

OPTICAL EFFECTS OF BLOOD CIRCULATION
WITHIN DENTAL PULP

by

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A Thesis

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INTRODUCTION

Dentists have used various means to evaluate the state of health of the dental pulp in order to permit selection of the proper treatment for teeth. Thermal, percussive, and electrical stimuli are widely used for these evaluations; yet no evidence has been presented to demonstrate a direct relationship between the patient's response to these stimuli and the actual histologic state of the pulpal tissue (1).

In general, the health of a tissue is related to the circulation of blood through its vascular bed. Interference with the supply of nutrients and removal of waste products place the viability of the tissue in jeopardy. Tissues which are inflamed or undergoing degenerative changes have markedly altered circulation. Knowledge of the characteristics of blood flow through a tissue could be of primary importance in estimating the health of that tissue.

LITERATURE SURVEY

Attempts have been made to assess circulation in the tooth pulp using histological methods (2). Observations have been reported on tooth pulp tissue which has been altered by application of chemical or physical stimuli. This sectioned tissue was compared with unstimulated control tissue. The process of extraction, fixation, sectioning and staining may introduce misleading artifacts. These techniques do not allow the tissue to be compared with itself. Other similar but unstimulated tissue is required. These comparisons capture but an instant of a continuum of the response of the tissue to the stimulating agent.

A significant advance in the study of pulpal circulation was achieved by techniques which permitted in vivo microscopic visualization of nearly exposed pulp tissue. The hard tissue covering a portion of the pulp was reduced to permit visual and microcinematographic observation of pulpal vascular dynamics. This approach allowed observation of changes in the caliber of blood vessels in response to directly and indirectly applied stimuli. Other phenomena concerning the dynamics of circulation were described as "ischemia," "thrombosis," "sludging," and "hemorrhage" (3). Alterations of blood flow were inferred by these authors. While this technique allows study of changes in circulation in pulp tissue, it also suffers from severe limitations. Only the most superficial vessels can be viewed. Furthermore, the trauma created by

the reduction of tooth structure may damage the pulp tissue and modify circulation.

Electrical conductance properties of moving blood change significantly in certain ranges of velocity (4). This principle has been used by Nyboer (5) to study blood flow in soft tissues. Liebman and Cozenza (6) applied the principle to teeth by implanting electrodes deep into the dentin and then directing a radio frequency current through the tooth. A cyclic change in impedance demonstrated a correlation between blood flow in the tooth and the heart rate. Application of various stimuli to the tooth brought about alterations in the amplitude of the impedance wave. These alterations were interpreted as changes in blood flow.

Meyer, Wiener, and Grimm (7) have reported an isotope dilution technique to estimate the blood flow through a tooth pulp. The fraction of cardiac output that flowed through the pulp of the cuspid teeth of young dogs was computed and they were able to estimate volume flow of blood through the pulp. Knowledge of blood flow at one moment in time, rather than over an extended period, is provided. Accurate measurement of radioactive decay rate necessitated the extraction of the tooth.

Sorenson (8) has explored a method of evaluating blood flow in the tooth which is based upon recovery of heat loss. The method measures the rate at which a tooth returns to its original temperature after it has been cooled approximately 2°C . The rate of return is assumed to be a function of blood flow. Although the cooling may alter blood flow

in itself, the effect must be minimal and reversible. This method of estimating blood flow in the dental pulp is one of the few which does not necessitate damage of tooth structure.

In 1962 Burnette and Hohn (9) showed that when a steady beam of light passed through a vital tooth which had had most of the dentin covering the pulp removed, it emerged as a faintly pulsating beam. The frequency of this pulsatile transillumination appeared to be related to the heart beat. They speculated that this simple transillumination technique might allow the blood flow characteristics of the dental pulp to be studied.

Upthegrove, Dorman, and Bishop (10) in 1965, reported a device which utilized the principles originally suggested by Burnette and Hohn. They employed a highly sensitive photo-resistive cell. The device was a marked improvement over that reported by Burnette and Hohn, since it was able to record strong optical pulsations even when there had been no reduction of hard tooth structure. This last item is an important one, since any technique used to measure blood flow should not in itself injure the tissue and thereby alter the flow. Of the techniques mentioned for studying blood flow in the pulp, only those based on thermal recovery and transillumination do not physically or chemically alter the tooth appreciably.

The optical technique shows promise as a method of continuously monitoring circulatory activity. However, a better understanding of this phenomenon is required before the method can be made fully useful.

PURPOSE

The study was divided into two phases. The purpose of the first, or in vitro phase was to test the sensitivity of a photoelectric apparatus in discriminating changes in optical properties of flowing blood in an in vitro model and to explain the mechanism by which these changes occur.

The purpose of the second, or in vivo phase was to determine the relationship of photoelectric responses to circulation within a vital tooth and to identify specific circulatory phenomena which cause those responses.

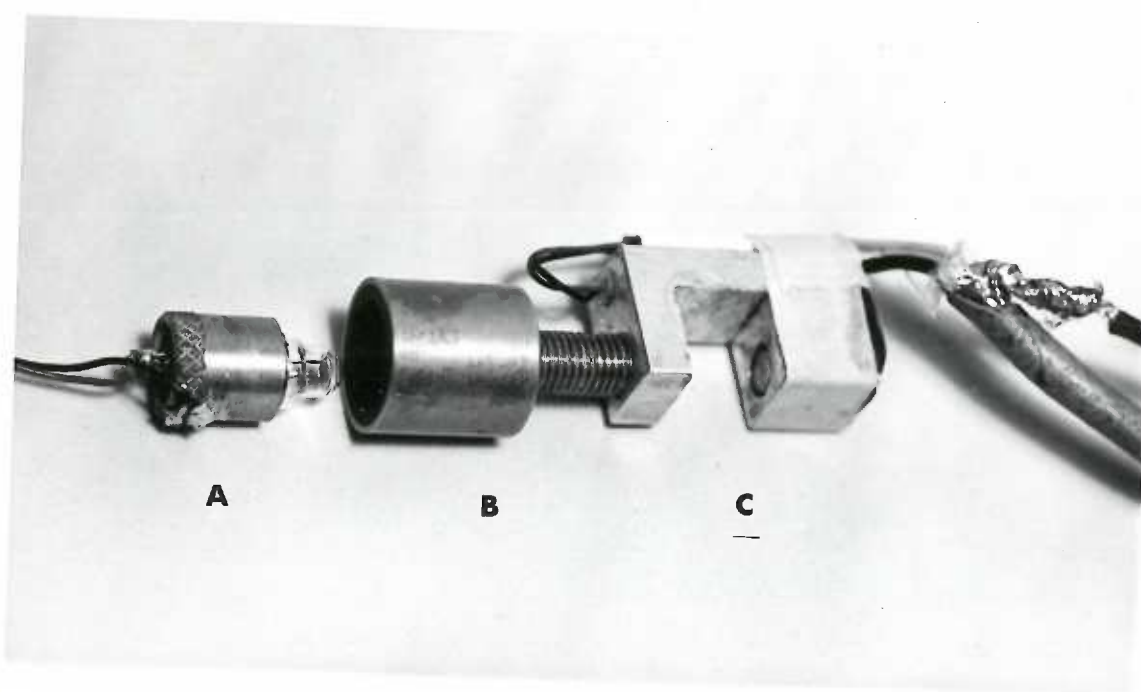


Figure 1: The Transilluminating Photoelectric Device (PED)
A. Light Source B. Hollow Shaft C. Photocell

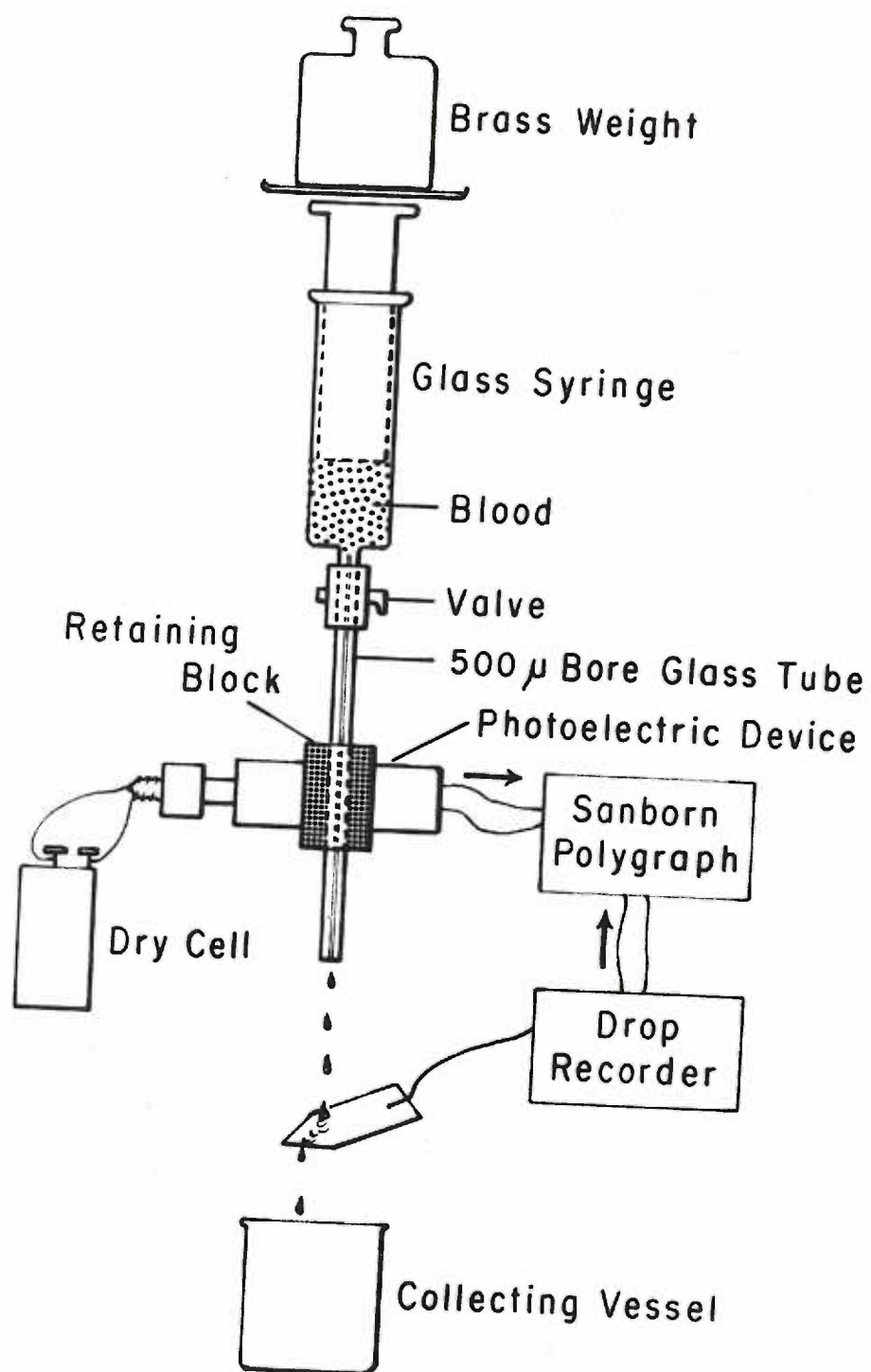


Figure 2: In Vitro Apparatus

MATERIALS AND METHODS - PHASE I

A photoelectric device (hereafter referred to as PED) was constructed similar to that described by Upthegrove¹ (11) (Figure 1).

An in vitro experiment was conducted. An apparatus of ultimate simplicity was constructed (Figure 2). A 20 ml. Luer-lok syringe was mounted vertically on a lab stand. The hub was fitted with a stop cock which in turn was connected to a six-inch length of 500u-bore glass capillary tube. Relative velocity of flow was recorded by a drop counter located just below the tip of the capillary tube. The PED was mounted approximately halfway down the capillary tube. Signals from the PED and the drop counter were directed to the Sanborn polygraph recorder². The velocity of flow through the capillary tube was regulated by the placement of various brass weights in a plastic dish cemented to the head of the syringe piston.

On three separate occasions the following procedures were carried out.

1. The syringe and capillary tube were filled with fresh defibrinated sheep's blood³. Care was taken to avoid the inclusion of air bubbles.

¹ A detailed description of the device can be found in Appendix #A.

² Model 67-500, Sanborn Company, Cambridge, Mass.

³ Fresh, sterile defibrinated sheep's blood obtained from:
Arthur N. Torland
8520 S.W. Avery Street
Tualatin, Oregon, 97062

2. The Sanborn recorder was engaged. (Time mark at bottom of tracings indicate one second intervals.)
3. With no weight in the plastic dish on top of the piston, the stop cock was turned from the off position to the on position, thereby allowing blood to slowly flow through the capillary tube.
4. The flow rate was then increased by placing a 200 gm. weight in the dish. After three seconds the 200 gm. weight was removed.
5. Following a brief delay, a 500 gm. weight was placed in the dish, thus causing the flow to surpass the rate achieved by the 200 gm. weight.
6. The experiment was concluded by the removal of the 500 gm. weight.

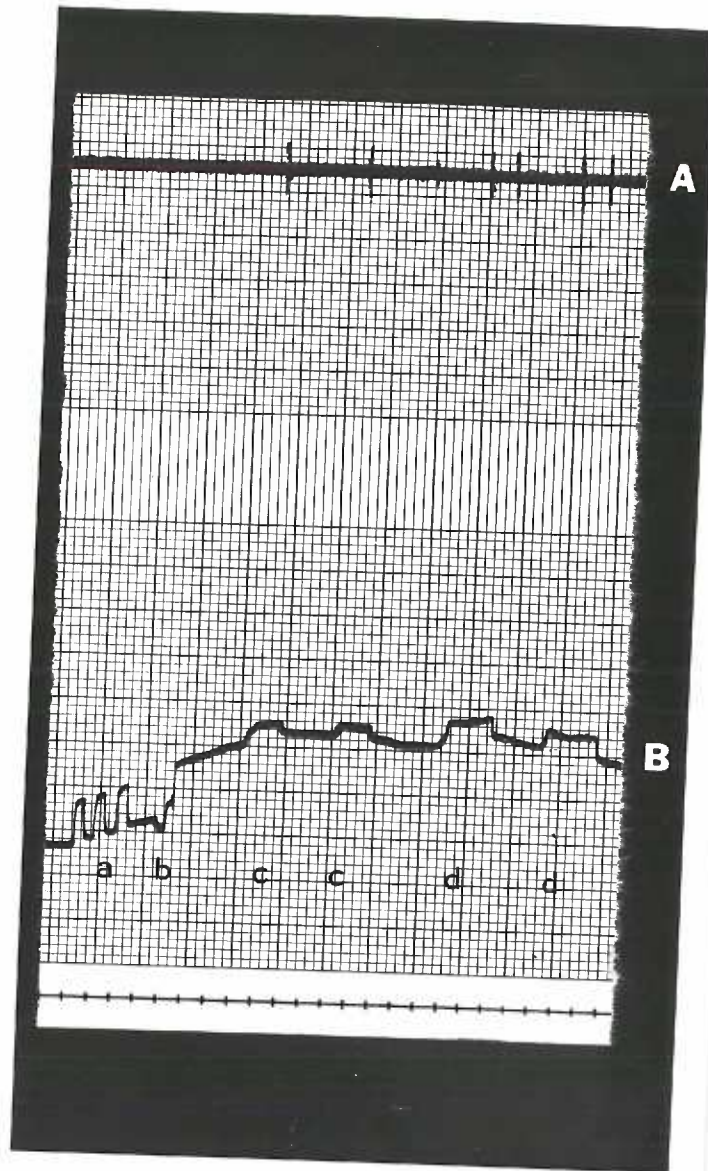


Figure 3: Relationship of velocity of blood flow to its transilluminosity in vitro

- A - drop recorder tracing
- B - photocell output
- a - calibration of deflection with known intensity outside light source
- b - open valve
- c - application and removal of 200 gm wt.
- d - application and removal of 500 gm wt.

RESULTS - PHASE I

The results of all three in vitro experiments indicated that the PED was sufficiently sensitive to register alterations in the transilluminosity of defibrinated sheep's blood flowing through a 500u-bore glass capillary tube at changing velocities (Figure 3). The degree of flowing transparency, as related to velocity was not quantitated. However, there appears to be a trend for transparency to be related to flow velocity.

The greatest increase in transilluminosity occurred when the rate was changed from zero to slow flow.

DISCUSSION - PHASE I

The PED used in this study is a relatively new and untested device. It was felt that sophisticated quantitation of the relationship of flow velocity to transparency would be premature at this stage of testing. Attention was focused on general characteristics, with hopes that future studies will focus on quantitation.

Rheologists have known for years that flowing blood has different optical properties than blood not in motion. The prominent British physiologist W.M. Bayless (12) wrote in 1952, "There is experimental evidence that the red cells are indeed oriented when blood is sheared. Direct observation of blood flowing in a capillary tube shows that it appears brighter, reflects more light, and absorbs less, when it is in motion than it does when at rest. This is particularly obvious when the flow is pulsatile."

Two phenomena occur in flowing blood which could account for these optical effects. One is the tendency for the red cells to concentrate in the axial stream. The other relates to the axis of orientation of the red cells to the vessel wall.

The phenomenon of axial concentration of red cells in circulating blood has been studied by a number of investigators. Taylor (13), for example, allowed blood to flow through capillary tubes (190 μ) at pressures of 10, 20, 40, 80, 160, 240, and 300 mm. Hg. He reported, "In all cases, with increasing rate of flow, there was an increase in the light transmission at the edge of the tube," he noted. In studies of microcirculatory pressures using micropipettes Wiederhielm (14) noted, "During the period of flow, large deflections were produced by the impingement of erythrocytes on the tip of the micropipette. No such deflections occurred when the tip of the pipette was within the peripheral plasma zone." In an attempt to explain the apparent decrease in viscosity of blood when it is flowing as opposed to when it is static, Burton (15) suggests "The explanation undoubtedly involves the phenomenon of axial accumulation of red cells." Bloch (16) used ultra-high speed microcinematography (7000 fps) to view circulating blood in mesenteric arterioles in frogs, mice, and rabbits. He found that the peripheral cell-free zone is not as well defined as some investigators have indicated. He did note, however, that there is a tendency for the population of red cells to be reduced at the peripheral portions of vessels containing flowing blood.

Orientation of red blood cells (RBC) parallel to the vessel wall could also affect the optical properties of flowing blood. The concept of orientation can be described by an analogous situation. If one were to place a log in a flowing stream he would find that the log would soon orient itself with its long axis parallel to the long axis of the stream.

Although (RBC) are not logs, they are biconcave discs which have been described to orient similarly in a flowing stream of blood.

Wiederhielm (17) wrote, "Quick freezing experiments have shown that there is a significant preferential orientation of the cells parallel to the walls,..." Burton (18) concluded from his experiments "... that a very small degree of flow of blood results in an overall orientation of the cells, where they (the cells) have room to be oriented." Bloch (19) reported from his study of microcirculation with high speed microcine-photographic techniques, "Behavior of flowing RBC's generally was a complex helical pathway. An apparent attempt was seen to align to the long axis. RBC's in the arteriole were most commonly oriented with their equatorial axes approximating a parallel position in relation to the long axis of the vessel." Bayliss (20) made a similar statement earlier when he said, "There will be a statistical orientation (of the RBC) since, on the average, each particle will spend most of the time with its plane nearly in the plane of shear."

The mechanism through which RBC orientation effects light transmission is not well explained in the literature, but nevertheless the optical effects are mentioned repeatedly. Elwell (21) noted that the scattering of light through cellular blood may be altered by different rates of flow through a cuvette. Taylor (22) noted "... an oriented system of particles would scatter less and so transmit more light than a random one." Kuroda and Fujino (23) studied the optical properties of dilute suspended erythrocytes in a glass apparatus and noted, "...transmitted light is more in-

tensive in the flowing state than while at rest." Suspensions of RBC's at physiologic concentrations however, result in a "negative streaming transparency" or in other words, a flowing opacity.

There are a number of unanswered questions. For example, Kuroda noted "a negative flowing transparency," i.e. flowing opacity, at concentrations of red cells above 10 per cent, while our in vitro experiments which used whole blood indicated a flowing transparency. The explanation for this disparity may be related to the major differences in experimental technique. Kuroda utilized a 700mu light source to transilluminate a swirling dish of suspended RBC's, while our method employed a broad spectrum mixed light source to transilluminate a thin column of defibrinated whole blood. Therefore, comparison of the deflection directions of these entirely different techniques may be of little value. It is interesting to note that when actuated by the signal from the PED, the deflection of the polygraph stylus can be in either direction, depending upon which side of the null point the Wheatstone bridge is balanced. It therefore was necessary to consistently balance the bridge in the same direction when several recordings were to be compared. Calibration was accomplished by placing a high intensity light source close to the PED so as to cause deflection of the stylus in spite of the opaque stabilizing block.

The explanation of the non-linearity of the flowing transparency in the model system might involve the possibility that the red cell orientation phenomenon may exert a relatively large effect at one flow range while the alteration of the peripheral cell-free zone may be most active at another flow range.

CONCLUSION - PHASE I

1. Change in the rate of blood flow through a capillary tube results in a change in optical properties of the blood; however, quantitation of these relationships was not pursued.
2. The PED is capable of responding to changing optical properties.
3. The changes in orientation of RBC with changes in velocity of flow described by other investigators would explain the optical effects observed.

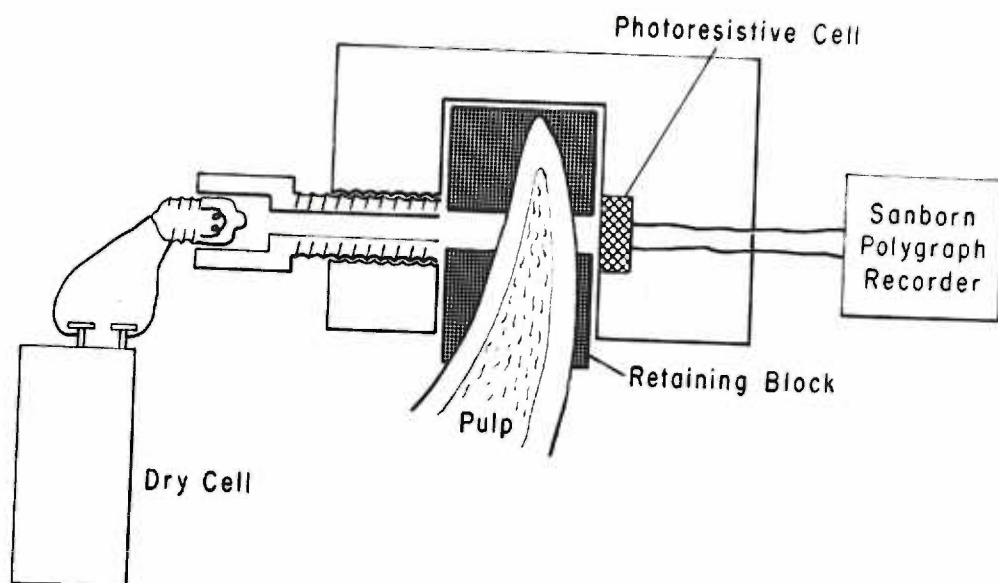


Figure 4: Schematic representation of canine tooth within the jaws of the PED

MATERIALS AND METHODS - PHASE II

Preliminary Testing

Preliminary testing of the PED on dogs under general anesthesia revealed that slight movement of the dog, due to cardioballistics, respiration, or the manipulation of an extremity, would alter the intensity of the light reaching the photo cell, and thereby introduce artifacts. Furthermore, variations in the extraneous room light created by the moving shadow of the author were found to introduce artifacts. These artifacts were eliminated by use of an opaque retaining block which held the PED tightly to the tooth¹. The apparatus is schematically illustrated in figure 4.

An experiment was performed which permitted the comparison of the PED response of a normal tooth to that of a similar, but pulpless tooth. A young mongrel dog was used. On the day before the experiment, the animal was tranquilized² to facilitate taking impressions of the teeth for fabrication of retaining blocks. Blocks were constructed to fit the mandibular right and left canines. On the day of the experiment, the animal was again tranquilized and approximately one hour later was placed under general anesthesia³.

¹ A detailed description of this procedure can be found in Appendix #B.

² Propiopromazine hydrochloride (TRANVEX) 50 mg/ml, Diamond Laboratories, Inc., Des Moines, Iowa. Dosage: 4 mg/kg, I.M.

³ Sodium pentobarbital (NEMBUTAL) 50 mg/ml, Abbott Laboratories, North Chicago, Illinois. Dosage: to effect, not to exceed 25 mg/kg, I.V..

The femoral blood pressure, ECG, and the PED responses were recorded simultaneously on a Sanborn polygraph¹. This allowed temporal comparison of any given point on the PED wave with any other point on the Blood Pressure or ECG waves.

Recordings of responses obtained with the PED were taken first on the right, and then on the left intact mandibular canines. The pulp tissue of the right canine was then removed and a root canal filling was inserted². Immediately upon completion of the root canal filling the PED was re-applied to both canine teeth and simultaneous recordings of blood pressure, ECG, and PED responses were again obtained.

Further Investigation

Further study of the relationship of photoelectric responses to circulation was carried out using six young mongrel dogs in the following manner.

1. The dogs were premedicated and anesthetized by the method previously described.
2. Simultaneous continuous recordings of blood pressure, ECG, and PED responses were obtained.¹
3. The right and left inferior alveolar nerve and artery were exposed and loosely ligated so as not to interfere with impulse transmission or blood flow³.

¹ A detailed description of this procedure can be found in Appendix #C.

² A detailed description of this procedure can be found in Appendix #D.

³ A detailed description of this procedure can be found in Appendix #E.

4. Heart rate was altered for several seconds by means of electrical stimulation of the vagus nerve (1 volt, 15 cycles/sec).
5. The right alveolar nerve was sectioned.
6. The right inferior alveolar artery was first partially occluded and then ligated tightly and sectioned distal to the ligature with respect to the heart.
7. The third and final bundle which included the right inferior alveolar vein, loose connective tissue and lymph vessels was then tightly ligated with a double ligature and sectioned between the ligature.
8. While recording responses from the PED on the right canine, the bundle containing the entire contents of the left mandibular canal was ligated and sectioned.
9. At the conclusion of the experiment, the recording apparatus was disengaged and the animal was sacrificed by the injection of an euthanasic¹ drug directly into the heart. A post-mortem examination was performed on the right canine².

¹ Sodium pentobarbital, propylene glycol, tetraethyldiaminotriphenylmethane sulfate (BEUTHANASIA), Burns Pharmaceuticals, Oakland, California. Dosage: 10 ml. injected into heart.

² A detailed description of this procedure can be found in Appendix #F.

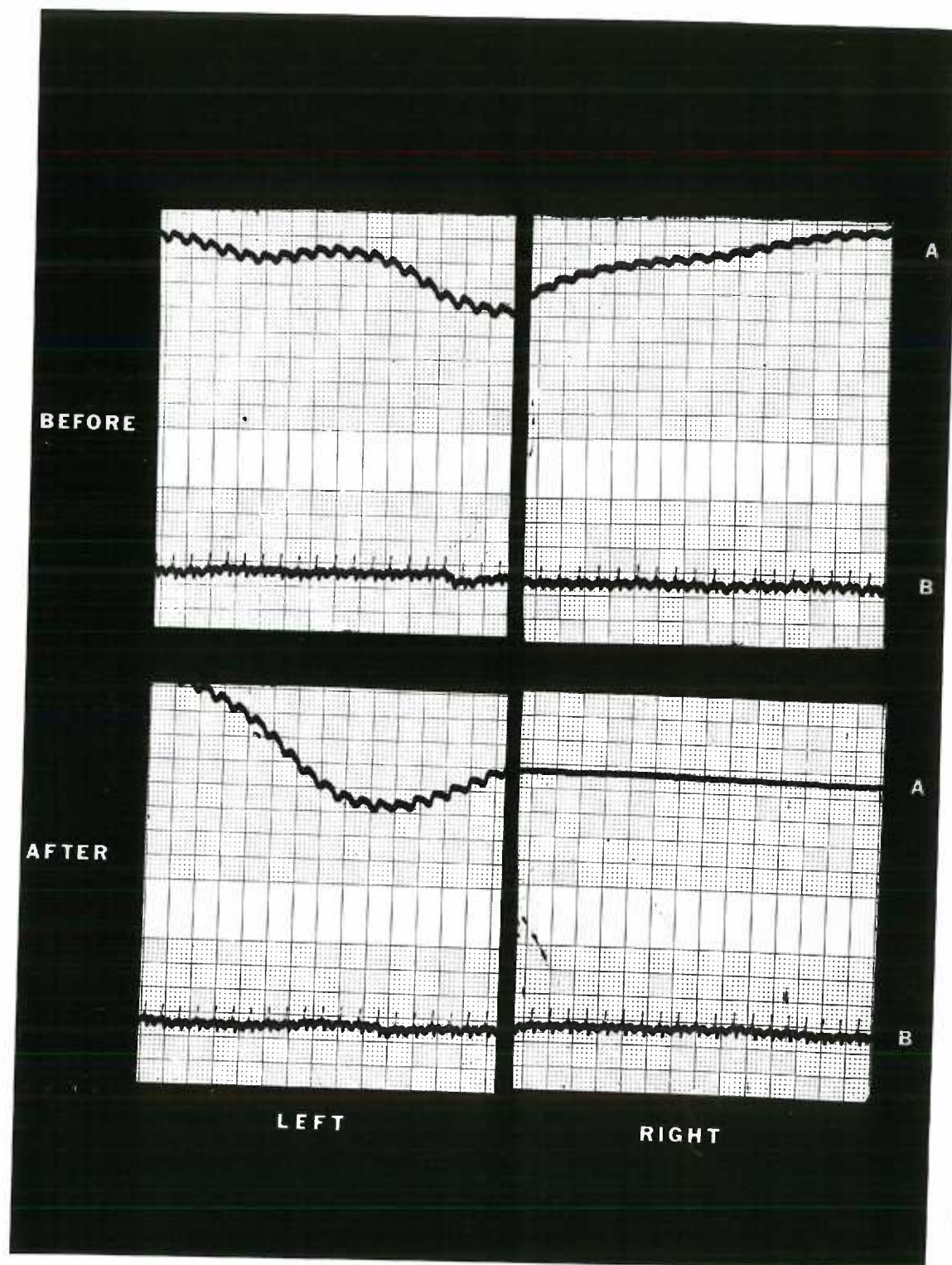


Figure 5: Endodontic procedure

A - PED output

B - ECG output

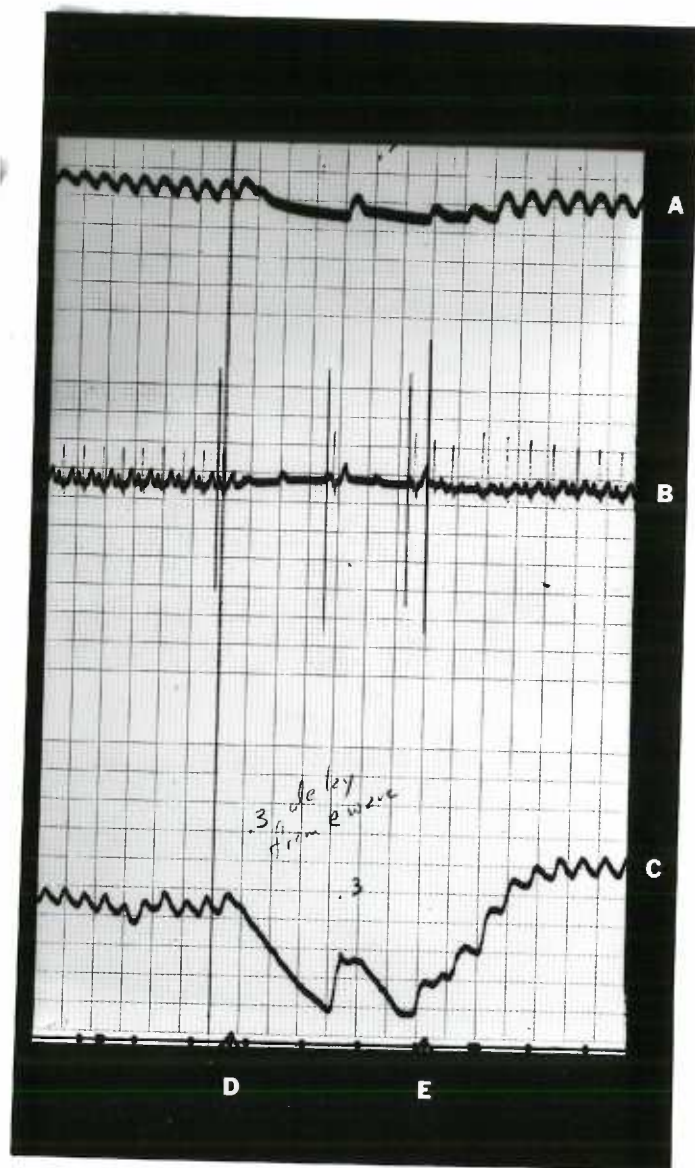


Figure 6: Delay of PED response after ventricular contraction

- | | |
|---------|-----------------------------|
| A - BP | D - Begin vagal stimulation |
| B - ECG | E - End vagal stimulation |
| C - PED | |

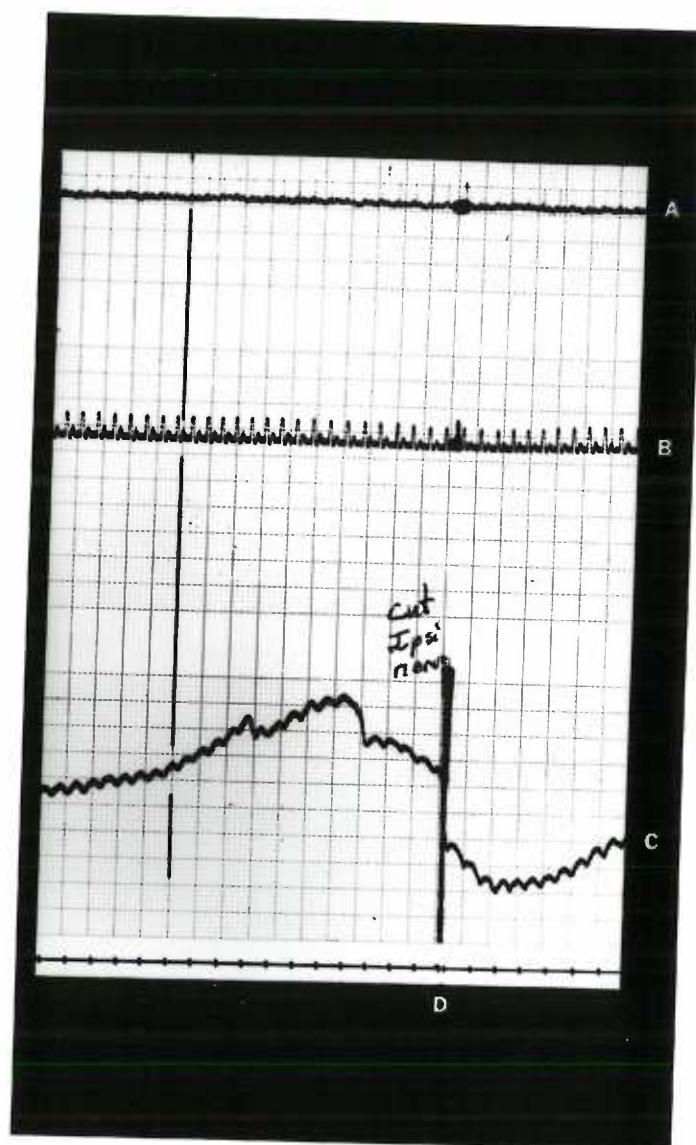


Figure 7: Section of Inferior Alveolar nerve

A - ECG

C - PED

B - BP

D - Section nerve

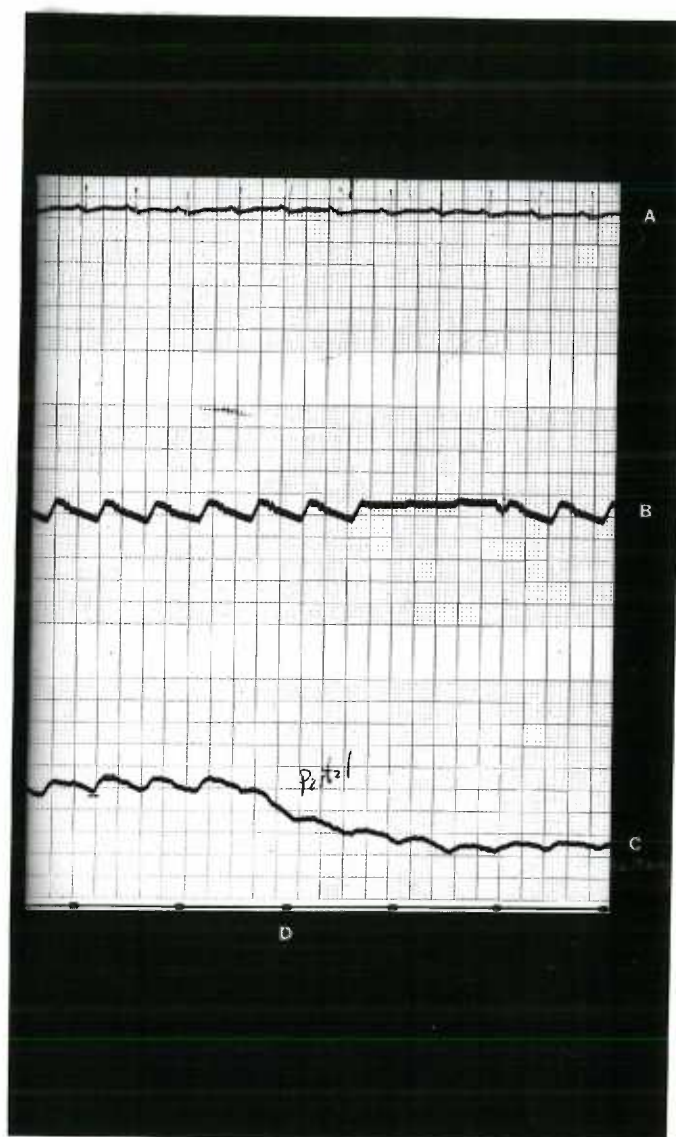


Figure 8: Partial occlusion of inferior alveolar artery

A - ECG B - BP C - PED

D - Begin partial occlusion

RESULTS - PHASE II

Preliminary Testing

1. The pre-operative recordings revealed close similarity in the PED waves obtained from the right and left canine (Figure 5).
2. The post-operative recording from the left canine was very similar to the pre-operative record; however, no wave was produced from the PED attached to the endodontically treated right canine (Figure 5).

Further Investigation

1. Stimulation of the vagus nerve was followed by a change in the PED wave frequency which closely followed change in heart rate. Furthermore, it was noted that there is a delay of approximately 0.3 seconds from the ventricular excitation (R wave of ECG) to the time the maximum opacity effect occurred within the tooth (Figure 6).
2. No alteration of the PED wave was noted when the right inferior alveolar nerve was sectioned (Figure 7).
3. Partial occlusion of the inferior alveolar artery was accompanied by a diminution in the amplitude of the PED wave (Figure 8). Quantitation of this relationship was not determined. Total ligation and section of the inferior alveolar artery was followed by an immediate cessation of the PED pulse

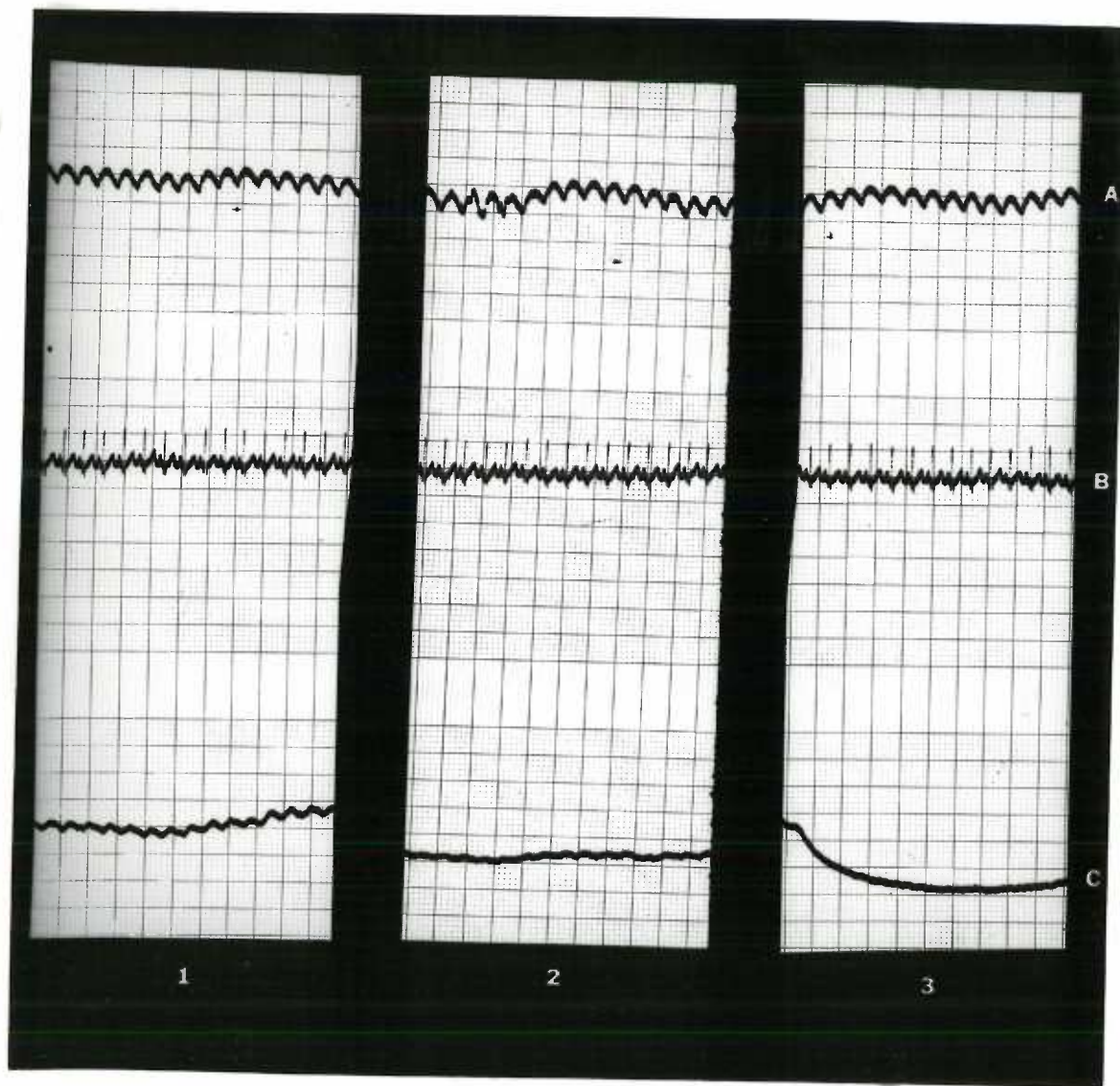


Figure 9: Section of ipsilateral inferior alveolar artery

A - BP

B - ECG

C - PED

1 - Before

2 - Shortly after section of ipsi-
lateral inferior alveolar artery

3 - After section of contralateral inferior alveolar
artery

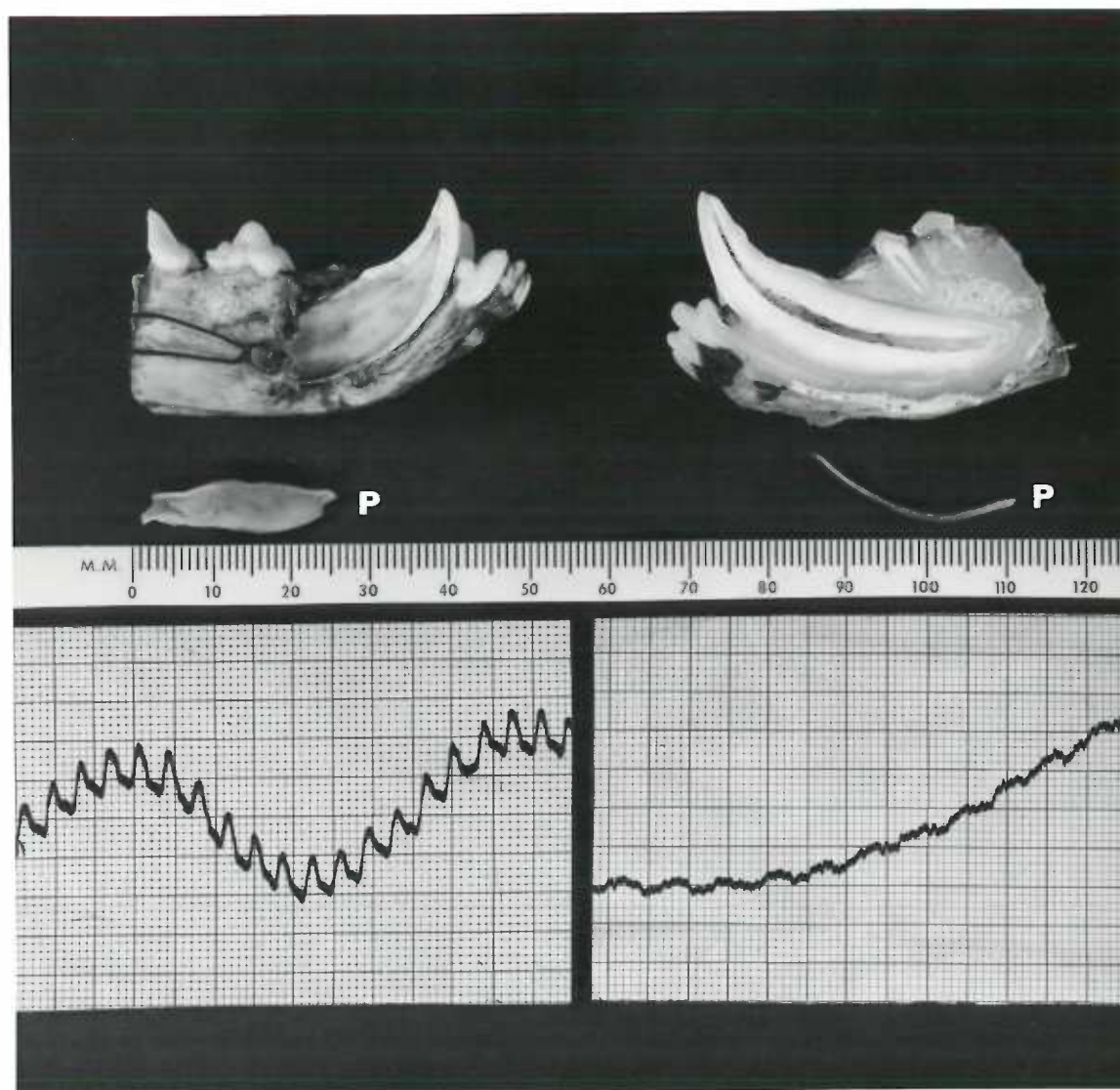


Figure 10: Relationship of size of pulp to amplitude of PED wave

P - extirpated pulp tissue



Figure 11: Relationship of pulp size to vessel size

while the systemic blood pressure and ECG tracing remained unchanged. After approximately one minute, a muted PED pulsation reappeared (Figure 9). At the same time, blood was seen to be issuing from the unligated severed distal end of the mandibular artery.

4. Ligation and section of the third bundle containing the mandibular vein and other tissues was not associated with a change in the PED wave.
5. Subsequent ligation of the contents of the contra-lateral mandibular canal was accompanied by total and permanent cessation of the PED wave. The flow of blood from the severed distal end of the right mandibular artery also ceased at this time. No alteration was seen in the systemic blood pressure or ECG wave.
6. The post-mortem study of the contents of the sectioned canines revealed great variation in gross size of the pulp organs. The younger dogs had the larger pulp chambers. The amplitude of the PED wave recorded from canines with large pulp organs was greater than those obtained from smaller organs (Figure 10).
7. A direct relationship of pulp size to vessel size was noted (Figure 11).

DISCUSSION - PHASE II

Since the author was conducting a general study of a relatively new device, quantitation of the relationship of the PED wave amplitude to the degree of partial occlusion of the inferior alveolar artery was not explored. Future studies might include the investigation of this relationship.

The obliteration of the PED pulse which followed total ligation and section of the right mandibular artery adds further support to the contention that the PED is responding to a circulatory phenomenon within the pulp. The reappearance of the PED wave, in an attenuated form, accompanied by simultaneous retrograde bleeding from the peripheral stump of the severed mandibular artery, indicates that the teeth may receive blood by collateral pathways. The delay may be related to the time required by the normally non-functioning channels to open. The fact that ligation of the contralateral mandibular artery caused total and permanent obliteration of this already muted PED pulse indicates that the route of the collateral circulation was probably via the mandibular symphysis.

The relationship demonstrated between pulp size and amplitude of the PED wave might be explained in several ways:

1. A tooth with a large pulp chamber has a relatively thin, transparent dentin wall, thus allowing relatively more light to pass through the tooth.
2. Inasmuch as a hemorheological phenomenon was shown to be responsible for the PED wave, it is reasonable to suppose that the larger vessels could be primarily responsible for a larger PED wave.

3. Part of the explanation for the relationship between pulp size and PED wave amplitude might also be explained in a third way. The beam of light in the PED which passes through the tooth is about 2 mm. in diameter. When the PED is placed on a tooth with a thread-like pulp, only a small portion of the entire width of the beam would be modulated; whereas when the PED is placed on a tooth with a large pulp the entire beam is more likely to pass through pulp tissue and thereby be modulated the maximum amount.

Histologic evidence for sympathetic and parasympathetic innervation of blood vessels in the dental pulp has been reported by Mathews and Dorman (24). According to Kuntz (25), most of these fibers travel to the teeth within the walls of the blood vessels, thus explaining why section of the mandibular nerve had no observable effect on transillumination in this experiment.

Identification of the influence of specific circulatory phenomenon

One plausible explanation for the alterations in transillumination is that they are related to alterations in blood vessel diameter caused by changing pressures associated with each heart beat. An alternative explanation for the pulsatile optical changes is that they are caused by fluctuations in the velocity of blood flow in the vessels. These variations in the velocity induce changes in orientation of blood elements, which in turn cause fluctuations in light transmittance.

According to Provenza (26), the arteries within the pulp are classified as arterioles. Recordings of pulsatile pressures related to heart beat at the level of arterioles have recently been reported by Wiederhielm, 1964 (27). When a glass micropipette (.5-5u tip diameter) was placed into an arteriole of the mesentery of a frog, he noted that "... a rapidly rising anachrotic limb, and a dichrotic notch were demonstrated down to the smallest branches of the arterial tree." Similar alterations in pressure have been demonstrated in the pulp chamber by Brown (28) via a cannula placed through the dentin to the pulp chamber. Although the tip of the cannula was probably in an extra-vascular portion of the pulp, the pulsations recorded were undoubtedly indirect effects from blood vessels within.

There is little mentioned in the literature to indicate that the pulsating pressures associated with the cardiac cycle actually induce alterations in vessel diameter at the arteriolar level. Krogh (29), in reporting on observations of vessels in the rabbit ear with a light microscope noted no visible movement of the arteriolar walls and variations only in the velocity of flow. Palmer (30) also observed the rabbit ear with a light microscope and was unable to detect change in the caliber of the capillaries.

Taylor (31), in 1950, reduced the dentin covering the pulps of rats to a very thin transparent section to facilitate visualization of the living pulp. He was unable to detect change in the caliber of the arterioles related to heart beat when using the light microscope.

Wiederhielm (32) in reporting on his microcannulation experiments in mesenteric vessels of the frog, commented on the absence of visible pulsations of vessels even when pulse pressures of 7-8 mm. Hg. can be recorded within them. Kirk, Rushmer, Woodbury and Wiederhielm (33), also noted the absence of visible pulsations in the minute arteries of the frog mesentery. Furthermore, Niedle and Liebman (34) have indicated that while vessels may be free to expand in unconfined soft tissues, this may not be possible when they are located within the relatively rigid confines of the dentin.

On the other hand, Dorman (35) revealed that he felt the PED was responding to diametrical changes of the blood vessels within the pulp. When questioned by the author about the incompressibility of the pulp chamber and its contents as suggested by Niedle and Liebman, Dorman suggested that intra-pulpal vessels could expand, and thus alter trans-illuminosity, by displacing extra-vascular fluid through the apical foramen and conversely they could contract by a return of the fluid back in the opposite direction. In essence, he suggested a to and fro movement of extra-vascular fluids from the apical foramen with each heart beat. In addition, Dorman said he had calculated the volume of fluid displacement necessary for sufficient blood vessel expansion to actuate the PED to be approximately 0.8 microliters. He has not yet published this finding.

The concept of the movement of extra-vascular fluid in and out of the apical foramen with each heart beat requires the assumption that the force exerted by the expanding blood vessels on the extra-vascular fluids

must be of sufficient magnitude to overcome the resistance to flow created by the cellular structure of the pulp and the narrow apical foramen. If pulsations in vessel size occur, it seems reasonable, based on the theory of Dorman, that they are more likely to occur in young teeth which have large open apical foramen, and therefore have more room for the apical "push-pull" of extra-vascular fluids, than in older, smaller, more confined pulps and foramen.

The investigations of Brown (36) suggest that the extra-vascular intra-pulpal fluids are not freely movable in and out of the apical foramen. He found that the hydraulic pressure necessary to inject 0.07ul of fluid through a cannula threaded into dentin to the pulp of young dogs required approximately 10 seconds to be dissipated. If it takes approximately 10 seconds to dissipate 0.07ul of fluid when injected into the extra-vascular space, it does not seem likely that approximately 10 times that amount of fluid (0.8u - Dorman) could be dissipated in approximately 1/10 of time, i.e., the heart rate. The discrepancy is in the vicinity of a factor of one hundred.

There is evidence, however, that the caliber of intra-pulpal blood vessels can slowly change due to vasomotor activity of prolonged generalized systemic pressure changes (37). The rate at which these vessels change caliber, however, is sufficiently slow to allow time for the displacement of fluid through the cellular elements of the pulp and apical foramen.

The presence of pulsatile pressures related to heart beat has been established at the arteriolar level. These pressures do not appear to cause pulsatile increases in vessel diameter. The possibility of pulsating changes in blood velocity, rather than in pulsating vessel diameter, is reviewed below.

As early as 1896 Howell (38) noted that, "The stream of red corpuscles in an arteriole is rapid and pulsating." Pohto and Scheinin (39), 1958, using the technique of Taylor (40), observed blood circulation within the pulp of a dog's cuspid. In connection with stasis they noted frequent occurrences of a rhythmic, jerking, back and forth movement, of red cells within the vessels. Widmer (41), using high speed microcinematography to study circulation in the rabbit ear also noted a pulsating velocity. Wayland (42) in a study of vessels 30-800 μ in diameter in the rabbit ear using a technique similar to Widmer's reported a pulsating velocity associated with systolic and diastolic pressures.

Johnson and Wayland (43), in 1967, related their observations using a two-slit method to determine velocity of RBC's in a loop of cat mesentery. They noted that, "In most preparations, red blood cell velocity changed in the same proportion as the arterial pressure".

While investigators have not yet quantitated the velocity of flow at various pressures, there does seem to be agreement that blood velocity changes do occur at the arteriolar level and are dependent upon pressure.

Either changes in velocity of blood flow, or changes in diameter of vessels related to heart beat must be caused by changes in intra-vascular

pressure. The intra-arteriolar pulsating pressures related to heart beat apparently do not cause observable pulsating increases in vessel diameter, but rather cause pulsatile variations in blood velocity. These findings are consistent with LaPlace's Law, which states, $P = T/R$; where P is transmural pressure in dynes/cm.², T , tension on the wall of the vessel in dynes/cm., and R is the radius of the vessel in cm. According to this law, the pressure necessary to distend the wall of a small blood vessel is greater than the force needed to distend a large one. Burton (44) notes, "The seemingly fragile structure of the capillary can withstand the distending force of the capillary pressure of 25 mm. Hg. because of its very small radius, which gives the tension in the wall a very great mechanical advantage over the pressure." Therefore, the smaller the artery, the less likely it is to distend due to the cyclic increase of internal pressure related to heart beat.

It appears that the major effect of pulsating intra-arteriolar pressures is not manifested by pulsations in vessel size, but rather by pulsations in the velocity of blood flow.

Apparently then, the pulsating pressures do not cause visible pulsating expansion of the arterioles, but rather appear to induce a pulsating velocity of flow. Velocity changes are likely to alter the transmittance of light through the vessel by either an alteration of the width of peripheral "cell-free" zone or both. The net optical effect of blood flowing, as opposed to not flowing, generally appears to be one of altered transilluminosity.

Even though the literature does not indicate that the diameter of arterioles changes with each heart beat, the possibility that this actually does occur is not precluded. It is therefore possible that the PED is responding to the optical effects caused by alterations of both vessel diameter and blood velocity. Based on the evidence presented in the literature, one might presume that if pulsating diameter changes related to heart beat do occur, their effects on transillumination are probably overshadowed by the effects related to pulsating velocity.

CONCLUSION - PHASE II

1. It was concluded that the response of the PED was due to intra-pulpal phenomena.
2. Changes in heart rate result in corresponding changes in PED wave frequency rate.
3. Reduction of the volume of flow through the inferior alveolar artery caused a reduction in the amplitude of the PED wave, although significant quantitation of this relationship was not established.
4. Total occlusion of both inferior alveolar arteries caused a cessation in PED waves.
5. The mandibular canine appears to have a source of collateral circulation.
6. Section of the inferior alveolar nerve had no demonstrable effect on the PED recording.
7. A direct relationship between the size of the pulp chamber and the amplitude of the PED was noted.
8. There appears to be a direct relationship of pulp size to the size of the vessels within.

GENERAL SUMMARY

A highly sensitive photoelectric device was constructed and tested. It was found to be sufficiently sensitive to detect the changes in optical density created by blood flowing through a capillary tube at changing rates of flow. The mechanism by which these optical changes were brought about in this study appeared to be velocity related orientation of the red blood cells within the column of flowing blood, although precise quantitation of this relationship was not established.

In an effort to determine the relationship of photoelectric responses to circulation within a vital tooth, the device was applied to the canine teeth of dogs. This device was shown to respond to circulatory changes related to heart beat which occur within the pulp. Misleading artifacts, due to extraneous light and various movements, were eliminated through the use of an individually constructed, opaque acrylic mounting block.

The following in vivo characteristics of pulpal circulation were noted through the use of the photoelectric device:

1. A change in heart rate caused a corresponding change in PED wave frequency.
2. Reduction of the volume of flow through the inferior alveolar artery caused a reduction in the amplitude of the PED wave. Quantitation of this relationship was not attempted in this study.
3. Total occlusion of the inferior alveolar arteries caused a cessation in PED waves.

4. The mandibular canine was shown to have a collateral source of circulation.
5. Section of the inferior alveolar nerve had no demonstrable effect on the PED recording.
6. Relationship between the size of the pulp chamber and the amplitude of the PED wave was demonstrated.

The explanations considered for the pulsatile optical phenomenon were:

1. Pulsatile increases in the intra-vascular pressure may produce pulsatile enlargement of the vessel. These changes in vessel size may produce alterations in the transillumination of the tissue.
2. Pulsatile increases in the intra-vascular pressure may produce pulsatile increases in the velocity of flow of blood. During the period of the cycle when rapid flow occurs, the orientation of the cellular elements within the blood is not the same as during the period of reduced flow. This pulsatile rearrangement of the cellular elements may produce pulsatile alterations in the transillumination of the tissue.

The preponderance of evidence in the literature suggests that those vessels in the rabbit ear, cat mesentery, etc. which are of similar diameter to those found in the pulp, but are not confined by dentin, do not appear to change diameter with each heart beat. Furthermore, the velocity of flow in many small vessels has been shown to fluctuate with

intra-vascular pressure. A number of authors have attested to the occurrence of alterations in the transmittance of light through blood which is flowing at changing velocities, and that these alterations may be due to changing orientation of the red cells.

GENERAL CONCLUSIONS

The PED used in this study was demonstrated to be sufficiently sensitive to register the optical effects created by blood flowing through a capillary tube at changing rates of flow. The mechanism by which these optical changes were brought about in vitro appeared to exhibit a trend towards velocity related orientation of the red blood cells within the column of flowing blood, although precise quantitation of this relationship was not established in this preliminary study.

Pulsatile alterations in the transilluminosity of vital canine teeth of dogs are recordable when the recording apparatus is sufficiently sensitive and stable. The frequency of these pulsations corresponds to the heart rate. The amplitude of the wave thus produced appears to be related both to the size of the pulp chamber and to the flow rate of the blood, although precise quantitation of these relationships has not been established. The intra-pulpal phenomena responsible for the pulsatile transilluminosity may result from a combination of factors, of which velocity related cellular orientation appears to be the major contributor.

At the present time the device is not a quantitative flow meter. It can, however, be utilized to determine the presence or absence of circulation and any relative changes in flow which occur.

BIBLIOGRAPHY

1. Seltzer, S., Bender, I.B., Ziontz, M. The dynamics of pulp inflammation: correlations between diagnostic data and actual histologic findings in the pulp. *J. of Oral Surg., Oral Med., Oral Path.* 16:846, 1963.
2. Provenza, D.V., Biddington, W.R. Effects of the topical application of a vasoconstrictor and a vasodilator on the capillary circulation of the dental pulp. *J. Oral Surg., Oral Med., Oral Path.* 11:1269, 1958.
3. Pohto, M., Scheinin, A. A microscopic observation on living dental pulp. *Acta Odont. Scand.* 16:303, 1958.
4. Liebman, F.M., Perl, J., Bagno, S. The electrical conductance properties of blood in motion. *Physics in Med. & Biol.* 7:2, 1962.
5. Nyboer, J. Electrical impedance plethysmography. Charles B. Thomas Pub. Co. Springfield, Illinois, 1959, p. 36-37.
6. Liebman, F., Cosenza, F. Adaptation of the electrical impedance method to the study of circulation in the tooth pulp. *Federation Proceedings Vol. 19, Part 1: 92*, 1960.
7. Meyer, M., Weiner, D., Grim, E. Blood flow in the dental pulp of the dog. *Proc. Soc. Exp. Biol. & Med.* 116:1038, 1964.
8. Sorenson, F.M. An indirect method for assessing pulpal blood flow. *Research annotations (I.A.D.R. abstr.)*, March 1967.
9. Burnett, E.W., Hohn, W.O. Investigation of possible circulatory changes in pulp. *J. Dent. Res.* 42:1038, 1963.
10. Upthegrove, D.D., Bishop, J.G., Dorman, H.L. A method of detection for blood flow in the dental pulp. *J. Dent. Res.* 45:1115, 1966.
11. Upthegrove, D.D., Bishop, J.G., Dorman, H.L. *loc. cit.*
12. Bayliss, L.E. Rheology of blood and lymph in deformation and flow in biological systems. Ed. A. Frey Wiesslig, Interscience Pub. Inc., New York, 1952, p. 355-418.
13. Taylor, M. The flow of blood in narrow tubes. *Aust. J. of Experimental Biol.* 33:1, 1955.

14. Wiederhielm, C.A., Woodbury, J.W., Kirk, S., Rushmer, R. Pulsatile pressures in the microcirculation of a frog's mesentery. *Am. J. Physiol.* 207:173, 1964.
15. Burton, A.C. Physiology and Biophysics of circulation. Yearbook Medical Pub. Inc., Chicago, Illinois, 1965, p. 54.
16. Bloch, E.H. A quantitative study of the hemodynamics in the living microvascular system. *Am. J. Anat.* 2:125, 1962.
17. Wiederhielm, C.A. Hemorheology. Proc. of First International Congress. Univ. of Iceland. Oxford, N.Y. Pergamon Press, 1967.
18. Burton, A.C. loc. cit.
19. Bloch, E.H. loc. cit.
20. Bayliss, L.E. loc. cit.
21. Elwell, L.H. personal communication.
22. Taylor, M. loc. cit.
23. Kuroda, K., Fugino, M. Fundamental conditions for measuring the streaming transparency of erythrocyte suspension. *Biorheology*, 2: 97, 1964.
24. Matthews, J.H., Dorman, H.L., Bishop, J.G. Fine structures of the dental pulp. *J. Dent. Res.* 38:940, 1959.
25. Kuntz, A. The autonomic nervous system. Lea & Febiger, 1945, p. 157-190.
26. Provenza, D.V. The blood supply of the dental pulp. *Circulation Res.* 6:213, 1958.
27. Wiederhielm, C.A. loc. cit.
28. Brown, A.C., Beveridge, E.E. The relation between tooth pulp pressure and systemic arteriole pressure. *Arch. Oral Biol.* 11: 1181, 1966.
29. Krogh, A. Anatomy and Physiology of capillaries. 2nd ed. Yale Univ. Press, New Haven, Conn., 1929, p. 4-7.

30. Palmer, A.A. A study in blood flow of minute vessels in the pancreatic region of the rat with reference to intermittent corpuscular flow in individual capillaries. *J. Experiment. Physiol.* 44:149, 1959.
31. Taylor, A.C. Microscopic observation of the living tooth pulp. *Science.* 111:40, 1950.
32. Wiederhielm, C.A. *loc. cit.*
33. Kirk, S., Rushmer, R.F. Woodbury, J.W., Wiederhielm, C.A. Pulsatile pressures in the microcirculation of frog's mesentery. *Am. J. Physiol.* 207:173, 1964.
34. Liebman, F.M., Cosenza, F. The study of blood flow in the dental pulp. *Physics in Med. & Biol.* 7:2, 1962.
35. Dorman, H.L. Personal communication. March, 1968.
36. Brown, A.C., Beveridge, E.E. *loc. cit.*
37. Asano, M., Yoshida, K., Tatai, K. Microphotoelectric plethysmography using the rabbit ear chamber. *J. Appl. Physiol.* 20:1056, 1965.
38. Howell, W.H. An American textbook of physiology. W.B. Saunders Pub. Co. Philadelphia, Pa., 1896, p. 375.
39. Pohio, M., Scheinin, A. *loc. cit.*
40. Taylor, A.C. *loc. cit.*
41. Widmer, L.K. Zur stromungs geschwindigkeit in Kleinsten peripheren arterien. *Arch. Kreislaufforsch.* 27:54, 1957.
42. Wayland, H. Rheology and microcirculation. *Biblio. Anat.* 5:533, 1965.
43. Wayland, H., Johnson, P.C. Erythrocyte velocity measurement in microvessels - a two-slit photometric method. *J. Applied Physiol.* 22:333, 1967.
44. Burton, A.C. *loc. cit.*

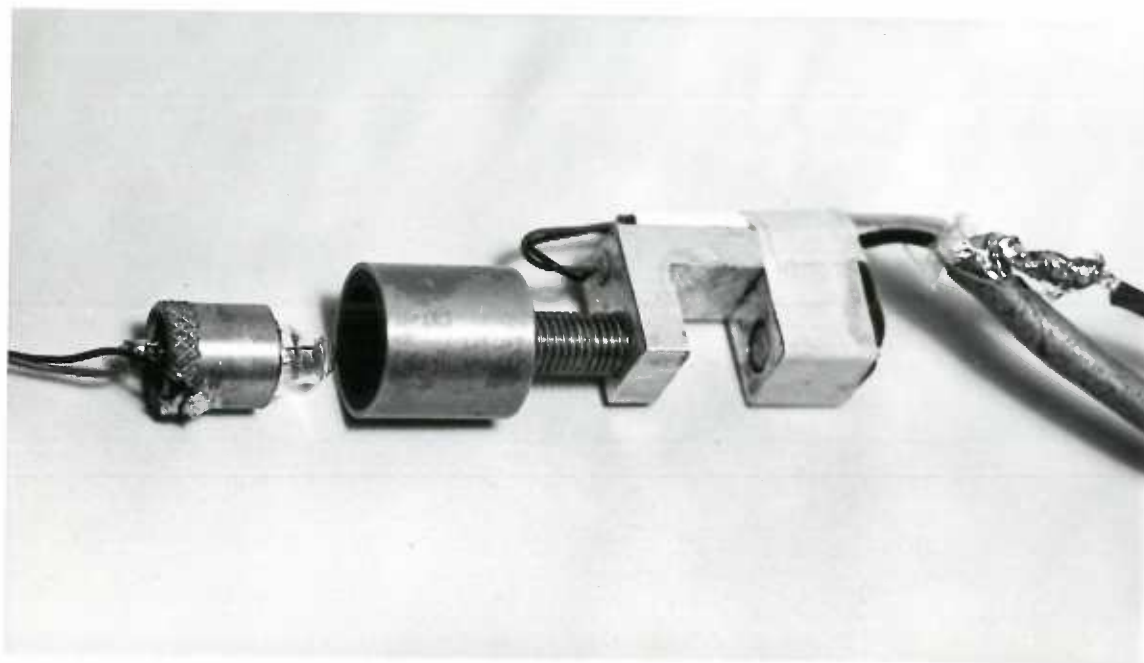


Figure 12: The transilluminating photoelectric device (PED)

A - Light source B - Hollow shaft C - Photocell

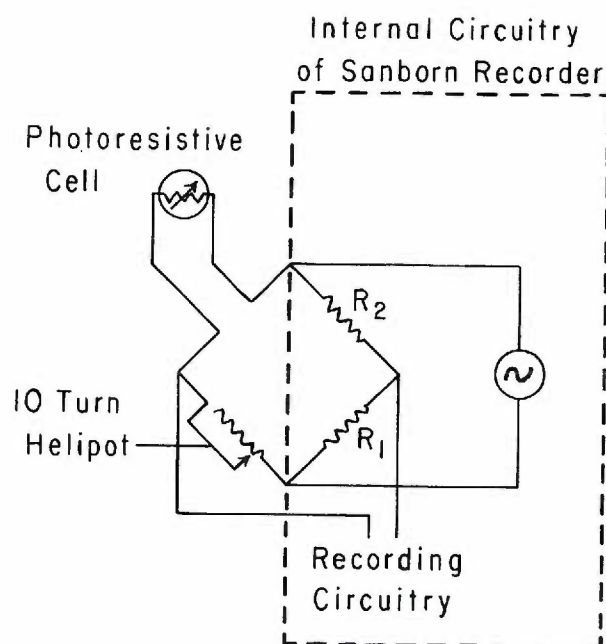


Figure 13: Wiring diagram of PED in wheatstone bridge

APPENDIX A

The Transilluminating Photoelectric Device and Related Circuitry

The PED has a design similar to that of a "C" clamp (Figure 12). A photo-resistive cell¹ was imbedded in the machined aluminum fixed jaw, and a light source² was incorporated into the machined brass movable jaw. The movable jaw was threaded into the fixed jaw and could be advanced securely against any object to be transilluminated. To insure a constant intensity of the light source, power was obtained from three 1.5V telephone-type dry cells wired in parallel. The photo-resistive cell was introduced as one leg of the Wheatstone bridge in a Sanborn strain gauge amplifier³ (Figure 13). Changes in resistance caused by variation in light falling upon the photo-resistive cell were recorded on one channel of a Sanborn polygraph⁴.

¹ Clairex CL604-L, Clairex Corp., 1239 Broadway, New York, N.Y.

² GE 1.5V, .3A - Flashlight type

³ Model 67-500 - Sanborn Company, Cambridge, Mass.

⁴ Model 67M - Sanborn Company, Cambridge, Mass.



Figure 14: Retaining block mounted on sectioned tooth

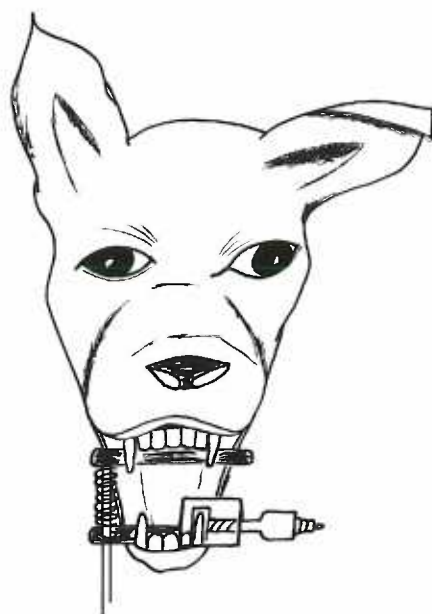


Figure 15: Retention of PED & Block with mouth gag

APPENDIX B

Stabilizing Block

The technique of the construction and application of the block is described here:

1. An alginate impression of the tooth to be transilluminated was obtained. Tranquilization of the dog was necessary for the above procedure.
2. Rapid setting stone was then poured into the impression.
3. After separation, a soft mass of opaque acrylic was then molded over the die and allowed to polymerize.
4. When hard, the block was removed from the die and trimmed so that its external dimensions were approximately the same as the internal dimensions of the PED.
5. Finally a hole, approximately 3 mm. diameter, was bored through the acrylic to provide a route for light to pass from the source through the tooth, to the photocell. (Figure 14).
6. After placing the block on the tooth, the PED was secured to the block by advancing the movable jaw.
7. Positive retention was further insured by pressing the block onto the tooth with a mouth gag (Figure 15).

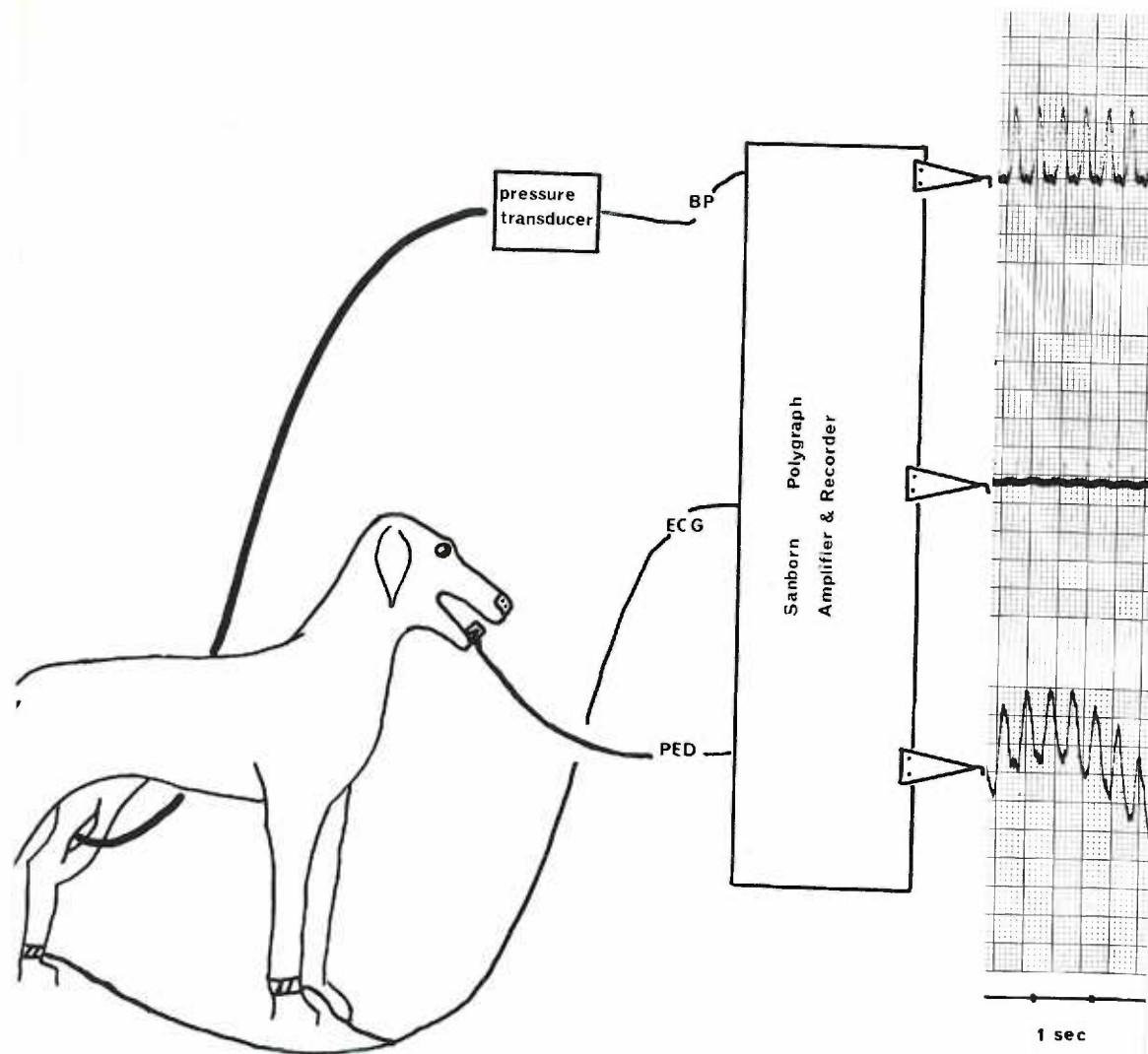


Figure 16: Schematic diagram of method for obtaining simultaneous recordings of B.P., ECG, & PED

APPENDIX C

Simultaneous Blood Pressure, ECG & PED Recordings

Continuous, simultaneous recordings of blood pressure, ECG, and PED were obtained by: (Figure 16)

1. Blood pressure was monitored via an indwelling glass cannula placed in the right femoral artery. The pressure was transduced¹ to electrical signals which were in turn fed to the Sanborn polygraph², and recorded on one channel.
2. An electrocardiogram was obtained from electrodes placed on the right foreleg and left hind leg of the animal. These electrodes were connected to the ECG pre-amplifier component of the Sanborn and recorded on a second channel.
3. The signal from the photo-resistive cell in the PED was transmitted to the Wheatstone bridge which is incorporated into the strain gauge amplifier component of the Sanborn, and recorded on a third channel.

¹ E and M Physiograph - Bourdon Photo-electric type #91-300-70.

² For convenience in making electrical connections, the Physiograph transducer was connected to a Physiograph amplifier, and the output signal was fed to the Sanborn.

APPENDIX D

Endodontic Procedure

This endodontic procedure was accomplished by cutting an opening at the tip of the cusp with a #170 bur in an air turbine handpiece. The coronal pulp tissue was removed through this opening by use of a barbed broach and a #12 reamer. The bleeding radicular pulp stump was cauterized with formocresol and the empty portion of the pulp chamber was filled with a zinc-oxide-eugenol paste.



Figure 17: Delineation of window
over mandibular canal

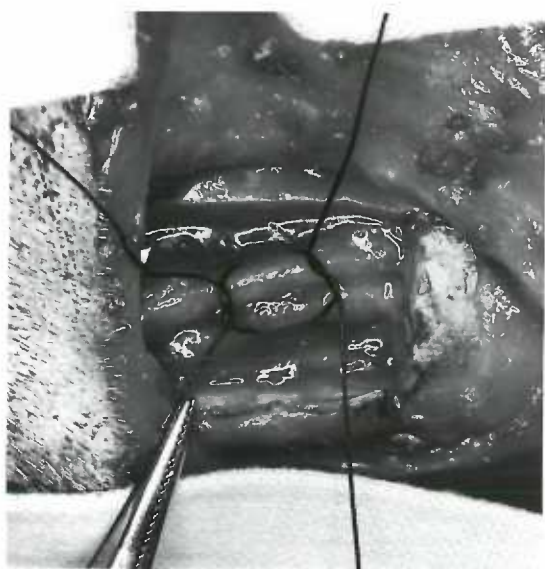


Figure 19: Ligation of vessels
within the mandibular
canal



Figure 18: Exposed contents of
the mandibular canal

APPENDIX E

Surgical Exposure of Contents of Mandibular Canal

1. A flap exposing a large portion of the right masseter muscle was created by making an incision along the posterior and inferior borders of the right mandible. The muscle and periosteum was reflected from the posterior lateral aspects of the mandible. A roughly rectangular area of denuded bone approximately 3 cm. x 5 cm. was thus exposed.
2. A shallow trench in the bone delineating an area of approximately 1 cm. x 2.5 cm. was cut with a dental air turbine (Figure 17). The depth of this trench was very carefully increased until the compact external bone was penetrated. The rectangular island of bone thus created was now detached from its base with a dental elevator, thus exposing a 2.5 cm. length of the mandibular canal and its contents (Figure 18).
3. The protective fibrous sheath surrounding the contents of the canal was gently teased away. The inferior alveolar nerve and artery were identified. The remaining contents consisted of loose connective tissue, venous, and lymphatic vessels. The latter, however, were not always clearly recognizable.
4. The inferior alveolar nerve was isolated and a loose pick-up type of ligature was placed around it. The same was done for

the inferior alveolar artery. The remaining contents of the mandibular canal, i.e., the connective, venous, and lymphatic tissues were loosely ligated in one common bundle (figure 19).

5. Procedures 2, 3, 4, and 5 were then performed on the left side of the mandible with the exception of an alteration of the ligating procedure. On the left side the entire contents of the mandibular canal were included in one large bundle and loosely ligated with a double ligature.
6. Simultaneous baseline recordings were obtained for blood pressure, ECG, and PED, as described in Appendix C.



Figure 20: Pulp tissue uncovered in situ

APPENDIX F

Post-Mortem

The entire anterior portion of the mandible was removed with scalpel and bone saw. Most of the soft tissue and alveolar bone were removed from the lateral aspect of the right canine by use of scalpel, chisel, and bone bur. The surface of the canine was scored with a #170 bur along its mid-sagittal plane. The tooth was then fractured in half by insertion of an instrument into the scored groove and the application of a twisting and prying force. The lateral section of the tooth split off, leaving the entire pulp tissue in the remaining section (Figure 20).