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The pulpal response to an experimental luting resin

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SUMMARY

An experimental luting resin became available for evaluation of its pulpal irritation potential. The material is a high molecular weight urethane-di-methacrylate which does not require a thinning monomer such as tri-ethylene-glycol-di-methacrylate (TEGMA). In each of 24 anaesthetized adult vervet monkeys labial cavities were cut in each of the 8 incisor teeth. In each monkey 2 randomly selected cavities were restored with one of 4 materials — the test resin, a silicate cement and 2 commercial zine oxide/engenol preparations. Specimens were recovered from 8 animals after 2 days, from 8 animals after 28 days and from a further 8 animals after 56 days. The specimens were prepared for histological examination. The resin was found to have caused severe inflammatory pulpal reactions in 2 day specimens. After 56 days no inflammatory cell infiltration of the pulp tissue was observable.

OPSOMMING

Die potensiële irritasie van die tandpulpa deur 'n eksperimentele sementeringshars is ondersoek. Die materiaal is 'n hoë molekulêre gewig uretaan-di-metakrilaat wat nie 'n verdunningsmonomeer soos tri-etileenglycol-di-metakrilaat (TEGMA) vereis nie. Vier en twintig volwasse blouape is in die ondersoek gebruik. Kawiteite is op die labiale vlakke van die 8 snytande van elke aap voorberei. In elke geval is 2 van die kaviteite met een van die volgende 4 materiale herstel: die toetshars, 'n silikaatsement en 2 kommersiële sinkoksied/eugenol preparate. Monsters is van 8 proefdiere na 2 dae verkry, van nog 8 na 28 dae en van die oorblywende 8 na 56 dae. Die monsters is behoorlik voorberei en daarna histologies ondersoek. Die ondersoek het getoon dat die hars 'n erge inflammatoriese reaksie in die pulpas van die 2 dae-groep veroorsaak het. In die 56 dae-groep is geen inflammatoriese selinfiltrasie in die pulpaweefsel waargeneem nie.

INTRODUCTION

In recent years a number of studies of the pulpal effects of various dental cements used as luting materials have been published. Attention has been largely focussed on zinc phosphate and especially on zinc polycarboxylate cements (Plant, 1970; Valcke, 1971; Smith, 1971; Barnes and Turner, 1971; Jendresen and Trowbridge, 1972; Plant and Jones, 1976). The effects of using cavity cleansers and pulp-protecting preparations in conjunction with luting cements have also been reported (Eames, Hendrix and Cleveland, 1978; Eames, Hendrix and Mohler, 1979). The general impression derived from reported studies of the pulpal reactions to luting materials is that the polycarboxylate cements are the least damaging.

Recently an experimental luting resin was made available for investigation of its effect on the dental pulp. It is well known that resins are irritant and even lethal to the dental pulp (Kramer and McLean, 1952; Manning, 1959; Heys *et al*, 1977; and Valcke *et al*, 1980). It was suggested that, since the experimental luting resin consists of a high molecular weight (about 500) urethane-di-methacrylate, which has a low diffusion coefficient, the resin would have a less severe effect on the pulp than other resins. Bis-GMA resins require a thinning monomer, tri-ethylene-glycol-di-methacrylate (TEGMA), and TEGMA is volatile. TEGMA, having a high diffusion coefficient, was considered to be responsible for pulpal damage (Michl, 1977).

This study was undertaken to test the hypothesis that the experimental resin might be less injurious to the pulp than other resins, the effects of which are already well documented.

MATERIALS AND METHODS

The method employed was based on the "Recommended standard practices for biological evaluation of dental materials" published by the Council on Dental Materials and Devices of the American Dental Association (1972). Twenty-four adult vervet monkeys (*Cercopithecus aethiops*) were used. These animals were immobilized with intramuscular phencyclidine hydrochloride*. Each monkey was examined and those exhibiting gross dental or periodontal defects were rejected and replaced with clinically normal subjects, which were then anaesthetized with

* Sernylan, Bio-ceutic Labs, Inc., St. Joseph, U.S.A.

intravenous pentobarbitone sodium**. In each animal a labial cavity was cut in each of the 8 incisor teeth (maxillary and mandibular). The instrument used was a plain cut inverted cone tungsten carbide friction grip bur size ISO 010 (U.S. 35) in an air turbine handpiece with water spray and no special finishing procedure was applied to the enamel margins of the cavities. A single operator prepared and restored the cavities in groups of 6 monkeys at each of 4 sessions. At each session 2 monkeys were allocated at random by drawing lots for specimen retrieval at each of 3 post-operative time intervals. Cavities to be restored with different materials were also allocated at random. The materials used were two commercial zinc oxide/eugenol (ZOE) cements as non-irritant negative controls, a silicate cement as an irritant positive control, and the unlined experimental luting resin. The materials were mixed in accordance with the manufacturer's instructions; in the case of the resin the powder was supplied in a predosed capsule and the liquid in a graduated syringe to facilitate accurate proportioning. All materials were mixed manually. Before insertion of the filling materials the cavities were debrided with an air/water spray and dried with compressed air. The materials were inserted with Oxicap*** anti-adhesive coated instruments to facilitate handling. The materials are identified in Table I.

On the expiry of the selected post-operative time intervals of 2, 28 and 56 days the specimens were retrieved. The monkeys were anaesthetized and their tooth pulps were fixed *in situ* by perfusion with phosphate buffered formol saline (Retief and Austin, 1973). Blocks of tissue in which the incisor teeth were included were recovered and decalcified in 0,5 M EDTA at pH 7,4 and 60 °C. Restorations present after decalcification were carefully removed with a needle. The teeth were then separated and embedded in Paraplast**** under vacuum.

Longitudinal labio-lingual serial sections were cut through the cavities at 7µm intervals and stained with haematoxylin and eosin. Using a Univar***** optical microscope the sections were examined independently by 2 assessors. At examination the sections were not identifiable in respect of either post-operative time interval or material used. Assessments of reactions were made using the criteria proposed by Stanley (1968). The remaining dentine thickness was measured with a measuring graticule along the shortest straight line between the cavity floor and the pulpal periphery. Three pulpal abnormalities were graded 0 — 3 ° according to severity. These abnormalities were displacement of odontoblast nuclei and superficial and deep inflammatory cell infiltration. Note was also taken of the presence or absence of reparative dentine.

Details of the post-operative time intervals and filling materials used were then retrieved and related to the mean gradings of the assessors, the information being entered in punch cards. The data was analysed with an I.B.M. 370/158 computer, using the Statistical Package for the Social Sciences (Nie *et al*, 1975).

RESULTS

The majority of the cavities restored with CAVITEC were either totally or largely empty when the specimens were recovered. The animals were fed a formulated cubed laboratory animal diet supplemented with fruit. On this diet the monkeys make little, if any, use of their anterior teeth in feeding. Wood Jones (1925) observed that "if an animal retains fairly generalised digits such as are possessed by the higher primates, toilet appliances will be few. The nimble, resourceful, inquisitive fingers have supplanted the specialised toilet implements". It seems likely that, in this case, the nimble and inquisitive fingers played a part in the loss of the fillings. CAVITEC is intended for use as a lining material and it is thus impossible to blame the manufacturers of the material in any way for the loss of these restorations. Heys *et al.*, 1977, used CAVITEC as a negative control in a pulpal study. However, they protected the material with a veneer of amalgam (Heys, 1978). The 48 CAVITEC specimens were discarded. Other specimens were also discarded for various reasons. 105 specimens were finally available for assessment.

Table II indicates the mean pulpal response to the test and control materials, while Table III shows the number and percentage of teeth in each experimental group showing odontoblast nuclei displacement, superficial inflammation, deep inflammation and reparative dentine formation.

The mean pulpal response scores according to Stanley's (1968) criteria (Table II) were statistically analysed

Table I Identification of materials

BRAND	TYPE	MANUFACTURER	BATCH NO
NOBETEC	Reinforced zinc oxide/ eugenol cement	BOFORS, Nobel-Pharma, Sweden	Liquid UT 1450 Powder Z 1409
CAVITEC	Two-paste zinc oxide/eugenol cement	KERR Mfg. Co. Romulus, Michigan, U.S.A.	Base 6 1356. Accelerator 7 1003
SUPER SYNTREX	Silicate cement	Amalgamated Dental, England.	Liquid PA 18 Powder T 1 E (Shade 30)
EXPERIMENTAL RESIN	Urethane-dimethacrylate	VIVADENT, Schaan, Liechtenstein.	230 178 588

** Sagatal, Maybaker (S.A.), Port Elizabeth, South Africa.
*** Vivadent, Schaan, Liechtenstein.
**** Sherwood Medical Industries, St. Louis, U.S.A.
***** C. Reichert A.G., Vienna, Austria.

Table II Mean pulpal response to test and control materials

Post-operative time interval	Material	No. of Teeth	Remaining Dentine Thickness x ± SD mm	Mean Odontoblast Displacement 0 - 3 °	Mean Superficial Inflammatory Response 0 - 3 °	Mean Deep Inflammatory Response 0 - 3 °
2 days	Nobetec	11	0,45 ± 0,23	0,4	0,4	0,1
	Syntrex	14	0,58 ± 0,24	1,3 } *	1,2 } *	0,4 } *
	Resin	15	0,45 ± 0,23	1,9	1,6	0,8
28 days	Nobetec	12	0,56 ± 0,25	0,1	0,3	0,1
	Syntrex	9	0,37 ± 0,11	0,5	0,4	0,3
	Resin	13	0,49 ± 0,20	0,1	0,3	0,2
56 days	Nobetec	11	0,47 ± 0,22	0,0	0,0	0,0
	Syntrex	9	0,49 ± 0,20	0,0	0,0	0,0
	Resin	11	0,60 ± 0,25	0,0	0,0	0,0

* $p < 0,01$ (Scheffe's test)

Table III Numbers of teeth (%) showing the presence of 1° or more of odontoblast displacement, superficial and deep inflammation and the formation of reparative dentine.

Post-operative time interval	Material	No. of Teeth	Odontoblast Displacement		Superficial Inflammation		Deep Inflammation		Reparative Dentine	
			No	%	No	%	No	%	No	%
2 days	Nobetec	11	4	36,4	3	27,3	1	9,1	0	0,0
	Syntrex	14	9	64,3	8	57,1	5	35,7	0	0,0
	Resin	15	13	86,7	11	73,3	9	60,0	0	0,0
28 days	Nobetec	12	2	16,7	1	8,3	1	8,3	6	50,0
	Syntrex	9	3	33,3	2	22,2	2	22,2	7	77,8
	Resin	13	2	15,4	2	15,4	2	15,4	8	61,5
56 days	Nobetec	11	0	0,0	0	0,0	0	0,0	8	72,7
	Syntrex	9	0	0,0	0	0,0	0	0,0	9	100,0
	Resin	11	0	0,0	0	0,0	0	0,0	7	63,6

using the one-way analysis of variance and Scheffe's multiple comparison test (Roscoe, 1975). The resin-filled specimens showed at 2 days significantly more odontoblast displacement and superficial and deep inflammation than NOBETEC ($p < 0,01$).

A non-parametric statistical analysis of the data reported in Table III was also carried out. The Fischer exact probability test (Siegel, 1956) showed the same pattern as the Scheffe's test. Odontoblast displacement and superficial and deep inflammation were significantly greater with the resin than with NOBETEC ($p < 0,05$).

Odontoblast displacement.

The most displacement of odontoblast nuclei occurred in relation to the resin after 2 days (Figs. 1 & 2). At that time interval the least displacement occurred in relation to NOBETEC. At 28 days the greatest displacement occurred in relation to SYNTREX and the least displacement in relation to the resin. In 56 day specimens no displacement was observed.

Superficial inflammation.

The resin specimens showed inflammatory cell infiltration into the odontoblast layer and subjacent pulpal tissue in a higher proportion of cases than with the other materials (Figs 1 & 2), such infiltration being least in the pulps related to NOBETEC, after 2 days.

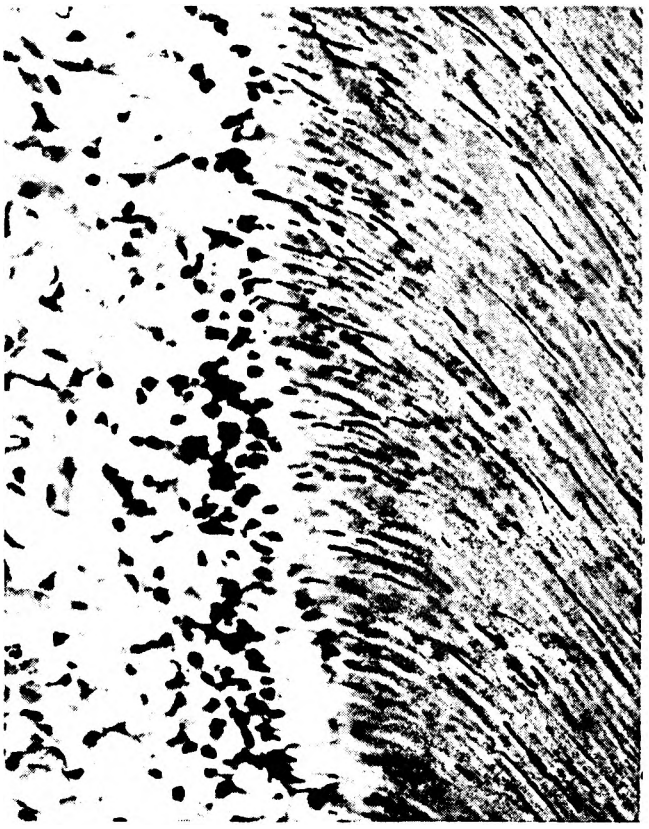

Fig. 1 Resin: 2 days: Odontoblast displacement, grade 3; Superficial inflammation, grade 3; deep inflammation, grade 2. x 480.

After 28 days the cellular infiltration was least in the NOBETEC specimens and greatest in the SYNTREX specimens, with the resin specimens displaying an intermediate degree of reaction (Figs 4 & 5). None of the 56 day specimens showed superficial inflammation.

Deep inflammation.

Inflammatory cell infiltration of the deeper tissue of the pulp after 2 days was similar to that recorded for the superficial tissue, while in the cases of 28 and 56 day specimens, the findings were identical (Figs. 1, 2, 3, 4, 5 & 6).

Reparative Dentine.

Progressive formation of reparative dentine was noted in the majority of 28 and 56 day specimens (Figs. 4 & 6).

DISCUSSION

The vervet monkey is considered a satisfactory experimental model for the study of pulpal reactions to restorative materials (Retief and Austin, 1973). A number of variables may distort the results of pulpal response studies. These include operative trauma, cavity toilet techniques, bacterial contamination and the variability of reactions to control materials, which have been discussed elsewhere (Valcke *et al.*, 1980).

The mistaken use of CAVITEC was an unsuccessful attempt to employ a less irritant negative control than NOBETEC and to establish a comparison between the 2 materials.

Fig. 2 Resin: 2 days: Odontoblast displacement, grade 3; Superficial inflammation, grade 3: Deep inflammation, grade 2. x 480.

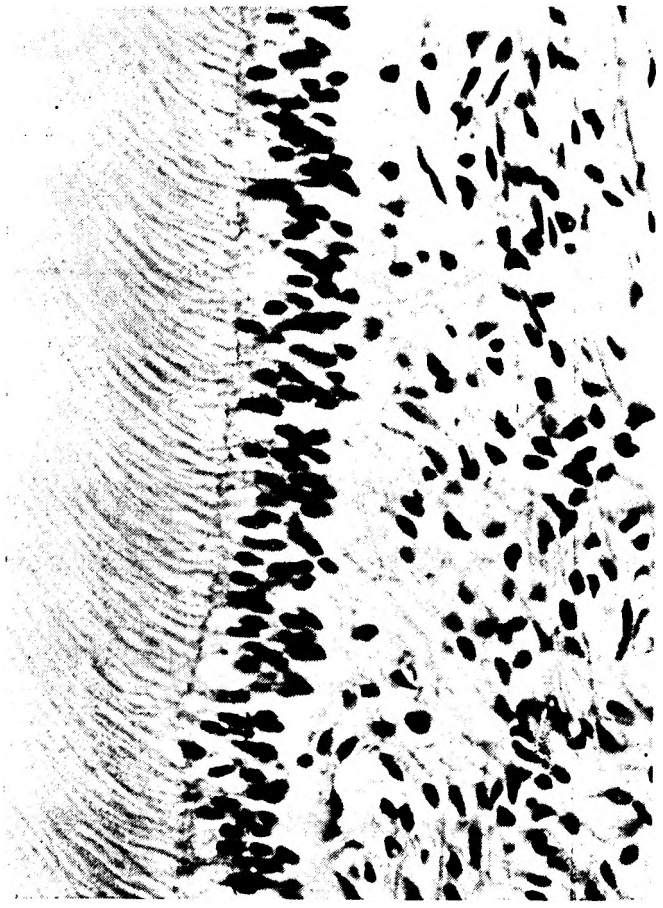

Fig. 3 Resin: 2 days: No inflammatory reaction. x 480.

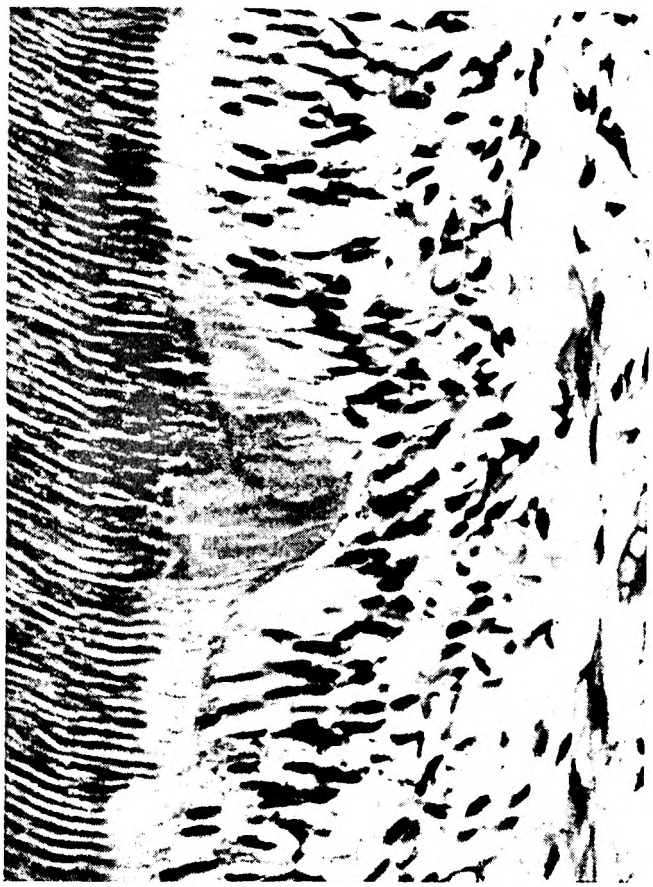

Fig. 4 Resin: 28 days: Reparative dentine: Superficial inflammation, grade 1: Deep inflammation, grade 1. x 480.

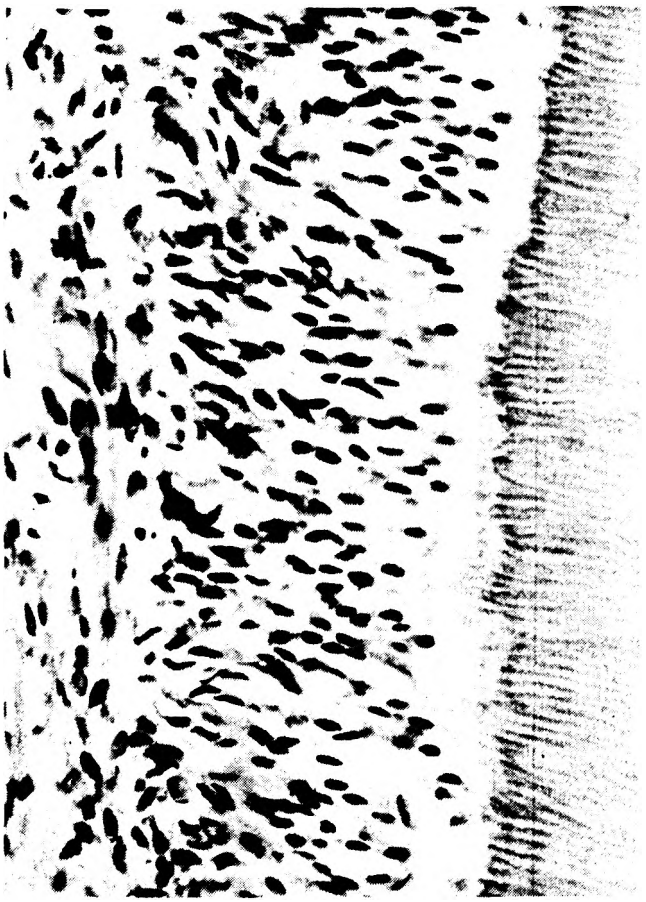

Fig. 5 Resin: 28 days: No inflammatory reaction. x 480.

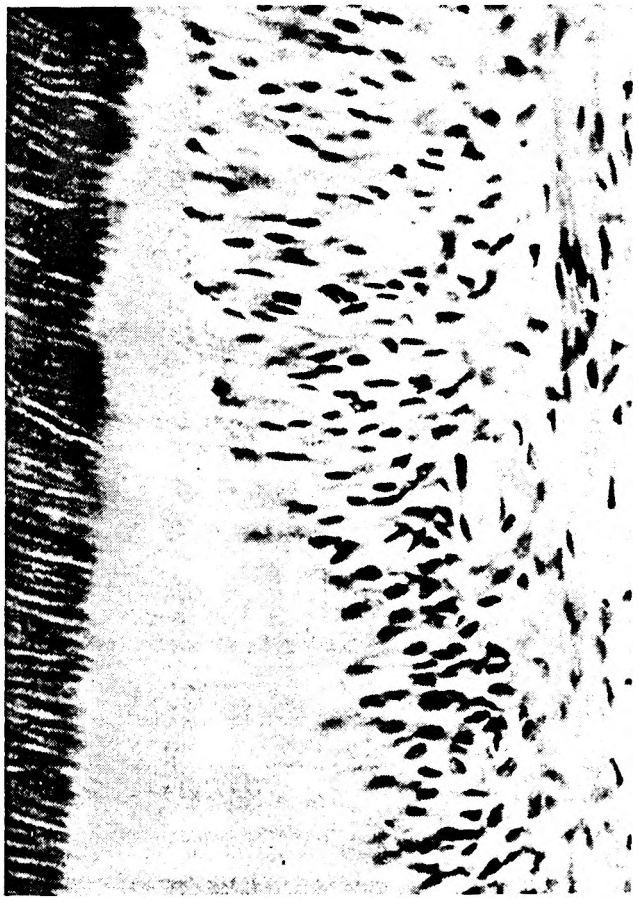

Fig. 6 Resin: 56 days: Reparative dentine: No inflammatory reaction. x 480.

The importance of another variable, the thickness of the remaining dentine between the test material and the pulp, is uncertain. The consideration of this point is complicated by the fact that pulpal studies have used human subjects, various other primates, and a variety of other animals. An assumption that the dentine is the same in all these animals may or may not be valid. Dowden (1970) and Plant and Anderson (1978) consider that the remaining dentine thickness is relatively unimportant. Other authors, for example, Brännström and Nyborg (1969) and Stanley *et al* (1969) take a contrary view. The precise mechanism by which an irritant placed in contact with cut dentine reaches the pulp is unclear.

Fish (1926) considered that there is a lymphatic circulation in the dentine and he named the anastomosing terminal branches of the dentinal tubules the "marginal lymph plexus". He also suggested that tissue fluid occupied a space between the odontoblast process and the wall of the dentinal tubule (Fish, 1932). Recently a space between the odontoblast process and the limiting membrane was demonstrated by Thomas (1979), who also demonstrated that the odontoblast process is limited to the pulpal third of the tubule in human coronal dentine. Thus it would appear that an irritant might reach the pulp by diffusion along a space, in which case the cavity depth would possibly be of little significance. Alternatively, it may be that irritants reach the pulp by way of the odontoblast process, in which case the cavity would have to extend into the pulpal third of the coronal dentine for the irritant to exert an effect. The possibility of the operation of both mechanisms, either together in some cases, or singly in some cases, can hardly be dismissed.

The postulate that the experimental resin might be less irritant than other dental resins was not confirmed. In fact, the resin elicited a greater response than either SYNTREX or NOBETEC in 2 day specimens. In 28 day specimens the response was reduced and no inflammatory cells were present in the 56 day specimens. In attempting to decide the clinical significance of these results, the intended purpose of a material must be borne in mind. Since this is a luting material, its effect on the pulp might well be reduced by the effect of sedative materials used to protect the prepared tooth during fabrication of the permanent restoration. Boyle (1955) stated that, after preparation, secondary dentine formation begins without delay.

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