

# In Vivo Study of Respiratory Depression of Pulp Tissue Resulting from Cavity Preparation and Dental Materials

Edmund R. Proctor, Jr., D.M.D., M.S.

Robert E. Sullivan, D.D.S., M.S.D.

Jerry F. Taintor, D.D.S., M.S.

## Abstract

*Pulpal respiration in second and third premolar dog teeth was measured by a radioisotope tracer determination. Dental amalgam, a zinc oxide and eugenol base, and a calcium hydroxide base were placed into a cavity preparation. Following a two-week period the teeth were extracted intact. The pulp tissue was removed and suspended in a phosphate buffer saline solution (PBS) to which  $^{14}\text{C}$ -succinic acid had been added. The  $^{14}\text{CO}_2$  produced was used as an index of the effect on pulpal respiration of the various dental materials and the cavity preparation. When compared to the control, the preparation and materials were found to have a significant effect on the pulpal respiration.*

## Introduction

An abundance of literature exists on the histological response of the pulp to restorative dental procedures<sup>1-5</sup> and dental materials.<sup>6-10</sup> However, little research has been done on the effects of dental materials on pulp tissue metabolism.<sup>11,12</sup>

The purpose of this investigation was to measure the changes in the pulp tissue respiration rate when dental amalgam, zinc oxide-eugenol, and calcium hydroxide (Dycal) were used as the restorative materials by using a  $^{14}\text{C}$ -labelled substrate and measuring the  $^{14}\text{CO}_2$  released as a consequence of substrate metabolism of the pulp tissue. It was hoped that the findings of this study would demonstrate the physiological response of pulp tissue respiration to routinely used dental materials and cavity preparation procedures. An attempt to establish a correlation between the thickness of remaining dentin and pulp respiration rate was also to be determined.

## Methods and Materials

The maxillary and mandibular second and third cuspid teeth of six mixed breed, five- to six-month-old dogs were used for this experiment. The 48 teeth in the sample were randomly divided into four groups: 12 teeth treated with zinc oxide-eugenol (ZOE) and an alloy restoration (Kerr Zinc Free Spheralloy); 12 teeth treated with calcium hydroxide (Dycal) and an alloy restoration; 12 teeth treated with no base and just an alloy restoration; and a control group which was subdivided into six untreated teeth and six teeth in which a cavity preparation was made just prior to extraction of the teeth.

Anesthesia using a 50-50 mixture of Nembutal and a 4% solution of Bio-tal<sup>a</sup> was injected intravenously at a dose of 1 cc/5 lb. of body weight.

The operative procedures were done by quadrants following debridement of the quadrant with gauze soaked with alcohol and isolation of the quadrant with gauze packs placed in the buccal vestibule. Using a high speed handpiece with a #33½ inverted cone bur under an air and water coolant, Class V cavity preparations were made on the center of the buccal surface using a template to assure uniform preparation size. Depth of the cavity preparation was determined clinically by bur head size. Actual depth was determined by histological sectioning.

In the indicated teeth, the pulpal floor was covered with ZOE (225 mg. zinc oxide + 30 mg. eugenol) or Dycal followed by condensation of amalgam, amalgam condensation without a base and control teeth left untreated. The mouth was again debrided and the gauze packs removed.

<sup>a</sup>Abbott Laboratories, North Chicago, Illinois

Two weeks following the operative procedures, the animals were again anesthetized and prepared for the extraction of the sample teeth. All teeth were infiltrated with a local anesthetic of 2% xylocaine with epinephrine 1:100,000, since the manipulation of the teeth was found to stimulate the animal. Half of the control group (six teeth) had Class V cavity preparations prepared in them without any restoration placed just prior to extraction. The experimental and control teeth were then atraumatically removed. The extracted teeth were immediately placed in individual vials of a phosphate buffered saline (PBS).<sup>b</sup>

Each tooth was then sectioned in a mesiodistal direction leaving the buccal half of the tooth intact for histological sectioning and measurement of the depth of the cavity preparation and the thickness of dentin remaining between the floor of the cavity preparation and the roof of the pulp chamber. The pulp was finally dissected out of the sectioned tooth using an excavator and cotton pliers and replaced in the vial of PBS. The buccal half of the tooth was placed in a separate vial with a decalcification solution (Decal).<sup>c</sup>

Each pulp sample, weighed to the nearest 0.1 mg., was then placed into a 16 mm x 150 mm test tube containing 2 ml of the reaction solution. The reaction solution consisted of 2 ml of phosphate buffered saline solution with a pH of 7.3, 20  $\mu$ m succinic acid disodium hexahydrate<sup>d</sup> and 1  $\mu$ Ci <sup>14</sup>C 1,4 succinic acid.<sup>e</sup>

The completed test tube assembly consisted of a tissue sample with the reaction solution, and a rubber stopper with a center well apparatus.

For the purpose of absorbing <sup>14</sup>CO<sub>2</sub> produced, 0.2 ml of hyamine hydroxide<sup>f</sup> was added to the center wells<sup>g</sup> and the rubber stopper placed to create a sealed system. Each tube was then placed in a Gyrotary water bath shaker<sup>h</sup> at 37°C for 30 minutes at 65 oscillations per minute. After 30 minutes shaking, 0.2 ml of 2M H<sub>2</sub>SO<sub>4</sub> was injected through the rubber stopper of each test tube to stop the pulp respiration. The test tubes were oscillated again for 30 minutes in the water bath in order to absorb any additional <sup>14</sup>CO<sub>2</sub> remaining in the system.

Each sample for counting, consisting of 0.2 ml of Hyamine plus four 0.2 ml methanol rinses, was placed

in a liquid scintillation counting vial<sup>i</sup> with Econoflour Premixed Scintillation Solution.<sup>j</sup> The vials were then placed in a Packard Liquid Scintillation Counter.<sup>k</sup>

Each sample was counted five times for a 10-minute period to reduce variation found in the individual samples. In order to account for background radiation incorporated during sample preparation, a laboratory control was made and counted with the appropriate sample. The background radiation count was subtracted from the average value of each test sample prior to calculating the test data.

The buccal halves of the teeth were decalcified, sectioned, mounted, and stained creating a series of tooth sections through the center of the cavity preparation. Using an ocular grid calibrated by a stage micrometer, the series of sections for each tooth was measured under a magnification of 10X and rounded off to the nearest 5 micrometers, then averaged, providing a mean thickness of remaining dentin between the pulp chamber and the floor of the cavity preparation of each tooth. Depth of the cavity preparation was also measured by this method.

Using an independent t test, it was determined if the pulp respiration rate in the experimental teeth differed significantly from the control teeth as well as between each experimental group. Correlation coefficients were used to determine if a correlation existed between the pulp respiration rate and the thickness of remaining dentin between the pulp chamber and floor of cavity preparation in the experimental groups. Range, mean and standard deviation were calculated from the depth of the cavity preparation as well as for the difference between cavity depth and bur size used in making the cavity preparation. It was then possible to determine if using bur head size as a clinical determinant for estimating depth of a cavity preparation was reliable.

## Results

The mean respiration rate for the control and experimental groups were as follows:

Group I (untreated teeth)	9.4 ct/min/mg
Group II (cavity preparation alone)	5.6 ct/min/mg
Group III (amalgam restoration alone)	3.2 ct/min/mg

<sup>i</sup>NEF-924—New England Nuclear, Boston, Massachusetts

<sup>j</sup>NEF-941—New England Nuclear, Boston, Massachusetts

<sup>k</sup>Model 2002—Packard Instrument Co., Downers Grove, Illinois

<sup>b</sup>#404—Grand Island Biological Company, Grand Island, New York

<sup>c</sup>Omega Chemical Company, Cold Springs, New York

<sup>d</sup>S-2378—Sigma Chemical Company, St. Louis, Missouri

<sup>e</sup>NEC-099—New England Nuclear, Boston, Massachusetts

<sup>f</sup>#882320—#882310—Kontas of Illinois, Evanston, Illinois

<sup>g</sup>NEF-921—New England Nuclear, Boston, Massachusetts

<sup>h</sup>Model G-76—New Brunswick Scientific Co., Inc., New Brunswick, New Jersey

Group IV (ZOE base with amalgam restoration) .....	8.4 ct/min/mg
Group V (Ca(OH) <sub>2</sub> base with amalgam restoration) .....	6.7 ct/min/mg

Using an independent t test to determine if a significant difference existed between the control and experimental groups showed that the mean respiration rate for the untreated teeth was significantly different from the mean respiration of each of the other four groups at the  $\alpha < 0.05$  level of confidence. The same statistical analysis was used to determine if a significant difference existed between the different experimental groups. Findings showed that a statistically significant difference at the  $\alpha < 0.05$  level of confidence existed between all groups.

This study also determined if a correlation existed between the thickness of dentin remaining between the roof of the pulp chamber and floor of the cavity preparation and the level of pulp respiration. Using correlation coefficients, the analysis revealed that no such correlation existed except in the experimental group with a zinc oxide-eugenol base (Group IV).

From the data comparing the depth of the cavity preparation to the bur head size, obtained by measuring the cervical wall of Class V cavity preparations, it was determined that clinical judgement in estimating the depth of cavity preparation using bur head size as the measure was unreliable when attempting to create a series of cavity preparations of uniform depth.

## Discussion

Dental amalgam, zinc oxide-eugenol (ZOE), and Dycal are used routinely in operative dentistry as a permanent restoration, base and temporary restoration, and base, respectively. However, by diffusing through the dentin via the dentinal tubules, these materials have been found to cause a corresponding pulpal response.<sup>6,7,13-17</sup> The cavity preparation itself, cut into the tooth for the purpose of receiving and retaining these dental materials has also been found to cause a pronounced pulp tissue reaction.<sup>1-3,18-24</sup> The pulp tissue response to dental materials and cavity preparation has routinely been evaluated by histological sectioning. A study by Fisher<sup>11</sup> showed the effects of certain dental drugs and materials on the oxygen consumption of bovine dental pulp tissue using a manometric O<sub>2</sub> uptake technique. Recent studies by Jones<sup>12</sup> and Labart<sup>25</sup> showed the effect of certain dental materials and orthodontic stress on pulp tissue respiration using a radioisotopic determination of carbon dioxide release. In this study, the latter technique, which was shown to be more sensitive than the manometric O<sub>2</sub> uptake,<sup>12</sup> was used to determine the effect of the three above-mentioned dental ma-

terials and the cavity preparation procedure on the pulp tissue.

The dogs used in the study were administered a general anesthetic composed of a Nembutal/Bio-tal mixture, as well as a local anesthetic of 2% Xylocaine with 1:100,000 Epinephrine prior to the extraction of the teeth. These two drugs would be expected to depress pulp tissue respiration,<sup>26,27</sup> but since the general anesthetic was administered proportionally to body weight and the local anesthetic equally distributed to the teeth by means of an infiltration technique, the amount of depression would be equal in each pulp specimen in the sample and would not be a factor impairing the findings.

The teeth in the sample were actively undergoing dentinogenesis and eruption during their operative dentistry procedure and surgical removal. Because of this finding, a higher pulp respiration rate could be expected than in teeth which were fully erupted and not undergoing active dentinogenesis.<sup>28,29</sup> Because of the relative proximity in the age of the animals used in the study, the stage of development in the extracted teeth and the consistency found in the narrow range of the pulp respiration rates in the control group seems to indicate homogeneity within the tooth sample.

The pulp respiration rate in Group II (cavity preparation alone) was depressed 40.42% when compared to the control. This resulted despite cavity preparation under ideal conditions of both an air and water coolant. Just how quickly the pulp recovers from this initial respiratory depression was not determined in this study. The results demonstrate, however, that conventional operative procedures can be expected to affect pulp tissue metabolism.

A surprising result was found in this study regarding the pulpal response to a dental amalgam restoration which conflicted with previous studies showing a mild to moderate pulpal response to amalgam condensation. This study showed a 65.96% mean reduction in the pulp respiration rate in teeth with a cavity preparation into which amalgam was condensed with no base or cavity liner. This could be caused by various components of dental amalgam which inhibit different cellular enzyme systems.<sup>30,31</sup> During the actual clinical procedure, a cavity varnish, liner, or base is usually placed prior to the condensation of the amalgam restoration. The role of this varnish, liner, or base was not investigated in this study, but it was demonstrated that dental amalgam is cytotoxic to the pulp tissue when placed in a cavity preparation without any type of pulpal protection.

Since the eugenol component of ZOE bases is believed to serve as the pulpal irritant, a pre-measured mix was made in an attempt to keep the residual eu-

genol constant with each mix. ZOE when used as a temporary restoration or base was found to cause little or no pulpal inflammation. And it has been claimed to reduce pulpal inflammation. That was confirmed by this study in which a ZOE base was found only to reduce the pulp respiration by 10.64%. As to the claim that ZOE can reduce pulp inflammation, one can only speculate about such a result when cavity preparation alone reduced the pulp respiration rate by 40.42%. This finding seems to indicate the healing effect of a ZOE base on the pulp tissue following cavity preparation. However, this outcome cannot be concluded from the findings of this study.

Dycal was also found to depress pulp respiration by 28.72%. This result also seems to indicate a reduction in the amount of depression in the respiration rate of the pulp occurring during cavity preparation. But the findings are not conclusive.

A uniform cavity depth was estimated clinically in a series of teeth using bur head size. A wide range in the cavity preparation depth was shown, 745-1080 microns, indicating the unreliability of clinical estimation in determining uniform depth of the preparation. A wide range of remaining dentin between the roof of the pulp chamber and the floor of the cavity preparation, 130-1095 microns, was also found. This was due to individual variation in the thickness of enamel and dentin in each tooth as well as the wide range in depth of the cavity preparations. Previous studies showed the effect of cavity preparation and dental materials on the dental pulp was related to the depth of the cavity preparation. No such correlation was found in this study comparing the thickness of dentin between the roof of the pulp chamber and the floor of the cavity preparation except in the ZOE group.

The results illustrate a physiological effect on cellular metabolism on the pulp tissue following cavity preparation and the placement of three routinely used dental materials. Dental amalgam was found severely cytotoxic to the pulp when condensed into a cavity preparation without any base or liner protecting the pulp regardless of the depth of this preparation. This finding suggests the placement of a base prior to condensation of amalgam into any cavity preparation. The base would prevent penetration of toxic amalgam materials from reaching the pulp.

It is hoped that this study leads to further investigations that will identify the specific irritants in dental materials and which enzyme systems are affected by these irritants. Such findings should provide information about how to prevent the deleterious effects of dental materials.

## Conclusions

The restorative materials used in this investigation and cavity preparation were found to depress respiration of the pulpal tissue by the following percentages:

dental amalgam .....	65.96%
zinc oxide-eugenol .....	10.64%
Dycal .....	28.72%
cavity preparation .....	40.42%

Less depression of tissue respiration was found when amalgam was condensed into a cavity preparation with a base present than when condensed directly into the cavity preparation causing an amalgam-dentin interface at the floor of the preparation.

A correlation between the thickness of dentin found between the roof of the pulp chamber and the floor of the cavity preparation and pulp respiration rate was not established in this investigation except for the zinc oxide-eugenol group.

Using bur head size for estimating uniform depth in a series of cavity preparations was found to be an unreliable method.

---

## References

1. Bodecker, C.: "Demonstration of Possible Ill Effects of Heat on the Pulp by Rapid Operative Technic," *JADA*, 26:527-532, 1939.
2. Brännström, M.: "Dentinal and Pulpal Response: I Application of Reduced Pressure to Exposed Dentin," *Acta Odont Scand*, 18:1-15, 1960.
3. Brännström, M.: "Dentinal and Pulpal Response: V Application of Pressure to Exposed Dentin," *J Dent Res*, 40:960-970, 1961.
4. Dachi, S. and Stigers, R.: "Pulpal Effects of Water and Air Coolants Used in High Speed Cavity Preparation," *JADA*, 76:95-98, 1968.
5. Goto, G., Imanishi, T. and Machida, Y.: "Clinico-Pathological Study on the Effect of High Speed Cavity Preparation on Human Deciduous Teeth," *Bull Tokyo Dent Col* 10:19-44, 1969.
6. Bhaskar, S., Cutright, D. and Beasley, J.: "Pulpal Response to Four Restorative Materials," *Oral Surg*, 28:126-133, 1969.
7. Brännström, M.: "Reaction of the Pulp to Amalgam Fillings," *Odont Revy*, 14:244-253, 1963.
8. Cotton, W.: "Pulpal Responses to Cavity Drying," *J Dent Child*, 38:85-92, 1971.
9. Dubner, R. and Stanley, H.: "Reaction of the Human Dental Pulp to Temporary Filling Materials," *Oral Surg*, 15:1009-1017, 1962.
10. Going, R. and Massler, M.: "Influence of Cavity Liners Under Amalgam Restorations on Penetration by Radioactive Isotopes," *J Pros Dent*, 11:298-312, 1961.
11. Fisher, A., Schumacher, E., Robinson, N., and Sharbondy, G.: "Effects of Dental Drugs and Materials in the Rate

- of Oxygen Consumption in Bovine Dental Pulp," *J Dent Res*, 36:447-450, 1957.
12. Jones, P. J., Taintor, J. F., and Adams, A. B.: "Comparative Dental Material Cytotoxicity Measured by Depression of Rat Incisor Pulp Respiration," *J Endod*, 5:48-55, February 1979.
  13. Applebaum, E.: "Lymph Channels in Dentin and Enamel Stained by Amalgam," *J Dent Res*, 9:487-502, 1929.
  14. Avery, J.: "Response of the Pulp and Dentin to Contact with Filling Materials," *J Dent Res*, 54:B188-B197, 1975.
  15. Brännström, M. and Soremark, R.: "The Penetration of <sup>22</sup>Na Ions Around Amalgam Restorations With and Without Cavity Varnish," *Odont Revy*, 13:331-336, 1962.
  16. Brännström, M. and Billberg, B.: "Pulp Changes Beneath Temporary Filling with Pharmatec and Zinc Oxide Eugenol," *Odont Revy*, 18:17-26, 1967.
  17. Brännström, M. and Nyborg, H.: "Pulp Reaction to a Temporary Zinc Oxide Eugenol Cement," *J Prosth Dent*, 35:185-191, 1976.
  18. Brännström, M.: "The Effect of Dentin Dessication and Aspirated Odontoblasts on the Pulp," *J Prosth Dent*, 20:165-171, 1968.
  19. Brännström, M. and Johnson, G.: "Movements of the Dentin and Pulp Liquids on Application of Thermal Stimuli: An *In Vitro* Study," *Acta Odont Scand*, 28:59-70, 1970.
  20. Fisher, C.: "Odontoblastenkerne in Dentinkand," *Dent Zahnmond Kiefrkh*, 18:39-46, 1953.
  21. James, V. and Schour, I.: "The Effects of Cavity Preparation and Zinc Oxide and Eugenol Upon the Human Pulp," *J Dent Res*, 35:421-426, 1956.
  22. Langeland, K.: *Tissue Changes in the Dental Pulp*, Oslo University Press: Oslo, 1957.
  23. Langeland, K.: "Histologic Evaluation of Pulp Reactions to Operative Procedures," *Oral Surg*, 12:1235-1248, 1959.
  24. Langeland, K.: "Tissue Changes Incident to Cavity Preparation: An Evaluation of Some Dental Engines," *Acta Odont Scand*, 19:397-430, 1961.
  25. Labart, W., Weimer, A. and Taintor, J. F.: "The Effect of Orthodontic Forces on Pulpal Respiration in the Rat Incisor," Submitted for Publication.
  26. Fisher, A. and Schwabe, C.: "Effects of Procaine Concentration and Duration of Contact on Oxygen Consumption in Bovine Dental Pulp," *J Dent Res*, 41:484-490, 1962.
  27. Kozam, G. and Burnett, G.: "The Effect of Local Anesthetics on the Respiration of Dental Pulp," *Oral Surg*, 13:229-235, 1960.
  28. Fisher, A., Belding, J., Opinsky, J. and Spinella, D.: "The Influence of the Stage of Tooth Development on the Oxygen Quotient of Normal Bovine Dental Pulp," *J Dent Res*, 38:208-215, 1959.
  29. Sasaki, S.: "Studies on the Respiration of the Tooth Germ," *J Biochem*, 46:269-279, 1959.
  30. Biesterfeld, R., Taintor, J. and Marsh, C.: "The Significance of Alterations of Pulpal Respiration," *J Oral Path*, 8:129-139, 1979.
  31. Maniloff, J., Coleman, J. and Miller, M.: *Effects of Metals on Cells, Subcellular Elements and Macromolecules*, Springfield: Charles C. Thomas, Publishers, 1970, pp. 291-340.

---

EDMUND R. PROCTOR is a Resident in Pedodontics, University of Nebraska College of Dentistry, Lincoln, Nebraska.

ROBERT E. SULLIVAN is Professor of Pedodontics, University of Nebraska College of Dentistry, Lincoln, Nebraska.

JERRY F. TAINTOR is an Associate Professor and Chairman of Endodontics, University of California School of Dentistry, The Center for the Health Sciences, Los Angeles, California.

Requests for reprints may be sent to Dr. Robert E. Sullivan, College of Dentistry, University of Nebraska, Lincoln, NE 68583.

---