AN IN VITRO STUDY OF THE PENETRATING AND SEALING PROPERTIES OF BIS-GMA RESIN PIT AND FISSURE COATINGS

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INTRODUCTION

There are many reports in the early dental literature to indicate that pits and fissures have long been regarded as sites which are particularly susceptible to dental caries. (20) (49) (93) (119) (143) (182)

Subsequent clinical surveys have also indicated the great vulnerability of pits and fissures to dental caries. Parfitt, (139) in a study involving 3,114 English school-children aged 2-15 years, observed that the greatest proportion of dental caries occurred on the occlusal surfaces of molars in both the deciduous and the permanent dentitions. In the 15 year old group, 81 per cent of the lesions were on occlusal surfaces while only 11 per cent were on approximal surfaces and 8 per cent on buccal and lingual surfaces. Surveys by Barr, Diodati and Stephens (10) and Grainger and Reid (69) showed a similar high incidence of dental caries in the pit and fissure surfaces of posterior teeth.

The early years after eruption appear to be the time when pit and fissure surfaces are at greatest risk when compared to the approximal surfaces. Berman and Slack, (17) in a 3 year longitudinal study of initially sound upper left permanent second molar teeth in English schoolgirls,
showed that within 3 years approximately 57 per cent of the occlusal surfaces were decayed or filled. In the same period, however, only 12.9 per cent of the approximal surfaces and 8.6 per cent of the buccal and lingual surfaces were decayed or filled. Similarly, the study by Walsh and Smart (186) in New Zealand and the study by Miller, Hobson and Gaskell, (127) in England, illustrate the extremely rapid onset of the initial carious lesion in the occlusal surfaces of permanent posterior teeth in the early years after eruption.

Despite the overall reductions in dental caries incidence produced by communal water fluoridation, (1) (9) (12) (95) (115) it is apparent that pit and fissure areas still remain as the most susceptible areas for the initiation of dental caries. Communal water fluoridation provides preferential protection to smooth surfaces and occlusal pits and fissures do not benefit to the same high degree. (11) (115)

In absolute terms, there is an overall reduction in the occurrence of pit and fissure caries in optimum fluoride areas. However, the proportion of cavities that occur in pits and fissures may greatly increase, due to the greater protection given to smooth surfaces. (11)

Typically, in the Tiel-Culemborg experiment (11) (12)
the low fluoride town (Culemborg) had 41.8 per cent of the total cavities detected in the pit and fissure surfaces compared to 51.5 per cent in the smooth surfaces of 15 year old children. In the optimum fluoride town (Tiel), 73.2 per cent of the total cavities detected were in the pit and fissure surfaces compared to 26.8 per cent in smooth surfaces for a similar group of children.

A similar situation has been observed when fluoride is ingested in the form of tablets (121) or fluoridated table salt, (122) or when fluoride is applied topically. (56) (66) (90) (98) (100) (128) (144)

The extreme susceptibility of pits and fissures to the initiation of caries has prompted investigations into methods specifically designed to reduce their vulnerability. Such methods have included alteration of fissure shape by grinding, (21) (49) early placement of restorations, (20) (92) chemical treatments (8) (99) and the placement of flexible or rigid coatings over the openings to pits and fissures.* In recent years the latter method, in particular, the use of rigid coatings based on Bowen's resin (BIS-GMA), (24) (25) has received much attention.**


Several of the clinical studies on BIS-GMA resin coatings have been of at least 2 years duration, (37)(87)(91)(162)(165) others have extended for periods of 12 months (67)(156)(158)(160)(161)(177)(188) while some have been of only 9 months (124) or 6 months (62)(116)(189) duration.

Except in a limited number of instances, (35)(37) fully retained BIS-GMA resin coatings were not removed in order to assess the soundness of the underlying fissure while only a few of the clinical studies (35)(37)(87) reported that bitewing radiographs were used to evaluate resin treated teeth at recall examinations.

Ideally, resins used to prevent caries in occlusal surfaces should completely fill and seal the entire fissure. From clinical studies it cannot be determined whether fissures are completely filled by resins or whether microleakage may occur. There have been conflicting claims* regarding the ability of BIS-GMA resins to completely penetrate fissures and there have been further conflicting claims** regarding the microleakage of these fissure coating resins. As far as can be ascertained from the literature, there have been no studies to show whether fermentable carbohydrates may penetrate between the resin and the tooth surface.


** (48)(50)(123)(146)(166)(190)
This in vitro study was undertaken to assist in clarifying some of these areas of contention, by determining the efficacy of BIS-GMA resins in penetrating and sealing pits and fissures. In the first part of the study an assessment was made of the depth of resin penetration into fissures in extracted teeth, whilst, in the second part, the fissure sealing properties of the resins were investigated using Na$_2^{14}$CO$_3$, sucrose-$^{14}$C and D-glucose-$^{14}$C. The latter two isotopes were used to determine the likelihood of fermentable carbohydrates penetrating the resin-enamel interspace.

This thesis presents a review of the literature relating to the development and investigation of pit and fissure treatments designed to prevent the initiation of dental caries. In addition, a description of the materials and methods used in the study, an analysis of the results obtained and a discussion of the results and the implications of these findings, are given.
REVIEW OF LITERATURE

The susceptibility of pits and fissures to dental caries has resulted in the introduction of several clinical procedures aimed at reducing the vulnerability of these areas. Such procedures have included prophylactic odontotomy, modification of fissure shape, treatment with chemical agents or coating the openings of pits and fissures with a variety of materials.

METHODS USED TO MINIMISE THE INCIDENCE OF PIT AND FISSURE CARIES.

PROPHYLACTIC ODONTO TOMY

Early practice encouraged the cutting out of retentive fissures and the placement of a restorative material as soon as possible after the tooth had erupted whether the fissure was carious or not. (20)(55)(65)(143) This operative procedure was strongly advocated by Hyatt (92) who termed the early restoration of such fissures "prophylactic odontotomy".

FISSURE ERADICATION

Bodecker, (18)(19) similarly, believed that the shape of certain fissures greatly predisposed them to caries and prevented adequate cleaning. Bodecker (21) supported the
concept of prophylactic odontotomy, but, in some situations, suggested that a process called "fissure eradication" was more appropriate. This process was an operative procedure whereby the fissural constriction was ground to a more favourable shape with round burs.

Prophylactic odontotomy and fissure eradication procedures, though apparently widely accepted, were not without their opponents. (31)(32)(183) Brucker, (31)(32) in particular, questioned the rationale of prophylactic odontotomy, and, unlike Hyatt, (92) found that a great number of fissures remained immune from caries. Adoption of a procedure which involved the removal of sound tooth structure, in anticipation of caries developing, was also regarded as not being truly preventive. (183)

CHEMICAL TREATMENTS

Silver nitrate (99)(125) and zinc chloride with potassium ferrocyanide (8)(142) have been used in attempts to inhibit pit and fissure caries. However, it was found that such treatments did not reduce the incidence of dental caries or aid in the arrestment of incipient lesions. More recently, a procedure involving fissure modification followed by chemical treatment with acetic acid and chromic anhydride has been tried, apparently with some success. (184)
It appears that the most successful form of chemical treatment for inhibiting caries in pits and fissures, has been the topical use of fluorides. However, despite overall reductions in occlusal caries incidence, the greatest benefits are imparted to smooth surfaces when fluoride is topically applied in a variety of forms. (56) (66) (90) (98) (100) (128) (144)

Dogon, Van Leeuwen and Kirklin (53) (54) and Pugnier (150) have used a fluoride containing cyanoacrylate coating to sustain high fluoride levels of acidulated phosphate fluoride solution applied to the occlusal surfaces. This technique showed considerable promise but ideally required 6 monthly applications. Fluoride containing polyurethane resins, specifically for the treatment of fissures, have been used also to increase the exposure time of the underlying enamel to the benefits of topically applied fluoride. *Epoxylite 9070 Topical Fluoride Coating and Fissure Sealant* (108) contains 10 per cent disodium monofluorophosphate as a fluoride component while *Elmex Protector** contains the hydro-fluoride of cetylamine. (161)

Only a limited number of clinical studies (124) (151) (160) (161) (162) have been reported on the efficacy of the fluoride containing polyurethane resins as fissure caries

* Lee Pharmaceuticals, South El Monte, Calif. Hereafter, referred to as "Epoxylite 9070".

** Vivadent, Liechtenstein.
preventive treatments. Only one trial, (151) involving *Epoxy light 9070*, has shown any reduction in fissure caries in the teeth treated with this product. All of the other trials have shown little or no benefits to be derived from the use of either product. The retention of these polyurethane coatings in the oral environment has been extremely short lived, with most coatings being lost within a few weeks. Although Lee et al., (108)(109) have reported that *Epoxy light 9070* showed promise in laboratory studies as a vehicle for applying fluoride to fissures, Forsten and Paunio (61) have shown little detectable fluoride to be available from *Epoxy light 9070* or *Elmex Protector* in the thicknesses normally applied to fissure areas.

PIT AND FISSURE COATINGS

a) Flexible Coatings

Covering fissure openings with flexible resin coatings, in the anticipation of preventing fissure caries, has generally proved to be quite unsuccessful. (124)(160)(161)(162) Both *Epoxy light 9070* and *Elmex Protector* polyurethane resins have shown limited durability in the oral environment. The majority of these coatings have been reported to be lost within several weeks. (124)(151) Both coatings contain fluoride, so in view of their limited durability, it appears that their main function is to expose the enamel in pits and fissures to a possible source of fluoride.
b) **Rigid Coatings**

Much attention has been given to the use of rigid coatings as barriers to occlude fissure orifices. Clinical studies have been conducted on such rigid fissure coatings as (i) dental cements, (118)(125)(185) (ii) alkyl-cyanoacrylate resins* and (iii) resins derived from the reaction product of bisphenol A and glycidyl methacrylate (BIS-GMA).**

(i) **Dental Cements**

Hyatt (92) and Bodecker (18) and other authors (65)(143) in the early dental literature, suggested, in some circumstances, coating fissures with oxyphosphate cements.

Some time later, Miller, (125) in 1951, reported the 2 year results of using copper cement in a clinical study. He found no significant reductions in fissure caries incidence as a result of covering fissures with copper cement at 6 monthly intervals.

More recently, further interest has been revived in the use of dental cements for protecting pits and fissures. Wallis (185) used a silico-phosphate cement, Petralit,***

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* (51)(141)(154)(178)(179)(180)
   (161)(162)(165)(177)(188)(189)
***Dental Fillings Ltd., London.
to cover fissures in upper and lower first permanent molars of 100 children aged 5-6 years. Forty one per cent of the treated fissures retained the material for an entire 6 months at which stage it was removed. At the final examination after 2 years, 75 per cent of the control sites were carious compared to 30 per cent of the treated sites. All the cement coatings were deliberately removed after 6 months which allowed direct examination of the treated pits and fissures.

McLean and Wilson (118) used a glass ionomer cement as a coating on fissures which had an orifice width greater than 100 microns. The fissures were pretreated with 50 per cent citric acid. After 2 years, 78 per cent of 138 treated teeth available for examination, still had cement coatings in situ. Only 5 of 138 treated teeth were carious at the end of 2 years. There were, however, no untreated controls to provide a comparison.

Fissure caries prevention employing dental cements would appear to have some value. However, to date, published clinical data is extremely limited and further clinical examination is required.
(ii) **Cyanoacrylates.**

There have been a number of clinical studies \(51, 141, 154, 178, 179, 180\) involving alkyl-cyanoacrylate resins as rigid fissure coating material.

The results of the studies of Takeuchi, \(178, 179, 180\) using an ethyl-1-2-cyanoacrylate resin coating, and the results of the studies of Cueto and Buonocore \(51\) and Ripa and Cole \(154\) using methyl-2-cyanoacrylate coatings, were encouraging. In these studies, the results varied but there were indications that cyanoacrylate fissure coatings may inhibit fissure caries. However, it was found that the retentive properties of the material were such that replacement was usually necessary every 6 months. Parkhouse and Winter, \(141\) in a clinical investigation involving a methyl-2-cyanoacrylate resin, found the retentive properties so poor that all fissure coatings were lost within 6 months and there was no significant reduction in fissure caries of the treated teeth.

Dogon, Van Leeuwen and Kirklin \(53, 54\) and Puginier \(150\) used a fluoride containing cyanoacrylate coating to augment the effects of a topical application of acidulated phosphate fluoride solution. Presumably, the cyanoacrylate coatings were used primarily to maintain high levels of fluoride at the enamel surface. The results from these studies were
quite encouraging with significant reductions in fissure
caries being recorded at the end of 2 years.

However, the American Dental Association Council of
Materials and Devices (6) has not recommended the routine
use of cyanoacrylate materials in dentistry. It reported
that cyanoacrylate polymers undergo degradation in the oral
environment and may form breakdown products that have a
potential to induce a toxic response. The Council further
noted that the most severe responses appear to be from
methyl-2-cyanoacrylate which breaks down rapidly and that
the higher cyanoacrylate homologues appear to undergo
slower degradation and produce less severe tissue reactions.

(iii) BIS-GMA Resins

BIS-GMA resins have received considerable attention
as rigid pit and fissure coatings. The basic resin was
developed by Bowen (25) and is essentially the reaction
product of bisphenol A and glycidyl methacrylate. The
viscosity of the reaction product is such that it is
usually diluted by the addition of methyl methacrylate or
other diluents to give the desired flow characteristics.(25)

A number of clinical trials* have been conducted to
test the efficacy of BIS-GMA resin coatings in preventing
pit and fissure caries. Some of these studies have

(161) (162) (165) (177) (188) (189)
extended for 2 or more years (37)(87)(91)(162)(165) but
most have not exceeded 12 months duration.* Although
the retention rates of BIS-GMA resin fissure coatings have
been shown to be high, (35)(37)(87)(91)(162) only one
investigator, Buonocore, (35)(37) has attempted to evaluate
the soundness of the fissures under the fully retained
resin coatings. He removed 27 fully retained resin
coatings on permanent teeth, to directly assess the
condition of the underlying fissures. Furthermore, only
Buonocore (35)(37) and Hinding and Buonocore (87) have
reported the use of bitewing radiographs to provide an
indication of the soundness of the coated pits and fissures
at recall examinations.

The varying degree of success obtained with BIS-GMA
resin fissure coatings in clinical trials and the use of
additives, in some of the coatings tested, (35)(37) has
resulted in the American Dental Association Council on
Dental Materials and Devices (2)(3)(4) assessing such
materials as only being "provisionally acceptable" as agents
for preventing pit and fissure caries.

Despite the inadequacies of many of the clinical studies,
the BIS-GMA resins have shown the most promise of the rigid
fissure coating materials.

LABORATORY STUDIES RELATED TO THE USE OF FISSURE COATINGS

In 1955, Buonocore (33) reported that the bonding of acrylic filling materials to enamel could be greatly enhanced by pretreating the enamel surfaces for 30 seconds with an 85 per cent solution of phosphoric acid.

Acid pretreatment techniques have since been used in such dental procedures as the repair of fractured incisal edges, (40)(159) the direct bonding of orthodontic brackets, (133)(187) the veneering of abraded or hypoplastic teeth with composite resin materials, (64)(85) the sealing of margins of restorations (43) as well as the coating of pit and fissure openings.

The popularity of such techniques has prompted laboratory investigations into the mechanisms of bonding resin materials to tooth structure.

Basically, in vitro research into pit and fissure coatings has been carried out in the following areas:

(1) The effect of acid solutions on enamel surfaces.
(2) The nature of the enamel-resin interface.
(3) The ability of resins to penetrate into pits and fissures.
(4) The sealing properties of pit and fissure coatings.
(1) THE EFFECT OF ACID SOLUTIONS ON ENAMEL

Since Buonocore (33) first pointed out the improvements in bonding gained by acid etching, many studies have been carried out to investigate the effect of acid solutions on enamel.* These studies have shown that when acid solutions are applied to clean enamel surfaces dissolution of the large inorganic component occurs. Such dissolution is, however, irregular and occurs at preferential sites related to the cores or peripheries of enamel prisms. (Figs. 1 and 2)

It has been suggested that preferential dissolution patterns may be influenced by enamel prism crystallite orientation, (7)(96)(136)(148)(169) the presence of organic material at various prism sites, (97)(136)(168) the presence of trace elements in diffusion pathways, (148) the time of exposure of the acid, (97)(187) the concentration of the demineralizing solutions (73)(97) and the type of acid solution used. (73)(138)

Hamilton, Judd and Ansell (80) suggested that the acid attack always started at the prism periphery. Subsequently Nichol, Judd and Ansell (136) suggested that the attack could then follow several directions either toward the prism core or along and around prism boundaries, thus explaining the various enamel dissolution patterns observed by themselves and other research workers.

Scanning electron micrograph showing preferential dissolution of the enamel prism cores after acid treatment. (Mag. x 4000 approx.)

(Fig. 3 Plate 2, Gwinnett (73))
FIGURE 2

Scanning electron micrograph showing preferential dissolution of the enamel prism peripheries after acid treatment. (Mag. x 4000 approx.)

(Fig. 4 Plate 2, Gwinnett (73))
FIGURE 3

Scanning electron micrograph showing 3 different enamel dissolution patterns adjacent to each other. Area (1) shows prism cores that have been etched preferentially by acid while area (2) shows prism peripheries etched preferentially. Area (3) shows pitted enamel resulting from acid treatment. (Mag. x 1200 approx.)

(Fig. 9 Plate 3, Gwinnett (73))
Although Gwinnett (73) and Retief (152) have observed different etch patterns on different areas on the same tooth (Fig. 3), both these investigators found prism cores to be preferentially dissolved more often than prism peripheries. However it does appear from a number of reports (73)(97)(129)(152) that the type of enamel etch patterns produced by acid solutions varies considerably.

It is noteworthy, however, that very few studies have been conducted on the effects of acid solutions on the fissure regions of occlusal surfaces. Gwinnett and Buonocore (77) and Hinding and Sveen, (88) after applying a solution of 50 per cent phosphoric acid and 7 per cent zinc oxide to occlusal surfaces, found that the prism cores were preferentially dissolved in most instances and that demineralization changes were confined mainly to cuspal inclines. In contrast, the deeper regions in pits and fissures were not usually affected.

Several studies, using tensile strength tests, have demonstrated the great improvement in bond strength between resins and enamel resulting from the prior treatment of enamel surfaces with acid solutions. (13)(29)(104)(105) In all cases, the tensile forces required to rupture the bonds formed with etched surfaces, far exceeded the forces required to break bonds to unetched surfaces. In many
instances where etching was employed, cohesive failures occurred within the body of the resin indicating the bond strength at the attached surface exceeded the tensile strength of the material itself. (104)(105) When resins attached to unetched surfaces were subjected to tensile loading, separation always occurred at the tooth-resin interface. (104)(105) The common use of 50 per cent concentrations of phosphoric acid in acid etch treatments has been justified by the results of tensile strength test studies (31)(138) and also by the results of surface etch profile studies. (52)(73)(174)

Buonocore (33) and Buonocore, Matsui and Gwinnett (41) have suggested that the improvements in bonding obtained by using acid solutions may be due to several factors:

(1) An increase in enamel surface area for attachment.

(2) The exposure of the organic framework of enamel, in and about which the resins can flow.

(3) The creation of spaces along interprismatic areas into which the resins can penetrate.

(4) The removal of the old, inert enamel surface, exposing a fresh, reactive surface more favourable for adhesion.

(5) The presence of an adsorbed layer of highly polar phosphate groups derived from the acid used.
However, in 1974, the American Dental Association Council on Dental Materials and Devices, \( (5) \) issued a statement regarding claims relating to "adhesion". The Council's opinion was that an "adhesive" dental material should bond to enamel "without reliance on interlocking effects." \( (5) \) In addition, the Council, after reviewing the status of pit and fissure coatings, ruled "that mechanical retention is the predominant mechanism of bonding and not adhesion." \( (4) \)

INFLUENCE OF PRISMLESS ENAMEL AND FLUORIDE TREATMENTS

Other factors which may affect the depth and pattern of etching by acid solutions are (i) the presence of prismless enamel and (ii) fluoride treatments.

(i) **Prismless Enamel**

Ripa, Gwinnett and Buonocore demonstrated the existence of an outermost layer of enamel composed almost entirely of crystallites whose c-axes are perpendicularly orientated to the outer enamel surface. Hence this layer of enamel does not present the usual prism structured appearance since there is no evidence of boundaries formed by abrupt changes in crystallite orientation. The presence of such a prismless outer layer of enamel has been demonstrated in both permanent and deciduous teeth. \( (70)(71)(94)(155)(164) \) All such observations, however, appear
mainly to have been limited to smooth surfaces and there appears to be little evidence to indicate that such layers are present in the fissure regions of teeth.

Sheykholeslam and Buonocore (171) remarked on the relative absence of prismless enamel on the occlusal surfaces of deciduous molars extracted from adult patients. Despite this, they suggested that the presence of prismless enamel may be responsible for the low retention rates of resin coatings in deciduous teeth observed in some of the clinical trials. (35)(37) They did consider, however, that the morphology and size of fissures present in deciduous teeth may also influence the relative lack of durability of resin coatings to deciduous teeth.

Gwinnett (76) has observed a pitted or smooth and relatively featureless type of dissolution pattern in prismless enamel after treatment with acid solutions. Furthermore, he suggested that such a dissolution pattern would provide a poor retentive surface for resin bonding.

(ii) Effect of Fluoride

Several investigators (110)(172)(192) have shown that fluoride treatment of etched enamel surfaces significantly reduced the bond strengths of applied resins. Fluoride appears to detrimentally affect bond strength whether applied to the enamel before (110) or after (172)
acids etching procedures. Deposition of reaction products within etched pores appears to be responsible for this reduction, when fluoride is applied subsequent to etching. (89)(172)

(2) THE NATURE OF THE ENAMEL-RESIN INTERFACE

Laboratory studies on the nature of the bond between resins and etched enamel surfaces have only established that bonding of the resins is primarily due to mechanical interlocking of these materials into micropores in the enamel surface created by acid dissolution. (73)(138)(152)(174) True adhesion involving molecular attractive forces of a chemical or physical nature are yet to be demonstrated. Utilization of such forces requires only very short distances of separation (1-2 Å) between adhesive and adherend and long term adhesion requires that this close proximity be maintained. (34)(74) Although adhesive forces may operate initially, it seems unlikely that such forces can be maintained in a moist oral environment due to the hydrophilic nature of the etched surfaces and the ability of water molecules to penetrate between adhering layers. (38)(153) It would appear, therefore, that mechanical bonding of resins to acid etched surfaces is the most likely mechanism of attachment and that resins flow into irregularly shaped microspaces created by preferential dissolution of the enamel prisms.
Studies of the enamel-resin interface have shown the presence of numerous projections emanating from the undersurface of the resin and interdigitating with the adjacent etched enamel irregularities.* The topography of the resin attachment surface appears to be influenced by such factors as the presence of prismless enamel and altered etch patterns produced by fluoride treatments. Apparently, these cause a reduction in the number and length of the resin tags (191) and a decrease in bond strength. (172)(192)

Silverstone (173)(174) has suggested that when resin coatings are lost, the loss may not be complete because resinous projections may remain embedded within the enamel. Furthermore, he suggested that these projections may then impart a resistance to subsequent acid attack. This factor has been cited as being responsible for the possible caries resistance of pits and fissures which have lost their resin coatings. (78)(86)

(3) THE ABILITY OF RESINS TO PENETRATE INTO PITS AND FISSURES

Ideally, materials used as fissure coatings should completely penetrate and seal the fissure constriction.

Lee and Swartz, (112) after examining a number of photomicrographs of fissures treated with polyurethane resin, claimed that the resin penetrated to the base of

the fissures in all instances and adapted closely to the fissure walls.

Lee et al., (110) in an examination of Epoxylite Fissure Sealant 9075* resin coatings, reported that the ability of this resin to penetrate deep fissures was "excellent." (111) After studying longitudinal sections of teeth treated with Epoxylite 9075 resin, Newhouse and Roydhouse (132) claimed that, in all cases, there was penetration of the resin to the base of the fissures where, they suggested, it intermingled with residual debris.

In marked contrast to these observations, other investigators have found that complete penetration of resins into deep fissures occurs infrequently. (39)(79) (149)(181)(190)(191)

It has been suggested that the degree of penetration may be influenced by the morphology (39)(181) and width (191) of pits and fissures and the entrapment of debris (39)(77) (181)(191) or air, (77)(181) within them.

In particular, Wright and Beck, (191) using light microscopy, and Taylor and Gwinnett, (181) using scanning electron microscopy, observed that narrow, deep fissures were usually incompletely filled by Nuva-Seal or Epoxylite 9075 resins. They also observed that debris was usually present

* Hereafter referred to as "Epoxylite 9075."
below the resin coatings over incompletely penetrated pits and fissures and that this debris appeared to preclude complete filling of the pits and fissures by the BIS-GMA resins.

(4) **THE SEALING PROPERTIES OF PIT AND FISSURE COATINGS**

**CYANOACRYLATE FISSURE COATINGS**

Buonocore, Matsui and Gwinnett (41) assessed the sealing properties of methyl cyanoacrylate coatings placed over the fissures of bicuspid and molar teeth. The specimens were stored for one year in saline solution before microleakage was assessed with 0.5 per cent basic fuchsin containing 10 μCi/ml. of radioactive sulphate. Dye penetration was assessed from sections of the teeth and radioisotope penetration was assessed from autoradiographs. The trial involved other materials but only 5 teeth were acid etched prior to the application of cyanoacrylate resin. No details are given but the investigators remarked on the "relative absence" (42) of penetration of the tracer solutions for this small group of specimens.

Figueroa et al. (58) examined the microleakage patterns in deciduous molar fissures covered with an ethyl-2-cyanoacrylate material. Thirty teeth were involved
in the study and no acid etching was employed prior to placement of the cyanoacrylate coatings. A 2 per cent solution of aniline blue dye was used to assess microleakage. Two groups of 10 specimens were subjected to temperature cycling while 10 specimens served as a control group maintained at 37°C. Microleakage occurred in 5 of the thermally cycled specimens but in none of the control specimens.

The microleakage of cyanoacrylate fissure coatings has also been assessed by Woody, Moffa and McCune (190) who used $^{45}\text{Ca}$ as tracer. They used extracted human molar teeth which were treated and stored for 6 months in distilled water at 37°C, and then thermal cycled prior to immersion in isotope solution. Other resin materials were also evaluated. Although complete details of their findings were not given the authors did indicate that the cyanoacrylate failed to seal the fissures against microleakage of $^{45}\text{Ca}$.

POLYURETHANE FISSURE COATINGS

Little work has been done on the assessment of microleakage occurring around polyurethane fissure coatings. However, Lee and Swartz, (112) using 30 extracted teeth, coated the fissures with polyurethane resin, stored them 7 days in water at 37°C and assessed microleakage by immersing the specimens in a $^{45}\text{CaCl}_2$ solution. The
autoradiographic findings showed that microleakage occurred in 7 of the 30 specimens.

Woody, Moffa and McCune (190) also assessed the sealing properties of a polyurethane fissure coating with $^{45}\text{Ca}$. The specimens were stored in water for 6 months and subjected to thermal cycling before being exposed to a $^{45}\text{CaCl}_2$ solution. Microleakage was found to occur.

BIS-GMA RESIN FISSURE COATINGS

Although BIS-GMA resins have been the subject of great interest in clinical trials of the fissure coating materials, there have been very few detailed accounts of the performance of these coatings in microleakage studies.

In 1971, Crowe (50) reported the results of a microleakage study involving the use of Epoxylite 9075 BIS-GMA resin as a fissure coating on extracted premolar and molar teeth. One group of teeth was placed in one per cent basic fuchsin solution for 30 minutes at room temperature. Another group was thermal cycled between crystal violet solutions, held at 5°C and 70°C, for 20 minutes. A third group of coated teeth was placed in a one per cent crystal violet solution and stored for 30 days at 37°C. Although no quantitative findings were presented the author indicated that the sealing properties were disappointing. Temperature cycling had no appreciable effect on the results.
Woody, Moffa and McCune, (190) in their assessment of several fissure coating materials using $^{45}$Ca as tracer, found that the ultraviolet light activated BIS-GMA resin was the only material which adequately prevented microleakage.

Pink, Corpron and Loesche (146) developed a technique to evaluate bacterial penetration occurring at the enamel-fissure coating interface. They covered extracted caries free human molar fissures with Nuva-Seal resin coatings. Sterile broth was inoculated with Strep. mutans or C. histolyticum and placed above the fissure coated area. After incubation for periods up to 8 days at 37°C the test organisms were identified as having penetrated the interface area in 42 of the 68 (61.8 per cent) specimens tested.

Later, Mednick, Loesche and Corpron (123) tested, in vivo, the ability of Nuva-Seal resin to prevent leakage of bacteria into shallow, sterile cavity preparations in fissures in human deciduous molars. Sterile paper points were placed under Nuva-Seal resin coatings in 25 teeth. Nineteen teeth were subsequently available for examination, the experimental period ranging from 21 to 116 days. Ten of these (52.7 per cent) demonstrated bacterial penetration to the base of the resin. This study also showed that
oral microorganisms purposely placed beneath Nuva-Seal resin coatings were viable, in a number of instances, after 4 to 16 weeks.

Craig (48) examined the sealing properties of Nuva-Seal and Epoxylite 9075 resins using a dye. All coatings exhibited some degree of dye penetration after thermal cycling.

Rudolph, Phillips and Swartz (166) used $^{45}$CaCl$_2$ as tracer to assess the sealing properties of Nuva-Seal, Epoxylite 9075 and an experimental BIS-GMA resin placed over pits and fissures in 180 sound extracted human molar teeth. Half the specimens were assessed for microleakage 7 days after fissure coating and the remainder were assessed after 3 months. At the end of each time period, 45 teeth were thermal cycled (15°C - 45°C) for a total of 2,500 cycles over an additional period of 5 days prior to microleakage assessment. The autoradiographic findings showed the presence of microleakage in 4 of the 60 teeth treated with Nuva-Seal resin, one of the 60 teeth treated with Epoxylite 9075 resin and none of the 60 teeth treated with the experimental BIS-GMA resin.
MATERIALS AND METHODS

1. FISSURE PENETRATION STUDY

MATERIALS

TEETH

Two hundred and sixty sound upper premolar teeth extracted for orthodontic reasons from patients, aged between 11 and 13 years, were used. Immediately after extraction, the roots of the teeth were removed and discarded. The crowns were then placed in Hanks' Balanced Salt Solution (83) and stored at 4°C until required.

ENAMEL PRETREATMENT SOLUTIONS

Nuva-Seal Tooth Conditioner* (50 per cent phosphoric acid containing 7 per cent by weight zinc oxide (35)) was used to etch the enamel prior to the application of Nuva-Seal* resin.

Epoxylite Fissure Cleanser 9075** (50 per cent phosphoric acid (110)(113)) was used to etch enamel and Epoxylite Fissure Primer 9075** (hydrolyzed trimethoxyorganofunctional vinyl silane in acetone solvent (110)(113))

* L.D. Caulk Co., Milford, Delaware, U.S.A.

** Lee Pharmaceuticals, South El Monte, California, U.S.A.
was used to cover the etched enamel prior to the application of *Epoxylite Fissure Sealer 9075*. **#

**RESINS**

a) *Nuva-Seal* (a BIS-GMA resin diluted 3:1 by weight with methyl methacrylate monomer (35)) was supplied in 1 millilitre quantities in stoppered glass bottles, whilst the initiator (2 per cent benzoin methyl ether (35) was supplied separately in a plastic dropper bottle (Fig. 4). A *Nuva-Lite* (an ultraviolet light source with a wavelength of approximately 3,600 angstroms (163)) was used to cure the resin (Fig. 5).

b) *Epoxylite Fissure Sealer 9075** (a blend of diacrylate resins (113) of which BIS-GMA resin is a component (161) was supplied in 2 separate bottles. One bottle (*Epoxylite Fissure Sealer 9075-A*) contained the resin plus added benzoyl peroxide catalyst (110) (113) whilst the other bottle (*Epoxylite Fissure Sealer 9075-B*) contained the resin plus a tertiary amine accelerator (110) (Fig. 6).

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* L.D. Caulk Co., Milford, Delaware, U.S.A.

** Lee Pharmaceuticals, South El Monte, California, U.S.A.

# Hereafter, referred to as "Epoxylite 9075".
FIGURE 4

Materials used for enamel preparation and subsequent application of Nuva-Seal resin.
The ultraviolet light source (Nuva-Lite) used to cure Nuva-Seal resin.
Materials used for enamel preparation and subsequent application of Epoxylite 9075 resin.

(Bottle 1 - Epoxylite Fissure Cleanser; Bottle 2 - Epoxylite Fissure Primer 9075; Bottle 3 - Epoxylite Fissure Sealer 9075-A; Bottle 4 - Epoxylite Fissure Sealer 9075-B; Bottle 5 - Benzoyl Peroxide Catalyst to be added to Bottle 3).
METHOD

The occlusal surface of each tooth crown was cleaned by applying a slurry of pumice and water on a rotating bristle brush in a right angled handpiece. A special attempt was made to remove deposits from fissures by scraping with a fine probe. The tooth crowns were then thoroughly washed with a water spray and assigned at random to 2 groups.

APPLICATION OF RESINS TO OCCLUSAL SURFACES

a) NUVA-SEAL

The tooth crowns from the first group were dried with a stream of air and Nuva-Seal Tooth Conditioner applied to the occlusal surfaces with a cotton pellet. After 60 seconds* the teeth were washed, dried under a stream of air and examined visually to determine whether the characteristic uniform, dull satin appearance of a properly etched** enamel surface was present. If any of the surfaces did not have the required appearance, a further application of Nuva-Seal Tooth Conditioner was given. This procedure was continued until the desired surface was achieved.

* Manufacturer's recommended etching time.

** As described by Buonocore. (37)
One drop of initiator was added to one millilitre of Nuva-Seal resin and stirred for 30 seconds and then the activated resin was worked into the fissures, using a fine camel hair brush. Polymerization of the resin was then initiated by a 30 second exposure to the Nuva-Lite. The surface was wiped with a cotton pellet and inspected for deficiencies or defects. The coated crowns were returned to Hanks' Balanced Salt Solution and stored at 37°C for 24 hours.

b) EPOXYLITE 9075

The tooth crowns in the second group were cleaned and dried in the same manner as those in the first group. Epoxylite Fissure Cleanser was applied to the occlusal surfaces following the same technique used for Nuva-Seal Tooth Conditioner and the same criterion for determining properly etched surfaces was also used.

A cotton pellet was saturated with Epoxylite Fissure Primer and applied to the etched areas. These areas were dried for 2 minutes under a stream of air.

Epoxylite Fissure Sealer 9075-A solution was applied to the prepared occlusal surface with a cotton pellet. Polymerization was initiated by adding Epoxylite Fissure Sealer 9075-B to the Epoxylite Fissure Sealer 9075-A
Photograph shows resin coated tooth crowns embedded in dental plaster prior to sectioning by a diamond edged blade under a continuous water spray. Cuts were made parallel to the lines marked on the plaster block.
already in situ on the occlusal surface. Once the resin had polymerized, its surface was wiped with a cotton pellet and inspected for deficiencies or defects. The resin coated tooth crowns were returned to Hanks' Balanced Salt Solution and stored at 37°C for 24 hours.

TOOTH SECTIONING

All the tooth crowns were embedded in dental plaster leaving the occlusal surfaces exposed. Longitudinal plano-parallel cuts were made bucco-lingually through each crown with a diamond edged blade* rotating at 1,425 r.p.m. under a continuous water spray (Fig. 7). Four sections, 200 microns in thickness, were cut from the tooth crowns. One section was taken from the mesial region of each fissure, another from the distal and two sections from the central region. All the sections were washed under running tap water for 30 minutes.

PREPARATION OF PHOTOMICROGRAPHS

Five hundred and twenty sections from each group were examined under incident light using a Leitz Wetzlar** microscope and photographed at 100 times magnification.

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* Jadem Dembijaack Blade No. 27283-E. -- James Dembitzer, Antwerp, Belgium.

** Leitz, Wetzlar, Germany
Diagram illustrating the template of a medium thickness probe (30) in situ in a shallow fissure. The point of the template touches the base of the fissure and the sides of the template do not touch the walls.
Diagram illustrating the template of a medium thickness probe (30) in situ in a deep fissure. The point of the template does not touch the base of the fissure and the sides of the template touch the walls.
ASSESSMENT OF RESIN PENETRATION

For assessment purposes, the fissures were divided into 2 types, "shallow" and "deep". To do this, a template 100 times the dimensions specified by the British Standard for medium thickness probes, (30) was constructed and superimposed over the photomicrographs. If the point of the template touched the bottom of the fissure without binding at the walls then the fissure was classified as "shallow". If the side of the template touched the outline of the walls before the template point reached the base, the fissure was classified as "deep". (Figs. 8 and 9)

The degree of penetration of the resins was determined from the photomicrographs. (Figs. 10 and 11) If the resin failed to completely fill a fissure, the width at which resin penetration ceased, was measured. (Fig. 12)

The Chi-square test was used to determine whether there were statistically significant differences between the abilities of the resins to penetrate into "shallow" and "deep" fissures. Differences were regarded as being statistically significant at the 5 per cent level of significance.
A photomicrograph showing complete penetration of BIS-GMA resin into a shallow fissure. (Mag. x 160).
FIGURE 11

A photomicrograph showing incomplete penetration of BIS-GMA resin into a deep fissure. (Mag. x 160)
Ordinate diagram showing the range of deep fissure widths at which resin penetration ceased.
2. - MICROLEAKAGE STUDY

MATERIALS

The enamel pretreatment solutions and resins used were identical to those employed in the Fissure Penetration Study. (pp. 32-33)

TEETH

One hundred and twenty contralateral pairs of sound premolar teeth extracted for orthodontic reasons from patients, aged 11 to 13 years, were used. Immediately after extraction, the roots of the teeth were removed and discarded. The crowns were then washed and any remaining pulp tissue removed. The paired crowns were then placed in Hanks' Balanced Salt Solution and stored at 4°C until required.

RADIOACTIVE TRACER SOLUTIONS

Carbon-14 labelled solutions of sodium carbonate, D-glucose (uniformly labelled) and sucrose (uniformly labelled) were selected as tracer solutions.*

All tracer solutions had a specific activity of 200 microcuries per millilitre and were adjusted to be isosmotic with normal saline by the addition of the necessary quantities of sodium chloride.

* Source of Origin: Radiochemical Centre, Amersham, Bucks., U.K.
METHOD

Thirty contralateral pairs of tooth crowns were assigned at random to each of the treatment groups. In each tooth pair, the occlusal surfaces were etched and coated with either Nuva-Seal resin or Epoxylite 9075 resin following the procedures outlined in the Fissure Penetration Study. (pp. 37-40)

TREATMENTS

(1) AGEING

a) 3 Months

The occlusal surfaces of the teeth in this treatment group were coated with the respective resins then stored for 3 months in Hanks' Balanced Salt Solution maintained at 37°C, the solution being changed daily.

b) 6 Months

The occlusal surfaces of the teeth in this treatment group were coated with the respective resins then stored for 6 months in Hanks' Balanced Salt Solution maintained at 37°C, the solution being changed daily.

(2) THERMAL CYCLING

The occlusal surfaces of the teeth in this treatment group were coated with the respective resins then subjected to thermal cycling by alternately immersing a chamber
Photograph shows equipment used for thermal cycling specimens.

A. Water bath maintained at 4°C.
B. Water bath maintained at 60°C.
C. Six volt battery power source.
D. Timing mechanism to control immersion times.
E. Battery powered motor with attached metal arm which oscillated between baths.
F. Perforated chamber containing the specimens.
containing the specimens in water baths maintained at temperatures of 4°C and 60°C. The immersion time in each temperature bath was 30 seconds and the group was subjected to a total of 200 cycles.

The cycling was carried out automatically. A metal arm, driven by a battery powered motor and supporting the tooth crowns in a chamber, oscillated between the temperature baths. A timing mechanism allowed the chamber containing the resin coated crowns to be immersed in the baths for the required 30 seconds (Fig. 13).

(3) 24 HOUR CONTROL

The occlusal surfaces of the teeth in this treatment group were coated with the respective resins then stored for 24 hours at 37°C in Hanks' Balanced Salt Solution.

At the completion of the experimental treatments the teeth were prepared for immersion in the various radioisotope solutions.

PREPARATION OF SPECIMENS FOR IMMERSION IN RADIOISOTOPE SOLUTIONS

The walls of the pulp chambers in the resin coated crowns, were smoothed with round stones then coated with 2 layers of varnish* which was extended to the edge of the

* Copalite Varnish, Cooley and Cooley Ltd., Houston, Texas.
cut root surface. Subsequently, the pulp chamber was filled with paraffin based wax* and the cut root surface covered with sticky wax.** The whole of the crown surface, except for an area approximately one millimetre wide around the resin coating, was then covered with a thin layer of nail varnish. When the first layer of varnish had dried, a second coating was applied. These procedures were undertaken in an attempt to limit radioisotope penetration to the vicinity of the tooth-resin junction.

While the crowns were being coated, the resin margins were protected by a coating of water soluble Addent Wax.*** This wax was subsequently washed off under running water.

**IMMERSION OF TOOTH CROWNS IN RADIOISOTOPE SOLUTIONS**

Within each treatment group equal numbers of contralateral pairs were immersed in the 3 radioisotope solutions.

The tooth crowns were placed, occlusal surfaces downwards, in 10 millilitre stoppered vials containing the appropriate radioisotope solutions.

After 48 hours at 20°C, the specimens were removed from the radioisotope solutions and washed in running water for one hour.

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** Ainsworth Dental Co., Sydney
*** 3M Company, St. Paul, Minnesota.
Photograph shows sections from resin coated teeth on dental x-ray film before being clamped between perspex blocks. Each block is lined with a layer of foam rubber and a disposable paper napkin.
TOOTH SECTIONING

The tooth crowns were embedded in dental plaster leaving the occlusal surfaces exposed. Longitudinal plano-parallel cuts were then made bucco-lingually, through each crown with a diamond edged blade at 1,425 r.p.m. under a continuous water spray. Four sections, 200 microns in thickness, were cut from each tooth crown. One section was taken from the mesial region of each fissure, another from the distal and two sections from the central region. The sections were washed under running tap water for 30 minutes.

PREPARATION OF AUTORADIOGRAPHS

After washing the sections were dried for 2 hours. Under illumination from a Kodak Watten Safelight, Series 6B,* the sections were placed directly on dental x-ray film emulsion** and clamped between perspex blocks lined with foam rubber covered with a disposable paper napkin. (Fig. 14). The assembled blocks were placed in light tight boxes for 7 days, and then the clamped blocks were disassembled and the films were processed by a standardized technique to produce the autoradiographs.


EVALUATION OF AUTORADIOGRAPHS

Each autoradiograph was placed in a photographic slide mount and projected onto a screen. The depth of penetration of the tracer along the resin-enamel interspace was then observed. If the autoradiograph showed radioisotope penetration along the entire resin-enamel interspace, indicating maximum leakage, the leakage was assigned a value of 2. If the autoradiograph showed radioisotope penetration along part of the resin-enamel interspace, indicating slight leakage, the leakage was assigned a value of one. A zero value was assigned in instances where there was no evidence of tracer penetration.

Although 4 autoradiographic images were obtained for each tooth crown, only the highest value was used for analysis.

A Split Plot Analysis of Variance was carried out on duplicate readings of the autoradiographs to determine if there were statistically significant differences between the two resins in preventing microleakage of the tracer solutions. The analysis also was used to evaluate the significance of the effects produced by the experimental conditions and also to determine if there were differences in the abilities of the 3 tracer solutions to penetrate any spaces between the resin coatings and the tooth surface.
The Analysis of Variance was carried out for duplicate readings of the autoradiographs to determine the consistency of the reading method. Differences were regarded as being statistically significant at the 5 per cent level of significance.
RESULTS

1. FISSURE PENETRATION STUDY

One thousand and forty sections were examined. Thirty hundred and ninety of the 1,040 sections examined had shallow fissures while the remaining 650 had deep fissures.

Table 1 shows the incidence of incompletely and completely filled shallow and deep fissures for Nuva-Seal resin and Epoxylite 9075 resin.

<table>
<thead>
<tr>
<th>RESIN</th>
<th>SHALLOW FISSURES</th>
<th>DEEP FISSURES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incompletely</td>
<td>Completely</td>
</tr>
<tr>
<td></td>
<td>filled</td>
<td>filled</td>
</tr>
<tr>
<td>Nuva-Seal</td>
<td>19</td>
<td>166</td>
</tr>
<tr>
<td>Epoxylite 9075</td>
<td>37</td>
<td>168</td>
</tr>
<tr>
<td>Totals</td>
<td>56</td>
<td>334</td>
</tr>
</tbody>
</table>

Shallow Fissures
Resin versus Degree of Penetration
$\chi^2 = 4.78 \ p < 0.05$
(significant)

Deep Fissures
Resin versus Degree of Penetration
$\chi^2 = 0.883$
(not significant)
RESIN PENETRATION INTO SHALLOW FISSURES

One hundred and sixty six of the 185 (89.7 per cent) shallow fissures coated with Nuva-Seal resin were completely filled by the resin while 168 of the 205 (82.0 per cent) shallow fissures coated with EpoxyLite 9075 resin were also completely filled by the resin. (Table 1)

The Chi-square analysis in Table 1 shows that there are statistically significant differences between the abilities of Nuva-Seal and EpoxyLite 9075 resins to penetrate and fill shallow fissures, (p<0.05).

RESIN PENETRATION INTO DEEP FISSURES

Only 12 of the 335 (3.6 per cent) deep fissures coated with Nuva-Seal resin were completely filled by the resin while only 16 of the 315 (5.1 per cent) deep fissures coated with EpoxyLite 9075 resin were also completely filled by the resin. (Table 1)

The Chi-square analysis in Table 1 shows that there are no statistically significant differences between the abilities of Nuva-Seal and EpoxyLite 9075 resins to penetrate and fill deep fissures.

DEEP FISSURE WIDTHS AT WHICH RESIN PENETRATION CEASED

The range of deep fissure widths and the frequencies with which these widths were involved in incomplete penetration of resin, are presented in Fig. 12. The majority
<table>
<thead>
<tr>
<th>TREATMENT GROUP</th>
<th>RESIN</th>
<th>SODIUM CARBONATE $^{14}$C</th>
<th>D – GLUCOSE $^{14}$C</th>
<th>SUCROSE $^{14}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of specimens</td>
<td>No. of specimens</td>
<td>No. of specimens</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(a) (b) (c)</td>
<td>(a) (b) (c)</td>
<td>(a) (b) (c)</td>
</tr>
<tr>
<td>24 HOUR CONTROL</td>
<td>Nuva Seal</td>
<td>10 (7) 0(2) 3(1)</td>
<td>10 7(5) 2(1) 1(3)</td>
<td>10 6(6) 2(2) 2(2)</td>
</tr>
<tr>
<td></td>
<td>Epox. 9075</td>
<td>10 4(4) 2(3) 4(3)</td>
<td>10 3(2) 1(1) 6(7)</td>
<td>10 7(7) 0(2) 3(1)</td>
</tr>
<tr>
<td>THERMAL CYCLING</td>
<td>Nuva Seal</td>
<td>10 4(4) 5(4) 1(2)</td>
<td>10 9(10) 1(0) 0(0)</td>
<td>10 9(8) 0(2) 1(0)</td>
</tr>
<tr>
<td></td>
<td>Epox. 9075</td>
<td>10 0(0) 6(5) 4(5)</td>
<td>10 8(9) 2(1) 0(0)</td>
<td>10 6(7) 0(0) 4(3)</td>
</tr>
<tr>
<td>3 MONTHS</td>
<td>Nuva Seal</td>
<td>10 2(4) 3(2) 5(4)</td>
<td>10 5(6) 4(3) 1(1)</td>
<td>10 3(3) 1(2) 6(5)</td>
</tr>
<tr>
<td></td>
<td>Epox. 9075</td>
<td>10 4(2) 1(3) 5(5)</td>
<td>10 3(4) 2(1) 5(5)</td>
<td>10 1(1) 2(3) 7(6)</td>
</tr>
<tr>
<td>6 MONTHS</td>
<td>Nuva Seal</td>
<td>10 1(3) 5(5) 4(2)</td>
<td>10 5(5) 1(1) 4(4)</td>
<td>10 6(5) 2(3) 2(2)</td>
</tr>
<tr>
<td></td>
<td>Epox. 9075</td>
<td>10 4(4) 6(6) 0(0)</td>
<td>10 3(3) 4(4) 3(3)</td>
<td>10 3(3) 1(1) 6(6)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses indicate the incidence of maximum, slight and zero microleakage scores obtained for the 2nd. reading of the autoradiographs.
TABLE 3

SPLIT PLOT ANALYSIS OF VARIANCE

<table>
<thead>
<tr>
<th>SOURCE OF VARIATION</th>
<th>DEGREES OF FREEDOM</th>
<th>SUM OF SQUARES</th>
<th>MEAN SQUARES</th>
<th>F. RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grand Total</td>
<td>1</td>
<td>644.03</td>
<td>644.03</td>
<td></td>
</tr>
<tr>
<td>Treatments</td>
<td>3</td>
<td>22.85</td>
<td>7.617</td>
<td>19.733 (p &lt; 0.001)</td>
</tr>
<tr>
<td>Radioisotopes</td>
<td>2</td>
<td>4.754</td>
<td>2.377</td>
<td>6.158 (p &lt; 0.01)</td>
</tr>
<tr>
<td>Treatments x Radioisotopes</td>
<td>6</td>
<td>25.963</td>
<td>4.327</td>
<td>11.210 (p &lt; 0.001)</td>
</tr>
<tr>
<td>Whole Plot Error</td>
<td>108</td>
<td>41.403</td>
<td>0.386</td>
<td></td>
</tr>
</tbody>
</table>

<p>| Resins                      | 1                   | 11.408         | 11.408       | 13.281 (p &lt; 0.001) |
| Resins x Treatments         | 3                   | 2.242          | 0.747        | 0.870 N.S.         |
| Resins x Radioisotopes      | 2                   | 1.179          | 0.589        | 0.686 N.S.         |
| Resins x Treatments x Radioisotopes | 6   | 13.371         | 2.229        | 2.595 N.S.         |
| Split Plot Error            | 108                 | 92.800         | 0.859        |            |
| Total within Resins         | 120                 | 121.00         | 1.008        |            |
| Total                       | 240                 | 981.00         | 3.583        |            |
| Residual                    | 240                 | 21.00          | 0.086        |            |
| Total                       | 480                 | 1002.00        |              |            |</p>
<table>
<thead>
<tr>
<th>TREATMENT GROUP</th>
<th>RADIOISOTOPE GROUP</th>
<th>SODIUM CARBONATE $^{14}$C</th>
<th>GLUCOSE $^{14}$C</th>
<th>SUCROSE $^{14}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nuva-Seal</td>
<td>Epoxylite 9075</td>
<td>Nuva-Seal</td>
<td>Epoxylite 9075</td>
</tr>
<tr>
<td>24 HOUR CONTROL</td>
<td></td>
<td>1.50</td>
<td>1.05</td>
<td>1.45</td>
</tr>
<tr>
<td></td>
<td>S.D. = 0.716</td>
<td>S.D. = 0.510</td>
<td>S.D. = 0.224</td>
<td>S.D. = 0.366</td>
</tr>
<tr>
<td>THERMAL CYCLING</td>
<td>1.25</td>
<td>0.55</td>
<td>1.95</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td>S.D. = 0.716</td>
<td>S.D. = 0.510</td>
<td>S.D. = 0.224</td>
<td>S.D. = 0.366</td>
</tr>
<tr>
<td>3 MONTHS</td>
<td>0.85</td>
<td>0.80</td>
<td>1.45</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>S.D. = 0.875</td>
<td>S.D. = 0.894</td>
<td>S.D. = 0.686</td>
<td>S.D. = 0.933</td>
</tr>
<tr>
<td>6 MONTHS</td>
<td>0.90</td>
<td>1.40</td>
<td>1.10</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>S.D. = 0.718</td>
<td>S.D. = 0.501</td>
<td>S.D. = 0.968</td>
<td>S.D. = 0.795</td>
</tr>
</tbody>
</table>

* Mean of the 1st and 2nd readings combined.
† S.D. = Standard Deviation
### TABLE 4B

**MEAN MICROLEAKAGE SCORES FOR TREATMENT GROUPS**

<table>
<thead>
<tr>
<th>24 HOUR \ THERMAL \ 3 \ 6 CONTROL CYCLING MONTHS MONTHS</th>
<th>Nuva-</th>
<th>Epoxylite</th>
<th>Nuva-</th>
<th>Epoxylite</th>
<th>Nuva-</th>
<th>Epoxylite</th>
<th>Nuva-</th>
<th>Epoxylite</th>
</tr>
</thead>
<tbody>
<tr>
<td>\ SEAL \ 9075 SEAL \ 9075 SEAL \ 9075 SEAL \ 9075 SEAL \ 9075</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.45</td>
<td>1.05</td>
<td>1.67</td>
<td>1.27</td>
<td>1.02</td>
<td>0.70</td>
<td>1.12</td>
<td>1.03</td>
<td></td>
</tr>
<tr>
<td>S.D. = 0.811</td>
<td>S.D. = 0.928</td>
<td>S.D. = 0.601</td>
<td>S.D. = 0.851</td>
<td>S.D. = 0.873</td>
<td>S.D. = 0.850</td>
<td>S.D. = 0.846</td>
<td>S.D. = 0.802</td>
<td></td>
</tr>
</tbody>
</table>

* Mean of the 1st and 2nd readings combined

### TABLE 4C

**MEAN MICROLEAKAGE SCORES FOR RADIOISOTOPE GROUPS**

<table>
<thead>
<tr>
<th>SODIUM CARBONATE \ D-GLUCOSE \ SUCROSE</th>
<th>Nuva- \ Epoxylite</th>
<th>Nuva- \ Epoxylite</th>
<th>Nuva- \ Epoxylite</th>
</tr>
</thead>
<tbody>
<tr>
<td>\ SEAL \ 9075 SEAL \ 9075 SEAL \ 9075</td>
<td>Nuva- \ Epoxylite</td>
<td>Nuva- \ Epoxylite</td>
<td>Nuva- \ Epoxylite</td>
</tr>
<tr>
<td>1.13</td>
<td>0.95</td>
<td>1.49</td>
<td>1.08</td>
</tr>
<tr>
<td>S.D. = 0.817</td>
<td>S.D. = 0.778</td>
<td>S.D. = 0.775</td>
<td>S.D. = 0.897</td>
</tr>
</tbody>
</table>

* Mean of the 1st and 2nd readings combined.

**Overall Mean Microleakage Score**

Nuva-Seal = 1.3 (S.D. = 0.829)  Epoxylite 9075 = 1.0 (S.D. = 0.879)
<table>
<thead>
<tr>
<th></th>
<th>Sodium Carbonate</th>
<th>Glucose</th>
<th>Sucrose</th>
<th>Treatment Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Error of differences = 0.139</td>
<td></td>
<td></td>
<td></td>
<td>Standard Error of differences = 0.081</td>
</tr>
<tr>
<td>24 Hour Control Group</td>
<td>1.28</td>
<td>1.03</td>
<td>1.45</td>
<td>1.25</td>
</tr>
<tr>
<td>Thermal Cycling Group</td>
<td>0.90</td>
<td>1.90</td>
<td>1.55</td>
<td>1.45</td>
</tr>
<tr>
<td>3 Months Aged Group</td>
<td>0.83</td>
<td>1.15</td>
<td>0.60</td>
<td>0.86</td>
</tr>
<tr>
<td>6 Months Aged Group</td>
<td>1.15</td>
<td>1.05</td>
<td>1.03</td>
<td>1.08</td>
</tr>
<tr>
<td>Isotope Groups</td>
<td>1.04</td>
<td>1.28</td>
<td>1.16</td>
<td></td>
</tr>
</tbody>
</table>

* The means were obtained from the combined scores of Nuva-Seed and EpoxyLite 9075 resins and the combined 1st and 2nd readings.
Examples of autoradiographs showing maximum, slight and zero microleakage.

A. Maximum microleakage (2 score).
   Carbon-14 has penetrated along the entire resin-tooth interspace.

B. Slight microleakage (1 score).
   Carbon-14 has penetrated along part of the resin-tooth interspace.

C. Zero microleakage (0 score).
   No carbon-14 has penetrated along the resin-tooth interspace.
Examples of autoradiographs of ground sections from specimens coated with Nuva-Seal or Epoxylite 9075 resin. Control, thermal cycled, 3 months and 6 months specimens had been immersed in sodium carbonate-$^{14}$C solution for 48 hours prior to preparation of the autoradiographs.
FIGURE 16

SODIUM CARBONATE-14C

Nuva-Seal  Epoxylite 9075

24 HOUR CONTROL

THERMAL CYCLING

3 MONTHS

6 MONTHS
Examples of autoradiographs of ground sections from specimens coated with Nuva-Seal or Epoxylite 9075 resin. Control, thermal cycled, 3 months and 6 months specimens had been immersed in D-glucose-$^{14}$C solution for 48 hours prior to preparation of the autoradiographs.
FIGURE 17

D-GLUCOSE$^{14}$C

Nuva-Seal  Epoxylite 9075

24 HOUR CONTROL

THERMAL CYCLING

3 MONTHS

6 MONTHS
Examples of autoradiographs of ground sections from specimens coated with Nuva-Seal or Epoxylite 9075 resin. Control, thermal cycled, 3 months and 6 months specimens had been immersed in sucrose-\textsuperscript{14}C solution for 48 hours prior to preparation of the autoradiographs.
FIGURE 18

SUCROSE-$^{14}$C

<table>
<thead>
<tr>
<th>Nuva-Seal</th>
<th>Epoxylite 9075</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 HOUR CONTROL</td>
<td></td>
</tr>
<tr>
<td>THERMAL CYCLING</td>
<td></td>
</tr>
<tr>
<td>3 MONTHS</td>
<td></td>
</tr>
<tr>
<td>6 MONTHS</td>
<td></td>
</tr>
</tbody>
</table>
of the widths involved lie in the range 30-200 microns for both BIS-GMA resins.

2 - MICROLEAKAGE STUDY

Table 2 shows the incidence of maximum (2), slight (1) and zero (0) microleakage scores. Examples of autoradiographs showing maximum, slight and zero microleakage are presented in Fig. 15 and examples of the autoradiographic findings for the radioisotope and treatment groups are shown in Figs. 16,17 and 18. The findings of the Split Plot Analysis of Variance for the microleakage scores are presented in Table 3.

Tables 4A, 4B and 4C show the mean microleakage scores (1st and 2nd readings combined) for the treatment and radioisotope groups for Nuva-Seal and Epoxylite 9075 resins. The mean microleakage scores (1st and 2nd readings combined) for the treatment and radioisotope groups, using values obtained by combining the microleakage scores for both Nuva-Seal and Epoxylite 9075 resins, are shown in Table 5.

DIFFERENCE BETWEEN RESINS

The overall mean microleakage score per tooth for Nuva-Seal resin was 1.3 (S.D.=0.829) whilst the overall mean microleakage score per tooth for Epoxylite 9075 resin
was 1.0 (S.D.=0.879), the standard error of the difference being 0.085. The analysis of variance (Table 3) shows that the difference in microleakage scores for Nuva-Seal resin and Epoxylite 9075 resin was statistically significant (p<0.001).

The analysis of variance (Table 3) also shows that there was no statistically significant interaction between either of the BIS-GMA resins and the treatments or between either of the BIS-GMA resins and the radioisotope solutions.

**DIFFERENCE BETWEEN RADIOISO TOPE TRACER SOLUTIONS**

Table 3 shows that the differences in the degree of microleakage observed for the 3 radioisotope solutions were statistically significant (p<0.01).

The table of mean microleakage scores for the resins combined (Table 5) indicates that, overall, a higher mean microleakage score was recorded for D-glucose tracer solution (1.28) than for sucrose and sodium carbonate tracer solutions (1.16 and 1.04, respectively). However, this same trend was not reflected within the treatment groups (Table 5) and the interaction effects were found to be statistically significant (p<0.001) from the analysis of variance (Table 3). Hence, care is needed in interpreting the differences in microleakage between the radioisotope groups.
DIFFERENCE BETWEEN TREATMENT GROUPS

Table 3 shows that the differences in the amounts of microleakage produced by the treatments to which the BIS-GMA resins were subjected, were statistically significant \((p<0.001)\).

The table of mean microleakage scores for the resins combined (Table 5) indicates that, overall, thermal cycling tended to increase the mean microleakage score whilst ageing tended to reduce it. The mean microleakage score for specimens subjected to thermal cycling was 1.45, whilst that for specimens aged for 3 months or 6 months was 0.86 and 1.08, respectively. By comparison, the mean microleakage score for the 24 hour control specimens was 1.25. However, this trend was not reflected within the radioisotope groups (Table 5) and the interaction effects were found to be statistically significant \((p<0.001)\) from the analysis of variance (Table 3). Hence, care is needed in interpreting the differences in microleakage between the treatment groups.

CONSISTENCY OF READINGS

The analysis of variance was conducted on values obtained from duplicate readings of the autoradiographs. The consistency of the reading method was evaluated by comparing the residual mean square with the whole plot
error mean square (Table 3). The value for the residual mean square (0.086) was found to be small in relation to the value for the whole plot error mean square (0.386), thereby indicating that the reading method was consistent.
DISCUSSION

Although, in this study, both Nuva-Seal and EpoxyLite 9075 penetrated shallow fissures far more readily than deep fissures, 14.6 per cent of the shallow fissures were still not completely penetrated. This, together with the low incidence of complete penetration (4.3 per cent) observed in deep fissures, is in marked contrast to the findings of Lee et al. (110) and Newhouse and Roydhouse. (132) Lee et al. (110) claimed that the ability of EpoxyLite 9075 resin to fill deep fissures was "excellent" (111) while Newhouse and Roydhouse (132) in an in vitro study of EpoxyLite 9075 claimed that the resin completely penetrated all the fissures examined.

The results of this study support the observations of Taylor and Gwinnett (181) who reported that shallow fissures were more often completely penetrated by BIS-GMA resins than narrow, deep fissures. Several other investigators (39)(79)(149)(190)(191) have also found incomplete penetration of deep fissures by BIS-GMA resins.

O'Brien, (137) using a model isolated capillary system, has indicated that maximum penetration of liquids into crevices is more favourable when the crevice is narrow. However, such findings do not seem applicable
to fissures in human teeth. Wright and Beck (191) found that BIS-GMA resins did not completely penetrate fissures with widths less than 60 to 100 microns. Similarly, in this study, very few narrow, deep fissures were completely penetrated by the BIS-GMA resins but, the range of fissure widths at which resin penetration stopped, (Fig. 12) was quite large (30-380 microns). Taylor and Gwinnett (181) suggested that debris within the fissures, air entrapment, certain properties of the resins (for example, viscosity), and the morphology of pits and fissures may all influence the degree of resin penetration. Clearly more work is needed to establish the significance of each of these factors in the penetration of fissures by BIS-GMA resins.

It appears as though conventional cleaning procedures with pumice or cleaning pastes together with the use of acids employed for etching enamel, are ineffective in completely removing deposits from deep fissures. Buonocore (39) and Gwinnett and Ripa (79) have commented on the inability of conventional cleaning procedures to remove deposits from such sites, while Gwinnett and Buonocore (77) confirmed this by using scanning electron microscopy to examine deep fissures that had been subject to conventional cleaning procedures and then treated with acid.
As far as can be ascertained from the literature, there have been few attempts to determine the composition of the deposits remaining within fissures incompletely penetrated by BIS-GMA resins. However, there have been suggestions that such deposits may contain microorganisms, remnants of the enamel organ and debris from the cleaning procedures.

Although the viability of microorganisms in carious dentine left below restorations has been shown to be maintained for prolonged periods, little attention has been given to the question of the viability of microorganisms in deposits remaining under resin coatings. Reports by Handleman, Buonocore and Heseck and Handleman, Buonocore and Schoute have indicated large reductions in the numbers of culturable microorganisms from occlusal carious lesions which had been covered with Nuva-Seal BIS-GMA resin for periods of up to 6 months. However, an in vivo study by Mednick, Loesche and Corpron indicated that bacteria, placed beneath Nuva-Seal resin fissure coatings, could remain viable after test periods of 4 to 16 weeks. Newbrun, Plasschaert and Konig, in an in vivo study involving rat molars, reported that there was some evidence of increases in caries rates in fissures treated with
*Nuva-Seal, Epoxylite 9075* and *Epoxylite 9070* resins. Although their findings suggest that the viability of entrapped microorganisms was not reduced, they departed from the normal cleaning protocol prior to application of the resins. Debris was only scraped from the fissures with a probe and acetone was applied to aid drying of the etched surfaces. These alterations in tooth preparation may have prevented adaptation of the resins to the fissure walls and could explain the increase in caries activity observed in the treated teeth.

Prolonged viability of bacteria, possibly resident in debris below resin coatings, would require that such microorganisms receive a sufficient supply of substrate. Wright and Beck (191) have suggested that this would not occur if fissure coatings maintained an efficient marginal seal.

Under the conditions of this study, microleakage did occur in both *Nuva-Seal* and *Epoxylite 9075* specimens. Although *Epoxylite 9075* resin showed less microleakage than *Nuva-Seal* resin, both resins exhibited a high incidence of microleakage, the mean microleakage score for *Nuva-Seal* resin being 1.3 and that for *Epoxylite 9075* being 1.0.

The differences in microleakage observed for *Nuva-Seal* and *Epoxylite 9075* resins may be related to the
enamel pretreatment techniques or the physical properties of the materials themselves. In particular, the lower degree of microleakage observed for Epoxylite 9075 specimens, may be related to the use of the silane primer solution (Epoxylite Fissure Primer 9075) after acid etching. The use of such special coupling agents has been suggested as a means of enhancing the bonding of polymeric resins to enamel. (28)(45)(107) It has been postulated that organofunctional silanes are able to act as coupling agents and improve the adhesion of polymeric resins to tooth structure. (28)(106)(107)

Silane coupling agents have been used to improve bonding of silica filler particles to a BIS-GMA resin binding matrix in composite restorative materials. (24) Silane coupling agents are also hydrophobic (28)(170) and there have been indications (106)(107) that such agents may provide improved resistance to the rupture of adhesive bonds in the presence of moisture. Adhesive bonds may be disrupted by moisture from saliva (34)(153) or enamel fluids. (14)(15)(16) The use of a silane primer solution and the lower viscosity (113) of Epoxylite 9075 may be factors which have influenced the lower incidence of microleakage of Epoxylite 9075 resin in this study. The effect of coupling agents on the sealing properties of resin fissure coatings warrants further investigation.
In this study, 64 of the 120 Nuva-Seal specimens (53.3 per cent) and 46 of the 120 Epoxylite 9075 specimens (38.3 per cent) exhibited maximum microlakage (Table 2). The high incidence of microlakage observed for resins in this study contrasts markedly with the findings of Rudolph, Phillips and Swartz (166) and Woody, Moffa and McCune. (190) Rudolph, Phillips and Swartz (166) found that none of the 60 Epoxylite 9075 specimens and only 4 of the 60 Nuva-Seal specimens showed maximum microlakage when the fissure sealing properties of these resins were evaluated with $^{45}$Ca. Woody, Moffa and McCune (190) did not present quantitative data, but indicated that the ultraviolet light initiated BIS-GMA resin, which they assessed, prevented microlakage of $^{45}$Ca.

The results of this study, however, support the findings of the microlakage studies of Crowe, (50) Craig, (48) Pink, Corpron and Loesche (146) and Mednick, Loesche and Corpron. (123) These studies also found that microlakage of BIS-GMA resin fissure coatings occurred, when their sealing properties were tested with dyes (48) (50) and bacteria. (123)(146)

The findings of this study also show that the treatments to which the fissure coatings were exposed, influenced the amounts of microlakage that occurred. The differences in the amounts of microlakage produced by the treatments were found to be statistically significant
but care should be taken in interpreting these results since the interaction between the treatment groups and the radioisotopes was also found to be statistically significant (Table 3). The results show that thermal cycling tended to increase the amounts of microleakage whilst there was a trend towards reduced microleakage after ageing. (Table 5). The incidence of maximum microleakage scores reflects these trends. (Table 2). Thirty six of the 60 specimens subjected to thermal cycling and 34 of the 60 specimens in the control group showed maximum microleakage. Furthermore, only 18 of the 60 specimens exhibited maximum microleakage scores after 3 months ageing whereas 22 of the 60 specimens showed maximum microleakage after 6 months.

Craig (48) gave no quantitative findings but indicated that both Nuva-Seal and Epoxylite 9075 resins exhibited some degree of dye microleakage after thermal cycling. Crowe (50) did not state what effects ageing produced in the microleakage patterns of the Epoxylite 9075 specimens but he did indicate that thermal cycling had little effect on the overall results. Rudolph, Phillips and Swartz (166) indicated that thermal cycling or ageing for 3 months had little effect on the microleakage of BIS-GMA resin fissure coatings. Woody, Moffa and McCune (190) gave no indication of the influence of ageing and thermal cycling
on the degree of microleakage observed for an ultra-violet light initiated BIS-GMA resin.

The results of this study also indicate that, in vitro, fermentable carbohydrates are capable of penetrating between BIS-GMA resins and the walls of fissures.

Forty three of the 80 specimens assessed for microleakage with D-glucose-$^{14}$C solution and 41 of the 80 specimens assessed for microleakage with sucrose-$^{14}$C solution, showed maximum microleakage. (Table 2). In contrast, 26 of the 80 specimens assessed for microleakage with sodium carbonate-$^{14}$C solution, showed maximum microleakage. Although the differences in the amounts of microleakage that were recorded by these tracer solutions were found to be statistically significant, care should be taken in interpreting the results since the interaction between the isotope solutions and the treatment groups was also statistically significant. (Table 3).

Carbon-14 labelled sugar solutions have been used previously in some studies to assess microleakage. Cantwell et al. (44) used carbon-14 labelled fructose to assess microleakage around amalgam restorations while Hardwick (84) and Fremlin and Mathieson (63) have used
carbon-14 labelled glucose to assess the permeability of enamel and dentine. As far as can be determined from the literature, there have been no microleakage studies in which carbon-14 labelled sucrose was used as a tracer.

All of the reported microleakage studies assessing BIS-GMA resin fissure coatings with radioisotope solutions have involved the use of $^{45}\text{Ca}$ as calcium chloride. (166)(190) There have been no previously reported attempts to assess the microleakage of fissure coating resins with labelled sugars as tracer solutions. Craig (47) found $^{45}\text{CaCl}_2$ to be the least effective of 5 tracers ($^{45}\text{CaCl}_2$, $\text{Na}_2^{35}\text{SO}_4$, $^{22}\text{NaCl}$, $\text{Na}^{131}\text{I}$, $\text{Na}_2^{14}\text{CO}_3$) used to assess microleakage of composite resin and silicate cement restorations and found in his study that $^{14}\text{C}$ as $\text{Na}_2^{14}\text{CO}_3$ was the most suitable. Carbon-14 labelled glucose and sucrose were selected for use in this study since both these sugars can act as substrate for various microorganisms. (120)

It is not known whether the radioisotopes used in this study underwent any degradation into smaller molecules and thereby facilitated carbon-14 penetration along the tooth-resin interspace. However, Fremlin and Mathieson, (63) in their study of the penetration of
enamel by $^{14}$C-labelled glucose, were able to demonstrate by the use of paper chromatography that the major part of the radioactivity observed was due to unaltered glucose molecules. No study has been carried out to determine whether $^{14}$C-labelled sodium carbonate or sucrose remains unaltered in similar circumstances.

In view of the findings of this study and other microleakage studies, (48)(50)(123)(146) it seems desirable that greater attention be given to the assessment of the soundness of fissures coated with BIS-GMA resins when these materials are used in vivo. The incidence of retention of fissure coatings is recorded in all of the clinical trials reported to date. However, retention of fissure coatings gives little indication of the soundness of the underlying fissures especially if there is a possibility of microleakage occurring. With one exception, (37) it appears that only those pits and fissures which have lost their resin coatings have been adequately inspected. Since only those teeth which have lost resin coatings have fissures accessible to probing, those teeth with fully retained fissure coatings can only be assessed for caries visually or radiographically. Miller and Hobson (126) have shown that radiographs rarely show carious lesions in fissures
before they can be detected by probing. Therefore, it appears that if radiographs of resin coated teeth are taken, early carious lesions in fissures may remain undetected.

Studies by Parfitt (140) and Boyd, Wessels and Leighton (27) have indicated that the time taken for an incipient occlusal cavity to progress to a more clinically apparent cavity, can be long and variable. Some of the 1,011 carious lesions in pits and fissures observed by Parfitt (140) remained in the early stages for as long as 4 years. If caries were to develop under fully retained fissure coatings then the initial stages would defy detection by probing and there is little likelihood that such early stages would be diagnosed on radiographs. Such findings would indicate that a true assessment of the caries inhibitory effects produced by rigid fissure coatings should ideally involve removal of all coatings for inspection of the underlying fissures, supplemented by a bitewing radiographic survey. Alternatively, clinical studies of rigid pit and fissure coatings should be of several years duration, if radiographs are to be the sole means of caries detection. To date, only 5 of the reported clinical trials, (37)(87)(91)(162)(165) involving BIS-GMA resins, have extended for 2 or more years and in only 2 of these studies (37)(87) have the investigators indicated that bitewing radiographs were taken
at recall examinations.

Buonocore (35)(37) has conducted one of the longest trials of BIS-GMA resin fissure coatings. However, "about half" (36) of the teeth involved in his study were treated with a resin formulation which differs from that of the commercially available BIS-GMA products. These coatings contained 2 additives, synthetic calcium hydroxyapatite and calcium fluoride. It is not known what influence these additives may have had on the treated pits and fissures.

The results of this and other studies of pit and fissure coatings indicate that further work is needed to clarify the following:

1. The effect of silanes or other coupling agents on the sealing efficacy of resin fissure coatings.
2. The value of the use of pit and fissure coatings on shallow fissures in teeth that have been exposed to an optimal level of fluoride during development. This is desirable in view of reports by a number of investigators (22)(23)(57)(101)(102)(145) who have indicated that shallow fissures are less susceptible to dental caries than deep fissures. In addition, it has also been suggested (1)(176) that a trend towards more shallow fissures, induced by the ingestion of fluoride, (1)(46)(68)(103)(114)(176) may, in part, be responsible for the
reduction in occlusal caries incidence in fluoridated communities.

(3) The presence or otherwise of viable microorganisms under fissure coatings and their ability to remain viable in these locations.

(4) The methods of assessing the caries preventive efficacy of fissure coatings in clinical trials. More consideration should be given to assessing the soundness of fissures underlying fully retained coatings since recording the retention periods of coatings and evaluating fissures that have lost their coatings, gives little indication of the true caries preventive efficacy of fissure coating materials.
SUMMARY AND CONCLUSIONS

A review of the clinical trials of BIS-GMA resins used as fissure coatings indicates that this technique shows some promise as a means of preventing pit and fissure caries. However, little attention has been paid to the assessment of the soundness of the pits and fissures covered by these resins. A review of the literature also shows that there have only been a limited number of in vitro studies of the penetrating and sealing properties of BIS-GMA resin fissure coatings, and that there have been conflicting claims regarding the ability of BIS-GMA resins to penetrate and seal pits and fissures.

This study was designed to evaluate, in vitro, the abilities of Nuva-Seal and Epoxylite 9075 resins to penetrate and seal pits and fissures.

The depth of penetration of these BIS-GMA resins into shallow and deep fissures was determined from 260 sound upper premolar teeth extracted for orthodontic reasons. Nuva-Seal and Epoxylite 9075 resins were applied to equal numbers of teeth. Four sections were obtained from each treated tooth and the resulting sections were observed by incident light microscopy to determine the degree of penetration of the respective resins into shallow and deep
fissures. Classification of fissures and assessments of complete or incomplete penetration were determined from photomicrographs at 100 times magnification.

The sealing properties of Nuva-Seal and Epoxylite 9075 resin fissure coatings were assessed in an in vitro study involving 120 contralateral pairs of sound upper premolar teeth extracted for orthodontic reasons. One of each tooth pair was treated with Nuva-Seal resin while the contralateral tooth was treated with Epoxylite 9075 resin. Equal groups of paired specimens were subjected to thermal cycling or ageing for periods of 3 or 6 months and one group was used as a control. The specimens in the treatment groups were immersed in radioisotope solutions of either sodium carbonate-$^{14}$C, D-glucose-$^{14}$C or sucrose-$^{14}$C for a period of 48 hours. Four sections were then obtained from each tooth and the amount of microleakage based upon the degree of carbon-14 penetration between the resin coatings and tooth structure, was determined from autoradiographs prepared from each section.

The results of this study showed that:

1. Shallow fissures were more readily filled by Nuva-Seal and Epoxylite 9075 resins than were deep fissures.

2. Nuva-Seal resin was better than Epoxylite 9075 resin in completely filling shallow fissures.
(3) There was no statistically significant difference in the abilities of Nuva-Seal and Epoxylite 9075 resins to completely fill deep fissures. The incidence of incomplete penetration of deep fissures by both resins was high.

(4) Nuva-Seal resin fissure coatings demonstrated greater degrees of microleakage than Epoxylite 9075 resin fissure coatings.

(5) There was a tendency toward increased microleakage of both Nuva-Seal and Epoxylite 9075 resins after thermal cycling.

(6) There was a tendency towards decreased microleakage of both Nuva-Seal and Epoxylite 9075 resins after ageing for 3 months and 6 months.

(7) There was a tendency for greater microleakage to occur when D-glucose-\(^{14}\)C and sucrose-\(^{14}\)C solutions were used as tracers than when sodium carbonate-\(^{14}\)C solution was used.
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