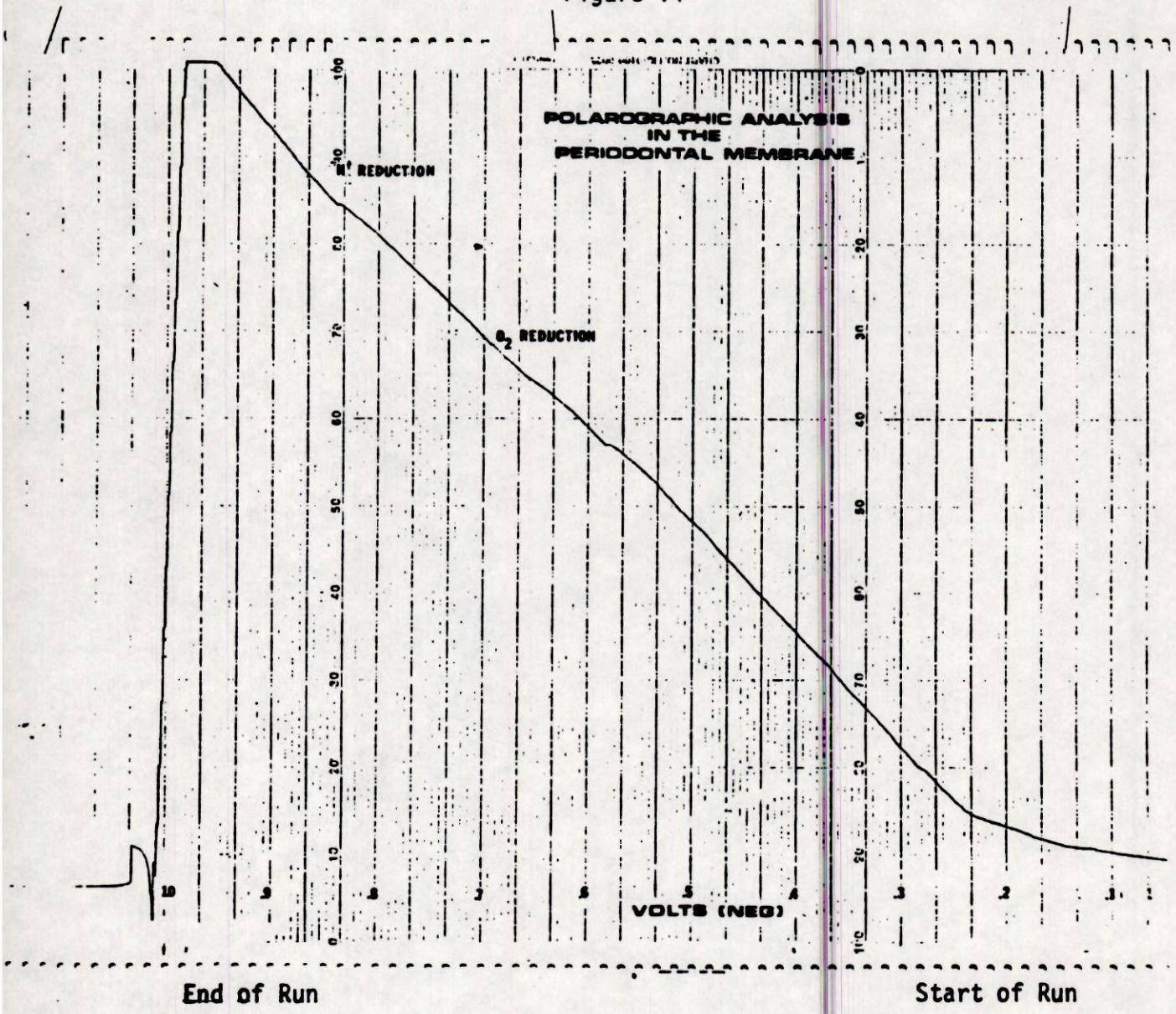
of a polarographic analysis conducted in the periodontal membrane of the maxillary central incisor of a hamster. This experiment was necessary in order to ascertain if this analytical technique could be utilized in a periodontal membrane to measure oxygen tension changes during force application. While such measurements had been conducted in other regions of the body, to this investigator's knowledge, none has ever been attempted in so small an area utilizing this particular electrode design. In Figure 14 the applied negative voltage is indicated on the abscissa. The run starts at zero applied voltage and proceeds by the gradual application of increasing negative potential on the electrode positioned in the periodontal membrane in an area similar to that seen in Plates VI - IX.

The increased current (which is depicted by a rise of the curve from 0 to -.25 volts) is expected in consideration of Ohm's Law. What this means is that as increased voltage is applied to a circuit, and the resistance is kept constant, an increase in current flow ensues. This is expressed in the classical formula:

$$\left(I = \frac{V}{R} \right)$$

Note the gradual slope and rise of current due to this relationship. In the region slightly more negative than -0.2 volts (approximately -0.225 volts), a sharp rise occurs, showing a

Figure 14


POLAROGRAPHIC ANALYSIS
IN THE
PERIODONTAL MEMBRANE
H^+ REDUCTION
O_2 REDUCTION
VOLTS (NEG)
End of Run
Start of Run

slope far beyond that expected at this voltage from Ohm's Law. This is due to the initiation of the reduction of oxygen, which is felt to proceed in two stages. In this stage dissolved oxygen was reduced to H_2O_2 at the potential approaching -0.6 volts. The slight break in the curve probably indicates the initiation of the second phase in the reduction of oxygen. If the chart speed were increased, these small breaks would be seen as well defined steps characteristic of polarographic current-voltage curves. In fact, this run was repeated at a faster chart speed, and this was demonstrated with great precision. Note that as the analysis approaches -0.9 volts, another break in the curve occurs, and a sharper rise in current had taken place due to the reduction of the hydrogen ion, with the liberation of hydrogen gas. This value for the reduction of hydrogen ion is in virtual agreement with the findings of Brighton and Friedenberg.[13] The exact voltage for reduction may vary slightly as a function of the pH of the interface surrounding the electrode. This experiment proved important to the present study for the following reasons:

1. It proved that the system and electrode used could reproduce a classical polarographic curve in periodontal tissue.

2. It permitted the determination of the ideal negative potential to charge the electrodes, in subsequent experiments, in order to record the oxygen tension response to force application.

An inspection of the curve generated reveals an ideal charging point for oxygen reduction at -0.7 volts. This approximates the center of the more stable phase in oxygen reduction and is safely removed from the reduction of the hydrogen ion. This voltage value agrees perfectly with that seen in the general literature. With the Clark Oxygen Electrode (used to measure dissolved oxygen) the reducing potential is set at approximately that value.

Results of Oxygen vs. Force Experiments in the Hamster Periodontium

The next series of figures are recordings of dissolved oxygen in the periodontal membrane of the maxillary central incisor in a male hamster. The histological appearance of the region in which the recording was made is shown in Plates IV and V.

The electrode used for sensing oxygen concentration was a #10 endodontic file coated with pure silver. The anode electrode is of silver/silver chloride construction. The approximate position relative to the periodontium of the silver electrode during the experiment are shown in Plates XIII and IX. In essence, the analytic circuit and instrumentation utilized for this analysis is identical to that in the previous experiments in this section. The one modification is that the voltage is not scanned in this series of recordings; instead it is set at -0.7 volts and applied to the silver coated electrode positioned in the periodontal membrane. This voltage was shown in the previous experiments to be ideal for an analysis of oxygen levels. It is also the value

usually utilized in commercial oxygen-sensing Clark electrodes. The abscissa on the scale represents time with a chart speed set at 20 cm./min. The speed was intentionally made at a fast rate to get information quickly and thus avoid the build-up of excessive polarization products on the electrode surface; also to minimize the time of force application so that many runs or repeat cycles could be conducted without system overload.

A detailed analysis of Figure 15 is given below. The significant portion of the run depicted in this figure 15 starts at point near 60 on the ascending scale. This represents a current flow (expressed on this scale in terms of -12.7 mv) due mainly to the reduction of dissolved oxygen in the vicinity of the electrode and also in part to the superimposed 0.7 volt potential (Ohm's Law flow). The slow decline in the level of the curve is caused by slight lowering of oxygen concentration in the vicinity of the electrode, and also the build-up of products of polarization as the voltage is maintained. Note the gradual slope of this pre-force drift. At the next point, a force is applied to the teeth by utilizing an electrically insulated hemostat (constructed by coating the metal with a high electrical resistance varnish). The hemostat applied a squeezing force between the two maxillary incisors. The oxygen sensing electrode was situated in the periodontal membrane between the two incisors. As force is applied, the curve shows an immediate drop, which continues as force was slowly increased. This drop reached a

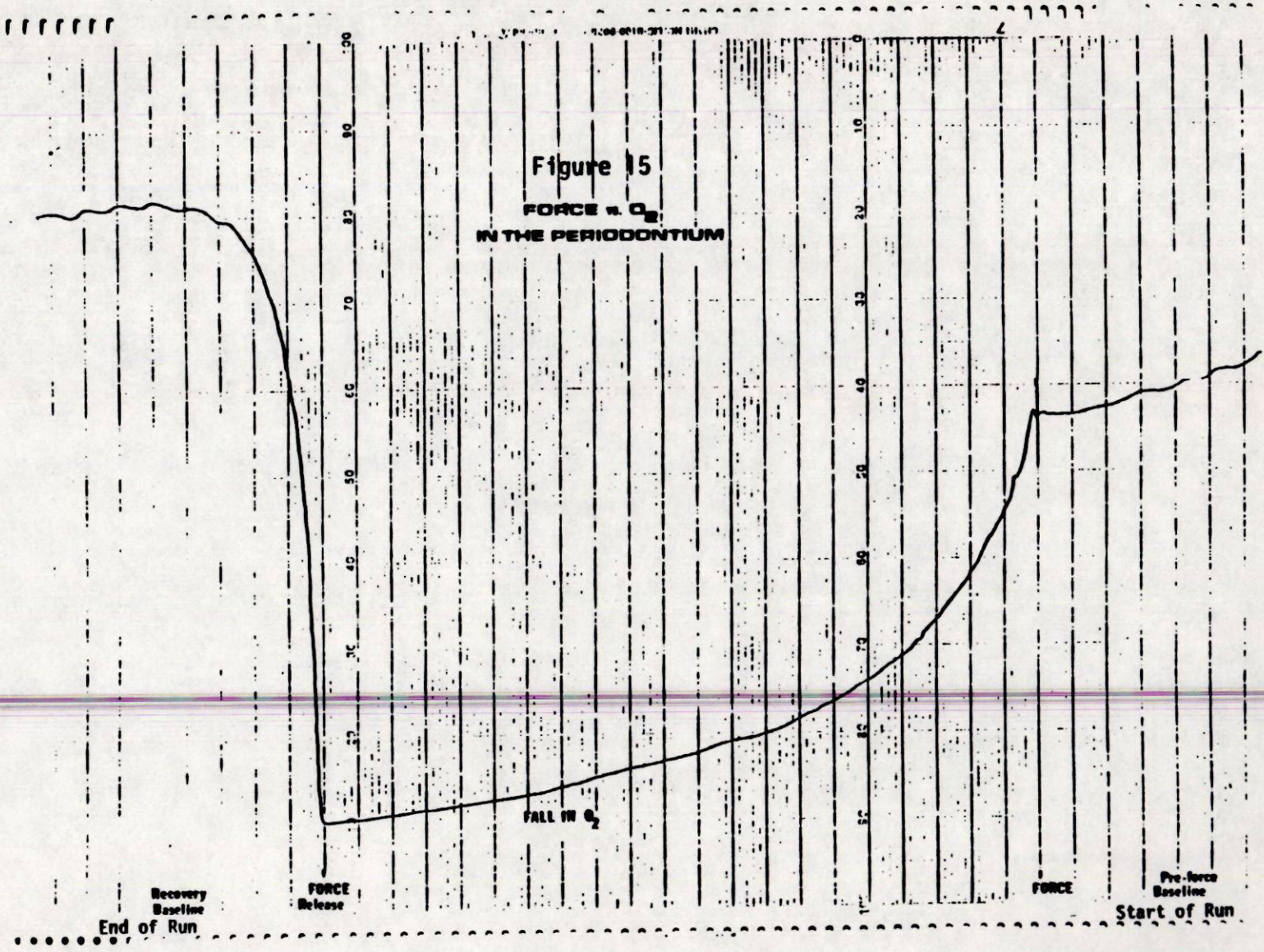

FORCE vs. O_2
IN THE PERIODONTIUM
FALL IN O_2
Recovery Baseline
End of Run
FORCE Release
FORCE
Pre-force Baseline
Start of Run

Figure 15

level of 10 on the ascending numbered scale, representing a value of -5.7 millivolts which expresses the amount of current flow in the circuit converted to millivolts by the measuring method. This represents a 55% drop in current output caused by the force application. The current reduction was mainly due to the reduced oxygen concentration in the vicinity of the sensing electrode, most probably caused by the vascular compression. Note the dramatic recovery as a result of the release of the pressure. The overall tendency was to establish a slightly lowered baseline with repeated runs.

This lowered baseline was due to the consumption of oxygen in the electrode vicinity and the build-up of polarization products during the run. This sequence of pressure application and release was repeated in two more runs, represented by Figures 16 and 17. The results were very similar.

Attempts were then made to apply a graduated force by means of the Dontrix Gauge perpendicular to the distal surface of one of the maxillary central incisors, while the other was held stationary with a rigid object. It was difficult to avoid slippage, and therefore the force values must be considered rough approximates. The results are seen in Figure 18. An inspection of this curve indicates a decrease in oxygen concentration as a result of a progressive increase in force application. The 4 oz force caused an oxygen drop equivalent to a

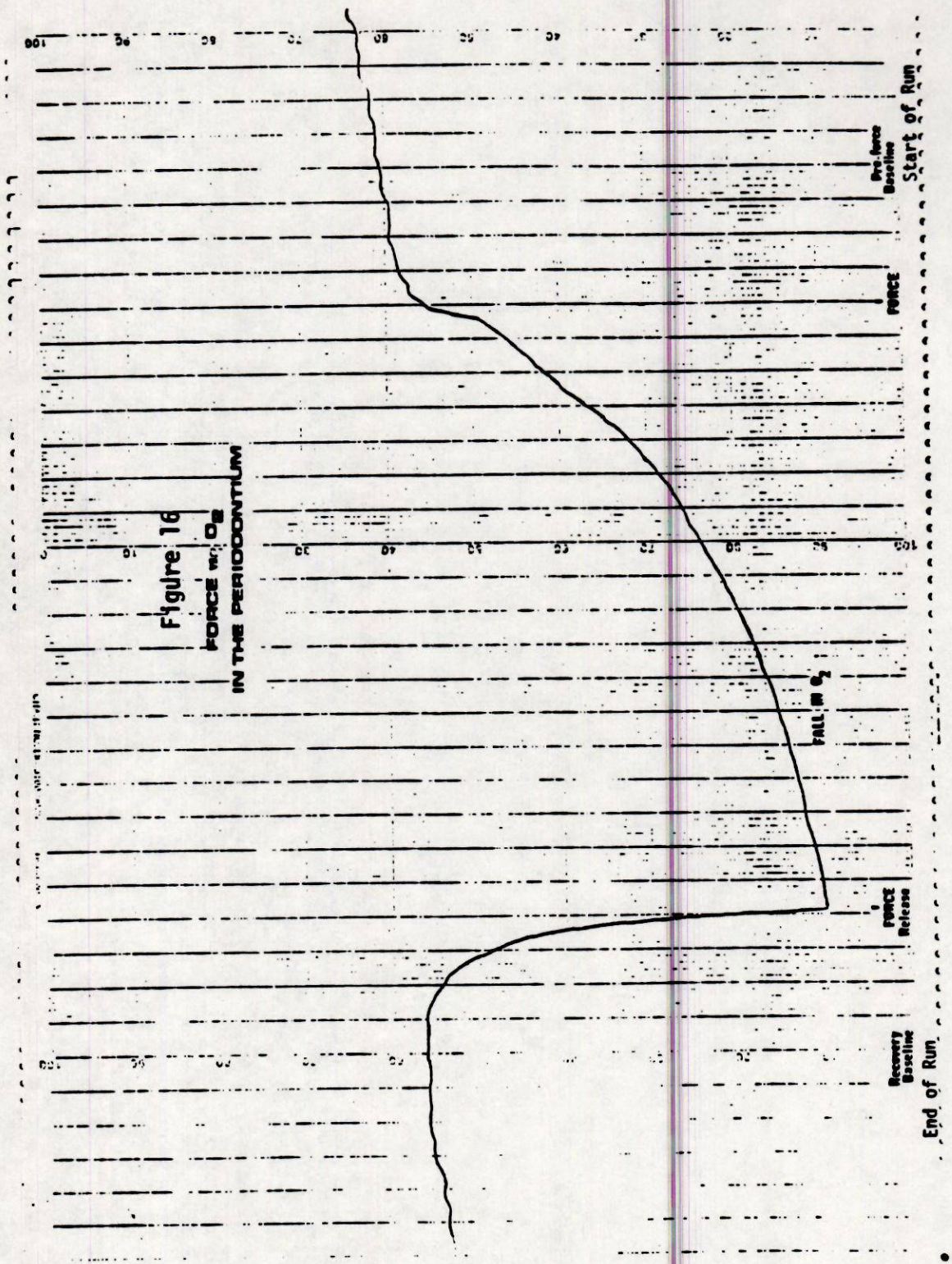


Figure 16
FORCE with O_2
IN THE PERIODONTIUM

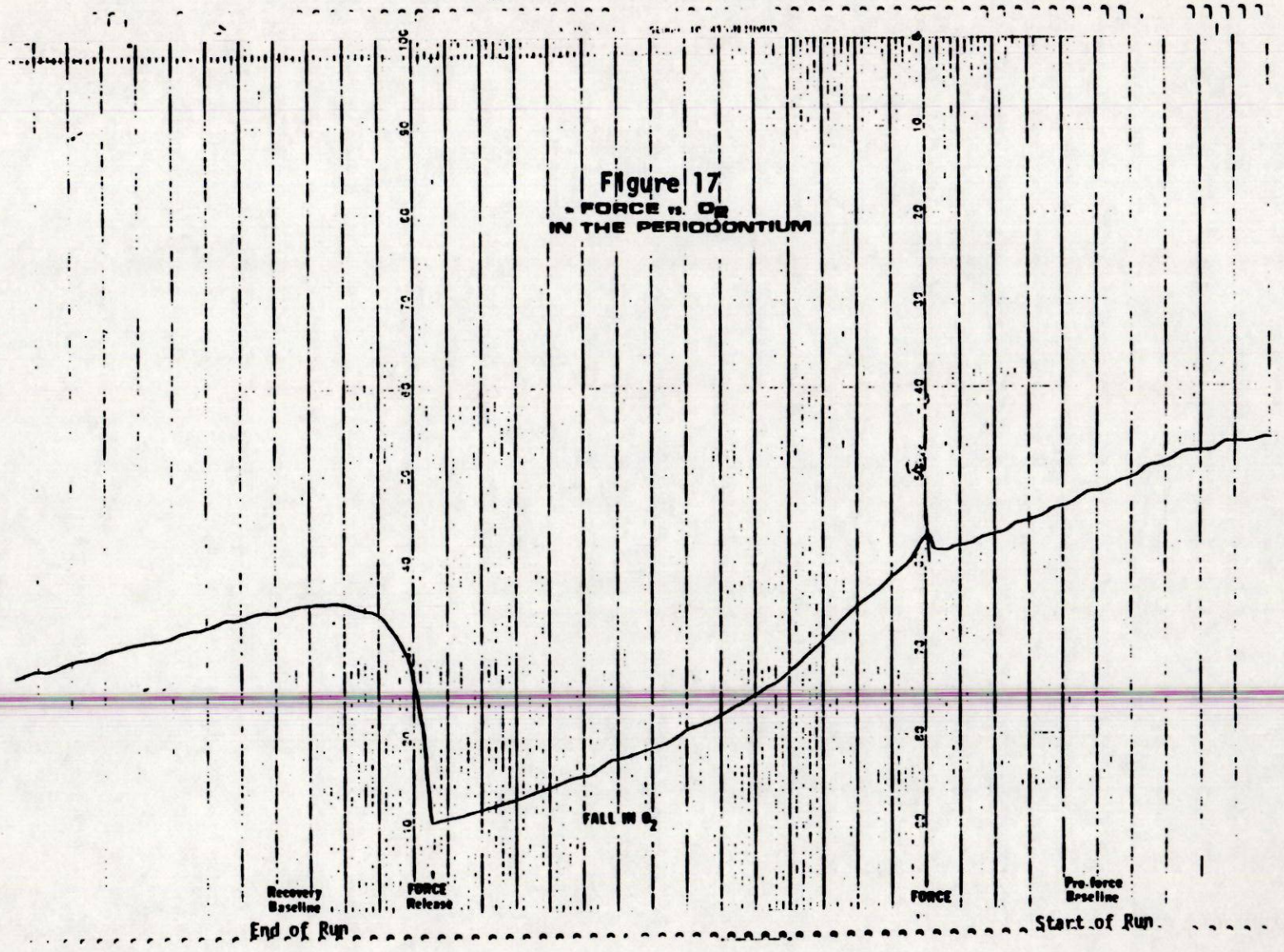
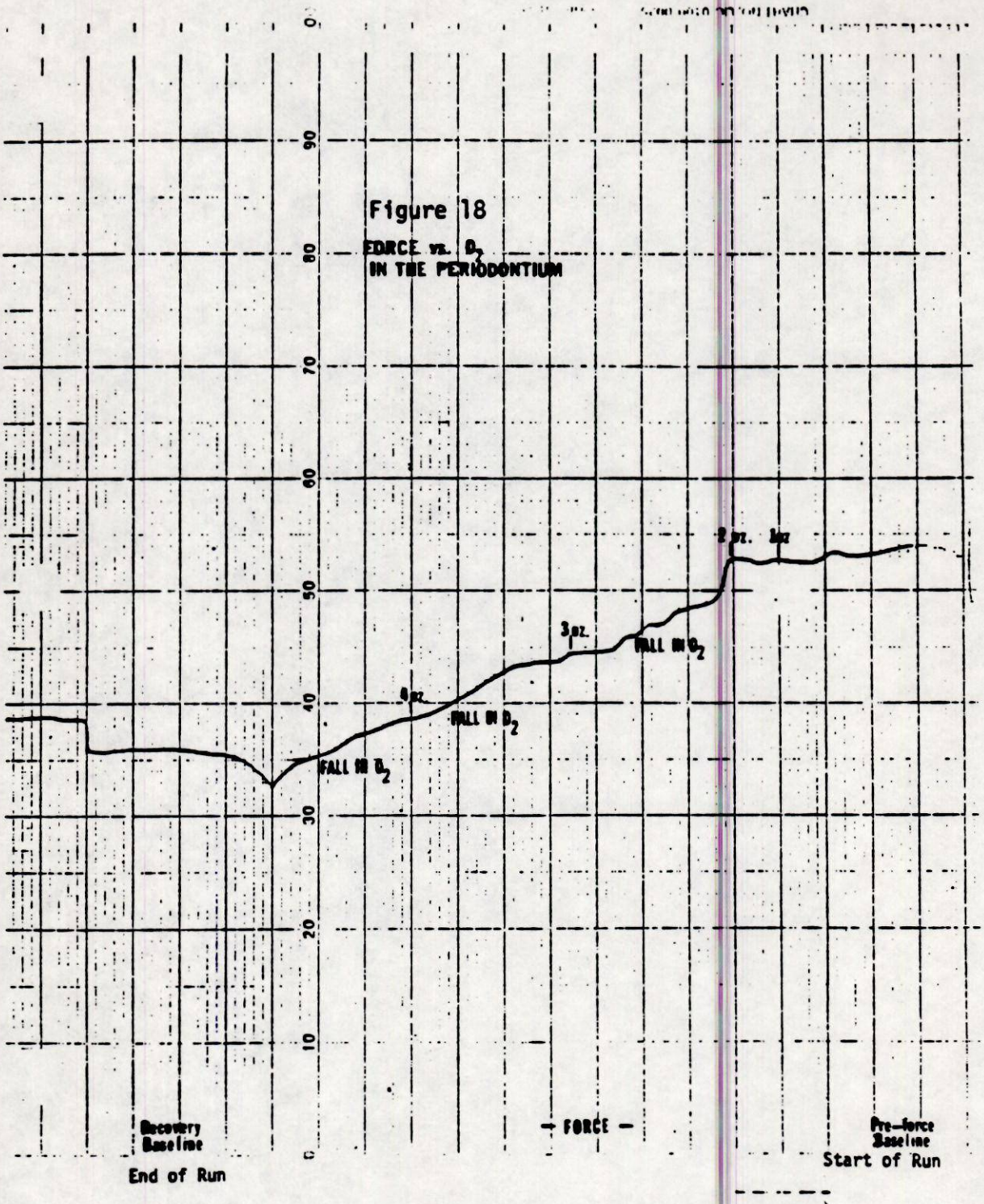


Figure 17
- FORCE vs. O_2
IN THE PERIODONTIUM


Figure 18
FORCE vs. D_2
IN THE PERIODONTIUM
FALL IN D_2
FALL IN D_2
FALL IN D_2
Recovery Baseline
End of Run
— FORCE —
Pre-force Baseline
Start of Run

curve displacement of approximately 20 chart units or -3 mv of current equivalents, representing a drop in oxygen tension which is only 24% as great as caused by the hemostat. This is not surprising since the hemostat delivered much greater forces causing more vascular compression.

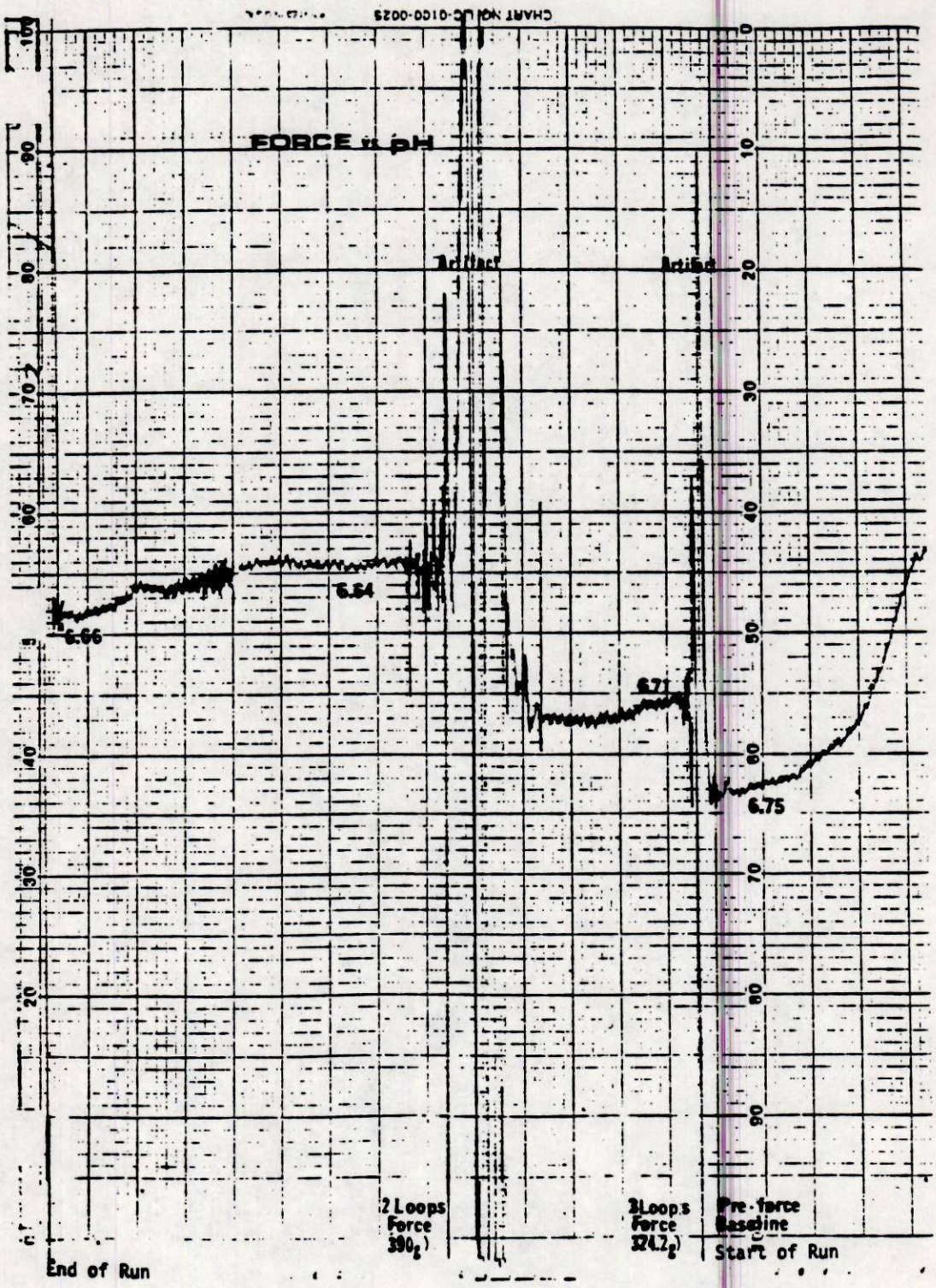
pH Measurements Results

The results of the pH experiment are shown in Figure 19. The pH readings were recorded at the terminal position of the root canal in proximity to the periapical extension of the tooth supporting apparatus.

The following observations are worthy of note. The pH of the system, before the application of force to the region, started at approximately 6.62. Soon there was an increase in pH which began to equilibrate at pH 6.75. This change was probably due to the mixing of the isotonic saline (which was placed in the canal to aid in establishing good contact with the sensing electrode and the periodontal membrane surrounding the apex) and the fluids of the periapical tissue.

At the point in the curve indicated as 3 loops of force, a 342 gram force was applied to the teeth via the 3 loop elastics. The pH electrode indicated an immediate, slight fall in the pH of the region to a value of 6.71. This represents an increased acidity of approximately 0.05 pH units. The pressure was maintained for a period of 2.4 minutes, and the curve shows a very

Figure 19


FORCE vs pH
Artifact
Artifact
6.66
6.64
6.71
6.75
2 Loops Force 390g)
2 Loops Force 124.2g)
Pre-force Baseline
End of Run
Start of Run

stable equilibrium at this value.

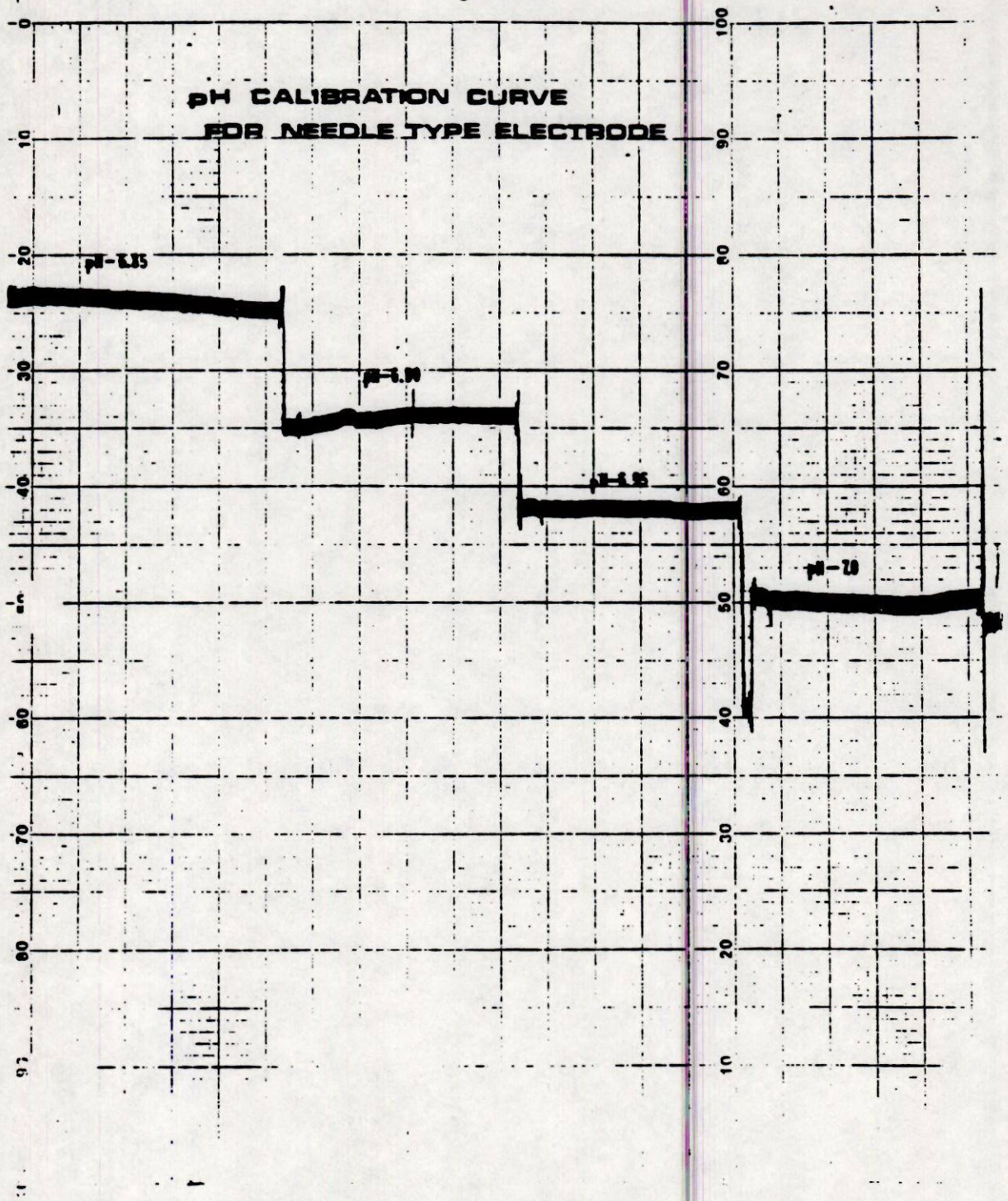
At the point indicated, a two loop force, equivalent to 390 grams of pressure was applied. The response again was immediate, and the pH changed to 6.64, which represents a 0.11 ph units greater acidic than the baseline. This force was maintained for a 6 minute period, and again the stability during this time was quite good (within 0.02 pH units). The vertical lines are artifacts that occurred during the application and stretching of the elastic loops.

Figure 20 shows the calibration curve for this experiment, which is particularly useful to determine the variation, stability and response of the recording system utilized in the pH experiment. This recording tends to establish that the system utilized can maintain a stability necessary for resolving minute pH changes within the range recorded in our pH experiment.

Temperature Results

The results of the temperature experiment, which were recorded in the periapical region of a female dog by means of a small opening through the buccal bony plate, are shown in Plate XXV. The findings are best summarized in the plot of values obtained of the temperature in the region during force application. It was not possible to follow these minute temperature changes on our recorder due to equipment incompatibilities. Therefore, the values were manually recorded as a function of

Figure 20


pH CALIBRATION CURVE
FOR NEEDLE TYPE ELECTRODE
pH-6.85
pH-6.90
pH-6.95
pH-7.0

time after the force application and plotted, shown in Figures 21-23.

What is seen is a small drop in temperature as a function of the applied force. The total fall in temperature was merely 0.77 after minutes of an applied force of 610 grams. The temperature sensing system can detect changes in temperature as low as 0.01C. The results of this are seen in Table III and Figure 23, which is a plot of Table III.

The technique to establish the force equivalents for the elastic loops stretched over the distance utilized in our pH and temperature experiments is shown in Plates XXI and XXII. The method proved very effective in providing an expression of force in terms of exact gram equivalents.

Figure 21

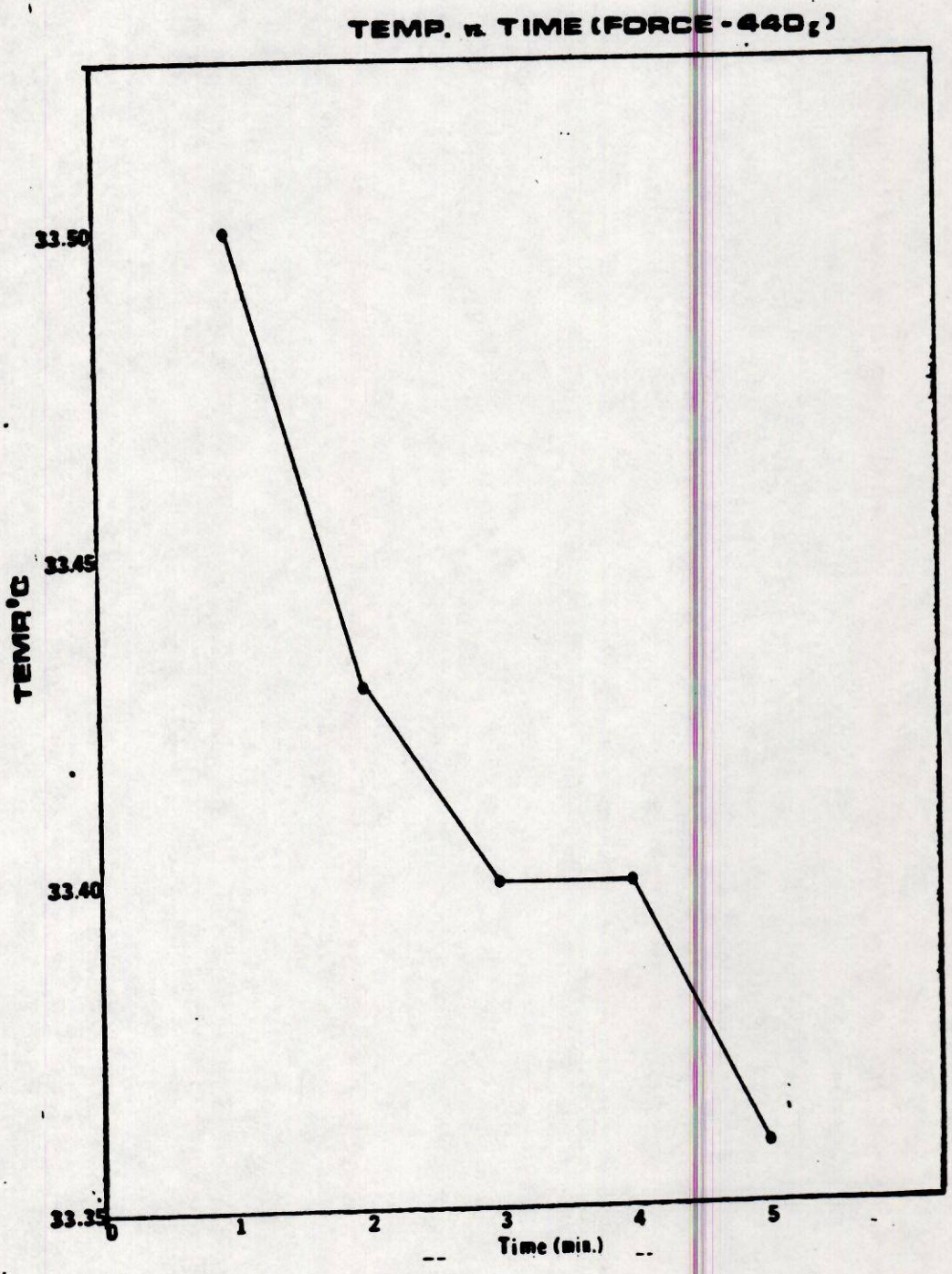

TEMP. vs. TIME (FORCE - 440g)
33.50
33.45
33.40
33.35
TEMP °C
0
1
2
3
4
5
Time (min.)

Figure 22

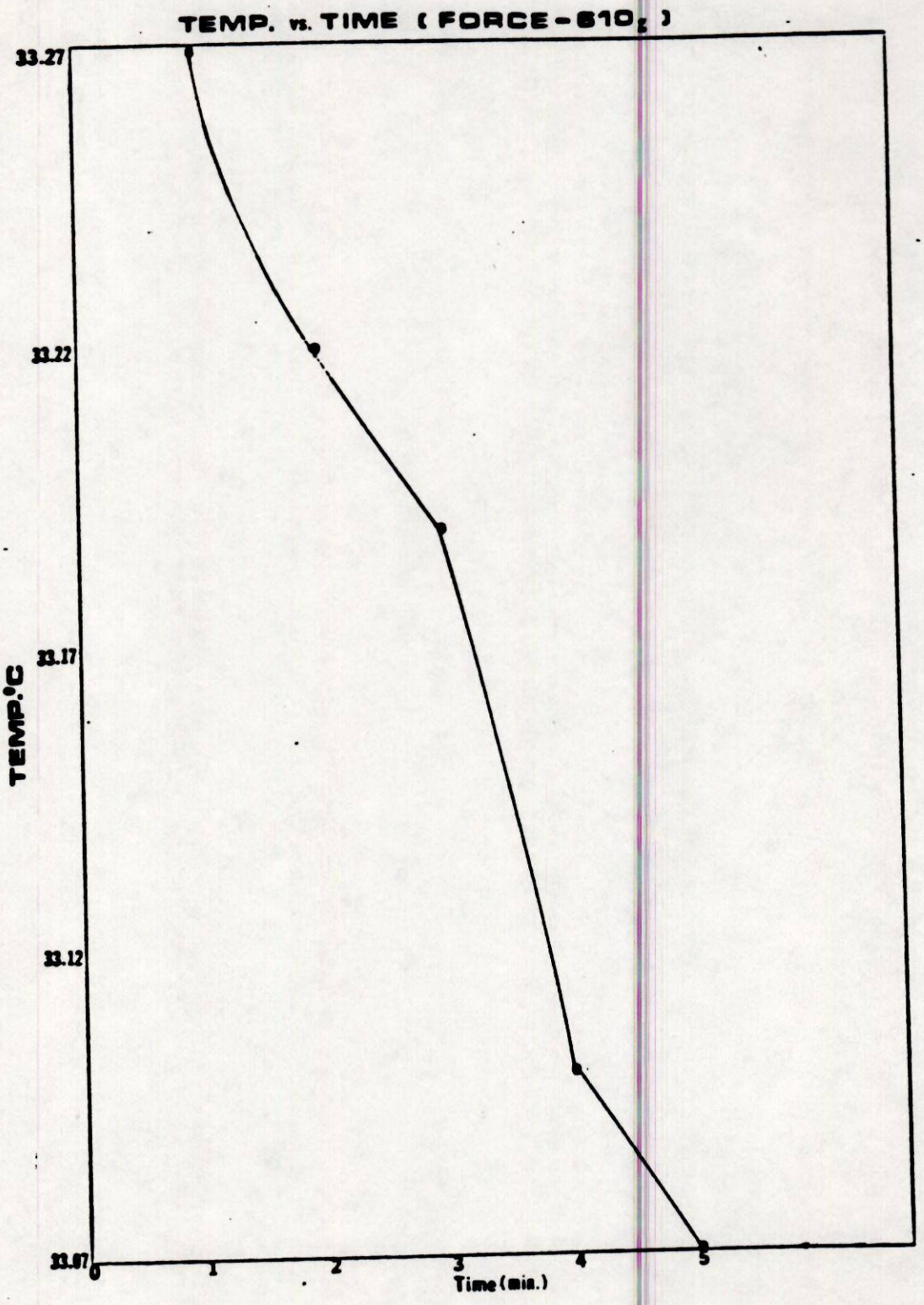
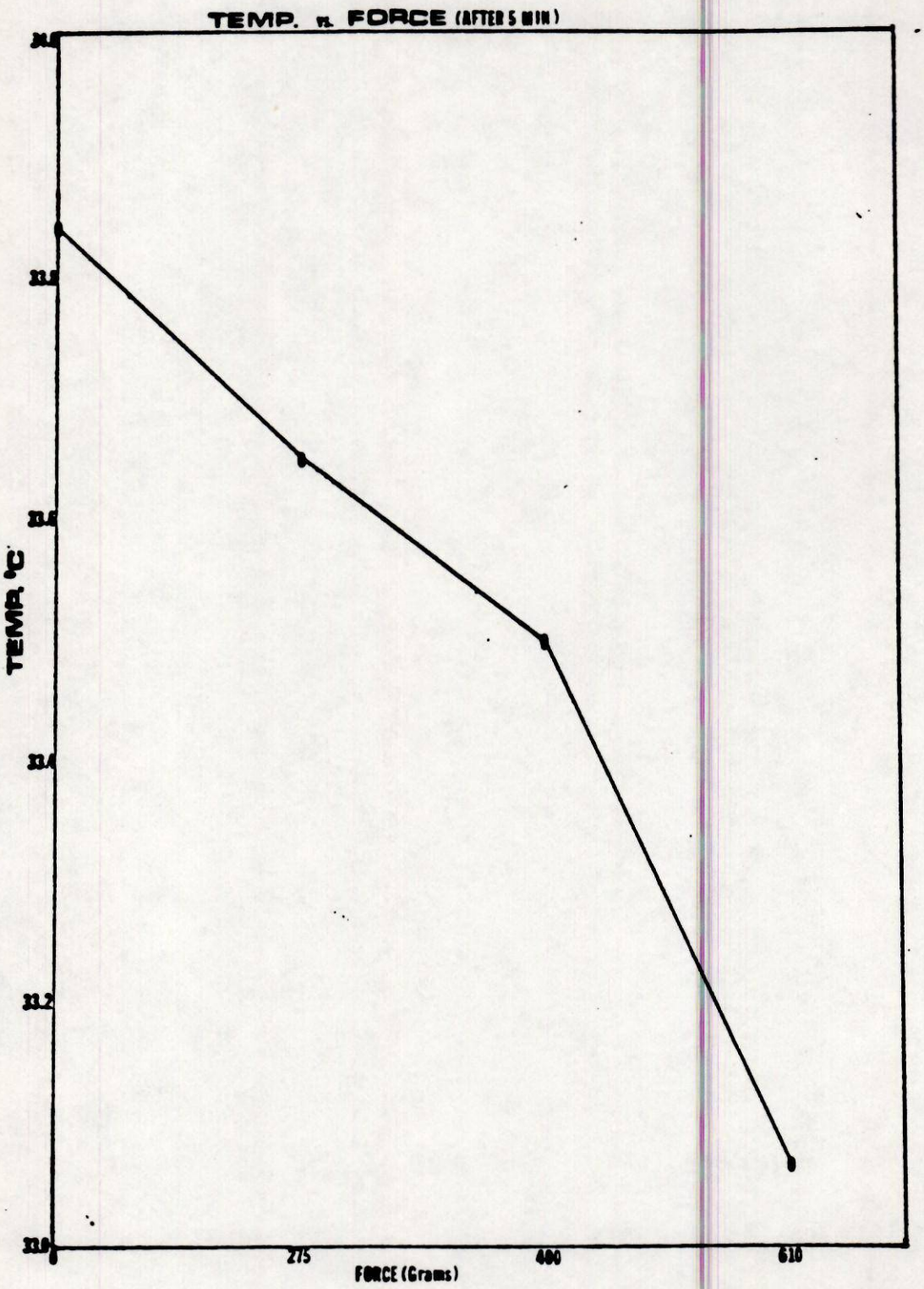

TEMP. vs. TIME (FORCE - 610g)
33.27
33.22
33.17
33.12
33.07
TEMP.°C
0
1
2
3
4
5
Time (min.)

TABLE III

FORCE vs. TEMPERATURE CHANGE

FORCE (in grams)	REDUCTION IN TEMPERATURE AFTER FIVE MINUTES FORCE DURATION
275	.19°C
440	.48°C
610	.77°C

Figure 23



DISCUSSION

Inasmuch as four distinct biological parameters were evaluated in this study in relation to force responses, the experimental results pertaining to each of the tested parameters will be discussed separately. Then an attempt will be made to interrelate the observations.

Oxidation-Reduction

It becomes apparent from the oxidation-reduction potential data that upon application of force, there were at least three distinct changes which occurred in the periodontal membrane around the area of the sensing electrode:

1. an immediate increase in the negative potential occurred;
2. this increase was maintained during the application of force and leveled off to a baseline value once the force was removed; and
3. the level of response exhibits a direct linear relationship to the amount of force applied, at least between the range of 1 - 4 ounces.

A correlation coeficient of 0.9 demonstrates the strong relationship of this association. A best fit line to depict the relationship is shown graphically in Figure 7. An inspection reveals that for each ounce of force applied, there is a general increase in negativity of approximately 122 mv within the 1 to 4 ounce

range. Converting ounces to grams, the range of linear response extends from 28.35 grams to 113.42 grams.

The term "force" is being used to describe what was applied to the tooth rather than the terms "pressure" or "tension". It is the investigator's belief that it is difficult to judge with certainty whether the region immediate to the sensing electrode was subjected to forces of pressure or tension. An examination of Plates VIII and IX showing the approximate position of the file relative to the periodontal structures, suggests that the redox sensing electrode is likely responsive to a combination of both pressure and tension forces within the tissue. Also, the presence of the electrode itself wedged between the cementum and the alveolar bone must have caused some compressive effect on the cluster of small vessels in the area. The presence of the electrode may also modify the force vectors generated during the applied stimulus, but this can not be avoided with an invasive type of measuring technique. The result must have been a reduced vascular profusion in this area.

Some of the cellular components shown by other investigators to reside in a rodent periodontal membrane must also have felt the physical insult and biochemical consequences of the lowered tissue perfusion due to the vascular constriction. A cell type known to respond quickly to changes in cellular

environment is the mast cell. Mast cells in general have intense metachromatic staining properties when stained with toluidine blue. This is due to products contained within their granules such as histamine, serotonin, other biochemical agents, and particularly acid mucopolysaccharides. There is an immediate response of mast cells to the application of force, according to Yamasaki et al.[19] This response is expressed as a loss of ability for staining with toluidine blue and was felt to be due to an immediate discharge of the previously mentioned mucopolysaccharides, amines, and other enzymes. As stated by these investigators, "Changes in the mast cells are recognized as representing one of the earliest stages of inflammation."[20]

Mast cells also respond to changes in pH in their surrounding environment and certainly to a fall in oxygen tension. It is thus suggested that immediate degranulation contributes to the negative surge in redox potential, observed in the present study, that occurred after pressure application in the region surrounding the sensor electrode. An important point emphasized by Bloom[21] is that the metachromatic reaction of the mast cells for toluidine blue is in most part due to the acid mucopolysaccharides which tend to possess a rather strong negative charge. This adds more information leading to a general suspicion that the speed of response, negative charge, and presence in the periodontal membrane indicts the mast cell as

one of the factors in the negative directional change in redox potential.

In addition to mast cells, the electrode was free to sense any or all of the electrical potentials reported to be emitted by any of the adjacent tissues during force application (e.g. piezoelectric potentials or injury potentials). However, the major potentials were thought to be those exerted at the electrode surface by the electrolytes or charged particles in the solution as an expression of their tendency to either accept or donate electrons. Therefore, redox measurements reflect the tissue chemistry of the area in addition to regional differences in potential due to states of polarization in immediate or remote regions. Finally the surge of negativity seems to coincide with the curves depicting the drop in oxygen tension. This seems logical since a drop in oxygen tension in a region should lead to an increased negative potential as seen in anaerobic microbial cultures. This most likely reflects the pile up of reduced products of metabolism that cannot be completely oxidized due to the reduced oxygen tension.

Oxygen Tension (Oxygen Concentration)

The fall in oxygen exhibited in the experiment on application of force in the hamster is probably due to a decrease in the tissue fluid perfusion to the area as a result of the

compression of the vasculature. Plate V reveals a rather extensive cluster of vessels located around the supporting bone of the periodontium continuous with the periodontal membrane. Also, the periodontal membrane itself has its own vascular plexes, so any decrease in vascular profusion is marked by a local decrease in oxygen tension. This is seen in other areas of the body, particularly in regions of the lower extremities, and presents a constant threat to diabetics with impaired vasculature due to narrowed, sclerotic arteries. It was not possible in the present experiment to ascertain the exact oxygen tension value, since there was no way for determining the total amount of surface area of the electrode exposed to the solution, as needed to calculate the exact oxygen tension level in terms of parts/million of dissolved oxygen. The method of exposing a silver metal oxygen-sensing electrode with polarographic circuitry, however, does permit an evaluation of rapid oxygen changes in very small regions of the body.

There are many examples of usage of an open type electrode for measuring oxygen tension,including studies by Larrabee and Bronk[22] (oxygen consumption of a perfused stellate ganglion of a cat); Urbach and Pierce [23] (open-type electrode into human skin to study cutaneous metabolism); Meyer et al[24] (oxygen tension in the brain, recording simultaneously from several open-type electrodes); Davies and Bronk[25] (open and

recessed electrodes for studying oxygen tension at the surface of the cerebral cortex of the cat). The research done with this general method for investigation is extensive, but apparently no one has heretofore recorded the rapid changes of oxygen in such a region of confinement as that of the periodontal membrane. It was not possible to obtain as exact a quantitative evaluation of the forces applied as in the redox experiment, and a compromise had to be made to minimize electrode movement. This is because electrode movement affects the recording of oxygen tension to a greater extent than redox sensing. In general, larger forces were used in the oxygen experiment than in the redox experiment, and the fall in oxygen increased with increasing force. Force application followed by a fall in oxygen tension, was consistently observed in repeated trials. In addition, the recovery to baseline was also a constant recording. Moreover, the rates of fall and recovery were very reproducible in repeated trials. When these observations are added to the fact that most electrode artifacts would have led to a movement in the opposite direction to that observed, a strong confidence can be placed in the validity of these recordings.

Given a fall in oxygen tension of the periodontal membrane on the application of force, an obvious question is then asked. What effect does this have on the biological structures in the area? Since oxygen serves as the terminal acceptor in

the oxidative phosphorylation pathway of energy formation, any biological function requiring an energy input for initiation will be affected. Tissues requiring a higher oxygen tension for proper function, such as brain tissue, would be affected to a greater degree. Those biological processes that utilize an anaerobic glycolysis pathway would be affected to a lesser extent.

As mentioned earlier, the work of Brighton and Friedenberg[27] on electrical stimulation and oxygen tension conducted in in vivo experiments on puppies, indicated that rapid growth was associated with a lower oxygen tension than that seen in the more slowly growing plate. Those in vivo studies also agreed with his in vitro studies in which he concluded that of the oxygen tension range tested (5% - 90%), the 5% level relates to the greatest rate of growth at the costochondral junction in 21 day old Sprague-Dawley rats. "The costochondral junction is a true enchondral growth plate, with a distinct bone cartilage junction that is readily identifiable under the dissecting microscope.)"[28]

He also found that high oxygen tensions were detrimental to growth. In a further extension of this elegant study, he charged metal electrodes in a chamber at various voltages. He observed that oxygen was reduced below 1.0 volt, and as the potential approached 1.0 volt or above, predominantly hydrogen

ions were released. This agrees with the polarograph recording in the present study (Figure 14) which shows a hydrogen wave rising at the same voltage. This is particularly gratifying in light of the fact that the polarographic run was attempted within the very narrow confines of a periodontal membrane in a complete living system.

pH Studies

Hydrogen ion changes in the periodontal membrane during force application are extremely difficult to measure. In retrospect, their measurement posed the most difficult problems encountered for any of the measurements. Considering the extremely narrow dimensions of the periodontal compartment and the fact that the sensing tip of the conventional hydrogen-ion electrode itself is usually wider than this narrow space, a new approach and animal model were selected. As noted in the Methods and Materials, an approach via the root canal was attempted. The reason for choosing a dog for the measurement of this parameter was the availability of a larger area surrounding the tooth, which gave more options for instrumentation. Also, with the root canal type of instrumental approach, a larger pulp canal was needed. During the course of the experiments, it became apparent that the region of the upper incisor in the hamster was better suited for measurements requiring the insertion of the

special redox sensing electrode, used in this study, into the periodontal tissue. The periodontal membrane (Plates VIII and IX) is reasonably wide and the density, and resiliency of the surrounding alveolar bone permitted reasonable ease of electrode insertion. Also, this special electrode makes more intimate contact with the periodontal membrane and hence senses the pressure more acutely.

The dog model further had an advantage in that it permitted a more exact method for measuring the applied forces by means of an elastic module system (previously described.) Moreover, it affords room to accommodate larger parameter sensing electrodes when used.

The results of the pH studies show a very slight drop in pH as the result of force application. An increased acidity is seen with an increase in force. The most probable explanation of this observation is that the response was in effect coupled to that of oxygen. One would expect that when the oxygen falls in the region, as was seen in the oxygen vs. force experiments, the area will become more acidic due to the slower rate of oxidation of the acids formed during respiration. Anaerobic glycolysis yields more acidic products than does aerobic respiration. It is also a common medical observation that in other tissue regions of the body (particularly in muscle tissue) oxygen deficiency leads to the build up of lactic acid and other organic acids, which in

turn can cause muscle cramping. As previously mentioned, acidity favors bone resorption, which may be due to a direct cellular effect or to an enhanced solubility of the mineral phase of bone or cementum. As also mentioned earlier, when the effects of buffers of varying pH on the solubility of enamel surfaces were tested, the fact that bone is very sensitive to acidity was noted. The rate of enamel surface solubility was measured by monitoring the rate of appearance of calcium in the acidic buffer solutions. This test also pointed to the sensitivity of even the most calcified tissue (enamel) to small differences in pH.

Temperature Studies

The measurement of temperature changes at the apex of a female dog's first bicuspid showed a slight drop in temperature in response to force application. This is to be anticipated in light of the fact that a compression of the vasculature in the region decreases the circulation and likewise the temperature in the area. This is certainly the case in diabetics with impaired circulatory flow to the lower extremities. Comparing the temperature of a limb with impaired circulation to that of one with good circulation, the impaired limb would most certainly be cooler. People with low blood pressure also frequently complain of cold hands and feet. It is proposed that the compression in the periodontium during force application and resultant decreased blood flow represents the primary cause of the slight drop in

temperature. Although the drop in temperature was slight, it can be viewed as the best reflection of the temperature changes measured. This was due to the difficulty encountered in ascertaining that the electrode was in fact in direct contact with the periodontal membrane, via the surgical approach utilized. Thus, the temperature changes could have been affected, or dampened by placement of the electrode in the approximate area but still not precisely within the periodontal membrane. It is desirable that this part of the study be repeated utilizing an electrode small enough to enter the pulp canal of one of the roots, with the electrode extending to the proximity of the apex, as was done in the pH study. This electrode would have to be specially constructed, since no commercially manufactured units were available at the time of this study.

SUMMARY AND CONCLUSIONS

This study deals with the biological responses of the tooth supporting apparatus to an application of force of sudden onset and short duration. Four parameters were selected, which were believed to be especially indicative of significant changes in tissue respiration and energy dependent biochemical reactions.

The parameters selected for evaluation were: oxygen tension; oxidation-reduction potentials; pH; and temperature. All of these factors are extremely important in a consideration of any biochemical reaction or chain of reactions that either requires or liberates energy.

Many reactions involved in bone remodeling and tissue repair would most likely fall into this category. A search revealed that, while these basic parameters were often mentioned or alluded to in many discussions of the biological response to force, no in vivo measurements have been undertaken, to the best of this investigator's knowledge. The lack of such measurement studies likely stems from the difficulty in obtaining electrode sensors small enough to reach the shielded and confined areas of the tooth supporting apparatus. Thus, this investigator's work also comprised the task of designing systems that could record

these parameters in the tooth supporting apparatus.

Once the electrodes and companion measuring systems were designed and tested, then they were applied to the in vivo model. The dentoalveolar region in two animal systems was utilized. One was the anterior two incisors of male hamsters. The periodontal membrane in these teeth was reasonably wide, and the bone sufficiently resilient to allow an extremely thin redox sensing electrode to penetrate.

The second animal model involved the mandibular bicuspid teeth in a female dog. This model permitted the application of calibrated forces and also the use of an access to the periodontal membrane via the root canal.

The key findings and conclusions are summarized below:

1. the periodontium in the hamster incisor undergoes changes in redox potential during sudden force application;

2. this alteration tends to drive the baseline value to increased negative potentials;

3. increase in redox negativity shows a linear response in the force range of 1 to 4 ounces;

4. the negative increase per ounce of force is in the range of approximately 122 millivolts;

5. recovery time after force removal, expressed as the time in seconds to restore the previous redox baseline value, tends to increase after 2 oz of force;

6. the 50% recovery rate of the increased redox value is markedly increased with a 4 oz load and shows evidence of having caused damage to the tooth

supporting apparatus in the hamster's maxillary incisors;

7. a technique to conduct polarographic analyses in the periodontal membrane of the hamster incisor was developed;

8. using this analytic electrochemical analysis, an oxygen concentration fall was demonstrated in the hamster periodontium during force application;

9. this oxygen fall was immediate on force application, and a recovery to normal oxygen level began as soon as the force was released;

10. there seemed to be an indication that this fall in oxygen was proportional to the applied force in the 1 to 4 oz range. The relationship was not as definite as that seen in the redox change;

11. there appeared to be a slight increase in the acidity of the periodontium in the mandibular bicuspid region in the dog during force application;

12. the increase in acidity was very slight, but positive. An increase in acidity equivalent to 0.11 pH units occurred after an application of a 390 gram force for 6 minutes duration;

13. a decrease in temperature in the periapical region in the mandibular bicuspid area in a dog was demonstrated;

14. the temperature fall varied with the level of force applied. Application of 610 grams caused a temperature reduction of 0.77°C after 5 minutes.

Tentative hypotheses formulated to attempt to consolidate and relate these observations are outlined below as a sequence of physiological events:

A. Force compresses the vasculature in the periodontium.

B. This vascular compression causes a fall in the oxygen concentration in the region.

C. The fall in oxygen concentration results in an increase in the negativity of the redox potential of the area.

D. This increased negativity is due to reduced molecules that are not oxidized at the same rate due to the oxygen deficiencies.

E. Increase in acidity is also a result of diminished oxygen tension.

F. The slight drop in temperature relates to diminished blood flow.

G. Since oxygen tension, acidic changes, and changes of redox potential are factors that influence bone resorption or bone deposition equilibrium, the periodontium reacts to these stimuli with the appropriate adjustment in the equilibrium.

This hypothetical sequence needs confirming study.

A new system for analysis has been developed that makes possible further experimental investigations in this previously untapped region, and a comprehensive determination of the biological dynamics of the periodontium at a fundamental level can be made.

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