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PULPAL CIRCULATION FOLLOWING REPLANTATION

OF TEETH IN MONKEYS

by

Ronald M. Milnarik, B.S.D., D.D.S., M.A.

A Thesis Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of

Master of Science

June

1977

The second balance model femer

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Ronald M. Milnarik, the son of Dr. Marshall W. Milnarik and Florence (Galvin) Milnarik, was born in Chicago, Illinois, on September 30, 1942.

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LIFE

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INTRODUCTION

When accidentally avulsed teeth are quickly returned to place, they are usually reattached and may function normally for many years. The pulps of such replanted teeth may undergo necrosis, but proper endodontic therapy will favor their retention. Occasionally the pulp will remain vital, surviving with only histologic alterations. The root surface can be affected by resorptive processes which may compromise the retention of the teeth.

Investigation of replanted teeth in humans is generally very limited. Radiographic evaluation is always possible, but histologic study can only be done when failure has necessitated extraction. A physiologic approach to replantation could yield valuable information about the mechanisms of reattachment. If this were done on laboratory animals, the replantations could be performed under controlled conditions and the healing process could be closely observed.

One physiologic method that has attracted attention recently is photoplethysmography, a photoelectric technique for recording blood flow. Since the circulation of blood within a tissue is the prime indicator of its vitality.

this technique can provide significant information. When used on teeth it detects changes in the ability of the tooth to transmit light. Pulsatile changes are noted which correlate with systemic circulatory activity and are thought to be indicative of blood flow in the dental pulp. The principle advantage of photoplethysmography is that it introduces minimum trauma to the subject teeth. Other methods require histologic section or <u>in vivo</u> removal of tooth structure to visualize pulpal conditions. This type of photoelectric blood flow recording device subjects the tooth only to the passage of a light beam.

This study was designed to evaluate photoelectrically the blood flow within replanted teeth of monkeys.

LITERATURE REVIEW

Pulpal Circulation

The dental pulp of the Rhesus monkey is supplied by small arteries and veins which pass into the tooth via one or more foramina. Normally a main foramen is located near the root apex, but accessory, lateral, and furcation canals may all make contributions to the pulpal vasculature. Kramer (1960) reported that the human pulpal vascular system originates with several arteries which run coronally through the central portion of the pulp. They give off many side branches that pass primarily to the periphery. There they arborize to form a dense plexus located just beneath the odontoblastic layer of cells. This plexus is drained by vessels which empty into several large central collecting veins. According to the microangiographic studies of Saunders (1967), the pulpal vascular pattern in the Rhesus monkey is very similar to the human.

Dahl and Mjör (1973) report that the small arteries entering the human pulp have diameters up to 150 microns. They branch into the arterioles which are about 20-40 microns in diameter. The tunica interna of the arterioles

is a single layer of endothelial cells with some elastin fibers, while the tunica media contains 1-3 layers of spirally-arranged smooth muscle cells. The terminal portions of the arterioles, the metarterioles, have occasional smooth muscle cells in their walls. The metarterioles seem to connect directly with venous channels and may serve as the preferred path for circulation through a tissue.

Each metarteriole gives off several sharply-angled capillary branches. The first portion of the capillary contains a ring of smooth muscle and is known as the precapillary sphincter. This sphincter, 15-20 microns in diameter, controls access to the capillary. The capillary itself is narrow, only 4-8 microns in diameter. The wall is 1 micron thick and consists of a single layer of flat endothelial cells. It seems to be designed for diffusion only. No muscle is present to diminish the size of the lumen and no elastin is present to permit expansion, so the capillaries are passive conduits for what the arterioles and pre-capillary sphincters direct to them.

Venules are larger (20-40 microns) and collect from several capillaries. They have discontinuous smooth muscle cells and contain a series of micro-valves (Gängler and Pilz, 1974b). These veins have thin walls and lumina several times the size of corresponding arteries. The tunica media is one muscle cell layer thick and this permits

the development of contractile pulsations which may aid in venous return.

Several authors have reported the presence of muscular artero-venous shunts in the pulpal tissue. Kramer (1960) demonstrated them between the large central vessels. Gängler and Pilz (1974b) described them in the peripheral plexus. In an enclosed organ like the tooth these shunts might be important in regulating blood inflow.

The basic function of the vascular system is of course to supply the tissues with their needs and to remove their waste products. In order for the blood to effect this exchange, the rapid intermittent flow produced by the heart must be dampened to a pace suitable for capillary diffusion. In humans the aortic blood velocity of 630 mm/sec is reduced to about 1 mm/sec in the capillaries (Whitmore, 1968). It is primarily the increased cross-sectional area of the arteriolar tree and capillary bed that accounts for this slowing. The total cross-sectional area of the arteries at 300 cm² increases to 2000 cm² for the arterioles and 4500 cm² for the capillaries (Berne and Levy, 1967). Blood pressure is similarly decreased, from near 100 mm Hg in the aorta to 30-85 mm Hg in the arterioles and 10-30 mm Hg in the capillaries. Henry and Meehan (1971) point out that the elastic arteries absorb the energy of the pulse peak and store it. Their recoil then keeps the blood flowing

between heart beats. McDonald (1960) reports that the damping effects of the freely elastic great vessels and the resistance of the less elastic arterioles combine with wave reflection patterns to diminish the pulsatile character of the pulse wave. Whitmore (1968) found almost no pulse wave in the capillaries.

Overall blood pressure is monitored by sensors located in the carotid furcation and aortic arch areas. Impulses from here are transmitted to the medullary vasomotor centers. Cardiac activity is then controlled by way of sympathetic and parasympathetic nerves. Blood pressure is further regulated by the sympathetic control of the degree of arteriolar constriction. Unmyelinated sympathetic nervous fibers are found in the walls of vessels down to arteriolar and metarteriolar sizes (Henry and Meehan, 1971).

Beyond the metarterioles no evidence of sympathetic control is seen. The regulation of blood flow to different capillary beds is primarily under local control. The precapillary sphincters seem to be affected most by their local environment. High levels of cell metabolites and low oxygen levels cause a relaxation of the ring of smooth muscle cells which normally keep the passage-way into the capillary closed. When the sphincter relaxes, blood is allowed to enter the capillary and the levels of metabolic substances are returned to normal. This opening and closing occurs in

a regular pattern and is known as vasomotion. It has a periodicity of every 30 seconds to several minutes (Zweifach, 1957).

Such combined sympathetic and local control over blood distribution appears to prevail in the dental pulp also. Provenza (1958) found that nerve fibers are intimately associated with blood vessels in the human dental pulp. Matthews <u>et al.</u> (1959) report that dense bundles of nerve fibers are contained in the tunica externa of dog pulps down to the pre-capillary level. They observed autonomic nerve fibers contacting smooth muscle cells in arterioles. In an electron-microscopic investigation Engström and Öhman (1960) found a profuse branching of unmyelinated fibers in company with the arterioles in the subodontoblastic plexus of human teeth.

Several investigations have demonstrated the function of these sympathetic fibers in the dental pulp. Neidle and Liebman (1964) reported decreased blood flow to the dental pulp of cats when the cervical sympathetic nerve was stimulated. Weiss <u>et al.</u> (1972) found pulpal blood pressure decreased when the cervical sympathetic chain was stimulated. When Taylor (1950) stimulated the mandibular nerve he observed no change in pulpal blood flow, though it did diminish when the cervical sympathetic chain was stimulated. The narrow lumen of most capillaries at 4-8 microns is too small to let red blood cells flow freely, since human erythrocytes have an average diameter of 8 microns. Red blood cells are readily folded and bent, however, and this permits them to creep through these narrow vessels.

The slow, passive blood flow of the capillaries stands in sharp contrast to the arterioles, where the arterial pulse wave strongly affects the blood flowing through a large lumen. A vastly stronger and more regularly pulsating pattern of blood flow in the arterioles is the result. The size of the lumen in arterioles is 20-40 microns and since this is much larger than the 8-micron red blood cells, certain peculiar flow characteristics of blood are noted here. These deviations from the normal flow of liquids were explained by Fahraeus and Lindqvist in 1931. Thev stated that in vessels from 20-30 microns in internal diameter blood flows in a laminar pattern with a central streamline. The external lamina is slowed by friction along the walls of the vessel. Successive internal layers are slowed less and less by this friction, so that the central lamina flows twice as fast as the mean velocity. The formed elements of the blood flow in this faster central streamline.

Bloch (1962) found that at the linear flow velocities usually encountered in arterioles regular laminar flow gives way to turbulent flow. He used high speed cinematography

to demonstrate that blood flow in such vessels is very disorderly. Red blood cells travel in a helical pattern, tumbling about their long and short axes. They are constantly bumping other cells and being compressed, lengthened, folded and twisted. All these contortions can occur within milliseconds. Most cells flow centrally in the vessel but many make excursions into the external plasma layers and bounce off the walls.

Blood Flow Studies

The study of changes in the volume of blood flow to an area is called plethysmography, from <u>plethys</u> - mass and <u>graph</u> - to write. In their excellent history of plethysmography Barcroft and Swan (1953) mention that methods for detecting changes in the volume of organs were in routine use in the seventeenth century. These employed closed tubes into which a limb was inserted. Changes in the height of an attached column of water were noted. Buison was the first to graphically record such findings. In 1905 Brodie reported that by blocking the vein exiting the plethysmograph chamber he could obtain a dynamic recording of blood inflow into the organ.

Because of its hard encasement the dental pulp was not accessible with such methods. An early attempt at studying

pulpal blood flow by Taylor (1950) involved grinding rats' incisor teeth until only a thin layer of dentin remained. Taylor found that he could microscopically observe circulatory changes through the transparent dentin. Pohto and Scheinin (1958) reduced the tooth structure of rats' incisors to within 15 microns of the pulp. They then used photographic techniques to study the effects of thermal stimuli on pulpal circulation. This technique was recently used by Gängler and Pilz (1974a) for observation periods up Beveridge and Brown (1965) introduced a techto 2 days. nique for recording intrapulpal pressure. This involved cutting a channel through tooth structure to the pulp surface. A minute cannula was screwed into this channel and dynamic pulsations synchronous with an electrocardiogram were obtained. Even dicrotic notches were visible in their recordings. Edwall and Olgart (1972) measured with great success the clearance rates of radioactive iodine deposited in deep dentinal cavities. In the recent report of Tönder and Aukland (1975) an electrode was inserted intrapulpally. It was used to measure the passage of hydrogen gas which had been introduced into the circulatory system via an endotracheal tube.

The electrical impedance properties of blood flow in teeth have been investigated by Liebman and Cosenza (1962) and Neidle and Liebman (1964). They report that when a

radiocurrent was applied to the teeth of dogs, reception of the signal fluctuated. These fluctuations corresponded with the electrocardiogram and were affected by heating and cooling the teeth and by occluding the carotid artery. They felt that the fluctuations were caused by the redistribution and reorientation of red blood cells within the vessels of the pulp. The electrodes in these experiments were attached to deep cavities prepared in the dentin of the teeth. In order to avoid tooth preparation on intact human teeth Brown <u>et al.</u> (1966) applied the electrodes directly to the enamel surface with a contact medium. They were unable to record significant activity with the electrical impedance technique.

All of the above techniques introduce trauma to the dental pulp so that the observations made are not of a normal pulp, but of an altered one. Bishop and Dorman (1968) review the techniques used in studying the circulation in teeth. They mention that only two methods are atraumatic. One based on calorimetry was employed by Adler <u>et al.</u> (1969). They discount it, as the heating effects of the periodontium overwhelm those of the pulp. The other method is a photoelectric one called photoplethysmography. Photoplethysmography is based on the very great absorbance of light in certain spectra by red blood cells. Weinman(1967) mentions that, whereas a certain thickness of

tissue would absorb 38% of the incident light, the same thickness of whole blood would absorb 99.3% of it. Because of this the volume of blood in an organ can have a very great effect on its optical density. This is particularly true in thin fleshy areas like the ear, nasal septum and finger. Recordings in such areas indicate increased tissue opacity as venous outflow is restricted or as flushing is observed. Under normal conditions a regular pattern of pulsatile waves can be noted in photoplethysmograph records. They can be correlated with systemic circulatory pulsations and are thought to be indicative of blood flow.

Photoplethysmography can serve as an excellent means of monitoring changes in blood flow within these types of soft tissues. In other areas it is more difficult to demonstrate pulsatile changes in tissue opacity. This is particularly true for teeth. The blood volume is small, only 0.003 ml in the incisor teeth of dogs (Kraintz and Conroy, 1960). In addition the pulp is shielded from direct passage of light by the dentin and enamel of the crown. Despite these difficulties photoplethysmography has been pursued in dental research because of the potential advantage of its atraumatic nature.

The very high absorption of light by blood is due to the presence of hemoglobin, which comprises 25% of the erythrocyte volume. Hemoglobin is most effective in

absorbing light when it is incorporated within an erythrocyte. Whole blood absorbs 5 times as much light as an equivalent hemolyzed solution (Kramer <u>et al.</u>, 1951). This is caused by the reflection and refraction of light within each red blood cell, so that the light encounters not just one, but a great number of hemoglobin molecules in its route through the cell. Another factor is the scattering of light by the erythrocytes as they tumble about in the flowing blood. Light thus may be bounced off or forced to pass through many erythrocytes before it can eventually traverse a column of blood. Loewinger <u>et al.</u> (1964) found in rabbit blood that scattering accounts for 20 times the increase in optical density that absorption would.

The type of light used in photoplethysmography is very important. Light of wave lengths less than 6000 angstroms is 100% absorbed by blood. Red light at 6400 angstroms is 100% absorbed by reduced blood, but only 30% by oxygenated blood. At 7350 angstroms reduced blood absorbs 70% and oxygenated blood 30% of the light. The wave length 8050 angstroms in the near-infrared portion of the spectrum is significant in that 50% of such light is absorbed by the blood regardless of its oxygen content (Fox and Wood, 1960; Nilsson, 1960). These differences permit the selection of a wave length that will either maximize or minimize the effect of the oxygen content of the blood in the tissue under study.

The first person to apply the photoelectric technique to research was Bonsmann (1934). He was interested in studying the effect of certain drugs on blood pressure. A miniature blood pressure cuff was placed around the tail of a rat and inflated. As the pressure in the cuff was reduced, he used a photocell and ammeter to determine when blood was once again flowing in the tail. The photoelectric apparatus thus substituted for a stethoscope in determining blood pressure.

Hanzlik <u>et al.</u> (1936) used a very bulky tungsten lamp and photronic cell to observe vasoconstriction and vasodilation in the rabbit ear. Deflections were graphically recorded and measured. Molitor and Kniazuk (1936) used a flashlight bulb and vacuum-type photocell. They included a thermocouple to give skin temperature and thus were able to distinguish between active hyperemia and passive congestion.

Hertzman was the primary exponent of photoplethysmography in the late 1930's and the 1940's. In his first report on the subject (Hertzman and Spielman, 1937) he describes a typical wave-form from a transilluminated human finger. It had a steeply rising anacrotic limb and a more gradually falling catacrotic limb containing a dicrotic wave. He found a particular form to be characteristic of an individual. Later (1937) Hertzman mentioned that variations in red blood cell number, shape and oxygen concentra-

tion, as well as the axial stream effect in small vessels, could affect the readings. In 1938 he reported on using scattered light instead of transilluminated light to observe vasomotor reactions in the skin at many different sites in humans. Hertzman and Dillon (1940) pointed out the advantages of photoplethysmography in comparison to mechanical volume plethysmography. These included speed, sensitivity, convenience and applicability to areas which could not be placed in a container. Attempts were made to quantify the deflections found in the finger with scattered light plethysmograms (Hertzman <u>et al.</u>, 1946).

In all these efforts by Hertzman photoemissive cells were used. In 1960 Weinman <u>et al.</u> introduced the photoconductive cell. These small cadmium selenide photocells rejuvenated research in photoplethysmography as their small size permitted application to many formerly inaccessible areas. Weinman and Manoach (1962) described their efforts with several types of photoconductive cells.

Giddon \underline{et} al. (1963) used narrow fiberoptic guides to transmit light to the gingival tissues and back to the photoconductive cell. They found pulsatile activity which was affected by vasoactive drugs.

In using a photoplethysmograph to evaluate blood flow in the ear and brain simultaneously, Heck and Hall (1964) obtained nearly identical recordings. This is significant

for dental pulp studies in that it demonstrates that plethysmography appears to be valid in rigidly encased organs.

Hocherman and Palti in 1967 reported on a brilliantly designed study. In this they recorded the photoelectric pulse from a finger, while it was encased in a water-type volume plethysmograph. Synchronous volume plethysmograph and photoplethysmograph waves were found, indicating that the finger expanded as photoelectric pulses occurred. When the water chamber was closed off, however, and no expansion of the finger was possible, the photoelectric pulsations continued with only slight diminution. This demonstrated the photoelectric apparatus was not just detecting gross enlargement of the organ.

In 1967 D'Agrosa and Hertzman developed a microscopic arrangement which enabled them to monitor pulsatile activity within individual vessels. They studied the mesentery of the frog and rat and found regular tissue opacity pulses only in arteries and arterioles. No such pulsing could be detected in capillaries or veins.

Pike (1969) conducted an <u>in vitro</u> investigation in which he photoelectrically monitored the flow of defibrinated sheep's blood through a capillary tube. When flow rates were varied, changes in the optical transmission of the blood were noted. He ascribed these alterations to changes

in the orientation of the red blood cells under different flow conditions. This explanation is consistent with earlier findings. Loewinger <u>et al.</u> (1964) stated that in flowing blood scattering by erythrocytes diminishes the light 20 times as much as absorption alone would. Bloch (1962) observed that turbulent flow occurs in arteries and arterioles. D'Agrosa and Hertzman (1967) localized pulsatile changes in tissue opacity to the small arteries and arterioles. Taken together these studies explain the operation of the photoplethysmograph. The key is that the amount of light scattered by the erythrocytes varies with the intermittently turbulent nature of blood flow in the arterioles.

Arfors <u>et al.</u> (1975) studied blood flow rates by means of a semi-conductor apparatus and electronic on-line velocity computers. A microscopic image of an individual vessel in the rabbit ear or mesentery was routed to an objective, to which were attached two photodiodes. As a blood cell passed one photodiode there was a change in the amount of illumination falling on the diode. The resultant alteration in the diode's electrical resistance was found to be characteristic of that particular red blood cell. They refer to this as the "electronic signature" of that erythrocyte. When this same response pattern was picked up by the other detector located just downstream, the flow

rate over that distance could be calculated. Recordings of the blood flow velocity in a 25 micron arteriole of a rabbit ear showed regular fluctuations between 2 and 4 mm/sec with time. Such a recording is actually another form of photoplethysmogram. Instead of merely recording pulsatile tissue opacity changes, the opacity changes noted in paired photodiodes are used to obtain a pulsatile recording of the rate of blood flow through a particular vessel.

Burnette and Horn (1963) were the first to apply the use of a photoelectric technique to teeth. They removed some tooth structure from a dog's tooth and applied a photoconductive apparatus. Small but rhythmic pulsatile changes in the tooth's optical density were found. Since they were synchronous with the electrocardiogram they interpreted these changes as resulting from circulatory pulsations within the dental pulp.

This technique was refined by Upthegrove <u>et al.</u> in 1966 so that it could be employed on intact teeth. Working with dog's canine teeth, they recorded regular waves which exhibited a rapid rise followed by a slower decline. A dip in this falling side resembled the dicrotic notch found in blood pressure recordings. In order to rule out the ballistic effects of cardiac contractions, the authors performed several tests. Occluding the carotid artery supplying the tooth caused a great reduction in the level of the recording

and the near disappearance of pulsatile activity. Injecting saline into this vessel produced an overall increase in translucency, while Cardio-green dye decreased the translucency. Neither solution affected the pulse waves themselves appreciably.

Fiberoptic guides were used by Christiansen and Meyer (1969) to record optical density fluctuations in human teeth. They mention that transillumination was much more effective than reflection and speculate that too much scattering and reflection of light within the tooth might be the reason for this.

Reese et al. presented their efforts with human pulpal photoplethysmography in 1971. In developing a dental apparatus they found that close adaptation to the tooth surface was important for stability and for excluding exter-Their final device was a custom acrylic tray nal light. incorporating the lamp and photoconductive cell. A rubber liner was used to retain the appliance and to mask out light. They observed changes in the pulse waves during heating and cooling of the teeth. A recording from an endodonticallytreated tooth showed no fluctuations in optical density at Reese et al. felt that the optical changes they all. observed were due to the turbulence and disruptions to laminar flow which occur as blood velocity is altered during pulpal circulation.

The changes in optical density of teeth caused by periapical injections of different solutions were presented by Zurawic (1972). He used a commercially available photoelectric transducer designed for use on the ear and adapted it to the maxillary central incisors of human subjects. The frequency of photoplethysmograph pulses and the electrocardiogram were reported to be identical and the tooth pulsations were said to be similar for each patient at different sessions. Injection of saline or Xylocaine did not appreciably affect the recordings, while epinephrinecontaining Xylocaine caused a noticeable decrease in pulse amplitude.

In 1973 Shoher <u>et al.</u> reported on their work with photoplethysmography in humans. They used a spring-mounted device containing the lamp and photoconductive cell so that it was readily transferable to different teeth. Rather than comparing their dental recordings with an electrocardiogram, they compared them with photoplethysmographic recordings of the finger and noted good correspondence. The authors ascribe the pulsations to light scattering by the blood in the course of its pulsatile distribution.

Blood flow during sympathetic stimulation in the maxillary canine tooth of the cat was studied by Beer <u>et al.</u> in 1974. They used a tube containing a lamp and photocell which could be slipped over the teeth. Pulsations were

recorded which had the same frequency as the electrocardiogram. Electrical stimulation of the cervical sympathetic chain produced a decrease in amplitude of the dental photoelectric recording.

Replantation

Replantation is defined by Dorland (1974) as "the reinsertion of a tooth into the alveolus from which it was removed or otherwise lost." Grossman (1966) suggests that the term intentional replantation be reserved for situations where a tooth is removed in order to perform endodontic therapy and is then reinserted. He distinguishes replantations from plantations, in which a natural or artificial tooth is inserted into a surgically prepared socket. Transplantation involves inserting a tooth into a different alveolus.

The literature on tooth replantation is voluminous and goes back to the Middle Ages. The majority of the articles are case reports, like Fauchard's recounting of his experience with five replanted teeth (1746). Many articles such as Healey's (1953) present a case and then discuss procedural and theoretical considerations. A third type reports on experimental investigations, where systematic observations can be of great value. The extensive literature on the subject of replantation has been reviewed thoroughly by Coburn and Henriques (1962), Costich <u>et al.</u> (1963), Natiella <u>et al.</u> (1970), and Kaplan and Ward (1971). In view of this only substantial works of particular significance to this research will be cited. Clinical studies will be presented first and experimental investigations later.

In the extensive report of Bielas <u>et al.</u> (1959) the authors recount their experience with the intentional replantation of 1030 posterior teeth. Root canal filling preceded the extraction and apicoectomy and reverse filling followed it. The tooth was returned to its socket and reduced from occlusion with no splinting employed. The extra-oral time varied between 3 and 60 minutes and averaged 12 minutes. They report that 59% of the teeth were still functioning after 5 years but that they were experiencing a 10% annual failure rate.

Traumatically exfoliated teeth were replanted by Lenstrup and Skieller (1959). The majority of the 45 teeth were maxillary central incisors in children. Normally root canal therapy was completed before replantation. The time the teeth were out of the mouth averaged about 1 hour. Splints were applied for 6-12 weeks. At the end of the observation period of 2 months to $5\frac{1}{2}$ years 25 of the 45 teeth were still functioning (56%). Of these all but 4 showed radiographic evidence of root resorption.

Two of these teeth had wide-open, immature apices at the time of avulsion and they were quickly replanted without root canal therapy. These teeth apparently recovered their vitality as the pulpal spaces had been narrowed greatly.

Deeb <u>et al.</u> (1965) reported on an extensive study of 274 intentionally replanted teeth. They used several different techniques. Extraction was followed by either root canal filling or by reverse amalgam filling. In most cases the periodontal membrane was carefully handled, but in some it was purposely scraped off. The teeth were splinted for 4-6 weeks. The best success rate at 5 years was 74% for the root canal-filled teeth with intact periodontal membrane. This compared with 10% for the root canalfilled teeth which had been scraped.

A group of 110 anterior teeth replanted after accidental loss were evaluated by Andreasen and Hjørting-Hansen (1966a). About half the teeth were root-filled prior to replantation. A further quarter had root canal treatment done 2-3 weeks later and the balance, having incomplete root end formation, did not receive any root canal therapy. In nearly all cases splinting was used. The teeth were observed clinically and radiographically for up to 13 years. Root resorptions were noted according to the classification of Andreasen (1972). <u>Surface resorption</u> involves isolated areas of cementum and sometimes dentin. It is a

normal finding and usually is repaired by cementum. This type of resorption is not visible radiographically and the teeth Andreasen and Hjørting-Hansen reported as having no resorption probably had surface resorption histologically. Replacement resorption indicates that alveolar bone tissue has eroded into the root, replacing it. This normally occurs in areas where the periodontal membrane was lost. It appears between 3 and 12 months, progresses at a variable rate, and eventually leads to tooth loss. Radiographic evidence of replacement resorption is erosion of the root without an accompanying radiolucency. Inflammatory resorption is characterized by rapid destruction of the root by inflammatory-type tissue. This can be traced to the presence of necrotic debris in the root canal. The authors found the process could be halted by performing adequate endodontic treatment. The root destruction is accompanied by a radiolucent defect. Such teeth are usually lost within In their study (1966a) Andreasen and Hjorting-1 year. Hansen reported no resorption for 25%, replacement resorption for 40%, and inflammatory resorption for 35% of the teeth. Of particular significance was the authors' study of the effect of the length of the extra-oral period upon resorption. No resorption was found in 90% of the teeth replanted within 30 minutes. After this the percentage dropped rapidly. It was 50% at 31-60 minutes, 38% at 61-90

minutes, 18% at 91-121 minutes, and 5% for longer than 120 Thirteen of the teeth had only partial root minutes. formation and were replanted without endodontic therapy. Of these, seven apparently were revascularized, showing gradual obliteration of the pulp chamber. This finding is consistent with the observation of Weine (1976), "Immature teeth rapidly replanted without pulp extirpation may require no further endodontic treatment. . . The open apex allows for a replacement and/or repair of the severed pulp vessels." The other six teeth with partial root formation in Andreasen and Hjørting-Hansen's study experienced pulpal necrosis and severe inflammatory root resorption. Six teeth with completed root formation never had root canal fillings per-Four of them demonstrated continued dentin formaformed. tion and in the other two the pulp necrosed. Stressing the importance of minimizing the extra-oral period, the authors suggest that all teeth be replanted as quickly as Root canal therapy should be undertaken later possible. for teeth with completed root end formation. Teeth with open apices should be observed closely and endodontics instituted if periapical breakdown develops.

In another 1966 study Emmertson and Andreasen reported on the intentional replantation of 100 molar teeth. Gutta percha or amalgam root fillings were placed extra-orally and great care was given to maintaining the viability of the periodontal fibers. Observations up to 13 years were carried out. No resorption was found in 68%, replacement resorption in 4% and inflammatory resorption in 27% of the recalls. They examined 32 teeth showing no resorptions past 5 years, 25 past 7 years, 12 past 9 years, and 5 beyond 10 years.

Grossman and Chacker (1968) studied 61 intentionally replanted posterior teeth. Root canal fillings were accomplished prior to extraction when possible. Otherwise reverse amalgam fillings were done extra-orally. Splints were used for 1 month. They report 57% of the teeth showed no signs of resorption up to 11 years.

In 1977 Kemp <u>et al.</u> reported on 71 accidentally avulsed and replanted teeth. These cases were done by many individuals with varying techniques. Only 20% of the teeth observed beyond 2 years had no root resorption.

The earliest significant experimental investigation was done with monkeys by Butcher and Taylor (1951). They applied intrusive forces to the mandibular central incisor teeth for 2 weeks. The authors claim that ischemia of the dental pulp resulted and that necrosis of the coronal pulp ensued. After removal of the force-producing appliance, they describe a migration of cells from the apical area into the necrotic tissue. The necrotic pulp appeared to serve as a matrix for tissue ingrowth. Hard-tissue production

which had ceased was begun by osteoblast-like cells that laid down first some structureless material and then atubular dentin. Later regular tubular dentin was once again formed. There was a gradient with the most tissue destruction and least normal dentin coronally and a more normal appearance apically.

In 1958 Flanagan and Myers published the results of their study of tooth replantation in hamsters. They found that, after healing, the epithelial attachment and the periodontal membrane appeared normal and that the dental pulp was often revascularized. The incidence of these favorable responses to replantation decreased markedly as the extraoral time increased. "Good takes" were reported for 60% of the teeth replanted within 30 minutes but for only 3% of those out of the mouth more than 6 hours.

Dogs and monkeys were the experimental animals used in a 1961 study by Löe and Waerhaug. They intentionally replanted teeth, varying the treatment of the periodontal membrane. Subsequent histologic examination showed extensive replacement resorption of those root surfaces denuded of fibers before replantation. Air-drying resulted in many small areas of replacement resorption. Teeth replanted immediately had small surface resorptive areas initially but these were all repaired by cementum at the 3-year observation period. The periodontal attachment was otherwise normal.

Rockert and Öhman (1962) used microradiograms to study the alterations in dentin formation brought about by extraction and immediate replantation. The subjects were human volunteers whose teeth were to be removed for orthodontic They found numerous areas of globular radioreasons. opacities near the pulpo-dentinal junction. Sometimes large demineralized areas were noted. Coarse irregular deposits of osteoid were occasionally seen as well. Tn 1965 Ohman reported on a series of 85 immediately replanted Histologic evaluation was done after up to human teeth. 1 year of retention in the mouth. At 1-2 days no pulpal changes were seen except for the residue of apical hemorrhage. On the third day cellular distortion and nuclear lightening were noted and by the sixth day complete pulpal necrosis In 12 day specimens spindle-shaped mesenhad occurred. chymal cells were observed infiltrating into the necrotic As this tissue ingrowth progressed, dentin-producing areas. cells differentiated. The product of these cells was at first very irregular with many cellular inclusions. Later it became more tubular and eventually appeared normal.

Andreasen and Hjørting-Hansen (1966b) had the opportunity to study 22 of the replanted anterior teeth included in their larger report. Six of these teeth had incomplete root development at the time of avulsion. Five of the six showed signs of replacement of the damaged pulp tissue by apical connective tissue.

The repair process in replanted hamster teeth was detailed by Costich <u>et al.</u> (1966). At 3 days there was necrosis of the pulp with an infiltration of polymorphonuclear neutrophils apically. Within a week there had been a replacement of the pulp by granulation tissue from the apical third and a gradual invasion of the coronal pulp by inflammatory cells. By the end of a month inflammation was greatly decreased and connective tissue had replaced the granulation tissue present before. Bone was being deposited in the root canal and coronal pulp. Within 6 weeks marrowcontaining bone had filled the entire pulpal space and ankylosis was occurring radicularly.

Sherman (1968) confirmed the 1961 finding of Löe and Waerhaug that replacement resorption occurred most frequently where the periodontal ligament had been disturbed.

In the experimental portion of their 1968 report Grossman and Chacker described the attachment apparatus of intentionally replanted teeth in Rhesus monkeys. Their conclusion was that a nearly normal relationship between the tooth and its supporting structures is reestablished within 4 months.

In dogs Anderson <u>et al.</u> (1968) compared the type of hard tissue produced by the pulp after replantation with the rapidity with which it was revascularized. Earlier revascularization of the apical tissue resulted in a
thicker, more regular layer of dentin there. The authors classify the type of dental hard tissue formed after replantation as: regular tubular reparative dentin, irregular reparative dentin, osteodentin, irregular bone, and regular bone or cementum. They found that the type of tissue formed was related to the type of formative cell observed. This in turn was influenced by the time lapse until the cell's nutrient supply was returned.

Grewe and Felts (1968) experimented with the immediate replantation of mandibular incisors in mice. One quarter of the 40 teeth were shed or underwent pulpal necrosis. The remaining 75% recovered and when observed 1-5 weeks later appeared normal except for certain changes at a level of the root corresponding to the replantation incident.

The maxillary central incisor teeth of 10 Rhesus monkeys were replanted by Shulman and Kalis (1969). They found the periodontal membrane normal in appearance by the fourth week post-operatively.

In 1970 Lemoine <u>et al.</u> reported on the immediate replantation of first molar teeth in 50 rats. By the third day the periodontal barrier to external fluids had been reestablished in the gingival sulcus. At 4 days polymorphonuclear neutrophils were observed in the pulp and periodontal membrane phagocytizing tissue debris. Multinucleated giant cells and osteoclasts were seen re-

moving bone. Fibroblasts were active at 1-2 weeks laying down collagen that was thicker and more irregular than normal. Later this collagen seemed to contract and to become more regularly aligned.

An excellent article by Monsour (1971) explains his research with the replantation of maxillary incisors in eight dogs. The teeth all had incomplete root development. Some were immediately replaced, while the others were stored first for 3 minutes in saline. Splinting was used for 2 weeks and the teeth were radiographed weekly and observed histologically through 6 months. Two of the immediately replanted teeth exhibited normal pulpal morphology and continued dentin formation. Only a calciotraumatic line betrayed the disturbance to nutrition which the extraction had produced. In the other immediately replanted teeth, and in all those whose replantation was delayed, the pulp slowly degenerated and was replaced by tissue growing in from the periapex. This tissue contained bone centrally, while peripherally osteoblast-like cells were laying down osteo-dentin. This hard tissue deposition completely eliminated the pulpal space in some instances. Monsour speculates that diffusion maintained the pulp tissue until it was revascularized in the two cases where the pulp survived. In the other cases the blood supply was not connected soon enough and the pulpal tissues slowly degenerated and were replaced.

Castelli <u>et al.</u> (1971) studied the revascularization of the periodontium in intentionally replanted teeth of Rhesus monkeys. They demonstrated by India ink perfusion that revascularization of the tissue adherent to the root had occurred by 6 days. During the interval they assume that diffusion through the blood clot supplied the replanted tissue.

A recent report (Barbakow et al., 1977) concerns replanted teeth in 32 vervet monkeys. Observation at 1 week showed granulation tissue bridging the cleft in the periodontium caused by the extraction. Where the pulps were not removed and replaced by root canal fillings necrosis occurred. Polymorphonuclear neutrophils were present at the junction of the necrotic pulpal tissue and the periapical granulation tissue. By the second week deposition of periodontal fibers had eliminated the former cleft and healing was underway. Some surface resorption of the root was seen. They found at 4 weeks that there was some downward growth of the epithelial attachment in about 20% of the teeth, particularly in areas where the cemental surface was crushed by forceps application. Root resorption and bony ankylosis were seen radicularly. Eight week observation showed extensive ankylosis in all teeth. Those replanted with intact pulps had vacant root canals and definite periapical abcesses. Aside from the periapical areas, the root-filled teeth and the teeth replanted

with intact pulps had identical appearances. Evidence is not given of the degree of apical development of the teeth. Some appear to have been immature and others fully developed.

MATERIALS AND METHODS

Subjects

Rhesus monkeys were chosen as the experimental animals because of the similarity of their teeth and supporting structures to those of humans. Developmentally and anatomically the pulp, root, and periapical tissues are very similar. The subjects used were obtained from India via an importer (Primate Imports, New York). They were 3 young males identified by tattoos as A, B, and C. All were in good health on arrival and remained so throughout the 1-year period of the research. Their weights and dental development indicated they were approximately 2 years old at the beginning of the experiment (Haigh and Scott, 1965).

The teeth found to be suitable for the application of the photoplethysmograph were the maxillary and mandibular central incisors. Root formation of these teeth was incomplete at the time of replantation. One central incisor was removed and replanted while the adjacent one remained as a control. In animal A the maxillary right and mandibular left central incisors were randomly selected for replantation. Referring to the teeth by animal code and tooth number. A's

experimental teeth were A8 and A24. Monkey B's experimental teeth were B9 and B25, while C had C8 and C24 replanted.

Equipment

Zurawic (1972) reported success with photoplethysmography of the human dental pulp using a modified form of a commercially available transducer. As this was on hand and was compatible with the available apparatus, it was decided to work with this proven instrument. The transducer (Model 780-16 Earpiece Plethysmograph, Hewlett-Packard) was designed to monitor the blood flow in the pinna of the human ear. The U-shaped device contains a light bulb which is directed through the intervening tissue toward a photoconductive cell located in the other arm of the U. The amount of light striking the cell affects its resistance to the passage of an electrical current. Changes in the amount of light passing through the tissue thus affect the strength of the current leaving the transducer. The particular photocell used (CL 703L, Clairex) was most sensitive to changes in the amount of red light having a wave length of 7350 angstroms.

The power for the transducer was supplied by a heart rate monitor (Model 780-7 Heart Rate Monitor, Hewlett-Packard). This unit also received the signal from the transducer and transmitted it to a filter (Model 3550 Filter. Krohn-Hite). The filter was set to eliminate all input with a frequency greater than 4 cycles per second. The filtered signal was preamplified (Hi-Gain Preamplifier, Narco) and fed to a four-channel polygraph (Physiograph Four, Narco). There it was further amplified (Mk VII Amplifier, Narco) and displayed on channel 3 with a rectilinear pen. Recording paper was advanced at a speed of 2.0 cm per second. The system was arranged so that decreases in the amount of light striking the photocell produced upward deviations in the tracing.

In order to permit correlation of the photoplethysmogram with systemic circulatory activity, an electrocardiogram was needed. This was obtained by strapping two electrodes on the animal's chest and connecting them to a pneumograph (Mk IV Impedance Pneumograph, Narco). One output of this device went through a preamplifier (Mk III Pre-Amplifier, Narco) and the amplifier to record the electrocardiogram on channel 2. A second output of the pneumograph went through the amplifier directly to channel 1. This was a record of respiratory activity. Channel 4 was used to indicate 1-second time intervals.

Procedure

The transducer was extremely sensitive to interference from any nearby electrical equipment, so all non-essential

electrical items in the experimental laboratory were disconnected. Since ambient light was also a problem, the door was closed and only one small incandescent lamp was used in the room. Movement of the transducer also produced erratic recordings, so extensive procedures were developed to try to stabilize it. A small plastic infant-carrier was modified to support the animal in a semi-upright position. His body was strapped in place. The head was stabilized in a specially constructed plastic tube. Custom-made biteblocks of a semi-hard denture reline material (Coe-Soft, Coe) were used to minimize jaw movements of the animal. This arrangement may be seen in Figure 1.

In order to apply the transducer to a particular tooth in the same manner each time, a special holder was fabricated for each dental arch. Its basis was a custom acrylic impression tray (Trayresin, Caulk) containing a dental rubber base-type impression (Coe-Flex Heavy Body, Coe) of the animal's mouth. The acrylic tray and rubber base material over the central incisor teeth were cut out. The tray was extended deeper into the vestibule and reinforced with additional acrylic or a metal brace. The opening in the tray was adjusted so as to permit the center of the transducer to be placed to the cervical extent of the involved teeth. The transducer was then aligned so that the beam would pass through the portion of the tooth where



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Figure 1: Photoplethysmograph Apparatus in Place.

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Figure 2: Subject with Restraint Collar in Cage.

radiographic evidence revealed the pulp chamber was located. The level of the gingiva facially and lingually placed some limitations on this positioning, as the beam could not be permitted to pass through any gingival tissue. Often this limitation prevented aligning the beam to pass through the very largest portion of the pulp chamber and restricted the target to a pulp horn. This best alignment for the transducer was marked with acrylic guides for later use.

Next a mold was constructed for the plastic splints that would be required later. Wax was molded to the desired shape on the stone model with clearnace for the transducer Rubber base adhesive was applied to adjacent areas head. of the acrylic tray and then additional dark brown rubber base impression material (Coe-Flex Regular Body, Coe) was placed over the lubricated model around one central incisor The lubricated transducer was then carried to place tooth. through the rubber base material. When the transducer was removed a firm seat for the transducer head had been developed in the surrounding rubber base material. This was checked visually to insure that no defects which might permit light passage were present. If any were found, this step was repeated to insure that no extraneous light could enter into the inside of the tray when the transducer was The same procedure was then carried out for the in place. adjacent central incisor. A heated copper tube corresponding in size to the transducer diaphragm was next used

to cut a shaft through the rubber base material. On the facial side this began at the bulb opening and it extended lingually to the receptor cell opening. The shaft was checked to ensure it would permit direct passage of the light beam through the tooth. Marks were then made on the stone model where these channels encountered the tooth surface. The sites were inspected to be certain that the beam would pass through the desired portion of the crown without encountering any gingival tissue.

Wire hooks were incorporated into the tray so that elastics could be used to further stabilize the transducer head. The tray was contoured and smoothed and a keyed pad was made to receive the custom bite-block. The resultant tray was bulky and it deformed the animal's lips, so that in the maxillary arch it tended to occlude the nostrils. Since this resulted in respiratory distress which interfered with the photoplethysmogram, lubricated segments of plastic tubing were inserted a short distance into each nostril to restore patency.

The transducer's standard separation of 4.4 mm between the bulb and the photocell was found to be inadequate to accomodate the monkey incisor teeth. The plastic bar connecting the two sides of the transducer was cut and the parts were reassembled to allow more space for the teeth. The orientation of the segments was maintained with a metal

tray, and acrylic resin was used to rejoin the parts. The elements of the modified transducer were separated by 9.1 mm. The inner corners of both bulb and photocell sides of the transducer were rounded to minimize the interference with palatal and vestibular soft tissues. Lead foil with a 1/8 inch diameter opening was glued over both openings to columnate the light beam and reduce the interference from scattered light.

Several tests were perforemd to determine the "noise" level of the system. One test involved blocking passage of the light beam through the tooth with lead foil. This was an ideal test as it included aberations due to movements of the animal. In a second method the transducer was placed in the tray on a plaster model of the animal's jaw. Later two solutions were injected into a subject's common carotid arteries to test the effect of lightening (saline) and darkening (Cardio-green dye) the blood flowing through the den-The carotids were also occluded to test the effect tal pulp. on the photoplethysmogram of reducing the blood flow. Standardization runs conducted by recording 1.0 mv impulses at different amplifications indicated the electronic circuitry was consistent.

A means of splinting the replanted teeth was required which would not interfere with passage of the light beam. It had to be very firmly affixed as it was anticipated that the animals would be curious and perhaps annoyed by such a

device. A clear acrylic splint, 2-3 mm thick, was used. It covered the central incisors with facial and lingual portions connected by acrylic passing through the distal embrasure areas. The incisal edge was adjusted so that the splint would not interfere with chewing. The splint extended to just short of the gingival margin.

In order to allow the animal to become accustomed to the splints they were placed several weeks prior to the actual replantation procedure. It was of course necessary to remove them to extract the tooth. Since this destroyed the splint, a set of similar splints was required so that pre- and post-replantation readings would be comparable. The mold for fabricating these splints had previously been incorporated into the tray by adding the final rubber base seating for the transducer over a wax model of the splint. Clear acrylic material (Flash Acrylic, Yates) was placed into the mold and then the tray was seated on the lubricated stone model. A series of four splints was made one after another in the same mold.

Photoplethysmograph tests were run with the splint in place, with the splint removed, and with a film of glycerine between tooth and splint to simulate the presence of an adhesive agent. These results were compared with those of the splints later cemented in place. The similarities throughout these tests indicate that the splints did not interfere with the photoplethysmograph operation.

Nine different methods of attaching the splints to the teeth were evaluated. The most effective was an orthodontic adhesive (Directon Adhesive, TP Laboratories) applied to the etched enamel surface. After placing an adhesive strip (Oradhesive, Squibb) to protect the gingiva from the etching agent and to control any hemorrhage, the manufacturer's directions for tooth surface preparation were carefully followed. The adhesive's liquid and powder elements were spatulated together and placed rapidly inside the splint. This was carried to place with pressure and then supported for 10-15 minutes. If the splint appeared to interfere in any way with the animal's bite it was adjusted, smoothed, and polished.

Early experience had shown the animals capable of removing the splints, so their access to the mouth was restricted with a large plastic collar as seen in Figure 2. It was custom-made of 0.060 inch low density polyethylene. The opening for the neck was made just small enough to prevent the animal from passing his hand between the collar and his neck. This inner border was padded and covered with a soft adhesive liner (Moleskin, Dr. Scholls). The collar was made large enough so that the animal could not reach around with his hands or feet to touch the oral area. The plastic sheet was circular with a radially-directed cut connecting the outer border with the central neck opening.

The edges of the cut were overlapped and bolted together. A strip of heavy tape covered the nuts and prevented the animal from releasing himself. The collar was attached at the front and rear to the vertical bars of the cage by twisted segments of wire. On the sides heavy wire pieces were fixed between the top and bottom of the cage and they were passed through grommets in the sides of the collar. This arrangement gave the animal considerable vertical travel while preventing him from traumatizing his teeth by biting the cage.

As the collar also prevented the animal from feeding in the normal manner, alternate means were developed. A water bottle was mounted on the front of the cage so that the animal could lap its spigot with his tongue. The monkey's biscuit diet (Purina Monkey Chow, Ralston Purina), supplemented with a multiple vitamin (Petamin-Plus, Hoffman-LaRoche), was softened with water and mashed. This was fed through a large plastic syringe to the animal.

In preparation for a testing session all restraint wires attached to the collar were removed so that the collapsible inner cage could be used to squeeze the animal against the front bars. A 12 mg dose of an immobilizing agent (Sernylan, Parke-Davis) was administered intramuscularly. This was supplemented as necessary in the procedural laboratory at a rate of about 4 mg every 2 hours.

In order to attain the near immobility required for the actual photoplethysmography, intra-venous injection of 20 mg of an anesthetic agent (Surital, Parke-Davis) was given as needed, approximately every 30 minutes. Normally two separate photoplethysmograph trials at least 45 seconds long were obtained. Settings for the electronic equipment were maintained the same at all sessions. The amplification for A was set at 9.0 and for B at 10.0 (maximum). It was 5.0 for C's maxillary teeth and 7.0 for the mandibulars.

To perform a replantation one of the splints was cut nearly through with a dental bur and gently pried loose. Any cement adherent to the tooth surface was removed. The area was evaluated as to gingival condition and tooth mobility. A #15 scalpel blade was used to sever the gingival attachment and then the tooth was carefully removed with a pedodontic forceps. A stop watch was activated at the moment of extraction. The tooth was placed on a sterile 2 x 2 gauze pad moistened with saline and was carried to a photographic apparatus. This assembly was designed to evaluate the degree of apical development but was found to be useless as blood always obscured the apical Prior to reinserting the tooth the socket was area. evacuated of blood with a small surgical aspirator tip. Care was taken not to touch the alveolar walls with the instrument. The extraction forceps were placed on the

tooth in the original manner and it was lifted from the gauze and returned to its alveolus 90 seconds after extraction. The new splint was seated over the replanted tooth and the adjacent control tooth. This ensured that the tooth was returned as closely as possible to its original position in the dental arch. The splint was hand-held with light pressure for 20-30 minutes to permit clotting to proceed in the radicular periodontal membrane. The splint was then carefully removed and the normal procedure followed to etch the teeth and cement the splint.

The photoplethysmograph monitoring equipment was again inserted, 20 mg of Surital was injected intravenously, and 1 hour after extraction another record was taken. The tooth in the opposite arch next underwent the same procedure, with pre- and 1-hour post-replantation tracings being obtained. Later the same day, recordings were made in a similar manner at 4 and 8 hours. The following day a 24hour study was done. Subsequently recordings were taken for all animals at 2, 4, and 7 days and at 2, 3, 4, and 5 weeks. Animal A was checked at 6 weeks also and subject C was followed through 8 weeks.

After the last post-replantation recording had been completed, the animals were sacrificed with a lethal intravenous injection of sodium pentobarbital. The anterior segments of the dental arches were quickly dissected away

with a scalpel and an automatic bone saw. They were placed in 10% formalin solution within 5-15 minutes after death. Within 1 hour all soft tissue was removed and excess cortical alveolar bone was reduced with a high speed dental bur and water coolant to facilitate fixation and decalcification of the specimen. Decalcification was accomplished in a solution of equal parts 50% formic acid and 20% sodium citrate. The histochemical procedures for paraffin embedding were carried out by an automatic processor. Sections were cut and stained with hematoxylin and eosin, silver impregnation, and periodic acid-Schiff methods.

RESULTS

The photoplethysmograph transducer used in this investigation was designed for use on the human external ear. It produces excellent results when applied to any thin soft tissue. Figure 3 is a recording of the pulsatile changes in the opacity of the cheek of one of the subjects. The top line indicates respiration, line 2 the electrocardiogram, line 3 the photoplethysmogram, and line 4 time. The large deviations in the base line of the photoplethysmogram are clearly coordinated with respiratory activity. The smaller plethysmogram, and line 4 time.

Though the transducer was modified in this experiment to accommodate the monkey teeth, these changes failed to suit it ideally to recording blood flow there. The light beam was columnated but much light was still scattered within the crown of the tooth. This light could follow a very indirect course through the tooth and arrive at the receptor cell much out-of-phase. The resulting irregularity and weakened signal strength necessitated an increase in system amplification in order to obtain sufficient activity on the recordings. Unfortunately this increase also had







Figure 4: Plaster Model of Subject B, Score -19, Amplification 10.0.

the effect of introducing into the recordings a great deal of electronic interference. Even with the receptor cell shielded from all light, activity on the photoplethysmogram could be noted at higher amplification levels (Figure 4). Such photoplethysmographic activity caused by electronic interference is obviously not correlated with the subject's cardiac activity. As a result the evaluation of the records of this experiment was complicated by the necessity to make an allowance for electronic noise.

This was done by devising a tracing overlay method of This method permitted the identification of evaluation. photoelectric pulse waves that were well correlated with cardiac activity and it discounted out-of-phase, random waves stemming from interference. A photocopy of the graph was placed on a mechanical drawing board and overlaid with tracing paper. A series of parallel vertical bars was drawn with transparent ink, one at the peak of each QRS complex on the electrocardiogram. This tracing was then transferred over the dental photoplethysmogram. It was moved laterally so that the ink bar indicative of a particular heart beat was positioned somewhere in the interval between its own R peak and the next one. The overlay was moved back and forth within the confines of this interval until the position was found where the most photoplethysmograph wave peaks fell within the overlaid ink bars. The

width of these ink bars of course influenced the percentage of pulse waves which could peak within them. A bar 90% as wide as the <u>R</u> interval would accommodate even many errant pulses. A narrow 10% bar might exclude even some true pulses which had perhaps been delayed momentarily due to respiration or bodily movement of the subject. Thirty percent was chosen as the standard for this experiment and the vertical ink bars were drawn approximately 30% as wide as the calculated <u>R</u> interval for each graph.

From the 6-20 pages of graphs available from each testing session for each tooth, one page was selected by visual inspection as having the best apparent correspondence between photoplethysmogram and electrocardiogram. On this graph a series of 25 consecutive \underline{R} peaks was chosen as having the best correlation with tooth opacity pulses. The overlay was then drawn and repositioned. The number of pulse wave peaks falling within the bars and the number falling outside the bars were next counted under a magnifying glass with rear illumination. This was done at least twice, until an identical within/outside tally was obtained. This tally was next converted to a percentage. The number within was divided by the larger of either the number of pulse peaks or the number of heart beats involved. From this percentage was subtracted 50% to obtain the score.

This was done in order to eliminate the influence of random noise pulsations. A sample calculation follows:

- 23 pulse wave peaks <u>within</u> superimposed <u>R</u> bars 2 pulse wave peaks <u>outside</u> superimposed <u>R</u> bars
- $\frac{23 \text{ within}}{25 \text{ total}} = 92\%$
- score = 92% 50% = +42.

Perfect score would be +50. Nil would be -50. A positive score is believed to be an indication of actual circulatory activity.

This scoring method was used for the experimental recordings seen in the following figures. Figure 5 demonstrates a photoplethysmogram well coordinated with electrocardiogram in a tooth 4 weeks after replantation. Figure 6 is of a control tooth 4 days after its neighbor was replanted. A poorly coordinated graph of a replanted tooth at 4 days is seen in Figure 7. The scores for all replanted and control teeth in the experiment are presented in Table 1.

This scoring system was proven suitable by certain tests for the electronic noise level. The transducer was placed in a tray on its plaster storage model. This sealed out all ambient light and blocked the passage of the light beam itself, so that absolutely no light could reach the photocell. The following scores were obtained: -24, -5, -8, -6, -6, +6, -14, -14, -4, -4, -10, -21, and -21.



Figure 5: Subject B, Replanted Tooth B25, 4 Weeks, Score +42, Amplification 10.0.



Figure 6: Subject A, Control Tooth A25, 4 Days, Score +30, Amplification 9.0.



Figure 7: Subject A, Replanted Tooth A8, 4 Days, Score -17, Amplification 9.0.



Figure 8: Plaster Model of Subject C, Score +6, Amplification 8.0.

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Table 1: SCORES OF PHOTOPLETHYSMOGRAPH TEST SESSIONS CORRELATED WITH HISTOLOGIC OBSERVATIONS

Subject		A				В				C				
Arch Tooth Treatment Session pre-op		maxilla	irv	mandibular		maxil	maxillary		mandibular		maxillary		mandibular	
		A9	AŠ	A25	A24	в8	B9	B24	B25	C 9	C8	C25	C24	
		con	repl	con	repl	. con	repl	con	repl	con	repl	con	repl	
		(<u>+5</u>)	- 8	-10	$(\overline{+9})$	-19	-16	- 7	-21	$(\overline{+14})$	-14	- 2	-22	
post- 1 op 4 8	hr	$(\frac{+6}{-3})$ -13	- 2 -18 - 4	$(\frac{+29}{-2})$ -29	$(\frac{-2}{+46})$	-12 (-13) (+18)	- 8 (<u>-37</u> (<u>+ 3</u>)	-18 (-12) (+2)	$(\frac{-11}{+10})$	$\left(\frac{\frac{-10}{+6}}{\frac{+22}{+22}}\right)$	$(\frac{-22}{+2})$	-26 -22 - 6	$(\frac{+26}{-6})$ -16	
1 2 4	da	$(\frac{-10}{+2})$	-17 - 6 -17	$-\frac{8}{(+30)}$	$(\frac{-18}{+9})$	-12 -16 -11	- 2 0 -16	- 5 -22 -19	- 2 -17 -17	$(\frac{+22}{+24})$ $(\frac{+24}{+26})$	$(\overline{+18}) \\ (\overline{+15}) \\ \overline{-10}$	-14 -22 -38	(+46) (+38) -22	
1 2 3	wk	$(\overline{\underline{+4}}) \\ (\underline{+10}) \\ \overline{-22}$	- 8 -18 -14	$(\frac{-14}{+18})$	$(\frac{+8}{+30})$ $(\frac{+30}{-14})$	-10 - 4 - 5	-13 -12 -23	$(\frac{-26}{+27})$	$-14 \\ -26 \\ (+4)$	-17 0 - 4	- 2 -26 - 6	-18 -22 -28	-10 -22 - 8	
4 5 6		$\left(\frac{\frac{\pm 12}{\pm 4}}{\frac{\pm 28}{\pm 28}}\right)$	(+4) -29 -10	- 8 - 6 - 6	$\begin{pmatrix} +42\\ +2\\ +2\\ -6 \end{pmatrix}$	-14 -13	- 9 -18	$(\frac{-3}{+6})$	$(\frac{+42}{-7})$	$\left(\begin{array}{c} +18\\ +10\\ +10\\ +10\end{array}\right)$	- 5 -25 -18	- 8 -18 -14	- 6 - 2 -14	
7 8										$\left(\frac{+10}{-2}\right)$	$(\frac{+34}{-22})$	-18 -10	$\left(\frac{1}{10}\right)$	
Histologic		D	I	D	I	D	I	D	I	D	P	D	I	
Indications		E	М	E	М	E	М	Ę	М	E	R	E	М	
of Coronal		F	Р	F	Р	F	Р	F	P	\mathbf{F}	0	\mathbf{F}	P	
Blood Flow			R		0		R		0		В		0	
Legend: con= DEF=		control repl=replanted definite PROB=probable			hr=hour IMPR=imp	y wk=w e IMPO:	wk=week ()=positive scores IMPO=impossible							

The highest score of +6 was calculated for the graph in Figure 8. It seems to have weak pulsatile activity, but the pulses can be lined up to some extent with the R bars. Once, the model with transducer in place was set in the animal's lap to see if respiration or bodily movement might account for pulse waves coinciding with heart beat. These scores were -19 and -17. In another test lead foil was placed over the receptor cell opening and the appliance was inserted into the animal's mouth. This method also permitted any bodily movement of the subject to affect the These graphs scored: -20, -8, -17, -16, -6, and -9. score. When the foil was removed and the light again permitted to pass through the tooth the scores were: -9, -8, -19, -19, In the 21 tests for electronic interference the and +16. average score was -12 with only one exceeding -4. A positive score was thus a strong indication that the photoplethysmograph activity exceeded the noise level and that actual tooth opacity pulses synchronous with heart beat were occurring. All such positive scores are encircled in Table 1.

Three physiologic experiments suggested by the work of Upthegrove <u>et al.</u> (1966) were conducted to test the validity of the information provided by the photoplethysmograph. Just prior to sacrifice the animal's common carotid arteries were exposed. Occluding the ipsilateral artery was found to reduce the score on recordings of the

animal's cheek from +34 to -6. When both carotids were blocked the score dropped from +42 to +6. Occlusion of the contralateral artery reduced the score only from +34 to +22. The results were less conclusive when tooth photoplethysmograms were compared. Control scores were very low to start with, ranging from -18 to +4, but it is very striking that in every case they decreased following carotid occlusion. Ipsilateral blockage brought decreases from -11 to -18, +2 to -13, and -2 to -10. Occlusion of both arteries produced the following drops: -10 to -14, -18 to -26, -10 to -18, -6 to -22, -9 to -20, +4 to -2, -9 to -30, -10 to -13, and -2 to -18. An injection of 5 ml normal saline into the ipsilateral carotid did not produce any consistent changes in the photoplethysmograms. The score for A's cheek went from +42 to +46 while the score for tooth A9 decreased from +30 to +21 and that for A25 increased from -22 to -7. The teeth of other subjects showed increases from -12 to -7 and -22 to -7, and decreases from +30 to +21, .-13 to -18, and -17 to -18. Injecting a darkcolored vital dye, Cardio-green, was similarly monitored photoelectrically. Observed changes were small. The score for the ipsilateral cheek dropped from +42 to +36. The teeth showed increases of -23 to -14, +22 to +26, and -9 to 0, and a decrease of -14 to -18. One test exhibited no change as the score remained -4.

During the normal histologic evaluation of the tissue sections particular attention was paid to any evidence of recent blood flow in the coronal portions of the pulp. Each tooth was rated according to the following scale on the probability of its having an effective coronal pulpal circulation:

definite

blood vessels normal in distribution and appearance and containing intact red blood cells

probable

vessels irregular in appearance and containing distorted red blood cells

improbable

vessels containing only vestiges of red blood cells

impossible atrophic vessels containing no red blood cells.

The results of this study are indicated at the base of Table 1.

All control teeth had evidence of normal pulpal circulation, though some histologic abnormalities were seen. The coronal pulp of one tooth (B8) was very fibrous and the odontoblastic layer had been greatly disturbed. Much irregular cementum was present in the coronal portion of the pulp chamber. Periapically, extensive resorptive areas had eroded through the cementum into the dentin and an area of ankylosis was observed. Tooth A9 had a wide band of cementum deposited beneath the dentin coronally (Figure 9). This was in turn underlaid by tubular dentin in the radicular



Figure 9: Subject A, Control Tooth A9, Coronal Pulp, 6 Weeks, H & E, x25, Coronal Blood Flow Definite.



Figure 10: Subject B, Replanted Tooth B9, Radicular Pulp, 5 Weeks, H & E, x100, Coronal Blood Flow Improbable. areas. Tooth C9 was normal in appearance except for a thickened layer of cementum on the root surface. The mandibular control teeth appeared normal. All control teeth were rated as having a definite, active coronal pulpal circulation.

All maxillary replanted teeth were revascularized apically, however only in one tooth (C8) did this recovery extend completely into the coronal pulp. When C8 was examined at 8 weeks, the coronal area contained many large blood vessels which were congested with normal-appearing Some breakdown of red blood cells had erythrocytes. occurred in the coronal pulp as hemosiderin-containing macrophages were seen. A calcio-traumatic line was evident in the dentin. In the apical half of the pulp normal tubular dentin, predentin, and odontoblasts were found beneath this line. This indicates that nutrition in the apical pulp was only slightly disturbed and that normal dentin deposition was soon resumed. Coronally there was evidence of greater disruption of pulpal circulation. The odontoblasts had degenerated and a layer of cementum containing many cellular inclusions was found. In all but the most coronal portion of the pulp the blood supply eventually returned, as odontoblasts were regenerated and tubular dentin was again laid down. The width of the band of cementum may be indicative of the length of time the

pulp had a sub-standard blood supply. This band was thick coronally and thinned toward the root portion of the tooth. The coronal area of tooth B9 was necrotic and considerable areas of extravasated red blood cells were present there at Many small vessels were noted beneath the necrosed 5 weeks. tissue. An odontoblastic layer was completely absent in the coronal portion of the tooth and only a thin predentin layer was found beneath the tubular dentin. No hard tissue had been deposited here since the replantation. In the root portion the odontoblastic layer was intact and regular dentin and predentin were seen (Figure 10). The coronal portion of the pulp had evidently undergone a degenerative process and it was being replaced by connective tissue growing in from the apical portion of the pulp. Tooth A8 also had undergone necrosis coronally when observed at 6weeks. The odontoblastic layer was missing and no new dentin formation was seen. In the radicular portion of the tooth normal odontoblasts were forming regular tubular dentin and predentin. The interior portion of the pulp contained many vessels with erythrocytes. In this tooth also, the coronal pulp was being repaired by apical tissue. It is probable that teeth B9 and A8, which were examined at 5 and 6 weeks, would have undergone further repair coronally to eventually appear more similar to tooth C8. the 8 week specimen.

Coronal blood flow was determined to be probable in C8 but improbable in A8 and B9. The root surfaces of the replanted teeth A8 and C8 displayed many minute shallow resorptive scars that had been repaired by cementum deposition. The root surface of tooth B8 was completely normal in appearance. The fibers of the periodontal membrane in all the replanted maxillary teeth were not functionally arranged. Significant numbers of inflammatory cells were not noted, either in the pulp or in the periodontal membrane.

All three mandibular replanted teeth had undergone pulpal necrosis and only fibrous remnants of the pulp remained (Figure 11). No dentin was deposited after the replantation, suggesting that blood flow never returned. Periapical inflammation was present in all cases. In specimen A24, granulomatous tissue could be seen protruding into the vacant root canal space (Figure 12). Many minute areas of root resorption were observed. In addition, tooth A24 had several deep defects.

Examination of radiographs taken just prior to replantation indicated that the apices of all teeth were less than completely developed. Estimated width of the apical foramen varied between 0.5 and 1.0 mm. At the time of subject A's sacrifice all four central incisor teeth had similar radiographic appearances with no appreciable



Figure 11: Subject C, Replanted Tooth C24, Coronal Pulp, 8 Weeks, H & E, x25, Coronal Blood Flow Impossible.



Figure 12: Subject A, Replanted Tooth A24, Periapical Area, 6 Weeks, H & E, x25, Coronal Blood Flow Impossible.

change from pre-replantation views. With animal B the only difference was a widening of the periodontal membrane space in both maxillary teeth. The lamina dura around the apex of replanted tooth C24 was widened, while the root canal of replanted tooth C8 was narrowed noticeably.

Gross examination of the experimental sites revealed no sinus tract openings or vestibular swellings. Marginal gingival tissues were moderately inflamed because of irritation by the splints. Gingival sulcus depths were about 1 mm deeper than previously. Mobility of replanted and control teeth was similar and less than 1 mm. The crowns of the central incisors had opaque whitish surface spots resulting from the acid etching in the splinting process.

DISCUSSION

The most significant observation of this study is that the pulps of all mandibular replanted teeth became necrotic, while those of the maxillary teeth regained their vitality. According to the work of Anderson <u>et al.</u> (1968) the absence of a calcio-traumatic line in the mandibular teeth indicated the circulation never returned to the pulp after replantation and that pulpal necrosis then ensued. On the other hand in the maxillary teeth the continued deposition of hard tissue beneath a calcio-traumatic line demonstrated that the pulp was reconnected to a source of nourishment. A revascularizing process was advancing coronally from the apical area and effecting pulpal repair.

The much more successful revascularization of the maxillary teeth might be explained by the less developed nature of their root apices. Radiographically the mandibular incisors were nearly fully developed apically while the maxillary incisors had not yet achieved full root length. The greater cross-sectional area of these teeth provided a larger surface for diffusion and vascular reconnection. Another possible explanation might lie in the richer blood supply afforded to the anterior portion of the
maxilla. The mandibular incisor teeth are supplied only by the terminal portion of the inferior alveolar artery, which courses through the much less vascular mandible.

The overwhelming defect in this experiment was the use of a photoelectric device that was not sufficiently sensitive to detect the minute opacity changes of the very small monkey teeth. In order to obtain a recording, the amplification was frequently increased to the point where excessive electronic interference was encountered. The basic problem was the small size of the coronal pulp in relation to the thickness of enamel and dentin. The light beam was columnated to 1 mm but this was inadequate to screen out the large amount of light which struck the photocell after a very indirect route through the crown of the tooth. Much refraction and reflection took place and the out-of-phase arrival of such light certainly interfered with the reception of the light that had taken the desired direct course through the pulp. With the instrument employed it was not possible to further restrict the size of the beam, as then it would have been too weak to produce any signal at all. It should be remembered that the separation between the photoelectric elements was increased from 4.4 mm to 9.1 mm to accommodate the monkey teeth. A narrower, more powerful beam would be required to obtain better readings. The exact alignment of the light beam between the bulb and photocell

should also be tested experimentally as the manufacturer admits they make no particular effort to align these components in their transducer. The spectral sensitivity of the photoconductive cell here was greatest at 7350 angstroms. At this wave length there is a considerable difference between the 30% light absorption of oxygenated blood and the 70% absorption by reduced blood. Some of the photoplethysmographic changes observed may have resulted from mere changes in the oxygen content of the blood, rather than from any actual blood flow. A photoconductive cell with its maximum sensitivity at 8050 angstroms, where the absorption is independent of oxygen content, would eliminate this problem.

Others (Upthegrove <u>et al.</u>, 1966; Reese <u>et al.</u>, 1971; Zurawic, 1972 and Beer <u>et al.</u>, 1974) have reported success with photoplethysmography in dental pulp studies. All of these except Zurawic used specially designed photoelectric devices. The Earpiece Plethysmograph as modified in this study does not appear to be suitable for use on teeth.

In view of the day-to-day variations in the plethysmogram scores, the stability of the experimental arrangement must be questioned. All possible measures were taken to use the same procedure each time. Room lights were turned off, all extraneous apparatus was disconnected, all equipment was allowed to warm up, and the same amplification settings were used each time. One factor might be the inability to duplicate exactly the transducer-to-tooth alignment at each session. The custom tray used here seems to offer the best stability and reproducibility that could be expected from a removable appliance. A fixed appliance would be more desirable but is impractical in a long term study. Variations in the depth of anesthesia was another problem. Sometimes there was quite a bit of respiratory activity and bodily movement which interfered with recording. The circulatory depressive effects of the anesthetic on the pulpal blood flow should be considered as well.

Zweifach (1957) described a periodic opening and closing of metarterioles and pre-capillary sphincters. He stated that this vasomotion occurs with a periodicity of every 30 seconds to several minutes. It is possible that during some of the 45-second experimental sessions a significant portion of the pulp being transilluminated was not receiving the same amount of blood inflow as at other times. If this were the case longer experimental trials might eliminate this factor. It is also possible that tissue repair and revascularizing efforts might be discontinuous. In this case successive trials might occur during periods of variable circulatory activity.

Cardio-ballistic effects as explained by Burton (1972) might account for photoplethysmograph waves. Vibrations might be transmitted to the tooth via the vessels in the

periodontal membrane even when no pulpal circulation was present. The carotid occlusion, saline, and dye tests used in this experiment to assess the cardio-ballistic effect were inconclusive because of electronic interference.

Looking at Table 1 it is striking that certain teeth had a series of positive scores while others were inconsistent or were always weak. The maxillary control teeth A9 and C9 had positive scores pre-operatively. They had relatively few lapses and finished with strong scores. Replanted tooth A24 had a similar series of scores but its pulp was found to have been totally necrotic since extraction. This tooth and C24, also necrotic, had the highest scores (+46) recorded.

It is interesting to speculate on what is responsible for such false scores. Aside from electronic problems it is possible that vibration of the tooth in its alveolus in response to the subject's bodily movements might be a cause. Another possibility is that the necrotic pulpal material transmitted the pulsations of the periapical vessels into the tooth, where they caused vibrations of the walls of the pulpal space. Van Hassel (1971) demonstrated that hydrostatic conduction like this might be possible in

a necrotic pulp, whereas such transmission would be impossible in intact pulpal tissue. A test of this hypothesis could be arranged by replanting a tooth wrapped in foil. The foil would prevent any true circulatory entry into the tooth. If photoplethysmographic pulsations were noted, it could be said they were caused by the tooth vibrating in concert with external blood flow.

Aside from these three teeth which had positive scores initially, all other teeth started out with negative scores. This is disconcerting since a tooth that does not exceed the noise level before treatment cannot be expected to do so after the experimental procedure. These nine teeth had a cumulative total of 20 positive scores, only 17% of the total, with an average of -8. This compares to the 25 positive scores, or 61% of all scores, and an average of +6 for those teeth which had positive scores pre-operatively. A solution for this problem would be to reposition the transducer at the beginning of the experiment until a location was found which gave a consistent positive response. None of the three maxillary teeth which were revascularized had positive scores initially, so it is not possible to follow the reparative processes in these pulps photoelectrically. Additional findings are that the two mandibular replanted incisors which had negative scores pre-operatively had several strong scores despite having a

totally necrotic pulp. Subject B had noticeably weak scores throughout.

The degree of amplification used appears to be inversely related to the quality of the recording scores. For C9 the amplification used was 5.0, where there was little electronic interference. This control tooth had the most positive series of scores recorded. The maximum amplification was used for all the teeth in subject B, for whom the weakest readings were obtained. An explanation for this might be that with animal B the amplification was increased in the absence of any actual dental optical activity until electronic interference began to produce activity on the graph.

One consistent finding is that the highest scores were all found on graphs having large deflections of the photoplethysmogram base line as in Figure 3. These large base line waves were co-ordinated with respiratory activity, and the smaller waves were almost exactly correlated with the electrocardiogram. It is unlikely that bodily vibrations associated with inhalation and exhalation are responsible for the small waves. Perhaps these deep respiratory maneuvers increased the force and thus the turbulence of blood flow to the pulp.

The histologic finding of a calcio-traumatic line and the deposition of intra-pulpal cementum in the maxillary

control teeth must be explained. This was not seen in the controls of the opposite jaw so it was evidentally not caused by any systemic condition. Local factors such as the repeated splinting procedures, traumatic occlusion, forceps injury during extraction of the adjacent tooth, and the animal's own abuse of the tooth must be considered.

The new technique for monitoring pulpal blood flow described by Tönder and Aukland (1975) would seem to be well suited to the study of pulpal revascularization in replanted teeth. This method involves measuring the passage of systemically-introduced hydrogen gas through the pulp. The gas is brought into the circulation via an endotracheal The sensor for the gas is implanted in the surface tube. of the pulp by means of a channel drilled through the enamel and dentin of the crown. If hydrogen gas were detected by the sensor in a recently replanted tooth, it could be said with certainty that blood flow to that area of the dental pulp had been reestablished. While this method would not give the results for replanting a normal tooth, the inflammatory effects of the drilling could be nearly discounted, as they would be minor compared to the extraction trauma.

SUMMARY

Six central incisor teeth of Rhesus monkeys were removed and replanted 90 seconds later. The blood flow in these and in control teeth was monitored with a photoplethysmographic device. This photoelectric apparatus was not appropriately designed for use in this location and excessive electronic interference obscured the recordings. No conclusions could be reached about the reestablishment of circulation in these teeth, based upon evaluation of the photoplethysmograms.

Histologic observation revealed that the pulps of all three mandibular replanted teeth had undergone necrosis, while those of the three maxillary replanted teeth had been at least partly revascularized. This revascularization was complete in the apical portion and it extended coronally a variable distance. In one tooth this reparative process was nearly completed and in the other two it had only reached the cervical level.

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APPROVAL SHEET

The thesis submitted by Dr. Ronald M. Milnarik has been read and approved by the following committee:

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The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

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Director's Signature